Capillary Electrophoresis Separations of Inorganic Anions, Cations and Small Molecules

by

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Abstract

Capillary electrophoresis (CE) is a powerful separation technique. The analysis of inorganic ions using capillary electrophoresis is becoming increasingly recognized for its high resolving power, low cost, and the simplicity of design, optimization, and execution of CE experiments. To achieve better resolution and separation efficiency and to minimize the analyte adsorption on the capillary wall, control of the electroosmotic flow (EOF) and capillary surface charge are essential.

Capillary wall coatings have been one of the major solutions to adjust the EOF and minimize analyte adsorption. This thesis demonstrates the optimization of inorganic ion separations using a variety of wall coatings including surfactant-based, cationic polymeric, and successive multiple ionic layered (SMIL) coatings. Specifically, the double chain zwitterionic surfactant 1,2-bis(10,12-tricosadiynoyl)-sn-glycero-3-phosphocholine (Diyne PC) was polymerized to a form neutral, semi-permanent bilayer coatings on the capillary wall which masked the surface charge of the fused silica capillary. A suppressed EOF (2.0×10^{-4} cm^2/Vs) and better peak shape and separation efficiency (N = 300,000 plates/m) were achieved for small amine compounds. Cationic polymers such as poly(diallyl dimethyl ammonium chloride) (PDADMAC) electrostatically adsorb onto the negatively charged capillary wall to yield a semi-permanent cationic coating, resulting in a reversed EOF. However, such coatings are not stable at high pH. It is demonstrated that this instability was due to a surface catalyzed conversion of the quaternary amines of the polycations to tertiary amines. Successive multiple ionic layer coatings prepared from alternating polycation/polyanion layes demonstrated greater alkaline stability as the multiple ionic layers shield the EOF generating final layer from the surface. Alkaline separations of inorganic anions is demonstrated on both the polycation and multiple layer coatings.

Fluorescence detection was investigated for inorganic anion and cation analysis for routine, in-field oilfield water analysis. Indirect fluorescence using 8-hydroxypyrene-1,3,6-trisulfonic acid as the electrolyte probe enabled detection of trace sulfate (0.5 ppm) in high salinity samples (Cl^-~1000 ppm) of oilfield waters . For cation analysis, a fluorescent chelating reagent, fluorescein-thiocarbamyl-1-(4-aminobenzyl) diethylenetriamine-N, N, N’, N”, N”-penta-acetic acid (FTC-AB-DTPA) is used to complex target cations in the sample, and then a cationic polymer, hexadimethrine bromide (polybrene), was added to background electrolyte to form ion association complex (IAC) with the metal-probe complex. The difference in the mobility of the IAC is the driving force for separation and the fluorescent chelating probe enables the direct fluorescence detection of cations. This method is capable of simultaneous detection of multiple heavy metal cations (Mn^{2+}, Pb^{2+}, Cu^{2+}, Cd^{2+}, Ni^{2+}, Zn^{2+} and Co^{2+}) at lower than 10^{-7} M in high calcium matrix sample (Ca^{2+}>1000 ppm).

Preface

This dissertation is submitted for the degree of Doctor of Philosophy at the University of Alberta. All the work was conducted in the Department of Chemistry, University of Alberta under the supervision of Prof. Charles A. Lucy. The thesis consists of six chapters.

This thesis is an original work by Lei Pei. Chapter One is an introduction to the thesis and a review of the associated background. A version of Chapter Two has been published as L. Pei, C. A. Lucy, “Polymerized phospholipid bilayers as permanent coatings for small amine separations using mixed aqueous/organic capillary zone electrophoresis”, *J. Chromatogr. A* 1267 (2012) 80-88. A version of Chapter Three has been published as L. Pei, C. A. Lucy, “Insight into the stability of (diallydimethylammoniumchloride) and polybrene poly cationic coatings in capillary electrophoresis”, J. Chromatogr. A 1365 (2014) 226-233. A version of Chapter Four has been accepted for publications in Anal. Methods as L. Pei, K. J. Schimdt, H. J. Crabtree and C. A. Lucy, “Determination of inorganic anions in oilfield water using capillary electrophoresis with indirect fluorescence detection”. I was responsible for all experimental work and data collection as well as the manuscript composition. Charles A. Lucy was the supervisory author and was involved with concept formation and manuscript composition.

Chapter Four and Chapter Five were collaborative work with Wilson Analytical and HJC Consulting Company. I was responsible for all the CE experimental work. Dr. Kenneth J. Schmidt from Wilson Analytical built the LED light source unit and provided the oilfield water samples. Dr. H. John Crabtree from HJC consulting was involved with concept formation. Dr. Shingo Saito from Saitama University provided the chelating agent used in Chapter Five. Chapter Four and Chapter Five are currently unpublished.

Chapter Six is a summary of the work in the thesis and a brief discussion of possible future projects.

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List of Abbreviations

Abbreviations	**Full Name**
AAS	atomic absorption spectrometry
AB	1-(4-aminobenzyl)
ACN	acetonitrile
AFM	atomic force microscopy
BGE	background electrolyte
C^4D	capacitively coupled contactless conductivity detector
CDTA	cyclohexanediamine tetraacetic acid
CE	capillary electrophoresis
CHES	N-cyclohexyl-2-aminoethanesulfonic acid
CZE	capillary zone electrophoresis
Diyne PC	1,2-bis(10,12- tricosadiynoyl)-*sn*-glycero-3-phosphocholine
DMPC	1,2-dimyristoyl-*sn*-glycero-3-phosphocholine
DMSO	dimethyl sulfoxide
DODAB	dioctadecyldimethylammonium bromide
DOPC	1,2-dioleoyl-sn-glycero-3-phosphocholine
DOTA	1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid
DR	dynamic reserve
DTPA	diethylenetriamine-N, N, N’, N”, N”-penta-acetic acid
EDTA	ethylenediamine tetra acetic acid
EOF	electroosmotic flow
ESI	electrospray ionization
FTC	fluorescein-thiocarbamyl
HPLC	high performance liquid chromatography
HPTS	8-hydroxypyrene-1,3,6-trisulfonic acid
IC	ion chromatography

ICP	inductively coupled plasma
ICP-MS	inductively coupled plasma mass spectrometry
ICP-OES	inductively coupled plasma optical emission spectrometry
I.D.	inner diameter
ISE	ion selective electrode
LD	laser diode
LED	light-emitting diode
LEDIF	light-emitting diode-induced fluorescence
LIF	laser-induced fluorescence
LOD	limit of detection
MBE	moving boundary electrophoresis
MeOH	methanol
MS	mass spectrometry
M.W.	molecular weight
NACE	non-aqueous capillary electrophoresis
O.D.	outer diameter
PC	phosphorylcholine
PDADMAC	poly(diallyldimethylammonium chloride)
PDMA	polydimethylacrylamide
PEEK	polyether ether ketone
PEG	polyethylene glycol
PEI	polyethylenimine
PHEA	poly(hydroxylethylacrylamide)
PMT	photomultiplier tube
ppb	parts-per-billion
ppm	parts-per-million
psi	pounds per square inch (1 psi = 0.06895 bar = 6.895 kPa)
PSS	poly(sodium-4-styrenesulfonate)
PTFE	poly tetrafluoroethylene

RSD	relative standard deviation
SAGD	stream assisted gravity drainage
SMIL	successive multiple ionic layer
S/N	signal-to-noise ratio
TR	transfer ratio
Tris	tris(hydroxylmethyl) aminomethane
UV	ultraviolet
RSD	relative standard deviation
SPB	supported phospholipid bilayer
tITP	transient isotachophoresis
XPS	X-ray photoelectron spectroscopy
ZE	zone electrophoresis

List of Symbols

Symbols	**Full Name**
a	molar absorptivity of analyte
b	optical pathlength
c	concentration of analyte
k_a	rate constant of adsorption
k_d	rate constant of desorption
k_{EMD}	electromigration dispersion factor
l_{inj}	length of injection plug
n	number of replicates
pI	isoelectric point
pKa	negative logarithm of the acid dissociation constant
q	electric charge of an ion
r	capillary inner radius
r_i	radius of an ion
t_m	migration time
v	velocity of analyte
A	Absorbance
A_S	illuminated area of detection window
B/A	peak asymmetry factor
C_{LOD}	theoretical limit of indirect detection
C_p	concentration of photometric probe
D	diffusion coefficient
E	electric field strength
E_t	spectral radiance of light source
I	electric current
K_f	formation constant

L_D	the length from the Inlet of capillary to the detection window
L_T	total length of capillary
N	number of theoretical plates
N_{sys}	number of theoretical plates based on Foley-Dorsey equation
P	power
P_0	intensity of incident light
P_t	intensity of light transmitted through analyte
P_f	Fluorescence intensity of a sample
R	resistance
R_s	resolution
R^2	correlation coefficient
T_f	transmittance of filter
V	applied voltage
W_b	peak width at baseline
$W_{1/2}$	peak width at half maximum height
$W_{0.1}$	peak width at 10% peak height
$\Delta\mu$	difference in electrophoretic mobility
ε_0	permittivity of vacuum
ε_c	transmittance efficiency of collection optics
ε_m	transmittance efficiency of light detection device
ε_r	dielectric constant of medium
ε_x	transmittance efficiency of detection window
ζ	zeta potential
η	viscosity
κ	electrical conductivity
λ	wavelength
μ_{app}	apparent mobility
$\overline{\mu}_{app}$	average apparent mobility of analytes

μ or μ_e	electrophoretic mobility
μ_{EOF}	electroosmotic mobility
ν_m or ν	migration velocity
σ^2_{diff}	variance due to longitudinal diffusion
σ^2_{ads}	variance due to solute-wall adsorption
σ^2_{EMD}	variance due to electromigration dispersion
σ^2_{EXT}	variance due to extra-column band broadening
σ^2_{Joule}	variance due to Joule heating
σ^2_{tot}	total variance of eluted peak
Φ	quantum yield of molecule
Φ_{col}	collection efficiency of fiber optics

Chapter One: Introduction

1.1 Motivation and Thesis Overview

Capillary electrophoresis (CE) has become a popular separation technique due to its high resolving power, low-cost and ease of operation. The analysis of inorganic ions using capillary electrophoresis has wide application in pharmaceutical, industrial, and forensic fields. Hence, a large portion of this thesis focuses on the modification and optimization of CE methods for inorganic anion and cation analysis.

Capillary coatings are commonly used to prevent cation adsorption and to modify the electroosmotic flow (EOF). Chapters 2 and 3 present improvements to the stability of coatings. Various types of semipermanent coatings including surfactant, adsorbed polyelectrolyte, and successive multiple ionic layered (SMIL) coatings, were studied.

In Chapters 4 and 5 the focus of the thesis shifts to detection of inorganic ions in a manner suitable for field analysis. Fluorescence detection based methods for anion and cation analysis are presented in Chapters 4 and 5. In Chapter 4, an indirect fluorescence detection based CE method was developed for anion analysis in high saline oilfield water. Chapter 5 explores a direct fluorescence detection method for transition metal analysis. Chapter 6 summarizes the studies performed in this thesis and suggests future areas of related project development.

1.2 History of Capillary Electrophoresis

Separation by electrophoresis is based on the differential migration of charged species in an electric field. The history of electrophoresis begins with the work of Arne Tiselius in 1937, where he separated a protein mixture in a U-shaped glass tube filled with buffer solution [1]. This technique was known as the “moving boundary electrophoresis” (MBE) technique. MBE only allows partial isolation of the fastest and slowest migrating ions. Zone electrophoresis (ZE), where analytes migrate as well-defined delimited zones, was introduced in the 1950s to address the shortcomings of MBE. Zone electrophoresis can be performed in various media including paper or cellulose acetate membranes. Zone electrophoresis using gel as supporting media was firstly reported by Oliver Smithies in 1955 [2]. Gel electrophoresis found widespread application in biochemistry after Raymond and Weintraub introduced polyacrylamide gel [3] and Hjerten introduced agarose gel [4] as the electrophoresis media. Nowadays gel electrophoresis has become the foundation for a wide range of modern biological analysis such as protein fingerprinting [5] and Southern blotting [6]. However, gel electrophoresis has the limitations of long analysis times and low separation efficiencies due to the use of viscous gel media and low applied voltage.

To improve separation efficiency, in 1967 Hjerten demonstrated free zone electrophoresis that used tubes with small inner diameters [7]. By using a 3 mm diameter quartz tube filled with free solution, he separated inorganic ions and macromolecules. However, this technique became a more feasible and high efficiency analytical tool only after the introduction of narrow diameter capillaries (inner diameter <200 μm) [8-10]. In 1981, Jorgenson and Lukacs achieved high separation efficiencies using 75 μm i.d. capillaries, demonstrating the great potential of modern capillary zone electrophoresis (CZE) [11-13]. The narrow capillaries greatly facilitate the dissipation of the heat that is generated by the passage of an electric current in solution. With this excellent heat dissipation, high electric fields can be applied for the separation, resulting in high separation efficiencies and short separation times. Since then, capillary electrophoresis has made remarkable progress in wide ranging applications such as biological analysis [14], pharmaceutical analysis [15,16] and forensics [17,18].

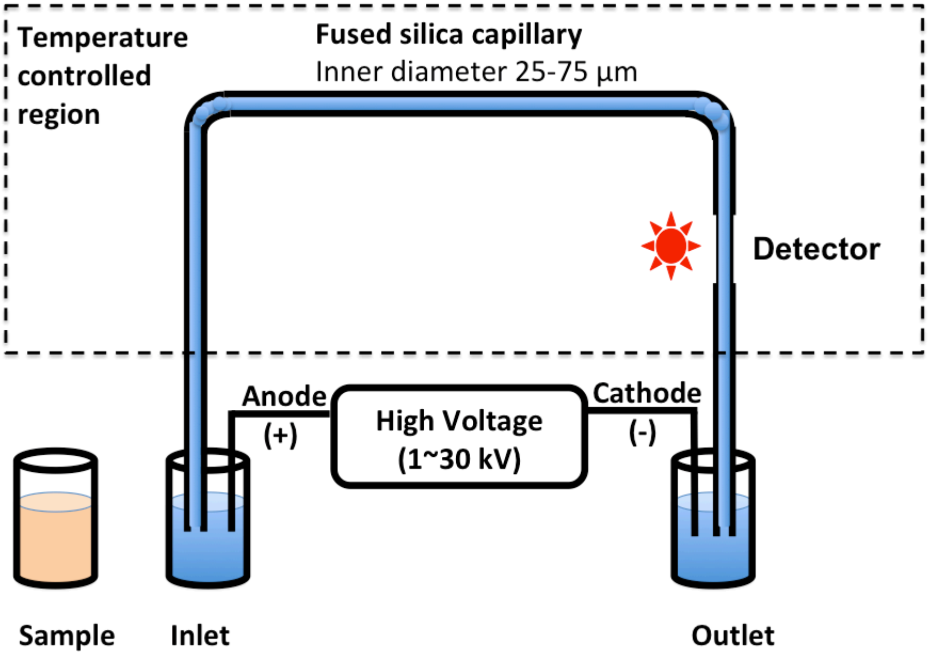

Figure 1.1 A schematic setup for capillary electrophoresis instrument

1.3 Fundamentals of Capillary Electrophoresis

1.3.1 Instrumentation

Figure 1.1 is a schematic diagram of a CE instrument [19]. The basic components are a capillary, a high voltage power supply, inlet and outlet buffer vials, a sample vial, an online detector and a data processing system. The capillary is commonly made of fused silica, externally coated with polyimide for flexibility and strength. Capillaries made from polymers such as poly tetrafluoroethylene (PTFE) [20-22], and polyether ether ketone (PEEK) [23] have also been reported. However, the polymer capillaries have not shown any specific advantages in separation compared to fused silica capillaries [22,24]. On the other hand, the mechanical and optical property of the polymers limited their application [22]. The polyimide is not UV transparent, so a few millimeters of polyimide coating near the end of the capillary is removed to create a detection window. Other external coating materials such as Teflon AF [25] have been used in some applications such as in Chapter 2, because the Teflon AF coated capillary is UV transparent. The capillary inner diameter is typically 25-75 μm and the capillary length varies from 20-100 cm. As many parameters in CE are temperature dependent, an efficient temperature control system, either air-based or liquid coolant-based, is essential to thermostat the capillary.

To perform a CE separation, the inlet end of the capillary is immersed into the vial containing sample solution. Then a small plug of sample (1-10 nL) is injected into capillary either hydrodynamically or electrokinetically. After sample injection, the inlet end of the capillary is re-immersed into the buffer vial and a high voltage is applied to initiate the separation. The voltage is provided by a high voltage supply (1-30 kV) connected to two electrodes, usually made of platinum, which are placed into the inlet and outlet vials. The polarity of the voltage can be switched to accommodate the direction of ion migration. Under the applied electric field, the analytes migrate at different velocities, related to their charge-to-size ratios. When the analytes pass through the detection window, the transmission of light is altered. The signal from the detector is then sent to a personal computer for data processing. The types of detectors used in CE will be discussed in Section 1.4.

In this thesis, two commercially available CE instruments were used. In Chapter 2, a Hewlett Packard (Agilent) ^{3D}CE instrument equipped with a photodiode array absorbance detector was used. The detection window was made 8.5 cm from the capillary outlet end. An air-cooling system was used to thermostat the capillary. In Chapters 3, 4 and 5, a Beckman Coulter (AB SCIEX) PA800 plus Pharmaceutical Analysis System was used. The detection window was made 10 cm from the capillary outlet end. A liquid coolant system was used to thermostat the capillary. Absorbance detection was used in Chapter 3 and fluorescence detection in Chapters 4 and 5.

1.3.2 Migration in Capillary Electrophoresis

1.3.2.1 Electrophoretic Mobility

When positioned in a uniform electric field, an ion experiences a driving electric force and a frictional retarding force. When the ion migrates steadily in a given medium, the driving electric force and the frictional retarding force are balanced. The electrophoretic mobility, μ_e, can be expressed as

$$\mu_e = \frac{q}{6\pi\eta r_i} \tag{1.1}$$

where q is the net charge of the ion, η is the viscosity of the medium, and r_i is the radius of the hydrated ion. Equation 1.1 is known as the Hückel equation. It predicts that the electrophoretic mobility of an ion in a given medium is determined by its charge-to-size ratio. Hence, electrophoretic separation occurs for analytes with different mobilities, or charge-to-size ratios.

1.3.2.2 Electroosmotic Flow (EOF)

An important phenomenon in CE is the bulk flow of the solution inside the capillary upon application of an electric field. This bulk flow of solution is known as the electroosmotic flow (EOF). The inner walls of fused-silica capillaries carry a negative charge due to the presence of weakly acidic silanol (-SiOH) groups (pKa~5.3). Cations in solution build up near the capillary wall to balance the negative charge of the wall, forming an electric double layer. This double layer includes an immobilized compact layer and a mobile diffuse layer [26]. When an electric field is applied, the excess cations in the diffuse layer migrate towards the negative electrode (cathode). Since the excess cations in the diffuse double layer are solvated, their migration draws the solution along with them, forming a bulk flow of solution within capillary.

Unlike a parabolic profile from a pressure driven flow, an important feature of EOF is the flat flow profile as the driving force for the EOF is uniformly distributed along the capillary wall (Figure 1.2). The flatness of the EOF flow profile reduces peak broadening compared to pressure driven flow.

The factors affecting the magnitude of the electroosmotic mobility is given by the Smoluchowski equation,

$$\mu_{EOF} = -\frac{\varepsilon_0 \varepsilon_r \zeta}{\eta} \tag{1.2}$$

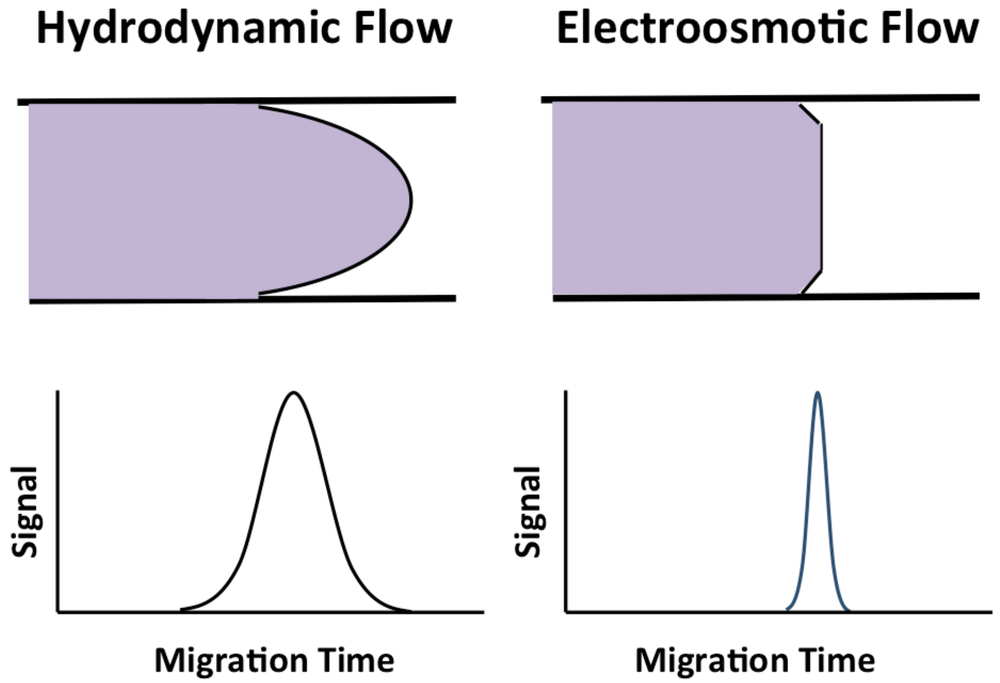

Figure 1.2 Flow profile and corresponding solute zone.

where ε_0 is the permittivity of vacuum, ε_r is the dielectric constant of the medium, ζ is the zeta potential of the capillary inner surface, and η is the viscosity of the medium. The negative sign in Equation 1.2 reflects the convention that a cathodic EOF has a positive value.

The zeta potential is a function of surface charge density at the silica surface. Hence it is affected by the deprotonation of the surface silanol groups, the ions adsorbed on the silica surface, and the ionic strength of the buffer. The deprotonation of the surface group is heavily dependent on the electrolyte pH. The pK_a of the surface silanol groups is around 5.3 [27]. The μ_{EOF} of a bare capillary approaches 0 at pH lower than 2.5 as the silanol groups are protonated; the μ_{EOF} increases with increasing pH in the vicinity of the silanol pK_a, and reaches a maximum around pH 8~9 as the silanol groups are fully deprotonated. Hence controlling the pH of the background electrolyte (BGE) is essential to maintain a stable EOF. The ionic strength of the BGE also affects the zeta potential. An increase of the ionic strength compresses the mobile diffuse layer and lowers the zeta potential, and as a result reduces the EOF.

1.3.2.3 Measurement of Mobility

When an electric field is applied in capillary electrophoresis, the apparent mobility (μ_{app}) of an analyte molecule is the sum of the ion's intrinsic electrophoretic mobility (μ_e, Equation 1.1) and electroosmotic mobility (μ_{EOF}, Equation 1.2).

$$\mu_{app} = \mu_e + \mu_{EOF} \tag{1.3}$$

The apparent mobility of an ion can be experimentally measured as:

$$\mu_{app} = \frac{L_T L_D}{t_m V} \tag{1.4}$$

where L_T is the total length of the capillary, L_D is the length from the inlet of the capillary to the detection window, t_m is the migration time of the analyte and V is the applied voltage. As shown in Equation 1.3, the apparent mobility of a neutral analyte (i.e., $\mu_e = 0$) is the electroosmotic mobility, μ_{EOF}. Hence the μ_{EOF} is usually calculated by measuring the migration time of a neutral analyte. For cationic analytes, their electrophoretic mobility is in the same direction of EOF. Hence cations elute ahead of the EOF peak, as shown in Figure 1.3. The higher the charge-to-size of the cation, the shorter its migration time. For anionic analytes, their electrophoretic mobility is in the opposite direction of the EOF. However most analytes separated by CE have an electrophoretic mobility less than the μ_{EOF} at neutral pH, and so the anionic analytes still migrate in the same direction with EOF but after the EOF peak. The greater the charge-to-size of the anion, the more it migrates against the EOF, and so the longer the observed migration time.

1.3.2.4 Background Electrolyte (BGE)

The selection of proper background electrolyte is key to a successful CE method. The BGE transports the electric current and buffers the pH in CE [28]. Based on the type of BGE utilized, CE can be categorized as aqueous CE and non-aqueous CE (NACE).

The regulation of pH is an important function of the BGE in order to maintain a constant EOF and mobility. Aqueous BGE are by far is the most adopted in CE for their high solvating power of electrolytes, their low cost, non-volatility and compatibility with detectors. Inorganic salt solutions such as phosphate and borate are commonly used in aqueous BGE [28]. From a practical point of view, the concentration of the BGE needs to high enough to ensure adequate buffer capacity. However, too concentrated a BGE would lead to Joule heating [29,30]. A typical aqueous BGE concentration in CZE ranges from 5 to 100 mM.

NACE is commonly used for the analysis of hydrophobic analytes, particularly hydrophobic pharmaceuticals [31-33], which are not soluble in aqueous solution. Moreover, organic solvents provide a good alternative when adjusting the separation selectivity in CE [34]. Separation parameters such as resolution, analysis time and selectivity can be altered easily with mere adjustment of the BGE composition [35]. Hence organic solvents have either been applied in CE as BGE directly or employed as BGE modifiers. The organic solvents applied in NACE must meet certain requirements: be able to dissolve the analytes and electrolytes; availability at low cost and as pure substances; and low or non-UV absorptive [31]. Commonly used NACE solvents include methanol, acetonitrile and dimethylformamide.

1.4 Detection in CE

Numerous detection methods have been applied in CE for analytes with different properties. Detectors in CE can be classified as on-line or off-line detection. On-line detection, including absorbance, fluorescence, conductivity and refractive index, is achieved by monitoring an on-column detection cell, such as in Figure 1.1, while the analyte zone migrates through the capillary. A major advantage of on-line detection is that extra-column band broadening is minimized. Off-line detection such as mass spectrometry monitors analyte after elution. This discussion will only focus on on-line CE detection techniques.

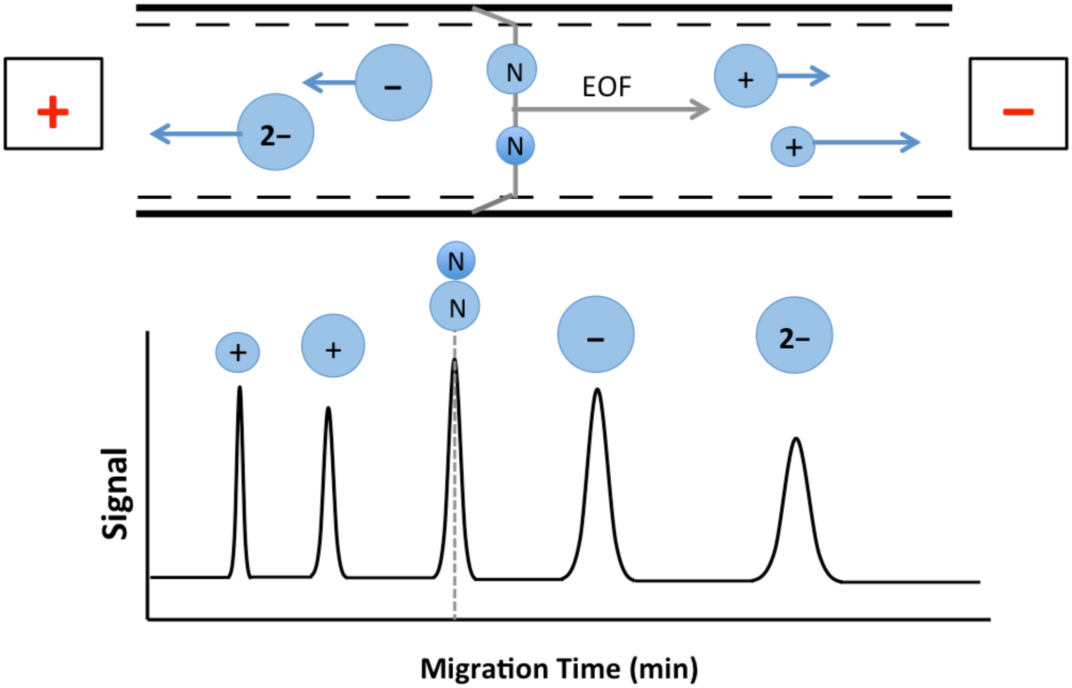

Figure 1.3 Migration order within a capillary under an electric field and the resultant electropherogram.

1.4.1 Absorbance Detection

On-line UV-visible absorbance detection is by far the most commonly used detection method in CE. Most organic compounds can be detected at 195~210 nm [36], making absorbance applicable to an abundance of analytes [36,37]. Also the detector components are robust, inexpensive and easy to operate.

According to Beer-Lambert's law, absorbance (A) is given by

$$\log\frac{P_0}{P_t} = A = abc \qquad (1.5)$$

where P_0 is the intensity of the incident light, P_t is the intensity of light transmitted after absorbance by the solution, a is the molar absorptivity of the analyte, b is the optical pathlength and c is the concentration of the analyte. As shown in Equation 1.5, the sensitivity of absorbance detection is proportional to the optical pathlength. Due to the small diameter of the capillary (25~75 μm), the sensitivity of on-line absorbance detection is low. Typical detection limits range from 10^{-5}~10^{-7} M [38]. The studies in Chapter 2 and 3 use this detector configuration.

To improve the sensitivity in the absorbance detection, numerous approaches have been applied to extend the optical pathlength [39]. Typical strategies include expanded detection cell such as bubble cells [37], Z-type flow cells [40], and multi-channel capillaries [41]. Detection limits of 10^{-8} M can be achieved by using these modifications. However these configurations can add extra-column broadening, and require customized and expensive capillaries or apparatus. Hence they are not used in this thesis.

1.4.2 Fluorescence Detection

Fluorescence detection, especially laser-induced fluorescence (LIF), is the most sensitive, small volume detection technique developed to date for CE. The detection limit of on-line CE-LIF system is 10^{-13} M [36,42].

1.4.2.1 Basic Principles

When a molecule absorbs visible/ultraviolet light, the molecule is excited from the ground electronic state to an excited electronic state that is also vibrationally and rotationally excited. At the excited vibrational level, several events can happen for the molecule to relax to the lower vibrational level. The molecule could either enter another excited vibrational level having the same spin quantum numbers through internal conversion, or enter another excited vibrational level with different quantum spin numbers through intersystem crossing. Both internal conversion and intersystem crossing are radiationless steps. The molecule can also transfer its energy to neighboring molecules through radiationless collisions. Moreover, the molecule could relax to the ground electronic state by emitting a photon, i.e. radiative emission. The relative rates of internal conversion, intersystem crossing, or radiation emission depend on the molecule, the solvent, and multiple conditions such as temperature and pressure. If the emission of a photon takes place in 0.5~20 ns after the excitation as a result of transition between electronic states of the same spin quantum number, this is called fluorescence emission. When self-absorption is negligible, the fluorescence intensity (P_f) of a sample is given by [43]

$$P_f = \Phi P_0 abc\varepsilon_x \varepsilon_c \varepsilon_m \varepsilon_{PMT} \tag{1.6}$$

where P_0 is the optical power of the incident light, Φ is the quantum yield of the molecule (photons emitted/photons absorbed), a is the molar absorptivity of the analyte, b is the optical pathlength and c is the concentration of the analyte. The ε terms are the efficiency of: the excitation optics, x; the detection window, c; the collection optics, m; and the light detection device (generally a photomultiplier, *PMT*), respectively. Equation 1.6 shows that the fluorescence intensity in CE depends on the power of the incident light (P_0), the nature of the molecule (terms Φ and a), the optical setup (b, ε_x, ε_c, ε_m, and ε_{PMT}) and the concentration of the analyte at the detection window (c). The different optical setups of sensitive fluorescence detection for micro-column systems have been reviewed by Johnson and Landers [44,45].

1.4.2.2 Fluorescence Excitation Light Source

Instrumentation for fluorescence detection in CE generally consists of an excitation light source, focusing optics, collection optics, emission filter, and detector (Figure 1.4). To excite the analyte from the ground to the excited state, a light source of appropriate wavelength, intensity, and spatial characteristics is required. The incident light intensity onto the capillary via fiber optics is given by [43]

$$P_0 = E_{t \bullet \Delta\lambda} A_S \Phi_{col} T_f \qquad (1.7)$$

where E_t is the spectral radiance of the light source in units of W/m^2/str at the spectral region $\Delta\lambda$, A_S is the illuminated area of the detection window, Φ_{col} is the collection efficiency of the fiber optics, and T_f is the transmittance of the filter used. Typical A_S values for a capillary are only 5×10^{-9} m^2 [43]. To increase the incident light intensity, light sources with high optical power are preferred in CE-fluorescence detection.

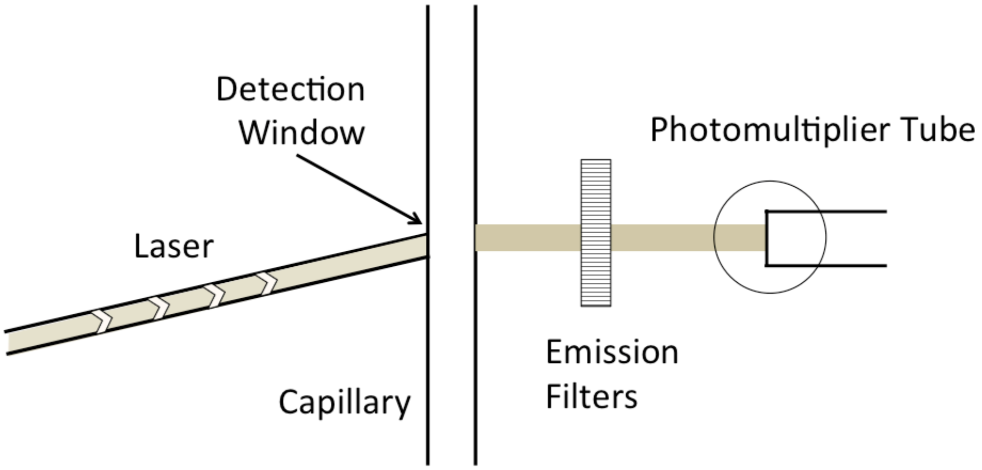

Figure 1.4 Schematic diagram of the optical setup of the Beckman PA 800 plus LIF detection system. Adapted from Beckman PA 800 plus manual.

A variety of excitation light sources can be used for CE-fluorescence, as discussed below. Jorgenson et al. firstly used a high-pressure mercury arc lamp for on-line fluorescence detection of fluorescent derivatives of amino acids, dipeptide and amines in CE [11]. More recently lasers and light emitting diodes (LED) are most commonly used.

1.4.2.2.1 Laser-induced Fluorescence Detection

The first on-line 325-nm HeCd laser-induced fluorescence detection for CE was reported by Zare and co-workers in 1985 [46]. Since then, laser-induced fluorescence (LIF) has been the major choice for fluorescence detection in CE. Laser beams are powerful, directional, monochromatic and coherent, making them particularly well suited for focusing intense light onto small capillaries.

Lasers can be classified as gas, solid-state and semiconductor lasers according to the material of the active medium used in the resonator to produce radiation [47,48]. The optical power and output wavelength of various lasers are summarized in Figure 1.5 [49].

Since the introduction of LIF detection, the gas-based lasers such as Argon-ion, HeCd and HeNe lasers have become very popular due to their compatibility with common fluorescent molecules. For example, the 488-nm Argon-ion laser matches well with the excitation wavelength of fluorescein. Detection of fluorescein labeled compound by CE-Argon-ion laser induced fluorescence was achieved in the early 1990s [50]. However, these lasers still suffer from a number of limitations including high cost, large size, low stability and limited lifetime (~5000 h).

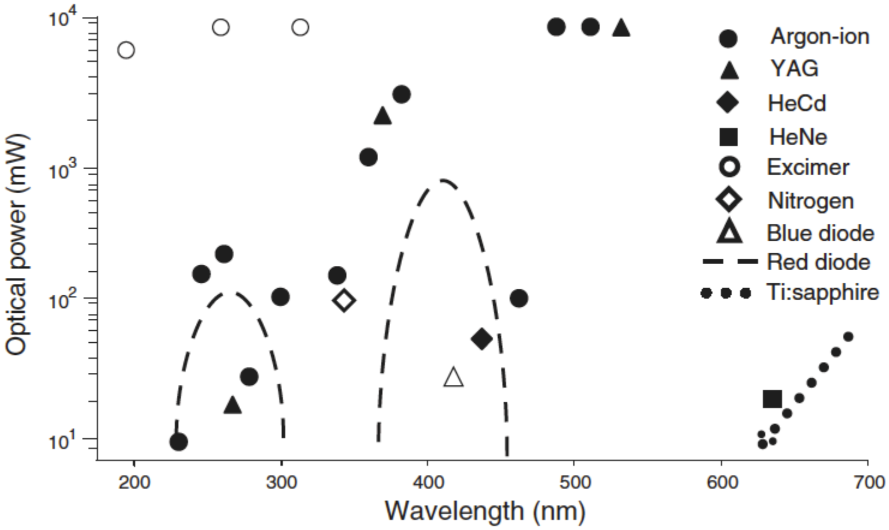

Figure 1.5 Output power and wavelengths of various laser systems used in LIF detection. Reprinted from Ref. [41] with permission from Elsevier.

Diode lasers, which are based on the semiconductor technology, have attracted much attention in CE-LIF system. When positive and negative potentials are applied to p-type and n-type semiconductors, electrons and positive holes are injected into the junction region. Due to a population inversion, strong stimulated emission occurs in the active channel of the waveguide [47]. The emission wavelength depends on the band gap of the semiconductor, which is determined by the materials of the semiconductor. Diode lasers are inexpensive (down to~$25 USD), energy efficient (can be powered by a battery) and highly stable (0.01% noise) [48]. Furthermore, due to their small size, diode laser based detectors can be extremely compact and miniaturized for micro-separation systems [49,51].

The first diode lasers had limited output which was confined to wavelengths at the near-IR and red region of the electromagnetic spectrum [51,52]. During the 1960s and 1970s, laser diodes evolved rapidly from homo-junction to hetero-junction diode laser devices. Numerous efforts have been made to shorten the output wavelength, matching up with the excitation spectra of commonly used fluorescent dyes. In the 1990s, the development by Shuji Nakamura of blue and ultraviolet lasers made from GaN materials greatly advanced diode laser application [53,54]. This also led to the rapid development of high-brightness blue/green light-emitting diodes (LEDs) [55].

1.4.2.2.2 LED-induced Fluorescence Detection

Recently LEDs have become an attractive alternative to lasers as an excitation source [56]. LEDs are also semiconductor-based diodes. Commercial LEDs are available from the deep-UV (AlGaN semiconductor) to the near-IR (InAs semiconductor).

The difference between LED and laser diode sources lies in their working principle, performance and structure, as summarized in Table 1.1 [57]. Comparisons of separations using CE-diode laser induced fluorescence with those using CE-LED induced fluorescence (488 nm) showed that CE-LEDIF has equivalent or better sensitivities and a lower noise level [58,59]. The detection limit reaches 10^{-11} M for a well-designed CE-LEDIF system [60]. Generally speaking, LEDs are gaining popularity in micro-column fluorescence detection due to their energy efficiency, low-cost, high-stability, low-noise, small-size and long lifetime (~10^5 h). However, most commercial LED's lack of monochromaticity results in stray light which can degrade the detection limit [56]. Direct fluorescence detection with an LED excitation source is used for determination of trace transition metals in Chapter 5.

1.4.3 Indirect Detection

Molecules that do not possess any chromophoric properties cannot be detected by direct absorbance or fluorescence. Indirect detection is based on the displacement of a detector-responsive species present in the BGE by the detector-nonresponsive analyte. Such displacement results in a decrease in the signal intensity. Hjerten et al. first employed indirect UV detection in CE for the analysis of organic ions in 1987 [61]. The reason for developing indirect detection schemes is that indirect detection is universal. As long as the analyte and the BGE have different response to the detector, the analyte can be detected by indirect detection [62]. Because of this, tedious sample preparation (e.g. chemical derivatization) can be avoided. Indirect detection based on absorbance [63], fluorescence [64,65] and conductivity [66,67] are most commonly reported in the capillary electrophoresis literature.

Table 1.1 Comparison of laser diodes and LEDs. Reprinted from Ref. [41]

Characteristic	**Laser Diode**	**LED**
Light output	Coherent	Non-coherent
Output power	Proportional to current above the threshold	Linearly proportional to drive current
Current	5-40 mA threshold current	50-100 mA drive current
Coupled Power	High	Moderate
On/Off speed	Higher	Slower
Bandwidth		
Use with fiber	Single mode, multimode	Multimode
Spectral width	0.0001-10 nm	40-190 nm
Longevity	Long	Longer
Cost	Higher	Lower

1.4.3.1 Fundamentals of Indirect Absorbance and Fluorescence Detection

For indirect absorbance and fluorescence detection in CE, an absorbing or fluorescent co-ion called the "probe" is added to the background electrolyte. Detection of analyte is accomplished by displacement of the co-ion leading to a measurable decrease in the background photometric signal (Figure 1.6). The theoretical limit of indirect detection (C_{LOD}) is given by [68]

$$C_{LOD} = \frac{C_p}{TR \times DR} \tag{1.8}$$

where C_p is the concentration of the probe, and *TR* is the transfer ratio (the number of probe molecules displaced by one analyte molecule). *DR* is the dynamic reserve, which is the ability to measure a small change on top of a large background signal, and is equal to the signal-to-noise ratio (S/N) of the background signal. The three parameters are not necessarily independent. For example, according to Equation 1.8, reducing the concentration of probe molecule should reduce the detection limit. This is because for a given amount of the probe being displaced, the fractional change of the signal will be larger if the background signal is derived from a lower concentration of the probe. However, as the background signal is decreased with lower *C*p, the background signal-to-noise ratio also decreases, resulting in a lower *DR*. Equilibrium and surface effects can further reduce the *TR*. Overall, decreasing the probe concentration will not necessarily improve the detection limit [62,68].

Several factors need to be considered when comparing different modes of indirect detection. The first consideration is the dynamic reserve, *DR*. Reducing the background noise increases the *DR*, and therefore lowers the C_{LOD}. In photometry, shot noise determines the background noise from the instrument. In fluorometry, the background

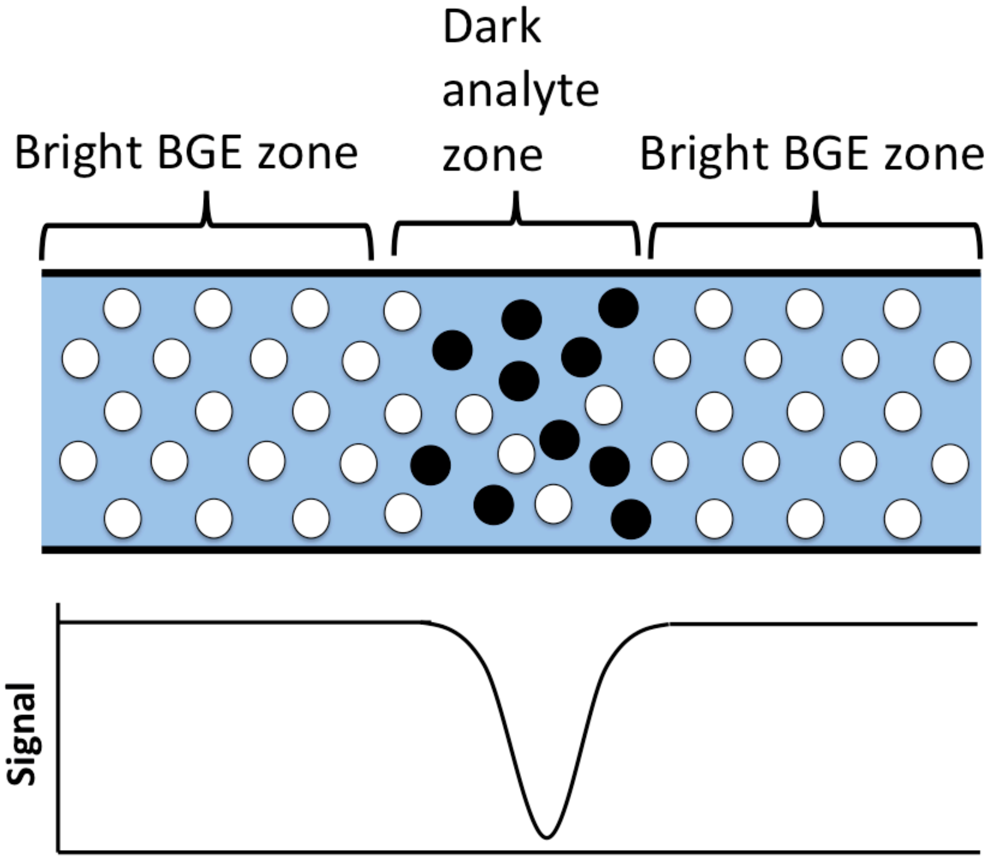

Figure 1.6 General scheme for indirect fluorometric detection.

noise consists of the intensity noise from the excitation light source and the detector shot noise. In the 1990s, the intensity stability of common gas-based lasers was roughly 1% (DR = 100), while the *DR* for absorption detector was around 10^4 [62,68]. As a result, the low intensity stability of the laser resulted in indirect laser-induced fluorescence detection having an equivalent detection limit with indirect UV absorbance detection. Typical *DR* are now >3000 with diode lasers [64]. Thus, nowadays, indirect fluorescence detection exhibits much lower detection limits (10^{-6}~10^{-8} M [64,69,70]) compared to indirect absorbance detection (10^{-5} to 10^{-6} M [71-73]).

Indirect fluorescence detection with an LED is used for determination of inorganic anions in Chapter 4.

Another advantage of indirect fluorescence detection is the capability of lowering detection limits by reducing C_p or using narrower inner diameter capillaries [68]. As absorbance detection is strictly pathlength dependent (Equation 1.5), *DR* for absorbance will degrade as optical pathlength or C_p decreases. Although fluorescence intensity also decreases with decreasing pathlength and C_p, there is such a strong signal that it is possible to maintain the sensitivity of the detection [68].

1.5 Efficiency and Band Broadening in Capillary Electrophoresis

1.5.1 Efficiency and Resolution

In capillary electrophoresis, a narrow sample plug (1-10 nL) experiences band broadening while migrating through the capillary. The band broadening processes in CE can be classified as: longitudinal diffusion; Joule heating induced band broadening; electromigration dispersion band broadening; solute-wall adsorption band broadening; and extra-column band broadening [74,75]. An important parameter in CE, efficiency, is used to measure the degree of the peak broadening. Efficiency is expressed by the number of theoretical plates (N) as

$$N = \frac{L_D^2}{\sigma_{tot}^2} \tag{1.9}$$

where L_D is the capillary length to the detector and σ_{tot}^2 is the variance of the eluted peak. Both L_D and σ_{tot}^2 are observable parameters. For Gaussian peaks, N can be experimentally calculated from the migration time (t_m) and from either the peak width at baseline (W_b) or peak width at half maximum height ($W_{1/2}$) as

$$N = 16\left(\frac{t_m}{W_b}\right)^2 = 5.54\left(\frac{t_m}{W_{1/2}}\right)^2 \tag{1.10}$$

If the peak is not symmetrical, Equation 1.10 should not be used to calculate N because the width measurements will not follow the predicted Gaussian distribution [76]. Foley and Dorsey discussed various figures of merit for ideal and tailed peaks, and based on an exponentially modified Gaussian developed the empirical formula [77]

$$N_{SYS} = \frac{41.7(t_m / W_{0.1})^2}{B / A + 1.25} \tag{1.11}$$

where $W_{0.1}$ is the peak width at 10% peak height, and A and B are measured at 10% peak the height as shown in Figure 1.7.

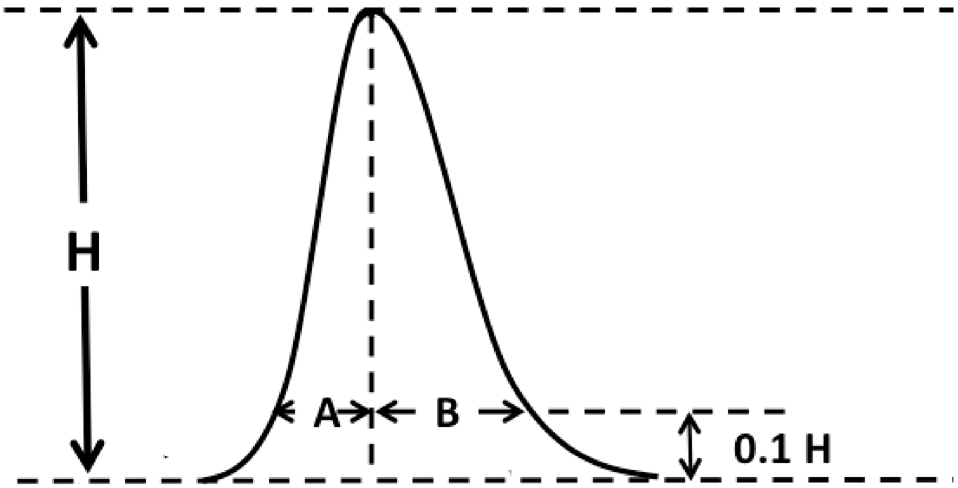

Figure 1.7 Figure defining A and B in Equation 1.11

Another important parameter for chromatographic separation is resolution, R_s. Resolution defines the degree of separation between two peaks. Resolution in CE is defined as

$$R_s = \frac{\sqrt{N}}{4}\frac{\Delta\mu}{\overline{\mu}_{app}} = \frac{\sqrt{N}}{4}\left(\frac{\Delta\mu}{\mu_{EOF} + \mu_e}\right) \tag{1.12}$$

where Δμ is the difference in the electrophoretic mobility between two ions and $\overline{\mu}_{app}$ is the average apparent mobility of the analytes. Equation 1.12 shows that if the EOF and the analyte ions are in opposite direction, which is known as counter-EOF situation, the resolution will be increased if their mobility values are close. On the other hand, if the EOF and ion mobility are in the same direction, the larger the EOF mobility, the larger the denominator term becomes. Hence achieving resolution is more challenging. Thus, control of EOF is vital to achieve excellent separation power in CE.

As shown in Equation 1.12, resolution is also proportional to the square root of the efficiency, *N*. Hence to improve the separation resolution, it is important to minimize band broadening and increase efficiency.

1.5.2 Band Broadening

The overall variance (σ^2_{tot}) in Equation 1.9 is the sum of the variances from all peak broadening processes. The contributions of all the independent band broadening processes to the total variance are additive [78]

$$\sigma^2_{tot} = \sigma^2_{diff} + \sigma^2_{Joule} + \sigma^2_{EMD} + \sigma^2_{ads} + \sigma^2_{EXT} \tag{1.13}$$

where σ^2_{diff} is the variance due to longitudinal diffusion band broadening, σ^2_{Joule} is the Joule heating-induced band broadening, σ^2_{EMD} is the electromigration dispersion band broadening, σ^2_{ads} is the solute-wall adsorption band broadening, and σ^2_{EXT} is the extra-column band broadening. The following sections will describe each of the individual processes and their contribution to the overall band broadening.

1.5.2.1 Longitudinal Diffusion

Longitudinal diffusion refers to the axial spreading of analyte molecules as a result of concentration gradient. Ideally, longitudinal diffusion is the only on-column band-broadening source in CE. The variance caused by longitudinal diffusion is expressed by

$$\sigma^2_{diff} = 2Dt_m \tag{1.14}$$

where *D* is the diffusion coefficient of the analyte and t_m is the migration time of

the analyte. Equation 1.14 shows that the analytes with lower diffusion coefficients experience less longitudinal diffusion than those with larger diffusion coefficients. That is, macromolecules such as proteins should display less broadening and higher *N* than small ions such as Cl^-. Also the longer an analyte spends inside the capillary (longer t_m), the broader its peak becomes.

Substitution of Equation 1.4 into Equation 1.14 gives

$$\sigma^2_{diff} = 2D\frac{L_D L_T}{\mu_{app} V} \tag{1.15}$$

Substitution of Equation 1.15 to Equation 1.9 gives theoretical maximum efficiency in CE , i.e., if longitudinal diffusion is the only band broadening source [79]

$$N = \frac{\mu_{app} V L_D}{2 D L_T} = \frac{\mu_{app} E L_D}{2D} \qquad (1.16)$$

where E is the electric field strength (V/L_T). From Equation 1.16, under ideal conditions, the separation efficiency is directly proportional to the applied electric field strength, E.

1.5.2.2 Joule Heating-Induced Band Broadening

The main advantage of performing electrophoresis in narrow-bore capillaries is the excellent heat dissipation due to the large surface-to-volume ratio of the capillaries. Heating is problematic since it can cause non-uniform temperature gradients, local viscosity changes, and subsequently band broadening.

Joule heating is generated by the passage of an electric current (I) through a conducting solution when an electric field, E, is applied. Joule heating originates with Ohm's law [80]

$$V = IR \qquad (1.17)$$

where V is the applied voltage, I is the current, and R is the resistance to current flow. The power, P, deposited within the capillary is given by

$$P = I^2 R \qquad (1.18)$$

The heat generated is dependent on the applied power. When the heat generation exceeds the heat dissipation, radial temperature gradients are generated due to the faster heat dissipation near the capillary wall and slower heat dissipation at the capillary center. While the absolute rise in temperature is generally not detrimental, temperature gradients are. The temperature gradients give rise to viscosity and diffusion coefficient differences within the running buffer, and hence lead to peak broadening [81]. A one-degree change in temperature results in a 2~3% change in viscosity, and thus a 2~3%

change in mobility. The variance caused by Joule heating-induced temperature gradients has been summarized in multiple different equations [82-85]. In general, the variance has a strong dependence on the capillary radius (*r*) and applied electric field (*E*)

$$\sigma^2_{Joule} \propto \frac{r^6 E^5 \kappa^2}{\lambda_b^2} \qquad (1.19)$$

where κ is the specific electrical conductivity of the electrolyte buffer and λ_b is the thermal conductivity of the electrolyte buffer. Equation 1.19 demonstrates the importance of performing high voltage electrophoretic separations in narrow-bore capillaries. Also it shows that the temperature control within the capillary can be affected by the electrolyte buffer content and concentration [86].

To minimize the Joule heating-induced band broadening, narrower capillaries or lower electric field should be used. However, as discussed in Section 1.4.2.1 a high electric field is needed to minimize longitudinal diffusion. The maximum voltage for a particular electrolyte buffer system can be experimentally determined from an Ohm's plot based on Equation 1.17. An Ohm's plot is the plot of the observed current versus the applied voltage for a specific buffer system. Linearity of the plot indicates constant resistance (*R*). A temperature rise within the capillary results in a decrease in the resistance. Hence the voltage at which the Ohm's plot deviates positively from linearity is designated the optimum voltage.

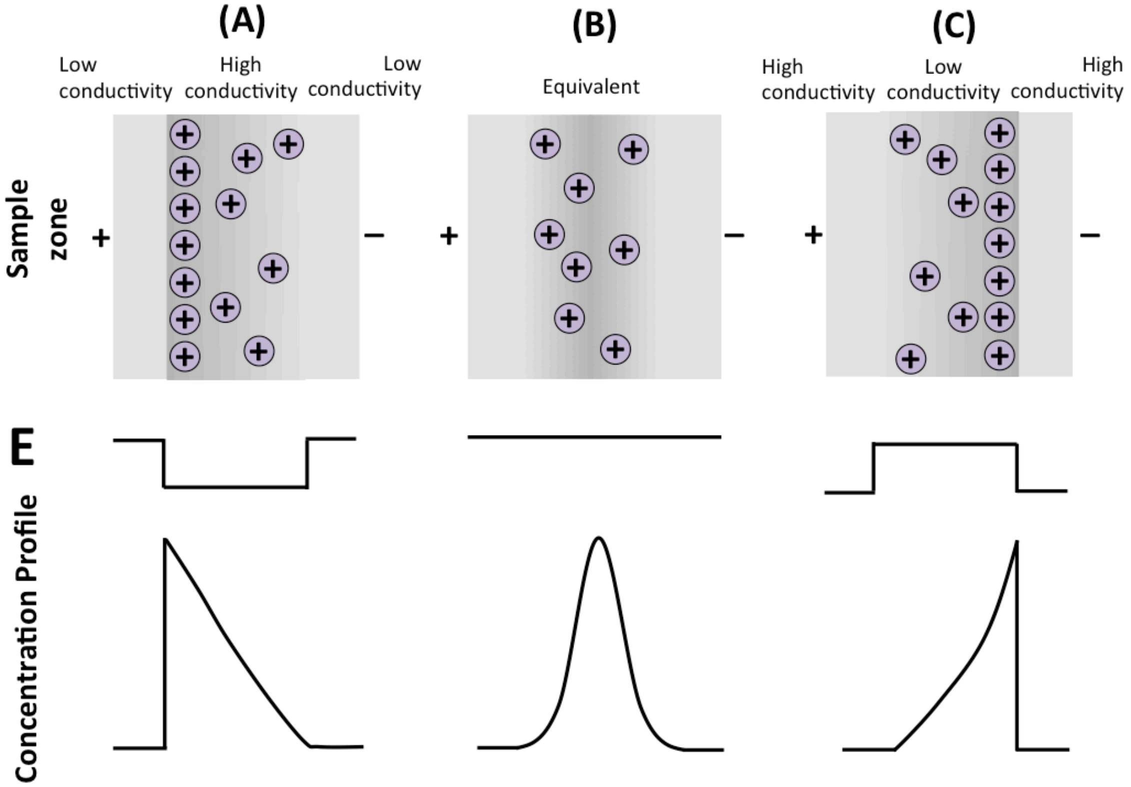

Figure 1.8 Schematic of Electromigration Dispersion (EMD) as a result of mismatched conductivity between the sample zone and the electrolyte buffer. Adapted from reference [19]**.**

1.5.2.3 Electromigration Dispersion

Electromigration dispersion (EMD) is caused by a significant difference in the electrophoretic mobility between the sample zone and the surrounding electrolyte. This mobility difference causes the local electric field strength to vary, which causes peak shape distortions. Figure 1.8 (A) shows an example of a sample with higher mobility (higher conductivity and lower resistance) than that of the BGE. The front edge of the sample zone, which diffuses in the direction of migration, encounters a higher voltage drop when entering the buffer zone [19]. Therefore the analyte velocity is higher in the BGE region than that in the sample zone. In other words, the analyte ions overrun the co-ion and leave the leading boundary of the sample zone and enter the buffer zone. As a result, a triangular analyte concentration profile with a diffuse leading edge and sharp trailing edge results. Conversely, when the analyte has a lower mobility than the BGE, as shown in Figure 1.8 (C), the leading edge will be sharp and the trailing edge diffuse. When the conductivities are equivalent (Figure 1.8 (B)), no peak distortions occur and a typical Gaussian peak shape (due to longitudinal diffusion broadening) is observed.

The variance due to electromigration dispersion is [74]

$$\sigma_{EMD}^2 = \frac{2El_{inj}C_s k_{EMD}}{9C_b v} L_D \qquad (1.20)$$

where E is the electric field strength, l_{inj} is the length of the injection plug, C_{s} and C_{b} are the concentrations of the ions in the sample and BGE respectively, v is the velocity of the analyte, and k_{EMD} is the electromigration dispersion factor which depends on the difference in effective mobilities of the constituent ions in the BGE and the sample. In CE, the concentration of the sample ions is kept low relative to the ions in the BGE to keep the conductivity difference between the two regions small. Under such conditions, the term $\frac{C_s}{C_b}$ is small, resulting in low electromigration dispersion variance.

When developing a CE method for most inorganic ions, indirect absorbance or fluorescence detection are commonly used. As described in Section 1.3.3.1, dilute buffers are often used in indirect detection to lower the detection limit because otherwise the co-ion in the BGE competes with analyte ion for displacement of the probe ion, resulting in a smaller signal change. Typical buffer concentrations of 2~10 mM are adopted in indirect detection methods, which are about 5~7 times less than those used with direct detection [87]. Due to the low BGE concentration and hence the larger $\frac{C_s}{C_b}$ term, electromigration dispersion is more pronounced in indirect detection [88], as will be evident in Chapter 4.

1.5.2.4 Solute-Wall Adsorption Band Broadening

While adsorption itself is the retention mechanism in chromatography, adsorption of analyte to the capillary wall in CE causes severe peak tailing and deteriorates the separation efficiency [89]. Moreover, adsorbed analytes change the local surface zeta potential, resulting in a nonhomogeneous EOF in the capillary [89]. Analytes interact with capillary walls by means of electrostatic attraction, hydrogen bonding and hydrophobic interactions [90,91]. The variance contribution from solute-wall adsorption can be expressed as [89,92]

$$\sigma_{ads}^2 = \left[\frac{K^2}{D} \frac{r}{(r+2K)} + \frac{4K}{(r+2K)k_d} \right] \bullet v_m^2 t_m \qquad (1.21)$$

where $K = k_a/k_d$ is the distribution coefficient between the wall and the solution, k_a and k_d are the rate constants of adsorption and desorption respectively, *D* is the diffusion coefficient of the analyte, v_m is the migration velocity of the solute and *r* is the inner radius of the capillary. Equation 1.21 shows that analyte with slow desorption kinetics results in a large contribution to the variance, thus large band broadening.

1.5.2.5 Extra-column Band Broadening

All of the band broadening phenomena discussed previously occur "on-capillary" during the migration process. Extra-column band broadening are those arising outside the migration process, such as the finite width of the injection zone, the finite detection window of the detector [89,93], and detector electronics. Ideally the extra-column band broadening should not decrease the separation efficiency by more than 10% [93,94].

It has been shown that injection can influence the separation efficiency, especially for long injection plug and short capillary [94]. The variance caused by a rectangular plug can be expressed as [95]

$$\sigma_{inj}^2 = \frac{l_{inj}^2}{12} \tag{1.22}$$

where l_{inj} is the length of the injected sample plug. To minimize the injection band broadening, the length of injection plug needs to be minimized. Also the quality of capillary cutting at the inlet is important. Cohen and Grushka demonstrated that when the capillary is cut straight (perpendicular to the capillary axial), the peaks are narrower and symmetrical [96]. If the capillary cut is slanted, the peaks are broader and likely to tail. This is because a slanted cut makes the injected sample plug not rectangular but of rhomboid shape.

For detector based band broadening, the common sources are detector rise time, detection window length and data acquisition rate. The detector rise time refers to the time span required for the output signal to rise from 10% to 90% of its maximum value when a steady input is instantaneously applied. The detector rise time should be reasonably short for fast moving peaks. The detection window length can also give rise to detector band broadening when the sample zone length is comparable to the detection window length [93]. Another source of detector band broadening is the data acquisition rate. Data acquisition rate refers to the data point collection frequency of the detector. The data acquisition rate should be fast enough to collect enough data points so all the details of the peak can be well defined [97]. Finally, care must be taken in selecting the data treatment method. Data handling software of some instruments, including the Agilent CE used in Chapter 2, employ hidden data smoothing algorithms that run parallel to the data acquisition [98].

1.6 Inorganic Ion Separations in CE

Inorganic analysis by CE has been widely applied since the 1990s. The motivation is that CE provides: high-efficiency separations; capability of simultaneous analysis of cations and anions; availability of alternative detection techniques and various preconcentration techniques to improve detection limits; and rapid and simple method development [99,100]. In practical inorganic analysis, CE is seen as a complementary analytical technique to chromatographic methods.

1.6.1 Separation Systems

One of the major interests in inorganic analysis is the separation performance using CE. Recent research has focused on the challenges of controlling EOF, of reducing analysis time, handling complicated matrix interferences, and improving limits of detection.

1.6.1.1 Coated Capillaries

Coated capillaries have been applied for both inorganic cation and anion analysis. Coatings used in CE can be generally classified as covalently attached coatings and non-covalent coatings. Non-covalent coatings can further be divided into dynamic coatings and semi-permanent coatings. Covalent coatings such as polyacrylamide [101], semi-permanent coatings such as surfactants [102] and ionic liquids [103], and dynamic coatings such as surfactants [104] improve the separation repeatability by improving the EOF stability in anion analysis. Moreover, coated capillaries improve separation efficiency in cation analysis by preventing cation adsorption to the fused silica wall [105,106].

1.6.1.2 Uncoated Capillaries

When using a bare capillary for inorganic analysis, BGE additives are commonly used to modify the selectivity and improve the resolution. For instance, for inorganic anion analysis, di-, tri- or tetra cationic ion-pairing reagents were added to the BGE to convert the target anions to positively charged cations [107,108]. For cation analysis, chelating agents were used to complex the target cation, forming anionic complexes. Cationic polymer BGE additives such as polybrene were then used to pair with the anionic metal complex [109].

Another common approach in inorganic anion analysis is counter-EOF separation. As shown in Equation 1.12, when the EOF and analyte mobility are equal and opposite in direction, the resolution can go to infinity in theory. In the case of counter-EOF, the analyte electrophoretic mobility is offset by the electroosmotic flow; the resolution power can be extremely high and can be used to separate isotopic anions [110]. However, the EOF of bare capillary above pH 4 is normally much stronger than anionic species, as a result all anionic species will be swept towards the cathode. To achieve suppressed EOF, acidic BGE ($pH<4$) can be used [64,111,112] for anion analysis.

1.6.2 Detection

Few inorganic anions have absorbance in the UV-vis range, limiting the application of direct absorbance detection [29,113] for anion analysis. Indirect absorbance detection, on the other hand, has been widely applied to both cation and anion analysis [63,114]. The fundamentals of CE-absorbance with indirect and direct detection were discussed in Section 1.3. When using indirect detection, buffering of the BGE is essential for reproducible migration times. In indirect absorbance detection, one common method of buffering is by utilizing the probe itself as the buffer. For instance, chromate and benzoate are commonly used probes for this method [115,116]. Typical photometric probes for cation analysis include imidazole, benzylamine, and 4-methylbenzylamine [117,118]. Another alternative to buffering electrolytes is the use of amphoteric electrolytes [114]. An ampholyte will buffer at its isoelectric point (pI) when the pK_a values of the two buffering groups are roughly within 1 unit of the pI [114]. Moreover, an ampholyte exists in a zwitterionic form with a net charge of zero at its isoelectric point. The zwitterions will not competitively displace the probe, making such ampholyte ideal for indirect detection.

Few inorganic ions are fluorescent. Therefore derivatization is required for direct fluorescence detection. CE with fluorescence detection is highly popular for the analysis of large biological molecules due its high sensitivity after derivatization [44]. However, it is challenging to utilize fluorescence detection for inorganic analysis. There is no generally applicable fluorescent derivatization agent for inorganic anions. However, in the analysis of transition metal cations, fluorophore tagged chelating agents can be used to complex the target cations, forming a fluorescent metal complex [109,119,120]. This approach is used in Chapter 5.

In the absence of a derivatization reagent for anions, indirect detection is used (Section 1.4.3.1). In indirect fluorescence detection, it is challenging to find a fluorescent probe with matching mobility of inorganic ions due to the large conjugated alkene structure of fluorescent compounds. Compared to the various photometric probes, only a few fluorescent probes have been reported. For instance, 2,5-dihydrobenzoate [121], 8-hydroxypyrene-1,3,6-trisulfonic acid [64,65] were used as fluorescent probe for anion analysis, cerium (III) was used for cation analysis [121], and fluorescein was used for cation-EDTA complex analysis [122].

Electrochemical detection, especially conductometric detection, which involves measuring the conductivity of the solution, is a universal detection technique requiring a relatively simple experimental setup. Recently the rapid development of capacitively coupled contactless conductivity detector (C^4D) has made conductivity a popular and fully developed detection method in inorganic analysis by CE [123-125]. Simultaneous detection of cations and anions in complicated matrices such as urine, serum and high saline can be achieved with C^4D [123,124]. However, in conductivity detection, the high separation voltage must be separated from the conductivity detector electronics, which complicates the system [125]. Therefore CE with C^4D detection was not used in this thesis.

CE with mass spectrometry (MS) has been explored for inorganic analysis [99]. Online detectors such as ICP-MS [126-128] and ESI-MS [129,130] enable simultaneous multi-elemental and multi-isotopic inorganic analysis in complex matrix such as blood plasma. However miniaturization and portability are the long term objective of the studies in Chapters 4 and 5. Miniaturization of MS systems is beyond the scope of the present work. Therefore CE-MS will not be discussed further.

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Chapter Two: Polymerized Phospholipid Bilayers as Permanent Coatings for Small Amine Separations Using Mixed Aqueous/Organic Capillary Zone Electrophoresis*

2.1 Introduction

Since Jorgenson and co-workers popularized zone electrophoresis in open-tubular glass capillaries in the early 1980's [1], capillary electrophoresis (CE) has become a powerful separation technique. The small diameter of the capillaries enhances heat dissipation, thus enabling the use of high voltages, which yield high separation efficiencies, high resolution, and rapid separations of compounds ranging from small inorganic ions [2,3] to large biological molecules [4,5]. CE is now well established for both small molecule pharmaceuticals and biopharmaceuticals [6]. Application areas include impurity testing, chiral separations and determination of drug properties and others [7]. However the separation performance for basic analytes in CE is compromised by analyte adsorption onto the negatively charged capillary wall.

Many approaches have been explored to solve the adsorption problem. These include using: extreme pH [8]; high salt concentrations [9]; non-aqueous background electrolytes [10]; and altering the chemistry at the silica wall or shielding the silanols with a coating. Such coatings can be categorized as either permanent or physically adsorbed coatings. In the former, derivatization of silanols on the capillary surface results in formation of a covalently bound coating [11-13]. Such covalent coatings often require multi-step synthesis, which can be both elaborate and time-consuming. Physically adsorbed coatings are non-covalently adsorbed to the capillary wall by magnetic [14] or more commonly electrostatic [15-17] interactions. Physically adsorbed coatings are generally easier to prepare than covalent coatings [18], and if necessary can be removed from the capillary and then regenerated. In this chapter I propose a

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surfactant based coating approach that maintains the ease of adsorbed coatings, with the permanence of covalent coatings.

Both cationic surfactants [17,19] and zwitterionic surfactants [20] have been utilized as coatings in CE. Two-tailed cationic surfactants, such as dioctadecyldimethylammonium bromide (DODAB) [19,21] and zwitterionic surfactants such as 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) [22] form “semi-permanent” coatings on capillary walls. After simply rinsing the capillary with a surfactant solution, multiple runs can be performed without the need for surfactants to be in the running buffer. Up to 300 injections have been performed on a DODAB coated capillary without regeneration of the coating [23]. However, cationic surfactants such as DODAB generate a strong anodic EOF, which overwhelms the mobility of most compounds, thereby reducing resolution. Further, acidic analytes interact electrostatically with the cationic coating, leading to adsorption and tailing.

Zwitterionic surfactants yield a net neutral coating that enables separation of both acidic and basic analytes. Phosphorylcholine (PC) based lipids have been studied extensively as zwitterionic surfactant coatings for CE, as they form self-assembled supported phospholipid bilayers (SPB) on the capillary surface [24]. Such SPB coatings display efficiency up to 1 million plates/m and better than 5% day-to-day reproducibility for proteins separations [20,22]. Unfortunately, to date phospholipid coatings have not displayed the stability of their cationic analogues. Thus, PC coatings must be regenerated regularly. Numerous techniques have been studied to improve the formation and stability of PC coatings, such as adding divalent cations to promote vesicle fusion onto the capillary wall [25], increasing the hydrophobicity of the surfactant [18] and using narrow bore capillaries [23,26]. Regardless, PC coatings must be regenerated regularly and can be removed from the capillary by a simple rinse with organic solvents such as formamide, chloroform and methanol [27-29].

Clearly this solubility in organic solvents limits the utility of surfactant-based coatings in non-aqueous capillary electrophoresis (NACE). NACE is useful for the analysis of hydrophobic analytes, particularly hydrophobic pharmaceuticals [10,28,30-32]. However NACE still suffers from the problem of analyte adsorption on the capillary wall. Further, dynamic coatings must be regenerated more frequently under NACE conditions than for aqueous conditions [28,29,33]. Therefore, developing a simple and stable coating for nonaqueous buffer conditions will help enable further utilization of the NACE technique.

Polymerizing a bilayer composed of unsaturated surfactants can enhance the stability of supported phospholipid bilayers. O'Brien and co-workers demonstrated that the stability of a bilayer membrane is significantly enhanced by polymerization of lipids containing either a diacetylene [34,35] or a dienoyl group [36,37]. Such polymerized bilayers have been used to reduce pyrite oxidation in solution [38], as a biocompatible membrane for biosensors [39], and polymerized dienoyl containing surfactants such as bis-SorbPC and bis-DenPC have been used to form cross-linked membranes to create ion channels and capillary coatings [40-42]. In these applications, a self-assembled bilayer is first formed from the surfactant monomer. The bilayer is then photo-polymerized *in situ* to form a cross-linked structure. All cross-linked bilayers show enhanced stability compared to the corresponding un-polymerized surfactant coating, while maintaining the ability to resist protein adsorption at the surface.

Here, we use the commercially available diacetylene containing surfactant 1,2-bis(10,12-tricosadiynoyl)-*sn*-glycero-3-phosphocholine (23:2 Diyne PC, 23-carbon chain, Figure 2.1) to prepare capillary wall coatings for mixed aqueous/organic capillary zone electrophoresis. This is the first example of a CE coating based on diacetylene surfactant bilayers. The cross-linked bilayer coating is generated by *in situ* photo-polymerization within an exterior Teflon-AF coated transparent capillary. This photo-polymerized coating was optimized and characterized, and the separation of small amines was performed both using aqueous BGE and ACN/water BGEs.

2.2 Material and Methods

2.2.1 Apparatus

All CE experiments were performed using a Hewlett Packard ^{3D}CE instrument (Agilent Technologies, Palo Alto, CA, USA) equipped with a UV absorbance detector. 254 nm was used for direct detection of the neutral marker (mesityl oxide), and 214 nm was used for the amine separations. Data acquisition was at 10 Hz using HP ^{3D}CE ChemStation software (Agilent) for Windows XP on a Pentium 4 computer. Untreated exterior Teflon AF coated, deep UV-transparent fused silica capillaries (50 µm I.D. x 360 µm O.D., Polymicro Technologies, Phoenix, AZ, USA) with a total length of 32 cm (23 cm to the detector) were used. As the capillary is UV transparent, it was not necessary to create a window for absorbance detection.

Polymerization was performed at 254 nm within a Rayonet^{TM} photochemical reactor (Southern New England Ultraviolet Company, Branford, Connecticut 06405) with ~$1.65x10^{16}$ photons/s/cm^3.

2.2.2 Chemicals

Nanopure 18 MΩ water (Barnstead, Chicago, IL, USA), HPLC grade acetonitrile (ACN) and methanol (Fisher Scientific, Nepean, ON, Canada) were used. Buffers were prepared from ultra-pure tris(hydroxylmethyl)-aminomethane (Tris; Sigma-Aldrich, St. Louis, USA) adjusted to pH 7.4 with hydrochloric acid (Fisher). The diacetylene phospholipid 1,2-bis(10,12-tricosadiynoyl)-sn-glycero-3-phosphocholine (Diyne PC, Figure 2.1) was purchased from Avanti Polar Lipids (Alabaster, AL, USA) as a white powder.

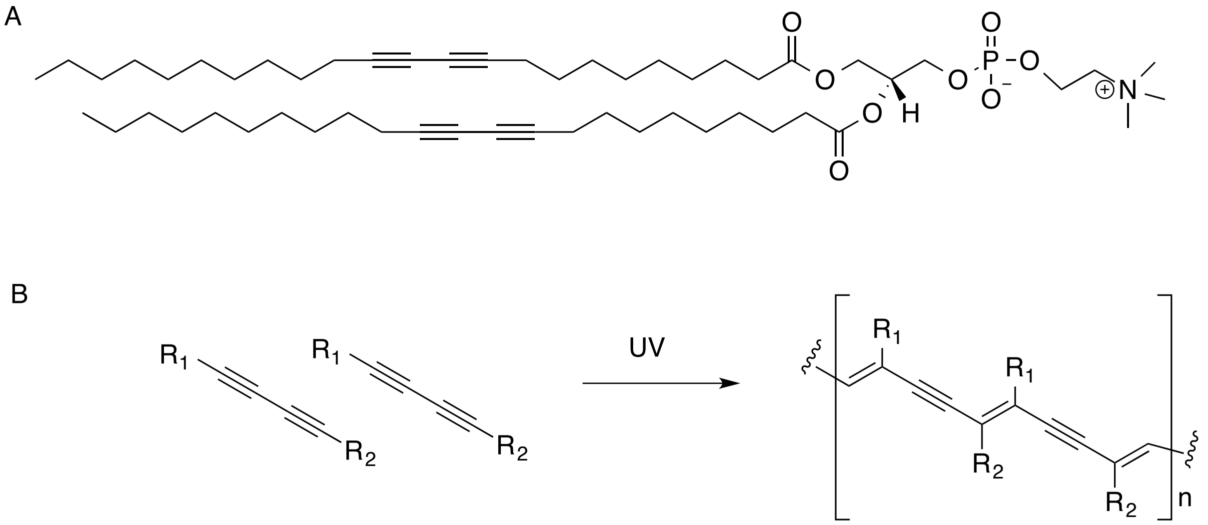


Figure 2.1 The structure (A) and the polymerization reaction (B) of 1,2-bis(10,12-tricosadiynoyl)-sn-glycero-3-phosphocholine (Diyne PC) [35].

Benzylamine, veratrylamine and 2-(p-toly)ethylamine (Aldrich, Milwaukee, WI, USA) and 2-phenyl ethylamine (BDH, Poole, England) were used as model analytes. Mesityl oxide (99%; Aldrich) was used as the neutral EOF marker.

2.2.3 Preparation and Polymerization of Diyne PC coating

The method used to prepare surfactant solution was similar to that described previously [18], except that all weighings were performed in a dark room equipped with a red light bulb (60 W) to avoid inadvertent photo-polymerization of the surfactant. 0.1 mM Diyne PC solution was prepared in 20 mM Tris buffer with 20 mM $CaCl_2$ at pH 7.4. The solution was sonicated (30 min at 60°C in an ultrasonic bath (Aquasonic Model 75HT, VWR Scientific Products) and stirred at room temperature for 15 min to form small unilamellar vesicles (Figure 2.2A) [22].This sonicate/stir cycle was repeated 2 - 3 times until the solution was clear. Vesicle solutions were used on the same day prepared.

New 50 μm I.D. capillaries (32 cm total, 23 cm to the detector) were mounted into a CE cartridge and rinsed (940 mBar) with 1 M NaOH for 15 min, 0.1 M NaOH for 15 min, Tris buffer rinse for 5 min, and finally with 0.1 mM Diyne PC in Tris buffer for 20 min (940 mBar). Non-adsorbed Diyne PC was removed from the capillary using a 1 min high-pressure rinse (940 mBar) with 20 mM Tris (pH 7.4) buffer leaving an adsorbed bilayer of Diyne PC on the capillary walls (Figure 2.2B). The capillary with the un-polymerized bilayer coating was removed from the cartridge, blocked at both ends with small rubber stoppers, placed into a 36 cm long quartz test tube filled with ~35 mL water, and cooled to 0°C (10 min) to rearrange the bilayer membrane into a crystal-like lattice to facilitate the photo-polymerization [34]. The frozen test tube including the coated capillary was then placed in the photochemical reactor (254 nm, ~1.65×10^{16} photons/s/cm^3) for 15 min to form the polymerized Diyne PC supported bilayer coating (Figure 2.2C). The test tube

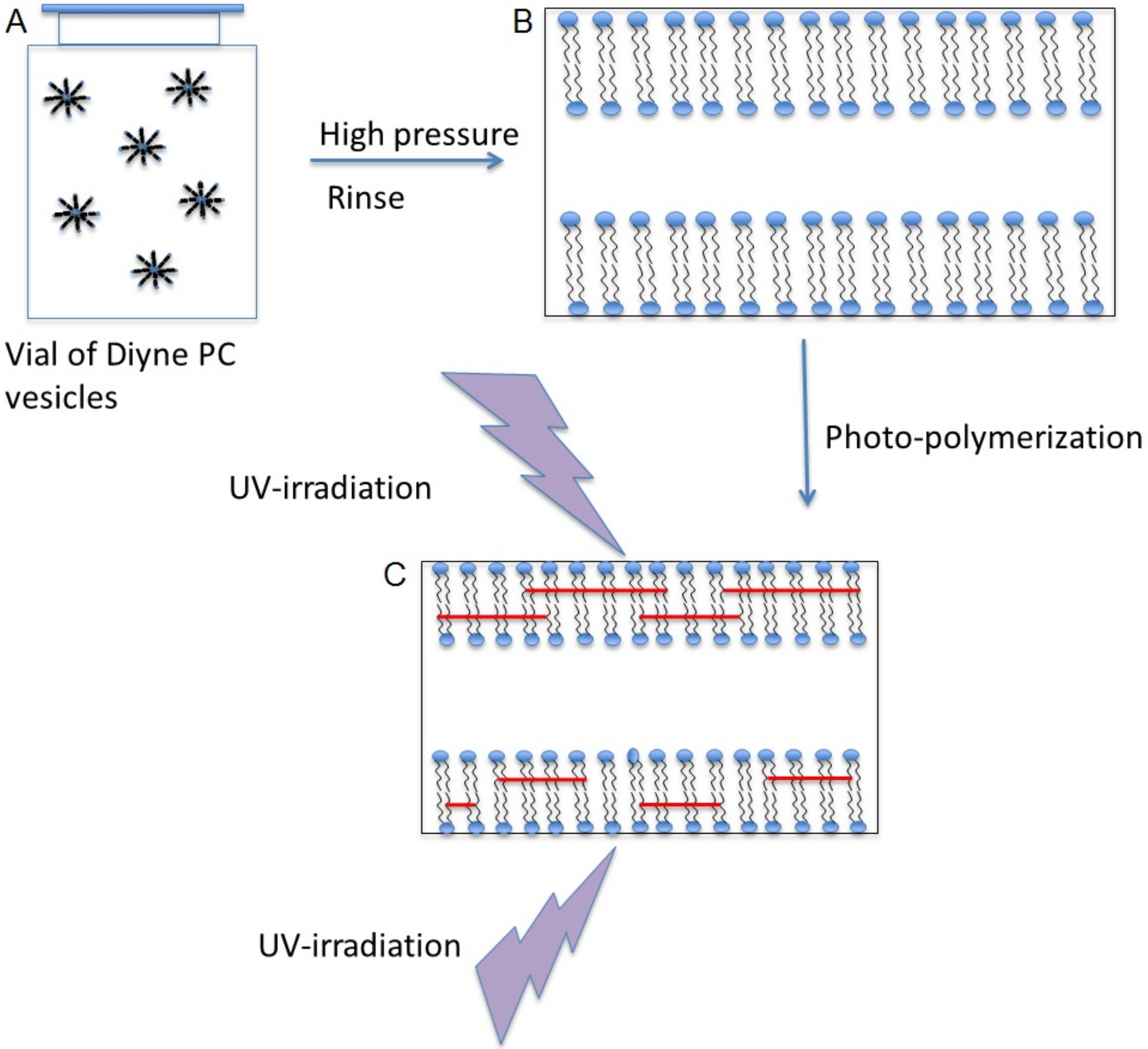


Figure 2.2 Schematic diagram of *in situ* preparation of photo-polymerized Diyne PC.

was removed from the photo-reactor, and the capillary was removed after the surrounding ice melted. The capillary with the photo-polymerized bilayer coating was then loaded back into the instrument cartridge for use in the Agilent CE.

2.2.4 Spectroscopic Monitoring of the Surfactant Polymerization Reaction

Vesicle solutions were prepared using the sonicate/stir method as described in Section 2.2.3. 10 mL aliquots transferred to a quartz test tube and sealed, cooled to $0^{o}C$, vacuum degassed and blanketed with N_2. The photo-polymerization kinetics was monitored using illumination times of 5 to 105 min within the Rayonet^{TM} photochemical reactor. After each illumination period, the UV-visible absorbance spectrum from 365 ~ 665 nm of the vesicle solution was monitored at room temperature using a 1.0 cm quartz cuvette and a Beckman Coulter DU800 UV/visible spectrometer (Beckman Coulter Canada, Inc., Mississauga, ON, Canada). Fluorescence emission spectra were collected at room temperature using a Photon Technology International MP1 fluorescence system (PTI, Birmingham, NJ, USA), controlled by FeliX32 software (PTI).

2.2.5 AFM Imaging of Polymerized Diyne PC Bilayer Formation

AFM images were obtained at room temperature using a Digital Instruments Dimension 3100 AFM (Veeco Metrology Group, Plainview, NY, USA) in tapping mode. Arrow NCR AFM tips with a resonant frequency of 285 kHz and a force constant of 42 N/m were acquired from NanoAndMore (Lady's Island, SC, USA). The silicon wafer substrate (University Wafers, South Boston, MA, USA) were pretreated with piranha solution (75% conc. H_2SO_4 (Caledon Lab Chemicals, Georgetown, ON, CA) / 25% (v/v) H_2O_2 (Fisher Scientific, Fair Lawn, NJ, USA) to remove the organic residue, and then cleaned with deionized water and dried under N_2.

2.2.6 Stability of Polymerized Diyne PC Coatings

The stability of the polymerized capillary coatings was characterized using the hydrodynamic rinse method [17,19]. The bilayer coating was prepared as described in Section 2.2.3. To test the stability of the coating, the EOF was monitored after successively longer rinses (940 mBar) of the coated capillary with either methanol or acetonitrile solutions. Rinse volumes are reported in terms of capillary volumes calculated using the Poiseuille equation. After each timed rinse, the capillary was flushed with 20 mM Tris (pH 7.4) aqueous buffer (940 mBar, 2 min) to fill the capillary with a consistent buffer, and the EOF was monitored by injection of 0.3 mM mesityl oxide (35 mBar, 2 s) and application of 20 kV.

2.2.7 Small Amine Separations

Amine separations were performed in bare and coated (Section 2.2.3) capillaries (50 µm I.D. 360 µm O.D. x 32 (23.5) cm long). Preliminary studies were performed on individual amines using mixtures of aqueous buffer (30 mM Tris, pH 7.4) with ACN; the optimized buffer condition was applied to the amine separations. An aqueous mixture of benzyl amine, veratrylamine, phenylethylamine and tolyethylamine (0.2 mM each) was injected hydrodynamically (35 mBar; 2.0 s) and separated at 20 kV. Efficiencies were based on the peak width at half-height method, as calculated by the ChemStation software (Agilent).

2.2.8 Storage Stability Study

A polymerized Diyne PC coated capillary was prepared as described in Section 2.2.3. After 30 days of use, it was stored dry at room temperature. After 2 months the capillary was rinsed with running buffer for 5 min at 940 mBar, and the amine separations were performed as described in Section 2.2.7. This process was repeated on the same capillary after another 2 months dry storage.

2.3 Results and Discussion

O'Brien and co-workers extensively studied the polymerization of unsaturated phospholipids, including sorbyl, diacetylenyl, dienoyl, methacryloyl and butadiene lipids, both in vesicle solutions and in supported phospholipid bilayers [35-37,43,44]. Applications of these polymerized bilayers include preparation of biocompatible ion channels [40,41,45], protein binding studies [46] and so on. Unfortunately the polymerizable bis-Sorbyl PC and bis-Dienoyl PC surfactants are not commercially available. Further their synthesis and purification is challenging. Of the other polymerizable surfactants, only the diacetylene containing amphiphiles are commercially available. This work is the first exploration of polymerized diacetylene bilayer coatings in separation science.

2.3.1 Preparation and Polymerization of Diyne PC by UV Irradiation

Free radical initiators are ineffective with diacetylene surfactants [44,47]. Thus, photo-polymerization was studied. Polymerization of the poly-diacetylene extends the overlapping π-orbitals within the system (Figure 2.1B) resulting in a visible change in solution color from transparent to red [48]. Attempts to photo-polymerize Diyne PC vesicle solutions at room temperature yielded little color change. Diacetylene containing amphiphiles can only be polymerized efficiently if the surfactant chains are well aligned in a solid-analogous phase or in other solid like assemblies [44]. Due to this topotactic nature, Diyne PC is best polymerized at lower temperatures [49]. Thus, as stated in Section 2.2.3 the vesicle solutions were cooled to ~0 ^{o}C prior to UV-irradiation. UV-visible spectra of a 0.1 mM Diyne PC vesicle solution during photo-polymerization (Figure 2.3A) show growth of two broad bands at 470 and 520 nm,

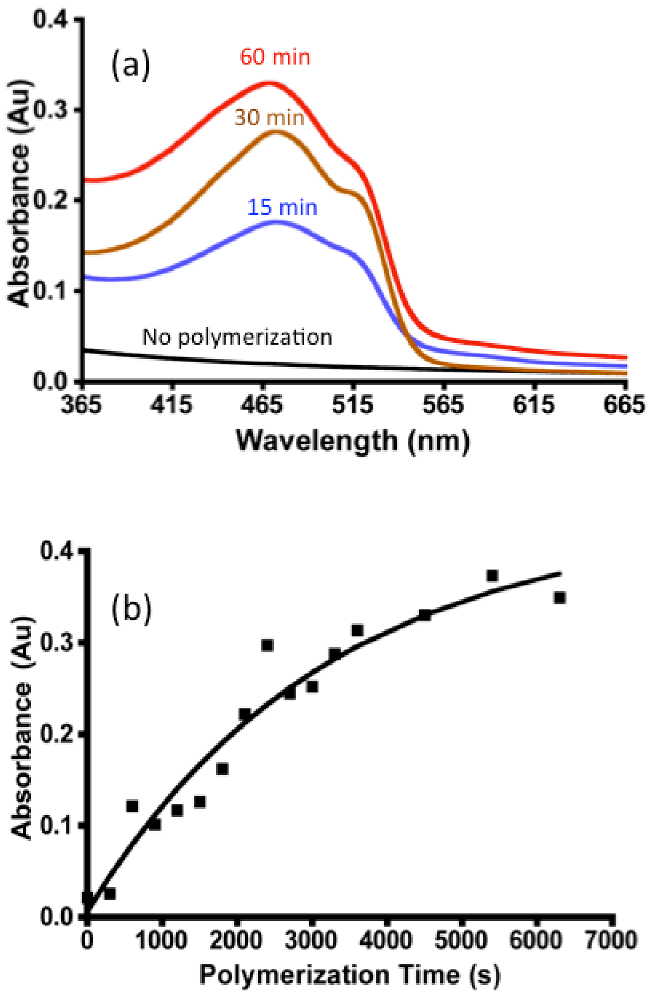


Figure 2.3 Characterization of the photo-polymerization of a Diyne PC vesicle solution. (a) UV-vis spectra of the 0.1 mM Diyne PC solution after different polymerization times; (b) kinetic monitoring of Diyne PC vesicle solution polymerization at 520 nm. The solid curve is the fit of the experimental data to first order. Conditions, as in Section 2.2.4.

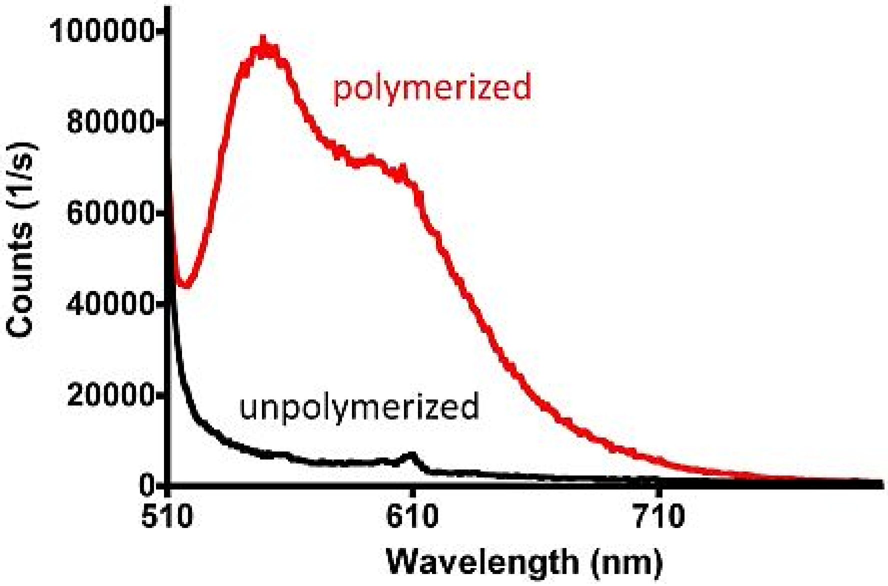


Figure 2.4 Fluorescence emission spectrum of polymerized and unpolymerized Diyne PC vesicle solution, conditions: 0.1 mM Diyne PC solutions, polymerized for 15 min at 0°C and detected at room temperature.

consistent with the literature [35,50]. Fitting the absorbance at 520 nm with irradiation time to a first order process (Figure 2.3B, R^2=0.94) yields a half-life of 37 min, consistent with reports that photo-polymerization was complete by 90 min [46]. Color changes for polymerization of Diyne PC solutions up to 0.5 mM indicate comparable kinetics. Fluorescence emission at 550 ~ 650 nm (Figure 2.4) increased with UV irradiation time, consistent with previous studies [51] and is also an indicator of polymer formation.

Atomic Force Microscopy has been widely used for imaging surfaces at the nanoscale, which facilitates the study of the physical properties of phospholipid bilayers and monolayers on solid surfaces [52,53]. Figure 2.5 are a series of images illustrating the formation of supported Diyne PC bilayers. Figure 2.5A is a control image performed on a wafer exposed to 20 mM Tris (pH 7.4) containing 20 mM $CaCl_2$. The control shows a smooth (0.3 nm vertical distance) surface. Figure 2.5B is the image of an unpolymerized Diyne PC bilayer formed by incubation of the substrate with 0.1 mM Diyne PC. The vertical distance associated with a defect on the surface indicates a depth of ~ 4 nm, consistent with the formation of a phospholipid bilayer [35]. Photo-irradiation of the bilayer in Figure 2.5B results in an *in situ* polymerized Diyne PC bilayer (Figure 2.5C) which again displays a ~ 4 nm thickness. The silicon surface in Figure 2.5B and 2.5C is largely covered by the bilayer coating. In contrast, Figure 2.5D is the image of a surface incubated with Diyne PC vesicles that have been photo-polymerized before incubating the substrate with the vesicles (*ex situ* polymerization). This surface is discontinuous with large defects. This indicates that the vesicles were too highly polymerized to rupture after adsorbing onto the substrate surface. Previously *ex situ* oligomerization was successfully used with the diene containing surfactant 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) [54]. However the success of ex situ polymerization with DOPC actually indicated that only limited oligomer formation (primarily dimers) had been achieved. Thus the failure of the *ex situ* polymerized

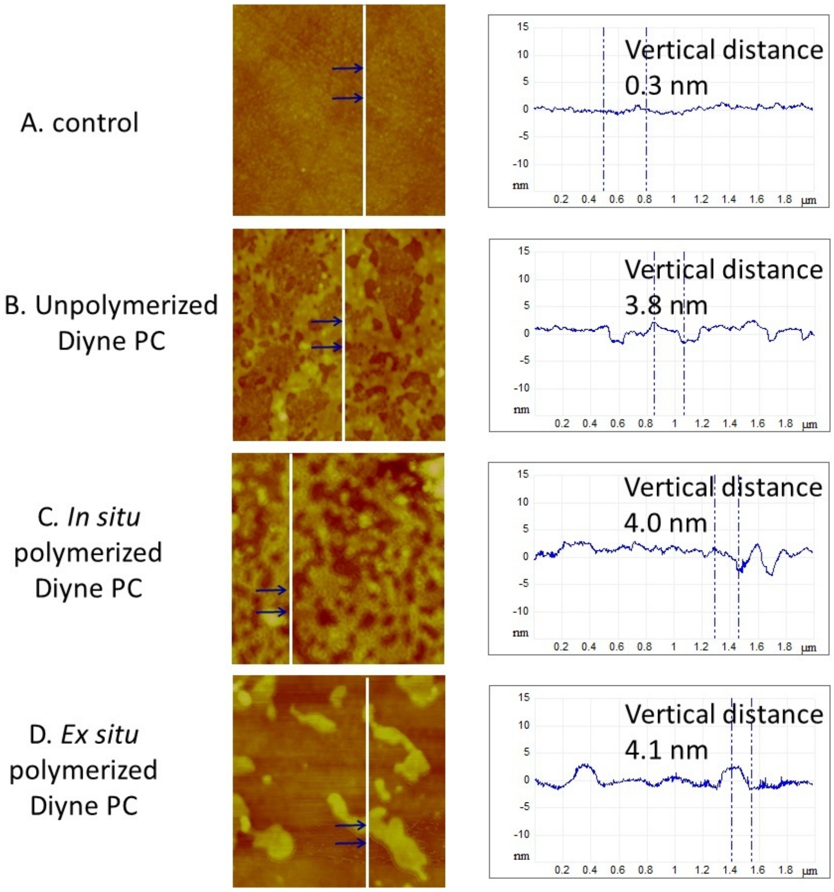


Figure 2.5 Tapping mode AFM height images (left) and depth profiles (right) for 2 µm × 2 µm (A) silicon wafer, (B) coated with unpolymerized, (C) *in situ* polymerized and (D) *ex situ* polymerized Diyne PC. Conditions: A) wafer exposed to the 20 mM Tris (pH 7.4) containing 20 mM $CaCl_2$. B) unpolymerized Diyne PC bilayer formed by incubation of the substrate with 0.1 mM Diyne PC in 20 mM Tris (pH 7.4) containing 20 mM $CaCl_2$ for 30 min. After removal of the vesicle solution, the substrate was rinsed with deionized water, dried under N_2, and then the bilayer was imaged as in Section 2.2.5. C) *in situ* polymerized Diyne PC bilayer was prepared as in b, dried with N_2 and cooled to 0 °C, and then UV-irradiated for 15 min in the Rayonet^{TM} photochemical reactor. The wafer was then rinsed and dried, and imaged. D) A solution of polymerized Diyne PC vesicle was prepared as described Section 2.2.4 (*ex situ* polymerization) and equilibrated with a wafer for 30 min, rinsed with water, dried and imaged.

Diyne PC in Figure 2.5D is indicative of a higher degree of polymerization. This result is also consistent with that of others who used the vesicle fusion method (*i.e.*, *in situ* polymerization) to prepare polymerized SPB films [55,56]. As the purpose of my research is to minimize the non-specific adsorption sites on capillary wall, higher surface coverage is preferred. Hence *in situ* polymerization is utilized in the remainder of my experiments.

2.3.2 Preparation and Characterization of Polymerized Diyne PC Coating

The *in situ* method was used to polymerize the Diyne PC coatings (Figure 2.2). The unpolymerized phospholipid vesicle solution (Figure 2.2A) was first rinsed through the capillary to form a non-polymerized supported bilayer membrane coating (Figure 2.2B), followed by UV irradiation of the whole capillary to cross-link the bilayer (Figure 2.2C). One challenge with this procedure is that the polyimide coating of a traditional CE capillary is not UV transparent. Aspinwall and co-workers removed sections of the polyimide coating to enable photo-polymerization of a bis-Sorb PC bilayer coating [57]. However this leaves the capillary fragile. Here I utilize the commercially available UV-transparent Telfon AF coated capillaries to facilitate in situ photo-polymerization within the capillary.

To compare the stability of the coatings, electroosmostic flow (EOF) measurements were taken as an indicator of the surface coverage. Bilayer coatings shield the surface silanols, and thereby suppress the EOF [3,17]. The EOF of a bare capillary in 20 mM Tris (pH 7.4) buffer is 5.7×10^{-4} cm^2/V-s. After equilibrating the capillary with 0.1 mM Diyne PC, the EOF decreases to 0.3×10^{-4} cm^2/V-s (square in Figure 2.6 at 0 capillary volume). This highly suppressed EOF is similar to that observed with other zwitterionic surfactants [20,22,58,59]. After polymerization, the EOF was 2.0×10^{-4} cm^2/V-s (triangle in Figure 2.6 at 0 capillary volume), indicating reduced surface coverage of the capillary wall. This phenomena also was observed for poly(bis-SorbPC) coatings [42], and is consistent with the AFM images (Figure 2.5).

Both the observed EOF and the former studies [42] indicated the polymerized bilayer membrane inside a capillary is not as continuous as the un-polymerized one, generating more film defects. As the nature of the micro-reaction performed inside a capillary, this topotactic reaction suffered from less uniformity and less UV irradiation intensity. Even worse, as it is impossible to dry and degas the capillary inside completely, the radicals that are the polymerization initiator would be scavenged by the oxygen remained in a capillary [60]. As a result, the polymerize reaction has lower yield compared to the reaction performed in solution or on open solid surfaces, resulting in formation of small membrane defects. Hence, the capillary surface charge was not fully masked and generates a less suppressed EOF.

The stability of the bilayer coating was enhanced by photo-polymerization. Previous studies have shown that two-tailed surfactant coatings such as dimethyldioctadecylammonium bromide are removed by formamide [28] or even 60% methanol-water [29]. There is no report of unpolymerized phospholipid coatings that can resist rinses with pure organic solvent. To characterize the stability of the Diyne PC bilayers coating, I hydrodynamically rinsed both the polymerized and non-polymerized Diyne PC coatings with pure methanol and acetonitrile. A series of solvent rinses were performed, with the EOF measured under aqueous conditions after each rinse. As shown in Figure 2.6A the non-polymerized Diyne PC coating was rapidly removed by methanol, as indicated by the EOF increasing from 0.3×10^{-4} cm^2/V-s to 5.3×10^{-4} cm^2/V-s within 80 capillary volumes of methanol rinse. On the other hand, for the polymerized Diyne PC coating, the EOF remained around 2.0×10^{-4} cm^2/V-s after exposed to over 1500 capillary volumes of methanol wash. Similarly for ACN wash (Figure 2.6B), the EOF of non-polymerized Diyne PC coated capillary returned to that of an uncoated capillary (5.7×10^{-4} cm^2/Vs) within 250 capillary volumes, while the EOF of the polymerized Diyne PC coated capillary remained unchanged after 600 capillary volume of ACN rinse. Compared to other non-covalent coatings in NACE, this polymerized Diyne PC coating does not require any

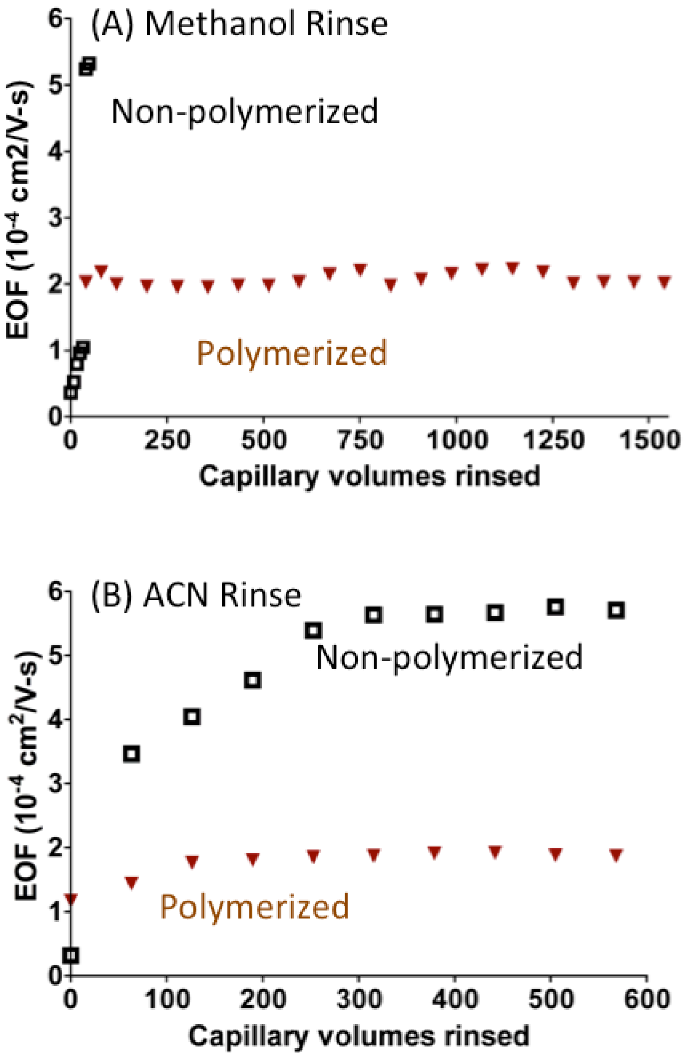


Figure 2.6 Stability of the EOF on non-polymerized Diyne PC coating (□) and a photo-polymerized Diyne PC coating (▼) upon hydrodynamic rinsing of the capillary with: (A) methanol; and (B) acetonitrile. Separation buffer: 20 mM Tris, pH 7.4. Conditions: non-polymerized Diyne PC and polymerized Diyne PC coated capillaries were prepared as described in Section 2.2.3; hydrodynamic rinses at 940 mBar for 5 to 200 min. Other conditions as described in Section 2.2.6.

regeneration [28,33,61]. Poly(Diyne PC) demonstrated stability similar to the poly vinyl alcohol (PVA) and poly ethylene glycol (PEG) covalent-linked polymer coatings previously used in NACE [62,63].

2.3.3 Amine Adsorption

Figure 2.7a illustrates the impact of adsorption on the peak shape of a small protonated amine (veratrylamine) on an uncoated capillary using BGE with various %ACN. A distinct and prolonged tail is observed in aqueous BGE, indicating analyte adsorption at the capillary wall.

When veratrylamine was run on the poly-Diyne PC coated capillary using aqueous BGE, the tailing due to adsorption was eliminated. Efficiencies calculated using the Foley-Dorsey equation (Equation 1.11) were only 60,000 plates/m [64], presumably due to hydrophobic interaction between the analyte and the cross-linked bilayer. Adding increasing amounts of ACN to the BGE resulted in improved peak efficiency and symmetry (Table 2.1). Table 2.1 also summarizes the performance observed for other amines using the poly-Diyne PC coated capillary. Other amine separations performed in NACE, which utilized electrochemical detection technique, generated comparable efficiencies of 180,000 and 700,000 plates/m for triethylamine and tripropylamine respectively [65]. Literature amine separations performed in methanol NACE showed ≤ 360,000 plates/m (our measure) using BGE containing chemical selectors and additives [61]; on a covalent-linked PVA coating on chip showed ~ 55,000 plates/m in DMSO [66]; and on a zirconia-coated capillary in ACN-methanol displayed ≤15, 000 plates/m (our measure) [67].

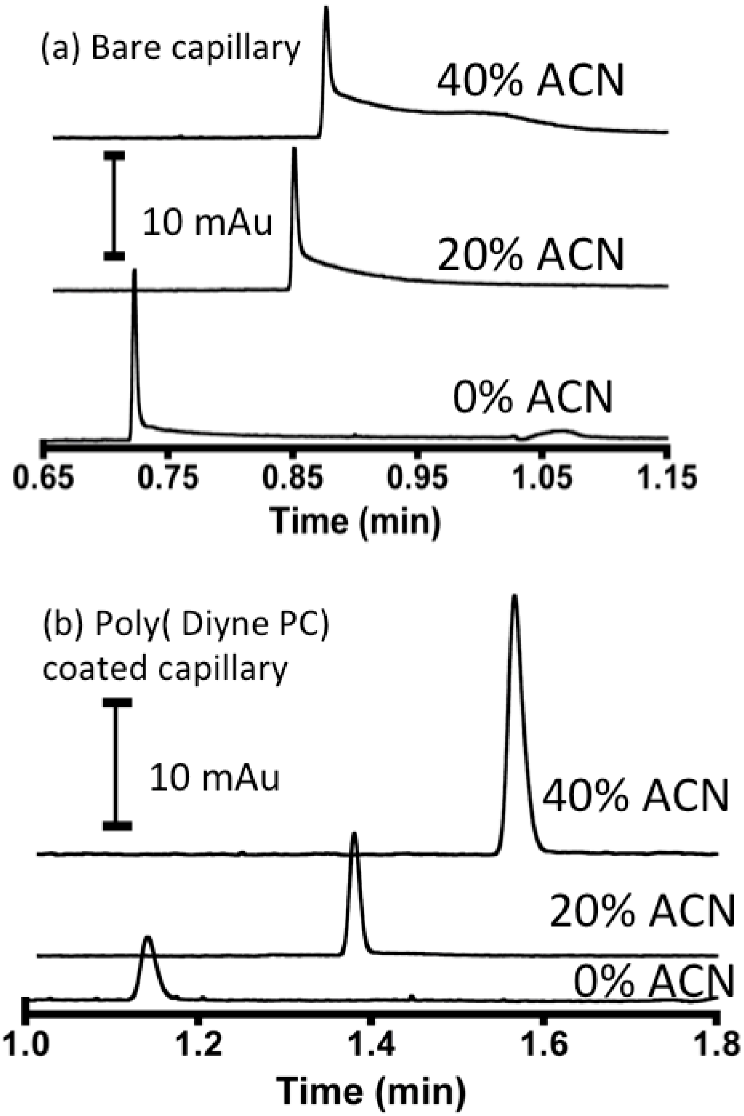


Figure 2.7 Separation of veratrylamine using: a) a bare capillary; and b) a polymerized Diyne PC coated capillary. Conditions: capillary, 50 μm I.D. x 32 cm (23.5 cm to detector) used as received or coated as in Section 2.2.3; injection, 0.2 mM veratrylamine at 35 mBar for 3 s; run buffer, 30 mM Tris, pH 7.4 mixed with ACN in v/v, ratio as indicated; voltage, 20 kV; detection, 214 nm.

Table 2.1 Amine separation comparison in bare and coated capillary

	Efficiency (plates/m)		Peak Asymmetry Factor	
	bare capillary[a]	poly-Diyne PC coated[b]	bare capillary	poly-Diyne PC coated
benzylamine	42	220,000	30	0.48
phenylamine	77	340,000	34	0.72
tolyethylamine	36	320,000	35	1.18
veratrylamine	80	370,000	45	1.06

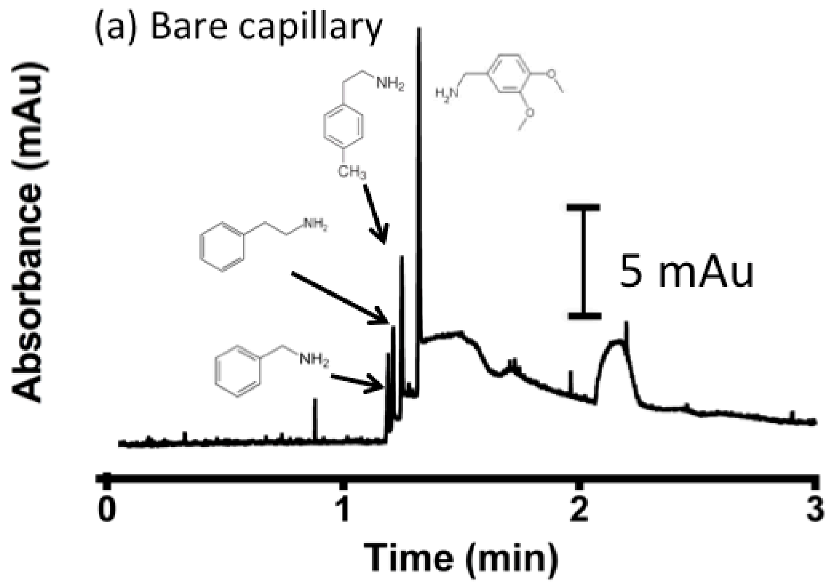
Conditions: 32 cm × 50 µm I.D. capillary; 20 kV; 60% 30mM Tris/40% ACN (v/v); pH 7.4

[a,b] efficiency calculated using Foley-Dorsey equation (Equation 1.11)

2.3.4 Amine Separation Study

To test the coating separation performance in NACE, four small amine compounds with similar charge-to size ratio were separated on the polymerized Diyne PC coated capillaries using 40% ACN-aqueous (30 mM Tris, pH 7.4; v/v) mixture as background electrolyte. Figure 2.8a shows that on a bare capillary adsorption of four compounds onto the capillary cause severe baseline distortions. With the poly(Diyne PC) coating (Figure 2.8b), the separation of all four amine compounds was achieved, yielding symmetric Gaussian peaks with high efficiency (Table 2.1). It should be noted the suppressed EOF also contributes to the improved peak resolution, compared to the co-EOF separation in Figure 2.8a. Also as shown in Figure 2.8, the elution order is the same on the poly(Diyne PC) coating and the bare capillary, indicating that hydrophobic interactions with the coating are not significant. As shown in Table 2.2, the migration time reproducibility, both on a single capillary and for multiple capillaries, were good.

Previous amine separations by CE required a pre-column analyte derivatization or introduced a pseudo-stationary phase to increase the amine mobility difference [66,68,69]. Here the four amine compounds were separated quickly on the poly(Diyne PC) coated capillary without any derivatization. Therefore this coating could be further applied for analysis of biogenic amines that are formed and broken down by typical metabolic processes in the cells of living organisms. These biogenic amines are expected to have similar mobility and are thus hard to separate.


(a) Bare capillary

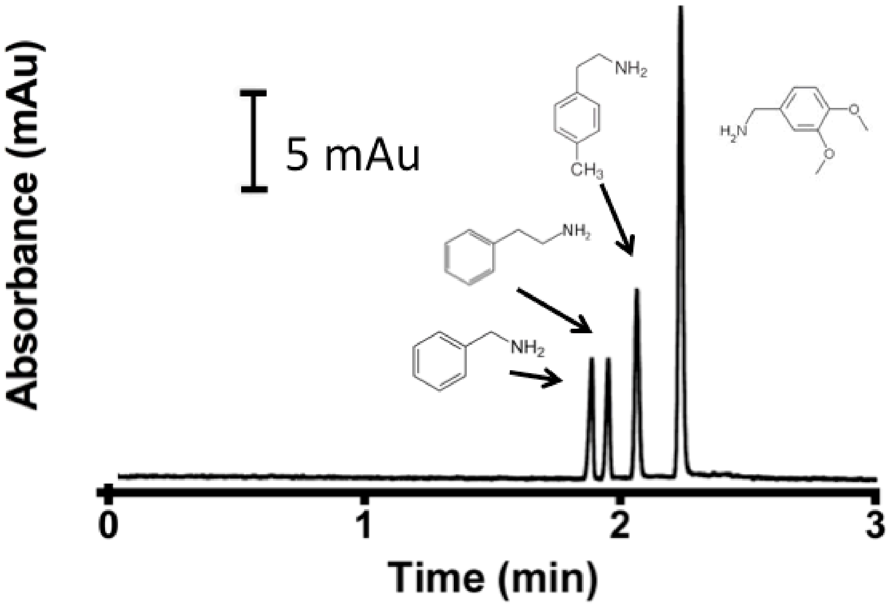

(b) Poly(Diyne PC) coated capillary

Figure 2.8 Separations of four amines on (a) bare capillary; and (b) polymerized Diyne PC coated capillary under NACE conditions. Conditions: applied voltage, 15 kV; capillary, 50 μm I.D. × 32 cm (23.5 cm to detector); buffer, 60% 30 mM Tris/40% ACN (v/v), pH 7.4; detection 214 nm; 0.2 mM amine sample dissolved in water and injected hydrodynamically at 35 mbar for 3 s.

Table 2.2 Separation reproducibility performed on poly-Diyne PC coating

	migration time RSD (%)	
	day-to-day (n=4)	capillary-to-capillary(n=4)
Benzylamine	1.8	4.1
Phenylethylamine	1.9	4.1
Tolyethylamine	2.0	4.3
Veratrylamine	1.9	4.8

Conditions: 32 cm × 50 μm I.D. capillary; 20 kV; 60% 30 mM Tris/40% ACN (v/v), pH 7.4

2.3.5 Storage Stability

Previously it has been demonstrated that the stability of surfactant bilayer coatings degraded rapidly with storage [42]. This degradation limited the direct shelf-bench use of the bilayer coatings. Riekkola and co-workers showed that unpolymerized phospholipid vesicle solutions were relatively stable for over 14 days [70]. However they did not investigate the storage stability of phospholipid coatings. I also investigated the CZE performance of polymerized Diyne PC coatings with storage time. Figure 2.9 shows electropherograms of the four amine compounds on a poly-Diyne PC coated capillary after two months and four months dry storage, respectively. The results clearly show efficient and repeatable separations of the amine compounds, with only a 10% change in migration time. This small change is presumed to be due to the gradual loss of the monomer Diyne PC that remained inside the polymerized bilayer coating, leaving more defects in the lipid film. As a result, the EOF increased from 2.0×10^{-4} cm^2/V-s to 3.4×10^{-4} cm^2/V-s, speeding up the separation.

2.3.6 Pharmaceutical Separations

Under aqueous buffer conditions the basic drugs metoprolol and chloropheniramine exhibit poor resolution and severe peak tailing on a bare capillary. To solve this problem CE separations of these basic pharmaceuticals have generally been performed under acidic buffer conditions [28,67]; however, Joule heating is a concern at such low pH. I separated the pharmaceuticals on a poly(Diyne PC) coated capillary using a mixed aqueous/organic solvent system. As shown in Figure 2.10a, on the bare capillary addition of organic solvent to the running buffer improved the resolution, but did not eliminate peak tailing. On the other hand, the electropherogram obtained using a poly(Diyne PC) coated capillary (Figure 2.10b) showed improved resolution and peak shape (efficiency: metoprolol 240,000 plates/m, chloropheniramine 210,000 plates/m).

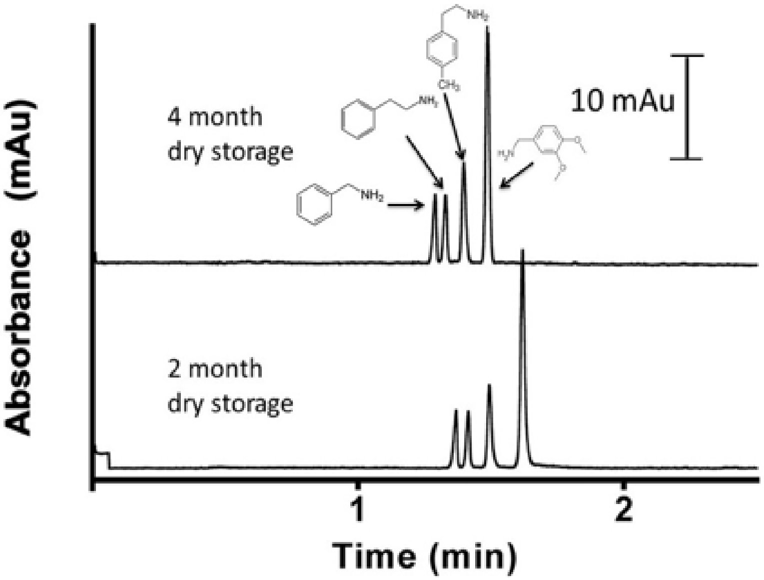
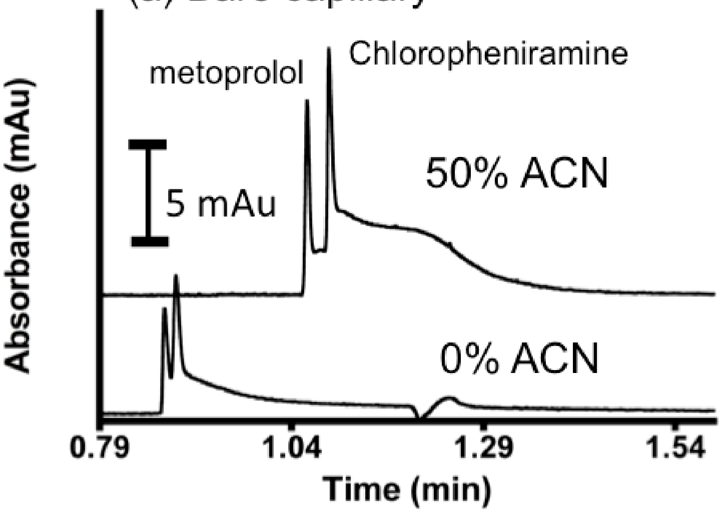


Figure 2.9 Effect of dry storage on separations performed with the poly-Diyne PC coated capillary. Conditions: applied voltage, 20 kV; capillary, 32 cm × 50 μm I. D. (23.5 cm to detector); buffer, 60% 30mM Tris/40% ACN (v/v), pH 7.4; detection 214 nm; 0.2 mM amine sample dissolved in water and injected at 35 mbar for 3 s.

(a) Bare capillary



(b) Poly(Diyne PC)

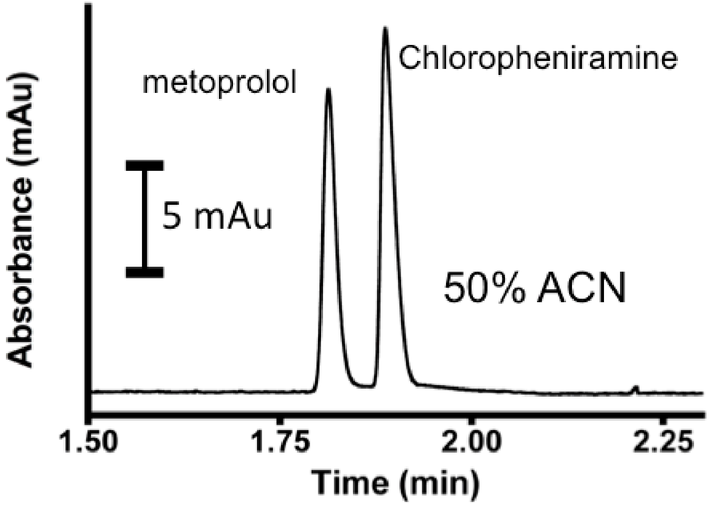


Figure 2.10 Separation of basic drugs on (a) bare capillary and (b) poly(Diyne PC) coated capillary. Conditions: applied voltage, 20 kV; capillary, 50 μm I.D. × 32 cm (23.5 cm to detector); buffer, 40 mM Tris, pH 7.4 mixed with ACN in v/v, ratio as indicated in figure; 0.5 mM metoprolol and chlorpheniramine sample was dissolved in deionized water and injected at 35 mbar for 3 s.

2.4 Conclusions

A highly stable cross-linked coating prepared by *in situ* polymerization of Diyne PC surfactant can be utilized in capillary zone electrophoresis. Under the optimized photo-polymerization conditions, Diyne PC can be polymerized effectively both *in situ* and *ex situ*. Using the *in situ* polymerization method, the monomer surfactant coating can be cross-linked and the stability was enhanced significantly. Moderately suppressed EOF can be maintained through organic solvent washes, and long-term dry-air storage. The EOF was not as suppressed as the monomer Diyne PC coating, consistent with the partial coverage noted using AFM studies. Nonetheless, the polymerized coating's ability to resist organic solvent rinse allowed the application of poly Diyne PC coating in an NACE separation. Also as the poly(Diyne PC) coating shielded the silanol groups in capillary wall, it reduced the adsorption of cationic analytes onto the capillary wall, and hence improved the peak shape and separation resolution. This newly developed coating can be further applied to separations of basic pharmaceutical compounds and biogenic molecules.

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Chapter Three: Insight into the Stability of PDADMAC and Polybrene Polycationic Coatings in Capillary Electrophoresis[*]

3.1 Introduction

Ionization of the silanol groups on the silica capillary wall is responsible for the electroosmotic flow (EOF) within capillary electrophoresis (CE). The deprotonation of the surface silanol groups fluctuates particularly in the pH range of 4~6 [1,2]. But even for a given pH, the EOF can vary due to hysteresis. The irreproducibility of the EOF has been one of the key challenges in acceptance of CE. A second challenge is that positively charged analytes, particularly polycationic species such as proteins, may adsorb onto the negatively charged surface [3-6]. Consequences of capillary wall adsorption include reduced resolution, band broadening [7], sample loss [8] and poor migration time repeatability and reproducibility [5,9]. Analyte adsorption may also change the zeta-potential of the capillary surface, and so the EOF is also affected.

The most common solution to these problems is to coat the capillary to shield the analyte from the surface, and vice versa [10,11]. The coating can be affixed to the capillary surface either through covalent bonds or physical adsorption. Covalent coatings are widely used but require complex and time-consuming synthetic steps, which increases the cost of a CE analysis [12-14]. A key limitation of covalent coatings is that they are limited to the pH 2-8 range [15,16].

An attractive alternative are physically adsorbed coatings due to the simplicity of their coating procedure and ease of automation [5,17,18]. One example is the zwitterionic surfactant coating demonstrated in Chapter 2. Another commonly used physically adsorbed coating is polymers. Physically adsorbed polymer coatings may be based on

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neutral polymers, such as poly(vinyl alcohol) [19], poly(ethylene oxide) [20] or poly(hydroxylethylacrylamide) (PHEA) [21]. However, these hydrophilic neutral polymer coatings show relatively poor stability, and so require coating regeneration between runs [20,22] or need to be present in the background electrolyte [23]. Therefore they are classified as dynamic coatings [10,11]. Increasing the hydrophobicity of the neutral polymer coating has been demonstrated to increase the stability of the coating [23]. However, the hydrophobic moieties in the polymer promote adsorption of hydrophobic proteins and other large biomolecules, limiting their application in bio-analysis [24].

In 1990, Towns and Regnier [25] and Wiktorowicz and Colburn [26] simultaneously introduced static cationic polymer coatings for protein separations. Due to the stronger interactions between coatings and fused-silica wall, static coating materials need not be present in the background electrolyte [10]. The polycationic polymers adsorbed onto the capillary surface through strong electrostatic interactions, making the adsorbed polycation coatings more stable than neutral polymer coatings. Adsorption of the cationic polymer reversed the wall charge [27,28]. Since then, cationic polymer coatings have been widely applied to CE, for biomolecules separation [29], isoelectric focusing and sample pre-concentration [30]. Subsequently, successive multiple ionic-polymer layer (SMIL) coatings were introduced [31-33]. In a SMIL, the capillary is first coated with a cationic polymer layer. Then an anionic polymer layer such as dextran sulfate was adsorbed through electrostatic interaction with the cationic layer. In this manner a pH independent normal EOF was achieved. Additional counter-charged polyelectrolyte layers can be added to yield reversed or normal EOF [34,35]. Adsorbed coatings have been applied to CE-mass spectrometric analysis of biological molecules [34,36-39]. Given the wide application of cationic polymers as physically adsorbed coatings, it is very important to understand their coating behavior.

The cationic polymer coatings in the literature are all poly-amines. Examples include: chitosan [40] which contains primary amines; linear polyethylenimine [41] (PEI) that contains secondary amines; branched PEI which contains primary, secondary and tertiary amines [25]; Polybrene [42] and poly(diallydimethylammonium chloride) (PDADMAC) [43] which possess quaternary amines. Generally speaking, the quaternary amine-based cationic polymers are preferred as the quaternary ammonium groups remain permanently charged regardless of the background electrolyte (BGE) pH [44]. Hence these coatings exhibit low variation in EOF with pH and can be operated over a wide pH range [43].

Surprisingly, the literature suggests that adsorbed quaternary amine-based polymers do not form stable coatings under alkaline BGE conditions. For instance, a drifting EOF was observed at pH > 8 for PDADMAC monolayer coatings [34,45]. Indeed a single layer of PDADMAC can be removed by a 1 M NaOH rinse [46]. Polybrene (hexadimethrine bromide) also showed similar EOF mobility drift (RSD~9%) at pH 8.3 [28]. In contrast, SMIL coatings consisting of PDADMAC and poly(sodium-4-styrenesulfonate) can endure extreme alkaline rinses [46]. Likewise a polybrene/poly(vinyl sulfonic acid) coated capillary demonstrated better separation reproducibility (RSD~0.68%) in pH 8.5 BGE [36] compared to the polycationic coating alone.

Thus, although the instability of the cationic polymer coatings at high pH is a well-known phenomenon, no systematic investigations have been carried out to explain this surprising behavior. In this chapter I investigate the underlying cause of this instability, using both electrophoretic methods and X-ray photoelectron spectroscopy.

3.2 Experimental

3.2.1 Chemicals

Poly(diallyl dimethyl ammonium chloride) (PDADMAC) of M.W. 0.085×10^5, 28 wt.% in water was from Polysciences Inc. (Warrington, PA, USA). PDADMAC of M.W. 7.6×10^4 (35 wt.% in water), 1-2×10^5, 2-3.5×10^5 and 4-5×10^5 (all 20 wt.% in water), N-Cyclohexyl-2-aminoethanesulfonic acid (CHES; purity 99%), hexadimethrine bromide (polybrene, purity ≥95%, M.W. not given), poly(sodium 4-styrenesulfonate) solution (PSS, M. W. 1.0×10^6, 25 wt% in water), potassium iodate (99.5%), sodium bromide (99+%) and benzoic acid (99%) were from Sigma-Aldrich (St. Louis, MO, USA). Sodium dihydrogen phosphate monohydrate (≥99.3%) and hydrogen peroxide were from Fisher Scientific (Fair Lawn, NJ, USA). Mesityl oxide (>90%) was from Aldrich (Milwaukee, MI, USA). Sodium hydroxide (>97.0%) was from Anachemia (Montreal, QC, CA). Sodium chromate (anhydrous, 96%) was from BDH (Poole, England, UK). Sodium chloride (purity > 99.0%) was from EMD Chemicals (Gibbstown, NJ, USA). Nanopure 18 MΩ water (Barnstead, Thermo Scientific, Marietta, OH, USA) was used throughout. Concentrated sulfuric acid was from Caledon Lab Chemicals (Georgetown, ON, CA).

Various background electrolyte solutions (BGE) were used to cover the pH 7.0~11.0 range. For pH 7.0, 7.5, 8.0, 8.5, 9.0 and 11.0, 20 mM phosphate buffers were prepared by adjusting the pH of a 20 mM sodium dihydrogen phosphate solution with NaOH. NaCl was added to the phosphate buffers to adjust the ionic strength to 62 mM, which matches the ionic strength of the 20 mM pH 11.0 phosphate solution. For pH 9.5, 20 mM CHES buffer was prepared by adjusting the pH of a 20 mM CHES solution with NaOH.

Coating solutions of PDADMAC (0.5% w/w) and polybrene 10% (w/w) [28,32] were prepared in deionized water unless otherwise noted.

3.2.2 Apparatus

Capillary electrophoresis was performed on a Beckman PA 800 plus Pharmaceutical Analysis system with a UV absorbance detector (Beckman Instruments, Fullerton, CA, USA). Untreated fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) with an inner diameter of 50 μm, outer diameter of 365 μm, and total length of 40.2 cm (30 cm to detector) were used. Data acquisition (4 Hz) and control were performed using 32 Karat software for Windows XP on a Lenovo Thinkcentre computer. The capillary was thermostated at 25°C.

3.2.3 Capillary Coating

New capillaries were used for all coating studies. Before coating, each new capillary was pretreated with methanol for 10 min (20 psi rinse, 1 psi = 68.95 mbar), 1 M NaOH for 10 min (20 psi rinse), 0.1 NaOH for 10 min (20 psi), and finally deionized water for 5 min (20 psi). The EOF was checked to confirm consistent initial conditions. To generate a PDADMAC coating, the pretreated capillary was rinsed with a PDADMAC coating solution (composition detailed in discussion) for 15 min (20 psi), followed by a 5 min rinse (5 psi) with the BGE to remove any excess coating solution [46]. Longer coating times resulted in the same EOF.

For polybrene coatings, the pretreated capillary was rinsed with a polybrene coating solution (composition detailed in discussion) for 15 min (5 psi), followed by a 5 min rinse (5 psi) with the BGE.

For SMIL coatings, a 5-layer PDADMAC/PSS coating was used, i.e., the last deposited layer was a PDADMAC layer [46]. Coating solutions were prepared by dissolving the cationic PDADMAC (M.W. 4-5x10^5) or the anionic PSS at 0.5 wt% in water. The cationic and anionic polyelectrolyte layer were alternatively deposited by rinsing the capillary with corresponding coating solution for 15 min (20 psi) with a 5-min rinse (20 psi) with BGE between coatings. EOF measurements were taken after each layer was deposited to ensure formation of each layer.

3.2.4 Coating Stability

The hydrodynamic stability method was used to assess the stability of the coatings [47]. Specifically, to evaluate the polycation coating stability in different BGE buffers, a series of 5 min high-pressure (30 psi) buffer rinses were performed. After each pressure rinse assault on the coating, an EOF measurement was made to assess the surface coating. The EOF was determined with 2 mM mesityl oxide as a neutral marker using a 0.5 psi 3 s hydrodynamic injection. The capillary was thermostated to 25 °C, detection was at 254 nm, and a constant voltage of ±18 kV was applied (depending on the direction of the EOF).

3.2.5 XPS Characterization

X-ray photoelectron spectroscopy (XPS) was performed using a Kratos Analytical AXIS 165 spectrometer with a monochromatic Al Kα source ($h\upsilon$ = 1486.71 eV). The spectrometer was calibrated by the binding energy (84.0 eV) of Au $4f_{7/2}$ with reference to the Fermi level. The pressure of the analysis chamber during experiments is better than 9.7×10^{-12} psi. High-resolution scans were conducted with a pass energy of 20 eV. The X-ray source was run at 14 kV and a current of 15 mA. All binding energies were referenced to the C 1s hydrocarbon peak at 284.8 eV.

The silicon wafer substrates (University Wafers, South Boston, MA, USA) were pretreated with piranha solution (75% conc. H_2SO_4 and 25% (v/v) H_2O_2) to remove any organic residue prior to use.

To mimic the capillary pretreatment procedure (Section 3.2.3), each silicon wafer was immersed in methanol for 10 min, in 1 M NaOH for 10 min and 0.1 M NaOH for 10 min. The substrate was then rinsed with water before use.

To investigate the surface chemical change of the polycation, the silicon substrates were immersed in polycation solutions for different periods of time (1, 24, 48 and 72 h), and then rinsed with deionized water, and dried using N_2 before XPS analysis.

Comparable experiments were performed on SMIL coatings. The SMIL coating was prepared by immersing the silicon wafer in 0.5 wt.% PDADMAC solution for 15 min and then immersing the wafer in 0.5 wt.% PSS solution for 15 min. This process was repeated until there were 5 layers of polyelectrolyte on the wafer. Then the wafer was rinsed with deionized water to remove excess polymer solution. As a control, a single layer of PDADMAC was deposited onto another silicon wafer by immersion in 0.5 wt% PDADMAC solution for 75 min. These wafers were rinsed with deionized water and dried with N_2 before XPS analysis.

3.2.6 Chemical Conversion of Quaternary Amine Functionality

To study the polycation conversion to a tertiary amine in solution, PDADMAC coating solutions were prepared in three ways: without any pre-treatment (i.e., as in Section 3.2.1); with alkaline pre-treatment; and with a pH 7 ionic strength control.

The alkaline treatment was performed by diluting PDADMAC (M.W. $4\text{-}5\times10^5$) to 1% (w/w) with 1 M NaOH, and allowing the solution to sit for 24 h. The solution was then neutralized to pH 7 with 1 M HCl, resulting in a solution of 0.5% (w/w) PDADMAC in 0.5 M NaCl. To make an ionic strength control solution, 0.5% (w/w) PDADMAC (M.W. $4\text{-}5\times10^5$) was prepared directly in 0.5 M NaCl. The untreated coating solutions were prepared by diluting the concentrated PDADMAC (M.W. 0.085×10^5, 0.76×10^4, $1\text{-}2\times10^5$, $2\text{-}3.5\times10^5$, $4\text{-}5\times10^5$) to a concentration of 0.5% (w/w) in deionized water. These solutions were both used in capillary coatings and XPS characterization.

3.2.7 Inorganic Anion Separation

A 50 μm I.D. × 60.2 cm (50 cm to detector) capillary was coated with a monolayer of M.W. 0.085×10^5 or $4\text{-}5\times10^5$ PDADMAC as per Section 3.2.3. The BGE was 20 mM CHES at pH 9.5. A 1.0 mM mixture of bromide, chromate, iodate and benzoate was injected at 0.5 psi for 0.5 s, and separated using -25 kV. The anions were detected using direct detection at 210 nm. Between each run, a 1-min rinse (20 psi) of BGE was performed to remove any excess analyte solutions. Comparable separations were also performed on the SMIL coated capillary detailed in Section 3.2.3.

3.3 Results and Discussion

Quaternary amines are permanently positively charged, regardless of solution pH. The pK_a of surface silanols ranges between 4 and 6 [1]. Thus, strong electrostatic attraction would be expected under alkaline conditions, and so it was expected that adsorbed quaternary amine polycationic polymers would have greater alkaline stability than covalent coatings, which are limited to pH ≤ 8 [13]. The literature observations of limited stability for polybrene and PDADMAC coatings at pH > 8 were therefore surprising [34,45,46].

There are no systematic studies of the pH effect on PDADMAC coatings in the literature. To verify the effect of BGE pH on polycationic polymer coatings, we tested the stability of a coating prepared with M.W. 0.085×10^5 PDADMAC over a range of pH (Table 3.1). To compare the stability of the coatings, the electroosmotic flow (EOF) mobility was used to indicate the surface charge. As expected, the initial EOF observed for PDADMAC coated capillaries was reversed and near pH independent. Previous literature has shown that PDADMAC forms stable coatings from pH 2.5 to 7.0 [46]. Indeed, Table 3.1 shows that after 50 min of high pressure (30 psi) rinsing with pH 7.0 buffer, the reversed EOF only decreased 8% in magnitude. However, as the pH of the BGE used to rinse the PDADMAC coated capillary became more alkaline, the change in the observed EOF become greater.

Table 3.1 Effect of pH on coating stability of PDADMAC (0.085×10^5)

	Initial EOF	EOF after pressure rinse	Variation of EOF mobility
	10^{-4} cm^2 V^{-1} s^{-1}	10^{-4} cm^2 V^{-1} s^{-1}	%
pH 7.0	-3.23	-2.97	8
pH 7.5	-3.32	-2.91	12
pH 8.0	-3.42	-2.82	17
pH 8.5	-3.38	-2.45	27
pH 9.0	-3.12	-1.87	40

Conditions: 20 mM phosphate buffers were adjusted to required pH with NaOH. The ionic strength was adjusted with NaCl to 62 mM (ionic strength of 20 mM pH 11 phosphate buffer). The PDADMAC coated capillaries were rinsed hydrodynamically with the buffer for 50 min at 30 psi between EOF measurements.

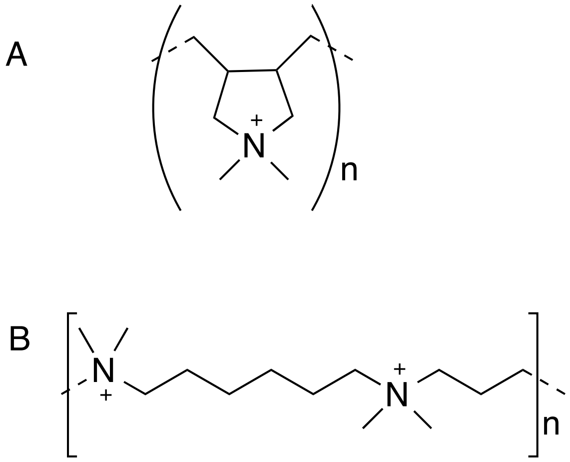

Figure 3.1 Structure of (A) PDADMAC and (B) polybrene.

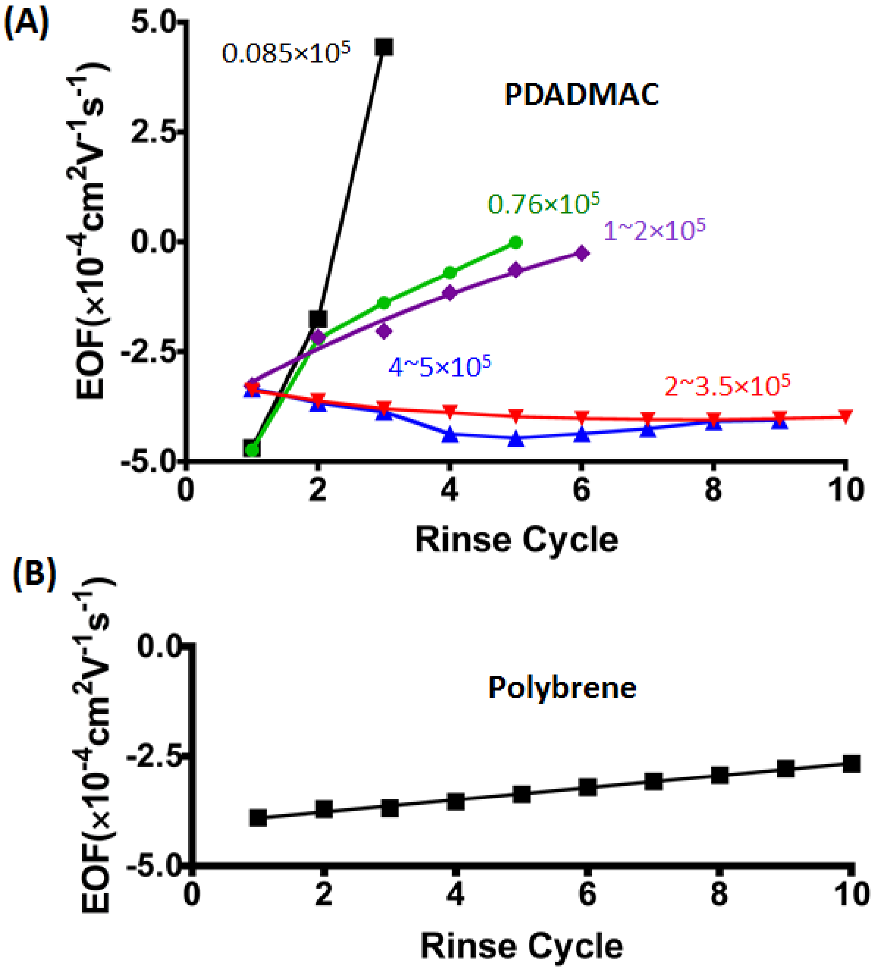

Figure 3.2 Stability of polycationic coatings in pH 9.5, 20 mM CHES solution. A) PDADMAC coatings with molecular weight indicated. B) Polybrene coating stability. Each "rinse cycle" refers to a 5 min 30 psi rinse of the coated capillary with the pH 9.5 buffer. Conditions: capillary, 50 µm I.D. x 40.2 cm (30 cm to detector) coated as in Section 3.2.3; after each pressure rinse the EOF was measured by 0.5 psi 3 s injection of 2 mM mesityl oxide, application of ±18 kV at 25°C, and detection at 254 nm.

Thus, the literature's observations of limited stability for polycationic-adsorbed coatings under alkaline conditions are confirmed. The studies below seek an understanding of the formation of adsorbed polycationic polymer coatings in CE, and of their instability under alkaline conditions.

3.3.1 Effect of Molecular Weight on Coating Stability

Many studies using polymers to form adsorption coatings in capillary electrophoresis fail to quote the molecular weight (M.W.) of the polymer used. As shown in Table 3.1, the alkaline conditions weakened the stability of the PDADMAC coatings, allowing the degradation experiments to be performed in reasonable time. This is despite Wang et al. reporting dramatically greater stability for coatings formed with high molecular weight PDADMAC (428 000) than low (30 000) [45]. To elaborate on the preliminary studies of Wang et al., we studied coatings prepared from PDADMAC with 5 different molecular weights (Figure 3.2A). These studies were performed at pH 9.5.

The EOF of a bare capillary in a pH 9.5, 20 mM CHES solution is + 5.0×10^{-4} $cm^2V^{-1}s^{-1}$. When the cationic polymer adsorbs onto the capillary, the surface charge becomes positive and the EOF reverses in direction (-3 to -5 $\times10^{-4}$ $cm^2V^{-1}s^{-1}$), as has been observed in the literature [46]. The reversed EOF observed immediately after coating are similar regardless of the M.W. of the PDADMAC (Figure 3.2A).

Figure 3.2A shows that the coating stability improves with increasing M.W. of the PDADMAC. For M.W. $0.085\text{x}10^5$, the EOF rapidly reverted to the normal direction, indicating fast desorption of the PDADMAC coating. The EOF associated with PDADMAC coatings prepared from M.W. $0.76\text{x}10^5$ and 1-2 $\text{x}10^5$ also drifted back to the normal EOF direction, with the drift being faster for the lower M.W. polymer. In contrast, coatings prepared from higher M.W. PDADMAC (M.W. 2-$3.5\text{x}10^5$ and 4-$5\text{x}10^5$), were more stable when exposed to over 10 rinse cycles (over 300 capillary volumes) of pH 9.5 buffer.

Polybrene (hexadimethrine bromide) possess linear quaternary amines while PDADMAC has cyclic quaternary amines. A polybrene coated capillary exhibit a similarly unstable EOF in pH 9.5 BGE (Figure 3.2B). This is consistent with previously reported polybrene performance [28,31].

3.3.2 Base-Catalyzed Instability of Polycationic Coatings

Section 3.3.1 demonstrates the importance of the molecular weight of the polycationic polymer on the stability of the semi-permanent capillary coating. However these studies do not explain the pH dependence of the stability evident in Table 3.1. A polycation based on quaternary amines should yield coatings whose EOF does not vary with pH, as the charge of the functional group would be +1 regardless of the pH. In contrast, the behavior in Table 3.1 is suggestive of a weak base functionality (i.e., primary, secondary, or tertiary amine), which have pK_a of 9-10.

There is precedence in the literature for base catalyzed conversion of quaternary amines to tertiary amines in the presence of hydroxide via Hoffman elimination [48,49]. Quaternary amines derived from β amino ketones have been observed to decompose rapidly at room temperature or lower [50]. However studies of adsorbed cationic polymer degradation at room temperature are limited [51].

PDADMAC polymer with M.W. $4\text{-}5\times10^5$ were exposed to strong alkaline conditions (0.1 M NaOH) for 24 hours, prior to neutralization of the solution and coating onto the capillary (Section 3.2.6). Two controls were used in this study. Firstly, the same PDADMAC was used without any pre-treatment (i.e., coated onto the capillary as in Section 3.2.3). Secondly, as neutralization of the NaOH increased the ionic strength, an ionic strength control was prepared with PDADMAC in 0.5 M NaCl.

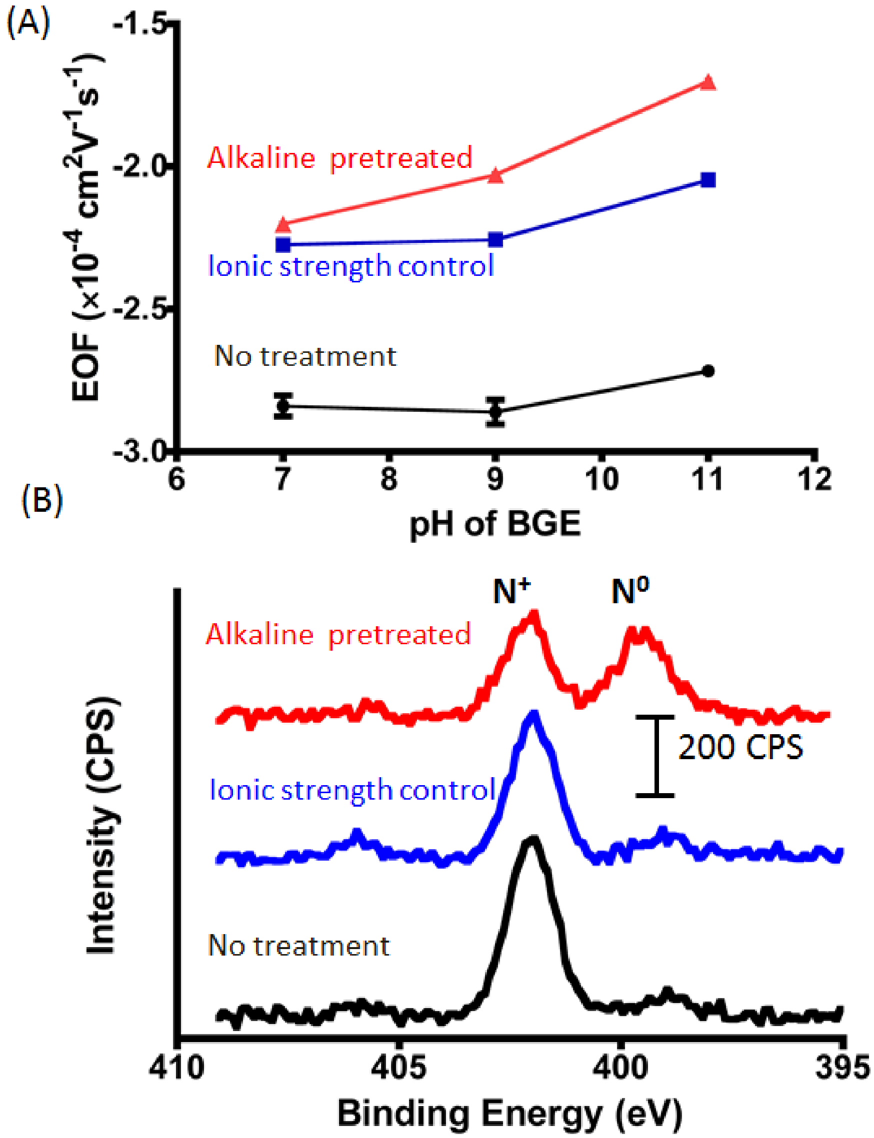

Figure 3.3 Impact of alkaline conditions on PDADMAC (M.W. 4~ 5×10^5) coating. (A) EOF on capillaries coated with: fresh 0.5% w/w PDADMAC (black); 0.5% w/w PDADMAC in 0.5 M NaCl (ionic strength control, blue); and PDADMAC stored in 1.0 M NaOH for 24 h, then neutralized with HCl. Capillaries and EOF measurements (n=3) as in Figure 3.1. In some cases the error (1 standard deviation) is smaller than the data point. B) High-resolution N 1s XPS spectra of the three PDADMAC coatings on silicon wafer. The silicon wafers were immersed in the 0.5% w/w PDADMAC coating solution for 1 h to form the coating.

Figure 3.3A compares the EOF behavior of the native PDADMAC, the alkaline treated PDADMAC, and PDADMAC in a comparable ionic strength solution. Consistent with Figure 3.2, the high M.W. PDADMAC provides a stable EOF up to pH 9, but the magnitude of the reversed EOF decreases at higher pH. In comparison, the NaCl pretreated PDADMAC coating showed a 0.7×10^{-4} $cm^2V^{-1}s^{-1}$ lower EOF value at all pH, but a similar stability profile.

Previous studies have shown that increasing the ionic strength of the coating solution decreases the magnitude of the reversed EOF [34,52]. The alkaline pretreated PDADMAC generates a similar EOF as the ionic strength control at pH 7.0, but an increasingly weaker EOF than the controls as the BGE pH increases. This behavior is consistent with the hypothesis that tertiary amines with pK_a ~ 9-10 have been formed within the PDADMAC by the alkaline pretreatment.

X-ray photoelectron spectroscopy (XPS) was used to confirm that tertiary amines are formed in PDADMAC under alkaline conditions. XPS provides both elemental and chemical state information for surface chemistry [53]. XPS has been used to study silica surfaces modified by a covalent bonded phase [54,55] or with physically adsorbed polymer layers [56]. XPS has been widely used for quantification of surface quaternary and tertiary amines [51,57-59]. Figure 3.3B shows the N 1s region of the XPS spectra for silicon substrate that was equilibrated with the three coating solutions used in Figure 3.3A. The peaks at 402.1 eV and 399.5 eV correspond to the N^+ (quaternary amine) and N^0 (tertiary amine) state, respectively [58-61]. PDADMAC should not contain any tertiary amines. Nonetheless, the N 1s XPS analysis of the untreated PDADMAC coatings (black trace) reveals ~ 10% neutral tertiary amine (N^0, 399.5 eV) relative to the quaternary amine (N^+, 402.1 eV). The XPS of the ionic strength control solution (blue trace) was comparable to the native PDADMAC (black trace). The alkaline treated PDADMAC (red trace) shows substantial (~40%) neutral amine. These results indicate that alkaline conversion of the quaternary amines within PDADMAC to tertiary amines is possible. However the degree of conversion and the severity of the conditions necessary to cause this conversion are inconsistent with the observed coating instability observed under the much milder conditions in CE (e.g., Figure 3.2A).

3.3.3 Surface-Catalyzed Instability of Polycationic Coatings

In 2011, Hsiao et al. studied quaternary amine-based cationic polymer lubricant behavior on silicon oxide using XPS [51]. Under their room temperature conditions, they observed that 30% of the quaternary amines were converted to tertiary amines. This was the first report showing degradation of quaternary amines on a SiO_2 surface at room temperature. They concluded that the degradation of the quaternary amine was induced by the interactions with the SiO_2 surface [51].

To determine whether the capillary surface could be accelerating the degradation of the quaternary amines within the polycationic coatings, PDADMAC (M.W. 4-5 x 10^5, 0.5 wt% in water) and polybrene (10 wt% in water) were deposited onto a silicon surface for 1 to 72 h. Figure 3.4 shows the resultant high-resolution N 1s XPS spectra of the coatings. Figure 3.4A shows that for contact times between PDADMAC and the silicon dioxide from 1 to 24 h, the decrease in the N^+ peak area and increase in N^0 peak area correspond to a 30% conversion of the quaternary amine. After 48 - 72 h interaction, about 45% of the quaternary amines were converted to tertiary amines. No obvious changes were observed in the peak area ratio after 72 h.

It should be noted that over time the cumulative N 1s signal in Figure 3.4A decreased. This is believed to be due to the weaker adsorption of tertiary amine on SiO_2 compared to quaternary amine. To test this, two substrates were exposed to PDADMAC (M.W. 4×10^5-5×10^5) coating solution for 24 h. After coating, one substrate was immersed for 1 min in

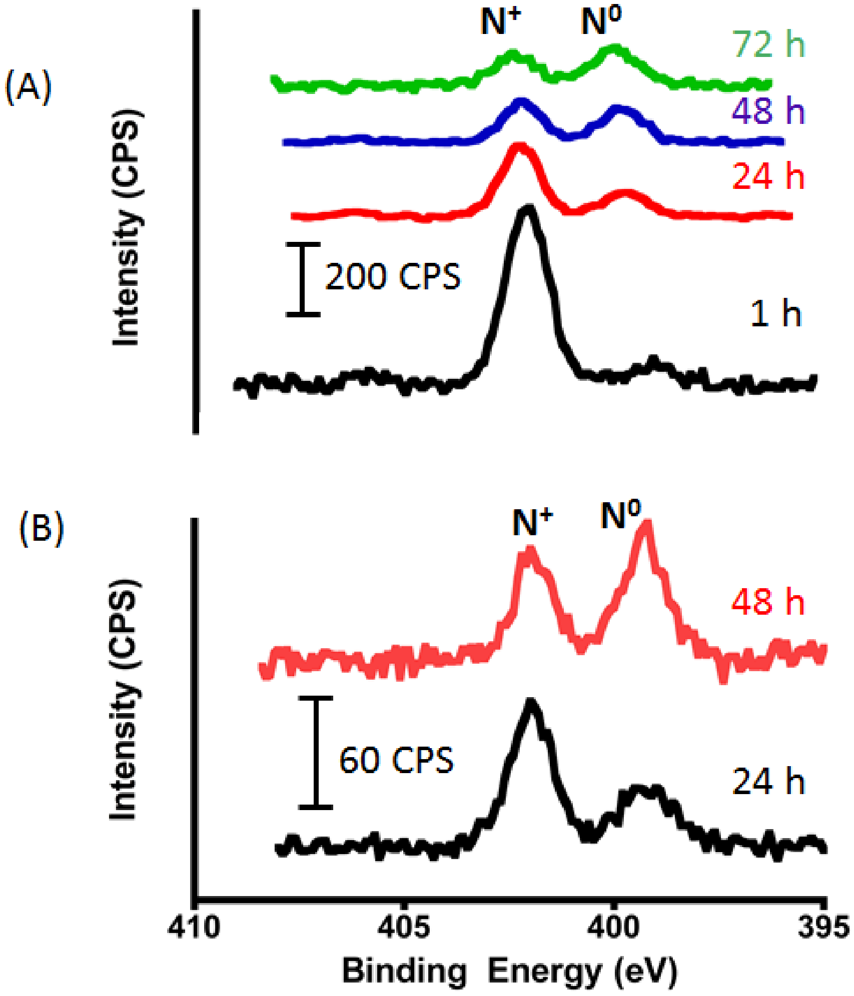

Figure 3.4 High-resolution N 1s XPS spectra of (A) PDADMAC (M.W. 4-5×10^5) and (B) polybrene on silicon wafers, after exposure of the wafers to the coating solutions for the periods of time indicated. Conditions as in Section 3.2.5.

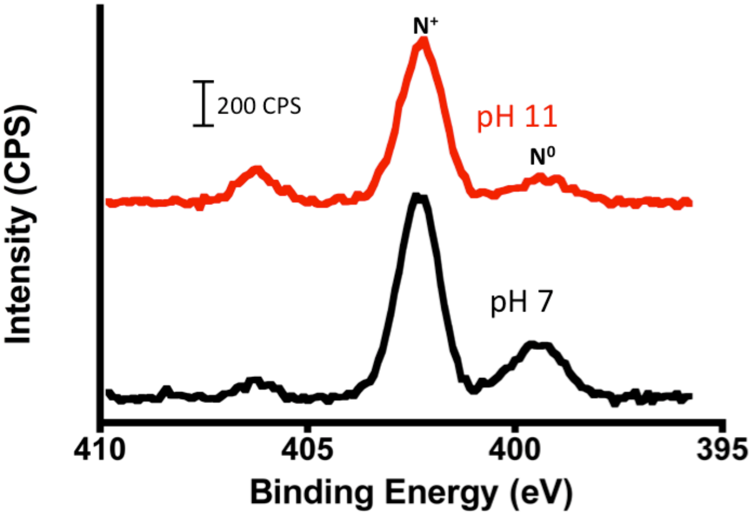

Figure 3.5 High resolution N 1s XPS spectra of PDADMAC (M.W. 4-5×10^5) coated on silicon wafers for 24 h. Then the silicon wafer was immersed in 20 mM phosphate buffer with different pH (as indicated) for 1 min before XPS analysis.

20 mM phosphate pH 7.0 buffer and the other in a comparable pH 11.0 buffer. Based on Figure 3.4A, the resulting adsorbed polycation would possess both quaternary and tertiary amines. XPS analysis (Figure 3.5) showed similar N^+ peaks for the two samples, indicating a similar quantity of quaternary amines on both surfaces. In contrast, the substrate immersed in pH 11.0 had a reduced N^0 peak compared to that immersed in pH 7.0, demonstrating less tertiary amines remained on the surface. Thus, the conversion of quaternary amines within PDADMAC to tertiary amines weakens the polymer adsorption at high pH. Polybrene showed similar behavior (Figure 3.4B). After 24 and 48 h adsorption on silicon dioxide, N 1s peaks characteristic of tertiary amines (N^0) are evident. As with PDADMAC, increasing the time that the polymer was on the surface increased the quaternary amine conversion.

3.3.4 Model of Degradation of Polycationic Coatings

Based on the above, the quaternary amines of polycationic polymers do convert into tertiary amines under alkaline solution conditions, but the rate is slow (Section 3.3.2). Adsorption onto a surface accelerates this chemical conversion (Section 3.3.3), consistent with the literature [51].

Figure 3.6 summarizes the degradation (instability) of polycationic polymer coatings in CE. Electrostatic interaction plays a main role in the adsorption of PDADMAC (or other polycationic polymers) onto the silica surface. The strength of the interaction depends the polymer M.W. (Figure 3.2A) and on the nature of the polycation [28,34], but should be independent of the pH. Quaternary amines within the polymer may undergo conversion to tertiary amines (shown as PDADMAC* in Figure 3.6) due either to: a slow base degradation in solution (route 2); or surface accelerated degradation that can occur at more modest pH (route 3). At pH ≤ 7 both the quaternary and tertiary amine functionalities would be positively charged. Hence the PDADMAC and the partially degraded

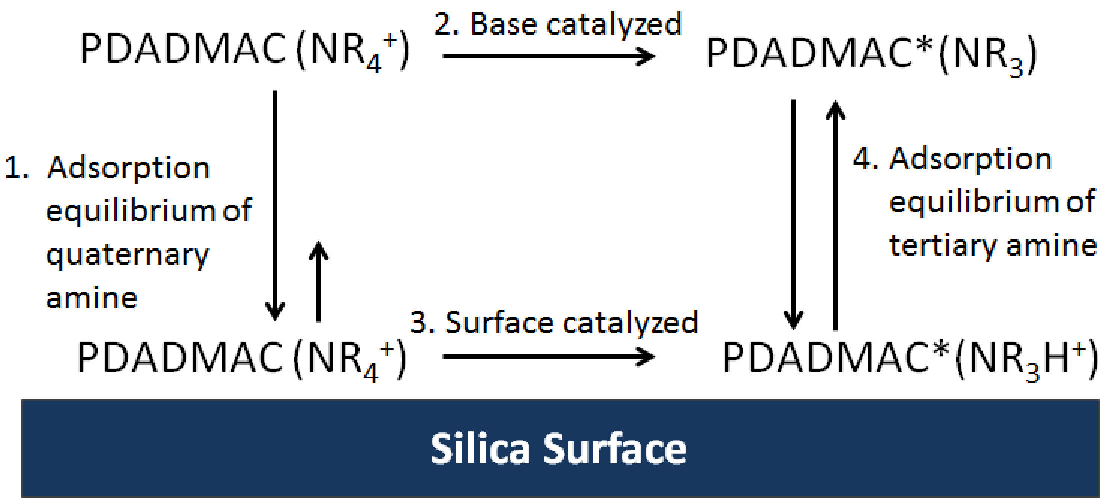

Figure 3.6 PDADMAC degradation in solution and in contact with silica surface.

PDADMAC* would adsorb onto the capillary surface with a strength comparable to the equilibrium arrows shown for step 1. However at pH approaching 9 (typical pK_a of tertiary amines), the tertiary amines within PDADMAC* would deprotonate – reducing the electrostatic attraction between the adsorbed polymer and the surface. Hence greater coating instability is observed for the PDADMAC coating at high pH.

3.3.5 Inorganic Anion Separations

Many separations must be performed at high pH. For instance the inorganic anion separation shown in Figure 3.7 was performed at pH 9.5 to ensure full deprotonation of chromate (pK_a = 6.51). Rapid (<4 min) separations are enabled by reversing the EOF with PDADMAC. However, as noted above alkaline conditions result in conversion of the quaternary amines of PDADMAC to tertiary amines, and thus a reduction in the magnitude of the reversed EOF. As a consequence, migration times for this co-EOF separation increase from run to run.

Higher M.W. PDADMAC show greater EOF stability (Figure 3.2A). Thus, as shown in Fig. 3.7 greater run-to-run migration time reproducibility, efficiency and baseline stability is achieved using 4-5x10^5 M.W. PDADMAC than 0.085×10^5. However, a small migration time shift (17% for benzoate) still was evident using the high molecular weight PDADMAC.

Successive multiple ionic polymer coatings (SMIL) have been reported to have greater alkaline stability than a single layer of polycation [34,36,37,46]. In Figure 3.8, a 5-layer PDADMAC/PSS SMIL coating was utilized to perform the anion separation. The SMIL coating showed better separation repeatability (migration time shift -2% for benzoate) compared to the monolayer PDADMAC coating (+17%). The enhanced stability of the SMIL coating under alkaline conditions is consistent with previous studies [46]. This greater stability can be rationalized based on Figure 3.6. In a monolayer coating, the polycation will be in intimate contact with the silica surface, and so will experience surface

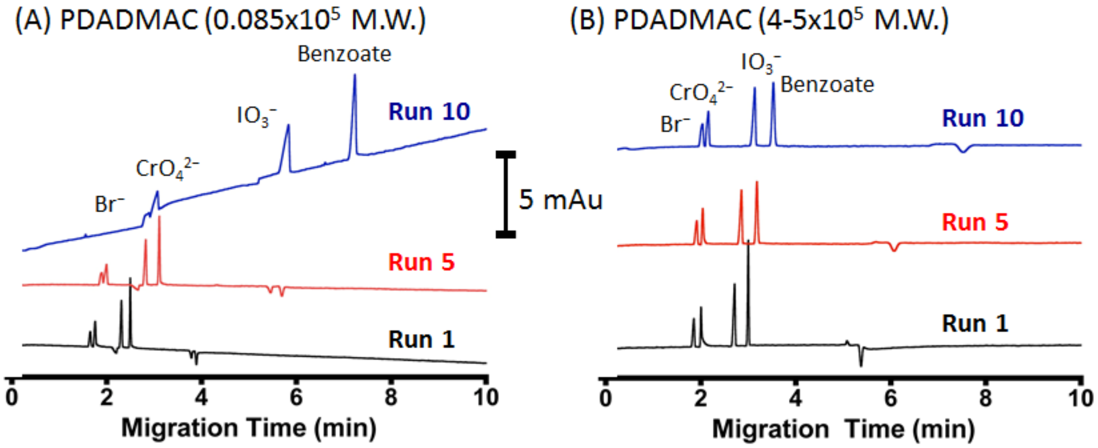

Figure 3.7 Inorganic anion separations using capillaries coated with PDADMAC of (A) 0.085 x10^5 M.W. and (B) 4-5x10^5 M.W. Conditions: capillary, 50 µm I.D. × 60.2 cm (50 cm to detector) coated with 0.5% w/w PDADMAC as in Section 3.2.3; injection, 0.5 psi for 5 s of 1 mM of each anion; BGE, 20 mM CHES at pH 9.5; voltage, -25 kV; detection, 210 nm. Between each run, a 1-min rinse (20 psi) of BGE was performed.

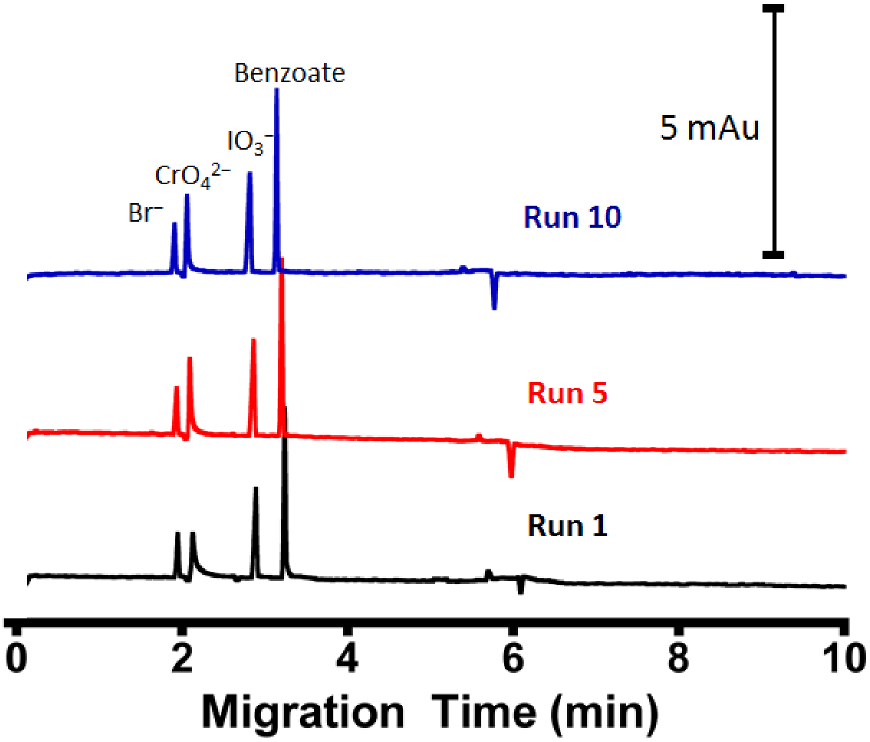

Figure 3.8 Inorganic anion separations using 5-layer SMIL coated capillaries. Conditions: capillary, 50 µm I.D. × 60.2 cm (50 cm to detector) coated alternately with 0.5% w/w PDADMAC and 0.5% w/w PPS as in Section 3.2.3; injection, 0.5 psi for 5 s of 1 mM of each anion; BGE, 20 mM CHES at pH 9.5; voltage, -25 kV; detection, 210 nm. Between each run, a 1-min rinse (20 psi) of BGE was performed.

accelerated conversion to the tertiary amine. In contrast in a multilayer coating, the outer EOF controlling polycationic layer is held away from the silica surface by the underlying layers. This reduces the surface induced conversion of the outer polycationic layer, and thus stabilizes the EOF (Figure 3.8).

To investigate the surface chemistry of the SMIL coating, a 5-layered PDADMAC/PSS coated silicon and monolayer PDADMAC coated silicon were analyzed using XPS. In the N 1s spectra (Figure 3.9A), the N^0 signal due to tertiary amines is lower in the SMIL coating than in the monolayer PDADMAC coating, consistent with the EOF studies above. In Figure 3.9B, the 232 eV peak in the S 2s spectra corresponds to the sulfur in the PSS (polystyrene sulfonate) layer of the SMIL [62].

3.4 Conclusions

In discussions of capillary coatings for CE, the limited pH stability of covalent coatings is often quoted as a reason to use an adsorbed polymer coating. This chapter provides a systematic study of the stability of adsorbed polycationic coatings. Herein capillary electrophoretic and X-ray photoelectron spectroscopic studies demonstrate that polycationic coatings such as PDADMAC and polybrene are not stable at higher pH. The quaternary amines within these polycations exhibit dual modes of degradation: slow conversion to tertiary amines in alkaline solutions; and accelerated conversion to tertiary amines when adsorbed on silica. Under alkaline BGE conditions these tertiary amines deprotonate, resulting in lower cationic surface charge and desorption of polymer from the capillary surface. Greater coating stability is achieved using higher molecular weight polycationic polymers or distancing the polycation from the capillary surface as in successive multiple ionic layer (SMIL) coatings.

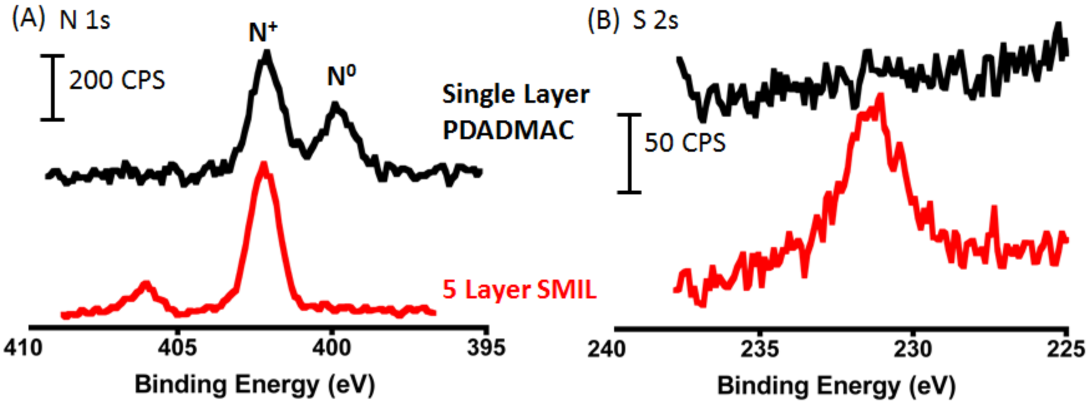

Figure 3.9 (A) High-resolution N 1s XPS spectra and (B) High resolution S 2s XPS spectra of 5-layer SMIL coating and monolayer PDADMAC. The SMIL coating was prepared by immersing the silicon wafer with 0.5 wt% PDADMAC (M.W. 4-5×10^5) and 0.5 wt% PSS solution for 15 minutes alternatively. The monolayer of PDADMAC was prepared by immersing the silicon wafer with 0.5 wt% PDADMAC (M.W. 4-5×10^5) for 75 min.

3.5 References

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Chapter Four: Determination of Selected Inorganic Anions in Oilfield Water Using Capillary Electrophoresis with Fluorescence Detection[*]

4.1 Introduction

Oil production is the foundation for worldwide energy supplies. During oil production, oilfield water, also known as oilfield-produced water, is generated in large quantities [1] with 15 to 20 billion barrels of water produced per year in the United States of America alone [2]. Oilfield production water consists of formation water, flood water, and condensed water [3]. *Formation water* is naturally present in the reservoir. When the pressure within a well is not high enough for oil production, *flood water* is injected into the reservoir to pressurize and displace hydrocarbons. *Condensed water* is that collected upon removal of water vapor from produced gas [4].

The chemical composition of oilfield water is very complex and varies from well to well. Oilfield water may contain various toxic compounds such as BTEX (benzene, toluene, ethylbenzene and xylenes), PAHs (polycyclic aromatic hydrocarbons), and organic acids [4]. Oilfield water may also contain very high concentrations of sodium chloride, and lower concentrations of anions such as sulfate and nitrate. The cost of producing, handling, and disposing of the oilfield water often defines the economic lifetime of a field and the actual hydrocarbon reserves [2,5]. For example, pure water is not very corrosive to steel, while saline water increases the water conductivity and corrosion [5]. As a result, the wear of oilfield equipment and replacement costs increase with salinity.

Oilfield water is analyzed for a variety of purposes, including characterizing aquifers, evaluating environmental compliance, and monitoring corrosion and scaling [2,4,5].

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[*] A version of this chapter has been accepted for publication in *Analytical Methods* as L. Pei, K. J. Schmidt, H. J. Crabtree, C. A. Lucy. K. J. Schmidt built the LED light source and provided the oilfield water samples. L. Pei conducted all CE experiments, compiled all data and did all writing.

Subsequent water treatment is guided by this analysis. Several classic methods of analysis are used to determine the anions in oilfield-produced water. Chloride, the major component of oilfield water salinity, is usually determined by Volhard titration where a measured excess of silver nitrate is added to an aliquot of the water sample, and the free Ag^+ is titrated [5]. However, any iodide or bromide present will be titrated as well. Sulfate, another major constituent of oilfield water, is measured gravimetrically after addition of barium to the sample. This gravimetric method is very time consuming [5]. Therefore, recent method development for oilfield water analysis has primarily focused on chromatographic methodologies. However such instrumentation is necessarily in the lab. Hence, there is increasing interest in the use of portable and field-deployable instrumentation for oilfield water sample analysis. Because the distance between sample collection and analysis can be minimized.

Ion chromatography (IC) is a very common tool for the determination of inorganic and small organic anions [6-8]. In chromatographic analysis, the high salinity of oilfield water sample presents a special challenge [9-14]. Analysis of sulfate and arsenate were affected by the presence of high concentrations of chloride, due to competition between the analyte ion and chloride for the active sites on the resin [11,15,16]. Cl^-, SO_4^{2-}, NO_3^-, I^- and Br^- determination in natural water matrix samples such as oilfield water, seawater and wastewater were achieved using a laboratory IC [14,17-19]. For heavy duty, on-location analysis, a portable and autonomous instrument is preferred. However, limited eluent capacity and the weight of high pressure pumps and components have limited the popularity of portable IC [20,21]. Currently there is no portable IC that is suitable for on-location oilfield water analysis.

Capillary electrophoresis (CE) is increasingly used for the analysis of a wide variety of analytes due to its speed and efficiency [22-25]. Compared to HPLC, CE has high resolving power, low cost operation, and simplicity in design, optimization and operation [26]. CE is also appropriate as a portable instrument as it has limited solvent consumption and lower weight. All of these advantages make CE a promising analytical technique for on-location oilfield water analysis.

CE has been widely used for inorganic ion analysis [26-28]. Recent developments in CE of inorganic ions have focused on high efficiency separations of anions and cations within complex sample matrices [29,30], use of alternative detectors [27,31], rapid and simple method development [32,33], and CE portability [34-36]. Several strategies have been employed for anion analysis in high salinity samples. UV absorbing anions (SCN^-, NO_3^-, I^-) were detected at ppm levels using a high chloride background electrolyte buffer (BGE) [37]. However this method cannot detect non-UV absorbing anions such as sulfate and chloride, which are of primary interest in oilfield waters. Trace anions have also been pre-concentrated using transient isotachophoresis (tITP) [38-40]. However, the leading electrolyte and terminating electrolyte need to be deliberately chosen for each analyte ion [39,40]. Hence, there is a need to develop a sensitive, universal anion analysis method suitable for portable CE of oilfield water samples.

To develop a universal method for all the inorganic anions, indirect detection was utilized in this work. As was discussed in Section 1.4.3, indirect detection is based on the displacement of a photometric (absorbing or fluorescent) ion present in the BGE by an analyte co-ion, resulting in a decrease in signal intensity [41-43]. Indirect UV absorbance detection has been widely used for inorganic anions [44,45]. Recently, Donkor et al. developed a CE-indirect UV detection method for inorganic anion analysis in highly saline oilfield water [46]. In general, the sensitivity of indirect UV absorbance (10^{-5} to 10^{-6} M [47-49]) is poor. Indirect conductivity can achieve comparable or lower detection limits (10^{-5} to 10^{-7} M) [34,50]. However, conductivity detection requires isolation of the conductivity detector electronics from the high separation voltage, which complicates the system [51]. On the other hand, laser-induced fluorescence detection has been widely used in CE, with exquisitely low detection limits [52,53]. To date, indirect fluorescence detection has seen very limited use in inorganic anion analysis. This is mainly due to the lack of appropriate fluorescent probes [45] and the stability of the light sources. Most fluorescent probes do not have mobilities that match those of inorganic anions, an important criterion in minimizing peak broadening [54,55].

In this chapter, an indirect fluorescence detection method was developed and optimized for common inorganic anions in high salinity samples. 8-Hydroxypyrene-1,3,6-trisulfonic acid (HPTS) is used as the probe. A UV-LED is used as the light source. LEDs' small size, robustness, stable light intensity and low power consumption [56,57] make them compatible with the needs of a portable capillary electrophoresis instrument.

4.2 Experimental

4.2.1 Chemicals

All solutions were prepared in Nanopure 18-MΩ water (Barnstead, Thermo Scientific, Marietta, OH, USA). 8-Hydroxypyrene-1,3,6-trisulfonic acid trisodium salt (HPTS), sodium sulfate, sodium bromide, sodium nitrate and sodium hydrosulfide hydrate were from Sigma-Aldrich (St. Louis, MO, USA). Sodium chloride (>99.0%) was from EMD Chemicals (Gibbstown, NJ, USA). Sodium thiosulfate was from Anachemia Canada (Montreal, QC, CA). Sodium iodide was from BDH Chemicals (Toronto, ON, CA). All the inorganic salts were prepared and stored as 10,000 ppm stock solutions. Formic acid, methanol and acetonitrile were from Fisher Scientific (Fair Lawn, NJ, USA). All solutions were filtered (0.22 μm) before use.

4.2.2 Apparatus

Capillary electrophoresis was performed on a Beckman PA 800 plus Pharmaceutical Analysis system with an LIF detector (Beckman Instruments, Fullerton, CA, USA). Data acquisition (4 Hz) with normal filtering and control were performed using 32 Karat software for Windows XP on a Lenovo Thinkcentre computer. Fluorescence emission was collected using a 520-nm band-pass filter with a bandwidth of 20 nm (Beckman). The sample was injected hydrodynamically using 0.5 psi for 3 s. Untreated fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) with an inner diameter of 50 μm, outer diameter of 365 μm, and total length of 60.2 cm (50 cm to detector) were used. The capillary was thermostated at 25°C.

4.2.3 Light Sources

The LED light source is a prototype Wilson Analytical High-Intensity Optically-Stabilized LED Light Source (Model: 405-2; Sherwood Park, AB, CA). The LED emitter (LED Engin, San Jose, CA, USA) was set to 150.9 mW full output, and the central wavelength was nominally 405 nm with a bandwidth of 20 nm. A collimation lens and a fiber coupler were used to efficiently transfer the light into the optical fiber. Round optical filters (12.5 mm) could be positioned between the LED and fiber coupler. A 450 nm O.D. shortpass filter (Edmund Optics) was used to remove light above 450 nm from the spectral output of the 405 nm LED [58]. A constant-current power supply was used to drive the LED. A visible-light measurement diode was placed facing the LED to create active stabilization. This diode is connected to an optical feedback circuit that adjusts the LED light output power so that it is very constant, as will be shown in Section 4.3.1.

A violet diode laser from Laserglow Technologies (Toronto, ON, CA) was also tested. The laser output was set at 20-39 mW, with a central wavelength of 406.4 nm with a wavelength tolerance of ±5 nm during warmup. The diode laser was warmed up for at least 30 min before use.

Both the LED and violet diode laser were connected to the Beckman instrument through a 2 meter-long, 3 mm O.D., 550/600 µm core/cladding fiber optic cable (OZ Optics Ltd. Ontario, CA). A SMA 906 connector mates with the Beckman instrument and a SMA 905 connector mates with the light source.

To compare the power stability of the two light sources, baseline stability tests were carried out. The light sources were warmed up for 40 min before use. The capillary was filled with 0.4 M formic acid and the detector response was adjusted to zero. Then the solution (10 µM HPTS and 0.4 M formic acid) was hydrodynamically rinsed through capillary at 5 psi. The electropherogram was collected for 3 min at 520 nm at a data acquisition rate of 4 Hz with normal filtering. The peak-to-peak noise of the baseline was calculated after correction of baseline drift. Drift was taken as the slope of the linear regression of the baseline, and the noise was defined as the difference between the maximum deviation and minimum deviation in the baseline (Figure 4.1).

4.2.4 Procedures

New capillaries were pretreated with 1 M sodium hydroxide for 10 min (20 psi rinse), 0.1 M NaOH for 10 min (20 psi), and finally deionized water for 5 min (20 psi).

Eppendorf tips were calibrated by weight prior to use, as recommended by the manufacturer. Chloride standard solutions of 50~800 ppm were prepared in BGE (0.4 M formic acid, 10 µM HPTS). For sulfate, standard solutions of 1~20 ppm were prepared in BGE containing 500 ppm chloride.

4.2.5 Oilfield Water Sample

Oilfield water sample 11188 was a coffee-colored steam-assisted gravity drainage (SAGD) produced water sample, containing greater than 100 ppm of chloride and 100 ppm sulfate as determined by Wilson Analytical analysis. Sample 11180 was a cloudy Bakken formation water sample with a pungent odor, which contains 20 000 ppm chloride, and 75 ppm sulfate as determined by Wilson Analytical analysis. The samples were preserved and transferred in Nalgene bottles at room temperature. In the Wilson Analytical analyses, chloride was determined by Volhard titration and sulfate was quantified by inductively coupled plasma-optical emission spectrometry (ICP) at 182.036 nm and 180.669 nm using a Varian (now Agilent, Santa Clara, CA, USA) ICP-OES instrument calibrated using 10 - 1000 ppm SO_4^{2-} standards. Prior to CE analysis, the samples were filtered (0.22 μm) and diluted with background electrolyte.

4.3 Results and Discussion

Inorganic anion analysis using capillary electrophoresis with indirect photometric detection has been widely studied [35,43,45,49,59]. However, most of these applications have dealt with simple water matrices. The high salinity and variable nature of oilfield water presents a challenge for anion analysis. To facilitate the oilfield production and reduce the environmental harm, a portable analytical instrument is demanded for heavy-duty field analysis for inorganic anions. Capillary electrophoresis is compatible with such requirements. Herein we develop a simple, easy-operation capillary electrophoresis protocol for anion analysis that would be suitable for field analysis of oilfield water samples.

4.3.1 Laser Stability Study

For indirect fluorescence detection, the power stability of the light source is crucial [42,43]. The theoretical concentration limit of detection (C_{LOD}) of CE indirect fluorescence detection is given by [42]

$$C_{LOD} = \frac{C_p}{TR \times DR} \tag{4.1}$$

where C_p is the concentration of the relevant mobile-phase component, *TR* is the transfer ratio (the number of probe molecules displaced by one analyte molecule), and *DR* is the dynamic reserve (the ratio of the background fluorescence intensity to the noise) [42]. The more stable the background signal (larger *DR*), the lower the C_{LOD} that can be achieved. Given that the fluorescence intensity is proportional to the excitation light intensity, any power fluctuation in the excitation light source will propagate into the baseline noise. Hence it is important to first develop a stabilized light source.

Both laser diodes (LDs) and light-emitting diodes (LEDs) have been employed in portable CE instrumentation [35,36,60-62], and are studied here. Laser diodes have been widely used for excitation in CE- LIF methods [63,64] due to their great power stability, cost-efficiency and portability [65]. LEDs have also been used as light sources for fluorescence detection (LEDIF) [66,67]. The light emission in both cases is based on the same principle (p-n junction and carrier recombination in a solid state semiconductor). For blue light, LDs can in principle have high efficiencies at much higher input power densities than LEDs [56]. However, when used as an excitation light source for fluorescence detection, LEDs are much less expensive, more energy efficient and more stable (six times lower C_{LOD} [57,66,68]) compared to diode lasers [56,66,69,70].

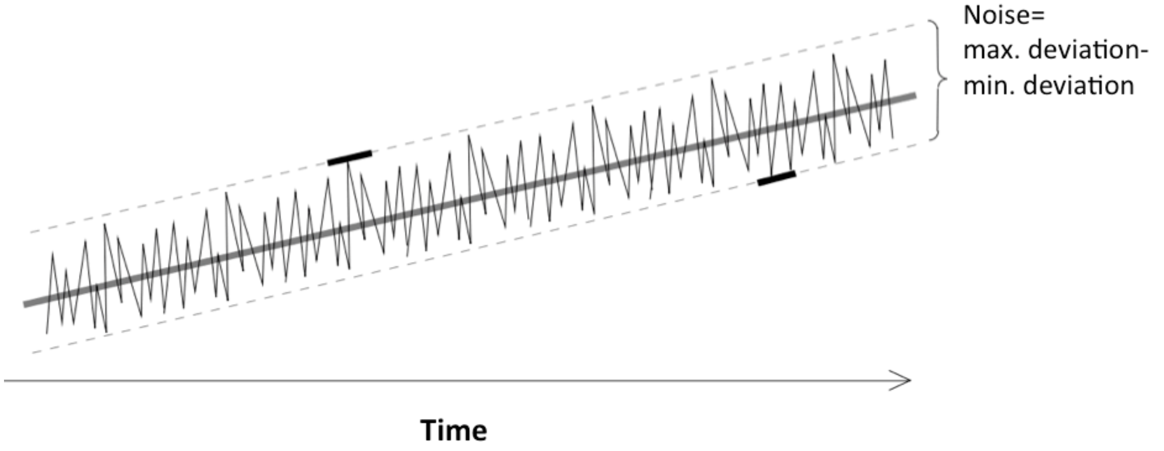

Figure 4.1 Demonstration of noise calculation used to assess stability of light sources (Section 4.2.3). Adapted from ref. [71].

To generate better stability with LEDs, an active stabilization was built into our LED light source, as mentioned in Section 4.2.3. To compare the power stability of the two light sources, 10 μM of the fluorescent probe HPTS was rinsed through the capillary and the baseline was monitored for both the diode laser and LED. Figure 4.2 shows a 180-s (720 data points) baseline segment void of any obvious perturbations. The diode laser yields a more intense signal (~0.63 RFU) compared to the LED (~0.44 RFU). However, the baseline noise, based on the drift-corrected peak-to-peak variation as per ref. [66], was 26 mRFU for the diode laser and 1.7 mRFU for the LED, consistent with the performance of LED in the literature [66,72-74]. Hence, the DR of the LED (~250) is 10 times better than the diode laser (~25). Due to the superior stability performance of the LED over the diode laser, we used LED-IF in all following experiments.

4.3.2 Detection of Inorganic Anions with HPTS

4.3.2.1 Selection of Indirect Fluorescent Probe

The selection of indirect fluorescent probes for small inorganic anions involves two factors: the probe must be compatible with the excitation light source; and the mobility of the probe must be comparable to that of the analytes to minimize electrodispersion [45,75]. This latter requirement ruled out many fluorescent compounds such as fluorescein, due to their low mobility resulting from their large conjugated system and low charge. 8-Hydroxypyrene-1,3,6-trisulfonic acid (HPTS) was selected as the probe. The three sulfonate groups of HPTS (Figure 4.3) compensate for its large molecule size, making HPTS's mobility comparable with that of inorganic anions [76,77]. HPTS has been used for the analysis of bromide, sulfate, nitrate and other inorganic anions [76,77]. When the pH is lower than 5, the maximum excitation wavelength for HPTS is 403 nm,

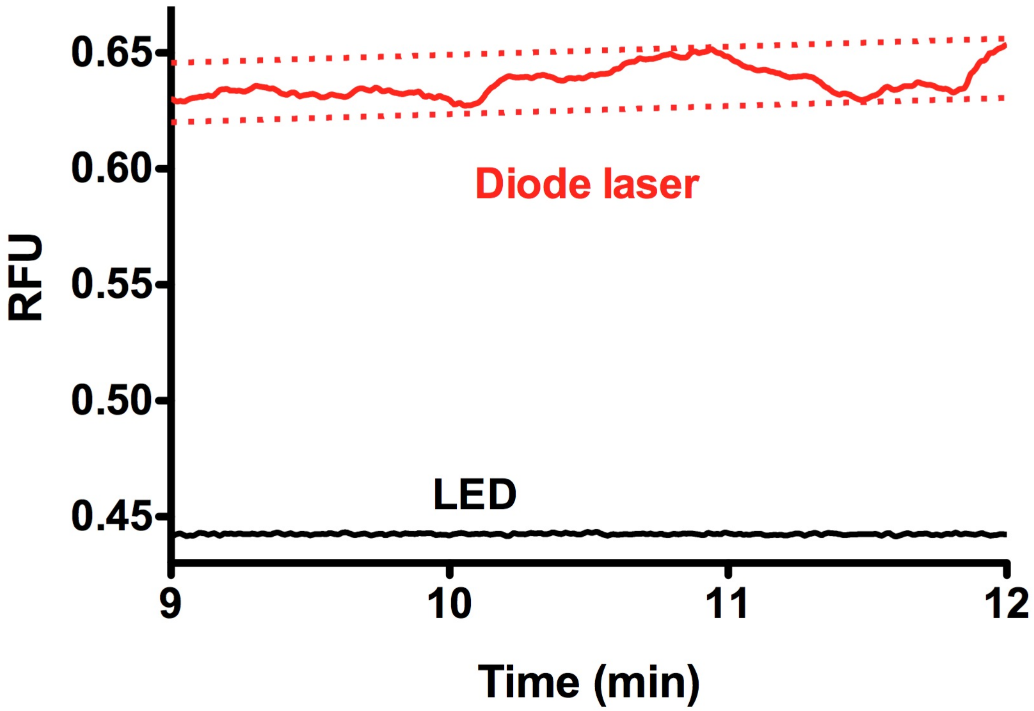

Figure 4.2 Comparison of baseline stability of diode laser and LED light source. Conditions: both light sources were turned on 40 minutes before use. Solution of 10 μM HPTS and 0.4 M formic acid, pH 2.0 was rinsed through capillary using 0.5 psi; capillary, L_T 60.2 cm, L_D 50 cm, I.D. 50 μm; detection, $\lambda_{excitation}$ = 405 nm and $\lambda_{emission}$ = 520 nm.

matching well with the nominal emission bandwidth of the LED light source (405 nm). Previous optimization studies of 1-100 μM HPTS determined 10 μM HPTS to be optimal. Preliminary studies herein corroborated these conclusions [77]. Hence 10 μM HPTS was used in the rest of this work.

4.3.2.2 Selection of Buffering Agent

Resolution between chloride and sulfate in CE is challenging, as they have similar electrophoretic mobility [78]. In oilfield waters resolution will be all the more challenging, given the large difference (10:1 - 2000:1 [79,80]) in the Cl^-/SO_4^{2-} concentration. Even addition of cationic BGE additives such as calcium [76] and phosphonium [32] generate low Cl^-/SO_4^{2-} resolution (<1 for calcium additive, no separation of Cl^-/SO_4^{2-} for phosphonium). Such low resolution is not suitable for heavy-duty, on-location field analysis of samples of varying matrix composition. A simpler, easy-to-operate method is needed in this case. One simple means of modifying inorganic anion mobility is pH. However, of the common anions, pH has generally only been used to modify the mobility of nitrite ($pK_a = 3.15$) [81-83]. Recently de Macedo et al. [30] showed that in pH 2.0 BGE the resolution between chloride and sulfate was enhanced. At this low pH, the sulfate is partially protonated ($pK_{a2} \approx 1.9$). Hence the mobility of sulfate is decreased. At the same time, the electroosmotic flow (EOF) is suppressed at pH 2.0, allowing the anions to counter-migrate against the EOF better and thus yield faster separations.

Given the concentrated nature of oilfield waters, the BGE requires a buffer anion that is similar in mobility to Cl^- (7.92×10^{-4} cm^2/Vs) to minimize electromigration dispersion (see below). At the same time, the BGE requires high buffering capacity. Nitrate ($pK_a = -1.4$, $\mu = 7.41 \times 10^{-4}$ cm^2/Vs) was investigated, but the system peak interfered with detection of chloride.

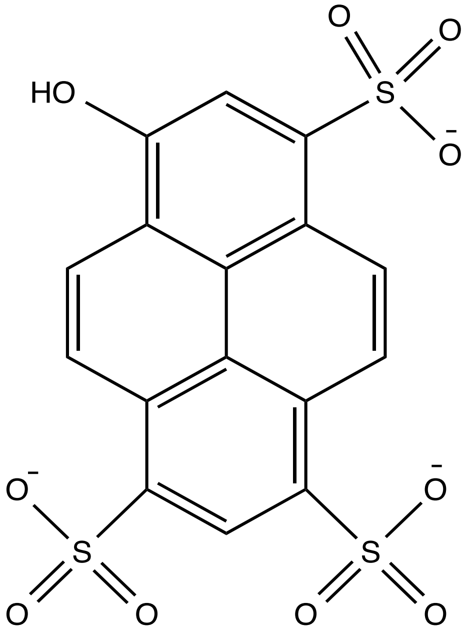

Figure 4.3 Structure of 8-Hydroxypyrene-1,3,6-trisulfonic acid (HPTS).

Formic acid (pK_a = 3.88, $\mu = 5.66 \times 10^{-4}$ cm^2/Vs) was previously used for low pH CE separations of chloride and sulfate [30]. As shown in Figure 4.4 (A), increasing the concentration of formic acid ten-fold increased the resolution from 0.95 to 5.4 between chloride and sulfate, but decreased the sensitivity 30% (based on peak area) for both anions. The reduced sensitivity is due to the added formate ions competing with the target anions for the displacement of HPTS. As a result, the TR in Equation 1 for the target anions is reduced. A 0.4 M Formic acid was chosen as the compromise between the resolution and buffering capacity provided by high BGE concentration and the sensitivity afforded by lower formic acid concentrations. Given this high BGE concentration, the current and voltage for the separation system were carefully monitored to prevent Joule heating (Section 1.5.2.2). The current was less than 30 μA and within the linear region of Ohm's plot in all experiments described below. Increasing capillary length (L_T) from 40.2 to 60.2 cm increased resolution between Br^- and Cl^- from 0.55 to 1.0 as shown in Figure 4.4 (B). In summary, the conditions used for oilfield water analysis were a BGE of 0.4 M formic acid (pH 2.0) with 10 μM HPTS, and a capillary length of 60.2 cm (50 cm to detector), and an applied voltage of -22.5 kV.

4.3.3 Figures of Merit

Figure 4.5 shows baseline separation was achieved at pH 2.0 for bromide, chloride, thiosulfate, nitrate and sulfate in less than 5 min. Iodide co-migrated with chloride and so cannot be resolved. Hydrosulfide and thiosulfate co-eluted at 3.34 min. Overall, the method is a relatively fast (~4 min) and simple (no additives or capillary coatings needed [26,33,84-86]) separation method for inorganic anions. Peak asymmetry was observed for the higher mobility anions (bromide, chloride) due to the increasing mobility mismatch between these anions and the formate buffer [76]. The efficiencies (64,000 plates/m for bromide) are comparable with other indirect LIF-CE methods [30,76,77].

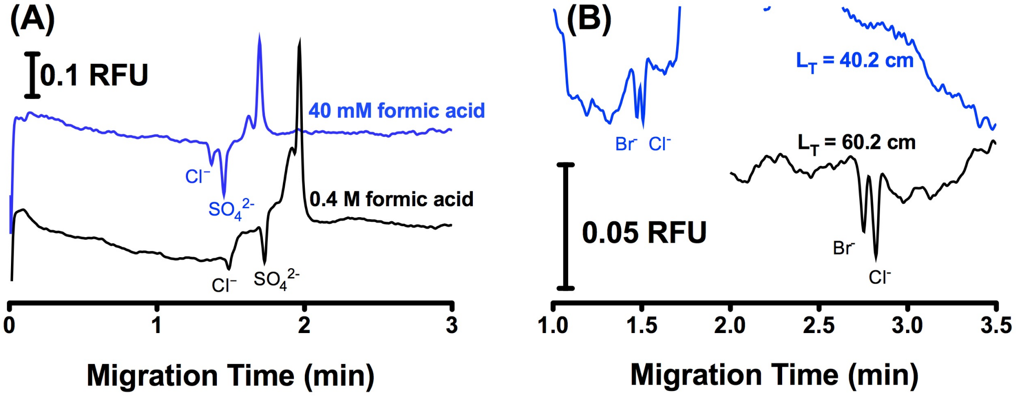

Figure 4.4 Optimization of (A) formic acid concentration and (B) capillary length. Conditions: (A) sample, 2 ppm Cl^-, 5 ppm SO_4^{2-}; capillary, L_T 40.2 cm, L_D 30 cm, I.D. 50 µm; voltage, -15 kV; electrolyte, 10 µM HPTS and formic acid, concentration as indicated in the electropherogram. (B) sample, 10 ppm Br^-, 10 ppm Cl^-; capillary, L_T as indicated in the electropherogram. $L_D = L_T - 10$ cm; voltage, -23 kV; electrolyte, 10 µM HPTS and 0.4 M formic acid, pH 2.0; detection, $\lambda_{excitation}$ = 405 nm and $\lambda_{emission}$ = 520 nm.

The focus of the anion analysis in oilfield water samples is the determination of sulfate. Given the high salinity of the samples, the resolution between chloride and sulfate is crucial for sulfate analysis. The resolution between chloride and sulfate in our method (8.5) is substantially better than indirect CE methods conducted at neutral to alkaline pH [46,76], and comparable to previous low pH indirect CE methods (resolution of 8.7, [30]). Furthermore the adoption of fluorescence detection in our method enabled ~10-fold lower LOD for both sulfate and chloride [30].

4.3.4 Anion Separations in High Salinity Samples

Figure 4.6 shows the peak shape of chloride upon injection of a high salinity sample. Electromigration dispersion was significant resulting in a fronting peak. As discussed in Section 1.5.2.3, electromigration dispersion is caused by a difference in the conductivity between the sample zone and the surrounding background electrolyte [87,88]. When injecting high chloride samples, the mismatched mobility between the chloride and the buffer ion (formate) caused the triangular peak shapes (asymmetry factor B/A = 0.1 in Figure 4.6 (A)) [46,66]. For other indirect CE method developed for high saline samples [27,30,46], the chloride peak asymmetry factor (B/A) is <0.2 for ≥200 ppm Cl^-, in agreement with Figure 4.6.

Injection time (volume) also impacts the peak size and the detection limit. For instance, Figure 4.6 shows injection of 500 ppm chloride and 0.5 ppm sulfate for varying injection times. Increased injection time increased the sulfate peak area. However, the chloride peak width also increased. This asymmetry ultimately limits the number of moles of chloride that can be injected without affecting sulfate detection. As shown in Figure 4.7, electrodispersion was significant above 30 ppm Cl^-. Increasing the chloride concentration from 400 to 1500 ppm (for a normalized injection time of 5 s) increases the

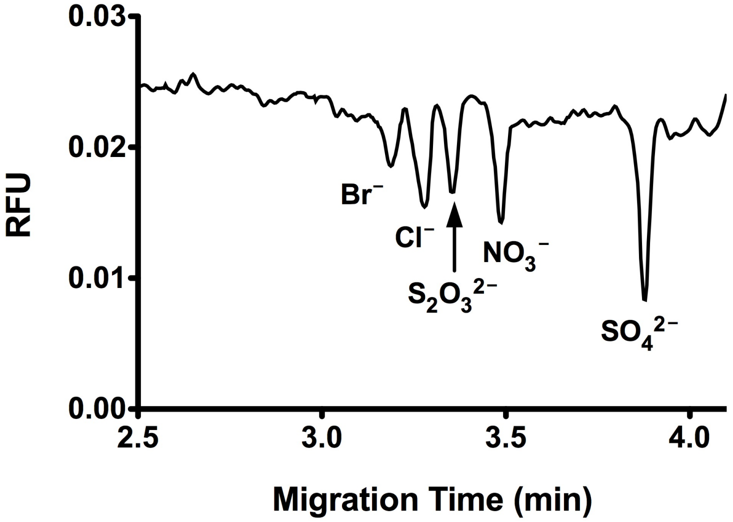

Figure 4.5 Separation of inorganic anions using HPTS at pH 2.0. Conditions: sample: 0.5 psi injection for 3 s of 10 ppm of Br^-, Cl^-, $S_2O_3^{2-}$, NO_3^- and SO_4^{2-}; electrolyte, 10 µM HPTS and 0.4 M formic acid, pH 2.0; capillary, L_T 60.2 cm, L_D 50 cm. I.D. 50 µm; voltage, -22.5 kV; and detection, $\lambda_{excitation}$ = 405 nm and $\lambda_{emission}$ = 520 nm.

peak fronting, with the asymmetry factor dropping to 0.05 (Figure 4.7 (C)).This severe asymmetry makes detection of nitrate (a non-priority anion) impossible and determination of sulfate increasingly difficult. As the chloride concentration in oilfield waters range from 100 to 100,000 ppm [79,80], sample dilution is needed prior to CE analysis. To determine the degree of dilution necessary for detection of trace anions in high salinity samples, the detection of bromide, nitrate and sulfate was tested with different chloride concentrations. Bromide was kept at 5 ppm, which is above the detection limit. The concentration of chloride was increased until the bromide peak could not be detected. Figure 4.7 (A) shows that bromide can be detected with a concentration ratio of 1:6 to chloride. Similar experiments were performed for nitrate at 1 ppm and sulfate at 1.5 ppm. Figure 4.7 (B) shows nitrate can be detected with a ratio of 1:400 to chloride, and Figure 4.7 (C) shows sulfate can be detected better than 1:1000 of the chloride concentration. The sulfate result is comparable with another indirect UV CE method developed specifically for oilfield waters [46], while our sulfate detection limit (0.4 ppm, see below) is lower due to the high sensitivity of fluorescence detection. The previous study did not investigate bromide or nitrate analysis in high salinity samples. In Figure 4.7 (A), a positive system peak appears at ~4 min. System peaks are common in indirect detection. The system peak is created by a vacancy of one component of the BGE and are aggravated by greater matrix concentrations [89].

To evaluate the effect of high chloride matrix on sulfate quantification, the calibration of sulfate was carried out as Section 4.2.4. LOD was determined based on 3 times the baseline noise level. Baseline drift was given as the slope of the baseline noise. Eight data points before the peak and after the peak were collected respectively, and the baseline was defined through these 16 data points. For standards containing 500 ppm chloride, the calibration curve for peak area of sulfate was linearly related to

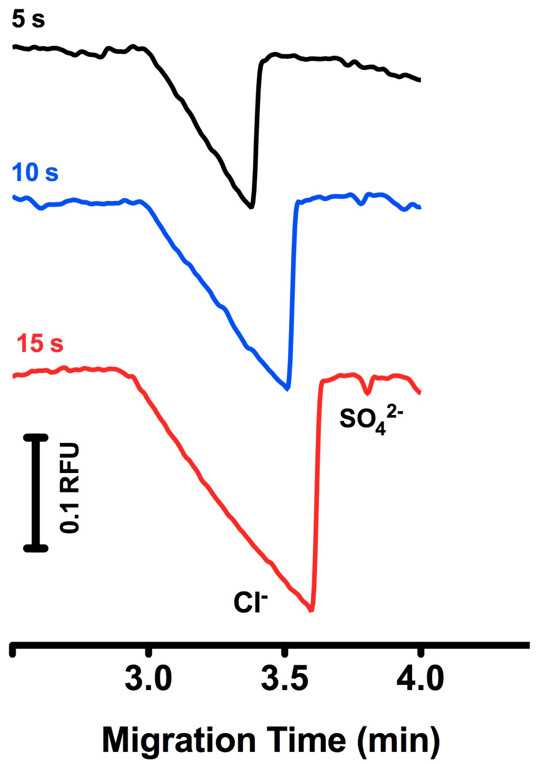

Figure 4.6 Effect of injection time on sulfate determination in high salinity sample. Conditions: sample, 0.5 ppm sulfate with 500 ppm chloride: injection, 0.5 psi for time indicated on the figure; electrolyte, 10 µM HPTS and 0.4 M formic acid, pH 2.0; capillary, L_T 60.2 cm, L_D 50 cm, I.D. 50 µm; voltage, -22.5 kV; and detection: $\lambda_{excitation}$ = 405 nm and $\lambda_{emission}$ = 520 nm.

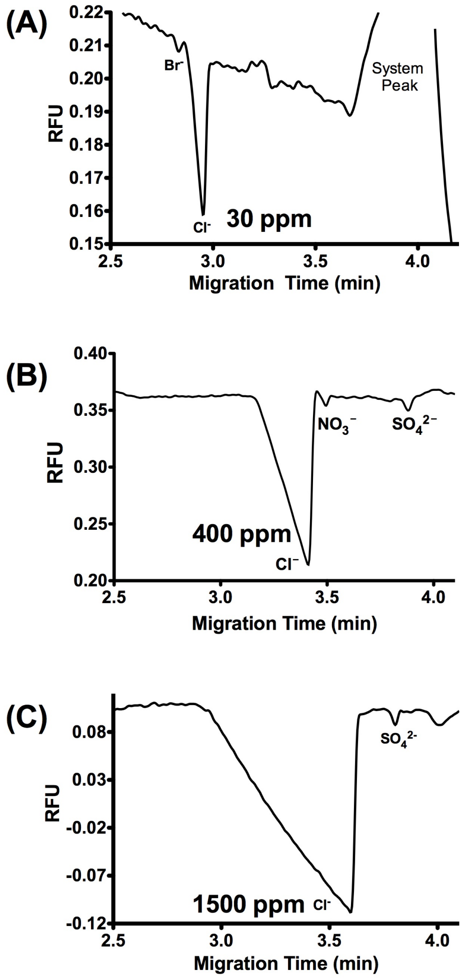

Figure 4.7 Anion analysis in high salinity samples. Samples (concentrations normalized to 5 s injection): (A) 5 ppm Br^- and 30 ppm Cl^-; (B) 1 ppm NO_3^- and SO_4^{2-} with 400 ppm Cl^-; and (C) 1.5 ppm SO_4^{2-} with 1500 ppm Cl^-. Conditions: injection, 0.5 psi; electrolyte, 10 μM HPTS and 0.4 M formic acid, pH 2.0; capillary, L_T 60.2 cm, L_D 50 cm. I.D. 50 μm; voltage, -22.5 kV; and detection, $\lambda_{excitation}$ = 405 nm and $\lambda_{emission}$ = 520 nm.

Table 4.1 Calibration curve from chloride and sulfate standard solutions[a]

Analyte	Regression Equation[b]	R^2	Calibration Range	LOD
Cl^-	Y = (640±6) X – (6200±2500) [c]	0.9987	50~800 ppm	1.4 ppm
SO_4^{2-} [d]	Y = (690±7) X + (370±70) [d]	0.9989	1~20 ppm	0.4 ppm

a. Conditions: BGE, 10 μM HPTS and 0.4 M formic acid, pH 2.0; capillary: L_T 60.2 cm, L_D 50 cm, I.D. 50 μm; injection, 0.5 psi for 5 s; voltage, -22.5 kV; detection, $\lambda_{excitation}$ = 405 nm and $\lambda_{emission}$ = 520 nm.

b. Regression equation: the dependent variable (Y) is peak area while the explanatory variable (X) is the analyte concentration in ppm.

c. Chloride calibration: The intercept is statistically equivalent to 0 within the 98% confidence interval.

d. Sulfate calibration: 500 ppm chloride was present in all standard solution to mimic the matrix effect. The intercept does not lie within the 99% confidence interval.

concentration over the 1-20 ppm range studied (Table 4.1), with a limit of detection of 0.4 ppm. A residual plot was checked to ensure the linearity of response. This range is comparable with other indirect LIF detection methods for samples without high salinity [43,59,76,77]. With those methods, sulfate could only be detected for <200-fold chloride:sulfate ratio due to the limited resolution (Rs<4 [87,90]).

4.3.5 Analysis of Oilfield Waters

Two oilfield water samples were analyzed using the developed method. Peaks were identified by comparison of migration times with those of standards. Quantification was done using peak areas compared to external calibration containing 50-800 ppm Cl^- and 1-20 ppm SO_4^{2-} as shown in Section 4.3.4. Figure 4.8 shows the analysis of a Bakken formation (A) and a stream assisted gravity drainage (SAGD) enhanced oil recovery (B) sample. For Bakken formation water at 50 times dilution, the sulfate peak was difficult to distinguish from the baseline. Hence the chloride and sulfate concentration were quantified with a dilution factor of 25. As shown in Table 4.2, the CE method yielded comparable chloride concentrations compared to the Volhard titration. Further dilution of the sample (total of 250 fold) enabled resolution of bromide and nitrate peaks from the chloride matrix. However no bromide or nitrate was detected (< 250 ppm in original sample). For the SADG sample (Figure 4.8 (B) and Table 4.2), there is excellent agreement in the chloride concentration with a RSD of 9%.

A systematic bias is evident in Table 4.2 in the analysis of sulfate by CE versus the ICP analysis, with the CE results being much lower. ICP measures the total sulfur in the sample. Thus other sulfur-containing species within the sample would also be reported as sulfate [5,14]. The negative bias in sulfate determination is also observed in a recent indirect CE-UV method designed for oilfield water analysis [46].

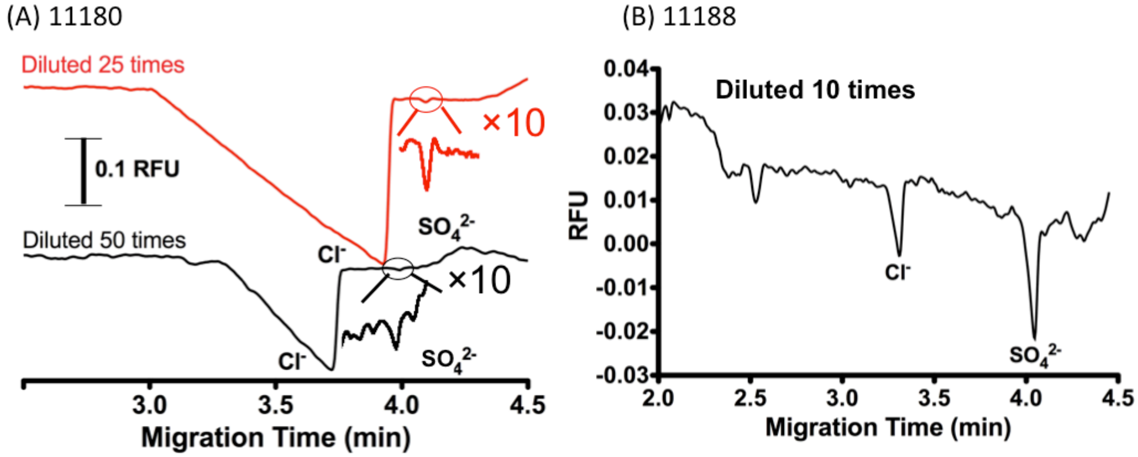

Figure 4.8 Electropherograms of a Bakken formation sample (A) and an enhanced oil recovery sample (B). Samples were filtered and diluted with BGE as per Section. 4.2.5. Dilution factors are indicated in the figures. Conditions: injection, 0.5 psi for 5 s; BGE,10 μM HPTS and 0.4 M formic acid, pH 2.0; capillary, L_T 60.2 cm, L_D 50 cm. I.D. 50 μm; voltage, -22.5 kV; and detection, $\lambda_{excitation}$ = 405 nm and $\lambda_{emission}$ = 520 nm.

Table 4.2 Conventional method versus CE quantification result of samples

Sample	**Conventional (ppm)**		**CE Mean ± S.D. (ppm) n=3**	
	Chloride (titration)	Sulfate (ICP)	Chloride	Sulfate
Bakken[a]	23600	75	28600 ± 400	15 ± 5
SAGD[b]	160	300	174 ± 15	127 ± 3

a. Bakken formation water sample was diluted 25 times before quantification.

b. SADG sample was diluted 10 times before quantification.

Spiking the Bakken formation sample with 10 ppm SO_4^{2-} yielded a recovery of 84%. Hence, we are confident that our sulfate quantification values are more accurate than the ICP results.

4.3.6 Method Compatibility with Sample Preservatives

To preserve and analyze oilfield water samples in winter, methanol and acetonitrile are often added to the sample as antifreeze [91,92]. The amount of organic additive can be up to 50% v/v. In this section I investigate the effect of these antifreeze additives on the inorganic anion analysis.

Figure 4.9 shows the effect of adding methanol and acetonitrile to the BGE. Thiosulfate eluted before nitrate in aqueous BGE (Figure 4.5). Figure 4.9A shows that addition of 10% methanol to the BGE results in thiosulfate and nitrate co-eluting. Upon increasing the methanol to 20%, the elution order of thiosulfate and nitrate were switched compared to Figure 4.5. Such changes in relative mobility are common in non-aqueous capillary electrophoresis [93,94]. Similarly, with 20% ACN in BGE (Figure 4.9B), the elution order was nitrate then thiosulfate. Also the analyte mobilities are much slower in organic-water mix compared to Figure 4.5. This might be due to the increased viscosity of the BGE and the diminished apparent ionic charge of inorganic anions [95,96]. Separation of bromide, chloride and sulfate was achieved with either 20% methanol or ACN. Resolutions are summarized in Table 4.3. Our method showed higher compatibility with organic solvents, compared with other CZE methods that adopted capillary coating [76] or buffer additives [97].

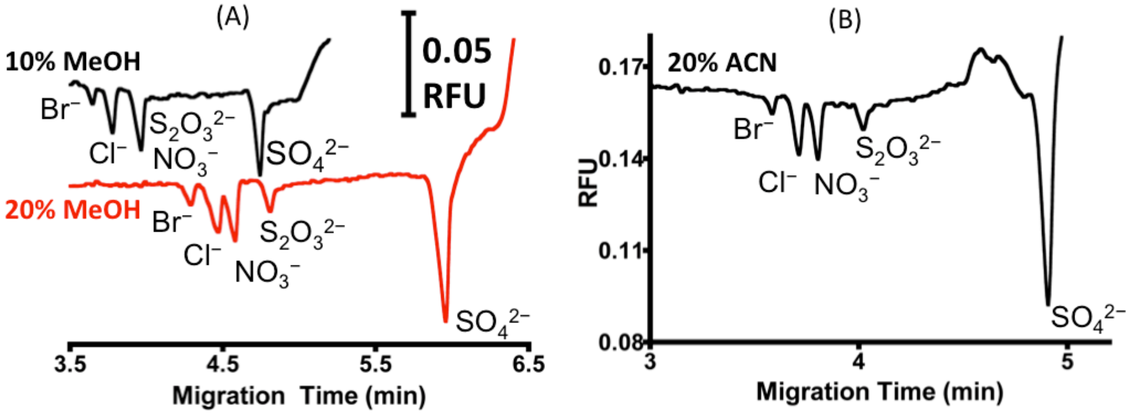

Figure 4.9 Separation of anion mixtures with methanol (A) or ACN (B) in the BGE. Conditions: sample, Br^-, Cl^-, $S_2O_3^{2-}$, NO_3^-, and SO_4^{2-} are 10 ppm each with the aqueous/ organic BGE; injection, 0.5 psi for 3 s; BGE, aqueous buffer of 10 µM HPTS and 0.4 M formic acid (pH 2.0) mixed with methanol or ACN (v/v ratio as indicated); capillary, L_T 60.2 cm, L_D 50 cm, I.D. 50 µm; voltage, -22.5 kV; detection, $\lambda_{excitation}$ = 405 nm and $\lambda_{emission}$ = 520 nm.

Table 4.3 Resolution in absence and presence of methanol and ACN in the background electrolyte[a]

Resolution BGE	**Br^-, Cl^-**	**Cl^-, SO_4^{2-}**
Aqueous	1.2	8.5
20% MeOH	1.4	9.5
20% ACN	1.6	11.9

a. Conditions: as in Figure 4.9

4.4 Conclusions

We developed and optimized an easy operating, simple-procedure anion analysis method using indirect fluorescent detection-capillary zone electrophoresis suitable for on-location oilfield water analysis. 8-Hydroxypyrene-1,3,6-trisulfonic acid (HPTS) was used as the fluorescence probe. The high stability of a 405 nm light emitting diode (LED) yielded a stable fluorescence background suitable for indirect detection. Baseline separation of bromide, chloride, thiosulfate, nitrate and sulfate under low ppm concentrations was achieved. Identification of bromide, nitrate and sulfate in high saline (≥30 ppm Cl^-) samples was accomplished. Analysis of oilfield water samples was achieved. This method also showed compatibility with organic preservatives in the sample and BGE.

4.5 References

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Chapter Five: Ultrasensitive Analysis of Heavy Metal Ions in Oilfield Produced Water Using Capillary Electrophoresis-Laser Induced Fluorescence Detection*

5.1 Introduction

During oil and gas production, large amounts of oilfield-produced water are generated. The 80 million barrels of oil produced per day globally results in 250 million barrels of associated wastewater [1,2]. The produced water contains a wide range of dissolved inorganic compounds including anions and cations, heavy metals and radioactive materials [1,3]. The numerous inorganic constituents can be potentially hazardous if discharged to the environment [4-6]. For example, trace quantities of heavy metal ions such as cadmium, copper, lead, zinc and nickel are common in oilfield water [3,7,8]. Heavy metals are environmental priority pollutants due to their persistence, toxicity, non-biodegradability, and ability to be incorporated into the food chain [9-12]. Information about the heavy metal ions in the oilfield water is vital for responsible oil production. From an environmental perspective, more than 40% of oilfield water is discharged into the environment [13]. Concentrations of heavy metal ions in discharged oilfield water need to be carefully monitored to meet the discharge regulations [14-16]. Also, from a production perspective, the presence of heavy metal ions must be monitored as they can be indicators of process upsets. For example, barium and strontium will precipitate with sulfate and be deposited to form scale in pipes [5,6].

Characterization of the metal cations in the oilfield water is challenging due to the high salinity of the produced water. Salt concentrations in oilfield water may vary from a few parts per million (ppm) to 300,000 ppm [1]. The high salinity of oilfield water is mainly from dissolved sodium, calcium, magnesium and chloride [1].

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Various detection methods have been applied to measure the heavy metals in oilfield water. Atomic absorption spectrometry (AAS) [7,17-19], inductively coupled plasma-optical emission (ICP-OES) or mass spectrometric (ICP-MS) [8,19-22] detection are most commonly used for heavy metal analysis in natural water samples as they are less prone to matrix effects [23]. Detection of heavy metal cations at parts per billion (ppb) levels in high salinity brine can be readily achieved [7,20,22,24,25]. However, there are major obstacles to the implementation of ICP to field analysis of oilfield water. A continuous supply of argon is needed to sustain the plasma, which limits the mobility of the instrument. Similarly AAS needs a continuous supply of flammable gas (Flame AAS) or argon (graphite furnace). Moreover, the atomic spectrometric detection methods suffer from polyatomic interferences, which requires extra separation or pre-concentration steps for trace metal analysis in high salinity brine waters [17-26]. Hence atomic spectrometric detection is appropriate for off-site laboratory analysis, but ill-suited for field analysis.

To facilitate oil production, in-field detection methods with portable instruments are needed in the oilfield. Portable technologies for metal detection may be electrochemical or chromatographic. Electrochemical detection methods are low-cost, simple to operate and provide prompt analysis [27-29]. Detection of heavy metal at nano-molar level can be achieved using electrochemical detection [27,30-32]. The most reported electrochemical techniques for heavy metal detection are voltammetry and potentiometry [27]. For voltammetric techniques, stripping techniques (i.e., anodic stripping voltammetry, ASV) are mostly used for heavy metal detection due to their high sensitivity and selectivity [31,33]. The disadvantage of stripping measurements is that only amalgam-forming metals can be determined. Also the technique is subject to various interferences, such as intermetallic compounds formed by other metal cations and the stripping wave disturbance by the presence of chloride [33,34]. For potentiometric techniques, ion selective electrode (ISE) are used due to their accuracy, fast response, non-destructive nature and low cost [35]. However, ISE sensors have narrow working ranges and suffer from significant interferences from some cations (e.

g. Cd^{2+} and Mn^{2+}) [36]. In general, each type of electrochemical device can only detect limited types of heavy metal ions [30,37-39]. Hence the electrochemical device is not suitable for heavy-duty, on-location oilfield analysis.

Chromatographic methods commonly applied to metal analysis are ion chromatography (IC) and capillary electrophoresis (CE). Chromatographic methods offer several advantages in metal cation detection such as multi-elemental capability in a single analysis and simplicity of operation [40-42]. The separation of major heavy metal cations (Fe^{2+}, Cu^{2+}, Zn^{2+}, Co^{2+}, Mn^{2+}, Cd^{2+}) at lower ppb level in 100 ppm of Ca^{2+} has been achieved on separation columns such as cation exchange column and cation/anion mixed-mode column [43-49]. However, the high concentration matrix cation (Na^+, K^+, Ca^{2+}, Mg^{2+}) in oilfield water would overload the capacity of ion-exchanging resin, resulting in peak distortion, poor peak resolution and irreproducible separation [44,45,50,51]. Hence extra sample preparation steps such as pre-concentration [52] or matrix ion removal [53] are required for metal analysis by IC.

As discussed in Chapter 4, capillary electrophoresis (CE) has been widely used for the analysis of ionic analytes due to its high resolving power, cost-efficiency and simplicity of operation [54,55]. CE and IC have been regarded as complementary separation techniques as they are based on different separation mechanisms [54,56]. In the past two decades, many approaches for heavy metal cation detection in CE have been explored [57-59]. For trace metal ions in complex matrices, an in-capillary concentration step such as transient isotachophoresis (ITP) can be used to pre-concentrate the analyte prior to separation [59-63]. Alternatively, detector formats such as contactless conductivity (C^4D) [64] and laser-induced fluorescence (LIF) [65,66] have also been implemented either to increase the instrument portability or detection sensitivity. Addition of complexing agent have also been utilized either to modify the separation selectivity or to facilitate photometric detection [62,67-69]. All these improvements have made CE of inorganic species mature. Hence CE has been widely applied in practical inorganic analysis [54]. Recent developments in CE of inorganic ions have focused on high efficiency separations of anions and cations within complex sample matrices [68,70] and CE portability [71-73]. Compared to HPLC, CE has high resolving power, low cost operation, and simplicity in design, optimization and operation. All of these advantages make CE a promising analytical technique for on-location oilfield water analysis. Hence CE was selected for our method development.

This chapter investigates CE protocols that would be suitable for robust, on-location field analysis. The method needs to be simple, insensitive to matrix effects and highly sensitive. Recently, Saito and co-workers achieved separation and sensitive detection of seven heavy metal cations (Cu^{2+}, Zn^{2+}, Ni^{2+}, Co^{2+}, Mn^{2+}, Cd^{2+} and Pb^{2+}) at sub-ppb levels with CE-LIF using fluorescent probe composed of a chelating moiety connected to a fluorophore (fluorescein) [65]. By careful adjustment of pH, the fluorescent probe did not complex with Ca^{2+} or Mg^{2+}, which would be the common matrix components in oilfield waters. Hence we investigated the method for heavy metal ions analysis in oilfield water samples.

5.2 Experimental

5.2.1 Chemicals and Materials

All solutions were prepared in Nanopure 18-MΩ water (Barnstead, Thermo Scientific, Marietta, OH, USA). Barium chloride dihydrate (≥99.999% trace metals basis), calcium chloride (≥99.99% trace metals basis), cadmium chloride (≥99.99% trace metals basis), lead(II) chloride (≥99.999% trace metals basis), nickel(II) chloride hexahydrate (≥99.999% trace metals basis), manganese(II) chloride tetrahydrate (≥99.99% trace metals basis), zinc chloride (≥99.999% trace metals basis), strontium chloride (anhydrous, ≥99.99% trace metals basis), copper(II) chloride dihydrate (≥99.99% trace metals basis), iron(II) chloride tetrahydrate (≥99.99% trace metals basis), sodium tetraborate (≥99.998% trace metals basis), hydrochloric acid (30%, TraceSELECT Ultra), sodium hydroxide (pellets, ≥99.99% trace metals basis), 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid

(DOTA, ≥97.0%), and hexadimethrine bromide (polybrene, ≥94%) were from Sigma-Aldrich (St. Louis, MO, USA). All metal stock solutions were prepared at 5 mM in 0.01 M HCl and filtered (0.22 µm) before use.

The fluorescent probe, FTC-ABDTPA (FTC = Fluorescein-thiocarbamyl, AB = 1-(4-aminobenzyl), DTPA = diethylenetriamine-N, N, N', N", N"-penta-acetic acid) shown in Figure 5.1 was provided by Professor Saito based on synthesis according to reference [65].

A 20% nitric acid bath was made by mixing 60 mL of nitric acid (reagent grade, Caledon Laboratories LTD, Georgetown, ON, Canada) and 200 mL deionized water. All glassware were soaked in the acid bath for 1 hour and then rinsed with deionized water prior to use.

5.2.2 Apparatus

Capillary electrophoresis was performed on a Beckman PA 800 plus Pharmaceutical Analysis system with a laser-induced-fluorescence detector (Beckman Instruments, Fullerton, CA, USA). Untreated fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) with an inner diameter of 50 µm, outer diameter of 365 µm, and total length of 60.2 cm (50 cm to detector) were used. New capillaries were pretreated with 1 M sodium hydroxide for 10 min (20 psi rinse), 0.1 M NaOH for 10 min (20 psi rinse), and finally deionized water for 5 min (20 psi rinse). The capillary was thermostated at 25 °C. Sample was injected using a 0.5 psi, 3 s hydrodynamic injection. Fluorescent detection used a Beckman Coulter 488 nm solid state laser coupled to a Beckman LIF module through a fiber optic connector. The output power at the end of fiber optic was 3 mW. Emission collected using a 520-nm band-pass filter with a bandwidth of 20 nm (Beckman) Data acquisition (4 Hz) and control were performed using 32 Karat software for Windows XP on a Lenovo Thinkcentre computer.

5.2.3 Procedures

5.2.3.1 Oilfield Water Sample Collection

Oilfield water samples were provided by Wilson Analytical. Sample 11123 was a rusty oilsands process water sample, and Sample 11124 was a clear oilsands process water sample. Samples were stored in capped PTFE bottles at 4°C. ICP-OES (Varian, now Agilent, Santa Clara, CA, USA) analyses of the samples were provided by Wilson Analytical. Ca, Mg, K, Na, Sr and Fe were quantified using 4 different wavelengths, and outliers were excluded upon result comparison. The sample was filtered (0.22 µm) and diluted with sample buffer (Section 5.2.3.2) before CE analysis. Standards could be run immediately after vortexing, but 1 hour reaction time is recommended for samples.

5.2.3.2 Capillary Electrophoresis Analysis

The background electrolyte (BGE) was based on that of Saito and co-workers, and consisted of 50 mM sodium tetraborate (pH 10.1) containing 1.0 mM DOTA and 0.05 wt% polybrene. The latter reversed the EOF and acted as an ion association mobility modifier (Section 5.3.1). The buffer used to prepare sample contained 5.0×10^{-6} M FTC-AB-DTPA, 10 mM sodium tetraborate (pH 9.9). Both the BGE buffer and the sample buffer were stored at 4°C. All metal cations standard solutions were prepared at 5 mM as standard stock solution in 0.01 M HCl. The metal cation stock solutions were stored at room temperature. Before injection, the metal cation stock solution was diluted to the designated concentration with sample buffer, and vortexed for 30 s to mix.

5.3 Results and Discussion

Inorganic cations may be separated and detected in capillary electrophoresis as the free ion, as weakly complexed ions, or as kinetically inert complexed ions [54]. Alkali metals are typically analyzed as free ions and detected using conductivity or indirect absorbance [64,67,69,74]. The separation of transition metals, whose free ions have similar mobility, is facilitated by the addition of a weak complexing agent such as α-hydroxyisobutyric acid [75,76] or acetic acid [77]. Chelating agents with large stability constants such as ethylenediamine tetra acetic acid (EDTA) or cyclohexanediamine tetraacetic acid (CDTA) form complexes that are kinetically inert during the time scale of a CE separation. Typically such metal-EDTA complexes are monitored by UV absorbance with 1~3 ppm (~10^{-5} M) detection limits [74,78]. With pre-concentration strategies such as sweeping, the detection limits can be further improved to sub-ppb level [62,79]. However, the pre-concentration techniques require elaborate optimization of experimental conditions for different sample matrices [80].

To achieve high sensitivity and selectivity, laser-induced fluorescence (LIF) was utilized in CE for metal ion detection. Indirect LIF detection has been applied to metal cation analysis [81,82]. However, as shown in Chapter 4, the low signal-to-noise ratio of the indirect detection makes it less appropriate for analysis of high concentration matrix ions. In this chapter we are interested in analyzing trace transition metals in the presence of a high concentration alkali metal and alkaline earth matrix. Thus, indirect fluorescence is not suitable for a developing a highly sensitive method. Fluorescent probes that can chelate metal ions have been used both with chromatographic and electrophoretic separations to aid LIF detection [83,84]. When using this approach, the kinetic stability of the complex is essential for the detection of metal ions [66,85-87]. As the fluorescent probe is the only source of the fluorescence signal in the sample, any cations associated with fluorescent probe could be detected. Hence the fluorescent probes would be detected in both its complexed and uncomplexed form. On the other hand, metal cations are only detected as the fluorescent metal-probe complex (Equation 5.1). Any kinetically unstable metal-probe complex would dissociate, resulting in non-fluorescent metal cations that cannot be detected. Hence, finding an appropriate fluorescent probe is the primary job of method development.

Table 5.1 Formation constants for metal-DTPA complexes[a]

Ion	**Log K_f**
Co^{2+}	19.3
Zn^{2+}	18.4
Cu^{2+}	21.03
Mn^{2+}	15.6
Fe^{2+}	16.5
Ni^{2+}	20.21
Cd^{2+}	18.93
Ca^{2+}	10.8
Sr^{2+}	9.68
Ba^{2+}	8.63
Mg^{2+}	9.03

a. from reference [88,89].

Diethylenetriamine-N, N, N', N", N"-penta-acetic acid (DTPA) is an octadentate chelating reagent which forms 1:1 complexes with most transition metal ions (Table 5.1). The stability constants of the metal-DTPA complexes are one to six orders of magnitude higher than those of ethylenediamine tetra acetic acid (EDTA). Hence DTPA forms more thermodynamically stable metal complexes than EDTA-like acyclic hexadentates [90,91]. A DTPA based fluorescent probe has recently been reported for CE detection of heavy metal ions. As shown in Figure 5.1, the probe consists of a chelating moiety (DTPA), a fluorophore (FTC: fluorescein-thiocarbamyl) and a spacer (AB: 1-(4-aminobenzyl) [65,92]. The probe, FTC-AB-DTPA, forms inert emissive complex with heavy and paramagnetic metal cations, while complexes with Ca^{2+} and Mg^{2+} were not detectable at alkaline pH [65]. The specific selectivity makes the probe highly suitable for oilfield water analysis as the matrix contains a high concentration of Ca^{2+} and Mg^{2+}.

5.3.1 Separation of Transition Metal–Probe Complexes

To verify the separation of the metal-probe complexes, CZE experiments of FTC-AB-DTPA complexes of Fe^{2+}, Cu^{2+}, Zn^{2+}, Ni^{2+}, Co^{2+}, Mn^{2+}, Cd^{2+}, Pb^{2+}, Sr^{2+} and Ba^{2+} were performed using a pH 10.05 BGE. The metal-FTC-AB-DTPA complexes are anionic. Addition of polybrene to the BGE results in ion association complexes (IAC, equation 5.2) between the anionic metal-chelate complexes and the polycationic polybrene [65].

$$M^{n+} + \text{FTC-AB-DTPA} \rightarrow MY^{n-5} \qquad \text{(metal-probe complexation in sample)} \qquad (5.1)$$

$$MY^{n-5} + \text{polybrene}^{m+} \rightleftharpoons MY^{n-5}(\text{polybrene}^{m+}) \qquad \text{(ion association in BGE)} \qquad (5.2)$$

The IAC enhances the differences in the electrophoretic mobilities relative to the metal chelate complex, resulting in improved resolution. The complexing agent 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) was also added to the BGE to mask trace metal ions in the BGE.

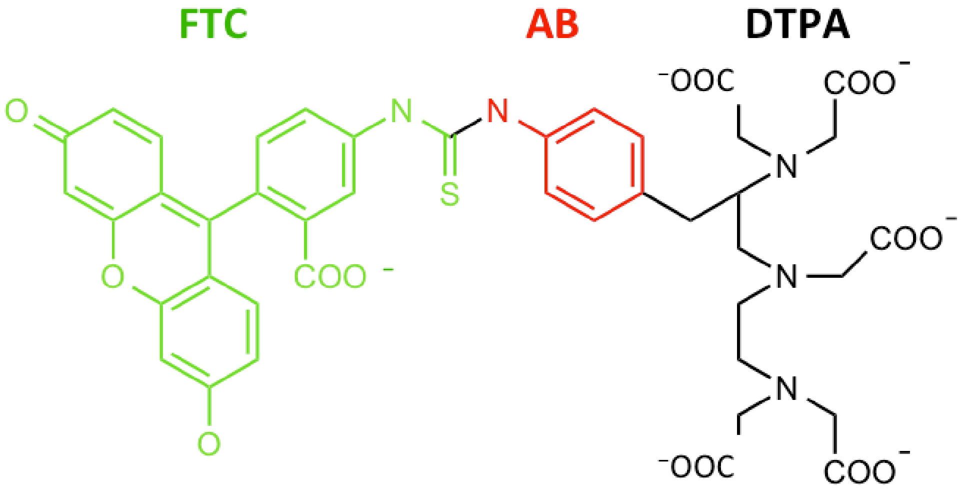

Figure 5.1 Structure of the fluorescent chelating agent, FTC-AB-DTPA.

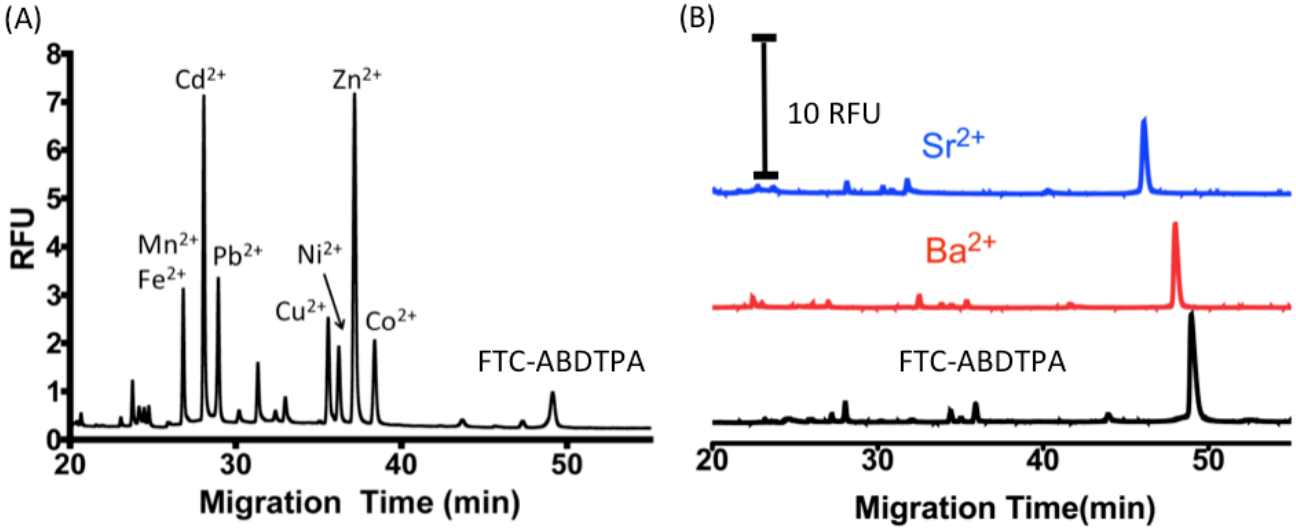

Figure 5.2 Electropherogram of metal FTC-AB-DTPA complexes with metal cations. (A) Transition metal cations that form stable complexes; and (B) Alkaline earth metal ions that are not detectable. Conditions: sample, all cations were prepared at 1.25×10^{-7} M, sample buffer contains [FTC-AB-DTPA]= 5.0×10^{-6} M, [borate] = 10 mM (pH 9.90); BGE: [borate] = 50 mM (pH 10.09), [polybrene] = 0.05 wt%, and [DOTA] = 1.0 mM.

Figure 5.2A shows baseline separation of Mn^{2+}, Cd^{2+}, Pb^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, and Co^{2+}. Unreacted FTC-AB-DTPA within the sample migrates slower than the metal complexes, and so does not interfere with the metal analysis. Fe^{2+} and Mn^{2+} co-elute, but this was not of concern as these ions are not of interest in oilfield waters. The separation efficiency was between 200,000 ~ 350,000 plates/m for each peak, which is better than the metal separation performed on IC column [45,47-49,93]. Compared to other CE separation methods that do not utilize a metal chelating agent, this method shows better separation efficiency and resolution, and does not require a pre-concentration step [57,59,64]. For other CE methods that adopted metal-chelation complexation in absorbance detection, the detection limit is only 10^{-5}~10^{-6} M without preconcentration [94-96]. Compared to literature CE methods that use a chelating agent, this FTC-AB-DTPA is capable of detecting 7 heavy metal cations. Previous literature has only reported simultaneous detection of 2~3 metal cations [58,85,86]. Hence this method is highly suitable for metal cation detection in oilfield water

5.3.2 Separations of Alkaline Earth Metals

Alkaline earth metals form much weaker DTPA complexes than transition metals (Table 5.1). As a result no metal complexes were observed for instance for injections of Sr^{2+} and Ba^{2+} (Figure 5.2B), or with Ca^{2+} and Mg^{2+}. Saito and co-workers presumed that this weak complexation resulted in labile complexes which decompose during the separation in FTC-AB-DTPA free BGE.

$$M^{n+} + \text{FTC-AB-DTPA} \rightleftharpoons MY^{n-5} \text{ (alkaline earth decomplexation)} \qquad (5.3)$$

However the concentration of Ca^{2+} and Mg^{2+} may reach 1000 times higher than the transition heavy metal cations in oilfield water samples [1]. It is possible that the concentrated Ca^{2+} and Mg^{2+} could compete with the trace heavy metal cations to chelate with the fluorescent FTC-AB-DTPA. To test the method compatibility with oilfield water sample, the separation of five transition metal cations

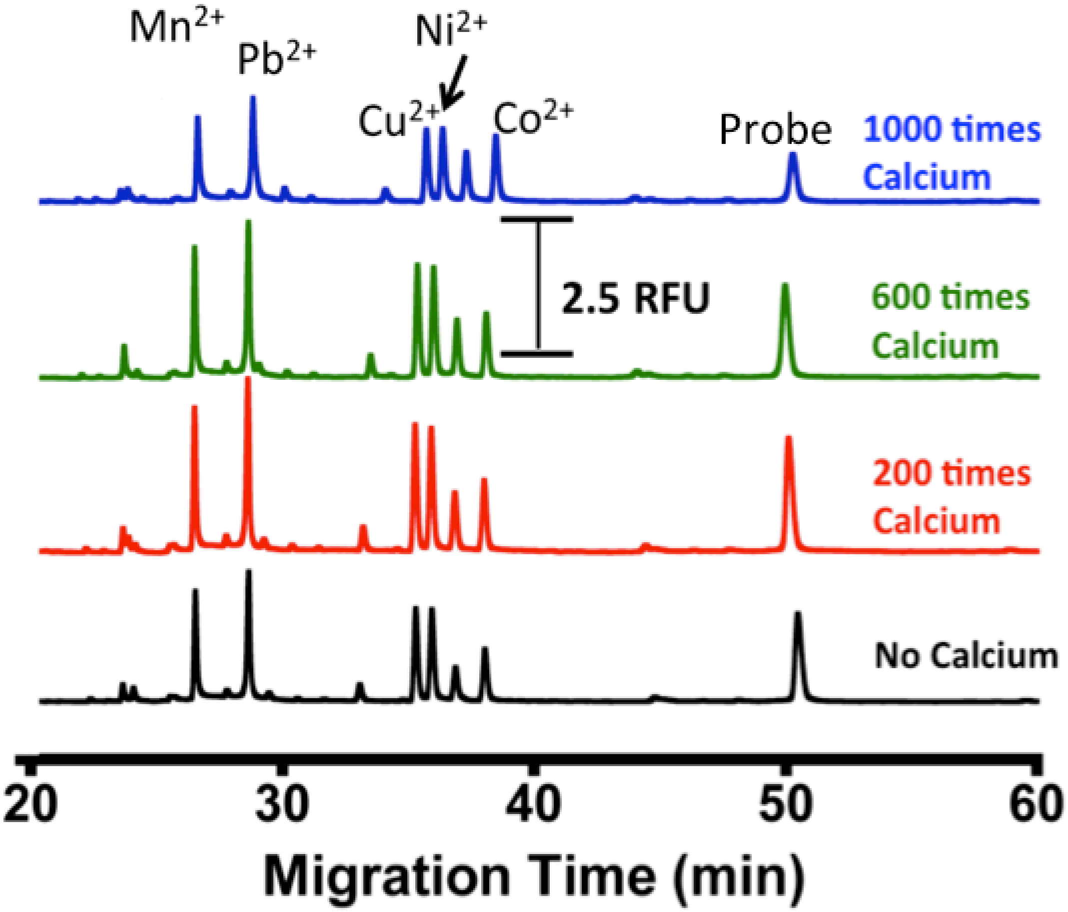

Figure 5.3 Separation of metal cations in high calcium matrices. Conditions: sample, all cations were prepared at 1.25×10^{-7} M, sample buffer contains [FT-AB-DTPA]= 5.0×10^{-6} M, [borate]= 10 mM (pH 9.90), and the calcium concentration indicated in the figure; BGE, [borate] = 50 mM (pH 10.09), [polybrene] = 0.05 wt%, and [DOTA] = 1.0 mM.

were performed with high concentration of calcium matrix. The concentration of each cation (Mn^{2+}, Pb^{2+}, Cu^{2+}, Ni^{2+}, Co^{2+}) was kept at 1.25×10^{-7} M while the calcium concentration was increased from 0 to 1.25×10^{-4} M. Figure 5.3 shows that the addition of calcium did not change the separation of heavy metal cations, i.e., the migration time RSD for Pb^{2+} is less than 1% and the resolution between Pb^{2+} and Mn^{2+} was unchanged. The compatibility with high calcium matrix sample demonstrated that our method is capable of handling samples with high alkaline earth metals.

5.3.3 Analysis of oilfield water samples

Two oilfield water samples were analyzed using the method. ICP-OES analysis indicated sample 11123 contained over 1660 ppm Ca^{2+}, 5660 ppm Mg^{2+} and an overall hardness expressed as mg/L $CaCO_3$ of 27400 ppm, and sample 11124 contained 48.6 ppm Ca^{2+} and 8 ppm Mg^{2+}. Figure 5.4A shows the analysis of sample 11124 after filtration and 4000-fold dilution. Peak identification was confirmed by spiking the sample with 2.5×10^{-7} M Mn^{2+} and Pb^{2+}. It should be noted that 1 h reaction time is necessary after addition of FTC-AB-DTPA to sample. Figure 5.4A shows the detection of Pb^{2+} was achieved in oilfield waters. Similarly in Figure 5.4B, Pb^{2+} was detected. The results showed that this method was capable of trace metal cation detection even in high alkaline earth metal sample. However, there is some migration time shift (RSD~ 1.5% for fluorescent probe) between run-to-run as shown in Fig. 5.4A. Hence development of an internal standard for migration time would be necessary [97]. Also metal contamination was observed in the blank. For example, in Figure 5.3, the peak at 37.4 min corresponds to Zn^{2+}. However, Zn^{2+} was not deliberately added to the sample. As stated in Section 5.2.1, all the chemicals used were trace metal level pure, all the glassware were soaked with nitric acid to remove metal residual, and all solutions were prepared using 18 MΩ deionized water. Despite all these efforts, contamination was still observed. It was unknown the source of contamination. As a result, it is impossible to quantify the LOD of the metal cations. Subsequent to this work being completed, the deionizer was determined to be faulty. Thus the deionizer may have been the source of the contamination.

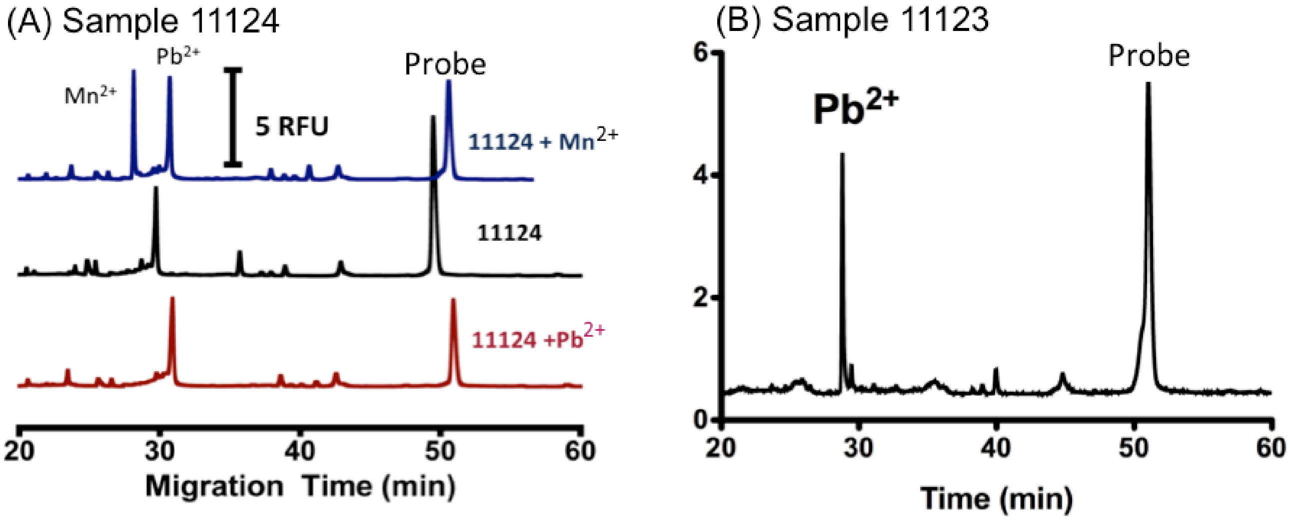

Figure 5.4 Analysis of oilfield water samples. (A) Sample 11124 diluted 5000 times. (B) Sample 11123 diluted 4000 times. Conditions: samples were filtered and diluted before injection. Sample buffer contains [FT-AB-DTPA] = 5.0×10^{-6} M, [borate] = 10 mM (pH 9.90); BGE, [borate] = 50 mM (pH 10.09), [polybrene] = 0.05 wt%, and [DOTA] = 1.0 mM.

5.3.4 Quantification

Linear calibration was carried out for Mn^{2+} over a range of 1-5 $\times 10^{-7}$ M. However, the peak area had limited run-to-run reproducibility (RSD of 15~30%). To correct for the irreproducibility, the peak area for the metal ion was ratioed by the total peak area. The total peak area in the electropherogram is only associated with the quantity of fluorescent species injected in capillary. The ratio between complexed probe and uncomplexed probe should be unaffected by factors such as injection volume [98]. Herein we defined the peak area ratio as:

$$Peak\ Area\ Ratio = \frac{Peak\ Area\ of\ Metal{-}Probe\ Complex}{Peak\ Area\ of\ Uncomplexed\ Probe + Peak\ Area\ of\ all\ Metal{-}Probe\ Complex} \qquad (5.4)$$

Recti-linear calibration curve (Figure 5.5) based on peak area ratio were best fit with a quadratic function with a correlation coefficient of R^2 >0.99 (n=4). The negative deviation of Figure 5.5 may be due to the Mn^{2+} concentration approaching the concentration of FTC-AB-DTPA (5×10^{-6} M) in the sample solution. The RSD of Mn^{2+} migration time was 0.5%. The RSD of peak area ratio for each concentration was 1~9%.

5.4 Conclusions

A sensitive CE-LIF detection method was applied for trace metal cation analysis in oilfield water. Complexation with the fluorescent chelating agent, FTC-AB-DTPA was adopted in the separation to selectively complex with metal cations but not with the alkaline earths, and to emit photometric signal. This method is very simple and easy to operate, and suitable for in-field analysis. Sub-μM detection of Mn^{2+}, Fe^{2+}, Pb^{2+}, Co^{2+}, Cu^{2+}, Zn^{2+} and Ni^{2+} was achieved with efficiencies 200,000 ~350,000 plates/m. High calcium matrix showed no effect on the separation resolution and efficiency. However, this method still needs further improvement, irreproducibility was observed in our experiment. To correct for those problems, an internal standard can be used for future work.

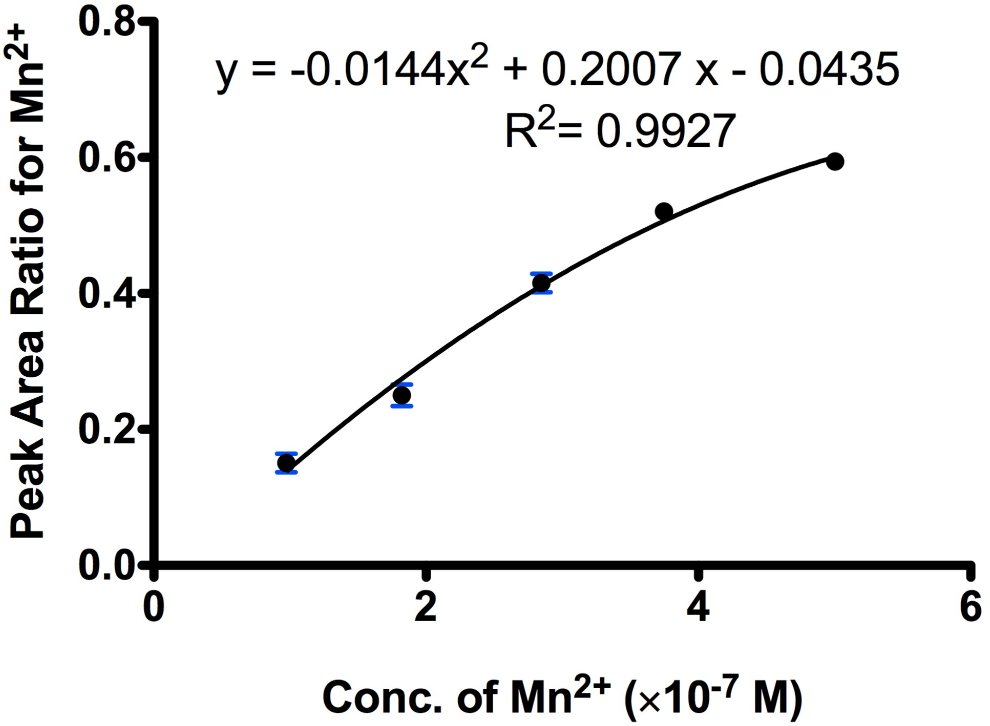

Figure 5.5 Calibration curve of peak area ratio vs. Mn^{2+} concentration

5.5 References

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Chapter Six: Summary and Suggestions for Future Work

6.1 Summary

This thesis enhances many aspects of capillary electrophoretic (CE) separations of small molecules. In Chapter 2, a polymerizable zwitterionic surfactant coating (Diyne PC) was utilized to block the surface adsorption for cationic molecules. In Chapter 3, poly-cationic coatings and successive multiple ionic layer (SMIL) coating were studied for their performance in the separation of small inorganic anions. The instability of the poly-cationic coatings was attributed to the degradation of the quaternary amines. Chapters 4 and 5 focused on development of fast, easy-operating separation protocols suitable for oilfield water inorganics analysis. Fluorescence detection approaches were applied to increase the sensitivity of the detection.

Coatings are commonly used in CE separations to address the issue of analyte adsorption onto capillary walls. Such adsorption causes peak broadening, migration time irreproducibility, and poor analyte recovery. Zwitterionic surfactant coatings have demonstrated great compatibility with biological molecules such as proteins [1]. However, zwitterionic surfactant coatings are not stable in mixed aqueous organic solvent needed for separation of hydrophobic organic ions. Chapter 2 presents a way to improve the stability of coatings prepared from the diacetylene two-tailed surfactant Diyne PC through crosslink-polymerization of the hydrophobic chains. The polymerized Diyne PC coating exhibits enhanced stability towards organic solvents such as methanol and ACN. Improved peak asymmetry and reduced analyte adsorption of small amines and basic pharmaceutical compounds were achieved on polymerized Diyne PC coated capillaries.

Another commonly used class of coatings are polycationic coatings such as poly(diallyldimethylammonium) chloride (PDADMAC) and hexadimethrine bromide (polybrene). Such polycations formed stable semi-permanent coatings at acidic to neutral pH, but were surprisingly unstable coatings at high pH. The stability behavior and degradation mechanism was studied in Chapter 3. Polycations with higher molecular weight formed higher stability coatings, as measured by the stability of the electroosmotic flow (EOF) and reproducibility of the migration times for repeated separations of inorganic anions. This stability decreased at pH >8. X-ray photoelectron spectroscopy (XPS) showed that the instability of polycationic coating was due to a silica surface-facilitated chemical degradation of the quaternary amine group in the polycationic coatings. To test this hypothesis, the pH stability of successive multiple ionic layer (SMIL) coatings were explored. SMIL coatings consist of multiple layers of poly-cations (PDADMAC) and poly-anions (poly(sodium 4-styrenesulfonate), PSS). SMIL coatings have demonstrated higher stability than just poly-cationic coatings [2,3]. It was hypothesized that the underlying ionic layers would distance the top polycationic layer that was responsible for the EOF from the silica surface. XPS demonstrated that less of the quaternary nitrogen in a SMIL was converted into the tertiary amine. Highly reproducible migration times (2% shift) were observed for inorganic anions at pH 9.5 using a SMIL coating.

To analyze inorganic ions in oilfield water, CE with fluorescence detection was explored in Chapters 4 and 5. In Chapter 4 I developed a CE method with indirect fluorescence detection for inorganic anions. The fluorescent dye 8-hydroxypyrene-1,3,6-trisulfonic Acid (HPTS) was utilized as fluorescent probe and was present in the BGE. A stabilized LED provided a more constant light source than a laser diode, so was used for indirect fluorescence detection. The capillary length, pH and buffer component were optimized to achieve a resolution of 8.5 between chloride and sulfate. High salinity sample (Cl^- range 30~1500 ppm) were tested to evaluate the electrodispersion effect on trace anion detection. The method achieved detection of trace amount of sulfate (0.5 ppm) in high salinity (1000 ppm chloride) samples.

Chapter 5 explored a chelation-based detection method for transition metal cations in oilfield water samples. The fluorescent chelating agent fluorescein-thiocarbamyl-1-(4-aminobenzyl) diethylenetriamine-N, N, N', N", N"-penta-acetic acid (FTC-AB-DTPA) was added to the sample and complexed with target cations, forming fluorescent cation-probe complexes. Addition of polybrene to the BGE results in ion association complexes (IAC) between the anionic metal-chelate complexes and the polycationic polybrene. The difference in the mobility of the different IAC is the driving force for the separation. Detection of trace level (i.e. 1.25×10^{-7} M) cations (Pb^{2+}, Cu^{2+}, Zn^{2+}, Co^{2+}, Cd^{2+}, Mn^{2+}, Ni^{2+}) was achieved with high calcium matrix (1.25×10^{-4} M, i.e.,1000 times higher concentration than the target cation) sample.

Generally speaking, this thesis has adopted multiple means to improve the inorganic analysis using CE, mostly by improving the separation repeatability through increasing the coating stability and by increasing detection sensitivity through use of fluorescence detection.

6.2 Future Work

Chapter Three demonstrated that the adsorptive polycationic coatings are unstable under alkaline condition while successive multi-ionic layer (SMIL) coatings remained stable [4]. Recent studies of the cationic surfactant dioctadecyldimethylammonium bromide (DODAB) also observed quaternary amine degradation is a silica-surface catalyzed reaction. To identify the exact degradation mechanism and factors affecting the reaction, XPS studies of quaternary amine and tertiary amine in SMILs under various experimental conditions are needed.

However, SMIL coatings can only yield strongly normal or strongly reversed flow. There remains a need for a robust and facile means of achieving suppressed electroosmotic flow over a wide range of pH.

Recently the Riekkola group reported a neutral double hydrophilic block copolymer-poly(N-methyl-2-vinylpyridinium iodide-block-ethyleneoxide) for capillary coating [5]. This copolymer consisted of a cationic block (poly(N-methyl-2-vinylpyridinium iodide) and a hydrophilic block (ethylene oxide). The cationic block adsorbed onto the negatively charged capillary wall via electrostatic interaction. This diblock copolymer coating effectively suppressed the EOF (2.0×10^{-6} cm^2/Vs) and was reported to be stable over a wide pH range (pH 4.0~10.5). This reported stability at high pH behavior is surprising compared to what has been observed in Chapter Three considering the cationic block is also a quaternary amine based polymer. In recent work from our group, DOBAB was observed to be unstable at alkaline pH, but a DODAB/polyoxyethylene (POE) stearate coating [6,7] was more stable. One possible explanation was that the POE may have a protective effect on the underlying quaternary amine adsorbed on the silica. This hypothesis would explain the greater alkaline stability of both Riekkola's coating and the DODAB/POE stearate coating. Alternately, the structure of the pyridine in Riekkola's coating may hinder the silica surface-catalyzed degradation of the quaternary amine, resulting in improvement of coating stability.

To test these theories, I propose to study the coating property of a quaternary amine-based cationic surfactant with a pyridine head group. The synthesis of pyridine-containing surfactant has been reported [8,9]. Also similar diblock polymers such as poly(styrene-b-2-vinyl pyridine-b-ethylene oxide) are commercially available from Polymer Source Inc. (Montreal, Quebec, CA). The Riekkola group has showed that the quaternization of pyridine can be achieved through a one-step reaction [5]. Hence it is reasonable to synthesize a cationic surfactant with a quaternized pyridine in head group. By investigation the coating performance of this surfactant, we can study the mechanism of the surface-catalyzed quaternary amine degradation.

6.3 References

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