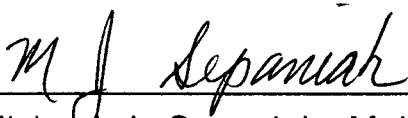


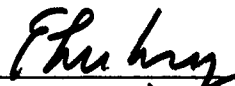
To the Graduate Council:

I am submitting herewith a dissertation written by Beth A. Colburn entitled "Determination of Environmentally Important Materials Including Rare Earth Metals, Uranium and Amines via Capillary Electrophoretic Methods Utilizing Indirect and Direct Spectrophotometric Detection." I have examined the final copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Chemistry.



Michael J. Sepaniak, Major Professor

We have read this dissertation
and recommend its acceptance:







Accepted for the Council:



Associate Vice Chancellor and
Dean of The Graduate School

**Determination of Environmentally
Important Materials Including
Rare Earth Metals, Uranium and Amines via
Capillary Electrophoretic Methods
Utilizing Indirect and Direct
Spectrophotometric Detection**

A Dissertation
Presented for the
Doctor of Philosophy
Degree
The University of Tennessee, Knoxville

Beth A. Colburn
August 1996

DEDICATION

To my loving family with gratitude

ACKNOWLEDGMENTS

I want to thank several people for their understanding and support during the process of my education. First, thanks to my parents for their sustaining love and confidence (Barbara and Donald Colburn), and to my brother, David Colburn, and his family for an occasional vacation. Second, to the Sepaniak research group (Mike, Kylene, Tim, Vika, Will and Jason) and other friends (Randy, Don, Dave, Bill, Dan, Dave and Rich), thanks for help when I needed it and the interesting discussions we've had. I greatly appreciate help and advice from former group members, including Dave, Rod, Tracy, Christy and Craig. I especially enjoyed Tracy and Sharon's invitations to their home as a retreat from the city and a relaxation. The person to whom I owe the most and want especially to acknowledge is Brian Clark. Without his unending support, love, understanding and encouragement I would not have achieved my goal.

Finally, I'd like to thank Dr. Michael Sepaniak for his guidance and assistance toward the research presented in this work.

ABSTRACT

The research presented in this dissertation concerns the analysis of certain environmentally important metals and compounds. In the analysis of lanthanum and lanthanide metals (referred to herein as "lanthanide metals"), the mobility of the background ion used in the indirect absorbance detection was determined to be very important. The best background ion found was 2-ethylimidazole. The sample matrix and method of injection also had an enormous effect on the analytical figures of merit. Hydrostatic injections from water produced the best LODs (ranging from about 1.7 to 5.4 ppm for the metals studied) and efficiency but had high asymmetry and coefficients of variation. Hydrostatic injections from running buffer exhibited good reproducibility but poor LODs. This method of lanthanide determination has limited applicability due to the necessity of stacking to achieve moderate LODs.

The analysis of the uranyl cation in groundwater developed herein exhibits high selectivity and good LODs (25 ppb) although a system peak is produced which could not be resolved from the analyte peak. Capillary length and diameter affected detectability and the resolution between the system and analyte peaks. This method is applicable to realistic samples and is a sensitive, rapid, inexpensive screening tool for the detection of the uranyl cation.

some impurities. The method developed herein lacked the necessary precision for the determination of the two major components. However, the LODs seen in the analysis of the impurities (from 2.5 to 14 ppb) were good. When a real liquid propellant sample was analyzed, the hydroxylamine peak obscured the possible ethanolamine and ammonia peaks; when diethanolamine was spiked into the sample at a concentration of 30 ppb, the signal was easily detected. Diethanolammonium nitrate can be present as an impurity in the triethanolammonium nitrate used in the formulation of the liquid propellant and this method is shown to be a speedy, inexpensive and sensitive determination.

Note: The term "rare earth metals" is used in the title due to paperwork requirements for graduation. The term "lanthanide metals" is used throughout the dissertation to refer to lanthanum and the lanthanide series.

TABLE OF CONTENTS

	page
Chapter 1. Introduction to Capillary Electrophoresis	1
Statement of Problem	1
Organization	2
Fundamentals	3
Brief Historical Perspective	6
Apparatus	10
Electroosmotic flow	12
Joule Heating	17
Theoretical Considerations and Advantages	18
On-column detection	25
 Chapter 2. Quantitative Aspects of Lanthanide Metal Determinations Using Capillary Electrophoresis with Indirect Absorbance Detection	 36
Introduction	36
Experimental	39
Apparatus	39
Chemicals	39
Procedures	40
Calculations	41
Results and Discussion	43
Background Ion Mobility	43
Chelating Agent Concentration	46

Sample Matrix	48
Injection Method and Matrix	50
Conclusions	55
Chapter 3. A Screening Protocol for the Direct Determination of Low ppb Levels of the Uranyl Cation Using Arsenazo III and Capillary Electrophoresis	
	58
Introduction	58
Experimental	63
Apparatus	63
Chemicals	63
Procedures	64
Results and Discussion	65
Stacking Mechanism	65
System Peak	68
Running Buffer Optimization	71
Sensitivity and Detectability	75
Optimum Column Length and Injection Plug length	76
Interferences and Selectivity	79
Conclusion	81
Chapter 4. Determination of Liquid Propellant via CZE with Indirect Absorbance Detection and MECC with Laser-Induced Fluorescence Detection	
	83
Introduction	83
Experimental	88

Apparatus	88
Chemicals	89
Procedures	89
Results and Discussion	91
Analysis of Hydroxylamine Sulfate and Triethanolamine	91
Analysis of Diethanolamine, Ethanolamine, and Ammonium Nitrate	99
Conclusions	108
Chapter 5. Concluding Remarks	109
References	117
Vita	125

LIST OF TABLES

	page
Table 1. Comparison of Efficiency and Asymmetry for Tested Background Ions	45
Table 2. Figures of Merit: Efficiency, Asymmetry, Limits of Detection with CVs	53
Table 3. Concentrations of Arsenazo III and Sodium Chloride Investigated	73
Table 4. Optimized Running Buffers	74
Table 5. Optimization of Effective Column Length and Injection Plug Length	78
Table 6. pK_a Values of Selected Propellant Components	92
Table 7. Determination of Optimal Dilution Factor for Best Peak Height Reproducibility of Hydroxylamine Sulfate and Triethanolamine	98

LIST OF FIGURES

	page
Figure 1. Diagram of basic CE apparatus	11
Figure 2. Velocity profiles resulting from a) hydrostatic flow and b) electroosmotic flow	14
Figure 3. Depiction of a) electroosmotic and electrophoretic mobilities and b) observed velocities for cations, neutrals and anions	16
Figure 4. Depiction of a) direct and b) indirect absorbance detection	27
Figure 5. Depiction of a cause of asymmetry in indirect detection	31
Figure 6. Structures of chemicals in lanthanide metal research	44
Figure 7. Electropherograms showing effects of HIBA	47
Figure 8. Electropherograms showing effect of stacking	49
Figure 9. Calibration curves for praseodymium	52

Figure 10. Calibration curves for a) lanthanum and b) dysprosium	56
Figure 11. Absorbance spectra of a) free Arsenazo III and b) the uranyl - Arsenazo III complex	62
Figure 12. Depiction of the stacking mechanism	66
Figure 13. Typical electrophoretic peaks	69
Figure 14. Calibration curve for uranyl cation	77
Figure 15. Standard addition calibration curve for the Poplar Creek sample	82
Figure 16. Separation of a standard mixture containing 100 ppm of each solute with imidazole as the background ion	93
Figure 17. Separation of a standard mixture using 1,1'-carbonyldiimidazole as the background	95
Figure 18. Dilutions of hydroxylamine sulfate and triethanolamine which exhibit the best reproducibilities	97
Figure 19. Derivatization procedure for NBD-Cl	100
Figure 20. Separation of derivatized diethanolamine, ethanolamine and ammonium	102

Figure 21. Calibration curves for NBD-diethanolamine, -ethanolamine and -ammonium	103
Figure 22. Analysis of derivatized liquid propellant sample	105
Figure 23. Analysis of triethanolamine containing diethanolamine and ethanolamine	107

LIST OF ABBREVIATIONS

A	absorbance
A'	area
a	absorptivity
A _s	asymmetry
b	path length
b'	base
C	concentration
C _{BGI}	concentration of background ion
CGE	capillary gel electrophoresis
CV	coefficient of variation
CZE	capillary zone electrophoresis
D	diffusion
DNA	deoxyribonucleic acid
DR	dynamic reserve
E	field
ELP	extended light path capillaries
h	height
HIBA	α -hydroxyisobutyric acid
HPLC	high performance liquid chromatography
i.d.	inner diameter
k	fraction of fluorescence detected
k'	capacity factor
k _b	thermal conductivity of the buffer

L_d	length to detector; also known as effective length
L_{inj}	length of injected sample plug
LOD	limit of detection
LDR	linear dynamic range
MECC	micellar electrokinetic capillary chromatography
N	efficiency or number of theoretical plates
n	peak capacity
o.d.	outer diameter
P_f	power of fluorescence emission
P_o	power of excitation for fluorescence
pK_a	negative log of the ionization constant of an acid
q	net charge
R	apparent hydrodynamic radius
R_{pg}	rate of power generation
R_s	resolution
t_0	time of an unretained species
ΔT	temperature gradient
t_{inj}	time of injection
t_m	retention time of the micelles
TR	transfer ratio
t_r	retention time of the solute
UV	ultraviolet
v_{eo}	electroosmotic velocity
v_{inj}	velocity of injection
v_{obs}	observed velocity

$w_{1/2}$	width of peak at 1/2 height
w_b	width of peak at baseline
w_l	width of left part of peak at 10% peak height
w_r	width of right part of peak at 10% peak height
wt %	weight percent

α	selectivity factor
ϵ	dielectric constant
η	viscosity
ϕ_f	quantum efficiency
μ	mobility
μ_{ave}	average mobility
μ_{eo}	electroosmotic mobility
μ_{ep}	electrophoretic mobility
μ_{obs}	observed mobility
$\Delta\mu$	difference in mobility between two analytes of interest
ζ	zeta potential

Chapter 1

Introduction to Capillary Electrophoresis

Statement of Problem

The research presented herein focuses mostly on analyses of inorganic species. Usual methods of analysis for metal ions include atomic absorption, inductively coupled plasma emission spectroscopy and inductively coupled plasma mass spectroscopy. These methods can be extremely expensive or cannot be readily optimized to quantitate more than one element at a time. Capillary electrophoretic methods can offer a cost effective methodology with the potential of simultaneous detection of many elements. The high speed analysis reduces the time necessary for costly technicians; low reagent and sample consumption reduces capital expenditures. In addition, instrumentation for capillary electrophoresis is considerably less expensive to maintain and operate than most of the aforementioned techniques.

Unfortunately, metals do not possess broad absorption bands; therefore, molecular spectrophotometric analyses must take advantage of indirect detection or complex formation. Certain metals present challenging separation problems because they exhibit very similar properties; for example, nearly identical mobilities, which result in poor selectivity. Resolution, directly

proportional to selectivity, suffers when analytes have similar mobilities.

This research presents difficult analyses of complex samples such as mixtures of lanthanide metals, the uranyl cation in ground water, and a liquid propellant sample. The determination of lanthanide metals is a suitable analysis for the elucidation of quantitative aspects involved in an indirect absorbance detection method. The determination of the uranyl cation is important due to its presence in water and minerals, and its toxicity. The composition of the liquid propellant directly affects its ability to perform and therefore must be known. The suitability of capillary zone electrophoresis (CZE) and micellar electrokinetic capillary chromatography (MECC), two techniques discussed herein, for the rapid, sensitive and selective determinations of these solutions is demonstrated.

Organization

Due to the variety of subjects presented in this work, each chapter will contain an Introduction, Experimental and Results and Discussion section. Chapter 1 is a general introduction to capillary electrophoretic techniques and provides the reader with a basic understanding of the theory and advantages of CZE and MECC.

Chapter 2 describes the quantitative analysis of lanthanide metals. The determination requires the use of a complexing agent to improve selectivity between the various members of the series.

Indirect detection is utilized as the metals do not possess suitable absorption bands.

Chapter 3 presents the development and testing of analytical methodologies for the analysis of the uranyl cation ($U(VI)$). The method presented is highly selective and sensitive.

In Chapter 4, methodologies for the determination of various organic amines in a liquid propellant sample are developed and tested. Indirect absorbance is utilized for the detection of the two major components, whereas derivatization followed by fluorescence detection is required for the minor components.

Finally, Chapter 5 summarizes the important conclusions reached in this work.

Fundamentals

In recent years, there has been an exponential growth in the development and use of capillary electrophoretic techniques for the analysis of complex samples. This increase in popularity originates from the ability to provide high resolving power, rapid analyses and low cost. Thus, these techniques offer unique advantages over conventional separation methodologies.

Capillary electrophoretic techniques comprise a family of techniques which have been successfully employed to separate a wide variety of analytes. Several different techniques are recognized; this diversity makes capillary electrophoretic techniques ideal candidates for most separation applications.

Techniques such as CZE, MECC and capillary gel electrophoresis (CGE) have extended the range of applications to include the analysis of small inorganic or organic molecules, peptides, proteins and DNA fragments. Each technique has a slightly different basis for separation; however, regardless of the technique, the fundamental requirement to achieve resolution of solute components is to impart significantly different mobilities to each component, i.e., to achieve selectivity. The mobility depends on the size-to-charge ratio of the analyte (1,2). For CZE, a difference in mobility for analytes of similar size can be achieved by changes in pH, which will alter the charge provided the analytes of interest have acidic or basic functionalities.

Because the mobility of the analyte depends on the size-to-charge ratio of the analyte, CZE cannot separate species which have no mobility or equal mobilities such as neutral molecules or certain biopolymers, respectively. In these cases, running buffer additives must be incorporated to impart a difference in mobility between analytes. For the analysis of neutral molecules for which the mobility is zero, the addition of a surfactant to the running buffer at a concentration greater than the critical micelle concentration causes the formation of charged micelles. A charge is imparted to the neutral molecules as they partition into the micelles. The differential equilibria between the free and the associated analyte molecules create the mobility differences

between the analytes. Separation of neutral molecules can be achieved, and this is the basis for MECC. (1,3-6).

Analytes which have equal mobilities, such as DNA materials, create special difficulties in separation. In these cases, as the size of a DNA fragment increases, so does the charge which results in no net change in the mobility of the fragment. Thus, separation cannot be achieved by CZE or MECC. For these particular separations, a difference in mobility can be achieved by the creation of a porous gel matrix inside the capillary column. Smaller fragments pass through the gel pores with less difficulty while larger fragments experience more friction and therefore have a longer migration time (1,3,4,7,8). Thus, separations based on size can be accomplished.

These approaches - free zone, micellar and gel electrophoresis - render capillary electrophoretic techniques especially comprehensive. Results of analyses of inorganic solutes compare well with results from ion chromatography in less time although with less sensitivity (9). This problem can be solved with a pre-concentration technique, examples of which will be discussed later. A wide variety of organic solutes can easily be analyzed from a variety of sources such as environmental samples, catecholamine metabolites, nerve agent hydrolysis products, herbicides and carboxylic acids found in wines. CZE often yields faster separations than high performance liquid chromatography (HPLC) and with less sample preparation (9). Chiral species, amino

acids, peptides, proteins, nucleic acids, oligonucleotides, carbohydrates, pharmaceuticals and clinical samples are also suitable for analysis by capillary electrophoretic techniques (9).

Even with the advances and significant improvements concerning analysis time, cost, detection and sample complexity, separation science still faces ever more stringent demands. Capillary electrophoretic techniques have short analysis times, require low sample and solution volumes, are quite inexpensive and can separate complex samples that are intractable by other techniques. Given these advantages, many research groups are working to overcome the few disadvantages that exist, and to apply the techniques to an even broader range of samples.

Brief Historical Perspective

Electrophoretic techniques can be traced back to 1791 to Faraday's laws of electrolysis (3,10). Tiselius published his thesis (11) in 1930 involving the separation of proteins utilizing moving boundary electrophoresis. In this technique, a sample consisting of a mixture of proteins is contained in one reservoir connected via a tube to a reservoir containing an electrolyte. As a voltage is applied, the sample proteins migrate according to their electrophoretic mobilities. As the analysis proceeds, the fastest migrating analyte emerges from the sample solution followed by successively slower sample constituents. However, full separation cannot occur because more sample is being injected continually,

and so each analyte is merely partially purified (10). Thermal convection arising from electrical, or Joule, heating often distorts the boundaries between different proteins (10). Approaches to minimize this effect include cooling the experimental apparatus (10), preventing convection by adding stabilizers such as gel or paper (3,10,12), and decreasing the diameter of the tube. Reducing the column diameter increases the surface area-to-volume ratio of the tube which improves heat dissipation. Furthermore, reducing the diameter diminishes the flux of ions through a unit volume of column. This reduces the absolute amount of heat generated as discussed in a later section (1,6).

The development of isotachopheresis occurred over a broad period, beginning in 1923 and extending to the present. A technique called capillary isotachopheresis is still undergoing development (10). The sample is placed between a leading electrolyte of high mobility and a tailing electrolyte of low mobility. The sample separates into adjacent zones and migrates toward the detector as voltage is applied. Thus, it is important that the mobilities of the leading and tailing electrolytes are higher and lower, respectively, than the mobilities of the analytes in the sample. All zones in the system migrate at the same speed (*iso tach* in Greek), leading to the name isotachopheresis (3,6).

In 1967, Hjerten developed free zone capillary electrophoresis (1,5,10). This technique uses no stabilizers or support within the tube nor does it use leading or tailing

electrolytes. Realizing the advantages of capillaries in reducing thermal convection, Hjerten was nevertheless forced to use 3 mm inner diameter (i.d.) tubes as no detector with adequate sensitivity was available (1,5). Unfortunately, electrophoresis in such large tubes generated excessive Joule heating caused by the passage of electric current through the running buffer (1,6). This will cause thermal gradients which lead to fluid convection if the gradients are of sufficient magnitude. Fluid convection tends to re-mix separated bands. To minimize dispersive effects, Hjerten rotated the tubes about their longitudinal axis thereby homogenizing thermal gradients and minimizing convective effects.

In 1979, Everaerts et al. reported free zone electrophoresis in a 200 μm i.d. Teflon capillary (13). Both ultraviolet absorbance and conductometric detection were performed on-column. High sample concentrations were required to achieve adequate detectability; however, such high sample concentrations lead to band broadening due to field inhomogeneities. The analyte of high ionic strength creates a region of high conductivity and low field. This distorted electric field results in a distortion of the analyte band such that the high efficiency inherent to capillary electrophoretic techniques remained undiscovered (1,3-5).

In 1981, Jorgenson and Lukacs published highly efficient separations using on-column fluorescence detection (2). In this work they used 75 μm i.d. capillaries which were effective in minimizing Joule heating. Furthermore, the sensitivity achieved

using fluorescence detection permitted the injection of sample concentrations well below the buffer concentrations, thus allowing high efficiencies to be attained. The authors also described the importance of electroosmotic flow as it relates to resolution and analysis time (1-5,14).

In 1984, MECC was developed by Terabe (1,3). The presence of a pseudo-stationary phase consisting of micelles allows the determination of both charged and neutral species in the same analysis (15-23). The neutral analytes partition into the micelle, gaining an effective charge due to the association. Different species will associate with the micelles to different degrees, resulting in a separation. MECC will be discussed in a later section.

The years since 1984 have seen advances such as maturation of new formats (use of gels, micelles, etc.) and an accompanying broadening in range of applications. Since the late 1980s, automation and commercial instruments have come into wide use (24,25). Application and method development publications have flourished due to the high efficiencies, the simple instrumental requirements and the high analysis speeds obtained in capillary electrophoretic techniques (1,4,5).

Detection in capillary electrophoretic techniques usually occurs on-column resulting in a path length approximately equal to the column i.d. Thus, detection by absorbance is limited by the short path length of the capillaries; however, use of other

detection methods has increased the utility of capillary electrophoretic techniques. Absolute detection limits are significantly improved by laser-induced fluorescence detection, to the attomole range (and lower) in ideal circumstances (1,15,16,26-29). On-line mass spectrometry provides another mode of sensitive detection (30,31). Electrochemical methods have been used to detect catecholamines in a single snail neuron among other applications (1,32,33). Indirect detection can be used to analyze components lacking chromophores and fluorophores, and will be discussed in detail in a later section.

Apparatus

CZE and MECC are the relevant techniques for the research discussed in this dissertation and the basic apparatus for both is portrayed in Figure 1. The separation capillary is usually made from fused silica coated with polyimide to increase flexibility. A small portion of the polyimide coating is removed via flame or hot sulfuric acid to expose the clear silica beneath to form a detection window for on-column optical detection. The capillary often has dimensions of 25-75 μm i.d. x 30-100 cm in length. These narrow diameter capillaries generate less Joule heating due to two factors. First, fewer ions are contained in the column which leads to less current and, hence, less Joule heating. Second, the small i.d. provides a high surface area-to-volume ratio resulting in efficient heat dissipation. Joule heating will be discussed in

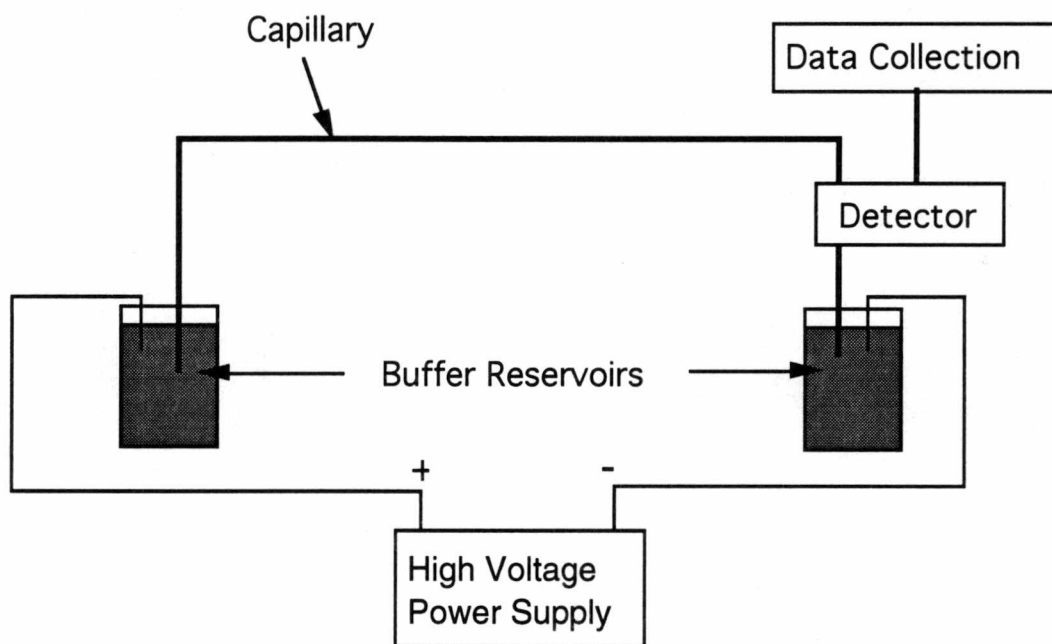


Figure 1. Diagram of basic CE apparatus.

detail later. Detection limitations and obstruction of the capillary due to airborne particles or precipitation restrict the minimum capillary i.d. to about 25 μm for common use. The length of the capillary is determined by the analysis speed required and by the efficiency and resolution needed. Longer columns provide better efficiency and resolution (see later discussion) but increase analysis time.

The capillary is filled with running buffer and positioned between two running buffer reservoirs. Electrodes placed in the reservoirs complete the circuit for a high voltage power supply to provide the driving force (i.e., electric field) for the separation. Normally, because of the cathodic electroosmotic flow (discussed below), the injections occur at the anodic end and detection near the cathodic end. Electrophoresis can also occur under opposite conditions known as reverse polarity. The most common detection modes are absorbance and fluorescence and these will be discussed later in this chapter. A data collection device records the electropherogram for later manipulation and analysis.

Electroosmotic flow

Electroosmotic flow is one of the distinguishing characteristics of many capillary electrophoretic separations. It is the bulk flow of the solvent through the column due to the negative charge of the capillary walls and the diffuse layer of hydrated cations next to the walls which balance the charge. As

voltage is applied, this diffuse layer of hydrated cations migrates toward the cathode dragging the bulk solvent in the same direction. The velocity of the electroosmotic flow (v_{eo}) is given by

$$v_{eo} = \frac{\epsilon \zeta}{\eta} E \quad (1)$$

where ϵ is the dielectric constant, ζ is the zeta potential of the liquid-solid interface, η is the viscosity of the buffer and E is the applied field (1). The term $(\epsilon \zeta / \eta)$ is often known as the electroosmotic mobility, μ_{eo} (1,4).

The bulk flow has a very different profile than that generated by pressure driven or hydrostatic modes which produce parabolic flow profiles (see Figure 2). In the parabolic profile shown in Figure 2a, the velocity in the center of the capillary is greater than that at the edges. Thus, an analyte ion at the center of the capillary migrates faster than one near the wall causing the analyte band to disperse. To minimize this contribution to band broadening, analytes must diffuse rapidly across the capillary diameter to average these different velocities. The radial diffusion is slow and contributes to band broadening. This is generally referred to as a resistance to mass transfer in the mobile phase. Obviously, the electroosmotic plug-like flow profile does not suffer from this type of band dispersion since the velocity profile is nearly constant across the entire diameter of the capillary (see Figure 2b) (34,35). An analyte ion at the center of

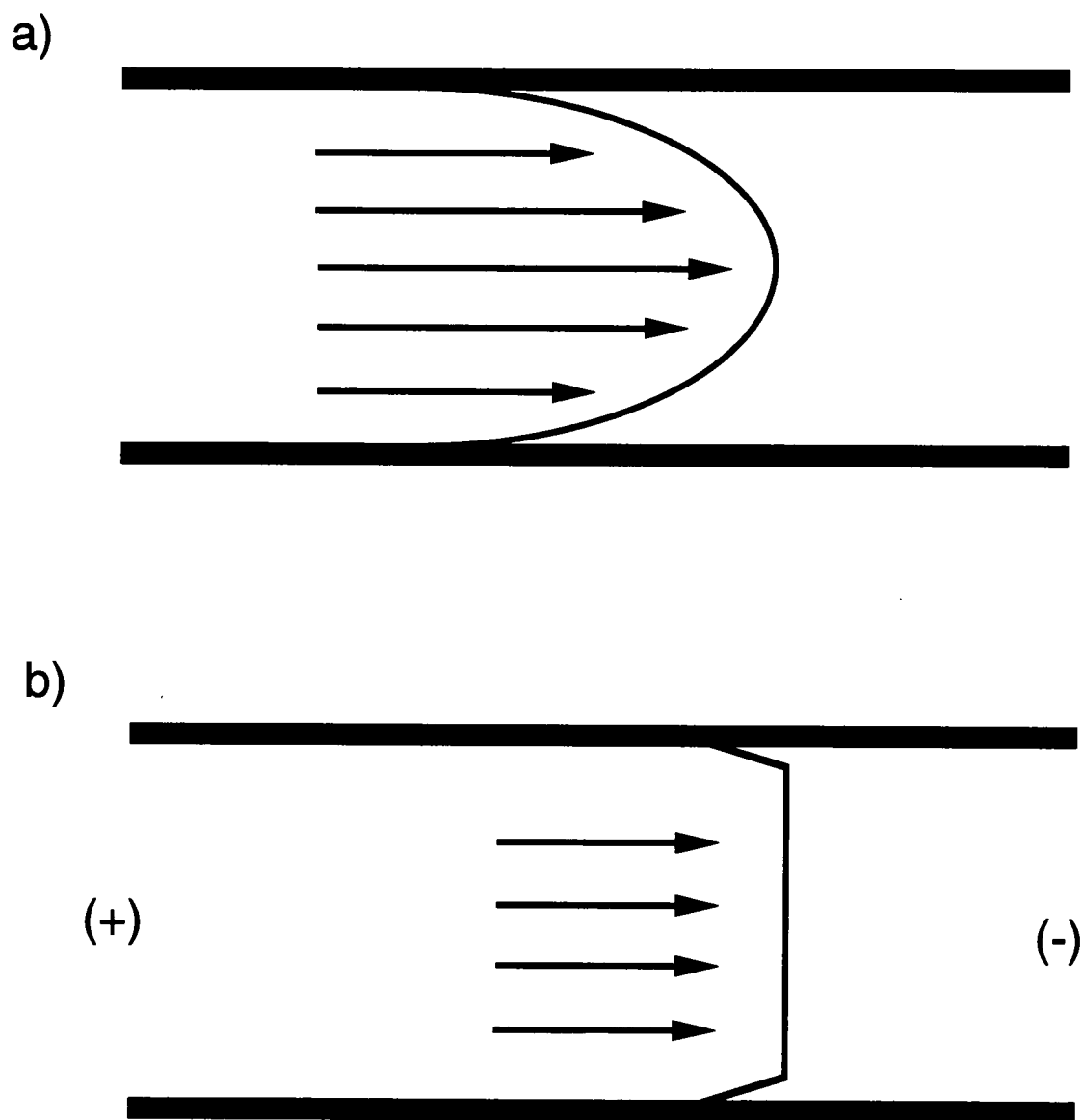


Figure 2. Velocity profiles resulting from a) hydrostatic flow and b) electroosmotic flow.

the capillary migrates at virtually the same velocity as one near the wall resulting in minimal band dispersion. Thus, band dispersion due to resistance to mass transfer in the mobile phase is minimized in most capillary electrophoretic methodologies. By minimizing this band dispersion phenomenon, highly efficient separations (10^5 plates) can be achieved for most solutes.

Electroosmotic flow also allows both cations and anions to be analyzed simultaneously. Each ion has an intrinsic electrophoretic mobility (μ_{ep}) and is attracted to either the cathode or anode. The observed velocity (v_{obs}) at which the ions appear to move is given by

$$v_{obs} = (\mu_{ep} + \mu_{eo})E \quad (2)$$

where μ_{eo} is the electroosmotic mobility and E is the applied field. The observed velocity is simply the vector sum of the electroosmotic and electrophoretic velocities. Under normal conditions, cations and electroosmotic flow move in the same direction (see Figure 3), resulting in a large observed velocity. Neutral species ($\mu_{ep} = 0$) elute together with a velocity equal to the electroosmotic flow. Anions try to migrate against the electroosmotic flow. However, the electroosmotic velocity is usually greater than the anion velocity and sweeps the anions toward the cathode resulting in a lower observed velocity. Thus, both anions and cations can be determined in the same analysis.

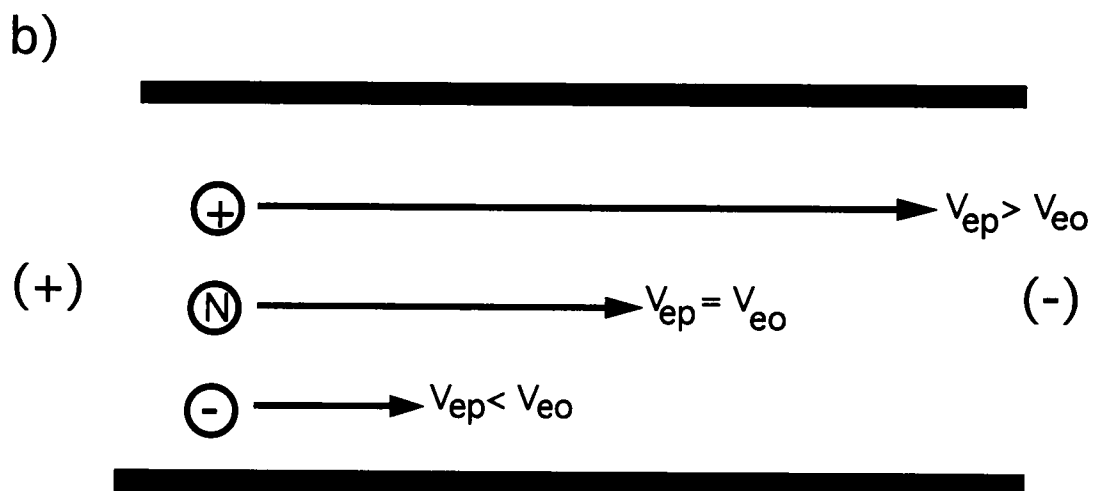
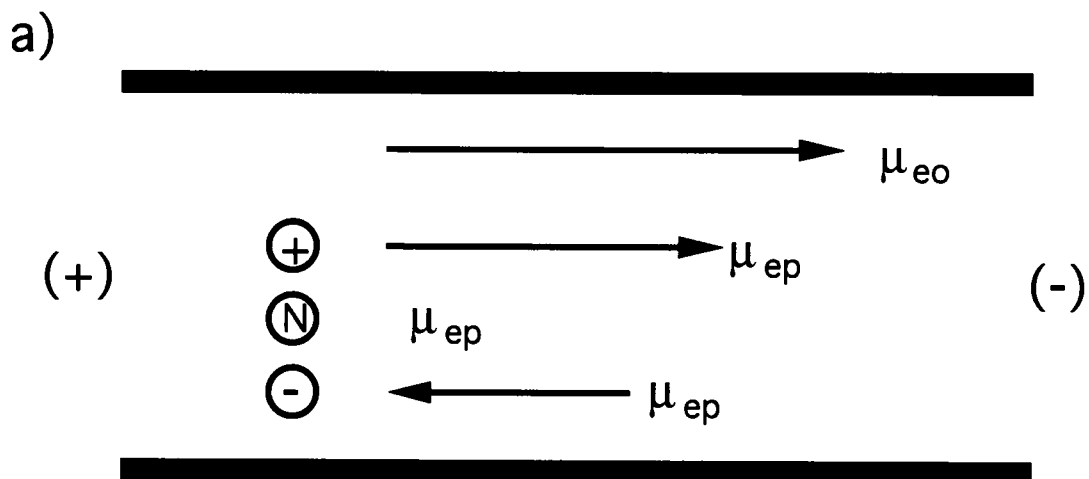


Figure 3. Depiction of a) electroosmotic and electrophoretic mobilities and b) observed velocities for cations, neutrals and anions.

Joule Heating

Joule heating results from the passage of electric current through the buffer (1,6). This heating creates a temperature gradient across the capillary diameter with the temperature at the center being higher than near the walls. This creates a viscosity gradient, which affects the mobility (μ) as shown below

$$\mu = \frac{q}{6 \pi \eta R} \quad (3)$$

where q is the net charge of the analyte, η is the solution viscosity and R is the apparent hydrodynamic radius of the analyte (6). Since mobilities are inversely proportional to the solution viscosity, solutes near the center of the capillary will travel faster than those near the wall. This leads to a parabolic flow profile and band broadening as previously discussed. Excessive Joule heating can lead to degassing or boiling of the solvent. This can cause a catastrophic breakdown of flow if a bubble breaks the electrical conduction path in the capillary. Parameters which affect the generation of Joule heating include capillary diameter, applied field strength and ionic strength of the running buffer.

The thermal gradient produced by Joule heating is proportional to the square of the capillary radius

$$\Delta T = \frac{(R_{pg})(i.d./2)^2}{4k_b} \quad (4)$$

where ΔT is the temperature gradient across the capillary radius, R_{pg} is the rate of power generation, i.d. is the capillary inner diameter and k_b is the thermal conductivity of the buffer (1,4,6). In addition, as the capillary radius is diminished, the surface area-to-volume ratio increases, resulting in better heat dissipation. Thus, reductions in the capillary radius minimize Joule heating effects by reducing the magnitude of generated heat and by dissipating the heat more effectively. This allows the use of high fields resulting in high-speed analyses. Other approaches to reducing Joule heating include decreasing the applied fields and using dilute running buffers; however, the lower voltages result in longer analysis times.

Theoretical Considerations and Advantages

Because of the electroosmotic plug-like profile and the efficient heat dissipation of capillaries, these techniques exhibit several unique advantages. The hallmark of capillary electrophoretic techniques is the high efficiency which can be attained. Contributions to total band broadening due to injection effects, Joule heating, analyte-wall interactions and field inhomogeneities can be minimized by the proper choice of experimental parameters. Thus, in the ideal case, the sole

contributor to band dispersion results from molecular diffusion. A measurement of band dispersion is provided by the number of theoretical plates (N) which is a measure of the efficiency. In experimental terms,

$$N = 5.54 \left(\frac{t_r}{w_{1/2}} \right)^2 \quad (5)$$

where t_r is the retention time and $w_{1/2}$ is the full width of the peak at half height. In theoretical terms,

$$N = \frac{\mu_{obs} E L_d}{2D} \quad (6)$$

where μ_{obs} is the observed mobility, E is the field, L_d is capillary length to the detector and D is the diffusion coefficient. Thus, increasing the field or capillary length will improve efficiency; also, analytes with a high mobility or low diffusion coefficient will exhibit high efficiency.

Other sources of band broadening exist in MECC. The presence of micelles as a pseudo-stationary phase causes resistance to mass transfer and micelle polydispersity to become important (1,5,6). Usually, the surfactants used in MECC are anionic and form negatively charged micelles which attempt to migrate in opposition to the electroosmotic flow. If the electroosmotic flow is of sufficient magnitude, the micelles are swept towards the

cathode and past the detector. Thus, as the analyte partitions into the micelle, it is retarded with respect to the analyte in the running buffer. This is the basis for separation but also can contribute to band broadening. If the analyte transfers rapidly between the running buffer and the micelles as well as between different micelles, little band broadening occurs. The rapid transfer between micelles will average out the various velocities of differently-sized (i.e., polydispersed) micelles. However, if the kinetics of intermicelle transfer are slow, the analyte band broadens, particularly when micelle polydispersity is considered.

The average number of surfactant monomers per micelle is known as the aggregation number. As the monomers exchange between the micelle and the bulk solution, the size of the micelle varies, potentially causing the electrophoretic mobility to change. This polydispersity contributes to band broadening. However, if intermicelle transfer kinetics are fast, this effect is decreased. Therefore high concentrations of surfactant reduce the distance between adjacent micelles and increase the rate of intermicelle transfer. This rapid transfer averages the disparate micelle velocities, thereby decreasing band dispersion; higher efficiency or plate numbers result. However, thermal effects can become noticeable at high concentrations of surfactant due to increased ionic strength (1,5,6).

High efficiency in an analysis can lead to good resolution in a separation. Parameters which effect resolution (R_s) are shown below for CZE

$$R_s = \left(\frac{N^{1/2}}{4} \right) (\Delta\mu) \left(\frac{1}{\mu_{ave} - \mu_{eo}} \right) \quad (7)$$

where N is the efficiency, $\Delta\mu$ is the difference in mobility between two analytes of interest, μ_{ave} is the average mobility and μ_{eo} is the electroosmotic mobility. Another way to improve resolution is to increase the second term of Equation 7, selectivity or $\Delta\mu$, which can be addressed through buffer additives or changes in pH. Buffer pH is often changed to increase selectivity by potentially affecting the charge of the analyte. Careful selection of the pH enables separation of species with different pK_a s by selective ionization of the analytes. System retention, denoted by the third term in Equation 7, is the final influence on resolution. When the electrophoretic mobility is equal in magnitude but opposite in direction to the electroosmotic flow, resolution will be maximized but at the expense of analysis time. A decrease in buffer pH can neutralize the SiO^- groups at the capillary walls resulting in a lower zeta potential and, hence, a lower electroosmotic mobility (2). At low electroosmotic mobilities, analytes with similar electrophoretic mobilities will have more different net mobilities. Unfortunately, the separation time will increase.

In MECC, resolution (R_s) is given by

$$R_s = \left(\frac{N^{1/2}}{4} \right) \left(\frac{\alpha - 1}{\alpha} \right) \left(\frac{k'_2}{1 + k'_2} \right) \left(\frac{1 - [t_o/t_m]}{1 + [t_o/t_m]k'_1} \right) \quad (8)$$

where N is the efficiency, α is the selectivity factor, k'_1 and k'_2 are capacity factors, t_o is the retention time of an unretained compound and t_m is the effective retention time of the micellar phase (1,5,6). The capacity factor for MECC is the ratio of the amount of solute in the micellar phase to the amount of solute in the mobile phase and is given by

$$k' = \left(\frac{t_r - t_o}{t_o [1 - t_r/t_m]} \right) \quad (9)$$

where t_o , t_r , and t_m are the retention times of an unretained solute, the analyte and the micellar phase, respectively. The selectivity factor (α) is given by

$$\alpha = \frac{k'_2}{k'_1} \quad (10)$$

where k'_1 and k'_2 are the capacity factors of two different solutes. Clearly, increasing efficiency improves resolution. If the retention time for the micellar phase approaches infinity (i.e., when the

second phase is stationary), the final term in Equation 8 drops out. This leaves an equation for resolution identical to the one used in conventional chromatography.

Maximum resolution occurs in MECC for capacity factors between 1 and 5 due to the presence of k' in the denominator of the last term in Equation 8 (6,36). The last two terms decrease for large capacity factors. The length of an elution window determines the optimal capacity factor. Because the pseudo-stationary phase actually migrates toward the detector, all the analyte associated with the micelles will eventually elute resulting in a limited elution range or elution window. If the micelles migrate "upstream" as efficiently as the electroosmotic flow migrates "downstream," the elution window is maximized, resulting in a greater optimum capacity factor. However, retention time reproducibility can be difficult to attain under such conditions (6).

Peak capacity, a measure of the overall separating power of a system (3), is also extremely high in CZE. Peak capacity depends on efficiency as shown below

$$n = 0.5 N^{1/2} \quad (11)$$

where n is the peak capacity and N is efficiency. Given the high efficiency attained in CZE, peak capacities of a few hundred can be reached as compared to capacities of about thirty with HPLC

methods. This allows for efficient separation of highly complex samples such as biological fluids or coal tars (3). In MECC, peak capacity is restricted by the limited elution window and is shown below

$$n = 1 + \frac{N^{1/2}}{4} \ln \frac{t_m}{t_0} \quad (12)$$

where N is efficiency and t_0 and t_m are the retention times of an unretained solute and the micellar phase, respectively (6). Assuming $N = 10^5$ and making no attempt to increase the elution window (in which case, $t_0/t_m \approx 0.3$), a theoretical peak capacity of less than 100 is calculated (6). This is a relatively high peak capacity, but it assumes equally spaced peaks. Under normal conditions, however, analytes are likely to bunch at one end of the elution range or the other due to similarities in charge or hydrophobicity (6). Thus, elution window elongation is necessary for complex mixtures of analytes.

Short analysis times and low volume requirements of both sample and buffer are further advantages of capillary electrophoretic techniques. The efficient heat dissipation of capillaries allows extremely high fields to be applied. Separations using 1000-2000 V/cm and requiring only one second or less have been reported (4). As for volume needs, a few microliters is enough to fill the capillary several times. Thus, expensive buffer

additives can be tested and used to improve selectivity without high cost. Furthermore, separation conditions can be optimized easily and quickly. Samples which are difficult to prepare in large volumes can be analyzed many times from a very small volume. Thus, in certain respects, capillary electrophoretic methods fill a role complementary to other separation techniques.

On-column detection

Absorbance and fluorescence detection are the two most common methods of detection in capillary electrophoresis. One of the greatest challenges in capillary electrophoretic techniques is detection. The small volumes of solutes (nL) which are injected make off-line collection and detection very difficult. Thus, on-column detection is almost exclusively used in capillary electrophoresis. The small volume of sample injected leads to very good mass, or absolute, limits of detection (LOD); however, with respect to concentrations, these LODs are about two orders of magnitude poorer than those attained by HPLC (4) with absorbance-based detection.

Absorbance detection is based on Beer's law as shown below

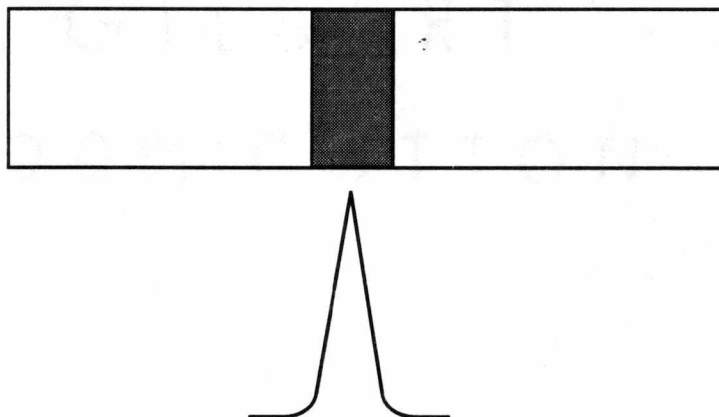
$$A = abC \quad (13)$$

where A is absorbance, a is the absorptivity of the solute, b is the path length and C is the concentration. Since the path length for

on-column detection is generally approximately equal to the capillary internal diameter (about 50 μm), the absorbance is low. Therefore, absorptivity or concentration must be relatively high for an analyte to be detected. The low level of light passed through the capillary due to its small diameter can allow detector shot noise to determine the LOD. A small amount of stray light decreases the impact of detector shot noise. However, excessive stray light leads to high background levels and reduces the linearity of the method (5,6,12,37). Thus, absorbance detection in capillary techniques is limited by short path lengths and poor light throughput such that only modest detectability can be achieved. Nevertheless, this method is commonly used because it is easy to implement and a broad variety of species absorb in the ultraviolet or visible spectrum.

Two different modes of absorbance detection can be used - direct or indirect (see Figure 4). With direct absorbance detection, a running buffer is chosen which does not absorb and thus provides a low background (see Figure 4a). Analytes that contain suitable chromophores absorb the incident radiation as they pass the detection window, thereby resulting in peaks on the electropherogram. In contrast, indirect detection is a more widely applicable method which allows the detection of analytes lacking chromophoric groups (38-42). A highly absorbing ion of the same charge as the analyte is added to the running buffer, thereby producing a constant high background signal (see Figure 4b). Due to

a)



b)

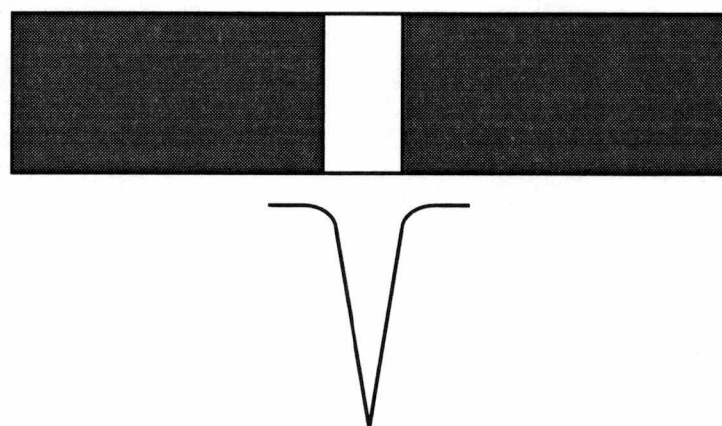


Figure 4. Depiction of a) direct and b) indirect absorbance detection.

charge repulsion, the analyte ion displaces the background ion from the analyte band causing a localized decrease in the signal. Thus, the analyte is detected by a diminished signal rather than by an increased signal. A large background signal leads to detection difficulties.

Detectors operate best when a sufficient power of radiation impinges on the detector, that is, an adequate light throughput is achieved. With indirect detection, the light throughput is deliberately attenuated by the inclusion of a highly absorbing species in the running buffer. Thus, shot noise from the detector has an even greater impact on detectability than in direct absorbance. Furthermore, the stability of the source also becomes very important. A noisy light source will produce a noisy background signal which will limit detection as discussed below.

In indirect detection, the LOD can be estimated by the following equation

$$\text{LOD} = \frac{C_{\text{BGI}}}{(\text{TR})(\text{DR})} \quad (14)$$

where C_{BGI} is the concentration of the background ion, TR is the transfer ratio and DR is the dynamic reserve (38). Each parameter is discussed below. With indirect detection, the objective is to displace a large relative number of background ions. This can be accomplished by using a low concentration of a highly absorbing

species as the background ion. Thus, as the background ion is displaced, a larger decrease of the signal is observed than would be the case with a high concentration of background ion; this enhances the signal-to-noise ratio. To achieve the best LODs, therefore, the only ion available to be displaced in the running buffer would be the background ion. However, higher concentrations of the background ion result in better peak symmetry allowing easier measurements of the peak. On the other hand, a high background ion concentration increases the ionic strength of the running buffer resulting in more Joule heating, as previously discussed, which decreases efficiency (42).

Another parameter to consider is the transfer ratio, which is a measure of the number of background ions displaced per analyte ion. Assuming that both the background ion and analyte ion have the same magnitude of charge (e.g., +1) a ratio of one background ion displaced per one analyte ion is desirable and can be approached although not quite attained. If buffer ions are present in the running buffer, the analyte ion could displace those ions instead of the background ions which would cause a lower signal. The transfer ratio can be calculated from the Kohlrausch functions or from the partial ionizations of the analyte and the background ions (38).

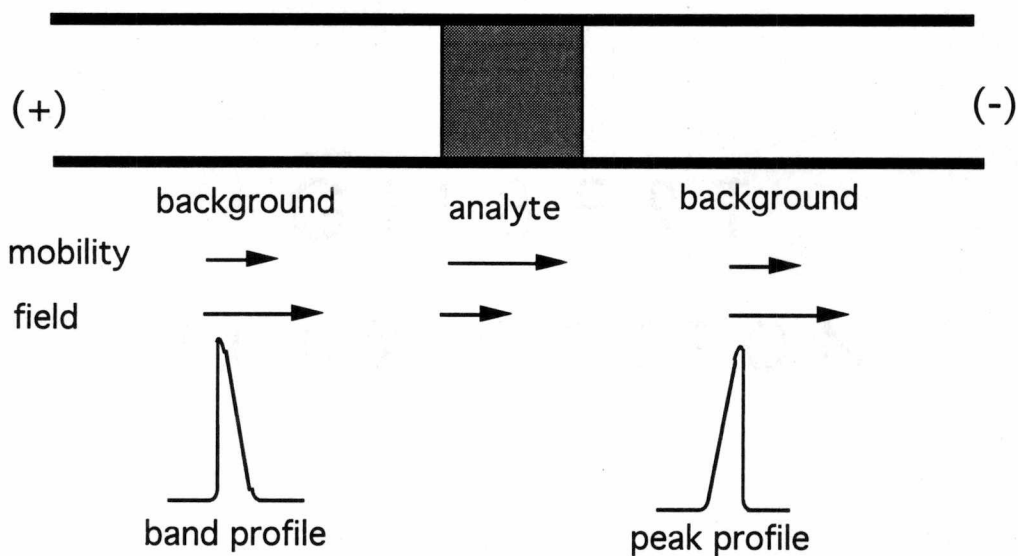
The final parameter in Equation 14 is the dynamic reserve, which is equal to the signal-to-noise ratio of the background signal. A high, steady background signal with minimal baseline

noise produces the best dynamic reserve. If the baseline is noisy, a small displacement peak would be difficult to detect.

Another parameter to be considered when employing indirect detection is the mobility of the background ion. If the mobilities of the solute and the background ion are approximately equal, the observed peak will be sharp and symmetrical. However, if the solute migrates faster than the background ion, a fronting peak will occur (refer to Figure 5a). This is because, when the analyte has a larger mobility than the background ions, the analyte conducts charge more efficiently (i.e., has a higher conductivity) resulting in a lower field within the analyte band. A solute ion diffusing ahead of the majority of the analyte band enters a region of higher field and migrates faster. Analytes which diffuse behind the analyte band also enter a higher field and migrate faster, returning to the bulk of the analyte band. These two effects cause the band profile shown in Figure 5a. As this band migrates past the detector, the fronting peak profile shown in the figure is produced on the electropherogram.

Similarly, if the analyte has a lower mobility than the background ion, the analyte will have a lower conductivity resulting in a higher field within the analyte band. If a solute ion migrates ahead of the majority of the band, it will enter a region of lower field and fall back to the analyte band. If the solute diffuses behind the bulk of the analyte band, it is left behind. These two effects result in the band profile shown in Figure 5b. As

a) $\mu_{\text{analyte}} > \mu_{\text{background ion}}$



b) $\mu_{\text{analyte}} < \mu_{\text{background ion}}$

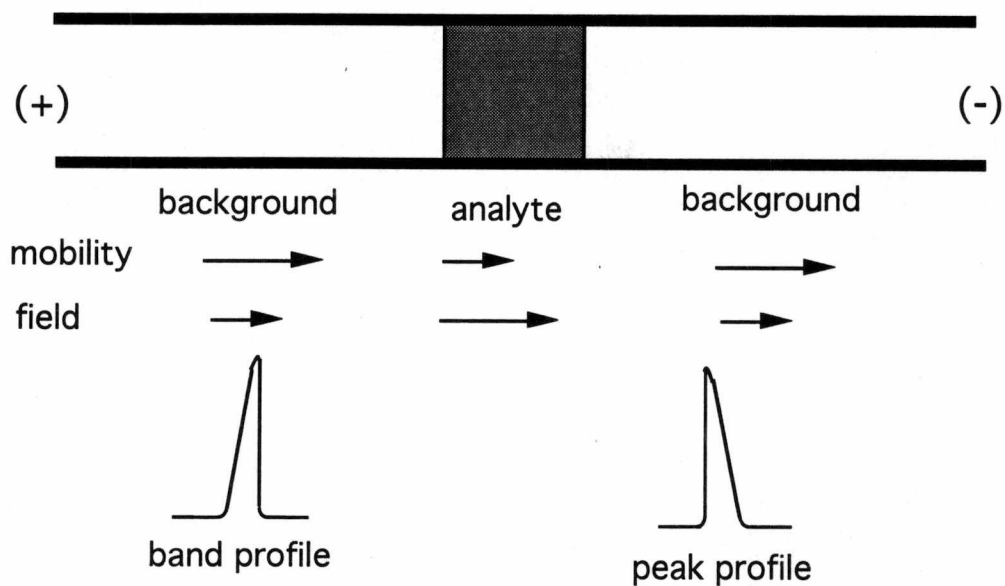


Figure 5. Depiction of a cause of asymmetry in indirect detection. The mismatch in mobility between the analyte and background ion leads to a) fronting when the analyte mobility is greater than the background ion mobility or b) tailing when the analyte mobility is less than the background ion mobility. See text for discussion.

this band flows past the detector, a tailing peak is produced on the electropherogram (6). The asymmetry thus produced reduces efficiency, quantitative reproducibility and increases the LOD due to difficulty in measuring the area of the peak. In principle, indirect absorbance detection is more widely applicable than direct detection but suffers from poorer reproducibility and detectability.

Fluorescence detection is more sensitive than absorbance detection and can achieve LODs on the order of 10^{-13} to 10^{-15} moles under normal conditions (6). With an ideal fluorophore, elaborate instrumentation and complicated signal processing, 10^{-18} mole LODs have been reached (5). Lasers enhance detectability due to the intensity of the light source and low background signal. They are powerful, highly collimated sources and can be focused tightly through the capillary which reduces scatter and stray light. However, the limited number of wavelengths available from lasers restricts the application of fluorescence detection. As seen below, the greater the excitation source power, the higher the resulting emission power

$$P_f = P_o \phi_f k a b C \quad (15)$$

where P_f is the measured fluorescence emission power, P_o is the excitation power, ϕ_f is the quantum efficiency of the solute, k is the fraction of emitted fluorescence that is detected, a is

absorptivity, b is path length and C is concentration. The above equation applies only to solutions having low absorbances. At high absorbance, the fluorescence emission intensity is no longer linearly proportional to the excitation intensity. Fluorescence detection also provides better LODs due to low background levels at the emission wavelength. This low background is due to the limited number of compounds which fluoresce. Running buffer components and impurities are not likely to be among those compounds which fluoresce and therefore do not interfere with the signal. While improving sensitivity, this aspect also limits applicability.

Approaches to alleviate these restrictions include indirect fluorescence detection and complexation. Indirect fluorescence detection, analogous to the indirect absorbance detection discussed above, can extend the applicability of fluorescence detection to species which are not fluorescent or which are not excited at easily attained wavelengths. Unfortunately, laser sources are generally not as stable as conventional sources. Thus, dynamic reserves are not particularly large and indirect fluorescence is usually not more sensitive than indirect absorbance detection.

Pre- or post-column derivatization to covalently attach a strong fluorophore to an analyte can greatly increase the applicability of fluorescence detection (6). However, pre-column derivatization greatly increases the difficulty of separation due to

the formation of multiple products. Not only is the desired product formed, but other products can also be created; that is, some analyte may remain underivatized and some may be multiply derivatized. These different ratios can lead to band dispersion and multiple peaks thereby making analysis and quantitation more difficult. The derivatization process also makes the analytes more similar by adding the same moiety to each. This results in more similar mobilities which increases the difficulty of the separation. Post-column derivatization reduces the resolution already attained during the separation. The normal analyte band volume (1 - 100 nL) leads to severe band broadening due to dead volume in the detector. Thus, on-column absorbance or fluorescence detection is usually utilized (37).

Another technique which avoids the problems of pre- or post-column derivatization is on-column complexation of the analyte. The derivative is added to the running buffer in sufficient concentration that the complexation of the analyte will occur as the separation proceeds.

While fluorescence is a sensitive detection method, it is more complicated than absorbance detection. Researchers are attempting to improve LODs achieved by absorbance methods by using longer path lengths or by pre-concentration or stacking techniques. Increasing the path length can be achieved by making a bubble in the capillary at the detection window (5,43), using

rectangular capillaries (1,44) or utilizing Z-shaped flow cells (1,3,44). Stacking methods will be discussed in Chapters 2 and 3.

Chapter 2

Quantitative Aspects of Lanthanide Metal Determinations Using Capillary Electrophoresis with Indirect Absorbance Detection

Introduction

An advantage of CZE is the ability to alter electrophoretic performance (e.g., improve selectivity) by adding various reagents to the running buffer. Specific interactions of solutes with reagents in the running buffer often enhance separations in CZE. Approaches to date include the addition of surfactants (45), organic solvents (46) and chelating agents (47). Ideally all chemical or physical interactions of injected solutes with system components occur exclusively with the species in solution, so novel reagents can be investigated without complications resulting from the preparation and use of stationary phases. In addition, the low volume of running buffer required by CZE facilitates the evaluation of rare and expensive substances for both applied and fundamental studies and minimizes disposal problems when toxic materials are involved.

A potential application for CZE is the separation and detection of lanthanide metals. Two obvious concerns in this application are the similarity of the lanthanide mobilities and their lack of suitable absorption bands for detection as discussed

below. The intrinsic mobilities range only from 72 to 67 x 10⁻⁵ cm²/V·s across the series from La³⁺ to Lu³⁺ (48). This small difference does not allow sufficient resolution. In this work, an anionic ligand (α -hydroxyisobutyric acid, HIBA), was added to the running buffer to reversibly form weak complexes with the lanthanide metals. This serves to differentially reduce the positive mobilities of the metal ions which improves resolution in two ways (see Equation 7). First, selectivity is improved by increasing the mobility differences of each lanthanide metal. Second, system retention is altered by reducing the observed electrophoretic mobilities, thus allowing slight differences in mobility to be magnified.

The lanthanide metals lack broad absorption bands in the visible spectrum which are necessary if an instrument with a relatively broad spectral bandpass is to be utilized. These absorption bands also lack the high absorptivity necessary for a method to be highly sensitive. The lack of suitable absorption bands for the lanthanide metals requires an alternative to direct absorbance detection which is the most common mode of detection in CZE. Indirect absorption is a more widely applicable detection method that permits the analysis of solutes which do not absorb at the selected wavelength as discussed in Chapter 1 (38). A highly absorbing ion is added to the running buffer and is displaced by the analyte due to charge repulsion. The signal, therefore, is caused by

the displaceable background ion and the analyte is seen as a negative peak due to the absence of the ion in the analyte band.

The mobility of the background ion is critical to the efficient separation of the analytes (39-42,48,49). If the mobility of the analyte ion is greater than that of the background ion, the peak will "front" (see Figure 5a). Conversely, if the mobility of the analyte ion is lower than that of the background ion, peak "tailing" will be observed (see Figure 5b). The fronting peak occurs for analyte ions with higher mobilities than the background ions. As the solute migrates ahead of the majority of the analyte band, it enters a region of higher field and migrates faster. Analyte ions which diffuse behind the band enter a region of higher field and migrate faster, returning to the analyte band. Tailing peaks occur for analytes with a lower mobility than the background ion. As solute ions migrate ahead of the band, they enter a region of lower field and fall back into the analyte band. Solute ions which diffuse behind the analyte band enter a region of lower field and are left behind (6). The resulting peak asymmetry will adversely affect the efficiency and resolution of the separation and the LOD and the linear dynamic range of the analyte measurement.

The ionic strength of the sample matrix is also important because it affects peak shape. A high ionic strength in the analyte band causes a region of high conductivity which results in a low field. The field inhomogeneities distort the band shape, and this degrades the analytical parameters by making the beginning and

end of the peak difficult to locate. Injection technique, whether based on electrokinetic or hydrostatic methods (50,51), will affect the asymmetry, efficiency, LOD, reproducibility and resolution. The goal of these experiments is to study the effects of these factors on quantitative figures of analytical merit.

Experimental

Apparatus

The CZE separation apparatus was similar to that described elsewhere (47) and consisted of the following. Electrical fields are provided with a Spellman high voltage power supply (Plainview, NY) electrically connected to inlet and outlet reservoirs (microcentrifuge vials) via platinum wire electrodes. Fused silica capillaries (75 μm i.d. x 365 μm outer diameter [o.d.]) surface modified with a C-8 phase were supplied by Supelco (Bellefonte, PA).

Ultraviolet absorbance detection at 214 nm was accomplished using a SSI (Scientific Systems Inc, State College, PA) Model 504 absorbance detector with a CZE flow-cell adapter. The electropherograms were recorded on a Varian (Palo Alto, CA) Model 4400 integrator.

Chemicals

The samples consisted of various lanthanide nitrates or oxides. Europium, terbium and dysprosium were purchased as

nitrate pentahydrates from Aldrich (Milwaukee, WI). The lanthanum and praseodymium oxides from Sigma Chemical Company (St. Louis, MO) were dissolved in concentrated nitric acid and evaporated to obtain the nitrates. The running buffer was HPLC grade water from Baxter Scientific containing 10 mM of an imidazole derivative (imidazole, 2-methylimidazole, 4-methylimidazole, 2-ethylimidazole, or 2-benzyl-2-imidazoline) as the background ion and 3 mM HIBA purchased from Aldrich. The running buffer was adjusted to pH 4.7 using acetic acid. The samples were dissolved in either water or running buffer.

Procedures

The capillaries used in this work were cut to 50 cm lengths and an optical window was produced by removing the polyimide coating 10 cm from one end of the capillary with hot 50% sulfuric acid. A syringe needle was affixed via epoxy to the opposite (inlet) end to facilitate rinsing.

The capillaries were initially treated with 0.01 M NaOH for a period of 10 minutes to gently remove impurities from the surfaces and rinsed with HPLC grade water. The capillaries were then filled with the running buffer and suspended between the two running buffer reservoirs which were positioned at equal height to eliminate hydrostatic flow. The operating voltage (15 kV) was applied across the capillary and the system allowed to equilibrate for thirty minutes.

Sample injections were made either hydrostatically or electrokinetically. Hydrostatic injections were accomplished by placing the inlet end of the capillary in the sample solution and raising it 10 cm above the outlet end for 26 seconds. The capillary was then removed from the sample, rinsed with HPLC grade water and placed in the inlet buffer reservoir followed by application of the separation voltage. Electrokinetic injections involved replacing the inlet reservoir with the sample container and applying a potential of 2 kV across the capillary for a period of 26 seconds. The capillary inlet was then rinsed and returned to the buffer reservoir prior to applying the separation voltage.

Calculations

The electropherograms were recorded at a high chart speed using an integrator and measured by hand for area, efficiency, resolution and asymmetry. This was done manually due to the tailing nature of the peaks which "confused" the integrator. The equations used for each calculation are given below.

Areas (A') were calculated for each peak by

$$A' = 0.5b'h \quad (16)$$

where the peaks were triangulated and the base (b') and height (h) were measured for the calculation of area. Efficiencies (N) were determined as shown below:

$$N = 5.54 \left(\frac{t_r}{w_{1/2}} \right)^2 \quad (17)$$

where the efficiency (N) is related to the retention time (t_r) of the analyte of interest and the width of the peak at half height ($w_{1/2}$). The degree of separation between peaks was calculated by

$$R_s = \frac{t_{r2} - t_{r1}}{w_{b2}} \quad (18)$$

where the resolution (R_s) is related to the difference in retention times (t_r) of the two analytes of interest and the baseline width (w_b) of the second. The extent of fronting or tailing was determined by calculating the asymmetry as shown below:

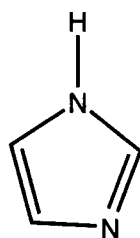
$$A_s = \frac{w_r}{w_l} \quad (19)$$

where the asymmetry (A_s) is calculated by dropping a vertical from the peak center and dividing the width of the right (w_r) part by the width of the left (w_l) part at 10% of the peak height.

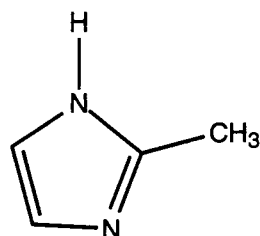
Results and Discussion

Background Ion Mobility

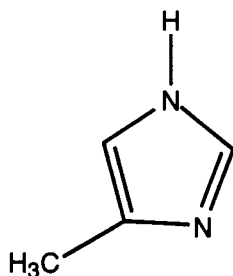
Imidazole was initially chosen to be the background ion because reports stated it had a mobility that was similar to the mobilities of the lanthanide metals (42,48). Moreover, imidazole absorbs strongly at 214 nm at pH 4.7 and does not interact with either the capillary walls or the analytes. However, it was observed in this work that its mobility is greater than the lanthanide analytes and, consequently, produced badly tailing peaks. Other imidazole derivatives were investigated with the goal of finding one with a lower mobility to improve efficiency and asymmetry (see Figure 6 for structures). Table 1 shows the efficiencies and asymmetries produced by each of the five lanthanide metals for each of the five imidazoles investigated for use as a background ion. Of the five imidazoles investigated, only 2-ethylimidazole and 2-benzyl-2-imidazoline seemed reasonable. However, the baseline for 2-benzyl-2-imidazoline was observed to be very noisy. Moreover, it produced extraneous peaks in the vicinity of the peak for lanthanum. This extra noise in the baseline decreases the dynamic reserve (see Equation 14) which raises the concentration limit of detection. With a noisy baseline, it is difficult to detect small peaks. The extraneous peaks in the vicinity of the lanthanum peak also exacerbate problems with peak identification. The baseline for 2-ethylimidazole is more stable and the asymmetry increases more gradually for the later eluting



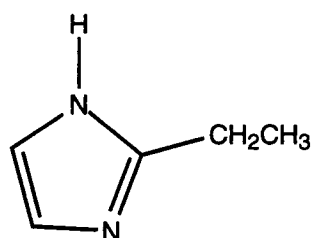
Imidazole



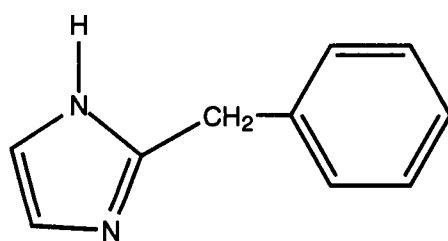
2-Methylimidazole



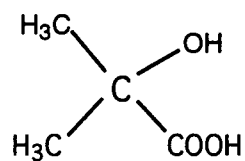
4-Methylimidazole



2-Ethylimidazole



2-Benzyl-2-imidazoline



α -Hydroxyisobutyric acid
HIBA

Figure 6. Structures of chemicals in lanthanide metal research

Table 1. Comparison of Efficiency and Asymmetry
for Tested Background Ions^a

Background ion	La	Pr	Eu	Tb	Dy
Efficiency^b					
Imidazole	17,000	14,000	13,000	11,000	11,000
2-Methylimidazole	34,000	23,000	18,000	17,000	14,000
4-Methylimidazole	27,000	17,000	14,000	12,000	14,000
2-Ethylimidazole	37,000	28,000	24,000	19,000	27,000
2-Benzyl-2-imidazoline	46,000	99,000	31,000	19,000	27,000
Asymmetry^c					
Imidazole	5.0	5.8	6.3	5.7	4.9
2-Methylimidazole	4.0	4.7	5.8	5.4	5.0
4-Methylimidazole	4.6	5.9	6.3	6.1	6.8
2-Ethylimidazole	3.4	4.8	5.4	5.0	4.9
2-Benzyl-2-imidazoline	1.9	4.6	6.3	5.9	4.4

a) Injected concentration of rare earth metals 50 ppm

b) N by Equation 17

c) A_s by Equation 19

analytes. The efficiency decreases drastically for the later eluting analytes with the other imidazole derivatives; that is, the mobility match is better for 2-ethylimidazole. Thus, 2-ethylimidazole was chosen as the background ion for these experiments.

Chelating Agent Concentration

Experiments were conducted to determine the appropriate HIBA concentration to improve resolution without increasing Joule heating. This acid differentially complexes the lanthanide metals (52), improving selectivity and resolution. Based on experiments using 1 to 9 mM HIBA concentrations in the running buffer, it was determined that 3 mM HIBA gave sufficient resolution, whereas lower concentrations did not provide adequate resolution. A higher concentration produced excessive Joule heating due to a higher running buffer ionic strength. The effect of HIBA concentrations on these lanthanide separations is demonstrated with the electropherograms shown in Figure 7. Notice that the resolution improves markedly as the concentration of added HIBA is increased from 1 mM to 3 mM. The complexation of the tripositive lanthanide cations reduces their charge slightly such that the transfer ratio, theoretically three background ions displaced per analyte ion, would correspondingly decrease. This may account for the small reduction in peak height in Figure 7b. System retention is also affected by the reduction in charge of the metals. The mobilities of the analytes are reduced, becoming more like the background ion

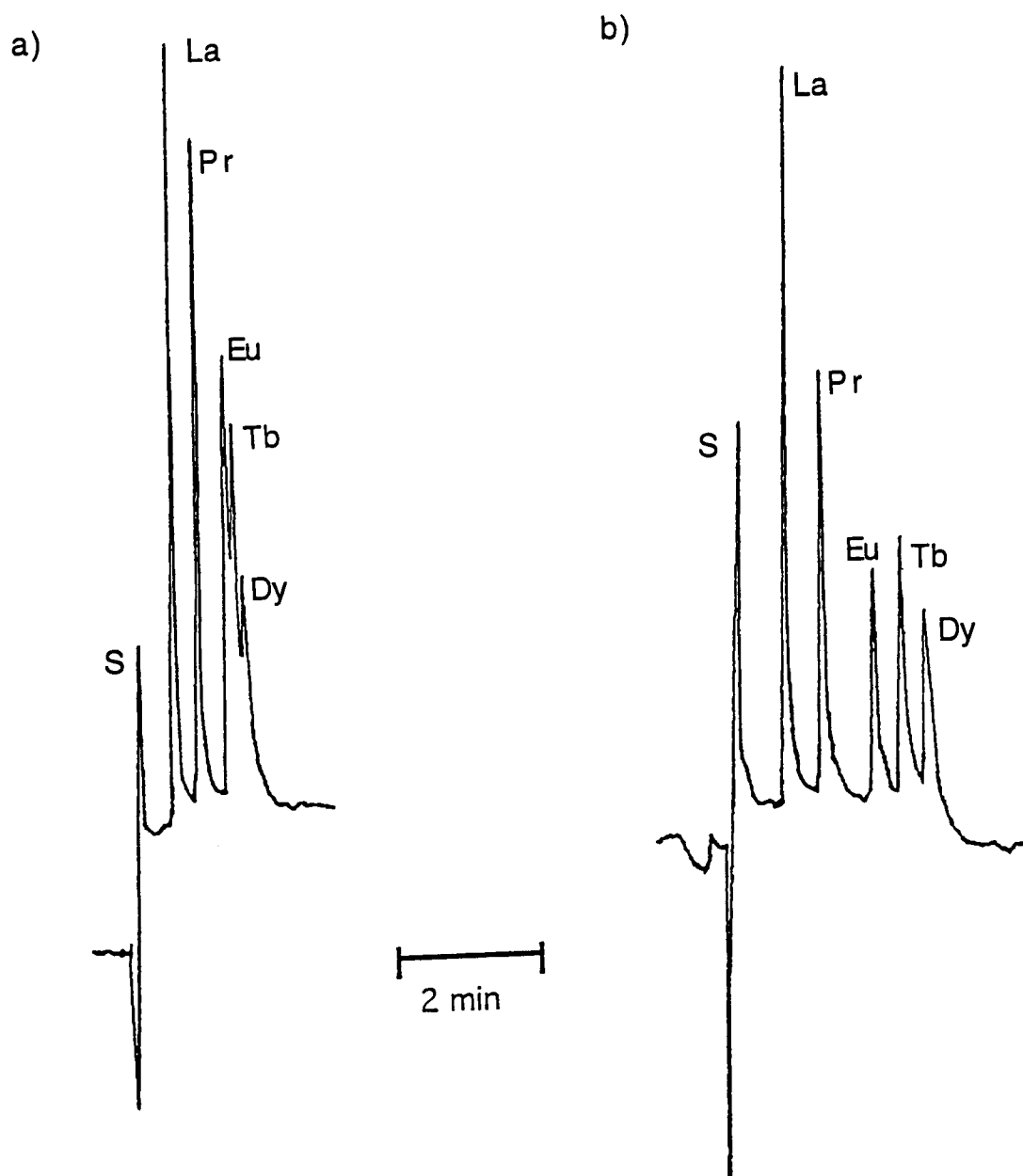


Figure 7. Electropherograms showing effects of HIBA. a) 1 mM HIBA b) 3 mM HIBA. The running buffer contained 10 mM 2-ethylimidazole and the sample contained 50 ppm each of La, Pr, Eu, Tb, and Dy nitrates (in order of elution following $\pm$ system disturbance [S]) dissolved in water.

mobility, resulting in increased resolution and longer analysis times.

Sample Matrix

The sample consisted of lanthanum, praseodymium, europium, terbium, and dysprosium nitrates dissolved in water and diluted to the desired concentration in either water or running buffer. The plug length injected (see "Experimental Procedures") into the column was about 5 mm (28 nL). This relatively large injection volume was necessary to detect moderately low concentrations. Large injection volumes or analyte concentrations can cause a decrease in efficiency (2). However, the poor match in mobilities between the background ion and the analyte ion is the predominant cause of tailing and poor efficiency in this work.

With a sample matrix of water, stacking or concentrating of the analyte at the inlet is generally observed (see Figure 8). When the sample matrix is the running buffer or a solution of comparable ionic strength, the stacking mechanism is not operative (see Figure 8a). Stacking occurs when a sample of low ionic strength is injected into a running buffer of higher ionic strength (44,50,51). When the voltage is applied, the field within the analyte band is higher than that in the running buffer due to fewer ions in the sample zone and a higher resistivity. The ions migrate toward the front of the band where they encounter the running buffer and a lower field. The result is a stacking (or concentrating) of the

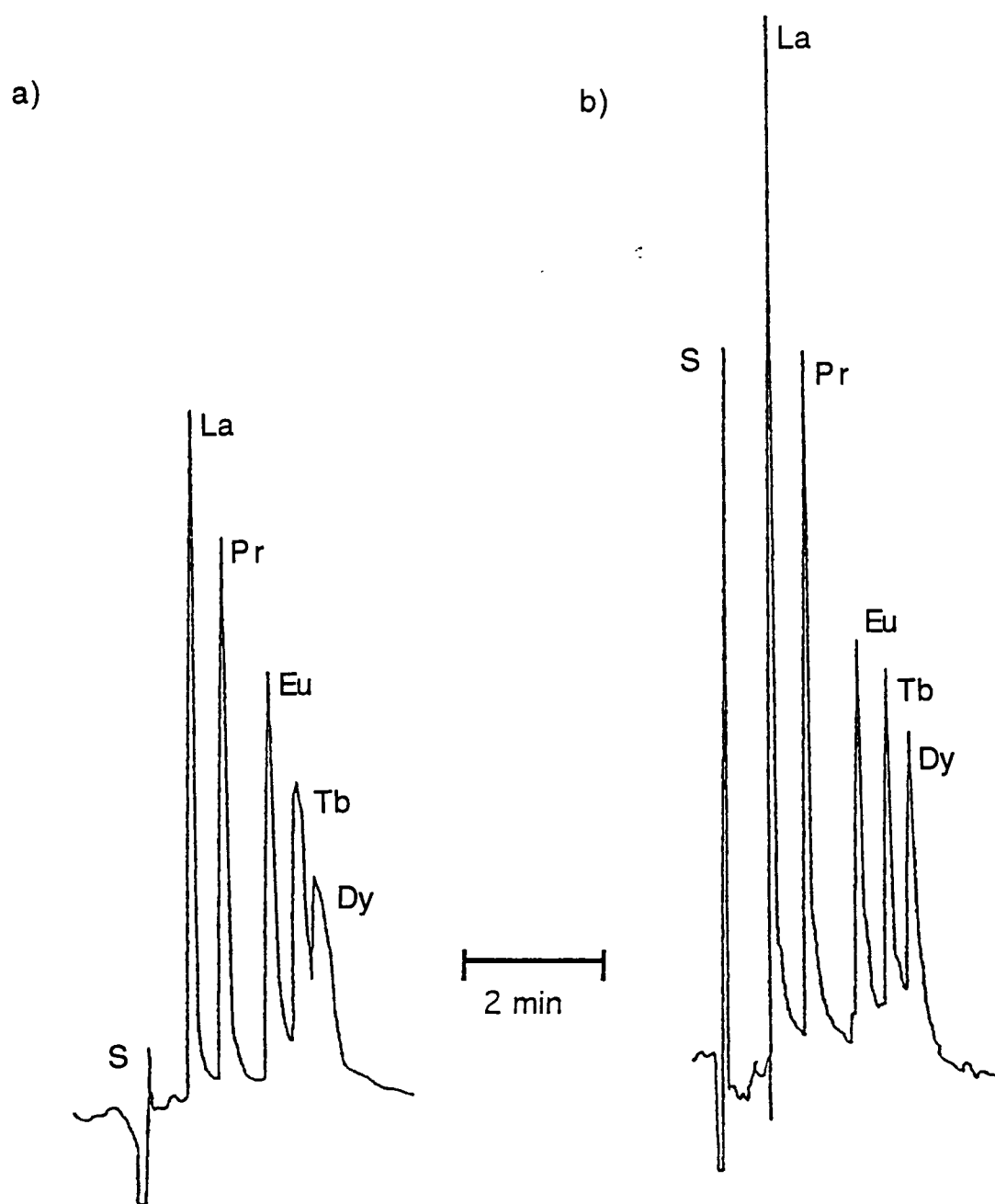


Figure 8. Electropherograms showing effect of stacking. a) injection from running buffer matrix (10 mM 2-ethylimidazole and 3 mM HIBA) b) injection from water matrix. "S" is a system peak.

sample ions at the front of the band resulting in better sensitivity and resolution (see Figure 8b). The stacking process can dramatically improve detectability and can be necessary to see moderately low concentrations (see the related discussion below).

Lanthanide LODs in the sub-ppm range have been reported with the assistance of sample stacking (48,49). Unfortunately, the practical utility of this process is questionable since most lanthanide metal analyses involve matrices that have moderate to high ionic strengths. Thus, there remains a need either to [1] develop methodologies to selectively reduce the concentration, perhaps via extraction, of extraneous ionic components in realistic samples containing lanthanide metals prior to injection or [2] improve current detection methods for CZE.

Injection Method and Matrix

The method of injection, in combination with the sample matrix, has a profound effect on the LOD and reproducibility of the separation. In this work, electrokinetic injections from a sample matrix of water provides better detectability than other methods or matrices. The lowest detectable concentration injected is approximately 0.5 ppm. Unfortunately, both the peak area and peak height reproducibility observed with this method are extremely poor. The analytes stack during the injection (sometimes dubbed "field amplified injection" (44)) as well as during the early stages of the separation. While this provides for a considerable

concentrating effect, it also means that slight variations in time, applied voltage, position of the capillary within the sample, etc., have large effects on the injection process (44). When a calibration curve of peak areas versus concentration for praseodymium is plotted for this method, a regression coefficient of 0.080 is obtained (see Figure 9a). This can be compared with a regression coefficient of 0.999 for praseodymium for hydrostatic injections from water (see Figure 9b). Thus, electrokinetic injection from water is a very sensitive technique but definitely lacks the precision required for quantitative work. Automated injection and rinse procedures may improve the regression coefficient for electrokinetic injections out of water. Better control of injection times, injection voltages and sample placement could possibly improve precision.

Table 2 summarizes results obtained in this work concerning the effects of injection method and sample matrix on efficiency, asymmetry, limits of detection, and peak area reproducibility. Many different influences act on these parameters in a complex interplay such that trends due to one influence or another are difficult to discern. One observation regarding Table 2 is that differences in mobilities between the background ion and analytes leads to a large asymmetry factor which degrades other parameters. Measuring the tailing peaks is challenging because the location of the beginning and end of the peaks is difficult to determine; therefore, higher coefficients of variation (CV) are

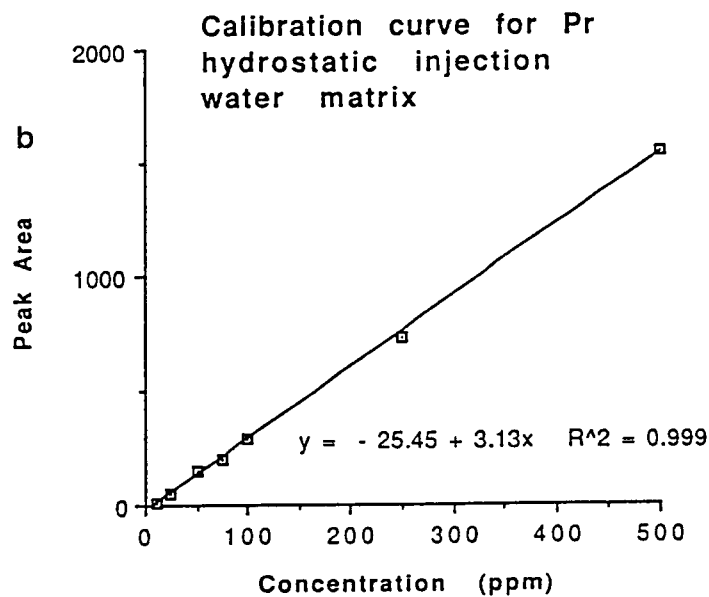
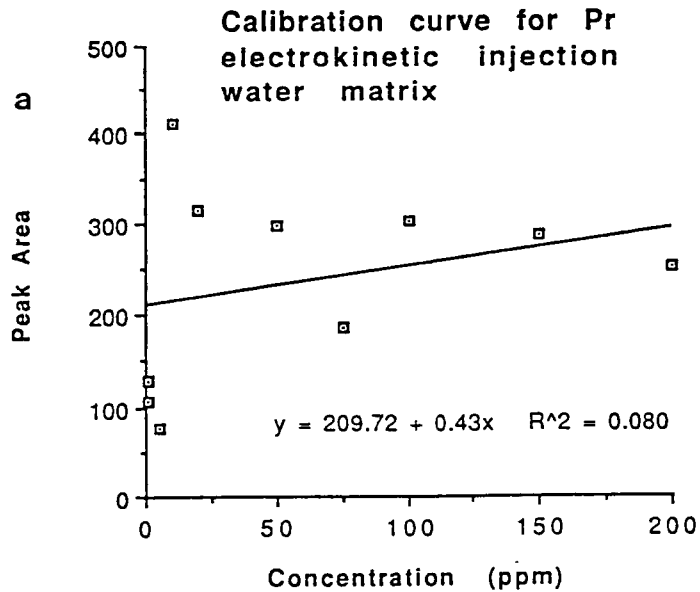


Figure 9. Calibration curves for praseodymium. a) electrokinetic injection from water (lowest injected concentration 0.5 ppm) and b) hydrostatic injection from water matrix (lowest injected concentration 10 ppm). Conditions as in Figure 7b.

Table 2 Figures of Merit
Efficiency, Asymmetry, Limits of Detection with CVs

Lanthanide	Efficiency ^a	Asymmetry ^b	LOD (ppm) ^c	CV of areas (%) ^d
Hydrostatic Injection from a Water Matrix				
La	79,000	4.3	1.7	17
Pr	28,000	8.7	2.7	13
Eu	11,000	11	3.9	12
Tb	19,000	13	4.1	14
Dy	20,000	10	5.4	42
Hydrostatic Injection from a Running Buffer Matrix				
La	13,000	2.7	2.9	5.8
Pr	11,000	7.3	2.9	7.0
Eu	10,000	10	7.7	5.7
Tb	6,900	8.5	15	6.0
Dy	4,000	6.0	24	13
Electrokinetic Injection from a Running Buffer Matrix				
La	78,000	9.0	7.9	13
Pr	15,000	8.3	13	19
Eu	13,000	7.1	27	32
Tb	14,000	7.1	43	26
Dy	19,000	6.0	50	42

a) N by Equation 17 at 50 ppm

b) A_s by Equation 19 at 50 ppm

c) LOD at a signal-to-noise ratio of 3 using peak height

d) CV determined at 50 ppm with 6 - 10 trials

obtained. Moreover, this uncertainty in measuring peaks creates a comparable uncertainty in the determination of other parameters shown in Table 2. The asymmetry also decreases efficiency and increases LODs.

Certain points regarding the efficiency data in the table can be made. Long injection plugs can limit the efficiency; the peak variance due to the injection should be less than ten percent of the total peak variance. The long plugs used in these experiments probably limit efficiency when injections were made from a running buffer matrix (see Table 2 - "Hydrostatic Injections from a Running Buffer Matrix" and "Electrokinetic Injections from a Running Buffer Matrix"). The stacking (see discussion above) that occurs due to the water matrix offsets this problem (i.e., efficiency is generally better for the "Hydrostatic Injections from a Water Matrix" compared to the other two sections in Table 2). The stacking effect has its limits, however, as a hydrostatic (or parabolic) flow caused by the mismatch between the sample band and the bulk electroosmotic velocity can ultimately lead to band dispersion. Thus, an optimum injection plug length exists (50,51).

The hydrostatic injections from water are fairly well behaved and produce reasonable efficiencies and the best LODs. The slightly higher peak asymmetry obtained for this mode of injection compared to other modes of injection is somewhat puzzling. During the stacking process, the ionic strength of the sample zone within the capillary increases. While highly

concentrated samples can produce field distortions and peak asymmetry (2), the stacking process should terminate before this occurs. However, because of stacking, the final concentration of the sample for the injection from water is clearly greater than that for injections from the running buffer. It is also possible that this stacking exacerbates the asymmetry problem caused by differences in mobility between the background ion and analyte ion.

Figure 10 shows the calibration curves for lanthanum and dysprosium, the first and last metals to elute. The linear dynamic range for each method studied is up to two decades of concentration above the LOD except in the case of lanthanum in the hydrostatic injections from water. That plot shows a roll-off, which is probably due to "overloading" the concentration of the background ion, 10 mM 2-ethylimidazole, in the running buffer. In every other case, the upper boundary of the linear dynamic range is determined by a lack of resolution at high injected concentrations and not by limitations in the linearity of the response.

Conclusions

In summary, sample stacking is necessary to achieve moderate detection limits for the lanthanide metals. Realistic samples, unless relatively concentrated, are not likely to be determined quantitatively using this technique due to the low ionic strength requirement of stacking. A sample of high ionic strength could be made suitable for this analysis by decreasing the ionic

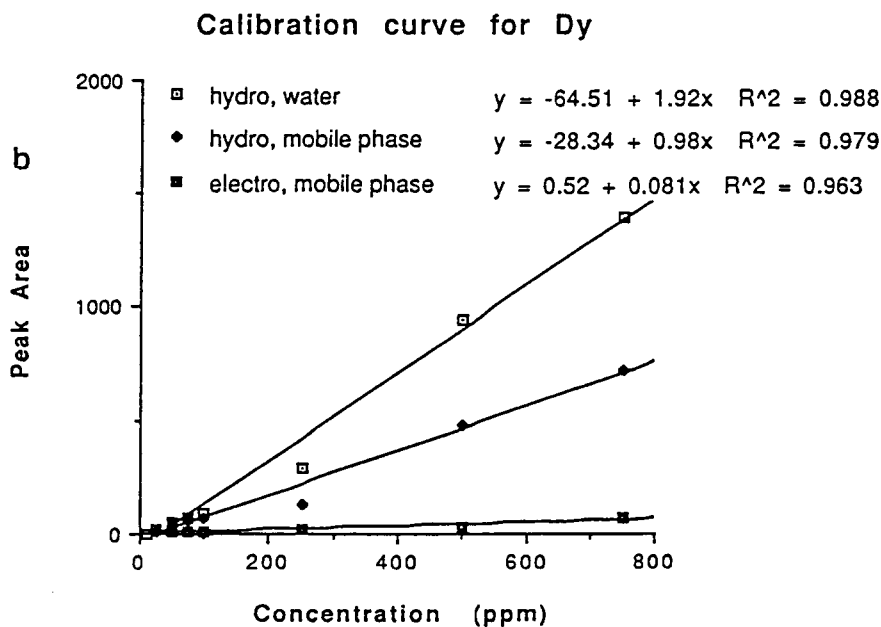
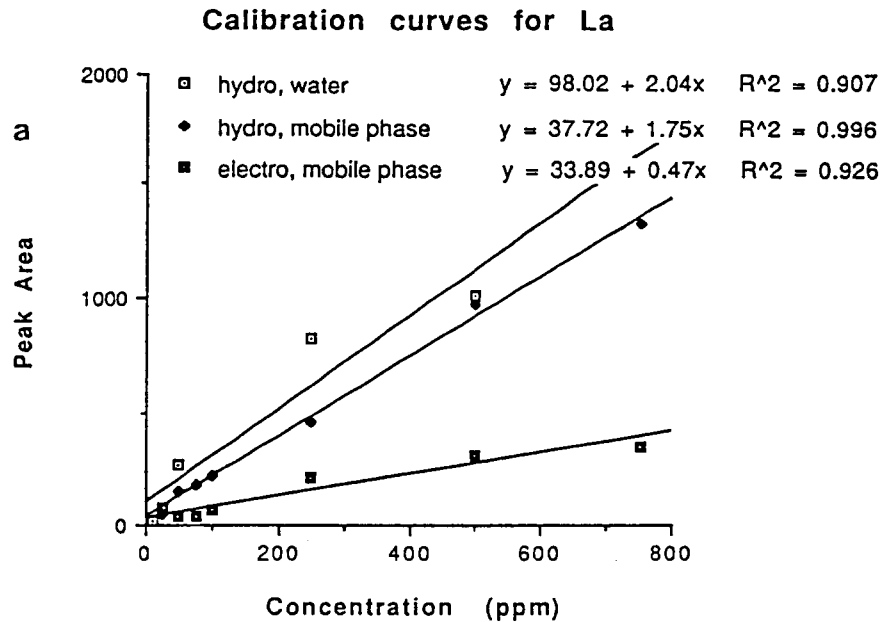


Figure 10. Calibration curves for a) lanthanum and b) dysprosium. Hydrostatic injections from water, hydrostatic injections from running buffer, and electrokinetic injections from running buffer shown. Conditions as in Figure 7b.

strength of the sample. This could be achieved by selective extraction or dialysis preceding analysis by CZE with stacking.

Future areas of study could include the use of a better background ion and the use of automated instrumentation. Using a background ion with a lower mobility would decrease the asymmetry of the analyte peaks, thus making peak measurement easier and more reliable. This, in turn, will improve efficiencies, reproducibilities and limits of detection. Automated instrumentation should improve reproducibility by removing human error and by yielding precise control of parameters such as injection time and voltage. This methodology is not optimized at this time for quantitation of lanthanide metals due to the modest efficiency and, more significantly, poor detectability, both of which should be improved by the approaches just discussed.

Chapter 3

A Screening Protocol for the Direct Determination of Low ppb Levels of the Uranyl Cation Using Arsenazo III and Capillary Electrophoresis

Introduction

There is a need within the geological, environmental and bioassay fields for a fast and simple analysis tool for uranium due to its presence in water and various mineral samples (53). The toxicity of uranium and its use in nuclear power stations and nuclear weapons creates the need for a cost effective analysis (54). Numerous methodologies have been developed for the analysis of uranium including time-resolved laser fluorescence (53,55-57), isotope dilution mass spectrometry (58), inductively coupled plasma atomic emission spectrometry (59) and flow injection analysis (60). Most of these approaches are expensive, time consuming and instrumentally complicated. Simpler methods which depend on spectrophotometry have been effective. These methods require either the use of masking agents or a separation such as liquid chromatography, ion chromatography or extraction. Most of these simpler methods, however, require large volumes of samples, mobile phases and solvent and long analysis times (57,61-67).

Capillary electrophoretic techniques, with their high efficiency, speed and small sample and reagent volume requirements, can be more cost effective and time efficient than the aforementioned methodologies. The small capillary diameters are very effective in dissipating Joule heating; thus, large fields can be used to decrease the separation time. The plug-like flow leads to highly efficient separations. Over the past few years, capillary electrophoresis has become a very useful separation tool. The high efficiency and speed make it an ideal choice for many applications. However, the diminutive size of the capillary creates challenges in detection (44,50,51,61,68).

Sample stacking is one of the simplest ways to concentrate a sample and improve detectability (see discussion in Chapter 2). In this approach, the analyte is dissolved in either water or low ionic strength buffer. The low ionic strength in the sample region results in a high field. Thus, the sample ions in the injection plug will migrate at a higher velocity. As the sample ions encounter the interface between the sample and relatively high ionic strength running buffer, velocity is reduced due to the presence of a lower field within the running buffer. Thus, the analyte stacks into a narrow band. Subsequently, the analytes are separated by free solution capillary electrophoresis (44,50,51,68). Both anions and cations can be analyzed by this technique.

Unfortunately, when stacking is performed, field inhomogeneities between the sample plug and the running buffer

cause the electroosmotic flow velocity to be different between the two regions. This causes a hydrostatic pressure resulting in a parabolic flow profile which can broaden the solute band and negate the concentrating effect. Longer sample plug lengths or greater disparity between the sample and running buffer ionic strengths will augment the dispersive effects of parabolic flow; however, more sample can be injected with a longer sample plug which will improve detectability. Thus, an optimum sample plug length exists to maximize the advantageous effects of sample stacking and to minimize the deleterious effects of band broadening (50,51,68). A good general review article concerning stacking and other methods of sensitivity enhancement has been written by Albin et al. (44). A mathematical treatise on stacking is provided by Burgi and Chien (68).

A variety of reagents can be used to form a complex with U(IV) or U(VI) for use in a spectrophotometric analysis; these include 4-(2-pyridylazo)resorcinol (PAR), 1-(2-pyridylazo)-2-naphthol (PAN), malachite green and 2,2'-(1,8-dihydroxy-3,6-disulfo-naphthylene-2,7-bisazo) bisbenzenearsonic acid (Arsenazo III) (54,66,69-71). Arsenazo III is the most widely used reagent for the determination of uranium (VI) although it also complexes with uranium (IV). There is a large red shift (about 140 nm) in the lowest energy absorbance band of free Arsenazo III when it forms a metal complex. Thus, excess Arsenazo III present in the system will have a very low background at the wavelength of maximum

absorbance for the complex (54,57,64,65,69-71). Typical spectra of free Arsenazo III and the uranyl - Arsenazo III complex appear in Figure 11. The complex is believed to be in a one-to-one ratio between the metal and the ligand and to have a +2 charge. Unfortunately, Arsenazo III is not selective and forms complexes with many other metals such as thorium, zirconium, lanthanide metals, barium, strontium, sulfur, palladium, scandium, lead, iron(III), copper, calcium and aluminum (64,65,69-71). Solution pH plays an important role in complex formation; the optimum for the uranyl ion - Arsenazo III analysis is between pH 1 and 4. At these moderately low pH values, some of the aforementioned metals do not form complexes. However, the general lack of selectivity still renders a separation necessary.

The method presented in this chapter uses field amplified CZE (51,72) for a fast, simple and sensitive determination of the uranyl ion. A wide variety of experimental parameters are assessed and discussed. These include capillary column i.d., effective column length or length to detector, running buffer composition and concentration and sample plug lengths. Analytical figures of merit and the results of analyses of local water samples are also presented.

Spectra of Free and Complexed Arsenazo III

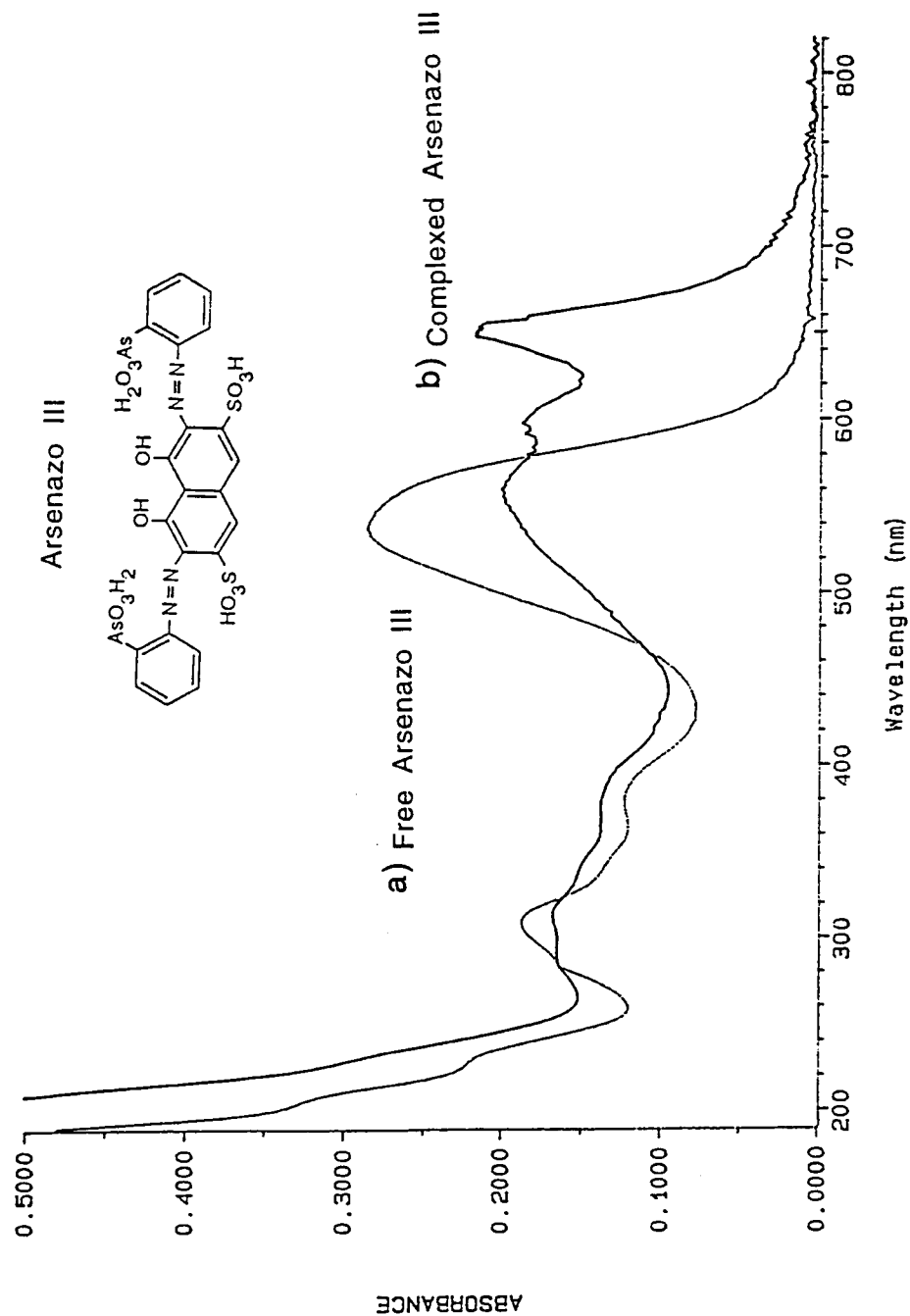


Figure 11. Absorbance spectra of a) free Arsenazo III and b) the uranyl-Arsenazo III complex.

Experimental

Apparatus

The apparatus used in these studies is the same as described in Chapter 2 "Experimental Apparatus", with the following exceptions. The absorbance detector was used at 650 nm. Fused silica capillaries (50 and 75 μm i.d., 360 μm o.d.) were obtained from Polymicro Technologies, Inc. (Phoenix, AZ) and extended light path capillaries (ELP) (25 μm i.d. with 125 μm window, 360 μm o.d., which will be referred to herein as 25 μm i.d. ELP) were purchased from Hewlett Packard (Palo Alto, CA). Running voltages were provided by a Hipotronics (Brewster, NY) high voltage power supply. Electropherograms were recorded using a chart recorder and a PE Nelson integrator Model 1020 S (Perkin-Elmer Corp., Cupertino, CA).

Chemicals

Concentrated stock solutions of uranyl nitrate hexahydrate (Mallinckrodt, Paris, KY) and Arsenazo III (Aldrich, Milwaukee, WI) were made in water and diluted to the appropriate concentration as desired. Sodium phosphate, sodium borate, sodium chloride, perchloric acid and methanol were obtained from Sigma (St. Louis, MO) and utilized as needed. Distilled water was deionized using a Millipore MilliQ filter (Bedford, MA).

Procedures

The columns were prepared and pre-treated according to the methods described in Chapter 2 "Experimental Procedures". Once a hydrostatic injection was performed, a negative voltage was applied for the separation (cathodic inlet, anodic outlet). The injection plug length was estimated by determining the linear velocity of the sample during the injection process. This was accomplished by continuously injecting a uranyl - Arsenazo III complex. Injection velocity (v_{inj}) is then given by

$$v_{inj} = \frac{L_d}{t_{inj}} \quad (20)$$

where L_d is the effective column length and t_{inj} is the injection time. The injection plug length (L_{inj}) is then determined by

$$L_{inj} = (v_{inj})(t_{inj}) \quad (21)$$

The following rinse procedure was utilized between separations: 5 minutes 100 mM NaOH, 3 minutes water, 2 minutes running buffer.

The samples were water based and contained uranyl cation, 0.1 mM Arsenazo III to form the complex and 0.1 mM HClO_4 to achieve optimum pH (about pH 2.7) for complex formation. The running buffer contained sodium phosphate, sodium borate, perchloric acid, Arsenazo III and sodium chloride and the optimum concentration for each constituent was experimentally determined.

Methanol (10% by volume) was occasionally included in the running buffer.

Results and Discussion

Stacking Mechanism

In this method for uranyl ion analysis, relatively long sample plugs (between 1-8 cm) were injected and subsequently stacked in a manner similar to that described by Chien and Burgi (50,51,72). The negatively charged complex has an electrophoretic velocity greater in magnitude and opposite in direction to the electroosmotic flow velocity. Thus, the sample buffer is backed out into the running buffer reservoir as the analyte stacks at the moving boundary between the sample and running buffer. This is shown in Figure 12.

In the experiment depicted in Figure 12, a long sample plug (13 cm) was injected from the outlet and negative voltage was applied. A very long sample plug is utilized to elucidate the stacking mechanism, although this long sample plug may overload the system and contribute to the triangular shape of the second peak in the electropherogram shown in Figure 12e. The effective column length was only 9 cm; therefore, the sample extended past the detector at the time the voltage was applied (see Figure 12a). Initially, the field in the sample zone is greater than that in the running buffer region due to the lower ionic strength in the sample

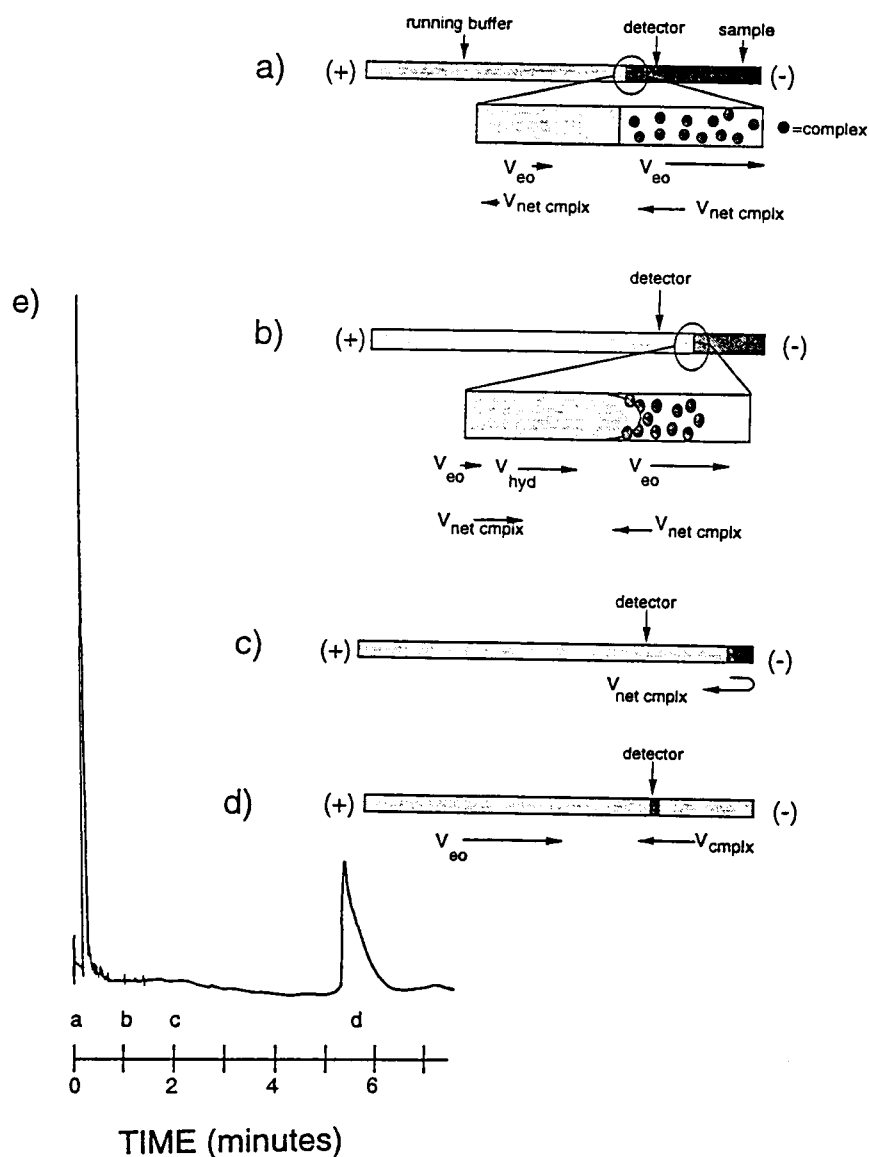


Figure 12. Depiction of the stacking mechanism. a) voltage initially applied, b) analyte stacking and removal of sample buffer, c) equilibration of the v_{eo} within the mobile phase and sample zones: analyte reverses direction, d) stacked analyte passes the detector, e) electropherogram from the experiment. Experimental conditions: $L_d = 9$ cm, i.d. $50\ \mu\text{m}$, applied field of $375\ \text{V/cm}$, injected plug length of 13 cm. See Table 4 for running buffer composition.

(see "Running Buffer Optimization" below). The electroosmotic flow velocity, therefore, is greater in the sample zone than in the running buffer region. However, the net electrophoretic velocity of the complex is greater than the electroosmotic flow in the sample zone, and sample ions begin to migrate towards the anode, stacking at the running buffer - sample interface.

A hydrostatic pressure forms because of the difference in electroosmotic velocities between the running buffer region and sample zone (see Figure 12b). The velocity due to this hydrostatic pressure makes up the difference between the two electroosmotic velocities. The analyte stacks at the boundary between the two zones while the hydrostatic pressure and electroosmotic velocity push the sample plug out the capillary inlet and into the running buffer reservoir. The injection plug length decreases and its ionic strength increases during this process which leads to a decrease of the field in the sample zone. The lowered field causes the electroosmotic velocity of the bulk solvent and the electrophoretic velocity of the complex to diminish in the sample zone. The complex attempts to migrate against the electroosmotic flow but is unable to do so against the combined electroosmotic flow and hydrostatic flow in the running buffer region. The portion of the sample which extended beyond the detector is quickly swept past the detector toward the capillary outlet as shown by the first peak (at approximately 30 seconds) in the electropherogram resulting from this experiment (Figure 12e).

By the time the sample plug nearly reaches the end of the column (Figure 12c), the hydrostatic pressure has diminished and the electroosmotic velocities in the sample and running buffer zones have become nearly equal. The analyte is now able to migrate against the electroosmotic flow (i.e., it reverses direction) and eventually migrates past the detector (Figure 12d). Considering the complexity of these electrophoretic processes, it is not surprising that the analyte band is asymmetric and not particularly efficient (see Figure 12e). In this experiment using a very short effective capillary, the Arsenazo III system peak and the complex peak show no separation (see below).

System Peak

Typical electrophoretic peak profiles are presented in Figure 13 in which the analyte appears as a shoulder on the system peak. Resolution between the system peak and the shoulder is dependent on the effective column length and is discussed below. A capillary with an effective length of 40 cm is used for this experiment. To assure complete complexation of the uranyl ion and any interfering metals present, excess Arsenazo III is added to the sample. Arsenazo III is negatively charged and exhibits a mobility only slightly greater than that of the uranyl ion - Arsenazo III complex. Although the absorbance of free Arsenazo III at 650 nm is minimal (Figure 11), stacking increases the concentration sufficiently to generate the system peak illustrated in Figure 13. The shoulder on

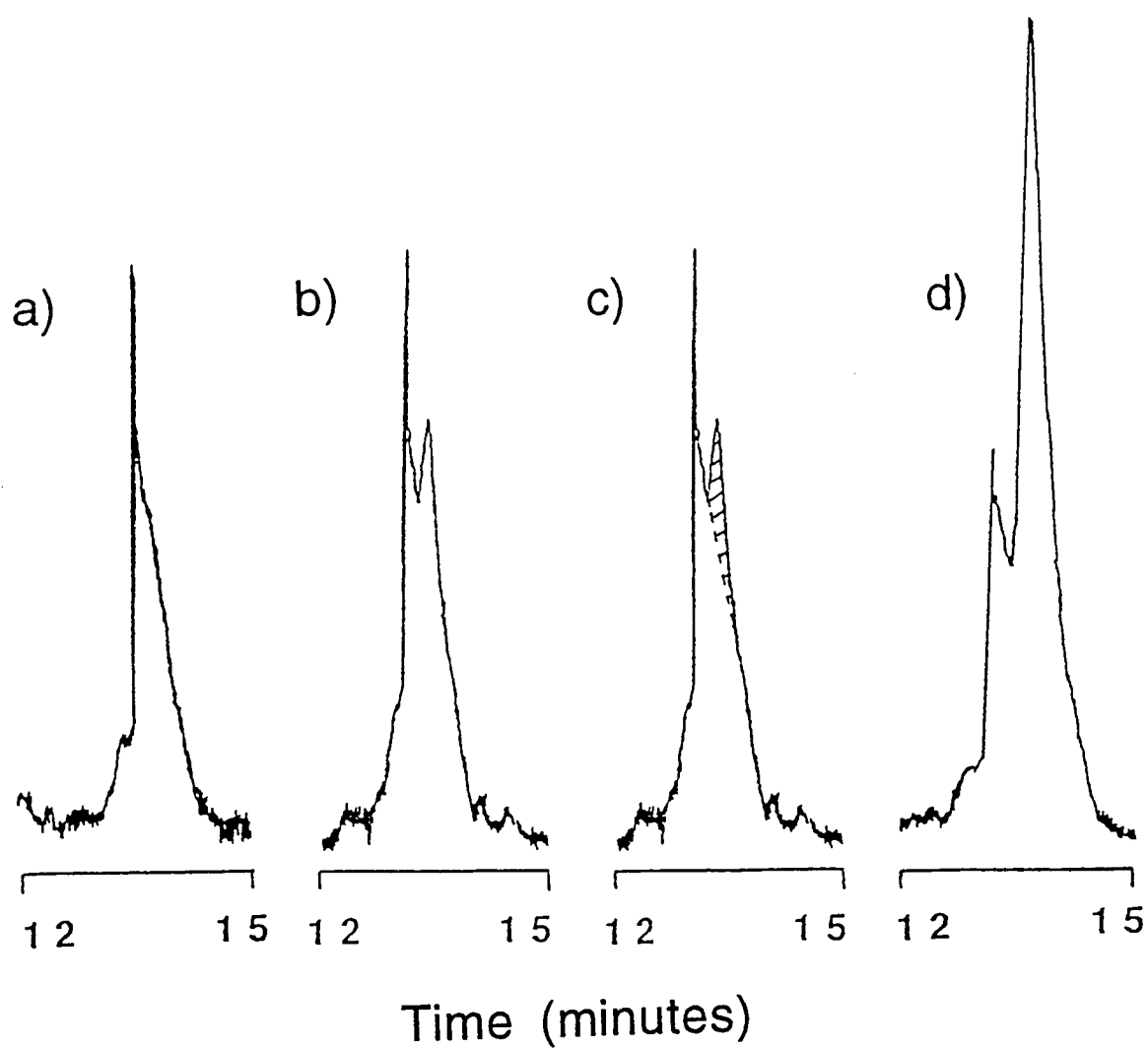


Figure 13. Typical electrophoretic peaks. a) system peak, b) 25 ppb injected uranyl complex, c) 25 ppb injected uranyl complex with analyte peak shaded, d) 200 ppb injected uranyl complex. See Figure 12 for experimental conditions.

the system peak (uranyl ion - Arsenazo III complex) grows with the concentration of the uranyl cation in the sample until it obliterates the system peak.

Many approaches were attempted to minimize the contribution of the system peak by increasing efficiency, improving the resolution between the system and analyte peaks or decreasing the magnitude of the system peak. One approach is to improve efficiency by increasing the stacking effect. Increasing the difference in ionic strength between the sample and the running buffer will enhance the stacking phenomenon. Unfortunately, at some point, band broadening from hydrostatic flow becomes excessive. Moreover, as the running buffer ionic strength is increased, efficiency decreases due to Joule heating. Another approach is to adjust system retention to increase resolution. Methanol was added to both the sample and the running buffer to decrease Joule heating (increase efficiency) and to slow electroosmotic flow. This would allow slight differences between the mobilities of the system peak and the analyte peak to be enhanced, thereby improving resolution. Unfortunately, this approach has minimal effect on efficiency and resolution. The concentration of Arsenazo III in the running buffer does not have a noticeable influence on the magnitude of the system peak. Ultimately, it was determined that the resolution between the system and analyte peaks appears to be best when longer columns

are used (see "Optimum Column Length and Injection Plug Length" below).

Running Buffer Optimization

Optimum running buffers had to be determined for each column i.d. Each buffer consisted of Arsenazo III, perchloric acid, sodium phosphate, sodium borate and sodium chloride in various concentrations. Methanol was also included on occasion.

A common buffer used in capillary electrophoresis (73,74) consisting of sodium phosphate and sodium borate was included in each running buffer. However, the concentration usually used (10 and 6 mM, respectively) seems to interfere with the complex formation between Arsenazo III and the uranyl cation. The absorbance of the complex decreases in the presence of this concentration of running buffer. It is believed that the uranyl ion forms a strong complex with the phosphate ion thereby preventing the Arsenazo III from complexing the uranyl ion. A tenfold decrease in concentration (i.e., 1 mM sodium phosphate and 0.6 mM sodium borate) appeared to cause no interference as determined by UV/Vis spectrophotometry, and was used in all experiments. The optimal formation for the Arsenazo III - uranyl complex occurs in an acidic medium; therefore, perchloric acid was added to the running buffer. Little difference was observed in the absorbance of the complex when the perchloric acid concentration was varied between 10 and 100 mM; thus, 10 mM HClO₄ was included in each

running buffer resulting in a measured pH of 2.7. The lowest perchloric acid concentration was used to minimize its contribution to Joule heating.

Arsenazo III and sodium chloride were doped into the running buffer to prevent complex dissociation and to improve stacking, respectively. However, excessive amounts of Arsenazo III will result in a high optical background, while high salt concentrations can produce excessive Joule heating. Therefore, an optimum concentration of each component was determined. These optimum concentrations vary with the capillary i.d. Table 3 shows the combinations of Arsenazo III and sodium chloride concentrations investigated for each column i.d., and Table 4 lists the optimum Arsenazo III and sodium chloride concentrations for each column with approximate LODs as experimentally determined. Although the optimum Arsenazo III concentration depends on the capillary employed, it is not known why this occurs. The optimum salt concentration varies as expected; that is, smaller i.d. columns tolerate higher salt concentrations. In each case, the thermal load is near the limit of causing significant thermal dispersion (75).

By adding methanol to the running buffer, the current could be reduced, thereby producing less Joule heating. Thus, higher fields could be employed to reduce separation time. The LODs represent a signal-to-noise ratio of three as described below and follow a trend opposite to that expected. The shortest optical path length has the best LOD and the longest optical path length has the worst

Table 3
Concentrations of Arsenazo III and
Sodium Chloride Investigated

Column i.d. (μm)	Arsenazo III (mM)	Sodium Chloride (mM)
75	0.01	10, 50, 75
	0.1	10, 50, 75
50	0.01	50, 75, 100
	0.1	50, 75, 100
25 ELP	0.01	50, 75, 100
	0.1	50, 75, 100, 125, 150, 200

Table 4
Optimized Running Buffers^a

Column i.d. (μm)	Arsenazo III (mM)	NaCl (mM)	L_d (cm)	LOD (ppb)	Thermal load (W/m)	Mobility (cm/min)
75	0.01	50	40	33	0.96	1.8
50 ^b	0.01	75	31	25 ^c	1.3	2.3
25 ELP	0.1	150	50	100	1-1.3	4.3

a All running buffers include 10 mM HClO_4 , 1 mM sodium phosphate and 0.6 mM sodium borate

b Optimum running buffer included 10% methanol

c Approximate LOD in a $L_d = 40$ cm column. Can detect 10 ppb in a $L_d = 60$ cm column.

LOD. This is probably an outcome of the complicated stacking process and interference by the partially resolved system peak.

Sensitivity and Detectability

Due to the presence of the system peak (Figure 13a), determination of the peak area was complicated. As shown in the figure, the peak shape changes with concentration. At low levels of analyte, the complex peak is a shoulder on the system peak. This is shown for a 25 ppb uranyl - Arsenazo III complex profile in Figure 13b and the appropriate portion used for quantitation is shaded in Figure 13c. As the analyte concentration increases, the shoulder grows as shown in Figure 13d. From 25 - 100 ppb, the area of the individual peak is determined by manually skimming the tail of the system peak (see Figure 13c). For higher concentrations, the average blank signal was subtracted from the total peak area to determine the peak area of the complex.

A reproducibility study was conducted by injecting the blank and a 25 ppb complex solution six times in a column with an effective length of 31 cm and 50 μm i.d. and performing the separation. The average peak area of the blank was 44 arbitrary units with a coefficient of variation of 8.5%. The average peak area of the 25 ppb uranyl cation peak, skimmed from the tail of the system peak as shown in Figure 13c, was 1.9 arbitrary units with a coefficient of variation of 51%. A coefficient of variation of 50% corresponds to a signal-to-noise ratio equal to three (76). Thus,

the LOD for this system is about 25 ppb. Lower concentrations can be detected if longer columns are used to improve resolution; however, separation time increases. Although a reproducibility study was not performed using the longer columns (total column length of 60 cm, effective column length of 50 cm), 10 ppb uranyl cation could be seen as a shoulder as clearly as the 25 ppb peak could be detected in the shorter column. Thus the LOD in the longer column is approximately 10 ppb.

The peak areas of duplicate injections were used to construct a calibration curve that exhibits a linear dynamic range of 2.8 orders of magnitude in injected concentration (see Figure 14). This linear dynamic range may be extended by increasing the concentration of Arsenazo III in the sample. However, increasing the Arsenazo III concentration in the sample will increase the system peak.

Optimum Column Length and Injection Plug Length

In screening applications, rapid separation times are necessary to increase throughput. In this study, longer columns exhibit better resolution and LODs at the expense of separation times (see Table 5). Therefore, various column lengths were evaluated using a 50 μ m column to establish a suitable compromise between resolution and separation time. At very short column lengths (effective column length of 9 cm), resolution between the system peak and the analyte peak was completely lost although the

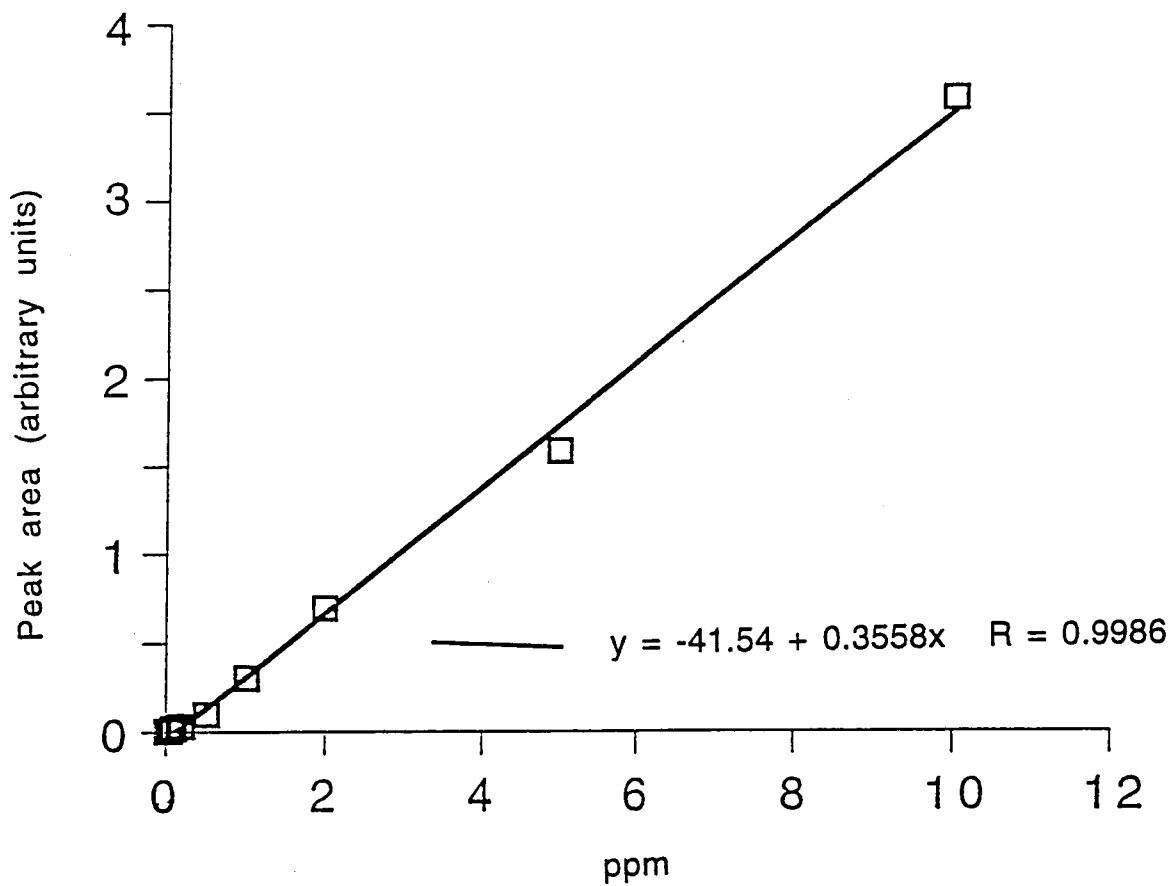


Figure 14. Calibration curve for uranyl cation. Injected plug length is 5.8 cm, $L_d = 31$ cm. See Figure 12 for other experimental conditions.

Table 5

Optimization of Effective Column Length and Injection Plug Length

Column diameter (μm)	Total column length (cm)	Effective column length (cm)	Optimum injection plug ^a (cm)	Approximate LOD (ppb)	Separation time (min)
75	50	40	3.5	33	22
50	60	50	7.8	10	20
	40	31	5.8	25	13
	40	9	3.9	200	6
25 ELP	60	50	1.6	100	12

a) Maximum injected plug length before excessive band broadening occurs

separation is rapid (6 minutes). A column with an effective length of 50 cm showed the best resolution obtained in these experiments at the expense of separation time (20 minutes). Thus, an effective length of 31 cm was used for most experiments as it represents a reasonable compromise between resolution and separation time (13 minutes).

The LODs listed in Tables 4 and 5 were determined using optimum injection plug lengths. By injecting more material into the column, detectability should improve provided efficiency and stacking remain sufficient for the purpose of the research. The best injection plug lengths were determined for each capillary with its optimum running buffer (see Table 4) by increasing the injection plug length until excessive band broadening occurred. The optimum plug length (see Table 5) is the best compromise between band broadening and improved LOD.

Interferences and Selectivity

A common limitation in uranyl cation analyses is inadequate selectivity. At a pH of 2.7, Arsenazo III forms complexes with several different metals including scandium, lanthanum, barium, lead, and iron(III) (64,65,69-71). Thus, extractions, pH adjustments, masking agents or some separation must be performed to provide the selectivity needed. CZE proves to be extremely selective in this case. The complex formed between the uranyl ion and Arsenazo III is negatively charged, while most other

complexes are neutral or positively charged. Thus, these complexes will be eluted into the running buffer reservoir during the stacking process described earlier. When some metals expected to be present in real samples (i.e., mercury(II), lead, or calcium) were added in excess, no additional peaks were observed, and the uranyl - Arsenazo III peak remained unchanged.

To assess the effects of a real sample on the LOD, water taken from the Pigeon River (Cocke County, TN) was filtered through a 8 μm polycarbonate filter to remove particulate matter and analyzed. Metal - Arsenazo III complexes formed at this pH are purple or bluish in color. All samples made with the Pigeon River water were a bright blue color, indicating a high level of complexes of various metals; yet these complexes did not interfere with the uranyl determination. A 50 μm i.d. capillary with an effective length of 50 cm was used for the analysis. A standard addition calibration curve was constructed which is linear with a coefficient of regression of 0.998. No uranyl cation above the LOD was found in the Pigeon River water. The river water matrix did not interfere with detectability of low concentrations of added uranyl (10 ppb).

An additional water sample was taken from Poplar Creek (Oak Ridge, TN) below the Oak Ridge Y-12 Plant and analyzed using the 50 μm i.d. column with a 50 cm effective length. Each of the samples made from this water was again blue showing a high concentration of complexed metals. Based on the standard addition

calibration curve (see Figure 15), about 18 ppb uranyl cation was found in the river water. This was the median as reported in the 1995 Y-12 National Pollution Discharge Elimination System Report (77) although collection occurred after a rain shower which may have the effect of stirring up the sediment and increasing the uranyl concentration.

Conclusion

In summary, this method of uranyl analysis is simple, rapid and inexpensive to perform. It has a separation time and LOD comparable with liquid chromatography methods (60) and requires far less volume of sample and reagents. However, detectability is limited by the presence of a large system peak. Attempts to increase resolution between the system and analyte peaks were of limited success. Improvements in detectability could be made if the system peak could be removed. Some reduction in the system peak may be accomplished by monitoring the separation at two wavelengths - one at the maximum absorbance of Arsenazo III (about 540 nm), the other at the maximum absorbance of the complex (about 650 nm) - followed by a mathematical manipulation to reduce the effect of free Arsenazo III background (see Figure 11). Unfortunately, this suggestion could not be evaluated due to limitations of the equipment. Nevertheless, the method presented herein is a good screening protocol for the uranyl ion at low ppb concentrations and exhibits a high degree of selectivity.

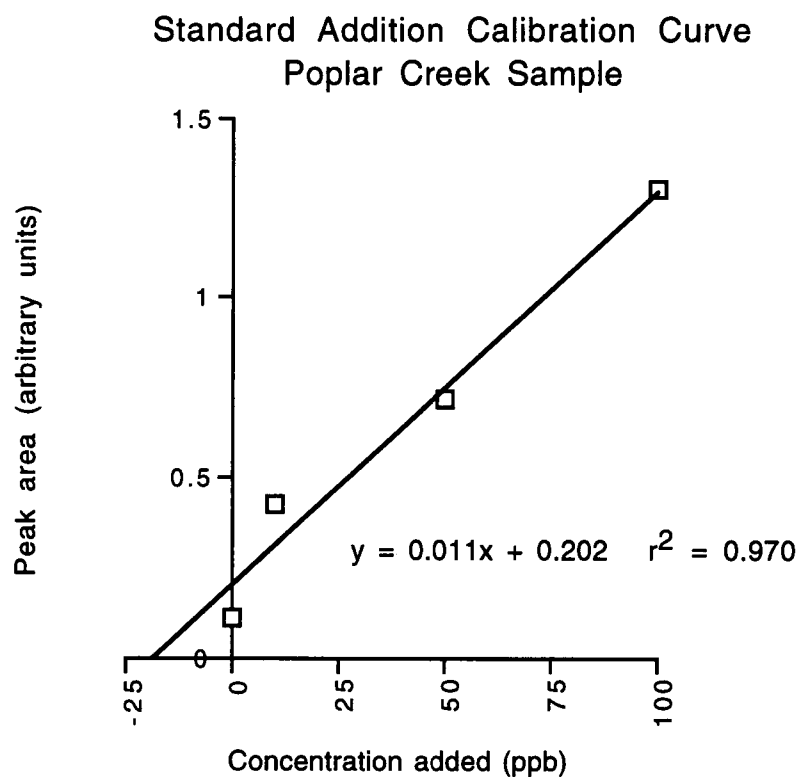


Figure 15. Standard addition calibration curve for the Poplar Creek sample. Experimental conditions: $L_d = 50$ cm, i.d. = 50 mm, applied field = -300 V/cm, injection plug length = 7.8 cm. See Table 4 for running buffer composition.

Chapter 4

Determination of Liquid Propellant via CZE with Indirect Absorbance Detection and MECC with Laser-Induced Fluorescence Detection

Introduction

The United States Army is investigating the possibility of utilizing liquid propellants, rather than solid fuel, for some of its equipment. The liquid propellants under consideration are hydroxylammonium nitrate-based, with the hydroxylammonium nitrate serving as the oxidizer and triethanolammonium nitrate serving as the fuel (78-84). Water is added as a solvent to achieve a homogeneous solution as well as to control the energy content of the propellant. The oxidizer and fuel are mixed in a seven-to-one molar ratio such that just sufficient oxygen is present to convert the propellant completely to water, carbon dioxide and nitrogen during combustion (78-82). This produces a force near the theoretical maximum and yields a gun with little or no muzzle flash and no secondary muzzle flash (81). The liquid propellant chosen for use in the 105 and 155 mm howitzers contains 60.79 weight percent (wt %) hydroxylammonium nitrate, 19.19 wt % triethanolammonium nitrate and 20.02 wt % water. With such high ionic content, the resulting mixture is more like a molten salt than an aqueous solution (83).

This type of liquid propellant decomposes with time, heat and pressure. Certain contaminants, especially nitric acid and iron(III), escalate the rate of decomposition. However, the propellants must be kept under heat and pressure to ignite and sustain combustion. Thus, if a stray shell should strike a storage tank (analogous to a fuel tank), the propellant should burn off in a relatively controlled manner rather than explode. This reduces the danger to nearby personnel and equipment (e.g., on an army base) (81).

Sealed storage tanks allow the build up of pressure and heat resulting from the decomposition and occasionally explode unexpectedly (85). Also, if the composition of the liquid propellant has changed, the propellant could have less thrust than required to propel the ammunition in the desired fashion, thus failing ballistic requirements or expectations. Thus, a need exists for the determination and monitoring of the composition of these propellants. The important species are hydroxylammonium nitrate, triethanolammonium nitrate, water and total nitrate content. Other impurities include ammonium nitrate, which is an important product of decomposition, ethanolammonium nitrate, diethanolammonium nitrate, nitric acid and assorted metal ions. This chapter presents the analysis of hydroxylamine sulfate, triethanolamine, diethanolamine, ethanolamine and ammonium nitrate, components which were used to mimic the actual constituents (hydroxylammonium nitrate, triethanolammonium

nitrate) and impurities (diethanolammonium nitrate, ethanolammonium nitrate and ammonium nitrate) of the liquid propellant because the exact chemicals were unstable or unavailable.

Currently, a battery of analyses must be performed to determine the composition of the liquid propellant. Hydroxylammonium nitrate and triethanolammonium nitrate are determined via acid-base titration (80,86). The pK_a s of these two species, 5.96 and 7.76, respectively are too similar for distinct endpoints to be observed (80). The first derivative of the titration curve makes these breaks more visible. Unfortunately, diethanolammonium nitrate, ethanolammonium nitrate and ammonium nitrate are not differentiated from triethanolammonium nitrate; thus, the concentration calculated for the latter is actually the total of the concentrations of all four of these components. Other analyses are performed on the liquid propellant sample as well. Water is determined by the Karl Fischer method, ammonium nitrate is analyzed by the Kjeldahl method, free nitric acid and total nitrates by titration, and metals by inductively coupled plasma-mass spectrometry (86). The total percentages thus obtained are subtracted from 100% and the remainder is labeled as the "total other impurities" (86). Adding the results of different methods and several different tests leads to error and uncertainty concerning the actual formulation. This large number of tests, and the lack of selectivity between the

triethanolammonium nitrate and the other ammonium nitrates, suggest the need for more selective and sensitive tests which can analyze a variety of solutes simultaneously.

Separation methods such as CZE and MECC are known for their ability to rapidly separate complex mixtures. The goal of the research reported in this chapter was to develop analytical methodologies capable of rapidly determining the concentration of the two major components (hydroxylamine sulfate and triethanolamine) as well as the presence and concentration of the impurities (ethanolamine, diethanolamine and ammonium nitrate). The ability to separate and quantitate all analytes would increase confidence that the propellant still had the proper formulation and that the propellant should conform to ballistic requirements and expectations. Two approaches were chosen for this determination. CZE with indirect absorbance detection preceded by extensive dilution was used to determine the highly concentrated species and MECC with laser-induced fluorescence detection following derivatization with NBD-Cl (4-chloro-7-nitrobenzofurazan, 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole) was used to analyze the minor components.

As previously discussed in Chapters 1 and 2, several factors influence the performance of indirect detection. The first factor is the choice of the background ion to be added to the running buffer. Since the analytes are cations, the background ion must be a cation possessing a high absorptivity and a mobility which

approximates the mobility of the analytes. It is desirable that a low concentration of the background ion produce a high signal such that the displacement of a relatively small amount of background ion will result in a large change in signal. To obtain symmetrical peaks, the mobilities of the background ion and analytes must be closely matched. Otherwise, fronting or tailing peaks will be observed and degrade the analytical figures of merit (see the discussions in Chapters 1 and 2).

This leads to a second factor to consider which can be a problem in any capillary electrophoretic method. The concentration of the sample should be about one hundred times more dilute than the running buffer to prevent field inhomogeneities. A homogeneous field is desired along the entire length of the capillary. If a band of high ionic strength exists in the middle of the capillary, the resistivity and thus the field will be low in that band. The field inhomogeneities will be reflected in distorted peaks which again degrade the analytical figures of merit. Thus the sample concentration must be kept low which can increase the difficulty in detection. This problem is exacerbated if the background ion is present at low levels to produce a large change in signal (as discussed above), yet is the major component of the running buffer.

A third factor which influences detection is the transfer ratio which should approach one background ion displaced per solute ion. The use of a low pH running buffer should help achieve

this goal by completely protonating background ions and solutes. This complete protonation will maximize the transfer ratio.

The pre-column derivatization necessary to form the NBD-amine complexes of diethanolamine, ethanolamine and ammonium nitrate can lead to multiple products which can degrade efficiency and resolution of the analysis. The complexes formed were neutral and fluorescent; therefore, MECC with laser-induced fluorescence detection was utilized for their analysis.

Experimental

Apparatus

The CZE separation apparatus used for the determination of the two major components was similar to that described in Chapter 2 with the following exceptions. Electrical fields were provided with a Hipotronics high voltage power supply (Brewster, NY) and electropherograms were recorded on a chart recorder.

The apparatus used for the MECC separation of the three impurities was similar to that described above differing only in the capillary dimensions (50 μm i.d. x 360 μm o.d. x 50 cm long). Laser-induced fluorescence detection was accomplished by focusing the output (488 nm, 15 mW) of an air cooled argon ion laser (Spectra-Physics Model 162A, Mountain View, CA) through the capillary and collecting the resulting fluorescence at 90° to the incident radiation. The fluorescence was transmitted to a photomultiplier tube Model HC 120-01 (Hamamatsu, Bridgewater,

NJ) via a 600 μm (core) plastic clad glass fiber optic purchased from General Fiber Optic (Fairfield, NJ) using an optical configuration built in-house (87). A 500 nm cut-on filter was placed before the photomultiplier tube to eliminate laser scatter, after which the signal was sent to a chart recorder.

Chemicals

Imidazole and various derivatives including 2-methylimidazole, 4-methylimidazole, 2-ethylimidazole, 1,1'-carbonyldiimidazole and 2-benzyl-2-imidazoline were purchased from Aldrich (Milwaukee, WI). Sodium phosphate, sodium borate, sodium acetate, sodium dodecyl sulfate (SDS), Triton X, ammonium nitrate, hydroxylamine sulfate, diethanolamine and ethanolamine were obtained from Sigma (St. Louis, MO). HPLC grade water and methanol was purchased from Baxter for use in the running buffer, the sample dilutions and the derivatization procedure. Triethanolamine was obtained from Fisher Scientific (Atlanta, GA). Carboxymethyl- β -cyclodextrins were received from Pharmatec, Inc. (Alachua, FL). A liquid propellant sample, diluted for safety, was obtained from Lockheed Martin Energy Systems, Inc. (Oak Ridge, TN).

Procedures

The capillaries were prepared and pre-treated as described in Chapter 2. For the indirect determination of hydroxylamine sulfate

and triethanolamine, the optimal background ion and concentration were determined experimentally and will be discussed below. A dilute liquid propellant-like sample was made containing approximately 12 and 4.1 wt % hydroxylamine sulfate and triethanolamine, respectively. Solutions containing 100 mM hydroxylamine sulfate, triethanolamine, diethanolamine, ethanolamine, or ammonium nitrate were prepared and used as standards. Hydrostatic injections were performed at a height differential of 20 cm for 20 seconds. Rinses of three minutes with 100 mM NaOH followed by water and re-equilibration with running buffer were performed between each injection.

The running buffer for the MECC determination of the minor components consisted of 10 mM sodium phosphate and 6 mM sodium borate buffer at pH 9 and either 50 or 90 mM SDS. Because this running buffer provided insufficient resolution, running buffers containing additives in addition to the SDS were investigated. The separations achieved utilizing these running buffers were also compared with other separations using running buffers containing cyclodextrins instead of SDS. Injections were made hydrostatically at a height differential of 10 cm for 20 seconds. The derivatization procedure of the amines was as follows: 5 mL of the sample at pH 9, 5 mL of methanol saturated with sodium acetate and 5 mL of 10 mg/mL NBD-Cl in methanol were mixed and heated at 60°C in a sealed container for one hour. The resulting

solution was diluted by 50- to 100-fold with either methanol or running buffer (88).

Results and Discussion

Analysis of Hydroxylamine Sulfate and Triethanolamine

Imidazole was initially chosen as the background ion in the analysis of hydroxylamine sulfate and triethanolamine and minor components. The running buffer consisted of 5 mM imidazole adjusted to pH 4 with sulfuric acid. This low pH should completely ionize the analytes (see Table 6) which will improve the transfer ratio (refer to discussion above). The results of a typical separation of a standard mixture of the five components is shown in Figure 16. The elution order was determined to be ammonium nitrate, hydroxylamine sulfate, ethanolamine, diethanolamine and triethanolamine.

As can be observed in the figure, the mobilities of analytes do not closely match the background ion. The ammonium peak was fronting, the hydroxylamine peak was symmetrical and the other peaks were tailing. Also, extraneous system peaks were produced which interfered with the identification and quantitation of the hydroxylamine sulfate and ethanolamine. With imidazole as the background ion, a negative system peak directly followed the negative hydroxylamine peak without baseline separation (see Figure 16). Another system peak (positive) followed the ethanolamine without separation. Although this separation

Table 6

pK_a Values of Selected Propellant Components

Constituent	pK _a
Hydroxylammonium Nitrate	5.96
Triethanolammonium Nitrate	7.76
Diethanolammonium Nitrate	*
Ethanolammonium Nitrate	*
Ammonium	9.24

* Value not available

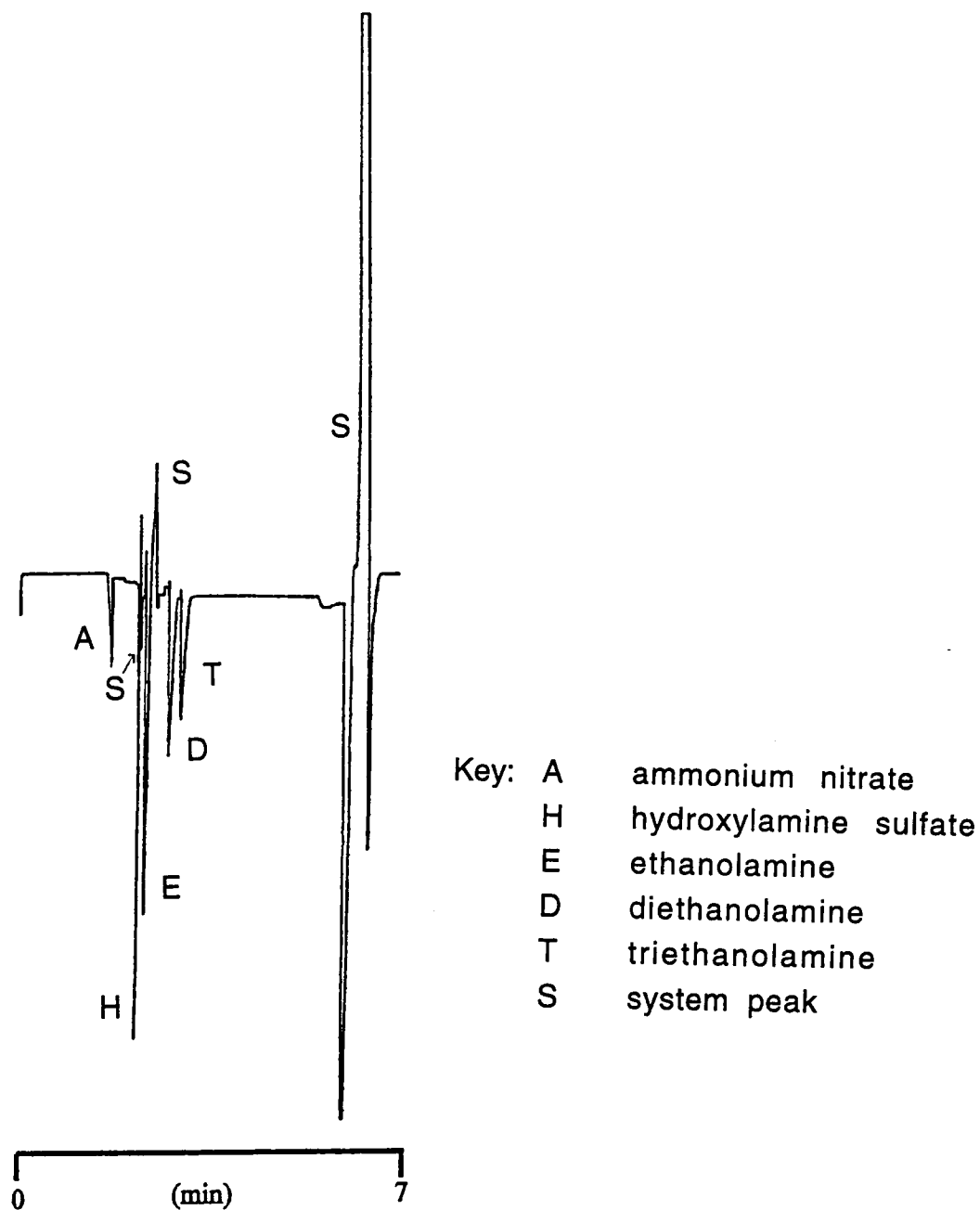


Figure 16. Separation of a standard mixture containing 100 ppm of each solute with imidazole as the background ion. Peak identification in order of elution is ammonium, hydroxylamine sulfate, ethanolamine, diethanolamine and triethanolamine. The running buffer is 5 mM imidazole at pH 4. System peaks interfere with the determination of hydroxylamine sulfate and ethanolamine.

exhibits better peak symmetry than seen in the electropherograms shown in Chapter 2, the peak symmetry is not good. Changes in the background ion identity should improve the peak symmetry and resolution by more closely matching mobilities. Reducing or moving the system peaks away from the analyte peaks will also improve the separation. Several imidazole derivatives were investigated in an attempt to meet these goals (see Figure 6 for structures). Of the derivatives tried, 2-methylimidazole, 4-methylimidazole, 2-ethylimidazole and 2-benzyl-2-imidazoline produced system peaks near the hydroxylamine sulfate peak and did not improve peak symmetry or resolution. The final derivative investigated, 1,1'-carbonyldiimidazole, allowed the determination of the hydroxylamine sulfate without interference from system peaks (see Figure 17). Moreover, the 1,1'-carbonyldiimidazole produced twice the background signal with the same concentration of background ion because it contains two imidazole groups. This improves the detection limit due to a low background ion concentration and a high dynamic reserve (see Equation 14).

At this point, an attempt was made to analyze a dilute imitation liquid propellant sample. Dilutions of samples of liquid propellant which contain impurity concentrations at expected detectable concentrations (ppm range) also contain the major components in the percent range. Thus, the major components of the sample were at concentrations that exceeded the background ion concentration, resulting in large inhomogeneities in the field.

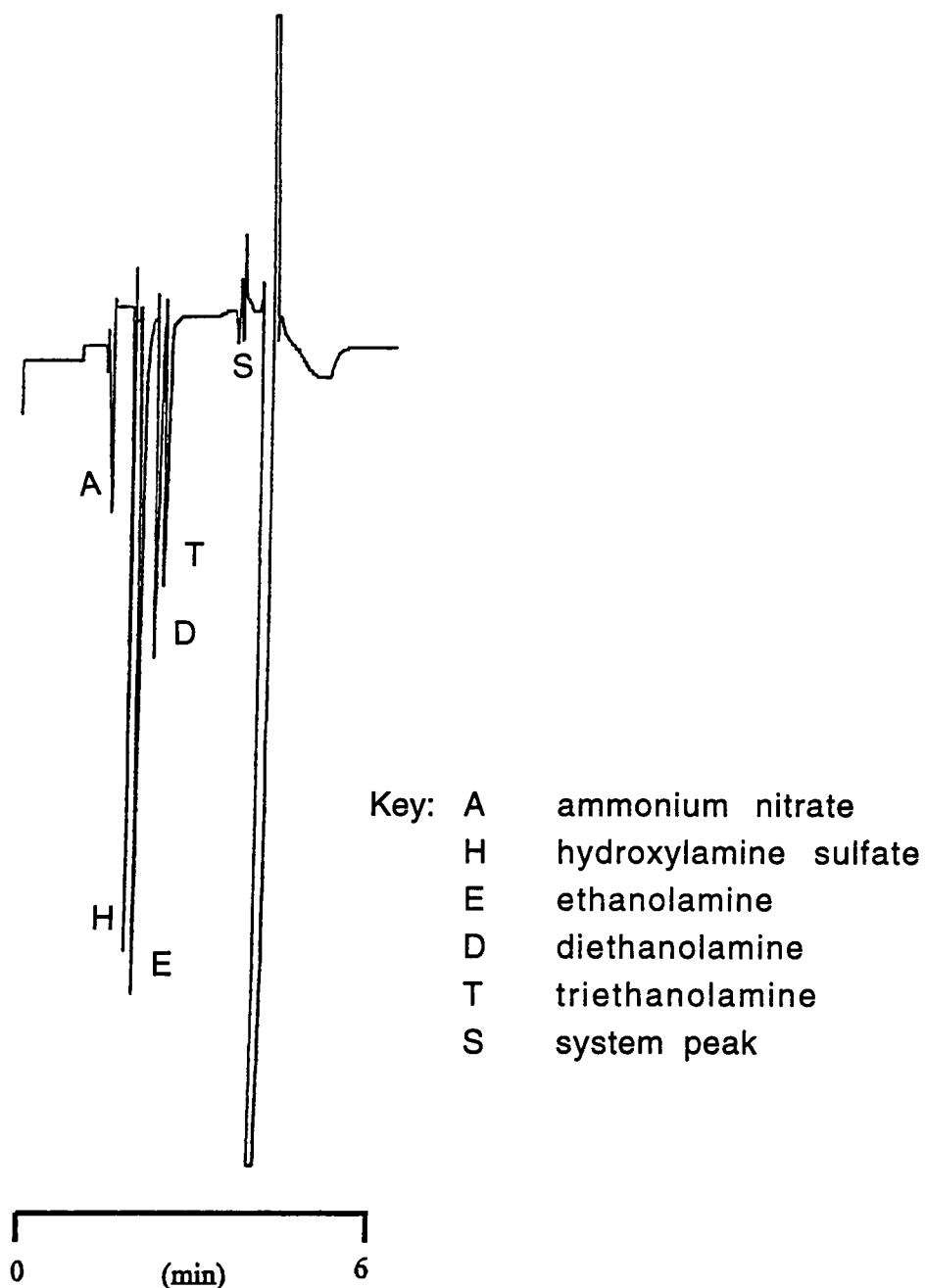


Figure 17. Separation of a standard mixture using 1,1'-carbonyldiimidazole as the background ion. The running buffer contains 5 mM 1,1'-carbonyl diimidazole and 10% methanol adjusted to pH 4. No system peaks interfere with the analysis.

This was reflected in a baseline which wandered from off-scale positive to off-scale negative and did not recover. In addition, the analyte concentration far exceeded that of the background ion such that insufficient background ions were present to be displaced by the analyte ions; therefore, any signal which could be produced would be beyond the linear portion of a calibration curve. The only way this method would be useful is to greatly dilute the propellant until the peaks for hydroxylamine sulfate and triethanolamine are on scale; of course, the impurities will be far below the limit of detection at this concentration. Decreasing the sample concentration will lead to poor reproducibilities as the LOD is approached. However, high concentrations result in field inhomogeneities. Thus, it was decided to use a second analysis for the determination of the impurities.

Studies were executed to determine what dilution factors of the propellant show the best reproducibility for the two major components. It appeared that 25,000-fold and 5,000-fold dilutions produced the best peak height coefficient of variation for hydroxylamine sulfate (1.2%) and triethanolamine (3.5%), respectively (see Figure 18 and Table 7). Figure 18 shows typical peaks for hydroxylamine sulfate and triethanolamine at these dilutions. Table 7 outlines the coefficients of variation produced at each dilution tested. Although dilutions other than those chosen for use exhibited better reproducibilities, they also produced rounded peaks due to high sample concentrations. Dilutions factors

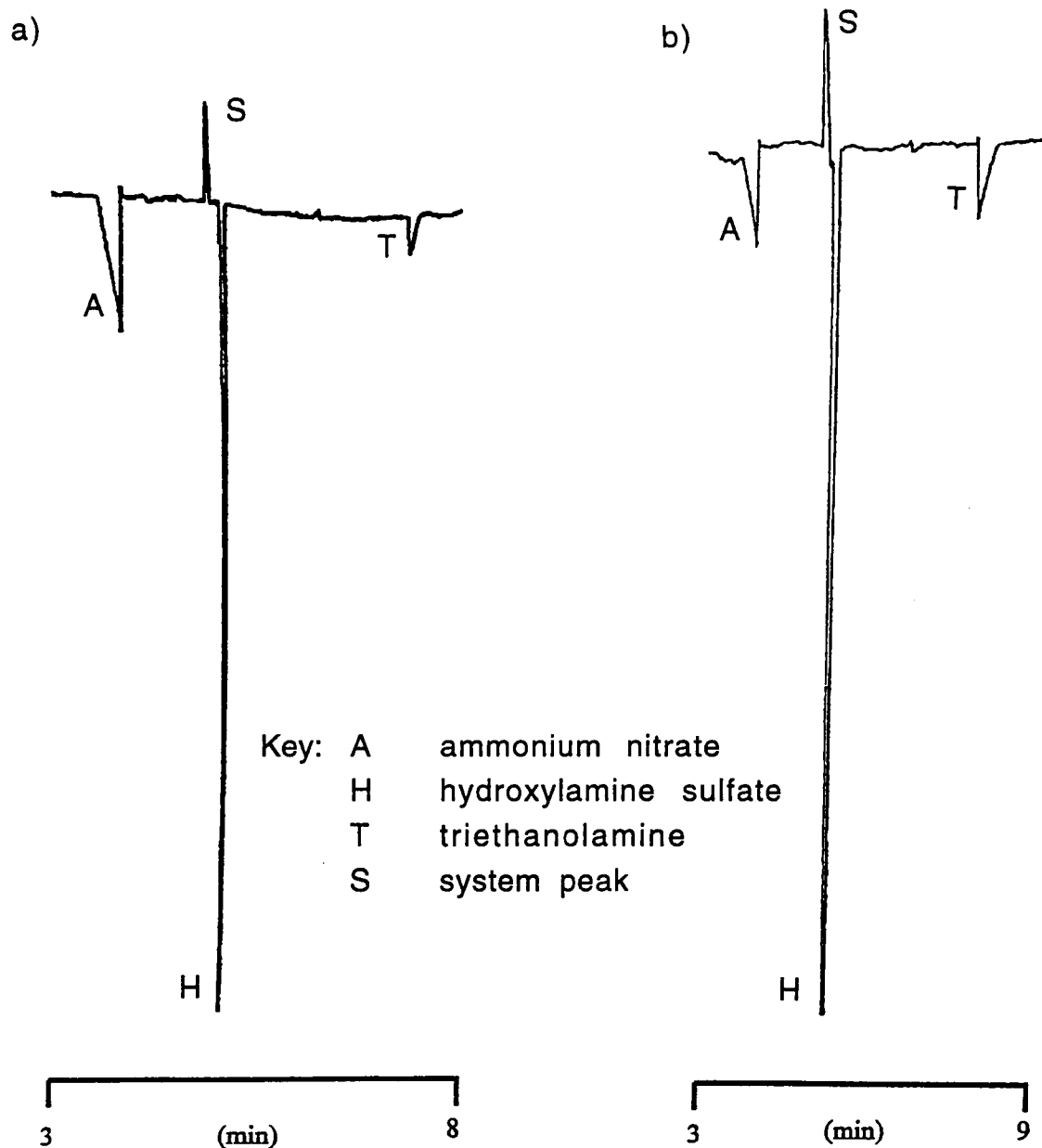


Figure 18. Dilutions of hydroxylamine sulfate and triethanolamine which exhibit the best reproducibilities. Running buffer as in Figure 17. a) hydroxylamine sulfate at 24 ppm which corresponds to a 25,000-fold dilution of liquid propellant b) triethanolamine at 41 ppm which corresponds to a 5000-fold dilution of liquid propellant.

Table 7

Determination of Optimal Dilution Factor
for Best Peak Height Reproducibility
of Hydroxylamine Sulfate and Triethanolamine

Dilution Factor	Concentration triethanol-amine (ppm)	CV triethanol-amine (%)	Concentration hydroxyl-amine (ppm)	CV hydroxyl-amine (%)
750	270	1.3	800	
1000	203	2.7	600	
1250	163	19	480	
1660	122	11	360	
2500	81	7.0	240	
5000	41	3.5	120	
7500	27	5.9	80	
10000	20	9.0	60	
12500	16	7.7	48	1.1
16667	12		36	9.2
25000	8		24	1.2
50000	4		12	4.3

were thus chosen which satisfied peak shape and reproducibility requirements. At these dilutions, the peaks exhibited reasonable signal-to-noise ratios. The hydroxylamine sulfate peak is off-scale at the 5,000-fold dilution utilized for the analysis of triethanolamine but not for the 25,000-fold dilution.

The concentration of background ion in the running buffer which exhibited the best reproducibilities and resolution between the system and hydroxylamine sulfate peaks was also determined. 1,1'-Carbonyldiimidazole concentrations of 2.5, 5.0 and 7.5 mM at pH 4 were tested and the 5 mM concentration appeared to generate the best reproducibility. The addition of 10% methanol to the running buffer improved the reproducibility somewhat further.

Analysis of Diethanolamine, Ethanolamine, and Ammonium Nitrate

Derivatizations were performed on separate solutions of diethanolamine, ethanolamine, ammonium nitrate, hydroxylamine sulfate and triethanolamine. The derivatization procedure can be used with primary and secondary amines but not tertiary amines as shown in the schematic in Figure 19. Thus, the triethanolamine was not derivatized and produced no peaks via fluorescence detection. After derivatization, the solutions listed above were diluted to 50 ppm in methanol. Using a running buffer consisting of 10 mM sodium phosphate, 6 mM sodium borate and 50 mM SDS at pH 9, four partially resolved peaks were produced. An increase of the SDS concentration improved resolution by increasing the capacity

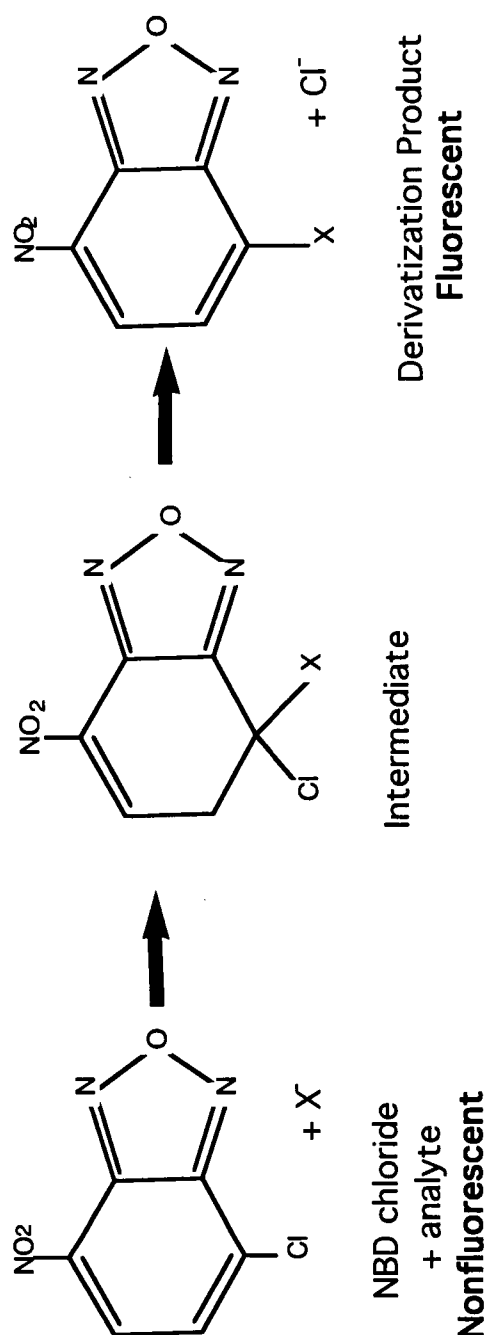


Figure 19. Derivatization procedure for NBD-Cl.

factor (see Equation 8). The capacity factor is the ratio of the amount of solute in the micellar phase to the amount of solute in the mobile phase. Increasing the micellar concentration thus increases the number of micelles available to the solute; therefore the amount of solute in the micellar phase increases. Increasing the capacity factor will thus improve resolution until the optimum capacity factor range is surpassed (6). The elution order was determined to be diethanolamine, ethanolamine and ammonium nitrate from injections of standard mixtures (see Figure 20). When injected singly, each derivative produced two peaks. The second peak from each solution had the same retention time and is assumed to be a common, unidentified by-product of the derivatization procedure.

Calibration curves for each of the three minor components were generated from the peak heights (mean of triplicate injections, see Figure 21). Ethanolamine and ammonium nitrate produced calibration curves with linear dynamic ranges of nearly two orders of magnitude with calculated LODs of 2.5 and 14 ppb, respectively (signal-to-noise ratio of three). The calibration curve for diethanolamine shows a roll-over beginning between 300 and 500 ppb resulting in a slightly shorter linear dynamic range and an LOD of 3.6 ppb. Nine injections of diethanolamine, ethanolamine and ammonium nitrate produced coefficients of variation of 2.2, 2.9, and 6.3%, respectively. These LODs and reproducibilities are sufficient for this methodology to be a good means of analysis.

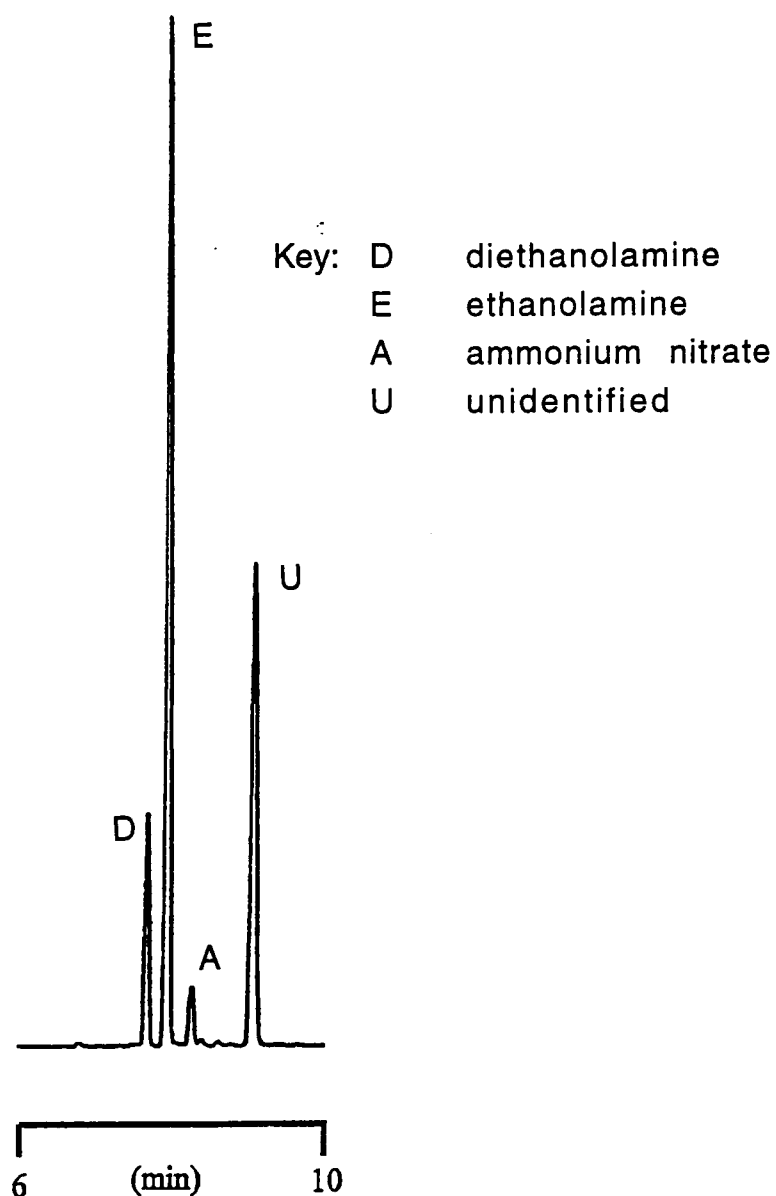


Figure 20. Separation of derivatized diethanolamine, ethanolamine and ammonium. Running buffer consisted of 10 mM sodium phosphate and 6 mM sodium borate at pH 9 and 90 mM SDS. Running voltage of 15 kV applied. Unidentified extra peak probably from derivatization process.

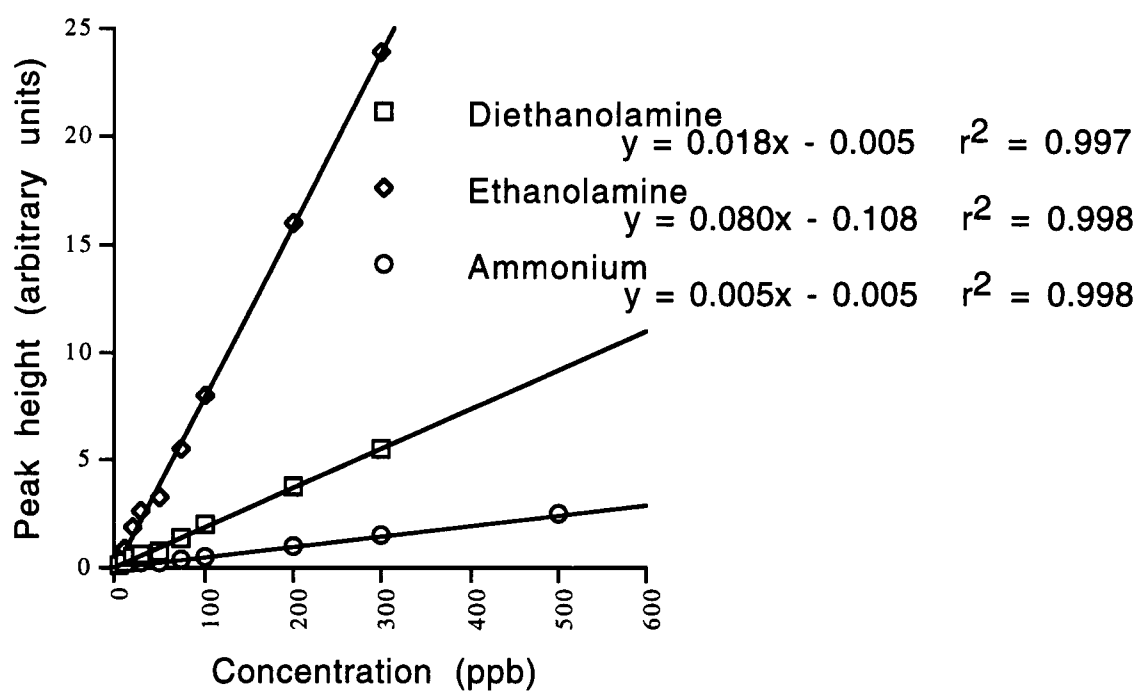


Figure 21. Calibration curves for NBD-diethanolamine, -ethanolamine and ammonium.

A sample of liquid propellant obtained from Lockheed Martin Energy Systems, Inc. was derivatized and injected (see Figure 22). Unfortunately, the hydroxylammonium nitrate is also derivatized by NBD-Cl and elutes just after ammonium nitrate under these conditions. At the high concentrations of hydroxylammonium nitrate present in the sample, the resulting enormous peak obscured any signals from ethanolamine and ammonium nitrate that may have been produced. No signal was observed for diethanolammonium nitrate in the liquid propellant sample (see Figure 22a), but when spiked at a concentration of 30 ppb, a peak was clearly seen (see Figure 22b). Thus, the particular sample analyzed did not have diethanolammonium nitrate present, or it was present at levels lower than the LOD.

Different running buffers were investigated in an attempt to move the hydroxylammonium nitrate peak away from the ethanolammonium nitrate and ammonium nitrate peaks. Increasing the amount of the micellar phase will increase the amount of analyte in the micellar phase, i.e., increase the capacity factor (see Equations 8 and 9). Increasing the capacity factor will improve resolution until the optimum is reached (see the discussion following Equation 10). Triton X, a neutral surfactant, was added in low concentrations to the running buffer containing 90 mM SDS to modify the SDS micelles. The mixed micelle which forms (SDS and Triton X) could have a greater phase ratio (micellar phase to mobile phase ratio) which would increase the capacity factor.

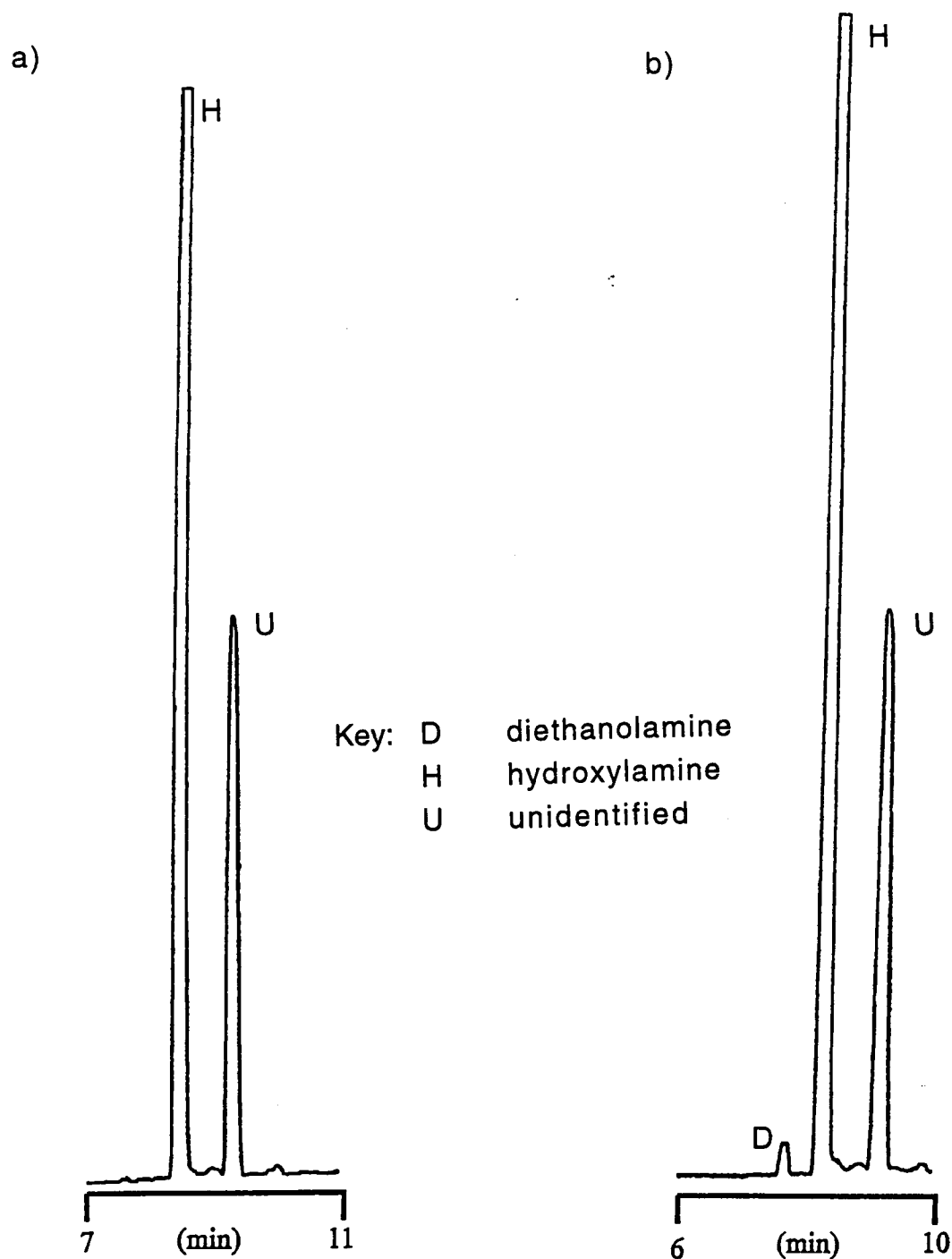
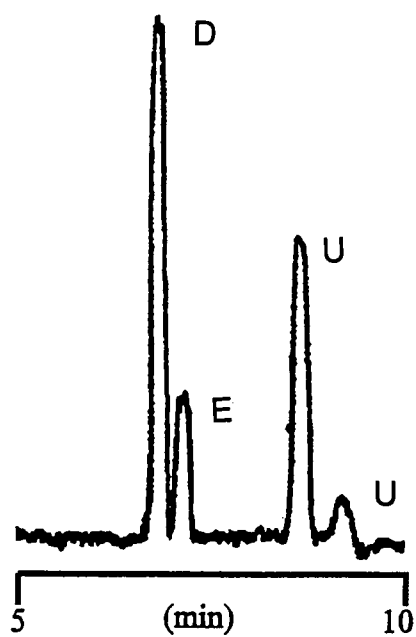


Figure 22. Analysis of derivatized liquid propellant sample. Running conditions as in Figure 20. a) unspiked sample and b) sample spiked with 30 ppb diethanolamine.

Neither the 0.25 or 0.5 % Triton X concentrations appeared to improve resolution. A running buffer consisting of carboxymethyl- β -cyclodextrins at 5 mM with 10 mM sodium phosphate and 6 mM sodium borate at pH 9 was also used to determine effects of a different type of buffer additive. Cyclodextrin-modified capillary electrophoresis uses cyclodextrins doped into the running buffer to separate the analytes. Cyclodextrins are cylindrically shaped, macrocyclic sugar molecules possessing a hydrophobic interior and a hydrophilic exterior. Cyclodextrins alter the retention of the analytes by forming inclusion complexes with the charged species and reducing the electrophoretic mobility (74). To reduce the excessive Joule heating generated, 30% methanol was added. Neither of these running buffers containing cyclodextrins improved the separation.

The maximum concentration of diethanolammonium nitrate allowed in triethanolammonium nitrate that is used in formulating the liquid propellant is 0.5 wt % (86) which is well above the detection limit. Thus, the derivatization and analysis of a triethanolamine sample was performed eight times to determine if diethanolamine was present as an impurity and its concentration if present. Both diethanolamine and ethanolamine were found in the sample at levels of 260 and 22 ppb with coefficients of variation of 8.7 and 9.0%, respectively (see Figure 23).



Key: D diethanolamine
E ethanolamine
U unidentified

Figure 23. Analysis of triethanolamine containing diethanolamine and ethanolamine. Running conditions as in Figure 20.

Conclusions

The indirect detection method developed in this work lacks the necessary precision for the analysis of the two major components of the liquid propellant. Coefficients of variation of less than 1% must be achieved for this to be applicable to that type of analysis. However, these methods are useful for the determination of low levels of impurities utilizing laser-induced fluorescence following derivatization with NBD chloride. The detection limits are more than sufficient (between 2.5 and 14 ppb for diethanolamine, ethanolamine and ammonia) for this application and the reproducibility is quite good. Improvements in reproducibility for both detection methods may be achieved with an auto sampler, commercial instrumentation and data smoothing. Further optimization of the running buffers may also improve precision.

Chapter 5

Concluding Remarks

The research presented in this dissertation develops and investigates novel analytical methodologies for the analysis of several environmentally important metals. A method is also presented for the analysis of amines which are of consequence as a liquid propellant. Significant conclusions are presented below.

When investigating the quantitative aspects of lanthanide metal determinations using CZE with indirect absorbance detection, the background ion identity and the injection method and matrix are of supreme importance. The mobility of the background ion must nearly match that of the analyte ions. If the two mobilities are not approximately equal, band distortion will occur. In this research, tailing peaks were observed because the analyte migrates more slowly than the background. The concentration profile produced causes a tailing peak on the electropherogram. If the two mobilities are approximately equal, the peaks will be symmetrical. Several attempts to find a background ion with a matching mobility for the lanthanide metals separated in this work were unsuccessful as the best one found (2-ethylimidazole) still produced tailing peaks. The main consequences of tailing and an increase in asymmetry include an increase in the LOD, a decrease in reproducibility and a decrease in efficiency.

In an effort to increase resolution between the lanthanide metals, an additive (HIBA) was doped into the running buffer. The mobilities observed for the lanthanide metals are very similar; however, HIBA differentially complexes the metals, thereby improving selectivity (see Equation 7). Each complex has a slightly different mobility. Resolution is also increased by changing the system retention by slowing the analyte mobilities. The complexes exhibit a lower mobility than the uncomplexed metals, thus improving resolution. Of the HIBA concentrations tried (1 to 9 mM), 3 mM was found to provide sufficient resolution without significant contribution to Joule heating.

The injection method, either hydrostatic or electrokinetic, and the sample matrix, either water or running buffer, were also of great importance in determining analytical figures of merit attained in this analysis. Electrokinetic injections of a sample dissolved in water produced very low LODs but lacked the precision necessary for an analytical method. Electrokinetic injections from a sample matrix of running buffer resulted in poor detection limits and reproducibilities. The poor reproducibility may be caused in part by the injection method. During an electrokinetic injection, the ionic strength of the analyte band is increased due to the concomitant increase in concentration of buffer cations within the analyte band. This distorts the peak shape as discussed above. Hydrostatic injections from a sample matrix of water produced the best LODs (approximately 1.7 to 5.4 ppm) due to the stacking which

occurs during the early part of the separation. This stacking can also cause problems with reproducibility and asymmetry. Nevertheless, the best efficiencies are seen when this method of injection is employed. Hydrostatic injections from the running buffer produced the best symmetry and reproducibility because of the matching ionic strengths between the sample and running buffer. No stacking or field inhomogeneities are present to decrease reproducibility. However, without sample stacking, detectability is inadequate for most applications.

This method of analysis for lanthanide metals is probably not sensitive enough for use with realistic samples. In most cases, it is expected that a realistic sample is likely to contain a matrix with a high conductivity, i.e., high salt concentrations. Under these conditions, stacking of the analyte ions will be diminished which will decrease the detectability of this method. However, if the high ionic strength of the sample can be reduced via ion chromatography or selective extraction, an electrophoretic technique could be suitable for the analysis.

Future studies could include searching for a background ion with a mobility which more closely matches that of the analytes and the use of automated instrumentation. These approaches should provide improvements in symmetry, thus efficiency; detectability may also be increased

While developing a screening protocol for the direct determination of low ppb levels of uranyl cation using Arsenazo III

and capillary electrophoresis, it was found that several different experimental parameters (i.e., running buffer constituents and concentrations, column length and injection plug length) strongly affected analytical figures of merit such as the LOD, reproducibility and interferences.

The stacking mechanism elucidated herein involves hydrostatic flow and a reversal in the direction of migration of the analyte complex. The excess Arsenazo III present in the running buffer stacks in the same manner as the analyte complex does, leading to a partially resolved system peak. Although the Arsenazo III has minimal absorbance at the wavelength used, the stacking causes a concentration sufficient to produce a system peak. The analyte peak appears as a shoulder on the side of the system peak; thus, the system peak reduces the detectability of the method.

The running buffer constituents and concentrations were optimized to maximize stacking without generation of excessive Joule heating. Optimal concentrations of running buffer constituents had to be determined for each column diameter used in these studies. For a 50 μm i.d. column, the running buffer contained 75 mM sodium chloride to enhance stacking and sufficient Arsenazo III to prevent dissociation of the complex. Methanol was also added to the running buffer to decrease Joule heating. The LODs found for each column did not follow the expected trend, i.e., the longer path length did not have the lowest

LOD. It is believed that this anomaly is related to the unresolved system peak.

Using the column which produced the best LOD (50 μm i.d.) and the optimal running buffer, the effects of column length and injection plug length were investigated. With a 9 cm effective length column, no resolution between the system peak and the uranyl - Arsenazo III complex peak was observed although the time for the signal to reach the detector was very short (6 minutes). A column with an effective length of 50 cm provided much better resolution; a concentration of 10 ppb of uranyl cation could be detected. A column with an effective length of 31 cm attained resolution sufficient to detect the injected complex in 13 minutes and was used for further studies. The LOD in this column was found by determining which concentration produced a coefficient of variation of 51% (i.e., a signal-to-noise ratio equal to three at 25 ppb uranyl). A calibration curve constructed for the uranyl-Arsenazo III complex exhibits a linear dynamic range of nearly three orders of magnitude of concentration. The optimal plug length, that is, the maximum amount which could be injected before excessive band broadening occurred, was found to be nearly 6 cm.

No other metals were found to interfere in the determination of the uranyl cation by this methodology. Several other metals do form complexes with Arsenazo III, but these are either neutral or negatively charged complexes and, consequently, do not interfere

with the measurement of the uranyl-Arsenazo III complex. Sufficient Arsenazo III is available to complex any metal present, thus avoiding competition between the metals for the ligand. "Real samples" from the Pigeon River and Poplar Creek were analyzed for the uranyl cation. None was found in the Pigeon River sample, although the Poplar Creek sample contained approximately 18 ppb. The other metal ions present, such as calcium and magnesium, did not interfere with the analysis.

The methodology developed herein is simple, rapid and inexpensive. It has separation times and detection limits comparable to a liquid chromatographic method. The removal of the system peak via mathematical manipulation and the use of an automated system should improve both detectability and reproducibility. Overall, this method is a good screening tool for the uranyl ion in realistic samples.

In the determination of the major components of a liquid propellant via CZE with indirect absorbance detection, the identity of the background ion and the dilution factor necessary to bring the analyte peaks on-scale were most important. For the MECC determination of the impurities in the liquid propellant utilizing laser-induced fluorescence detection, the concentration of micelles in the mobile phase was the most significant factor.

The background ion chosen (1,1'-carbonyldiimidazole) for the indirect determination of hydroxylamine sulfate and triethanolamine, was well matched to the analytes with respect to

mobilities, i.e., the peaks produced were efficient. The relatively low pH of the running buffer (pH 4) helped to protonate the analytes which increased the transfer ratio for the indirect detection. The liquid propellant could not be analyzed without great dilution of the sample. It was determined that a dilution factor of 25,000-fold for the hydroxylamine sulfate and 5,000-fold for the triethanolamine produced the best coefficients of variation (1.2 and 3.5%, respectively). Methanol added to the running buffer also improved the reproducibility by decreasing Joule heating.

The analysis of the impurities in the propellant proceeded via derivatization with NBD followed by laser-induced fluorescence detection. The procedure is useful in derivatizing primary and secondary amines. The separation utilizing MECC achieves baseline resolution if a high concentration of micelles is present in the running buffer. Calibration curves constructed for derivatized diethanolamine, ethanolamine and ammonium exhibit linear dynamic ranges of nearly two orders of magnitude of concentration. LODs for each are 3.6, 2.5 and 14 ppb, respectively, with coefficients of variation of about 2.2, 2.9 and 6.3%, respectively.

In a real liquid propellant sample, the hydroxylammonium nitrate is also derivatized and completely obscures the signals produced for ethanolammonium nitrate and ammonium nitrate. However, diethanolamine is well resolved from the major components. Other running buffers were investigated to improve the resolution between the hydroxylammonium nitrate, the

ethanolammonium nitrate and the ammonium nitrate peaks without success.

A small amount of diethanolammonium nitrate is allowed in the triethanolammonium nitrate used in the formulation of the liquid propellant. A sample of triethanolamine was analyzed and diethanolamine and ethanolamine were found. This analysis procedure does not exhibit sufficient resolution for the determination of the major components but would be a good tool for the determination of the purity of the triethanolammonium nitrate with respect to the presence of diethanolammonium nitrate. Future studies could include the use of automated instrumentation in an effort to improve the precision of both the CZE and MECC analyses and the investigation of other micelles and buffer additives in an effort to improve resolution in the MECC determination.

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