IS-T--1819

Separation of Ions in Acidic Solution by Capillary Electrophoresis

by

Thornton, Michelle

PHD Thesis submitted to Iowa State University

Ames Laboratory, U.S. DOE

Iowa State University

Ames, Iowa 50011

Date Transmitted: October 8, 1997

PREPARED FOR THE U.S. DEPARTMENT OF ENERGY

UNDER CONTRACT NO. W-7405-Eng-82.

DISCLAIMER

This report has been reproduced directly from the best available copy.

AVAILABILITY:

To DOE and DOE contractors: Office of Scientific and Technical Information
P.O. Box 62
Oak Ridge, TN 37831

prices available from: (615) 576-8401
FTS: 626-8401

To the public: National Technical Information Service
U.S. Department of Commerce
5285 Port Royal Road
Springfield, VA 22161

TABLE OF CONTENTS

Reprint — Removed for Separate Processing

ABSTRACT

Capillary electrophoresis (CE) is an effective method for separating ionic species according to differences in their electrophoretic mobilities. Separations of anions are usually achieved by adding a quaternary ammonium salt to the electrolyte solution to reverse the electroosmotic flow (EOF). It is now shown that excellent separations of inorganic anions such as the anionic chloro complexes of the platinum group elements (PGEs) and anionic metal oxides are obtained using an acidic electrolyte carrier. The EOF is minimized at an acidic pH which eliminates the need to reverse it. The acidic solution also helps to stabilize some analytes such as metal ions.

CE separations of amino acids by direct detection are difficult due to their similar electrophoretic mobilities and low absorbances. However, native amino acids can be separated by CE as cations at a low pH by adding an alkanesulfonic acid to the electrolyte carrier which imparts selectivity to the system. Derivatization is unnecessary when direct UV detection is used at 185 nm.

Capillary electrophoresis can be used under more acidic conditions than previously used for the detection of metal cations. CE can be used to detect UV absorbing metal cations directly in an aqueous electrolyte carrier near pH 2 with a detection wavelength of 185 nm or 214 nm. Simultaneous speciation of metal cations such as vanadium (IV) and vanadium (V) can easily be performed without complexation prior to analysis. An indirect UV detection scheme for acidic conditions was also developed using guanidine as the background carrier electrolyte (BCE) for the indirect detection of metal cations.

CHAPTER 1. GENERAL INTRODUCTION

Dissertation Organization

This dissertation begins with a general introduction containing a review of pertinent literature. This is followed by a research paper that has been published in a scientific journal and two other research papers which will soon be submitted for publication. Permission from the publisher extending reproduction and distribution rights has been obtained. Each paper is similar to the published version, although additional figures and tables have been added. Figures and tables are contained in the text of the paper at the appropriate location. References cited within each paper are listed after the conclusions of each paper. A general conclusion section follows the three papers.

Literature Review

Principles of capillary electrophoresis

Experimental setup

The capillary electrophoresis (CE) system is comprised of a high voltage power supply connected to electrodes that are placed in two separate vials filled with an electrolyte solution. The power supply is capable of delivering up to 30 kV. The electric circuit is completed by a fused silica capillary filled with the electrolyte solution. One end of the capillary is placed in the sample vial, and the other end is placed in the collection vial. For the determination of inorganic ions, the capillary has a 50 or 75 μm

i.d. and is 50 to 75 cm in length. The capillary passes through a fixed detector. Only a spectrophotometric detector is available on many commercial CE instruments. Figure 1 shows a diagram of a CE system with a high voltage power supply.

Prior to an analysis, a small volume of the sample is introduced into the left end of the capillary. There are two common modes that are used. In hydrostatic injection, the sample is introduced into the capillary by applying a pressure across the capillary while the capillary end is dipped into the sample solution at a given depth for a certain amount of time (e.g. 5 to 50 s). With electrokinetic injection, a voltage is applied across the capillary while the capillary end is dipped in the sample solution.

Theory

After a sample is injected into the capillary, a voltage potential is applied across the capillary which creates an electric field. Charged analytes respond to the electric field by migrating along the capillary. CE separates ions or charged analytes on the basis of their different velocities in the electrolyte filled capillary under the influence of the electric field. The differential migration of the charged analytes moves toward the cathode and passes through the detector before reaching the collection end of the capillary.

The velocity of an analyte is described by the following equation:

$$v = \mu E \text{ (cm/s)} \qquad (1)$$

where

v = velocity of the analyte (cm/s)

μ = mobility of the analyte ($cm^2/V \cdot s$)

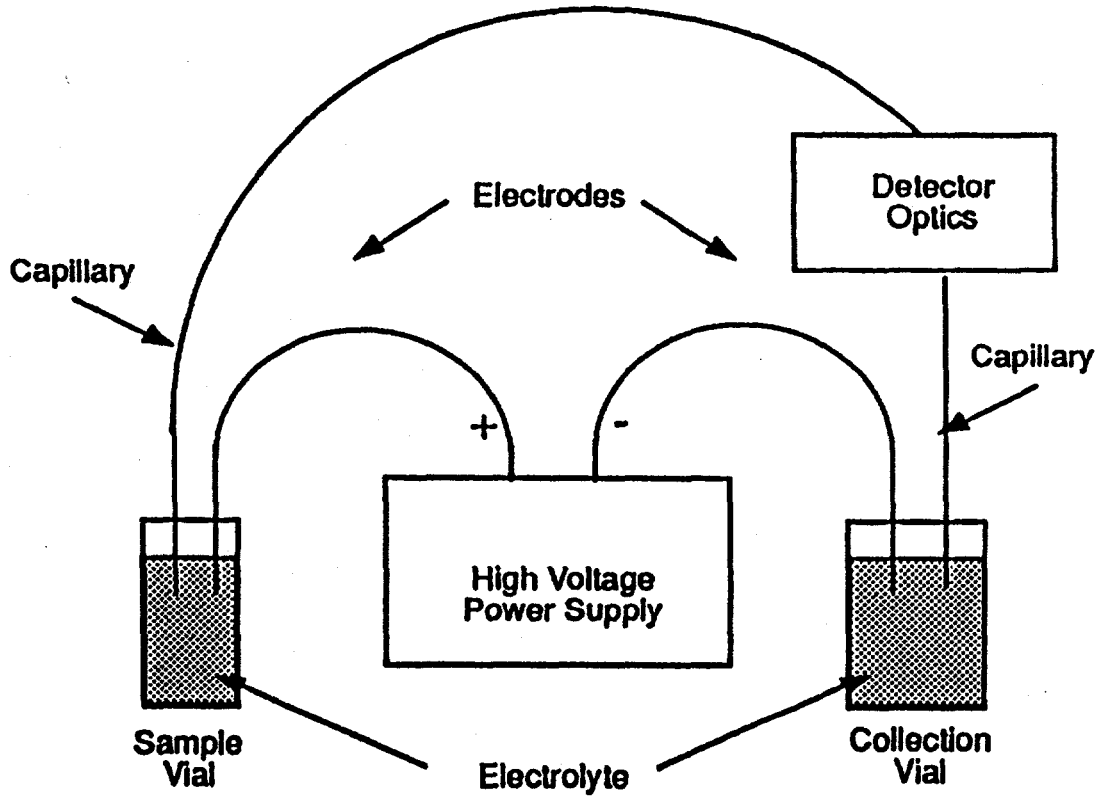

Figure 1. CE apparatus with a positive power supply.

E = strength of the electric field (V/cm)

The field strength, E, is described by the equation:

$$E = V/L \text{ (V/cm)} \quad (2)$$

where

V = applied voltage (V)

L = length of the capillary (cm)

The velocity of an analyte can also be determined by observing its migration time to the detector. The velocity for an analyte can be calculated from the following equation:

$$v = L_d/t_d \text{ (cm/s)} \quad (3)$$

where

v = velocity of analyte (cm/s)

L_d = length of the capillary to the detector (cm)

t_d = observed migration time to the detector (s)

Types of flow

Since the applied voltage and capillary length are the same for all analytes during a separation, the factor that determines the separation is the mobility of a particular analyte. The analyte's total mobility (μ) has two components:

$$\mu = \mu_{ep} + \mu_{eo} \text{ (cm}^2\text{/V·s)} \quad (4)$$

where

μ_{ep} = electrophoretic mobility (cm^2/V·s)

μ_{eo} = electroosmotic mobility (cm^2/V·s)

The overall mobility of any analyte (μ) is the sum of the electrophoretic mobility (μ_{ep}) and the electroosmotic mobility (μ_{eo}).

The analyte's total mobility can also be determined using the observed migration time. The equation for the mobility of an analyte is:

$$\mu = L_d L_t / t_d V \ (cm^2/V \cdot s) \qquad (5)$$

where

μ = total mobility of analyte ($cm^2/V \cdot s$)

V = applied voltage (V)

The electrophoretic mobility (μ_{ep}) is the component that causes the separation of analytes. Negatively charged analytes migrate toward the anode (+), and the positively charged analytes migrate toward the cathode (-). An analyte's electrophoretic mobility is a function of its charge and its size. Analytes with a higher charge have a higher mobility than the analytes with a lower charge. Large analytes move at a slower rate than small analytes.

Theoretical selectivity of ion separations in CE can be predicted on the basis of the equivalent ionic conductivities [1] of the ions, λ_i. The equivalent ionic conductivities are directly related to the electrophoretic mobilities [2], μ_{ep}, of the ions:

$$\mu_{ep} = \lambda_i / F \ (cm^2/V \cdot s) \qquad (6)$$

where F is the Faraday constant. The closer the equivalent ionic conductivities are for each ion, the more difficult the separation. The differences in the equivalent ionic conductivities for anions are sometimes large enough that selective separations are possible [3]. The opposite is true for most cations. Their equivalent ionic conductivities, and thus their mobilities, are usually too similar to expect selective separations.

The second component of analyte mobility is the migration resulting from electroosmosis (μ_{eo}). For most ions, the electroosmotic vector is larger than the electrophoretic vector. This causes the analytes, both cations and anions, to migrate towards the cathode. The cations migrate faster than the electroosmotic flow, and the anions migrate slower than the electroosmotic flow. Separation occurs through differences in the electrophoretic velocities of the ions. Figure 2 shows a vector diagram of analyte migration in a fused silica capillary.

The velocity of the electroosmotic flow (EOF) is dependent on the charge of the capillary wall or the zeta potential. The polarity of the charge on the wall determines the direction of the flow, while the amount of the charge, or zeta potential, determines the magnitude of the flow. The zeta potential is affected by the pH of the solution. Since the silanol groups on the capillary wall are weakly acidic (pK_a = 7-8) [4], the degree of their ionization is dependent on the pH of the electrolyte. The number of dissociated silanol groups increases as the pH increases, and the EOF increases also. The EOF is low up to pH 4.5, rises rapidly between pH 4.5 to 8, and levels off at still higher pH values [5,6]. At about pH 2, the dissociation of the surface silanol groups is completely suppressed, and the value of the zeta potential approaches zero [7]. At this low pH, the EOF should be minimal or approaching zero.

Peak shape

Mikkers et al. [8] studied peak shapes in CE. They found that the peak shape is symmetrical only when the mobility of the carrier electrolyte ion closely matches the mobility of the analyte ion and they have the same charge. Peak tailing occurred when

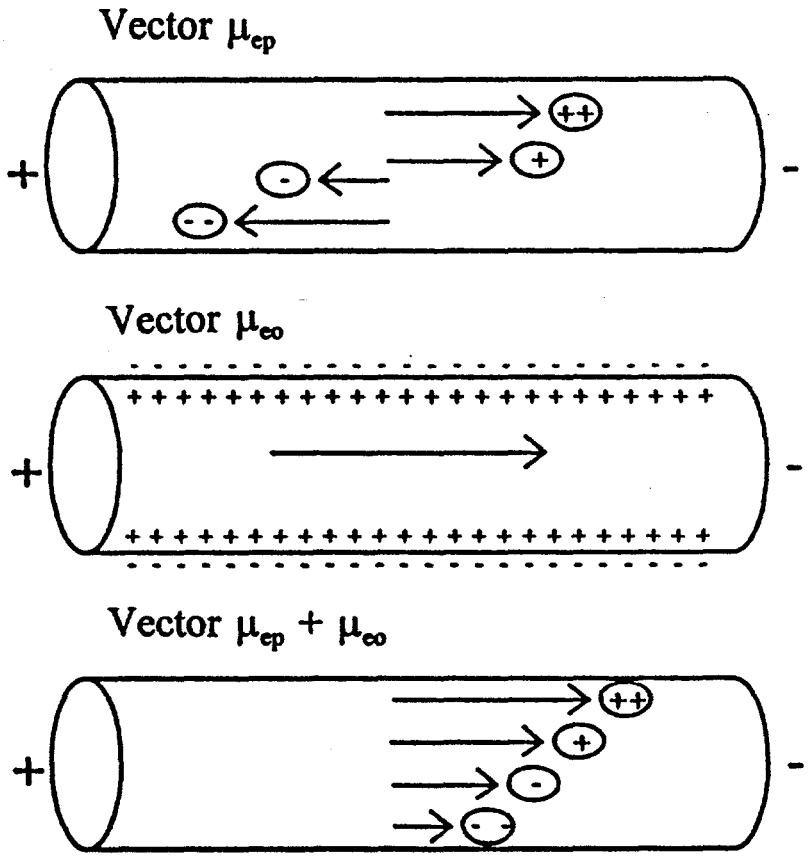

Figure 2. Vector diagram of analyte migration in a fused silica capillary under typical CE conditions.

the mobility of the analyte ion was slower than that of the carrier electrolyte ion. They found that peaks will front when the mobility of the analyte ion is faster than that of the carrier electrolyte ion. They reasoned that the analyte ions must alter the electrical field strength in order for the tailing and fronting to occur. They found that the ratio of buffer to sample concentration affected the degree to which tailing and fronting occurred. They concluded that the buffer concentration needed to be at least 100 times greater than the analyte ion concentration, or else there was a decline in the peak efficiency.

Hjérten [9] discussed peak asymmetry in regards to a conductivity difference observed at the boundary between the migrating analyte zone and the carrier electrolyte. The conductivity difference (Δk) is described by the following equation:

$$\Delta k = c_B[(\mu_A - \mu_B)(\mu_R - \mu_B)]/\mu_B \qquad (7)$$

where

c_B = analyte ion concentration

μ_B = mobility of the analyte ion

μ_A = mobility of the electrolyte co-ion

μ_R = mobility of the electrolyte counterion

According to Equation 7, peak shape in CE can be optimized not only by matching the mobilities of the analyte and co-ion, but also by selecting the correct counter-ion for the carrier electrolyte. The equation also indicates that peak asymmetry increases with an increasing concentration of the analyte ion in the migrating zone.

Electrostacking

Electrostacking, also known as sample stacking, [10-13] is a technique that causes a concentration of the analyte zone into a sharp band. It occurs when a voltage is applied along the capillary tube containing a sample plug with a higher electric resistivity, and thus a lower ionic strength, than that of the surrounding carrier electrolyte. An example of electrostacking is when the sample is dissolved in a low-conductivity solution like water.

The lower ionic strength of the sample zone creates a higher resistance which produces a higher field strength compared to that of the electrolyte. The increased field strength of the sample zone forces the analyte ions to migrate rapidly toward the boundary between the plug and the carrier electrolyte. Once the ions pass the concentration boundary between the sample plug and the carrier electrolyte, they immediately experience a lower electric field and slow down. The net effect of the difference in migration rates is the accumulation of the sample ions inside a very narrow zone at the sample-carrier electrolyte boundary. This leads to an increase in the number of theoretical plates (N):

$$N = L/H \tag{8}$$

where

L = length of the capillary (cm)

H = plate height (cm)

due to the decrease of the height of the analyte zone.

The difference in the concentrations inside the capillary also generates an electroosmotic pressure at the concentration boundary. A laminar flow results from the

electroosmotic pressure and causes extra peak broadening [14]. Sample stacking and the laminar broadening work against each other, so the best matrix for the preparation of the analyte is somewhere between pure water and the normal concentration of the carrier electrolyte used for the separation [13].

Separation of inorganic anions by capillary electrophoresis

Introduction

Capillary electrophoresis (CE) has been used mainly for applications involving the separation of non-metal analytes. In comparison, there have been very few publications dealing with inorganic separations. The majority of these papers involve the separation of inorganic anions. The methods developed for the separations of anions by CE are highly sensitive and efficient with limits of detection in the nanogram range and theoretical plate numbers between 20,000 to 1,000,000 [11,15-17]. These methods very often require the use of an alkaline pH. Direct or indirect spectrophotometric detection has been used, although conductivity [18], and other techniques have also been employed for detection.

Very few papers have investigated the separation of inorganic anions at an acidic pH [10,19,20]. The majority of publications have concentrated on using chromate in the electrolyte carrier at an alkaline pH for indirect detection of inorganic anions [21-24]. In Chapter 2, a method is developed to separate and detect inorganic anions directly using an acidic pH with excellent results.

Direct detection

The complete complexation of metals with ligands to form anions has been investigated [2,25,26]. Aguilar et al. [27] and Buchberger et al. [28] have separated metal-cyanide complexes in CE followed by direct UV detection. Aguilar et al. [27] separated hexacyanoferrate (II) and (III) ions using an electrolyte solution of a 20 mM mixture of NaH_2PO_4 and Na_2HPO_4 at pH 7. The authors stated that the ionic mobilities of the highly negatively charged metal cyanides are so large that they will migrate towards the anode and against the EOF. Buchberger et al. [28] separated 12 metal-cyanide complexes using a 20 mM phosphate buffer containing 1-2 mM sodium cyanide as the electrolyte solution.

A variation of this approach is to add the complexing ligand to the carrier electrolyte [2,29]. This establishes an equilibrium between the free and complexed metal ions. Swaile and Sepaniak [30] reported the separation of Ca (II), Mg (II), and Zn (II) with the chelating agent, 8-hydroxyquinoline-5-sulfonic acid (HQSH). In aqueous solution, the anion, HQS^-, is formed and binds with metal ions to form fluorescent complexes. The electrolyte solution contained 2.5 mM HQS^-, 10 mM Na_2HPO_4, and 6 mM $Na_2B_4O_7$ and was adjusted to pH 8. HQS^- was added to the carrier electrolyte, so that the metal-HQS complexes were formed within the capillary, and detected on-column by laser-based fluorescence.

Indirect detection

Anions are usually analyzed by indirect detection. A background absorbance is achieved by adding a low concentration of an anion that absorbs strongly in the visible

or UV region to the electrolyte solution. The visualization reagent passes through the detector at a fixed rate and establishes the background absorbance. In the sample zone, the concentration of the visualization reagent is reduced by an amount proportional to the sample ion concentration in order to maintain a constant ionic current in the capillary. This results in a negative detection peak due to the decrease in the concentration of the visualization reagent within the sample zone.

The most commonly used anion for indirect detection is chromate [21-24]. The use of chromate for indirect detection was introduced by Jones and Jandik [17] in 1990. A concentration of 5 mM is used usually, and the indirect detection is done at a wavelength of 254 nm. The chromate solution is buffered at pH 8, so it is completely in its ionized form.

In the vast majority of publications, separations of anions are carried out at an alkaline pH to ensure that the analytes and the chemical used for indirect detection will be in the anionic rather than the neutral form. Romano et al. [21] optimized conditions involving the use of chromate to separate inorganic anions, organic acids, or alkylsulfonates. Jandik and Jones [22] separated 30 anions in 3 minutes using a carrier electrolyte adjusted to pH 8.0 and containing 5 mM chromate and an EOF modifier. Wildman et al. [24] used chromate in their carrier electrolyte at pH 8.0 for indirect detection of the weak acid anions, oxalate and citrate, and the inorganic anions, chloride, sulfate, nitrate, phosphate and carbonate, in diluted urine. They were also able to detect the oxyanions of arsenic, arsenite and arsenate, in a matrix of diluted urine that had been spiked.

Electroosmotic flow modifiers

When analyzing for anions, injection is made at the cathode so the anions migrate toward the anode (+) and the detector. The electroosmotic flow (EOF) is in the opposite direction towards the cathode (-). If the electrophoretic mobility of the anions is less than the EOF, a flow modifier is required to reverse the EOF towards the anode so the anions can be detected.

Quaternary ammonium salts (Q^+) are used to modify the EOF. The positive charge of the Q^+ is attracted to the negatively charged silanol groups. The long hydrocarbon chains of the quaternary ammonium salts stick out from the wall and attract additional Q^+ molecules. They are attracted hydrophobically to the hydrocarbon tails, so the charge of the N^+ sticks out and away from the wall. This mechanism provides a net positive charge on the capillary surface which reverses the direction of the EOF. The most common Q^+ reagents are cetyltetraammonium bromide (CTAB) and tetradecyltetraammonium bromide (TTAB).

Timerbaev et al. [25] studied the use of 8-hydroxyquinoline-5-sulphonic acid (HQS^-) for the precolumn formation of negatively charged chelates. By adding HQS^- to the borate buffer at pH 9.2, they were able to separate a mixture of six transition metals in about ten minutes followed by direct UV detection at 254 nm. The fused-silica capillary column needed to be pretreated with a tetraalkylammonium salt to modify the electroosmotic flow. The salt reversed the direction of the electroosmotic flow (EOF) so that the complexes migrated in the same direction as the EOF. Otherwise, the electrophoretic mobility (EPM) could not overcome the magnitude of the EOF.

Zhang et al. [10] were able to separate and detect palladium (II) ($PdCl_4^{2-}$) within

three minutes in the presence of rhodium (III), ruthenium (III), osmium (III), and iridium (III) chloro complexes with an applied voltage of -17 kV and direct detection at 214 nm. They used a carrier electrolyte containing 50 mM HCl-KCl and 0.2 mM CTAB at pH 3.0.

Water-miscible organic solvents are used also to modify the EOF [31-33]. Methanol and acetonitrile are common solvents. Organic solvents increase the viscosity of the solution initially and decrease the dielectric constant [34] and the zeta potential [35]. These factors contribute to the reduction of the EOF.

Benz and Fritz [36] used butanol in conjunction with Q^+ to reverse the EOF. Under typical experimental conditions, a concentration of greater than 0.25 mM Q^+ is required in the carrier electrolyte to reverse the direction of the EOF. They found that by adding a low concentration of 1-butanol, less Q^+ was needed to reverse the EOF. A combination of 4-5% 1-butanol and 0.03 mM Q^+ gave optimum separations of complex mixtures of anions.

Chloro complexes of metals

The initial goal of the research presented in Chapter 2 was to study the separation of the chloro complexes of gold (III) and the platinum group elements (PGEs) ruthenium, rhodium, palladium, osmium, iridium, and platinum. An acidic electrolyte solution containing chloride was needed to form stable chloro complexes and avoid extensive hydrolysis. By operating at a very acidic pH, the silanol groups of a fused silica capillary are less ionized. They become completely protonated at pH 2 [7]. Due to a large number of the silanol groups of the capillary not being ionized, the EOF

would be minimal by working at an acidic pH. This would eliminate the need to use a flow modifier which is used commonly when anions are separated at an alkaline pH with a negative power supply. The flow modifier could also cause precipitation of the metals.

The difficulty is that if a high chloride concentration is present, the electrolyte carrier will have a high ionic strength which results in high conductivity, a large current, and large Joule heating. As the research was being completed, the publications by Baraj et al. [19,20] were noted. They separated $AuCl_4^-$, $PdCl_4^{2-}$, and $PtCl_6^{2-}$ within 18 minutes using an electrolyte solution containing 0.1 M HCl and 0.4 M NaCl. The high concentration of chloride appeared to decrease hydrolysis, but only a low applied voltage of -7 kV and a longer capillary column of 80 cm could be used due to the high conductivity of the carrier solution caused by its acidity and high ionic strength.

Separation of amino acids by capillary electrophoresis

Introduction

The research presented in Chapter 3 of this dissertation involved the separation of the twenty common amino acids as cations and detecting them directly without derivatization. A major challenge in analytical biochemistry is the separation of the amino acids commonly found in protein hydrolysates and physiological fluids. Only a few amino acids like tryptophan, tyrosine, phenylalanine, and cysteine absorb strongly in the range of 230 to 300 nm. Other amino acids only absorb at wavelengths below 220 nm, so many derivatizing reagents have been developed.

Derivatized amino acids

The most widely used detection method for amino acids uses pre- or post-column derivatization of the analytes with fluorescent probes and detection by measuring fluorescence or laser-induced fluorescence (LIF). The most popular derivatization reagents have been those that have already been used with HPLC like o-phthalaldehyde (OPA), fluorescamine, dansyl chloride (DNS), and phenylthiohydantoin (PTH) [37]. Naphthalenedialdehyde (NDA), fluorescein isothiocyanate (FITC), and fluorescamine have also been used. These reagents react with the ionic amino group, so the amino acids need to be separated by micellar electrokinetic chromatography (MEKC).

Liu et al. [38] designed a new fluorogenic reagent called 3-(4-carboxybenzoyl)-2-quinolinecarboxaldehyde (CBQCA) that had optimal migration behavior in CE, reactivity with a variety of peptides, adequate stability of the reaction products, and a matching excitation spectrum of the reaction products with the 442-nm output of the helium/cadmium laser. It formed highly fluorescent derivatives with amino acids and peptides, but required a reaction time of at least one hour prior to sample injection. They were able to separate 17 of the primary amino acids that are commonly found in proteins within 30 minutes using an electrolyte carrier containing 50 mM 2-[N-[tris(hydroxymethyl)methyl]amino]- ethanesulfonic acid (TES) and 50 mM sodium dodecyl sulfate (SDS) at pH 7. They were able to detect quantities in the low attomole (10^{-18} mol) range.

Cheng and Dovichi [39] reported detection limits of less than 6000 molecules by derivatizing the amino acids with fluorescein-5-isothiocyanate and using LIF detection. Albin et. al [40] evaluated the derivatizing reagents fluorescein isothiocyanate (FITC),

fluorescamine, 9-fluorenylmethyl chloroformate (FMOC), and o-phthaldialdehyde (OPA) using both pre- and postcapillary derivatizations with fluorescence detection in CE for the separation of six amino acids. The optimum conditions for precapillary derivatization were with FMOC, 25 mM SDS, and 20 mM sodium tetraborate at pH 9.5. The concentration limit of detection with FMOC was 10 ng/mL. The optimum conditions for postcapillary derivatization were 3.7 mM OPA, 0.5% β-mercaptoethanol (β-ME), and 2% methanol at 40°C. The concentration limit of detection with OPA was 60 ng/mL.

Cladrowa-Runge and Rizzi [41] derivatized amino acids with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC). They investigated the use of native and five modified β-cyclodextrins (β-CD) as buffer additives for enantioseparation of the AQC-derivatized amino acids. The electrolyte carrier contained 5 mM of the β-CD and 10 mM 1,3-bis-[tris(hydroxymethyl)methylamino]propane (BTP) at pH 7.0.

Derivatization with PTH has been popular [42-44] because PTH amino acids absorb strongly around 254 nm, and most commercial CE systems are equipped with UV detection. Otsuka et al. [42] separated 22 PTH amino acids using an electrolyte carrier containing 50 mM SDS. Terabe et al. [43] added 4 M urea to the electrolyte carrier to increase the migration window. Little and Foley [44] added Brij 35 which is a neutral surfactant to the anionic surfactant, SDS, which increased the separation efficiency but decreased the migration window.

Underivatized amino acids

The derivatization process requires considerable additional work which may be time consuming. Derivatization also changes the native electrophoretic mobility of the analytes. The detection of underivatized amino acids can be accomplished by indirect fluorescence [45-48], indirect amperometric [49], refractive index gradient [50], and indirect absorbance [51-54] detection methods.

Olefirowicz and Ewing [49] separated three amino acids using 0.1 mM dihydroxybenzylamine (DHBA), 25 mM 2-morpholinoethanesulfonic acid (MES), 10% ethanol at pH 5.5 with indirect amperometric detection. They achieved detection limits in the range of 500-attomoles. Pawliszyn [50] used a refractive index gradient to detect three amino acids using a 10 mM phosphate buffer at pH 7.

Ma et al. [53] used quinine sulfate for the indirect UV detection of amino acids at 236 nm. The electrolyte carrier contained 8 mM quinine sulfate and 20% ethanol at pH 5.9. Bruin et al. [52] used salicylate for the indirect UV detection of seven amino acids at pH 11.

Lee and Lin [51] separated 17 amino acids within 25 minutes using 10 mM 4-(N,N-dimethyl)aminobenzoic acid (DMAB) at pH 11 with indirect absorbance detection. They separated 19 amino acids using 10 mM p-aminosalicylic acid (PAS) and 0.05 mM Mg^{2+} at pH 10.3 within 50 minutes. Lee and Lin [54] improved their separations by adding cyclodextrins (CD) to the electrolyte carrier. They could separate 20 amino acids except leucine and isoleucine within 35 minutes using 10 mM PAS and 20 mM α-CD at pH 11. They obtained better resolution by replacing PAS with DMAB; however, a 55 minute analysis time was required. Complete CE separation of leucine and isoleucine

could only be achieved by replacing the α-CD with 15 mM β-CD, but it resulted in worse separations for some of the other amino acids.

Little has been published on the analysis of underivatized amino acids using direct absorbance detection. Bergman [55] separated six amino acids using a 50 mM phosphate buffer at pH 2.5 with an applied voltage of 25 kV and direct absorbance detection at 214 nm.

Separation of inorganic cations by capillary electrophoresis

Introduction

The research presented in Chapter 4 of this dissertation involved the detection of metal ions under acidic conditions using direct or indirect UV absorbance. Capillary electrophoresis (CE) has been used for many applications involving the separation of organic analytes. There are a few papers dealing with inorganic separations, but they mainly deal with anions. There are a few reasons for the lack of research involving the use of CE in the separation of metal cations. Other sensitive techniques are available for the detection of metal ions like ion chromatography [21,56-59], suppressed conductivity [60], atomic spectroscopies, and electrochemical methods [61-63]. The lack of a detection scheme was also a problem until Foret et al. [29] solved it by using indirect UV detection.

The first publication involving inorganic cations was in 1967 by Hjérten where he described the separation of bismuth and copper [64]. There were very few publications in the 1970's and 1980's [18,65,66]. The 1990's have shown some activity in this area

with an increase in the number of publications.

Experimental parameters

Research has been done in various areas concerning metals separation using CE. Factors that affect the separation like pH, ionic strength, viscosity, and composition of background carrier electrolyte (BCE) have been investigated. Many papers have dealt with enhancing the separations of metal cations with similar electrophoretic mobilities through complexing agents.

Beck and Engelhardt [67] studied several background carrier electrolytes (BCE) or UV-visualizing reagents for indirect UV detection. They found imidazole to be suitable for the separation of metal ions. Beck and Engelhardt [68] have also reported the use of p-aminopyridine and 2-hydroxybutyric acid for the separation of a few metal ions. Beck and Engelhardt [67] and Weston et al. [69] determined that the mobilities of analyte ions must match that of the BCE in order to achieve good separations.

Weak complexing agents

The addition of weak complexing agents in CE was introduced in the early 1990's by Foret et al. [29]. They used α-hydroxyisobutyric acid (HIBA) as the complexing reagent to separate 14 lanthanide cations within five minutes using creatinine for indirect UV detection. The weak complexing agents work by partially complexing the metal cations so there is an equilibrium between free and complexed metal ions. This increases the differences in the effective mobilities of the metal cations.

Weston et al. [70] used 4.0 mM α-hydroxyisobutyric acid (HIBA) at pH 4.4 to

separate 19 alkali, alkaline earth metals, and lanthanides in less than two minutes using 10 mM Waters UVCat-1 as the UV background-providing component of the electrolyte at 214 nm. They also studied the use of ammonia and citric acid as weak complexers.

Shi and Fritz [71] studied systems containing phthalic acid, tartrate, lactate, HIBA, malonic acid, and succinic acid. They separated 27 metal ions, including the 13 lanthanides, in six minutes using 15 mM lactic acid as the weak complexer at pH 4.25 and 8 mM 4-methylbenzylamine as the UV-visualization agent at 214 nm.

Shi and Fritz [72] reported being able to separate K^+ and NH_4^+ by adding 18-crown-6 ether to the lactate buffer. They also investigated the effect of methanol on the separation. They found that the electroosmotic mobility decreased linearly as the percentage of methanol in the buffer was increased.

Lin et al. [73] studied acetic, glycolic, lactic, hydroxyisobutyric, oxalic, malonic, malic, tartaric, succinic, and citric acids as weak complexing agents for separating a mixture of six alkali and alkaline earth metals using imidazole for indirect UV detection at 215 nm. They all worked, but lactic, succinic, hydroxyisobutyric, and malonic acid gave the best overall performances.

Lee and Lin [74] reported methods of separating cations using glycolic acid, α-hydroxyisobutyric acid, or succinic acid as the complexing agent and imidazole, benzylamine, ephedrine, or pyridine as the carrier buffer and the UV-visualizing agent. Silver (I) and aluminum (III) could not be separated from a mixture of 17 other cations using 10 mM pyridine as the background carrier electrolyte (BCE) and 12 mM glycolic acid at pH 4.0. The same result could also be achieved using 10 mM benzylamine and 16 mM glycolic acid at pH 4.0. Al^{3+} and Ag^+ could only be separated from the 17 ions

using 5 mM pyridine with the pH adjusted to 3.2 by sulfuric acid which also acts as a complexing agent.

pH

Lin et al. [73] looked at the role of the buffer pH. They found that the optimum pH for separating ions in the presence of a complexing agent is around the pK_1 of the acid. They found that operating above the pK_2 of di- and triprotic acids resulted in a decrease in the mobility of the divalent ions and a decrease in the number of theoretical plates due to complex formation.

Weston et al. [69,70] also investigated the role of pH. They found that lowering the pH increased the migration times due to the decreasing electroosmotic flow. This results from a change in the number of dissociated, negatively charged silanol groups on the inside of the capillary wall.

Strong complexing agents

Complete complexation or chelation has also been investigated [25,26,75]. The mobility of the metal cations can be selectively moderated due to the formation of metal complexes which have different stabilities within the capillary. Aguilar et al. [27] and Buchberger et al. [28] have separated metal-cyanide complexes in CE followed by direct UV detection. Motomizu et al. [76] used the UV-absorbing chelate, ethylenediaminetetraacetic acid (EDTA), to form complexes with alkaline earth metals at pH 9.2.

Timerbaev et al. [25] studied the use of 8-hydroxyquinoline-5-sulphonic acid (HQS)

for the precolumn formation of negatively charged chelates. By adding HQS to the borate buffer at pH 9.2, they were able to separate a mixture of six transition metals in about ten minutes followed by direct UV detection at 254 nm. The fused silica capillary column needed to be pretreated with a tetraalkylammonium salt to reverse the electroosmotic flow.

Cationic chelate complexes have also been used to separate metal ions. Timerbaev et al. [77] used 2,6-diacetylpyridine bis(N-methylenepyridiniohydrazone) (H_2dapmp) in a 10 mM sodium borate buffer at pH 9.0 to separate 14 metal ions within 12 minutes followed by direct UV detection at 254 nm. A micellar buffer system was used by adding tetradecyltrimethylammonium bromide to the running buffer to improve the resolution. The ion-pairing counterion was sodium n-octanesulfonate.

Noncomplexing agent

Shi and Fritz [72] reported a method where a complexing agent was not used, and the buffer was also used as UV-visualizing co-ion for indirect detection. They used a 8.0 mM nicotinamide-formate buffer at pH 3.2. They were able to detect Al^{3+} and VO^{2+} indirectly.

Other detection methods

Indirect fluorescence detection has been used in CE. Gross and Yeung [48] used indirect fluorescence detection to look at several metal cations. Bachmann et al. [78] used Ce (III) as a fluorescent carrier electrolyte and was able to detect ammonium, alkali, and alkaline earth metal ions. Swaile and Sepaniak [30] reported the separation

of three divalent cations with the chelating and fluorescent reagent, 8-hydroxyquinoline-5-sulphonic acid (HQS).

Mass spectrometry has also been tried as a detection scheme for CE [79,80]. Corr and Anacleto [79] have used mass spectrometry with an ion spray source to detect metal cations after separation by CE. They evaluated several separation buffers like creatinine, ammonium acetate and tris[hydroxymethyl]aminomethane.

References

1. P. R. Haddad and P. E. Jackson, *Ion Chromatography, (Journal of Chromatography Library, Vol. 46)*, Elsevier, Amsterdam, New York, 1990.
2. A. Weston, P. R. Brown, P. Jandik, W. R. Jones and A. L. Heckenberg, *J. Chromatogr.*, 593 (1992) 289.
3. W. R. Jones, P. Jandik, and A. Weston, *J. Chromatogr.*, 546 (1991) 445.
4. M. L. Hair and W. Herte, *J. Phys. Chem.*, 74 (1970) 91.
5. K. D. Lucas and J. W. Jorgenson, *J. High Resolut. Chromatogr.*, 8 (1985) 407.
6. R. M. McCormick, *Anal. Chem.*, 60 (1988) 2322.
7. P. Jandik and G. Bonn, *Capillary Electrophoresis of Small Molecules and Ions*, VCH Publishers, New York, 1993.
8. F. E. P. Mikkers, F. M. Everaerts and Th. P. E. M. Verheggen, *J. Chromatogr.*, 169, (1979) 1.
9. S. Hjérten, *Electrophoresis*, 11 (1990) 665.
10. H. W. Zhang, L. Jia and Z. D. Hu, *J. Chromatogr. A*, 704 (1995) 242.
11. F. E. P. Mikkkers, F. M. Everaerts and Th. P. E. M. Verheggen, *J. Chromatogr.*, 169, (1979) 11.
12. R. L. Chien and D. S. Burgi, *J. Chromatogr.*, 559, (1991) 141.

13. R. L. Chien and D. S. Burgi, *Anal. Chem.*, 63, (1991) 2042.

14. R. L. Chien and J. C. Helmer, *Anal. Chem.*, 63, (1991) 1354.

15. F. Foret, M. Deml, V. Kahle and P. Boček, *Electrophoresis*, 7 (1986) 430.

16. L. Gross and E. S. Yeung, *J. Chromatogr.*, 60 (1989) 169.

17. W. R. Jones and P. Jandik, *Am. Lab.*, 22 (1990) 51.

18. X. Huang, T. J. Pang, M. J. Gordon and R. N. Zare, *Anal. Chem.*, 59 (1987) 2747.

19. B. Baraj, A. Sastre, A. Merkoci and M. Martínez, *J. Chromatogr. A*, 718 (1995) 227.

20. B. Baraj, A. Sastre, M. Martínez and K. Spahiu, *Anal. Chim. Acta*, 319 (1996) 191.

21. J. Romano, P. Jandik, W. R. Jones and P. E. Jackson, *J. Chromatagr.*, 546 (1991) 411.

22. P. Jandik and W. R. Jones, *J. Chromatagr.*, 546 (1991) 431.

23. W. R. Jones and P. Jandik, *J. Chromatagr.*, 546 (1991) 445.

24. B. J. Wildman, P. E. Jackson, W. R. Jones and P. G. Alden, *J. Chromatagr.*, 546 (1991) 459.

25. A. R. Timerbaev, W. Buchberger, O. P. Semenova and G. K. Bonn, *J. Chromatogr.*, 630 (1993) 379.

26. M. Iki, H. Hoshino and T. Yotsuyanagi, *Chem. Lett.*, (1993) 701.

27. M. Aguilar, X. Huang and R. N. Zare, *J. Chromatogr.*, 480 (1989) 427.

28. W. Buchberger, O. P. Semenova and A. R. Timerbaev, *J. High Resolut. Chromatogr.*, 16 (1993) 153.

29. F. Foret, S. Fanali, A. Nardi and P. Boček, *Electrophoresis*, 11 (1990) 780.

30. D. F. Swaile and M. J. Sepaniak, *Anal. Chem.*, 63 (1991) 179.

31. K. Salomon, D. Burgi and J. Helmer, *J. Chromatogr.*, 559 (1991) 69.

32. S. Fugiwara and S. Honda, *Anal. Chem.*, 59 (1987) 487.

33. J. Gorse, A. T. Balchunas, D. F. Swaile and M. J. Sepaniak, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 11 (1988) 554.

34. G. M. Janini, K. C. Chan, J. A. Barnes, G. M. Muschik and H. J. Issaq, *Chromatographia*, 35 (1993) 497.

35. A. D. Tran, S. Park, P. J. Lisi, O. T. Huynh, R. R. Ryall and P. A. Lane, *J. Chromatogr.*, 542 (1991) 459.

36. N. J. Benz and J. S. Fritz, *J. Chromatogr.*, 671 (1994) 437.

37. W. G. Kuhr and D. Perrett, in P. Camilleri (Editor), *Capillary Electrophoresis: Theory and Practice*, CRC Press, Boca Raton, FL, 1993.

38. J. Liu, Y.-Z. Hsieh, D. Wiesler and M. Novotny, *Anal. Chem.*, 63 (1991) 408.

39. Y. F. Cheng and N. J. Dovichi, *Science*, 242 (1988) 562.

40. M. Albin, R. Weinberger, E. Sapp and S. Moring, *Anal. Chem.*, 63 (1991) 417.

41. S. Cladrowa-Runge and A. Rizzi, *J. Chromatogr. A*, 759 (1997) 157.

42. K. Otsuka, S. Terabe and T. Ando, *J. Chromatogr.*, 332 (1985) 219.

43. S. Terabe, Y. Ishihama, H. Nishi, T. Fukuyama and K. Otsuka, *J. Chromatogr.*, 545 (1991) 359.

44. E. L. Little and J. P. Foley, *J. Microcol. Sep.*, 4 (1992) 145.

45. E. S. Yeung and W. G. Kuhr, *Anal. Chem.*, 63 (1991) 275A.

46. W. G. Kuhr and E. S. Yeung, *Anal. Chem.*, 60 (1988) 1832.

47. T. W. Garner and E. S. Yeung, *J. Chromatogr.*, 515 (1990) 639.

48. L. Gross and E. S. Yeung, *Anal. Chem.*, 62 (1990) 427.

49. T. M. Olefirowicz and A. G. Ewing, *J. Chromatogr.*, 499 (1990) 713.

50. J. Pawliszyn, *Anal. Chem.*, 60 (1988) 2796.

51. Y.-H. Lee and T.-I. Lin, *J. Chromatogr. A*, 680 (1994) 287.

52. G. J. M. Bruin, A. C. van Asten, X. Xu and H. Poppe, *J. Chromatogr.*, 608 (1992) 97.

53. Y. Ma, R. Zhang and C. L. Cooper, *J. Chromatogr.*, 608 (1992) 93.

54. Y.-H. Lee and T.-I. Lin, *J. Chromatogr. A*, 716 (1995) 335.

55. T. Bergman, B. Agerberth and H. Jörnvall, *FEBS Letters,* 283 (1991) 100.

56. D. T. Gjerde and J. S. Fritz, *Ion Chromatography*, Hüthig, New York, 1987.

57. R. E. Smith, *Ion Chromatography Applications*, CRC Press, Boca Raton, FL, 1988.

58. W. R. Jones, P. Jandik and R. Pfeifer, *Am. Lab.*, May (1991) 40.

59. S. C. Grocott, L. P. Jefferies, T. Bowser, J. Carnevale and P. E. Jackson, *J. Chromatogr.*, 602 (1992) 249.

60. N. Avdalovic, C. A. Pohl, R. D. Rocklin and J. D. Stillian, *Anal. Chem.*, 65 (1993) 1470.

61. W. Lu, R. M. Cassidy and A. S. Baranski, *J. Chromatogr.*, 640 (1993) 433.

62. D. A. Skoog and J. J. Leary, *Principles of Instrumental Analysis*, Saunders, New York, 4th ed., 1992.

63. Z. Yi, G. Zhuang and P. R. Brown, *J. Liq. Chromatogr.*, 16 (1993) 3133.

64. S. Hjerten, *Chromatogr. Rev.*, 9 (1967) 122.

65. T. Tsuda, K. Nomura and G. Nakagawa, *J. Chromatogr.*, 264 (1983) 385.

66. J. L. Beckers, Th. P. E. M. Verheggen and F. M. Everaerts, *J. Chromatogr.*, 452 (1988) 591.

67. W. Beck and H. Engelhardt, *Chromatographia*, 33 (1992) 313.

68. W. Beck and H. Engelhardt, *Fresenius' J. Anal. Chem.*, 346 (1993) 618.

69. A. Weston, P. R. Brown, P. Jandik, A. L. Heckenberg and W. R. Jones, *J. Chromatogr.*, 602 (1992) 249.

70. A. Weston, P. R. Brown, P. Jandik, W. R. Jones and A. L. Heckenberg, *J. Chromatogr.*, 593 (1992) 289.

71. Y. Shi and J. S. Fritz, *J. Chromatogr.*, 640 (1993) 473.

72. Y. Shi and J. S. Fritz, *J. Chromatogr. A*, 671 (1994) 429.

73. T.-I. Lin, Y.-H. Lee and Y.-C. Chen, *J. Chromatogr. A*, 654 (1993) 167.

74. Y.-H. Lee and T.-I. Lin, *J. Chromatogr. A*, 675 (1994) 227.

75. S. Motomizu, S. Nishimura, Y. Obata and H. Tanaka, *Anal. Sci.*, 7 (1991) 253.

76. S. Motomizu, M. Oshima, S. Matsuda, Y. Obata and H. Tanaka, *Anal. Sci.*, 8 (1992) 619.

77. A. R. Timerbaev, O. P. Semenova, G. K. Bonn and J. S. Fritz, *Anal. Chim. Acta*, 296 (1994) 119.

78. K. Bachmann, J. Boden and I. Haumann, *J. Chromatogr.*, 626 (1992) 259.

79. J. J. Corr and J. F. Anacleto, *Anal. Chem.*, 68 (1996) 2155.

80. Y. Lui, V. Lopez-Avila and J. J. Zhu, *Anal. Chem.*, 67 (1995) 2020.

CHAPTER 5. GENERAL CONCLUSIONS

The method presented in Chapter 2 was successful. Excellent separations were obtained for the metal chloro complexes as well as for a number of metal-oxygen anions. This is the first time that all of the platinum group elements as their chloro complexes have been separated by CE. Speciation of Ir (III) and (IV) and Pt (II) and (IV) was achieved also.

This method offers significant advantages because metal anions can be analyzed at an acidic pH and detected directly. This is important because the pretreatment of many metals involves the use of mineral acids to dissolve them and keep their solutions stable. Another advantage of this method is the absence of an electroosmotic flow modifier, so there is no possibility of the metals precipitating within the capillary. The advantages of using direct detection are that it offers higher sensitivity and there is no need to match the mobilities of the analytes with that of the visualization reagent. Direct detection, thus offers better peak efficiency in symmetry of shape and sharpness. The method gives excellent results for the separation and detection of the chloro complexes of the noble metal anions and also for metal-oxygen anions. Better separations and baselines with faster analysis times are achieved than have been published previously. Separations of noble metal chloro complexes are better with CE than by HPLC with greater baseline resolution.

Amino acids can be separated in their native form as cations under acidic conditions without being derivatized and can also be detected directly with UV absorbance. The addition of ethanesulfonate, adjusted to an optimum pH, helps to resolve a majority of

the common amino acids from each other. Separation and resolution of the amino acid mixture are achieved through two mechanisms with ethanesulfonate. Selectivity is achieved through ion-pairing interactions between the amino acids and the ethanesulfonate in solution and also through the ethanesulfonate-coating of the capillary wall to prevent adsorption of the amino acids to the bare fused-silica capillary wall.

Capillary electrophoresis can be used under more acidic conditions than have previously been pursued for the detection of metal cations. Chapter 4 presents a method for CE that can be used to detect UV absorbing metal cations directly near pH 2 with a detection wavelength of 185 nm or 214 nm. Simultaneous speciation of metal cations such as vanadium (IV) and vanadium (V) can easily be done without complexation prior to analysis. Of the UV visualization reagents studied, guanidine was found to perform the best under acidic conditions for the indirect UV detection of metal cations.

ACKNOWLEDGEMENTS

I would like to extend my thanks to Dr. Fritz for accepting me into his group and being my major professor. I greatly appreciate the guidance and advice I have received.

I would like to thank all the professors who have served my POS committee. for their patience and support: Dr. Dennis Johnson, Dr. Robert Angelici, Dr. R. S. Houk, Dr. Tim Ellis, Dr. Charles Oulman, and Dr. Patricia Thiel.

I would also like to thank all the past and present Fritz group members including Ron, Wei, Nancy, Phil, John, Luther, Tom, Youchun, Dianna, Xue, and Jie for their help and friendship. It was a pleasure to work with such great people.

I am very grateful for the support I have received from my family and friends. I especially thank my parents, Joe and Denise, for always believing in me and loving me. I want to thank my friends from college, Angie and Cheryl, for their constant support, love, and friendship over the years.

I would also like to thank all the friends that I have made during my time in Ames for adding a dimension of fun to graduate school including Ron & Ann, Charles, Pat, Jay, Steve, Dave, Teresa & Patrick, JeNell & Morgan, Dave, John, Leslie, Nancy, Wei, Karl and Kristi.

I would also like to acknowledge my advisors at the College of St. Catherine, Sr. Mary Thompson and Sr. Betty Shakal, for their encouragement to go into the field of chemistry.

Most importantly, I thank God for all the gifts He has given me to make all of this possible.

I also thank Ames Laboratory and the Department of Education for financial support.

This work was performed at Ames Laboratory under Contract No. W-7405-Eng-82 with the U.S. Department of Energy. The United States government has assigned the DOE Report number IS-T 1819 to this thesis.