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The Separation of Aqueous Iron Species Found in Stepwise Complexation by Capillary Electrophoresis with Atomic Absorption Spectroscopy Detection

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THE SEPARATION OF AQUEOUS IRON SPECIES FOUND IN STEPWISE COMPLEXATION BY CAPILLARY ELECTROPHORESIS WITH ATOMIC ABSORPTION SPECTROSCOPY DETECTION.

by

Robert E. Sutton

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THE SEPARATION OF AQUEOUS IRON SPECIES FOUND IN STEPWISE COMPLEXATION BY CAPILLARY ELECTROPHORESIS WITH ATOMIC ABSORPTION SPECTROSCOPY DETECTION

Robert E. Sutton, Ph.D.
Western Michigan University, 1995

A capillary zone electrophoresis system was interfaced to an atomic absorption spectrometer to permit the separation and detection of various species of iron found in stepwise complexation in aqueous solutions. The samples were introduced at the anodic end of the column employing the electroosmotic flow to drive all the species to the detector at the cathodic end. Aqueous solutions of iron(II) and iron(III) chloride salts were among the compounds studied. The buffer employed was a 20 millimolar (2-[N-morpholino]ethanesulfonic acid adjusted to a pH of 6 with 6.6 millimolar potassium hydroxide. The detection limit was determined to be less than 0.23 parts per million iron.
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Robert E. Sutton
# TABLE OF CONTENTS

ACKNOWLEDGEMENTS .................................................................................................. ii

LIST OF FIGURES .............................................................................................................. iv

LIST OF TABLES ................................................................................................................ v

INTRODUCTION ................................................................................................................. 1

  General Analytical Requirements .............................................................................. 4

SURVEY OF ANALYTICAL TECHNIQUES ........................................................................ 7

LITERATURE REVIEW ................................................................................................... 13

  Capillary Electrophoresis ........................................................................................... 13

    Historical ..................................................................................................................... 13

    Theory ......................................................................................................................... 16

  Atomic Absorption Spectroscopy ......................................................................... 34

EXPERIMENT .................................................................................................................. 39

  Preliminary Study ..................................................................................................... 39

  Equipment ..................................................................................................................... 40

  Procedure ..................................................................................................................... 41

    Data Processing ......................................................................................................... 41

    Sensitivity ................................................................................................................. 47

    Analysis of Samples ............................................................................................ 54

RESULTS .............................................................................................................................. 58
Table of Contents-Continued

CONCLUSIONS .................................................................................................................. 64

APPENDICES

A. Equilibrium Expression for the Stepwise Formation of Complex Ionic Species ............................................................. 73
B. The Specifications for the Bertan Series 230-30R .......................................................... 75
C. The Coefficients Used in the Savitsky-Golay Algorithm ........................................ 77
D. Top of Capillary Electrophoresis Cell/AAS Interface .............................................. 79
E. First Electrophoresis Cell/AAS Interface ................................................................ 81
F. Burner Head-Capillary-Electrode Assembly ........................................................... 83
G. Modified Capillary Electrophoresis Cell/AAS Interface Top ................................ 85
H. Block Diagram of Capillary Electrophoresis/AAS System .................................... 87
I. A List of All Single Peaks From Iron(II) Chloride Samples ................................... 89
J. Distribution Plot of Iron(III) in Chloride Species ...................................................... 91
K. 1 PPM Iron(III) Chloride in 20 Millimolar Potassium Hydroxide ........................... 93
L. The List of Chemicals Used in This Study .............................................................. 100

BIBLIOGRAPHY ................................................................................................................ 102
LIST OF TABLES

1. The Number of Data Points Used in the Filter
   Versus the Dark Current Noise ............................................................... 43

2. The Number of Data Points Used in the Filter
   Versus the Average Signal Noise ............................................................ 44

3. Data Sampling Rate per Second Versus the Dark Current Noise ........... 46

4. Iron Concentration Versus the Average Signal ....................................... 48

5. Microliters of a 1 Part Per Million Iron(III) Chloride Solution
   Versus the Average Signal ....................................................................... 50

6. Time of Pressurization Versus the Weight of Water Eluted ................... 55

7. Data From Electropherograms of Iron(II) Chloride ............................... 59

8. Data From the Electropherogram of Iron(III) Chloride Prepared in a
   20 Millimolar Potassium Hydroxide Solution ......................................... 63
LIST OF FIGURES

1. The Number of Data Points Used in the Filter
   Versus the Dark Current Noise ................................................................. 44

2. The Number of Data Points Used in the Filter
   Versus the Average Signal Noise .............................................................. 45

3. Data Sampling Rate per Second Versus the Dark Current Noise ................ 47

4. Iron Concentration Versus the Average Signal ............................................ 49

5. Microliters of a 1 Part Per Million Iron(III) Chloride Solution
   Versus the Average Signal ........................................................................ 50

6. Time of Pressurization Versus the Weight of Water Eluted ......................... 55

7. Electropherogram of a 0.7 PPM Iron(II) Chloride Sample............................. 60

8. Electropherogram of a 100 PPM Iron(III) Chloride Sample ........................ 61
INTRODUCTION

Metals and their impact on the environment have been a major concern in developed countries for most of the last half of this century. The concern about metals has developed into a need to detect their presence, monitor their movement, and identify their chemical forms, i.e., chemical speciation, in complex aqueous matrices. Approximately 80 of the 109 elements found on the periodic table can be classified as metals. Less than 30 metals have been identified in compounds that produce toxicity in humans.1 The presence of metals in environmental waters occurs from natural erosion and human activities. Many instances of this have resulted in injury or death to living systems being reported.1-2 Pollution incidents involving mercury illustrate the diversity of effects caused by different chemical forms of the metal. Mercury exists in three forms: metallic, organic compounds and inorganic salts, all of which are found in the environment. While all three forms show varying degrees of toxicity, the most toxic form is organic, e.g., methyl mercury,1 followed by metallic mercury and the inorganic species. The inorganic and metallic species toxicity is dependent upon the mode of exposure and the specific chemical properties of the compound.3 Observations such as these support the conclusion that, the toxicity of a metal has more to do with the "chemical speciation" and the reactions involved in its biochemical

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1 Goyer, R.A. Casarett and Doull's Toxicology 1986 3rd Ed. 582-635.
2 Forestner, U., Wittman, G.T.W. Metal Pollution in the Aquatic Environment 1979
3 Fetter, C.W. Contaminant Hydrology 1993 267
transformation than with the total metal concentration. Current methods for reporting a metal's presence are as the total concentration. The focus of this research will be to develop a technique that will separate and identify the different chemical species of a metal in aqueous samples.

In water many metals form multiple species as the metal ions act as electron-pair acceptor (Lewis acids) toward electron-pair donors (Lewis bases) in an important type of chemical reaction, which may be generalized by the equation:

\[ M^{a+} + n[L]^{b-} \rightarrow M[L]_n^{(a-nb)} \]

The resulting product may be an ion pair, or coordination compound. In water the free metal ion may exist as a hydrated ion. These water molecules can be displaced by other Lewis bases. This displacement occurs in a step-wise manner similar to the protonation of polyprotic acids. The step-wise equilibrium constants or \( \beta \) values have been experimentally determined for many metal complexes. The equilibrium constants and concentration of ligand can be used to calculate the ratios of the free metal and each "metal complex species" in that system to the total metal concentration (see Appendix A). Plotting the fraction of the chemical species versus the concentration of the ligand is referred to as an alpha plot or distribution diagram. Where there are multiple systems in which a metal can form a complex, the free metal concentration could be reduced to levels that prevent precipitation. A technique able to separate and

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4Ebbing, D.D., Wrighton, M.S. General Chemistry 1993 4th ED. Chapter 23 980

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identify the different chemical species of a metal could aid in establishing the environmental importance of these species in a complex aqueous matrix.

Iron is the metal selected for study in this research. There are reports of some toxicity of iron\(^5\), however, these were not the reason for this selection. This metal was selected after examination of the results of work by Passero, Kehew and Rudder (1988)\(^6\) on the KL Landfill, west of Kalamazoo, Michigan, a current superfund site. The modeling of chemical analysis from the groundwater data collected at the landfill, using the software "Wateq's", revealed an oversaturation of iron minerals. In the explanation of the chemistry of the groundwater they proposed the possibility of metal-ligand complexation of iron. Rudder attempted to identify possible ligands present in the samples using high pressure liquid chromatography (HPLC) with ultraviolet detection.\(^7\) While metal-ligand complexation is a reasonable conclusion, there is also the possibility that the high levels of iron result from a hydrophilic colloidal dispersion with humic and fulvic substances. This research will pursue the development of an analytical technique based on identifying the species in a step wise complexation. The question addressed is: Can different species of iron complexes or other metal complexes be separated and uniquely identified in an aqueous sample?

\(^5\) Goyer, op. cit., p 613-14
General Analytical Requirements

The interest in the speciation of metals as an environmental concern has spurred many new analytical efforts. These efforts must meet the objectives of general analytical methods, i.e., selectivity, sensitivity, accuracy, precision, speed and cost. In the attempt to develop a technique for the chemical speciation of a metal, all these factors will be considered.

Selectivity for most chemists refers to the degree to which the analytical technique is free from interference by other species found in the sample matrix. One way to obtain selectivity is by the choice of separation technique. Often by using some chemical or physical property, the analyte can be isolated from the possible interferences. Selectivity can also be achieved through the detection technique. The more specific detection techniques will respond to only a limited class of compounds eliminating many interferences. The use of both separation and detection should provide the optimal selectivity for this project.

When considering sensitivity, the chemist looks at an instrument or a method and measures its ability to detect small changes in analyte concentration. This must be dependent on the precision of the measurements as well as the slope of the standard curve. When two methods have the same precision, the standard curve with the greater slope will have the greater sensitivity. Where the slopes of the two standard curves are equal then the method with the greater precision is more sensitive. Other factors must be met to obtain the quantitative results needed to evaluate sensitivity.
The work here will consider first the detection limit of the technique. The detection limit as defined by Skoog$^8$ is "the minimum concentration or weight of analyte that can be detected at a known confidence level". This detection limit depends on the blank signal from the instrument measurements. The equation for the minimum signal is:

\[ S_m = S_{bl} \cdot k \]

where \( S_m \) is the minimum acceptable signal from the analyte, \( S_{bl} \) is the mean blank signal, the standard deviation in the blank signal is \( s_{bl} \), and \( k \) the multiple of the standard deviation of the blank signal. Kaiser$^9$ argued for the value of \( k \) equal to three, and pointed out that the confidence level will be 89% or higher in all cases. This will be the criterion used for the data in this study.

The reproducibility of measurement is the precision of the technique or method. The figures of merit for precision include absolute standard deviation, relative standard deviation, standard deviation of mean, coefficient of variation and variance. Precision usually refers to the interpretations of quantitative data, however in this study it will be applied to qualitative data, i.e., retention times.

Accuracy is the closeness of a measure to the true or actual measure. This is a quantitative measure that can be evaluated with a laboratory sample using a given technique. Accuracy can be tested with a fortified sample and the technique evaluated. This is the procedure that will be considered in this study.

$^8$Skoog, D.A., Leary, J.L. Principles of Instrumental Analysis 4th Ed. p 7
The cost of analysis directly affects the use of the technique or method. The cost can be in time, equipment, or reagents. To keep the cost at a minimum, currently available equipment will be used and reagents that are easily obtained. The time in sample preparation will also be considered as every effort will be made to make this a cost-effective technique. As the technique meets the initial requirements set for it the other objectives will be considered.
SURVEY OF ANALYTICAL TECHNIQUES

As a preliminary step in this investigation a survey of various analytical techniques was made to decide which technique might offer the greatest advantage. The techniques were considered for their ability to separate the chemical species of metal under study. The detection technique was considered for its selectivity and ability to quantitate.

The simplest technique reported for the separation of metals is ultrafiltration. The differentiation of particulate and filtrate is determined by the pore size of the filter medium. The 0.45 micron filter is a common choice. Particulate materials include solids, metal crystals, metals absorbed onto humic substances or other surfaces and metals incorporated into organisms. The filtrates consist of free metal ions, inorganic complexes or complexes with small organic ligands. To change the make up of the particulate and filtrate a different pore size can be selected and even used in tandem.

Solid phase extraction is a technique that can also separate species. The separation is based on the affinity of the analyte for selected solid sorbants. The commercially available sorbants are anion, cation exchange materials, and a variety of organic phases, to extract neutral species. This technique has been successfully used with environmental samples.\textsuperscript{10-11}

Gas chromatography (GC) is a technique used for the separation and quantitation of multiple species. The resolution power of this technique has been a key in many current analytical methods. The resolution is determined by the difference in affinities of the analytes for the stationary solid or liquid phase versus the mobile gas phase. There are many solid phases and gases available for a variety of compounds that can be analyzed with this technique. Another significant advantage of gas chromatography is its ability to be interfaced with a variety of detection systems. Most of these detection techniques have good sensitivity and selectivity.

The resolution power of the high pressure liquid chromatography (HPLC) is theoretically greater than gas chromatography. High pressure liquid chromatography as an analytical technique has fewer sample restrictions than found in GC. This increases the number of compounds that can be analyzed by HPLC. High pressure liquid chromatography is also based on the affinities of the analytes for the liquid mobile phase versus a liquid or solid stationary phase. The degree of polarity of each phase is dictated by type of compounds analyzed. Interfacing of this technique to others must consider the elution volume of mobile phase containing the analyte.

Capillary electrophoresis (CE) has shown great resolution power comparable to both gas chromatography and high pressure liquid chromatography. Capillary electrophoresis has several different operational modes for the varied classes of compounds analyzed by this technique. Most of the separations are based on the

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charge-to-mass ratio of the analytes. There are a variety of detection techniques that can be interfaced with this technique.

Conductivity is a universal technique of detection based on Ohm's Law. The charged particles passing a set of electrodes, at a constant voltage, cause a change in the resistance and current measured. This technique requires special adaption for use with a capillary and may be useful in this research.

For detecting trace metals, i.e., between $10^{-5}$ and $10^{-9}$ molar, anodic stripping voltammetry is well adapted. The metals are reduced and deposited on to a stationary electrode. This electrode is often a hanging drop of mercury, a thin film of mercury on nickel or platinum wire, or a solid microelectrode. The electrolysis of one or more electroactive species can be done simultaneously using a slow linear voltage sweep. Identification is based on the potential measured at which Faradaic oxidation or reduction occurs. Quantitation is effected by the measured Faradaic current at a particular potential.

Mass spectroscopy (MS) is a detection technique that provides both sensitivity and selectivity. The analyte must be volatile and is commonly used for the analysis of organic molecules. The requirement of vaporization of the analyte makes metals difficult to analyze. It has also been reported that occasionally some MS detectors suffer degradation due to contamination from metals.\textsuperscript{12}

Ultraviolet/visible (UV/vis) spectroscopy is primarily used for quantitation of organic and colored substances at the detection stage of a method. This is often

\textsuperscript{12}Kramer, J.R., Allen, H.E. Metal Speciation Theory, Analysis and Application 1988 Ch. 8 p 158
described as a universal detector as there are many compounds that exhibit an absorbance spectrum in the UV/vis region. The quantitation is limited to the concentration range over which Beer's Law applies. The UV/vis technique can be applied to flowing analytes for direct detection of absorbing compounds or indirect detection with a solvent that absorbs and analytes that do not.

Fluorescence spectroscopy is a sensitive detection technique. There are fewer compounds that luminesce than absorb in the ultraviolet region. The combination of the excitation and emission wavelengths increases the technique's selectivity. The position of the detector relative to the source reduces background and increases sensitivity. The sensitivity can be increased even more through laser induced fluorescence.

Atomic emission spectroscopy (AES) is a highly selective detection technique with quantitative capability for all elements emitting in the UV/vis region of the spectrum. This technique traditionally uses a flame as the energy source, although other more costly sources are available. Some metals are particularly suited for this method since they exhibit strong emissions. Multiple elements can be analyzed in a single sample. The flame method does however require concentrations greater than the low trace levels anticipated in this study.

Inductively coupled plasma is an emission technique but it has a more energetic and costly source. The source is an argon plasma sustained by a high-frequency field. This results in a higher signal to noise ratio and detection levels are in the parts per billion range. The increased energy enables the monitoring of the more intense ion
lines for some compounds rather than the neutral atomic lines. This further increases the detection limits for some metals.\textsuperscript{13}

Atomic absorption spectroscopy (AAS) has both the selectivity and precision of AES for metals. The wide variety of source lamps enables the analysis of many metals. The use of an element specific lamp, often a hollow cathode lamp, eliminates many possible interferences and enhances the selectivity. The sensitivity of this technique has made it a choice for many metal analyses.

Examination of the techniques surveyed, produced the following conclusions. The technique of ultrafiltration is simple but does not provide the selectivity needed for the separation of the chemical species of iron anticipated to exist in natural aqueous samples. Solid phase extraction can separate compounds based on charge. It is within a charged group that additional separation is required. Anodic stripping voltammetry has the sensitivity but information on the voltages for the different species of iron may be difficult to ascertain, particularly with samples of complex matrices. Conductivity detection offers the possibility of establishing some data on standard solutions. However, its usefulness on samples with complex matrices may be limited. Gas chromatography is limited by the requirement that the analyte be volatile. Only a few metals are reported to produce volatile hydrides and fluorides that can be analyzed by this technique.\textsuperscript{14} The use of high pressure liquid chromatography presents a problem with the detection technique. These techniques suffer due to the dilution

\textsuperscript{13}Skoog, op. cit., Ch. 11 p 236
\textsuperscript{14}Kramer, op. cit. Ch 8 p 158

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effect by the considerable elution volumes of mobile phase containing the analyte. Mass spectroscopy's requirement of vaporization and potential for detector degradation make metals' analyses difficult. The ultraviolet detectors are too general and many analytes anticipated in this study do not exhibit significantly strong absorbance bands in this range. Fluorescence has great sensitivity, but the compounds selected for this study are not noted for luminesce. Atomic emission spectroscopy is not the suggested technique for iron analysis due to its modest sensitivity and significant potential for interferences. There was no inductively coupled plasma instrument available for this study and the high cost prohibited its purchase. The techniques that appear to have the most promise are capillary electrophoresis separation coupled with atomic absorbance spectroscopy detection. The resolution power of capillary electrophoresis should be adequate for selectivity, as the charge and mass of the species will change as the water molecules are replaced with ligands. These differences will be the basis for attempting the separation. Electro-focusing of the analyte by capillary electrophoresis should decrease the detection limits. The specificity of the atomic lines used in atomic absorption spectroscopy will eliminate many possible interferences. The sensitivity of this technique is often instrument dependent. The interfacing of these techniques will pose a challenge, but if accomplished, should give the desired capability.
LITERATURE REVIEW

Capillary Electrophoresis

Historical

The original work on electrophoresis for separation is credited to Arne Tiselius' Ph.D. thesis in 1930. The title of this work was "The Moving Boundary Method of Studying the Electrophoresis of Proteins".\textsuperscript{15-16-17} Later, in 1948, Tiselius received the Nobel Prize for the partial separation of a protein mixture in free solution. The separation was as contiguous bands in U-shape quartz tubes and detected using ultraviolet (UV) absorbance.\textsuperscript{18} There were inherent limitations to this moving boundary method that included incomplete separation of the sample, a requirement of large volumes of sample, thermal diffusion and convection. These limitations caused the development of this method to be carried out in anti-convection media, such as polyacrylamide or agarose gels. These types of materials in slabs or tubes were used for the size dependent separation of biological macromolecules, i.e., nucleic acids and proteins. While these methods have become the standard separation techniques, slab

\textsuperscript{15}Heiger, D. High Performance Capillary Electrophoresis-An Introduction \textbf{1992}, p 15
\textsuperscript{17}Vesterberg, O. History of Electrophoretic Methods Journal of Chromatography, \textbf{1989}, 480 3-19
\textsuperscript{18}Landers, J.P. Introduction to Capillary Electrophoresis: Handbook of Capillary Electrophoresis \textbf{1994} , Ch 1 p 1
gel electrophoresis suffers from long analysis time, low efficiencies and problems with detection and automation.

To address some limitations, new approaches were considered. The use of smaller bore tubes, also called open tube electrophoresis, was initially reported by Hjertén in 1967. In the instrument's description, Hjertén used quartz capillary tubes with a 1- to 3-millimeter internal diameter (I.D.) and immersed them in a cooling bath. The cooling bath was an attempt to minimize the convective mixing by continuously rotating them along their longitudinal axis. To accomplish this there was a movable carriage on to which the electrode reservoirs and capillary were mounted. The detector was a fixed UV monitor that the capillary was driven past. This "scanning" detector enabled the peak profile to be continuously monitored during the separation. To reduce the adsorption of the analyte on to the tube surface and the electroosmotic flow (EOF), the internal surface of the tube was coated with a nonpolar compound. With this instrument Hjertén accurately determined the mobilities of analytes and demonstrated the ability to separate inorganic ions, proteins, nucleic acids, and microorganisms by "free zone electrophoresis" and isoelectric focusing. The performance of the system was limited due to variations in the optical quality along the capillary axis and the large internal diameter of the tube.

The problem with the internal diameter of the tube was addressable with the development of tubes with smaller internal diameter. It was Virtanen and then

19 Ibid. p 2
20 Ibid. p3
Mikkers\textsuperscript{21} who made and used 200 \(\mu\)m i.d. capillary columns from glass and Teflon, respectively. Virtanen's use of these smaller diameter capillaries, demonstrated that Hjertén's 1967 prediction that smaller i.d. tubes could obviate the need for rotation of the capillaries to eliminate convection. This alternative to the slab-format offers the advantage that these narrow tubes are themselves anti-convective and the gel media was no longer necessary.

The next advances in capillary electrophoresis were in the early 1980s when Jorgenson and Lukacs\textsuperscript{22} began using 75 \(\mu\)m i.d. fused silica capillaries. The theory of electrophoresis was clarified as they described the relationship between the operational parameters and the separation quality. They demonstrated the potential of capillary electrophoresis as an analytical technique. The work by Jorgenson and Lukacs has been identified as the beginning of the modern era of capillary electrophoresis. Their papers included descriptions of simple research instruments consisting of a high voltage power supply, fused capillary column, electrode reservoirs and often a modified HPLC optical detector. The methods of sample introduction reported were siphoning, e.g., raising of one end of the column a predetermined height or dipping the end of the column into the sample and applying a voltage for a preset time interval. The columns identified were similar to those used in GC with an i.d. of 75 - 100 \(\mu\)m and a polyimide outer coating. The columns were underivatized silica surfaces and caused high electroosmotic flows. This electroosmotic flow along with the

\footnotesize
\textsuperscript{21}Heiger, op. cit. p 12


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electrophoresis mobility caused the motive force to drive anions, neutral species and cations past the detection point. For the UV detection, a section of the coating, "a window" had to be burned away. This simple system provided excellent separation of both large and small molecules. Today there is substantial growth in CE commercial instrumentation and publications in this area are expanding exponentially.

**Theory**

Electrophoresis has been defined as the differential movement of charged species (ions) by attraction or repulsion in an electric field. The three basic categories of electrophoresis are moving boundary, high-resolution electrophoresis, and zone. Each category offers advantages in the analysis or sample preparation.

"Moving boundary electrophoresis is a frontal separation method that usually involves migration of ionic solutes in a U-shaped tube. This technique involves the continuous sample addition onto the tube, resulting in a series of concentration boundaries forming as each solute migrates at a different rate. Frontal analysis is often used as a preparative rather than an analytical technique.

High-resolution electrophoresis involves "steady state" methods such as isoelectric focusing or isotachophoresis. Isoelectric focusing is carried out in a medium with a pH gradient and is commonly used to separate proteins. The pH gradient is formed within the capillary using ampholytes. These molecules are zwitterionic having both acidic and basic moieties. The solutes and ampholytes are

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23 Wallingford, op. cit.
introduced into the column with a basic solution at the cathode and an acid solution at the anode. Within the capillary the pH values can be varied as required by the experiment. This is determined by the pH of the acid and basic solution in the reservoirs and the ampholytes. When the voltage is applied the solutes migrate until they reach the pH where they become uncharged, i.e., the isoelectric point (pI). Any other protein passing through a zone will migrate to the pH region equal to its pI. This process is known as "focusing". Completion is established when a steady state is reached and current no longer flows. The solute and ampholytes are removed by pressure or changing the reservoir by adding salt. Isotachophoresis involves the use of electrolyte ions with higher and lower mobilities leading and trailing the plug of solute. To analyze cations for example the cations in the leading electrolyte must have a greater mobility than the analyte and the trailing electrolyte a lower mobility. When a voltage is applied, the cations in the leading electrolyte move fastest into a zone followed by zones each of the slower moving cations. The velocity of these zones is established by the mobility of all the leading electrolytes' cations. If a cation diffuses into another zone its velocity changes and returns to its own zone. This is a preconcentration step commonly employed before analysis by a mode of zone electrophoresis.

Zone electrophoresis introduces a narrow solute plug (or spot) into the potential field and is the technique most analogous to chromatographic techniques. Modes of zone electrophoresis that use capillaries are micellar electrokinetic chromatography (MEKC), capillary gel (CGE), and capillary zone electrophoresis.
Micellar electrokinetic chromatography can be used to separate neutral and charged solutes. This is accomplished by the addition of surfactants in the running buffer. Surfactants at concentrations above the critical micelle concentration form aggregates of individual molecules and micelles. Micelles are formed as the hydrophobic tails of surfactants are oriented toward a center producing a spherical shape. The neutral solutes' interaction with the micelle provides the charge and a electrophoretic mobility. The solutes then can move with the electroosmotic flow for cationic micelles and against the EOF for anionic micelles. These neutral solutes will partition between the micelle and the buffer and this effects separation. The interaction of the more hydrophobic the solute with the micelles results in more mobility.

Capillary gel electrophoresis is principally used as size-based separation. In the biological sciences this technique is used for the separation of macro-molecules, such as proteins and nucleic acids. This technique is directly comparable to slab or tube gel electrophoresis since the principles for separation are the same.

Capillary zone electrophoresis offers the basic advantages of MEKC and CGE, and was selected for this research. This mode can be used to separate solutes based on size and charge. Those solute particles with the same size and charge move into a narrower band, increasing their concentration. This separates them from particles of different sizes and/or charges and other bands or zones are produced. CZE accomplishes this without surfactants or gels. This combination of abilities was essential to the goals of this research, and CZE offered other advantages as well.
These advantages include the speed of analysis, efficiency, sample size, and the ability to reduce convection. Some of these advantages help to meet the general analytical requirements. Capillary electrophoresis' analysis time is usually in minutes. The efficiency results from the capillary producing up to three million theoretical plates. Sample size is usually in nanoliter volumes with nanograms of solute. An ability to reduce convection with heat dissipation overcomes the major limitation in normal electrophoresis. The parameters that affect these advantages are the electrokinetic effects, the pH, the ionic strength of the buffer, the viscosity of the solvent, the radius of the capillary, and the field gradient. These parameters will be considered for examination and their impact on obtaining the desired analytical results.

Speed of analysis is one key advantage of CZE. The speed or migration rate of the analyte is the result of the electrokinetic effects. The factors that affect the electrokinetics are the electrophoretic mobility, electroosmotic mobility, and electric field gradient. The apparent velocity or migration rate is expressed in the equation:

$$v_{\text{app}} = \mu_{\text{app}} E = (\mu_{\text{ep}} + \mu_{\text{eom}}) E$$

where $v_{\text{app}}$ is the apparent velocity, $\mu_{\text{app}}$ is the apparent mobility, $E$ is the field gradient and is the ratio of the voltage applied to the total length of the column, $\mu_{\text{ep}}$ is electrophoretic mobility and $\mu_{\text{eom}}$ electroosmotic mobility.\(^{24}\) The relationship to analysis time is expressed as follows.

\(^{24}\)Landers, op. cit. Ch 2 p 17
where $L$ is the length of the capillary, and $V$ is the applied voltage. The electrophoretic mobility is dependent on the particular analyte. The electroosmotic mobility affects all particles both charged and uncharged. Equation [3] shows that the shortest analytical time to achieve separation is facilitated by short columns and high voltages.

It is the differences in electrophoretic mobility of each analyte that allows the separation of the solutes. The electrophoretic mobility ($\mu_{\text{ep}}$) of a charged particle can be approximated by the Debye-Hückel-Henry equation:

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

where $q$ is the charge of the particle in coulombs, $\eta$ is the viscosity of the buffer in poise (gm cm$^{-1}$ sec$^{-1}$) and $r$ is the Stokes' radius of the particle in cm. The charged particles of concern in this study are metal ions and metal-ligand complex ions. Metals are reported to exist as hydrated ions but the water molecules may be replaced by other ligands. This can change the overall charge and mass of the ion. The viscosity of the solution is altered by the addition of organic solvent. Changes in the viscosity affect both charged and uncharged particles. The relationship between the Stokes' radius of a particle or molecule and its mass can be expressed by the following:

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25 Jorgenson, op. cit
26 Landers, op. cit. p 15

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\[ M = \frac{4}{3} \pi r^3 V_{sp} \]  \hspace{1cm} [5]

where \( V_{sp} \) is the spatial volume of the particle and \( M \) is the mass.\(^{27}\) The relationship between the radius cubed and mass and applying equation [4] shows that species with smaller mass will have greater electrophoretic mobility than larger molecules of the same charge. Empirical data suggests that modifications of the Debye-Huckel-Henry equation must be made to allow for non-spherical shapes, counter ion effects and nonideal behavior of biological molecules.\(^{28}\) Differences in particle mobilities can provide the power to even resolve chiral compounds using capillary electrophoresis. Use of the electrophoretic mobility to differentiate between analytes demands control of the electroosmotic mobility to ensure ample time for separation.

The electroosmotic mobility is an electrokinetic effect and causes the bulk flow of solution. This type of flow is called electroosmotic or electroendosmotic flow (EOF). There are three conditions that must be satisfied to generate electroosmotic flow. These conditions are the application of a d.c. voltage along the column, the presence of charges on the inner surface of the column, and the presence of a conductive solution within the column. The electroosmotic flow has been described as the "wall effect" and the "pumping effect". Electroosmotic flow was described by Helmholtz in the late 1800s when he carried out experiments where an electric field was applied to a horizontal glass tube containing an aqueous salt solution.\(^{29}\)

\(^{27}\) Landers, op. cit p 15
\(^{28}\) Ibid
\(^{29}\) Vesterberg, op. cit. p 5

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Helmholtz reported that negative charges imparted to the inner surface of a glass tube led to the movement of the ions toward the cathode. In a capillary the hydrated cations draw solvent along with them as they migrate toward the cathode. This movement of solvent causes the flow of neutral and negative charged particles in a column as well. The flow direction from the anode (+) toward the cathode (-) of the capillary is considered "normal" polarity for electroosmotic flow. The electrophoretic mobilities cause the cations to migrate faster than the EOF. The neutral species migrate at the same rate as the EOF since they have no electrophoretic mobility. The negatively charged particles migrate slower than EOF as they are subjected to the attraction from the anode and have in effect a negative electrophoretic mobility. All particles are eventually moved toward the cathodic end of the column where the detection device is typically located.

The charge on most solid surfaces is the result of an excess of negative particles. This can be the result of ionization of the surface, i.e., acid-base equilibrium, the adsorption of ionic species at the surface or the nature of the surface. For glass all these processes take place. On glass or silica the ionization of the silanol groups (SiOH) to the anionic form (SiO⁻) is a major source of charge. The number of silanol groups on a glass surface has been estimated to be between three and seven per 10 Å.³⁰ When the charged surface is in contact with a buffer, it results in a double layer. Counter ions (cations) are attracted to the solid's surface to maintain a charge balance. The closest or inner layer is called the Stern layer or inner Helmholtz plane and the

³⁰Landers, op. cit Ch 22 p 564
outer layer is called the outer Helmholtz plane or the diffuse double layer. This double layer creates a potential difference very close to the wall. This potential is called the zeta potential ($\zeta$). Electroosmotic mobility can be expressed by the equation:

$$\mu_{\text{EOF}} = \frac{\epsilon \zeta}{\eta}$$  \hspace{1cm} \text{[6]}

where $\epsilon$ is the dielectric constant of the solution, $\zeta$ is the zeta potential and $\eta$ is the viscosity.\textsuperscript{31} To influence the electroosmotic flow, changes can be made in the voltage applied to the column, the buffer solution, or the column's surface.

The electroosmotic flow can add to the electrophoretic velocity of the analyte and reduce the migration time until the desired separation may not be achieved. Any changes in zeta potential will produce proportional changes in electroosmotic flow. The zeta potential changes with changes in pH and ionic strength of the buffer. A buffer's pH determines the amount of ionization of the silanol groups and the surface charge. Over the pH range of three to eight, electroosmotic flows vary in glass, silica and Teflon.\textsuperscript{32} At a pH below three, most of the silanol groups are protonated so that surface charge and EOF are minimized. When the pH is greater than eight most of the silanol groups are dissociated causing the surface charge and EOF to be maximized.

The zeta potential also changes with the ionic strength of the buffer. Increased ionic strength of the buffer caused compression of the double layer and results in a decreased zeta potential and reduction in EOF. The charged surface of the capillary is

\textsuperscript{31}Landers, op. cit Ch. 22 p 569
\textsuperscript{32}Wallingford, op. cit. p 10

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an essential condition to obtain electroosmotic flow. Alteration of the surface of the column will cause changes in the potential between the surface and the ions in the Stern layer of the buffer. This approach was used by Tiselius when he coated his column with methylcellulose. By bonding a nonpolar compound to the column's surface effectively eliminates the electroosmotic flow. During analysis polycations can be adsorbed onto the capillary surface altering the electroosmotic flow as shown by Towns and Regnier. The zeta potential can be altered by the addition of organic solvent to the buffer. Schwer and Kenndler showed the decrease in zeta potential as the mole percent of organic solvent increases. Electroosmotic flow has been manipulated by the modulation of the field gradient by Demana, et. al, and application of radial voltages by Hayes, et. al. Analytical conditions have been designed to even reverse the direction of the electroosmotic flow through the addition of a cationic surfactant. Electroosmotic flow used to control the migration time of the analyte is a key tool in capillary zone electrophoresis.

33Ibid. p 13
The electroosmotic flow offers another advantage in capillary zone electrophoresis. The EOF has an almost flat flow profile that does not contribute to dispersion of the analytes. This near flat profile is due to the uniform distribution of the driving force along the capillary and the absence of a pressure drop within the capillary. This differs from other chromatographic techniques that use pressurized flow and have a laminar parabolic profile. The parabolic shape is caused by the resistance to the flow from the walls that results in broader peaks. This has a significant impact on the resolution obtained by CZE.

The efficiency and resolution of capillary zone electrophoresis have made it a technique often compared to HPLC. Resolution is the ability to separate to successive solutes bands or peaks. Efficiency is a measure of the extent of band spreading. In chromatographic techniques selectivity is also considered, however this is not so for CZE. In CZE the separation is driven by efficiency and due to the sharp zones created small differences in solute mobility (<0.05 % in some cases) are often sufficient for complete resolution.\(^{40}\) The resolution between peaks is directly related to the mobility of the solutes. Resolution can be defined by the equation:

\[
R = \frac{2 (t_2 - t_1)}{w_1 + w_2} = \frac{t_2 - t_1}{4 \sigma}
\]  

[7]

where \(t\) is the migration time to the detector, \(w\) is baseline peak width (in time), \(\sigma\) is the temporal standard deviation, and the subscripts refer to two solutes.\(^{41}\) The

\(^{40}\)Heiger, op. cit Ch. 2 p 38  
\(^{41}\)Ibid
efficiency of the column can be expressed by the number of theoretical plates and is defined as:

\[ N = \frac{L^2}{\sigma^2} \]  

where \( L \) is the column length. The flat flow profile and minimal diffusion keep the temporal standard deviation small and the number of theoretical plates high.

The use of capillary columns in electrophoresis offers the advantage of efficient dissipation of heat. The heating is caused by the passage of current through the capillary. Excessive solution heating leads to a parabolic temperature gradient across the capillary and an increase in the electrophoretic mobility of about 2% per degree Celsius. Capillaries eliminate the problem of convection and reduce the temperature gradient without an anticonvective medium, i.e., agar or gels. Agars and gels were beneficial in the early work in electrophoresis but had limits. The analyte suffered band spreading as the result of adsorption onto the gel or agar. Heating was still a concern even without convection. Capillary columns are very efficient in dissipating energy and minimizing convection. The dissipation of electric power per unit of length of the column can be expressed in the equation:

\[ \frac{P}{L} = \frac{kC \rho^2 V^2}{L^2} \]

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43 Wallingford, op. cit. p 5

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where $P$ is power, $L$ is the capillary length, $k$ is the molar conductance of the solution, $C$ is the buffer concentration, $r$ is the column internal radius, and $V$ is the applied voltage. At a constant voltage and buffer concentration the power dissipation per length can be improved by increasing the capillary length or smaller radius. The smaller radius increases the surface area to volume ratio and improves the ability to dissipate heat. The surface to volume ratio for a slab gel (14 x 11.5 x 0.15 cm) is 1.3, while a 57-cm capillary with a 75-μm I.D. has a ratio of 53. In heat dissipation the capillary thickness is also important when air cooled because the column serves as a heat sink. Thin columns are preferable for columns using a thermostated bath since the bath serves as the heat sink.

While there are many advantages in the use of small diameter capillary columns there are also limitations. A limitation of Tiselius' original work was the sample size. This is even more of a problem with the continued reduction in column diameters. Capillary electrophoresis requires samples in the nanoliter range and less than 50 milliliters of buffer solution for many commercial systems. To introduce samples of this size with precision into a capillary of 100 microns or less is currently done by electromigration or hydrodynamic flow. The other techniques, i.e., electric splitters, rotary type injectors, microinjectors, their requirements of specialized equipment limited their usefulness in this study. Electromigration or electrokinetic sample introduction is achieved by removing the anodic end of the capillary and electrode.

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44 Ibid
45 Landers, op. cit p 13
from the buffer and placing them into the sample vial. An injection voltage is applied for a brief period causing the sample to enter the end of the column. The flow is due to electrophoretic migration of the charged particles and electroosmotic flow of the sample solution. This technique is biased to the most mobile ions. To eliminate this bias the electrophoretic mobility must not be a factor. This can be achieved through the "use of an on-column fracture" as done by Linhares and Kissinger, Bao and Dasgupta. The electric field is connected before the inlet and at the inlet only the mass movement of solvent causes the sample flow onto the column.

Hydrodynamic flow sample introduction is caused by hydrostatic pressure. To create a hydrostatic pressure the anodic of the capillary is placed into the sample vial below the surface of the solution and the vial is raised vertically to a specified height for an interval of time. This causes a flow toward the lower (ground) vial. Alternative to this type of sample introduction are the uses of a vacuum at the ground end of the capillary or a positive pressure at the anodic end of the column. The technique shows no bias between ions.

Rose and Jorgenson compared manual and automated electromigration and hydrodynamic sample introduction. The automated methods of sample introduction were more reproducible than the manual techniques. There was a greater reproducibility for the hydrodynamic technique than the electromigration.

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Reproducibility as measured by the amount of the sample introduced into the column and determined using peak areas of a neutral marker mesityl oxide. While the electrophoretic mobility of the analyte can be compensated for, this method of sample introduction is also affected by the conductivity of the solution. Huang et al. compared electrokinetic to hydrostatic injections in solution with differing resistance. The electrokinetic injections showed a significant increase in peak areas with increased solution resistance for lithium and sodium compared to hydrostatic injections. These techniques of sample introduction differ in method, neither is reported to have a significant impact on separation efficiency. The relative standard deviations of separation efficiency for electromigration and hydrodynamic sample introduction are 4.1% and 2.9% respectively.

The small sample size in capillary electrophoresis is limited by the column size as the columns can easily be overloaded. To avoid overloading the column with sample, the length of the sample introduced should be between 1 and 5 percent of the column length. The sample concentration is recommended to be one thousandth of the buffer concentration to reduce distortions in the applied electric field. It has been established that high buffer concentrations reduce or eliminate electroosmotic flow and low buffer concentrations are preferable. The optimal buffer concentration can be determined by the voltage versus current, an Ohm's Law Plot. Any attempt to exceed these limitations on sample size will greatly affect the chromatography. Too large of a

50 Wallingford, op. cit. p 16 &18
sample plug, exceeding 5% of the column length limit reduces the column efficiency. Substantial increases in the analyte concentration require an increase in buffer concentrations. This causes a reduction in electroosmotic flow and an increase in analysis time. The sample size is limited by these factors and makes detection of the solute a potential problem. For example, "With a 75 μm i.d. capillary 100 cm long, a peak with a migration time of 10 minutes and 500,000 theoretical plates would have a zone length of 5.8 mm and a volume of 26 nanoliters." These small amounts make on column detection techniques most effective.

Detection in capillary electrophoresis has been called the "significant challenge" to this technique's development. Most of the current detection techniques used with capillary electrophoresis were included in the survey of analytical techniques. There have been many advances in CE but the lack of sensitivity in the detection techniques has been a major limitation. This is due primarily to the minute sample sizes as it approaches the detection limit for a technique. The detection limit is the lowest concentration of the sample that can be distinguished from the random signal of the system, i.e., noise. The requirement is the signal change for the sample must be at least three times the system's noise (equation 1). These requirements for the detection of the analyte may be in direct contrast to the parameters for optimal column efficiency.


Wallingford, op. cit p 23
The applications of capillary zone electrophoresis have grown over the years. Bioscience is the area where the most applications can be found. The compounds analyzed include peptides, proteins and drugs. CZE has also been very useful in the separation of small molecules and ions. The studies involving the ions and metals in particular will be the area of concentration for this review. There are 147 ions characterized. The groups of ions included inorganic anions, organic anions, alkali metals, alkaline earth metal, transition metals, Lanthanides, organic cations and a nonmetal cation, ammonium. The search terms, capillary electrophoresis and metal speciation, produced works on the separation of different metals, inter-metal separation, i.e., zinc, copper, iron, cobalt. The original work on the separation of inorganic cations, bismuth(III) and copper(II) within three minutes, is credited to Hjertén in 1967. The detection was by *in situ* UV scanning of the tube. Tsuda et. al. separated copper(II) and iron(III) within eight minutes and found the use of UV detection adversely affected the electropherogram due to a lack of strong absorbance at 254 nm. Aguilar et. al. found metal ion complexes in electroplating solutions by CZE and UV detection. Complexes of iron(II) and (III) cyanide, copper(I) cyanide and zinc hydroxide were studied and separated. This was the only study that reported intra-metal speciation, e.g. the separation of hexacyanoferrate(III) and

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53 Landers, op. cit, p 210
hexacyanoferrate(II). To address the problem of metals not exhibiting strong absorbance in the UV region of the spectrum, an alternative was indirect UV absorbance detection. Quang and Khaledi credited Hjerten et. al as the first to use of indirect UV detection. Quang and Khaledi worked to predict and optimize the separation of metal cations by CE using indirect UV detection. The electrophoretic mobilities of metals were the focus in this paper. The two experimental parameters were pH and concentration of the complex to determine the impact on the metals' mobilities. When 2-Hydroxyisobutric acid (HIBA) was the complexing agent they found that the mobilities of the transition metals nickel(II), cobalt(II), copper(II) and zinc(II) decreased with increasing pH and/or HIBA concentration. At higher pH values the ligand has a greater ability to complex while at lower values the ability of HIBA to complex is lost. Alkali metals do not exhibit these tendencies due to their lack of complexation. Alkali, alkaline earth metals and ammonium ions were separated and detected by indirect UV absorbance by Simunicova et. al. The addition of 18-crown-6 enabled the separation of these metals. However, under non-complexing conditions, their ionic mobilities are very similar. This modification of the mobilities of metals through the formation of complexes has become a key element used in CZE. Other ligands used to form metal complexes include:

8-hydroxyquinoline-5-sulfonic acid, 1,10-phenanthroline, 2,2'-bipyridyl, ammonia,

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4-(2-pyridylazo)resorcinol, acetate, gycolate, lactate, oxalate, malate, tartarate, succinate and citrate ions. These are used to perform inter-metal speciation with determination of millimolar concentrations of metals using direct or indirect UV as the detection method. Lithium in serum was analyzed by CZE with an on-column conductivity detector by Huang, et. al. They found a linear correlation of 0.988 between the lithium concentration determined by conductivity and atomic absorption spectroscopy. The use of capillary electrophoresis for metal analyses has greatly increased.

The separations of charged species, i.e., metals, based on their mobilities and the efficiency of capillary columns, were fundamental in the advances of CZE in this area. Choices of detection techniques and sensitivity are the principle limits. The most sensitive detection techniques are general and require some modification of the analyte. Specific detection of an unknown sample is problematic at best. The study of a system at equilibrium would also pose problems, if any changes during the analysis causes a shift in the equilibrium. The problem in this study was to select a sensitive detection technique and to balance the sample size required to achieve ample signal, with the effect the sample size can have on the column's performance.

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Atomic Absorption Spectroscopy

Atomic absorption spectroscopy (AAS) is one of the most widely used single-element techniques for the determination of metals. The phenomenon was first noted by Wollaston and Fraunhofer and later explained by Kirchhoff and Bunsen in the nineteenth century. The description of flame atomic absorption spectroscopy was published by Walsh in 1955. The first commercial AAS instruments appeared around 1959. Since then, many innovations have occurred. These included the methods of atomization, burner design, excitation, electronic systems to process the signal, and the types of lamps. These have greatly enhanced the performance of the AAS.

Atomic absorption spectroscopy is based on the absorption of discrete radiation from metal atom vapor. The frequency of radiation is proportional to the energy needed for the transition of an electron from one energy level to another. When the transition arises from or terminates in the ground state, the line is called a resonance line. For most elements this is where the largest population of the metal atoms exist. The other wavelengths associated with electronic transitions are usually non-resonance lines and ion lines. The proportion of atoms that meet these conditions in a flame is small.

Ingle, J.D., Crouch, S.R. Spectrochemical Analysis 1988, Prentice Hall, Inc. Ch. 10 p 273

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The processes involved in the analysis of a solution containing a metal using flame atomic absorption spectroscopy was described by Hieftje and Malmstadt. The solution is introduced as an aerosol. These droplets can be produced continuously or discretely. The continuous introduction at a constant rate results in a constant signal with time. The discrete introduction is as a plug that produces a maximum signal and then returns to zero, or baseline. The process to take the sample solution and convert it to a mist of finely divided droplets by a jet of a compressed gas is nebulization.

There are several techniques used to produce an aerosol. Ultrasonic and electronic systems produce small uniform droplets through induced pulsing of an electro-mechanical transducer. Droplet formation can take place in a premix chamber for laminar flow or at the base of the flame in a total consumption burner. The aerosol is desolvated as it moves upward toward the flame or other excitation source. The flame was one of the earliest sources but inductively coupled plasmas have also become popular. After the solvent is removed this leaves a crystal that is then vaporized. The vaporized molecules then dissociate into atoms. These ground state atoms may be excited by the thermal energy of the flame or plasma or the radiation of an external light source. The lamp's intensity is attenuated by absorbance in the flame and then strikes the photomultiplier tube causing a photocurrent that is measured with a preamplifier/amplifier system. The amplifier signal is commonly monitored by a computer.

One of the most common sources of radiation for AAS is a "hollow cathode lamp". This lamp consists of a tungsten or platinum anode and a cylindrical cathode sealed in a glass tube and filled with neon or argon at a pressure of 1 to 5 torr. The cathode is constructed of the metal whose spectrum is desired or a metal that serves to support a layer of that selected metal. With the application of a voltage ranging from 200-400 volts and a current between 10-20 milliamps the inert gas will ionize at the anode, be accelerated to the cathode and collide with some surface metal atoms. This produces a cloud of excited atoms by a process called sputtering. As these excited atoms return to ground state, they emit their characteristic radiation. New high intensity lamps are available which have a secondary current to the anode shield. This increases the number of excited metal atoms and the lamp's intensity. This is done without extending the excitation region to where it could begin to cool down. A concentrated cloud of both cool excited and relaxed atoms can cause shifting of the emission line known as self-reversal. This results in a loss of sensitivity and selectivity. A narrower line results in better sensitivity.

The key to selecting the desired wavelength lies in the optics of the system. Optics will determine the wavelength and the bandwidth. The bandwidth is defined as the width of the radiation at one-half peak height. There is an inverse relationship between bandwidth and quality of the optics. The two types of wavelength selectors are filters and monochromators. Filters, while being inexpensive, are broad bandwidth wavelength isolation devices. Consequently, they are not suitable for isolation of narrow linewidths required in AAS from the continuum produced by many lamps.
However, if a lamp, such as a hollow cathode lamp is used, the linewidth is determined primarily by the lamp and the bandwidth of the filter may be satisfactory to simply isolate the narrow line from its adjacent neighbors. However, any degree of line broadening present in the hollow cathode lamp will be transmitted to the sample and result in some degree of loss of sensitivity and selectivity. It is a rare occasion that a hollow cathode lamp can be operated under conditions that do not produce some small degree of line broadening. As a result, most manufacturers incorporate relatively sophisticated prism or grating monochromators as the principle wavelength isolation devices. This type of instrumentation has been available for many years because of the evolution of emission spectrographic instruments. These devices have sufficient resolution to be able to isolate narrow line widths to a degree that more than adequately satisfies the needs of an AAS spectrometer.

A monochromator consists of an entrance slit, a collimating lens or mirror, a prism or grating, a focusing element, and an exit slit. The Czerny-Turner is a classic grating monochromator while the Bunsen is an example of a prism monochromator. The two types of gratings are transmission and reflection. The reflection gratings are the more commonly used. It is a hard, optically flat, polished surface with a large number of closely spaced parallel grooves ruled into it. These grooves have been cut using a sharp diamond tool and the more identical the grooves are the better and usually more costly the grating will be. Laser technology has made holographic gratings available with greater perfection with respect to line shape and dimensions. This is because the gratings are made by an optical rather than a mechanical technique.
These improved gratings are less expensive and are more free of stray radiation and ghosts (double images). The increase in intensity in the radiation improves the sensitivity and aids in establishing a linear relationship between optical signal and concentration of the analyte.
EXPERIMENT

Preliminary Study

The preliminary study was started with a search of the literature on natural water and compounds that could complex with iron. A study by Malcolm et. al. reported on the complexation of iron and cobalt with fulvic acid. The formation constants were determined using solid phase extraction.\(^{62}\) Smith and Martell also studied the formation of metal complexes and carboxylic acids.\(^{63}\) The success with iron complexing with carboxylic acids and preconcentration of trace organic acids with solid phase extraction\(^{64}\) made it the initial choice of available techniques. The intent was to separate the species in a complex matrix and later identify the ligands of the iron complexes. The solid phase extraction was carried out using XAD-2 resin from Rohm and Haas. This resin was cleaned to obtain an analytical grade by the procedure described by Macolm.\(^{65}\) It was then used in an attempt to isolate the smaller more hydrophilic organic acids known to complex with iron. These attempts were unsuccessful for the smaller molecules. Solid phase extraction was proven successful


in the separation of the anions, cations, and neutral species that contain iron. A further separation within these classes is desirable but not directly obtainable using this technique. Without the ability to achieve the desired separation, little effort was put into studying the derivatives to produce volatile compounds to be analyzed by GC/MS. All efforts to employ solid phase extraction were suspended in favor of trying capillary electrophoresis with atomic absorption spectroscopic detection (CE/AAS).

Equipment

The capillary electrophoresis system consisted of a power supply, a capillary column and one or two reservoirs. The system's power supply was a Bertan High Voltage Series 230-R. The specifications are included in the Appendix B. This is a constant voltage system with a maximum voltage of 30 kilovolts. The operating current was limited to 25% of the system's maximum outlet. The high voltage cable employed a platinum electrode during the initial work but later was replaced by a gold electrode. The ground cable's electrode was platinum throughout the study. Capillary columns used were 75 micron internal diameter. The outer diameter varied between 360 and 363 microns. The columns with 363 micron outer diameters were the polyimide coated and the 360 micron columns were the uncoated UV-transparent type. Column lengths were 50 centimeters for each initial run. The columns were obtained from Supelco, Inc. (UV-transparent and polyimide coated) and the Restek

66Bui, C. op. cit

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corporation (polyimide coated). The buffer reservoirs were glass vials or low form wide mouth bottles.

The detection system was a Jarrell-Ash 82-360 Series Atomic Absorption/Flame Emission Spectrophotometer equipped with hollow cathode lamps. One was a Westinghouse high purity iron cathode with neon fill gas, part number WL 22837. The other lamps were iron Photron Super Lamp, Type: P826-S supplied by Starna Inc. This "super" lamp employed a dual power supply system. The secondary current source was a Starna Boost Control for super lamps, part number HCL-1800. The wavelength of choice for iron was 248.3 nanometers. The lamps had a quartz window on the Pyrex body. The amplified signal from the photomultiplier tube was connected directly to a data handling station that consisted of an analog-digital interface board, PCL-812PG, supplied by PC-Labcard connected to a 486-DX-33 computer. The software used to monitor the digitized signal Labtech Notebook, version 7.0.0. This software was used to filter the data and store it as a print file. The data was analyzed and plotted with a DOS version 5.0 of Borland, Inc.'s Quattro Pro.

Procedure

Data Processing

The first step of the evaluation of the selected techniques was to determine the method of data collection. Methods available to monitor the signal from the photomultiplier (PM) tube of the AAS were a strip chart recorder and an analog meter.
reading in percent absorption. The strip chart recorder was a Honeywell Electronik 194 that had an input range of 1 to 10 millivolts. The initial estimates of the noise in the system was approximately 3 to 5 millivolts. These results made this an unacceptable method to collect the data. The percent absorption meter was noisy and deemed too subjective. Neither of these methods was acceptable and subsequently the 486 computer/PCL-812PG and Labtech Notebook was purchased and used to collect the data. The output from the PM tube was amplified by factor of 100 using an inverting operational amplifier to approximately negative one volt. This greatly increased the signal to noise ratio. However, a parameter of greater concern was the noise level. As described earlier in the general requirement, the minimum detection level has been set at 3 times the standard deviation in the blank signal, e.g., noise level.

Two parameters considered in data collection were the sampling rate and signal processing. Even with the increased signal, the PM tube had a high noise level. To reduce the noise level, signal averaging, block averaging, moving average, Fast Fourier Transform, and a digital filter were considered. All of the averaging techniques reduced the noise but did not discriminate between the baseline and the analyte signal. The Fast Fourier Transform as established in this software transformed the time domain data to frequency domain data but did not allow the inverse transform back to the time domain to give the processed electropherogram. The method that provided the best results was the digital filter. This digital filtering function was used to implement Finite Impulse Response filters. The function multiplied the last \("r"\) data points on the block \("X"\) (data block) by a set of \("r"\) coefficients, and sum the \("r"\)
products to calculate the filtered value. The coefficients used were for the Savitsky-
Golay algorithm for smoothing data. This is a widely used technique in many
analytical chemistry applications such as chromatography. The values used are
contained in Appendix C. An error was detected in the coefficients for the 11 point
technique and therefore it was not used in this study. The dark current is a measure of
the current from the PM tube when no radiation is striking the detector. The signal
was measured and processed through the filtering algorithms (see Appendix C for
equation). Typical results can be found in Table 1 and graphically represented in
Figure 1. The evaluation was performed with the Super Iron Lamp at optimal
intensity. The results are shown in Table 2 and a graphic representation of the data in
Figure 2.

Table 1

<table>
<thead>
<tr>
<th>Number of Data Points</th>
<th>Average Signal (volts)</th>
<th>Noise ((\sigma)) (volts)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.02378</td>
<td>0.001696</td>
</tr>
<tr>
<td>5</td>
<td>0.023772</td>
<td>0.001443</td>
</tr>
<tr>
<td>7</td>
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</tr>
<tr>
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<td>0.023772</td>
<td>0.001338</td>
</tr>
<tr>
<td>13</td>
<td>0.02377</td>
<td>0.001303</td>
</tr>
<tr>
<td>15</td>
<td>0.023761</td>
<td>0.001293</td>
</tr>
</tbody>
</table>

Figure 1. The Number of Data Points Used in the Filter Versus the Dark Current noise.

Table 2
The Number of Data Points Used in the Filter Versus the Average Signal Noise

<table>
<thead>
<tr>
<th>Number of Data Points</th>
<th>Average Signal (volts)</th>
<th>Noise (σ) (volts)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>-0.95537</td>
<td>0.0157</td>
</tr>
<tr>
<td>5</td>
<td>-0.95535</td>
<td>0.00463</td>
</tr>
<tr>
<td>7</td>
<td>-0.95535</td>
<td>0.00535</td>
</tr>
<tr>
<td>9</td>
<td>-0.95535</td>
<td>0.00432</td>
</tr>
<tr>
<td>13</td>
<td>-0.95536</td>
<td>0.00352</td>
</tr>
<tr>
<td>15</td>
<td>-0.95508</td>
<td>0.00332</td>
</tr>
</tbody>
</table>
The data and plots indicated that the lowest noise and the maximum signal with minimal standard deviation in the signal occurs with the 15 point Savitsky-Golay data smoothing. This parameter was used in the processing of all data collected throughout the study.

The other parameter was the rate of collection of data points. Labtech Notebook has a range of $1 \times 10^{-30}$ to $1 \times 10^3$ data points per second. The rates 1, 5, 10, 20, and 30 data points per second were considered in this study. These rates were selected because they bracket the suggested rate of 10 hertz and produced manageable file sizes. The data was collected and filtered using Savitsky-Golay 15/point data

Figure 2. The Number of Data Points Used in the Filter Versus the Average Signal Noise.
smoothing. The noise level was a primary consideration in the data collection. There were also concerns about the size of the data files for each electropherogram and the number of data points generated for the iron-containing species detection. The data are contained in Table 3 and displayed graphically in Figure 3.

Table 3

<table>
<thead>
<tr>
<th>Sampling Rate Data Points per Second</th>
<th>Dark Current Noise (σ) (volts)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.032754</td>
</tr>
<tr>
<td>5</td>
<td>0.025046</td>
</tr>
<tr>
<td>10</td>
<td>0.026602</td>
</tr>
<tr>
<td>20</td>
<td>0.026412</td>
</tr>
<tr>
<td>30</td>
<td>0.026854</td>
</tr>
</tbody>
</table>

* All sample sizes were 600 data points.

This data was obtained separately for each rate and the lamp's signal varied. Consequently the signal to noise ratio was not used and the focus was solely on the noise level. It was decided to collect the data at a 10-hertz rate. The data file for a 30 minute electropherogram consisted of eighteen thousand data points. This required that the data be separated into smaller files that the analysis software could handle. The decision was made to make the data file three thousand data points. At this sampling rate a peak with a baseline width of two seconds would consist of twenty points. A test on the noise level was subsequently repeated and the selection of 10
data points per second was supported as an appropriate choice. Later tests, done after other improvements to the instrumentation, were made which further reduced the noise level.

**Sensitivity**

After the parameters on the data collection were established, the sensitivity of the detection system was determined using the Westinghouse conventional lamp for the initial calibration plot. The concentration range of the solutions varied from one to twenty parts per million iron. For these initial studies the signal was offset, (using software) by one volt to give a baseline signal of zero. Each solution was introduced
by conventional AAS aspiration and measured for 30 seconds then the signal averaged. The data showed that the one part per million solutions was clearly detectable. The results are contained in Table 4 and displayed graphically in Figure 4.

Table 4
Iron Concentration Versus Average Signal

<table>
<thead>
<tr>
<th>Iron concentration (ppm)</th>
<th>Average signal (volts)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.045</td>
</tr>
<tr>
<td>1</td>
<td>0.0888</td>
</tr>
<tr>
<td>2</td>
<td>0.1447</td>
</tr>
<tr>
<td>4</td>
<td>0.2226</td>
</tr>
<tr>
<td>5</td>
<td>0.2414</td>
</tr>
<tr>
<td>10</td>
<td>0.40095</td>
</tr>
<tr>
<td>15</td>
<td>0.51596</td>
</tr>
<tr>
<td>20</td>
<td>0.62017</td>
</tr>
<tr>
<td>25</td>
<td>0.71616</td>
</tr>
<tr>
<td>30</td>
<td>0.79073</td>
</tr>
<tr>
<td>50</td>
<td>0.99368</td>
</tr>
</tbody>
</table>

* multiplier = -1

This range of iron concentrations was repeated with the presence of organic compounds known to complex with iron. These compounds were malonic acid and 1,10-phenanthroline. Even under these conditions iron was detectable at the one part
per million levels. Repeated calibration plots verified the ability to detect iron at the one part per million levels with conventional AAS aspiration to introduce the sample.

![Graph showing iron concentration versus average signal.](image)

**Figure 4. Iron Concentration Versus Average Signal.**

The detection system was further calibrated for small volumes of low sample concentration. These volumes were in the one-half to four microliter range rather than the continuous flow of milliliters used for traditional AAS calibration curves. These volumes of iron solution (1 ppm) were injected using a five-microliter syringe. The one-half microliter injection gave a detectable signal. The data from the range of injections are shown in Table 5 and displayed graphically in Figure 5.
### Table 5

Microliter of a 1 Part Per Million Iron(III) Chloride Solution

Versus the Average Signal

<table>
<thead>
<tr>
<th>Volume of 1 ppm iron solution (µL)</th>
<th>Average signal (volts)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.052</td>
</tr>
<tr>
<td>0.5</td>
<td>0.102</td>
</tr>
<tr>
<td>1.0</td>
<td>0.134</td>
</tr>
<tr>
<td>1.5</td>
<td>0.171</td>
</tr>
<tr>
<td>2.0</td>
<td>0.230</td>
</tr>
<tr>
<td>2.5</td>
<td>0.267</td>
</tr>
<tr>
<td>3.0</td>
<td>0.315</td>
</tr>
<tr>
<td>3.5</td>
<td>0.354</td>
</tr>
</tbody>
</table>

**Figure 5.** Microliter of a 1 Part Per Million Iron(III) Chloride Solution

Versus the Average Signal.

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These results show that the system can detect small volumes of low levels of iron introduced into the AAS. The AAS had both the sensitivity and speed required for the projected amounts of iron to be eluted from the separation by electrophoresis on the capillary.

A new lamp was purchased which was reported to have an increased intensity due to a dual power source. This "super lamp" employed the traditional current supply but also a secondary current source to increase the excitation of the element and the intensity of the line generated. The new calibration curve was comparable to the result previously shown.

The construction of the capillary electrophoresis system was initially modeled after a commercial instrument observed at a laboratory of the Food and Drug Administration. This instrument was the Applied BioSystems Model 270A Capillary Electrophoresis System. It had a vacuum system for rinsing and filling the column. The instrument constructed for this study used a nylon cap with ports for a vacuum, a column, an electrode, a vent to atmosphere, a filling tube, and one to the aspirator of the AAS (see Appendix D). The solvent loss to the AAS was replenished through the filling tube connected to a Rainin Rabbit peristaltic pump and buffer solution. A U-shaped glass tube was used to connect the capillary column to the capillary aspirator of the AAS (see Appendix E).

In the first series of tests a UV-transparent column from Supelco, Celect™ UVT75, Cat. No 7-7552 was selected. The column was rinsed with 10% methanol, 0.001 molar NaOH solution, milli-Q water and the 20 millimolar (MES)
(2-[N-morpholino]ethanesulfonic acid)/histidine buffer pH 6.1. The analyte was a tris 1,10-phenanthroline iron(II) sulfate solution at approximately 195 ppm iron. The sample was introduced using gravity siphoning by raising the sample vial 10 centimeters for 30 seconds. The voltage to the power supply was set at 25 kilovolts and the initial current registered 0.015 milliamps. The colored sample's migration through the column was visually observed and arrived at the cathode in approximately 5.5 minutes. However, no iron was detected in the flow to the AAS. Promptly after the power to the CE was turned off, a response on the AAS was noted. Several modifications to this type of interface between the CE column and the AAS were unsuccessfully attempted. A new interface design was required.

The idea for a different type of interface came after the review of literature on capillary electrophoresis and other analytical techniques. The article Capillary Electrophoresis/Mass Spectrometry by Smith et. al. on CE/MS seemed to offer an option. This work had eliminated the cathodic reservoir and used the electrode position to electrostatically produce flow in the column. The first position of the cathode was above the Jarrell-Ash burner with the capillary column inserted through the capillary aspirator. The nylon cap previously used with the vacuum for rinsing and loading the buffer was abandoned. All the rinsing and filling of the columns were done with a vial and septum. The column was inserted through the septum and a vacuum pump used to partially evacuate the vial. The sample was loaded onto the column by

siphoning. Sample could be observed as it moved through the column but still no iron was detected. An old Perkin-Elmer burner head was then modified by drilling an opening in the burner head and placing the electrode inside the burner mixing chamber. A second opening was made for the insertion of the capillary column (see Appendix F). The sample was monitored as it traveled through the column but again iron was not detected by the AAS. Since it was certain that the analyte was being delivered to the flame, the question of flame kinetics is suggested as the source of the problem.

In an examination of the CE system it is expected that species are separated on a charge to mass basis. If the iron is being introduced as an ion, without a suitable counter ion, the reduction to ground state may be slow and occur in the cooler upper regions of the flame outside the optical path. To increase the reduction of the iron, iodomethane was introduced as an electron rich material into the premixed gases. The analysis of a 50-ppm iron solution as iron(III) chloride with this modification resulted in the detection of four peaks at 335, 550, 730, and 880 seconds. The run time was set for 15 minutes and consequently later peaks were not observed.

In addition to the problems with the interfacing of the two techniques there was a problem with the sample introduction. The use of siphoning to introduce samples was acceptable with colored solutions since visual observation assured the sample's presence. Loading of weakly colored samples could not be ascertained until after the electropherogram had been obtained. It was observed that removing the column from the septum caused some loss of a couple of millimeters of solution from the column. This loss was often sufficient to prevent the siphoning of sample on to the
column. This method was eventually replaced with a pressurized system that introduced the sample. This system (see Appendix G) consisted of a nylon top plate with a screw cap cemented into it. A block diagram of the system is shown in Appendix H. The cap contained ports for the column, electrode and gas line fabricated with HPLC fittings. Sample introduction was accomplished by pressurizing approximately 30 centimeters of one-quarter inch tubing and 50 centimeters of 0.010 inches internal diameter peek® tubing with a nitrogen flow of twenty pounds per square inch. The valve to the nitrogen tank was closed. The pressure in the tubes was used to force sample on to the column. To calibrate the amount of solution forced into the column as a function of time, milli-Q water eluted off the column was weighed. The assumption that at time zero no flow should occur was used in the regression analysis. The data from these studies are contained in Table 6 and plotted in Figure 6. The optimal time of 3 seconds should deliver approximately 0.1 mg of solution. At the average room temperature of 25.1°C the volume would be 0.1 microliters or approximately 5 percent of the 2.2 microliter volume of a 50-cm column. This seems to fit the condition described in the literature to obtain the optimum column efficiency.

Analysis of Samples

The technique for the analysis of iron solutions by CE/AAS was started by the filtration of all solutions through a 0.22 micron millipore filter. The column was placed in a brass capillary nut with a graphite ferrule. The column length was 72
Table 6

Time of Pressurization Versus Weight of Water Eluted

<table>
<thead>
<tr>
<th>Duration of pressure applied in seconds</th>
<th>Weight of water (mg)</th>
<th>Average weight of water in mg (std dev)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>0.6, 0.9, 0.8, 0.7</td>
<td>0.75 (0.13)</td>
</tr>
<tr>
<td>30</td>
<td>1.8, 2.0, 1.9, 1.9</td>
<td>1.9 (0.081)</td>
</tr>
<tr>
<td>45</td>
<td>3.5, 2.9, 3.3, 3.0</td>
<td>3.1 (0.28)</td>
</tr>
<tr>
<td>60</td>
<td>4.3, 3.9, 4.3</td>
<td>4.1 (0.23)</td>
</tr>
</tbody>
</table>

Figure 6. Time of Pressurization Versus the Weight of Water Eluted.

millimeters from the top of the nut. The port for the electrode was closed off with a nut containing a septum. To prepare a new column it was rinsed for 2 minutes with 10 percent methanol in Milli-Q water, followed by 0.5 molar potassium hydroxide
solution for 5 minutes, then flushed for 5 minutes with Milli-Q water, and finally 5 minutes with the new electrolyte. Between runs rinses were made with 0.1 molar KOH for 2 minutes followed by Milli-Q water for 1 minute before rinsing with the electrolyte for 5 minutes. Each solution was contained in a separate vial and was screwed into the cap in the nylon top plate. All solutions were delivered using the same nitrogen flow of twenty pounds per square inch with the valve to the tank open to give a continuous pressure. The sample was introduced as described above using time introduction of a pressurized nitrogen flow.

The electropherograms were produced through the analysis of collected data by the Quattro Pro software. The initial run times were 15 minutes, as the literature commonly reported elution times of less than ten minutes. Later the run times were increased to between sixty and ninety minutes. The data collected using Labtech Notebook and the parameter described earlier were "PRN" files. The files were closed after the collection of a predetermined number of "records". These files were imported to the Quattro Pro program for processing. The files were imported as a "comma-and-quote delimited file". Each file consisted of three columns; the time from the start of the run, the raw signal, and the filtered signal. The graph type selected was "X-Y". The time was plotted along the X-axis. The filtered signal was designated "first series", on the Y-axis. The graph formats were lines only, symbols were not used due to the large number of data points. The file size was limited by the Quattro Pro program at 6000 records, which represented a 10 minute electropherogram.
The first 1.5 seconds or 15 records were not included because of the number of points required in the filtering. Examples of electropherograms are included in the results.
RESULTS

The initial iron compounds selected for study were the chlorides of iron(II) and iron(III). Iron(II) oxidation state was selected for study because it is the form likely to be found in a reducing environment as the one reported at the KL Landfill. Iron(II) and chloride ions were not reported to form a complex and the iron therefore should exist as a single species. Thus iron(II) hexahydrate can be used to establish a detection range and apparent electrophoretic mobility. The iron(III) chloride had two formation quotients and should produce species with one or two chloride ligands in addition to the hexahydrated form. This system was selected to attempt the separation of these three species.

The iron(II) chloride samples ranged from 0.23 parts per million to 100 parts per thousand iron. All the samples were analyzed using a 20 millimolar MES (2-[N-morpholino] ethanesulfonic acid) buffer solution as the electrolyte. The solution was adjusted to a pH of 6 with potassium hydroxide. The separation voltage was 20 kilovolts and the column length was 50 centimeters with an internal diameter of 75 microns. The AAS employed an acetylene/air flame with iodomethane vapor introduced through the capillary aspirator. The migration times ranged from 1 to 14 minutes with a mean time of 7 minutes and a standard deviation of 3 minutes for 40 trails. This trail data was used to establish a two tailed 95 percent confidence interval (see Appendix I). The critical "t" value selected for the sample size was 1.960 and this
resulted in a reduction of the sample size to 13 measurements with a mean migration
time of 7 minutes and a standard deviation of 0.4 minutes (see Table 7).

Table 7

Data from Electropherograms of Iron(II) Chloride

<table>
<thead>
<tr>
<th>Conc Iron (ppm)</th>
<th>Migration Time (minutes)</th>
<th>Peak Height (millivolts)</th>
<th>Peak Width Baseline (sec)</th>
<th>Width 1/2 Max (sec)</th>
<th>Peak Area (mv sec)</th>
<th>Student &quot;t&quot; values</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4</td>
<td>6.4</td>
<td>60</td>
<td>22</td>
<td>20</td>
<td>1320</td>
<td>1.27</td>
</tr>
<tr>
<td>100</td>
<td>6.5</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>1.07</td>
</tr>
<tr>
<td>10</td>
<td>6.6</td>
<td>55</td>
<td>15</td>
<td>11</td>
<td>825</td>
<td>0.97</td>
</tr>
<tr>
<td>0.9</td>
<td>6.6</td>
<td>250</td>
<td>4</td>
<td>2</td>
<td>1000</td>
<td>0.93</td>
</tr>
<tr>
<td>10</td>
<td>6.7</td>
<td>50</td>
<td>19</td>
<td>16</td>
<td>950</td>
<td>0.73</td>
</tr>
<tr>
<td>1</td>
<td>6.9</td>
<td>20</td>
<td>2</td>
<td>4</td>
<td>80</td>
<td>0.33</td>
</tr>
<tr>
<td>1</td>
<td>6.9</td>
<td>50</td>
<td>1.5</td>
<td>3</td>
<td>150</td>
<td>0.26</td>
</tr>
<tr>
<td>0.7</td>
<td>7.1</td>
<td>70</td>
<td>1.5</td>
<td>3</td>
<td>210</td>
<td>-0.11</td>
</tr>
<tr>
<td>0.23</td>
<td>7.1</td>
<td>50</td>
<td>10</td>
<td>6</td>
<td>500</td>
<td>-0.14</td>
</tr>
<tr>
<td>10</td>
<td>7.2</td>
<td>50</td>
<td>30</td>
<td>26</td>
<td>1500</td>
<td>-0.34</td>
</tr>
<tr>
<td>0.2</td>
<td>7.3</td>
<td>75</td>
<td>13</td>
<td>11</td>
<td>975</td>
<td>-0.54</td>
</tr>
<tr>
<td>0.4</td>
<td>7.3</td>
<td>70</td>
<td>28</td>
<td>24</td>
<td>1960</td>
<td>-0.54</td>
</tr>
</tbody>
</table>

A typical electropherogram of a 0.7 ppm iron(II) as an iron(II) chloride solution in
milli-Q water is seen in Figure 7. Pretreatment of the column involved rinsing with 0.1
molar KOH followed by the buffer for 5 minutes each. Sample was introduced onto
the column from a four dram vial sealed with a septum. The column was inserted
Figure 7. Electropherogram of a 0.7 PPM Iron(II) Chloride Sample. 
The sample was introduced by siphoning (10 cm elevation for 1 minute).
The capillary length was 50 cm with a 20 kilovolt separation voltage.

through the septum and 5 microliters of air was added for 15 seconds. The
electropherogram exhibited a 70 millivolt iron peak with a 7 millivolt standard
deviation in the signal before and after the peak. The baseline peak width was 3
seconds. The low frequency signal in the electropherogram's baseline appears in all
data collected by hardware and software modifications. After much effort its origin
could not be determined. Attempts to remove the irregular signal were unsuccessful.

The formation quotients of iron(III) chloride and the "material balance"
function, $F_o$ were used to develop a distribution diagram. The distribution diagram of
iron(III) chloride shows (Appendix J) the relative fraction of each iron containing
species involved in the successive steps of the complex ion equilibrium system. This indicates the presence of three species of iron, e.g., the free metal, a monochloride and a dichloride (see Appendix A). This system was used to determine the ability to separate and detect the various species of iron in iron(III) chloride. Figure 8 is the electropherogram of a 100-ppm iron solution in milli-Q water. This sample was introduced by siphoning by raising the sample vial 10 centimeters for 1 minute. There were 3 iron peaks detected with elution times of 2.5, 4.7, 6.5 minutes. The respective peak heights were 100, 80, and 110 millivolts with a standard deviation in the baseline signal before and after the peaks of 5 millivolts.

Figure 8. Electropherogram of a 100 PPM Iron(III) Chloride Sample. Sample was introduced by the introduction of 5 microliters of air for 15 seconds into a sample vial. The separation voltage was 20 kV. Chloride concentration = 0.005 molar
The electropherogram of the iron(III) chloride sample changed as the pH of solution was changed. To examine, this a 1 ppm iron sample was prepared in 20 millimolar potassium hydroxide solution. There were two formation quotients for the formation of complexes of iron(III) hydroxide. The electropherogram detected the presence of 14 iron containing species. This electropherogram was run for approximately one hour. The data from the electropherogram is contained in Table 8. This electropherogram required six separate files to contain all the data points and is not reproduced here, (a copy appears in the Appendix K).

Potassium hexacyanoferrate(II) and potassium hexacyanoferrate(III) solution were prepared and analyzed. The formation quotient data suggest the existence of only a single species existing for both compounds. The electropherogram from both compounds exhibited multiple peaks. Iron(III) nitrate however, was a compound exhibiting a single peak as expected. The single observed peak is likely the hydrate iron(III) ions (a list of the chemicals used in this study can be found in Appendix L).
<table>
<thead>
<tr>
<th>Migration Time (minutes)</th>
<th>Peak Height (millivolts)</th>
<th>Peak Width Baseline (seconds)</th>
<th>Peak Width at one half height</th>
<th>Peak Area (mv*seconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td>19.4</td>
<td>65</td>
<td>3</td>
<td>1</td>
<td>195</td>
</tr>
<tr>
<td>22.7</td>
<td>65</td>
<td>3</td>
<td>1</td>
<td>195</td>
</tr>
<tr>
<td>29.0</td>
<td>45</td>
<td>2</td>
<td>1</td>
<td>90</td>
</tr>
<tr>
<td>32.1</td>
<td>40</td>
<td>3</td>
<td>1.5</td>
<td>120</td>
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<tr>
<td>35.4</td>
<td>50</td>
<td>2</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>38.5</td>
<td>50</td>
<td>2</td>
<td>1</td>
<td>100</td>
</tr>
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<td>41.7</td>
<td>45</td>
<td>2.5</td>
<td>1.5</td>
<td>112</td>
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<tr>
<td>44.7</td>
<td>65</td>
<td>3</td>
<td>1</td>
<td>195</td>
</tr>
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<td>47.7</td>
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<td>2.5</td>
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<td>125</td>
</tr>
<tr>
<td>50.7</td>
<td>65</td>
<td>2</td>
<td>1</td>
<td>130</td>
</tr>
<tr>
<td>53.8</td>
<td>45</td>
<td>2</td>
<td>1</td>
<td>90</td>
</tr>
<tr>
<td>55.1</td>
<td>30</td>
<td>2.5</td>
<td>1</td>
<td>75</td>
</tr>
<tr>
<td>56.6</td>
<td>35</td>
<td>1</td>
<td>0.5</td>
<td>35</td>
</tr>
<tr>
<td>59.7</td>
<td>55</td>
<td>3</td>
<td>1</td>
<td>165</td>
</tr>
</tbody>
</table>
CONCLUSIONS

The different aqueous complex species of iron were separated but not uniquely identified. This technique detected solely iron-containing substances but these were not identified beyond this criteria. There were several limitations experienced in this study which suggested further study should be done. These areas include the columns, buffer selection, interface design, sample introduction method and a detection system.

The fused silica columns have strongly influenced many advances in electrophoresis. A problem experienced with this study was the inability to uniquely identify the species detected. This inability to uniquely identify these species results from the lack of reproducibility in the migration time of the iron species.

Electroosmotic flow (EOF), a key element in this study, is greatly dependent on the condition of the column. Along with the electrophoretic mobility of the cations, the EOF provides the driving force for the neutral species and anions toward the cathode. In this study, relying on the formation quotients, the iron(III) chloride species are all cations. Most of the iron(III) chloride analyses' electropherograms had three species detected. These are concluded to be Fe(H₂O)₆⁺³, FeCl(H₂O)₅⁺², and FeCl₂(H₂O)₄⁺¹, from the formation quotients. The species expected to have the greatest mobility or lowest migration time was the Fe(H₂O)₆⁺³. In equation [3] the mobility was shown to be directly proportional to the charge on the particle and inversely proportional to the Stokes' radius. The Stokes' radius and mass were shown
to be proportional in equation [4]. Each chloride ion that replaces a water molecule increases the mass by 17.435 amu and decreases the charge by one. It would indicate an order of migration of Fe(H$_2$O)$_6^{+3}$ followed by FeCl(H$_2$O)$_5^{+2}$, and then FeCl$_2$(H$_2$O)$_4^{+1}$. The migration of the neutral species FeCl$_3$(H$_2$O)$_3$ would be next and indicative of the EOF followed by any negatively charged species. There were occasions when more than three species of iron detected. These were with new columns or when the analysis time of an electropherogram was extended beyond the 15 minutes initially selected. The increases in migration times with successive runs and additional peaks were causes for concern. These increases are believed to be the result of the interaction between the column and the iron ions.

The effects of metals and particularly iron(III) ions on the columns require additional study. Several electropherograms found that no iron was detected. After elimination of many possible explanations, it appeared that iron ions might have adsorbed on the column. The observation of both oxidation states of iron showed similar effects on the fused silica columns, although it seemed much more pronounced with iron(III). The migration times of the iron species increased with each successive analysis. Iron(II) ion migration times became more reproducible after reconditioning the columns between analysis. After several different variations of rinse protocols the optimal reconditioning resulted when the columns were rinsed with a 1 molar KOH solution, followed by a 0.1 molar KOH solution, then the buffer solution. Each solution passed through the column for five minutes. This procedure gave comparable migration times, but the rinses soon began to dissolve the columns. None of the
reconditioning methods suggested in the literature nor any variations including a rinse with a basic ethylenediaminetetraacetate solution proved successful for the iron(III) samples. This problem may be related to iron's affinity for silica surfaces. This affinity would have only been increased under the conditions of this study as the silica surface was highly charged and the iron separated into charged zones. The ionic attraction between the column surface and the iron(III) species, would be similar to the technique used to reverse electroosmotic flow. Surfactants such as C.T.A.B. (cetrimonium bromide) reverse the electroosmotic flow in a capillary column through the ionic interaction. The surfactant causes the surface to take on a positive charge and the Stern layer a negative charge. This would cause the electroosmotic flow to be toward the anode. If this were the case the electroosmotic flow is being first slowed, stopped, and eventually reversed. This would account for the longer migration times and lack of detection of iron within the set analysis time, 20 minutes, after a few attempts on a column. There were reported to be between 3 and 7 silanol groups per 10 Å of a column. It was calculated that at a 1 ppm iron(III) chloride solution with 1 percent free iron, hexaaqua, this would be 2 iron(III) ions present per silanol group. A better understanding of this phenomenon could lead to further improvements in this technique. This would begin with a profile of the EOF. Determination of the EOF is achieved by the detection of a neutral species. The potential for the shifting the equilibrium of the system with the addition of iron, in any form, is a concern, no suitable neutral iron containing compound could be identified. Work in the laboratory has begun to determine the EOF of a column with an organic marker, such as mesityl
oxide, using a UV detection system. This would help to characterize any changes that resulted from the iron analyses. When reproducible migration times are obtained these species can be collected and an elemental analysis can be performed to uniquely identify each species.

The further study on columns could be done to develop a technique to quantify the effects of iron ions on the electroosmotic flow in fused silica columns. Determination of the amount of iron retained on the fused silica column per run would be of particular interest. Additional studies of the different types of column surfaces, i.e., Teflon, glass, or modification of the surface of the fused silica columns could be conducted to observe metal ions migration and retention on the columns. The modifications to the surface would attempt to maintain the electroosmotic flow while having less affinity for metals.

Capillary zone electrophoresis has shown the ability to separate complex iron species and that the focusing of the capillary column makes the detection of sub part per million amounts of iron readily distinguishable from the background noise of the instrument. Another area where additional work should be done is with the choice of buffer. The buffers reported in the literature for metal analysis were MES, 2-hydroxyisobutyric acid (HIBA), phosphate and acetate buffers. The buffer selected for this study had to consider the pKa of the buffer. First the pKa should be as high as possible to obtain the maximum electroosmotic flow. The limit of concentration of iron must consider the fact that iron(III) can form hydroxide precipitates at pH value as low as 2. The phosphate buffers had pH values that were either too low or too
high. Acetate and HIBA form complexes with iron (III). All analyses in this study used MES as the buffer or electrolyte with potassium hydroxide to adjust the pH. Possible buffers should be considered which have a pKa of approximately five, to reduce the possible precipitation of the metal ion as a hydroxide, yet have some EOF.

The interface between the CE and AAS did prove successful. There are still problems with this design. The first problem was the electrode in the burner. It is a simple platinum loop fashioned by wrapping the wire around the tip of a pair of needle nose pliers. Attempts to have the loop ends joined to form a complete circle were unsuccessful. This loop would not produce the uniform electric field that could optimize the focusing of the metallic species into the flame. If an electrode could be obtained which was machined into the correct shape and thickness this should enhance the detection limits and resolution of this technique.

Another area of study is the angle between the flow off the capillary and the flow into the flame. In the present design this is a right angle. This drastic change in direction suggests a high probability to have some iron lost to the walls of the burner. A burner designed for this type of interface would minimize problems due to the angle and the flow off the capillary. Several unsuccessful attempts to achieve this were made. The distances between the site where the capillary column was inserted into the burner and the location of the electrode was less than 1.5 centimeters. This short distance prevented any opportunity to bend the capillary and have the flow off the column directed toward the flame. A burner designed with a longer vertical column could allow ample distance to direct the flow off the capillary as wanted.
The burner used in this study had limited allowance for vertical adjustment. The limits for adjusting the burner position relative to the lamp's radiation prevented optimization of the optical system. This limited the study of other metals as observed when trying to test the system with copper. The potential formation of oxides or other products makes flame position a key in metal detection in AAS. The iodomethane used to aid in the reduction of iron could have had the potential to form a molecular copper(II) iodide. Because of this, the reduction to ground state of copper likely occurred in a different position of the flame. These are problems that should be further investigated.

The method of sample introduction was another area with problems. The critical importance of restricting the amount of sample introduced on the column became apparent as different techniques were tried. It was reported that for quantitation the use of an automated sample introduction method gave the best results. In this study good resolution was achieved with siphoning of samples. One option in the future would be to make the entire cathodic end of the system a component that could be raised to use siphoning. The anodic end of the column would be stationary in the burner of the AAS. Another change would be with the lines from the nitrogen tank giving greater flexibility in raising the vials. The screw cap vial could be replaced with the type of vial used in carousels of commercial instruments. These fit into an O-ring and are raised to provide a seal. The vial containing the desired solution could then be rotated into position.
The small amounts required were introduced by nitrogen pressure using a vial and a microliter syringe and air. Continuous problems with septum leaks resulted in the decision to end the use of the syringe and to try an alternate methods of sample introduction. The technique of pressurized introduction using the nitrogen flow showed promise and was linear over the range tested (see Table 6). However, at the lower volumes, where the desired amount would be introduced no data was obtainable. The small amounts of liquid delivered from the column were lost during transport to and after being placed on the analytical balance. A method to calibrate and refine this technique may enable the correct and quantitative introduction of samples. Equipment is commercially available and being used in Super Critical Fluid Analysis as well as in CE for reproducible introduction of small sample volumes.

The Jarrell-Ash system, while being thirty-years old, worked well. The tubes in the amplifier and power source may be minor contributors to the system's noise. Replacement of these components with new solid state power supply and transistorized operational amplifiers likely would lower the noise levels. This could lower the amounts of detectable iron or other metals. These changes could also aid in the identification of the source of the low frequency signal found in the baseline. This erratic signal of approximately 0.33 hertz is another contributor to the noise of the baseline. Eliminating this could reduce the noise from ten millivolts down to the single millivolt range. Another change to the AAS system that should improve the signal would be to eliminate the periscope alignment, which currently serves no useful purpose. The radiation from the lamp is reflected off a pair of mirrors to lower it on to
the slit to the PM tube. This change is a simple one but the advantages gained from it were not deemed sufficient to compensate for the instrument down time.

The future directions of this study include the separation and identification of the varied species of metal found in a complex aqueous matrix. This should provide addition information in the development of any remediation plans. It could also offer an opportunity to identify the specific toxicity of the varied species of metal. These areas of concern were the justification for this study, along with the desire to understand why the apparent oversaturation without precipitation at the KL Landfill.

There is another possibility that could come from this study, the establishment of additional formation quotients. Those found on the limited systems studied in this project do not offer values for all the species expected to be found. It would also provide some explanation for the additional species of iron detected. The solution of iron(III) chloride had three reported forms as did the hydroxide complexes of iron(III), In combination there were 12 possible species that could be formed. To test this 1 ppm iron(III) chloride was diluted in 20 millimolar KOH. There were 14 peaks detected in the duration of the analysis. The additional peaks could be the trichloro, or trihydroxo, or a mixture of these to produce a neutral species. The ligand concentrations were not varied to increase the production of the negative species because of the problems with the electroosmotic flow. The analyses of potassium hexacyanoferrate(II) and potassium hexacyanoferrate(III), while both are reported to exist as a single species, more than one species of iron was detected. Since all these
species can be separated, if the migration times were reproducible, collection of the
species with their ligands would aid in the unique identification.

With funding this study could also be enhanced by attempting to combine CE
with an inductively coupled plasma with a mass spectrometer, ICP/MS. This
instrument has the power to vaporize the metals and identify and quantitate the other
elements present in the species. It could even allow for simultaneous analysis of
different metals.
Appendix A

Equilibrium Expressions for the Stepwise Formation of Complex Ionic Species
Appendix A

Equilibrium Expressions for the Stepwise Formation of Complex Ionic Species

Complex formation

Step 1 \[ M + L \rightleftharpoons ML \]
Step 2 \[ ML + L \rightleftharpoons ML_2 \]
Step 3 \[ ML_2 + L \rightleftharpoons ML_3 \]
 etc

\[ Q_1 = \frac{[ML]}{[M][L]} \quad Q_2 = \frac{[ML_2]}{[ML][L]} \quad Q_3 = \frac{[ML_3]}{[ML_2][L]} \]

\[ \beta_1 = Q_1 \quad \beta_2 = Q_1 Q_2 = \frac{[ML_2]}{[M][L]^2} \quad \beta_3 = Q_1 Q_2 Q_3 = \frac{[ML_3]}{[M][L]^3} \]

\[ C_m = [M] \]

\[ C_m = [M] + [ML] + [ML_2] + [ML_3] + \ldots \]

\[ C_m = [M] + Q_1 [M][L] + \beta_2 [M][L]^2 + \beta_3 [M][L]^3 + \ldots \]

\[ F_o = \frac{C_m}{[M]} = 1 + Q_1 [L] + \beta_2 [L]^2 + \beta_3 [L]^3 + \ldots \]

\[ \alpha_o = \frac{[M]}{C_m} = \frac{1}{F_o} \]

\[ \alpha_1 = \frac{[ML]}{C_m} = \beta_2 [L]^2 \alpha_o = \frac{\beta_2 [L]^2}{F_o} \]

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Appendix B

The Specifications for the Bertan Series 230-30R

75
Appendix B

The Specifications for the Bertan Series 230-30R

The output voltage range was 0 to 30 kilovolts.
The output current range was 0 to 400 microamperes.
The ripple at maximum output was 5 volts.
Polarity reversal could be achieved by the internal connectors.
The input power 115 Volts A.C. @ 0.5 ampere. 50-60 hertz.
Temperature coefficient (0-50°C): 100 ppm of maximum per °C.
Stability (after 1/2 hour warm-up) 0.01% pr hour: 0.02% per 8 hours.
Front panel meter: Front panel 3-1/2 digit LCD meter, switch selectable from reading either output voltage or current. Meter accuracy for voltage was 0.5% ± 200 volts and for current 2% ± 2 microamperes.
Current Limit: All units provide short circuit current limiting to less than 120% of the maximum rated output current. An optional rear panel switch that can select a current limit of 25% of maximum output can be provided.

***** All information provided here was obtained from the specifications reported by the manufacturer, Bertan High Voltage, 121 New South Road, Hicksville, NY 11801

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Appendix C

The Coefficients Used in the Savitsky-Golay Algorithm
Appendix C

The Coefficients Used in the Savitsky-Golay Algorithm

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In the 5 points Savitsky-Golay the equation appears as follows:

\[ F(x_i) = c_5 \ p_{i-4} + c_4 \ p_{i-3} + c_3 \ p_{i-2} + c_2 \ p_{i-1} + c_1 \ p_i \]

Where \( c_x \) are respective coefficients from the table above and the \( p_i \) values are from the converted analog signal.
Appendix D

Top of Capillary Electrophoresis Cell/AAS Interface
Appendix D

Top of Capillary Electrophoresis Cell/AAS Interface
Appendix E

First Electrophoresis Cell/AAS Interface
Appendix E

First Electrophoresis Cell/AAS Interface
Appendix F

Burner Head - Capillary - Electrode Assembly
Appendix F

Burner Head- Capillary - Electrode Assembly
Appendix G

Modified Capillary Electrophoresis Cell/AAS Interface Top
Appendix G

Modified Capillary Electrophoresis Cell/AAS Interface Top
Appendix H

Block Diagram of Capillary Electrophoresis System
Appendix H

Block Diagram of Capillary Electrophoresis System
Appendix I

A List of All Single Peaks From Iron(II) Chloride Samples
### Appendix I

#### A List of All Single Peaks From Iron(II) Chloride Samples

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<td>2</td>
<td>4</td>
<td>360</td>
<td>P-2.5uL/15S</td>
<td></td>
</tr>
<tr>
<td>1.12</td>
<td>412</td>
<td>20</td>
<td>2</td>
<td>4</td>
<td>80</td>
<td>P-2.5uL/15S</td>
<td></td>
</tr>
<tr>
<td>0.91</td>
<td>133</td>
<td>180</td>
<td>2</td>
<td>4</td>
<td>810</td>
<td>P-2.5uL/15S</td>
<td></td>
</tr>
<tr>
<td>0.91</td>
<td>116</td>
<td>80</td>
<td>2</td>
<td>4</td>
<td>320</td>
<td>P-2.5uL/15S</td>
<td></td>
</tr>
<tr>
<td>0.91</td>
<td>394</td>
<td>250</td>
<td>2</td>
<td>4</td>
<td>1000</td>
<td>P-2.5uL/15S</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>414</td>
<td>50</td>
<td>1.5</td>
<td>3</td>
<td>150</td>
<td>NF-5psi/15S</td>
<td></td>
</tr>
</tbody>
</table>

Mean Time of 422 sec

Standard Deviation of 193 sec
Appendix J

Distribution Plot of Iron(III) in Chloride Species
Appendix J

Distribution Plot of Iron(III) in Chloride Species

- $A_0$ (free metal)
- $A_1$ (monochloro)
- $A_2$ (dichloro)

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Appendix K

1 PPM Iron(III) Chloride in 20 Millimolar Potassium Hydroxide
Appendix K

1 PPM Iron(III) Chloride in 20 Millimolar Potassium Hydroxide
Appendix K

1 PPM Iron(III) Chloride in 20 Millimolar Potassium Hydroxide

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1 PPM Iron(III) Chloride in 20 Millimolar Potassium Hydroxide

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Appendix L

The Chemicals Used in This Study
# Appendix L

## The Chemicals Used in This Study

<table>
<thead>
<tr>
<th>Chemical Name</th>
<th>Grade</th>
<th>Description</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boric Acid</td>
<td>A.C.S.</td>
<td>Crystals</td>
<td>Spectrum Chemical</td>
</tr>
<tr>
<td>Ferric Nitrate</td>
<td>98%</td>
<td>Crystals</td>
<td>EM Science</td>
</tr>
<tr>
<td>Ferrous Oxalate</td>
<td>98%</td>
<td>Powder</td>
<td>MCB reagent</td>
</tr>
<tr>
<td>DL-Histidine</td>
<td></td>
<td></td>
<td>Sigma Chem. Co.</td>
</tr>
<tr>
<td>2-Hydroxy-isobutyric acid</td>
<td>99%</td>
<td></td>
<td>Aldrich Chemical Co.</td>
</tr>
<tr>
<td>Iodomethane</td>
<td>Fine Chemicals</td>
<td></td>
<td>Kodak Eastman</td>
</tr>
<tr>
<td>Iron(II) chloride</td>
<td>99%</td>
<td></td>
<td>Aldrich Chemical Co.</td>
</tr>
<tr>
<td>tetrahydrate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iron Reference Solution</td>
<td>Certified Atomic</td>
<td>1000 ppm ± 1%</td>
<td>Fisher Scientific Company</td>
</tr>
<tr>
<td></td>
<td>Absorption Standard</td>
<td>Solute: Ferric Chloride</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Solvent: Dilute HCl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MES (2-[N-morpholino]ethanesulfonic acid)</td>
<td>SigmaUltra &gt;99.5% (titration)</td>
<td>Monohydrate</td>
<td>Sigma Chemical Co.</td>
</tr>
<tr>
<td>Potassium Thiocyanide</td>
<td>Analytical Reagent</td>
<td>Crystals</td>
<td>Mallinckrodt Chemical Wks.</td>
</tr>
<tr>
<td>Potassium Ferrocyanide</td>
<td></td>
<td></td>
<td>Baker</td>
</tr>
<tr>
<td>Standard pH Buffers</td>
<td>Certified ± 0.01 pH@ 25°C</td>
<td>4,7,10</td>
<td>Conning</td>
</tr>
</tbody>
</table>
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