SENSOR DEVELOPMENT FOR THE NUCLEAR FUEL CYCLE:

ELECTROCHEMISTRY, SPECTROELECTROCHEMISTRY,

SPECTROSCOPY, AND CHEMOMETRIC ANALYSIS

By

AMANDA MARIE LINES

A dissertation submitted in partial fulfillment of
the requirements for the degree of

DOCTOR OF PHILOSOPHY

WASHINGTON STATE UNIVERSITY
Department of Chemistry

MAY 2016
To the Faculty of Washington State University:

The members of the Committee appointed to examine the dissertation of AMANDA MARIE LINES find it satisfactory and recommend that it be accepted.

Sue B. Clark, Ph.D., Chair

Samuel A. Bryan, Ph.D.

Kenneth L. Nash, Ph.D.

Nathalie A. Wall, Ph.D.
ACKNOWLEDGEMENT

This dissertation is truly the product of all the help given to me by several kind and exceptional people over the past four years. I hope this work and the resulting outcomes justify all of their very-much appreciated support.

First and foremost I must thank my husband Alex, whose endless patience, generous sense of humor, and creative problem solving has helped me accomplish many things. His support and confidence in my ability to succeed have been steadfast despite distance and my own concerns. I hope that I can be half the best friend/spouse/partner-in-crime that he is.

The support of my family has also been indispensable. I know many of them felt that I was a little crazy to choose this path, but they were also proud of my goals and accomplishments. They have helped me put things in perspective and find balance between my various priorities. There are many routes to take through life, and they have helped me understand those routes can be adjusted to include the things most important to you.

I should also thank Jen Braley and Luther McDonald for being mentors of a unique sort. They are perhaps my favorite examples of profoundly capable scientists who seamlessly meld the two worlds of being professional and having a good time. They helped me start my graduate career at WSU and have continued to help me throughout my time as a student by answering questions with both honesty and humor.

My advisors Sam Bryan and Sue Clark have been vital to the success of my PhD career. Without their encouragement and support, this dissertation would never have been written. Both their suggestions for improving my scientific approach as well as their tireless motivation during times of personal doubt have been essential to my growth as a scientist.
Dr. Bill Heineman has also been incredibly generous with his time and knowledge, without which the quality of my spectroelectrochemistry work would be significantly diminished. His student Shirmir Branch should also be recognized for providing the tools necessary for high-quality spectroelectrochemistry, so I did not have to rely on my ugly-but-functional MacGyver versions.

Finally I would like to acknowledge my committee: Sue Clark, Sam Bryan, Nathalie Wall, and Ken Nash. Their flexibility in working out “committee meetings” that were often one-on-one discussions over a poster or after a talk at an ACS conference has been hugely helpful. Their suggestions, comments, and willingness to share their knowledge and experience has drastically improved the quality of the work reported in this dissertation. Thank you.
SENSOR DEVELOPMENT FOR THE NUCLEAR FUEL CYCLE:

ELECTROCHEMISTRY, SPECTROELECTROCHEMISTRY,

SPECTROSCOPY, AND CHEMOMETRIC ANALYSIS

Abstract

by Amanda Marie Lines, Ph.D.
Washington State University
May 2016

Chair: Sue B. Clark

Fast, robust, and cost-effective means of detecting various species in complex solution environments are needed throughout the nuclear fuel cycle. Several techniques have the potential to meet this need and this manuscript will cover two spectroscopic based methods for accomplishing this.

Spectroelectrochemistry will be the first method discussed, and is a technique that can specifically quantify lanthanides and transition metals by simultaneously monitoring at least two physio-chemical properties. Application of this technique can be limited by both redox chemistry and spectral characteristics of analytes of interest; which is particularly apparent in species like the lanthanides and some free transition metals which have very weak spectral signatures. It is possible to circumvent these limitations and successfully apply spectroelectrochemistry to the analysis of these hard-to-detect species by capturing them in complexes with improved spectral characteristics. This is demonstrated with europium and ruthenium; these elements were chosen due to their spectroscopic and electrochemical characteristics as well as their relevance within
the fuel cycle and industrial fields. The electrochemical and the spectroelectrochemical characteristics of Eu(bpy)$_2$ type complexes will be discussed. As will the *in situ* electrochemical generation of Ru(bpy)$_3$ complexes and their subsequent spectroelectrochemical sensing within a singular spectroelectrochemical sensor device.

The second method discussed will be Raman spectroscopy utilized in tandem with chemometric analysis. A novel micro-Raman probe was developed and tested to monitor streams within microfluidic cells, allowing for characterization of small sample sizes either in-line or through grab samples. This system was tested on simple and complex systems containing HNO$_3$, NaNO$_3$, and/or UO$_2$(NO$_3$)$_2$. Chemometric modeling has been paired with this to build predictive models capable of identifying and quantifying these species based on Raman signatures. Initial testing on larger cell path lengths was successful and translates well to preliminary studies with a 250 µm path length microfluidic device.

Overall, these two methods have been used to successfully characterize and quantify examples of transition metals (Ru), lanthanides (Eu), and actinides (U) and have significant potential to be applied to other species of interest.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGEMENT</td>
<td>iii</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>v</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>x</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xi</td>
</tr>
<tr>
<td>CHAPTER ONE: INTRODUCTION</td>
<td></td>
</tr>
<tr>
<td>1. INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>2. OPTICAL DETECTION OF SPECIES WITH LOW MOLAR ABSORPTIVITY OR LOW QUANTUM YIELDS</td>
<td>3</td>
</tr>
<tr>
<td>3. SPECTROELECTROCHEMISTRY</td>
<td>9</td>
</tr>
<tr>
<td>4. RAMAN SPECTROSCOPY AND IN-LINE REAL-TIME PROCESS MONITORING</td>
<td>14</td>
</tr>
<tr>
<td>5. CHEMOMETRIC MODELING FOR QUANTITATIVE ANALYSIS</td>
<td>18</td>
</tr>
<tr>
<td>6. REFERENCES</td>
<td>22</td>
</tr>
<tr>
<td>CHAPTER TWO: ELECTROCHEMISTRY AND SPECTROELECTROCHEMISTRY OF LUMINESCENT EUROPIUM COMPLEXES</td>
<td></td>
</tr>
<tr>
<td>1. ABSTRACT</td>
<td>27</td>
</tr>
<tr>
<td>2. INTRODUCTION</td>
<td>28</td>
</tr>
<tr>
<td>3. EXPERIMENTIAL</td>
<td>33</td>
</tr>
<tr>
<td>Materials</td>
<td>33</td>
</tr>
</tbody>
</table>
CHAPTER THREE: IN-SITU ELECTROCHEMICAL FORMATION AND SUBSEQUENT SPECTROELECTROCHEMICAL DETECTION OF LUMINESCENT RUTHENIUM COMPLEXES

1. ABSTRACT .................................................................................................................. 67
2. INTRODUCTION ........................................................................................................ 68
3. EXPERIMENTAL ......................................................................................................... 71
   Materials .................................................................................................................. 71
   Equipment ............................................................................................................... 71
4. RESULTS AND DISCUSSION .................................................................................... 72
   Optimizing solution conditions ............................................................................. 73
   Ru solution chemistry and choice of Ru starting material ..................................... 78
   Cyclic Voltammetry .............................................................................................. 79
   Absorption and Fluorescence Spectroscopy .......................................................... 85
   Spectroelectrochemistry ....................................................................................... 89
   Spectroelectrochemical Quantification ................................................................. 103
LIST OF TABLES

1. CHAPTER TWO: ELECTROCHEMISTRY AND SPECTROELECTROCHEMISTRY OF LUMINESCENT EUROPIUM COMPLEXES

Table 2.1: Selected peaks demonstrating expected shifts and their assignments .................. 35

Table 2.2: Results of electrochemical characterization .......................................................... 46

Table 2.3: Spectroscopic characterization .............................................................................. 52

2. CHAPTER THREE: IN-SITU ELECTROCHEMICAL FORMATION AND SUBSEQUENT SPECTROELECTROCHEMICAL DETECTION OF LUMINESCENT RUTHENIUM COMPLEXES

Table 3.1: Characterization of electrochemically generated species and standards ............ 85

3. CHAPTER FOUR: DEVELOPMENT AND TESTING OF A NOVEL MICROSCOPIC RAMAN PROBE AND APPLICATION OF CHEMOMETRIC ANALYSIS

Table 4.1: Peaks of interest and limits of detection............................................................. 120

Table 4.2: Solution Sets 1 and 2 .......................................................................................... 122

Table 4.3: Model details....................................................................................................... 135
LIST OF FIGURES

1. CHAPTER ONE: INTRODUCTION

   Figure 1.1: figure 1 from Richardson et al. depicting mechanism of forming a spectroscopically identifiable Fe species from the hard to detect free Fe$^{2+}$ species[29] ................................................................. 5

   Figure 1.2: Figure 4 from Whan and Crosby depicting energy transfer within chelated rare earth systems. Straight arrows indicate radiative decay and wavy arrows indicate radiationless decay [33]. ........................................................................................................ 7

   Figure 1.3: Top: Sensing platform consisting of thin polymer film coated ITO electrode capable of applying three layers of selectivity for analytes of interest. Middle: full spectroelectrochemistry device incorporating sensing platform. Bottom: Orange probability function indicating penetration ability of evanescent wave from ATR probing technique. ........................................................................................................ 11

   Figure 1.4: Adapted from Andria et al [44] where the Top: absorption response at 520 nm of a SEBBS film containing Fe(bpy)$_3^+$ and Ru(bpy)$_3^+$ as a function of time while applied potential was stepped to selectively oxidize and reduce the Fe and then the Ru species. Bottom: Cyclic voltammogram indicating the redox couples for [Fe(bpy)$_3^+$]$^{2+/3+}$ and [Ru(bpy)$_3^+$]$^{2+/3+}$ .................................................................................................................. 13

   Figure 1.5: Mechanism of excitation and emission of Raman and fluorescence spectroscopy. Taken from Figure 2.2 of [50]. In plot B the green spectrum with the broad band is the absorption spectrum to be compared with the dotted red line which is the emission spectrum. The green spectrum with several sharp peaks presents a resonance Raman spectrum. ........................................................................................................ 16

   Figure 1.6: Application of chemometric modeling for real time analysis of data. Models are built from spectroscopic training sets and used to develop a chemometric model. This model can then be applied to data collected in-line at a flowing process stream to provide real time analysis. ........................................................................................................ 21

2. CHAPTER TWO: ELECTROCHEMISTRY AND SPECTROELECTROCHEMISTRY OF LUMINESCENT EUROPIUM COMPLEXES

   Figure 2.1: Schematic indicating the in situ formation of the luminescent Eu(III) complex and the electrochemical modulation of that complex between the luminescent Eu(III) and non-luminescent Eu(II) species. Both Reaction A and B can be monitored spectroscopically................................................................. 30

   Figure 2.2: Structures of ligands of interest and the formulas of their complexes with Eu. Blue coloration indicates the atoms that bind to the metal center. Red coloration indicates the most electron-donating group................................................................. 32
Figure 2.3: Normalized FTIR spectra of free ligands and complexes. .................................................. 36

Figure 2.4: Top row: pictures of spectroelectrochemistry setup #1 with instruments components labeled. Bottom row: schematic and picture of the spectroelectrochemistry thin cell. ........................................................................................................... 39

Figure 2.5: Top: Overlay of CVs of complexes aligned to the $E^{o}$ of the internal standard (ferrocene). CVs were collected at 100 mV/s using a Pt disk WE, Pt wire Aux, and an Ag wire quasi-RE. Bottom: Overlay of DPV responses of complexes. Black lines represent the background runs of the MeCN and 0.1 M TABPF$_6$. All complexes were at approximately 1 mM for CVs and 0.01 mM for DPV ....................................................... 42

Figure 2.6: Data is organized into four boxes for each of the four complexes, where within a box the top plot presents the CVs collected at multiple scan rates (900-20 mV/s) which were collected in MeCN and 0.1 M TBAPF$_6$ using Pt disk WE, a Pt wire Aux, and an Ag wire quasi-RE and the bottom plot presents the peak current vs the square root of the scan rate and the best fit lines used for Randles-Sevcik analysis. .................. 47

Figure 2.7: Top: Excitation spectra (dashed lines) and emission spectra (solid lines) of Eu (III)-based complexes, exciting at 330 nm for emission spectra. Bottom: Excitation spectra (dashed lines) and emission spectra (solid lines) of Eu(II)-based complexes. ................................................................................................................................. 49

Figure 2.8: Comparison of the emission spectra of EuCl$_3$ in water and Complex I in acetonitrile. Different solvents were required due to solubility constraints and Complex I was measured at half the concentration of EuCl$_3$ due to detector limits. The right plot presents the same data as the left plot, but zoomed in to show the emission peaks from EuCl$_3$ in water ................................................................................................................ 51

Figure 2.9: Plots of Intensity of the 613 nm Peak versus Concentration of the Complex in Solution. Clockwise from top left: Complex I, Complex II, Complex III, and Complex IV ........................................................................................................................................... 53

Figure 2.10: Spectroelectrochemical modulation of complexes: Data is organizing into four boxes for each of the four complexes, within a box the top plot presents the applied potential over time, the middle plots the spectra over time for the duration of the modulation, and the bottom plots the intensity of the 613-nm peak (red) and the 450-nm peak (black) over time. Baseline subtraction methods were used to extract 613-nm peak from shoulder of 450-nm peak. Results were normalized to allow for ease of comparison. .................................................. 56

Figure 2.11: Spectroelectrochemical modulation of complexes: Data is organizing into four boxes for each of the four complexes, where within a box the top plot presents applied potential over time and the bottom plots the intensity of 613 nm peak over time as applied potential was modulated. Red dots indicate points at which spectra were collected ........................................................................................................................................................................ 57
Figure 2.12: Top) overlay of emission spectra from the reduced, Eu(II) species, of the complexes (solid lines) and the emission spectra of the free ligand (dashed lines) where $\lambda_{\text{ex}}$ was 330 nm. Bottom) Fig. 7 from reference Yan et al. [42] .......................... 60

3. CHAPTER THREE: IN-SITU ELECTROCHEMICAL FORMATION AND SUBSEQUENT SPECTROELECTROCHEMICAL DETECTION OF LUMINESCENT RUTHENIUM COMPLEXES

Figure 3.1: Schematic detailing the sensor mechanism. Free Ru$^{3+}$ will be reduced to Ru$^{2+}$ in the presence of excess ligand which will allow for the formation of the luminescent [Ru(ligand)$_3$]$^{2+}$ species which can be oxidized to the non-luminescent [Ru(ligand)$_3$]$^{3+}$ species. Note Ru$^{3+}$(aq), Ru$^{2+}$(aq), free ligand, and [Ru(ligand)$_3$]$^{3+}$ species all lack the strong luminescent characteristics of [Ru(ligand)$_3$]$^{2+}$................................ 70

Figure 3.2: HySS speciation diagrams indicating speciation as a function of pH for 2,2′-bypridine (top left), 1,10′-phenanthroline (top right), an Fe(III)/acetate/bpy system (bottom left), and an Fe(II)/acetate/bpy system (bottom right). Data was taken from the NIST database. ............................................................................................................. 76

Figure 3.3: Examples of RuCl$_3$ solutions from left to right 20 mM RuCl$_3$ in 0.1 M acetate buffer, 20 mM RuCl$_3$ in 0.1 M NaCl, 0.1 mM RuCl$_3$ in acetate buffer, 0.1 mM RuCl$_3$ in 0.1 M NaCl .......................................................................................................................... 77

Figure 3.4: Top: CVs of solutions containing the bpy ligand. Bottom: CVs of solutions containing the phen ligand. CVs of both electrochemically generated complexes were collected after a total reduction time of 70 min (with exception of the teal line where potentials were applied in excess of 24 hrs). Data were collected using an ITO WE, Pt Aux, Ag/AgCl, 3 M NaCl RE in a 0.1 M acetate buffer. RuCl$_3$ was at 0.5 mM in all cases and ligands were at roughly 15 mM.................................................. 82

Figure 3.5: Top: CVs of experiments utilizing the bpy ligand collected between 10 min periods of applying a reducing potential of 0.05 V after a total reduction time of 70 min. Ru before the addition of ligand in grey, and successive CVs for the electrochemical generation are show from light to dark blue as total reduction time increases. Arrows indicate the direction of peak behavior as a function of time. Bottom: plot of peak current for the anodic (circles) and cathodic (triangles) waves of the Ru II/III (light blue), Ru II/IV (grey), and Ru(bpy)$_3$ II/III (blue) couples from CVs collected between periods of applying a reducing potential. Data were collected using an ITO WE, Pt Aux, Ag/AgCl RE in a 0.1 M acetate buffer. RuCl$_3$ was at 0.5 mM in all cases and ligands were at roughly 15 mM.................................................. 83

Figure 3.6: Top: Excitation (dotted lines) and emission (solid lines, excitation at 404 nm) profiles for electrochemically generated complexes and standards. Bottom: Absorption spectra (normalized for concentration differences) of electrochemically generated complexes and standards where [Ru] was constant at 0.01 mM and free ligand was at approximately 15 mM. ................................................................. 86
Figure 3.7: Top: emission spectra collected over the span of 40 min as free ligand (15 mM) and then RuCl₃ (0.1 mM) were added to the 0.1 M acetate solution and applied potential was stepped between 1.3 V and 0.05 V. Bottom: overlay of emission intensity at 605 nm for the spectra presented in the top panel (red, left axis) with the applied potential (black, right axis) versus time.

Figure 3.8: Top: absorption spectra collected over the span of 40 min as free ligand (15 mM) and then RuCl₃ (0.1 mM) were added to the 0.1 M acetate solution and applied potential was stepped between 1.3 V and 0.05 V. Bottom: overlay of absorption at 490 nm for the spectra presented in the top panel (red, left axis) with the applied potential (black, right axis) versus time.

Figure 3.9: Top: emission from the 591 nm band when electrochemically generating Ru(phen)_3 species by applying wave form 3 (grey line, where red dotted line indicates maximum ingrowth behavior), applying waveform 2 constant potential (light pink line), and wave form 1 no potential (purple line, light purple solid line is same experiment measured 48 h after storing solution on the bench top, light purple dotted line is same experiment measured 48 h after storing solution in dark). Three bottom plots show applied wave forms for modulation experiment (3), constant potential experiment (2), and no potential experiment (1), respectively.

Figure 3.10: Top: Reaction mechanism for the formation of [Ru(ligand)_3]^{2+/3+} from Ru(III) at the surface of electrode. Bottom: depiction of majority species present at the surface of the electrode when applying potentials in give ranges. The listed potentials are the E° for the Ru (II)/(III), Ru (III)/(IV), and [Ru(ligand)_3]^{2+/3+} couples from left to right. Note the printed 1.08 V will vary depending on ligand used.

Figure 3.11: Top: emission from the 605 nm band when electrochemically generating Ru(bpy)_3 species when applying potentials necessary to generate complex (grey), and what is observed when applying potentials capable of reducing complex but not capable of generating more complex (blue solid), the blue dashed line indicates the expected maximum emission intensity at 605 nm when applying potentials capable of reducing complex but not capable of generating more complex. Bottom: applied potential waveforms corresponding to top plot.

Figure 3.12: Top) the Δ emission between the final oxidizing and reducing steps of several experiments where the solution ratio of Ru and the respective ligand was varied. Blue circles correspond to experiments completed with bpy and red circles correspond to experiments completed with phen. Bottom) Overlay of luminescence spectra from the final reduction step. Blue spectra correspond to the bpy experiments and red spectra correspond to the phen experiments. Color variation from dark (dark blue and red respectively) to lighter (light blue and light pink) colors corresponds to increasing ligand concentration.

Figure 3.13: Top: Emission at 605 nm as a function of time for electrochemical generation experiments at varying [Ru] and constant M:L with the bpy ligand. Middle: Emission at 591 nm as a function of time for electrochemical generation experiments at
varying [Ru] and constant M:L with the bpy ligand. Middle: Emission at 591 nm as a function of time for electrochemical generation experiments at varying [Ru] and constant M:L with the phen ligand. Bottom: Applied potential as a function of time for the electrochemical generation experiments................................................................. 104

Figure 3.14: Δ emission at 605 nm for Ru(bpy)$_3$ species (standard and electrochemically generated species) and at 591 nm for the Ru(phen)$_3$ species versus the total concentration of Ru in the system. Inset depicts linear range for the electrochemically generated species. Note this data corresponds to Figure 3.13 where electrochemical generation potentials were only held for 47 minutes. ................. 106

4. CHAPTER FOUR: DEVELOPMENT AND TESTING OF A NOVEL MICROSCOPIC RAMAN PROBE AND APPLICATION OF CHEMOMETRIC ANALYSIS

Figure 4.1: A) Example of microfluidic chip B) schematic of microscopic Raman probe focused on chip C) picture of microscopic Raman probe focused on a flow cell D) Picture of focused excitation beam from microscopic Raman probe ......................... 116

Figure 4.2: Top: comparison of Raman spectra of a sample of 3 M HNO$_3$ and 2 M UO$_2$(NO$_3$)$_2$ collected in a 4 cm path length cell (black) and in a 1 cm path length cell (grey) where the inset presents the NO$_3$ peak of a 3 M HNO$_3$ solution in the three different cells; Bottom: Raman intensity versus concentration for UO$_2$(NO$_3$)$_2$ in the 4 cm path length cell (blue circles) the 1 cm path length cell (dark blue triangles), and the response for the HNO$_3$ species in the 4 cm path length cell (red circles) and the 1 cm path length cell (dark red triangles) and the 250 µm path length (pink squares). Error bars are marked at 3σ. ...................................................................................... 121

Figure 4.3: Top: Raman spectra as HNO$_3$ is held constant at 2 M and UO$_2$(NO$_3$)$_2$ is varied from 0 to 2 M. Bottom: Raman spectra as UO$_2$(NO$_3$)$_2$ is held constant at 2 M and HNO$_3$ is varied from 0 to 6 M. ........................................................................................................ 124

Figure 4.4: Top: Raman spectra over the course of a flow experiment (8 µL flow cell with a 1 cm path length) where concentrations of HNO$_3$ and NaNO$_3$ were varied. Bottom: double axis plot, left axis corresponds to the concentration profile for HNO$_3$ (black) and NaNO$_3$ (grey dotted), right axis corresponds to the intensity of the NO$_3^-$ band at 1048 cm$^{-1}$ (red). .................................................................................................................. 128

Figure 4.5: PLS modeling results for HNO$_3$ (top) and total NO$_3^-$ (bottom) where the black circles represent the calibration set (solution set 2 in 4 cm path length vials), the black lines indicate the best fit lines for the models, the red dashed lines indicate the 95% confidence limits of the models, and the red triangles indicate the prediction set (flow experiment in 1 cm path length cell, spectra presented in Figure 4.4). The insets show the model predictions (red triangles) as a function of time over the course of the flow test experiments (compare to Figure 4.4). ........................................ 130

Figure 4.6: Top: Raman spectra from flow cell experiment where solutions of different HNO$_3$ and UO$_2$(NO$_3$)$_2$ concentrations were injected into a flow cell of 1 cm path
Figure 4.7: Model performance data comparing the predicted versus the known concentrations for HNO₃ (top), UO₂(NO₃)₂ (middle) and total NO₃⁻ (bottom) solution components. where the black circles represent the calibration set (solution set 1 in 4 cm path length vials), and the red triangles indicate the prediction set (flow experiment in 1 cm path length cell, spectra presented in Figure 6). ........................ 132

Figure 4.8: The Raman excitation point (red dot) focused within the microfluidic channel. The edges of the channel can be seen along the top and bottom of the photo. Not the channel depth (path length) is 250 µm, while the channel width is 300 µm. ....... 137

Figure 4.9: Top: Raman spectra over the course of a flow experiment within the microfluidic device where concentrations of HNO₃ and NaNO₃ were varied. Bottom: double y axis plot, left axis corresponds to the concentration profile for HNO₃ (black) and NaNO₃ (grey dotted), right axis corresponds to the intensity of the NO₃⁻ band at 1045 cm⁻¹ (red).................................................................. 140

Figure 4.10: PLS modeling results for HNO₃ (top) and total NO₃⁻ (bottom) where the black circles represent the calibration set (solution set 2 in 4 cm path length vials) and the red triangles indicate the prediction set (flow experiment in microfluidic device, spectra presented in Figure 4.9). The insets show the model predictions (red triangles) as a function of time over the course of the flow test experiments (compare to Figure 4.9). ................................................................. 141
CHAPTER ONE

INTRODUCTION

Nuclear energy is a key component in the energy portfolios of many nations, including the United States where it accounts for approximately 20% of the power produced[1, 2], and France where it accounts for approximately 75% [3]. Additionally, countries such as China and Russia are planning to shift some of their energy needs to nuclear power in the coming years[4]. While nuclear energy has the benefits of a significantly smaller carbon footprint than fossil fuel sources, and significantly higher dependability compared to renewable sources such as photovoltaic and wind sources, there are concerns regarding its use [5, 6]. Safeguards concerns regarding the mis-use of nuclear material as well as the safe disposal of wastes and limits on minable sources of Uranium are primary concerns [7, 8].

Reprocessing used fuel is one available option that can alleviate the latter two concerns by shortening lifetimes of wastes as well as allowing for the recycling of U [2, 9]. However, in discussions of reprocessing, the concern for proper safeguards and radioactive material monitoring again pop up. Overall, one of the primary difficulties facing the nuclear industry is the need for detection methods capable of monitoring species of interest throughout the fuel cycle. The current methods for accomplishing this tend to be costly and slow. Furthermore, current methods often involve the practice of collecting grab samples, which can expose workers to radiation and may not provide a representative analysis of the systems studied.
There is a strong need for robust sensors that can provide fast analysis throughout the nuclear fuel cycle. Spectroscopic analysis is one option to meet this need due to its fast response and the known spectroscopic activity of many of the expected waste components [10-16]. Furthermore, spectroscopic equipment can be effectively plumbed in-line to reprocessing streams to supply in-line real-time analysis [10, 14].

There are, however, difficulties in applying spectroscopy to various aspects of fuel cycle analysis. For example, within reprocessing streams, there are likely to be numerous species that may have interfering spectroscopic signatures where matrix effects will not necessarily allow for linear spectroscopic response to concentration relationships. Additionally, the species of interest might not have strong or identifiable spectroscopic signatures. This has the potential to make detection and quantification of analytes difficult to impossible. Fortunately, spectroscopy can be paired with other techniques to circumvent some of these limitations. For example, spectroscopy can be paired with electrochemistry in spectroelectrochemistry to provide a method isolating analyte signature from an environment where direct interferents are present. Spectroscopy can also be paired with chemometric analysis to provide robust and fast means for quantifying species in complex environments where interferents and matrix effects make application of simple Beer’s Law type methods difficult. For those situations where analytes of interest do not have strong spectroscopic signatures, spectroscopy can be paired with clever chemistry to change the optical characteristics of those analytes. Overall, spectroscopy is a versatile technique that can easily be combined with other techniques to address the need for detection mechanisms throughout the nuclear fuel cycle.
OPTICAL DETECTION OF SPECIES WITH LOW MOLAR ABSORPTIVITY OR LOW QUANTUM YIELDS

When looking for fast and cost effective methods of identifying and quantifying analytes, spectroscopy is an excellent option. Spectroscopy has the added benefit of being incredibly versatile in how it can be used to probe a system. Several reports demonstrate the effectiveness of plumbing spectroscopic probes for Raman, FTIR, and UV-vis-NIR analysis directly in-line to flowing systems for real-time process analysis and monitoring [10, 11, 17]. Other reports demonstrate the applicability of either absorbance or fluorescence spectroscopy to directly probe electrochemical cells in either solution environments or more complex polymer film environments [18-20]. Furthermore, the spectroscopic equipment needed for Raman, absorbance, or fluorescence measurements can be designed in portable and robust packages, making it possible to have field deployable instruments.

However, the spectroscopic characteristics of many fission products do not lend themselves easily to effective spectroscopic analysis. The lanthanides for example have very low molar absorptivities and therefore undesirably high limits of detection in absorbance based measurements [21-23]. Additionally, while all lanthanides are luminescent, their low molar absorptivities result in low emission intensities, again leading to high limits of detection. Similarly, many of the d-block fission products do not have strong spectroscopic features as either free metal ions in solution or in some of their more common complexes. One example is technetium, where its most commonly expected form in processed waste and as an environmental contaminant is pertechnetate, which does not have a uniquely identifiable absorption signature and is not emissive [24]. Yet another example is ruthenium, which despite
the huge variety of species it can form within nuclear fuel reprocessing schemes, is not easily identified spectroscopically in solution [25-28].

Fortunately, many of the spectroscopic limitations of fission products can be circumvented through chemistry. By complexing the metal species of interest to ligands that enhance or change the spectroscopic characteristics of the metal, a previously hard to detect species can become significantly easier to detect. This approach has been tested and used throughout the literature both old and new. Earlier examples include the use of arsenazo III to bind thorium, uranium, and zirconium in complexes with large molar absorptivities [29]. A newer example of this was reported by Richardson et al, where the absorbance characteristics of Fe^{2+}(aq) were changed by complexing the metal ion to 2,2'-bypyridine (bipy) [30]. Figure 1.1 depicts the mechanism used in that work. Fe^{2+} (aq) does not have a strong or characteristic absorption spectrum, but the [Fe(bipy)_3]^{2+} has a well-known absorption spectrum and a large molar absorptivity. In all these examples, hard to detect species were captured in complexes that enabled or vastly improved the absorption based detection of the target analyte.

This method can be further improved by utilizing fluorescence instead of absorbance spectroscopy. Fluorescence based measurements often allow for improved spectroscopic selectivity as well as a significant reduction in limits of detection. Numerous luminescent complexes containing lanthanides or d-block metal ions have been thoroughly explored in the literature [22, 24, 31, 32]. This suggests that it could be possible to capture target metal ions within luminescent complexes and enable the improved spectroscopic detection of those species.
Figure 1.1: figure 1 from Richardson et al. depicting mechanism of forming a spectroscopically identifiable Fe species from the hard to detect free Fe$^{2+}$ species[29]
In the case of the lanthanides, complexed chromophores can be used to deliver light to the metal center and significantly improve emission of the metal species [22, 33, 34]. The mechanism for this has been discussed in several reports and Figure 1.2 presents a Jablonski diagram of energy transfer within rare earth-chromophore ligand complexes. These chromophores are often referred to as sensitizing or antenna ligands and numerous lanthanide complexes have been reported [22, 32, 33]. In the case of some transition metals such as Tc or Ru, luminescent complexes that utilize a slightly different mechanism of emission have also been reported in the literature [24, 35, 36]. For Ru the mechanism is fairly complex and arguments have been made to suggest the emission is a result or either a charge transfer or a \(^1T_{1g} \rightarrow ^1A_{1g}(d-d)\) transfer from the octahedral mode [35, 37]. Whichever mechanism is utilized by the complex, characteristics such as quantum yield and wave lengths of emission will vary depending on the bound ligand. This indicates that choosing the appropriate ligand is an important step. Interestingly, ligand choice is effected by several factors when determining what ligand will be appropriate for a particular metal species. These factors include:

1) Ligand energetics
2) Kinetics of formation/thermodynamic stability of complex
3) Solubility of ligand and complex

Ligands must display the appropriate orbital energetics to effectively sensitize the metal species. The donating orbitals of the ligands must fall within a certain energy range of the accepting orbital of the metal species [38]. As an example, the accepting orbital of Eu, \(^5D_0\), is significantly different in energy than the accepting orbital of Yb, \(^2F^{7/2}\). A ligand that could effectively sensitize Eu would not fall within the appropriate range to sensitize Yb [22]. While
Figure 1.2: Figure 4 from Whan and Crosby depicting energy transfer within chelated rare earth systems. Straight arrows indicate radiative decay and wavy arrows indicate radiationless decay [33].
this does indicate that a sensor meant for quantifying lanthanides would need added complexity to meet the ligand needs of each metal species, it also indicates that ligand choice can be used as a form of selectively changing spectroscopic characteristics of only a few target analytes.

The kinetics and thermodynamics of complex formation are also an important factor in ligand choice. If the goal is to change the spectroscopic characteristics of species and analyze those species within a short period of time, then ideally the kinetics of formation should be fast and the binding constants should be large. This would allow for the quantitative capture of the metal ion in a reasonable amount of time and would be necessary for field deployable sensors based on *in situ* formation of luminescent species. Similarly, if the goal is to develop a sensor based on the *in situ* generation of luminescent species, then both the ligand and resulting complex must be soluble within the sensor environment.

One of the primary limitations of spectroscopy as a method for identifying fission products is the poor spectral characteristics of many of those products. Fortunately this problem can be addressed through several methods. Aside from choosing the appropriate type of spectroscopy to best detect the analytes of interest, chemistry can be used to improve the spectral characteristics of otherwise hard to detect species. In these cases it is possible to bind the species of interest to ligands that will alter the spectroscopic characteristics of those species. Ligands can be chosen to enhance different types of spectroscopic response, for example they can be used to improve either absorption or emission signals. Overall, with appropriate ligand choice, the spectral characteristics of a target analyte can be drastically changed to allow for successful detection and analysis of the analyte.
SPECTROELECTROCHEMISTRY

Spectroelectrochemistry combines spectroscopy and electrochemistry to create a unique and powerful detection mechanism capable of isolating analyte signature based on two physio-chemical characteristics. The spectroelectrochemistry method involves monitoring a system spectroscopically while using applied potential to control the oxidation state of species present in that system. This results in a system where detection is solely based on a change in optical response due to a change in oxidation state. This essentially allows for multiple levels of selectivity for analytes of interest including but not limited to the redox potential and spectroscopic finger print of the analyte of interest [20, 39-41]. By employing multiple layers of selectivity for target analytes, spectroelectrochemistry offers a method a detection were target analyte signal can be isolated without completing prior separations of the samples.

The top box of Figure 1.3 presents a schematic that demonstrates how spectroelectrochemistry works and how the multiple levels of selectivity are applied. All spectroelectrochemical sensors have at least two levels of selectivity but additional layers can be added by altering the experimental setup. Spectroelectrochemistry setups vary but most setups require the use of an optically transparent electrode that will allow for spectroscopic probing of the species on the surface of the working electrode [18, 24, 42]. A common spectroelectrochemistry setup utilizes working electrodes that consist of a glass slide substrate that has been coated with conductive layer of material thin enough to be optically transparent. An example would be indium tin oxide (ITO) coated electrodes. This type of electrode is particularly advantageous because the optically transparent electrode can then be coated with an ion
exchange polymer film that can add another layer of selectivity to the spectroelectrochemical
device.

The top schematic of Figure 1.3 presents an example of an ITO electrode that has been
coated with a thin polymer ion exchange film and demonstrates examples for the layers of
selectivity provided by this type of spectroelectrochemistry device. The goal for this example
device in the top schematic of Figure 1.3 is to detect the species D in a complex system
containing other interfering species A, B, and C. The ion exchange film can be used to
selectively absorb either cations or anions. The example in Figure 1.3 absorbs cations,
eliminates species A; nafion and SSEBS films can accomplish this [24, 30, 43]. Of the
appropriately charged species that migrate into the film, only the species that are
electrochemically active in the potential range of interest will meet criteria to be detected, in
figure 1.3 that eliminates species B. Of the species that migrate into the film and are
electrochemically active, only the species with the appropriate spectroscopic signature will meet
the criteria to be detected, this eliminates species C. After applying the three layers of selectivity,
only the D species will be detected. Furthermore, the system can be spectroscopically probed by
utilizing the glass slide substrate of the working electrode as an attenuated total reflectance
(ATR) light guide. This is depicted in the middle and bottom schematics of Figure 1.3. At the
internal points of reflectance (reflectance points at the glass edge contacting the thin film) an
evanescent wave can penetrate the into the system a short distance to probe a limited portion of
the system [44]. The evanescent wave can penetrate a distance of one wavelength into the
system, which will predominantly limit the probed area to the thin film. This method can be used
for either absorbance or fluorescence based measurements as indicated by the middle schematic
of Figure 1.3.
Figure 1.3: Top: Sensing platform consisting of thin polymer film coated ITO electrode capable of applying three layers of selectivity for analytes of interest. Middle: full spectroelectrochemistry device incorporating sensing platform. Bottom: Orange probability function indicating penetration ability of evanescent wave from ATR probing technique.
Several reports demonstrate the power of this analytical technique where spectroelectrochemistry can be used to isolate desired analyte signal in systems that contain direct spectroscopic interferents [45, 46]. An example from Andria et al. can be seen in Figure 1.4 [45]. In this work, a solution containing a mixture of [Fe(bpy)$_3$]$^{2+}$ and [Ru(bpy)$_3$]$^{2+}$ was exposed to a SEBBS polymer film which selectively absorbs cationic species. The system utilized by Andria et al. only monitored the absorbance at 520 nm where both [Fe(bpy)$_3$]$^{2+}$ and [Ru(bpy)$_3$]$^{2+}$ absorb light. With only spectroscopic analysis it would be impossible to identify or quantify the species present based on the confounded absorption measurements. However, both [Fe(bpy)$_3$]$^{2+}$ and [Ru(bpy)$_3$]$^{2+}$ are electrochemically active (with notably different formal reduction potentials) and can be oxidized to species that do not absorb at 520 nm, [Fe(bpy)$_3$]$^{3+}$ and [Ru(bpy)$_3$]$^{3+}$. Andria et al. took advantage of this and selectively oxidized only the Fe complex. By doing this, the absorption signature from the [Fe(bpy)$_3$]$^{2+}$ species was turned off and a delta absorbance was observed where the delta represented the light absorbed by only the Fe species. Signal from the Ru species could also be turned on and off with applied potential to isolate the magnitude of light absorbed by only the Ru species. Via this process the spectroscopic signal from both the Fe and Ru target analytes were isolated despite the presence of direct spectroscopic interferents. Species could then be quantified based on the change in emission with applied potential and standard Beer’s Law analysis.

Spectroelectrochemistry is therefore a powerful technique capable of isolating desired analyte signal in complex systems. Furthermore, it is fairly adaptable to different detection schemes. Examples shown in Figures 1.3 and 1.4 focused on sensor designs utilizing thin polymer films, but spectroelectrochemistry devices have been designed to probe thin layer
Figure 1.4: Adapted from Andria et al [44] where the Top: absorption response at 520 nm of a SEBBS film containing Fe(bpy)$_3$ and Ru(bpy)$_3$ as a function of time while applied potential was stepped to selectively oxidize and reduce the Fe and then the Ru species. Bottom: Cyclic voltammogram indicating the redox couples for [Fe(bpy)$_3$]$^{2+/3+}$ and [Ru(bpy)$_3$]$^{2+/3+}$.
solution environments [20, 39], aqueous microdrops [42, 47], and thick layer solution environments [48]. Overall, spectroelectrochemistry has been applied to the detection of a wide range of analytes in a variety of environments from ferrocene in Hanford tank waste [18], to lanthanides in molten salts [15].

One particularly interesting application of spectroelectrochemistry had been seen in the work by Richardson et al [49]. In this work Richardson and co-workers developed a method to capture aqueous Fe$^{2+}$ within the sensor environment and complex it with a ligand that drastically changed its spectroscopic characteristics before using spectroelectrochemistry to analyze the Fe species. This is particularly interesting because aqueous Fe does not have a strong or uniquely identifying absorption of fluorescence signature, making it difficult to detect with systems that depend on spectroscopy, such as spectroelectrochemistry. However, by complexing the Fe, Richardson and co-workers bypassed that spectroscopic limitation and made a spectroelectrochemistry sensor capable of detecting an otherwise hard-to-detect species. Figure 1.1 presents the mechanism used in the Richardson work.

This work opens up a new and potentially extremely valuable field of sensor design. By combining methods for altering the spectroscopic signatures of analytes with the spectroelectrochemistry technique, new detection mechanisms can be developed to analyze hard to detect analytes in complex environments.

**RAMAN SPECTROSCOPY AND IN-LINE REAL-TIME PROCESS MONITORING**

Raman spectroscopy is a form of vibrational spectroscopy that is particularly useful for identifying structures and polyatomic ions within samples. The mechanism of Raman spectroscopy is presented in Figure 1.5 which was taken from the text “Introduction to the
Figure 1.5 demonstrates the difference in Raman versus fluorescence measurements, where in Raman spectroscopy an analyte is excited to a virtual energy state before emitting a photon to return to the ground state while in fluorescence spectroscopy an analyte is excited to a new electronic state before emitting a photon to return to the ground state. The Raman mechanism is essentially an inelastic scattering of light and typical Raman setups are focused on detecting the 180°, or backscattered, inelastically scattered light.

As stated above, Raman spectroscopy is particularly useful for structural analysis and identification of Raman-active polyatomic ions within a sample. Raman spectroscopy also presents a level of selectivity akin to fluorescence spectroscopy where the system is designed to detect inelastically scattered light at 180° to the excitation source. This system allows for a reduction of measured noise from the excitation source (which allows for lower limits of detection) where only Raman active species (and sometimes fluorescent interferents) are detected. This makes Raman spectroscopy a powerful tool for the selective analysis of species.

Raman spectroscopy has been applied in numerous areas and has the potential to be integrated into several new fields. Nuclear fuel reprocessing schemes include many Raman active species, making Raman spectroscopy very applicable to the analysis of those streams. Additionally, Raman response tends to be fast and can more quickly supply characterization information than many other forms of analysis used within the nuclear fuel cycle.
Figure 1.5: Mechanism of excitation and emission of Raman and fluorescence spectroscopy. Taken from Figure 2.2 of [50]. In plot B the green spectrum with the broad band is the absorption spectrum to be compared with the dotted red line which is the emission spectrum. The green spectrum with several sharp peaks presents a resonance Raman spectrum.
The idea of applying Raman spectroscopy to the analysis of nuclear reprocessing schemes leads into the goal of developing in-line real-time process monitoring systems. Methods for in-line real-time process monitoring are already successfully utilized in a variety of fields including the pharmaceutical and food industries [51-53]. Examples often utilize spectroscopy due to its non-destructive nature, versatility, and fast response. There are several fields that could benefit from the application of in-line real-time process monitoring, including nuclear fuel reprocessing where there is no in-line monitoring technology currently available and in use for determining the chemical composition within fuel reprocessing streams. With proper application and system design, Raman spectroscopy can be applied to the in-line real-time analysis of reprocessing streams. Several reports have presented the effectiveness of utilizing in-line real-time process monitoring on systems similar to nuclear fuel reprocessing schemes [10, 11, 14, 17].

Reprocessing currently is a controversial subject for many reasons, including the difficulties in safeguarding materials to ensure radioactive materials are not misused. Safeguards objectives cannot be met by material accountancy, containment, and surveillance alone [54]. These objectives could be more easily met if the reprocessing scheme could be continuously monitored/analyzed to determine stream compositions. Monitoring the processing of nuclear materials using grab samples is often too slow and expensive to be effective and is particularly difficult due to the high level of radiation present. In-line real-time process monitoring, however can provide fast (real-time) analysis of material while eliminating the difficult and dangerous need to collect grab samples (in-line). This form of process monitoring can help meet safeguards objectives while improving the safety and efficiency of process monitoring. In-line spectroscopic analysis can be paired with software analysis packages to provide real-time analysis and even system control as indicated in Figure 1.6.
CHEMOMETRIC MODELING FOR QUANTITATIVE ANALYSIS

Chemometric analysis provides a method for utilizing mathematical models to correlate observed data to select sample characteristics. Various forms of chemometric analysis have been applied to a wide range of analysis problems including archeological studies of pottery to determine pottery origin based on inductively couple plasma atomic emission spectroscopy [55] as well as monitoring rainwater pollution based on pH, conductivity, and spectroscopic data [56]. Chemometric analysis is a powerful method of elucidating various sample characteristics (sample origin, analyte concentrations/speciation, etc.) because it takes a comprehensive and all-inclusive look at observed data within its model.

When chemometric analysis is applied to spectroscopic data and used to quantify species in solution, it tends to be more powerful than typical calibration curves. In typical Beer’s Law analysis for example, calibration curves run into difficulty outside a limited linear range and with increasing system complexity/number of components (resulting in overlapping of bands or various matrix effects such as ion-pairing) because it is used only to look at select peaks. Chemometric analysis however, can utilize the entire observed spectrum. By doing this, chemometric analysis is not necessarily limited to the linear response range of a particular peak and can tickle out enhanced details indicating matrix and ionic strength effects, allowing for more sophisticated analysis and quantification of analytes.

While there are multiple forms of chemometric analysis, the form that is often most useful for quantifying analytes based on spectroscopic data is partial least squares (PLS) regression. The mathematics and theory behind PLS regression are described in detail elsewhere [57-59] but a brief explanation of the math is included below [60]. The primary strengths of PLS are its
ability to determine covariance between two datasets. In this case PLS can be used to find covariance between spectroscopic data and concentrations of analytes. Furthermore, PLS performs very well in cases where there are more variables (wavenumbers) than observations (samples measured). PLS regression can be presented in the form of:

\[ Y = XB + F \]  \hspace{1cm} \text{Eq 1.1}

Where \( X \) represents a matrix of the spectral data. This matrix is of the size \( I \times J \) where \( I \) is the number of spectra collected (ideally there will be multiple spectra of a single sample) and \( J \) is the number of wavelengths or wavenumbers measured. The matrix \( Y \) contains all the concentration information and is of the size \( I \times K \). \( I \), again is the number of spectra collected and \( K \) is the number of analytes of interest; where the matrix is populated by the concentration data for each analyte for each spectra. \( B \) then contains the regression relations and is of size \( K \times J \) and \( F \) is the residual error matrix. Note, \( X \) is referred to as the calibration set.

To build the matrix \( B \), the matrices \( Y \) and \( X \) must be decomposed to obtain the eigenvalues (scores) and eigenvectors (latent variables indicating variance). The matrices are broken down into the equations:

\[ X = TP^T + E_X \]  \hspace{1cm} \text{Eq. 1.2}
\[ Y = UQ^T + E_Y \]  \hspace{1cm} \text{Eq. 1.3}

Where the matrices \( T \) and \( U \) contain the scores, \( P^T \) and \( Q^T \) contain the latent variables, and \( E_X \) and \( E_Y \) contain the residual errors. Note, the superscript \( T \) indicates the matrix is transposed. The covariance between \( X \) and \( Y \) can then be determined using the equation:

\[ \lambda w_1 = X^TYY^T X w_1 \]  \hspace{1cm} \text{Eq. 1.4}
Where $w_1$ is the weighing vector. Note, the number of weighing vectors is dependent on the number of latent variables. These weight vectors then dictate the character of the size-weight matrix, $W$, which builds into the matrix of regression coefficients ($B$), through the following equation:

$$B = W(P^T W)^{-1} Q^T$$

Eq. 1.5

$B$ can then be used to predict characteristics of interest using the equation:

$$Y_{new} = X_{new}B$$

Eq. 1.6

Where $X_{new}$ is a matrix of new or unknown spectra and $Y$ is the measured/predicted characteristic of interest. Note, $X_{new}$ is referred to as the prediction set. Traditionally, $Y_{new}$ is referred to as a set of predictions. This can lead to some confusion regarding the accuracy and precision of the values obtained through PLS regression analysis. This is because concentrations calculated via other methods such as Beer’s Law or typical calibration curves are traditionally referred to as measured values. To clear up this confusion and indicate that the calculated $Y_{new}$ values are as reliable as values obtained via other calibration curves (given co-calculated errors) they will be referred to as measured/predicted values within this manuscript.

Chemometric modeling follows the pattern where a model is developed from a calibration set and the used to calculate characteristics of interest in a prediction set. After the model is built it can be integrated into software for real-time analysis of data. By further enhancing monitoring software, this system can also be used for process control, where process parameters can be automatically adjusted based on measurements/predictions made by the chemometric model. See Figure 1.6 for an example flow chart of this application.
Figure 1.6: Application of chemometric modeling for real time analysis of data. Models are built from spectroscopic training sets and used to develop a chemometric model. This model can then be applied to data collected in-line at a flowing process stream to provide real time analysis.
REFERENCES


7. Machiels, A; Sowder, A; Electric Power Research Institute, 2010.


24. Chatterjee, S; Del Negro, AS; Edwards, MK; Bryan, SA; Kaval, N; Pantelic, N; Morris, LK; Heineman, WR; Seliskar, CJ (2011) Luminescence-Based Spectroelectrochemical Sensor for [Tc(dmpe)(3)](2+/+) (dmpe=1,2-bis(dimethylphosphino)ethane) within a Charge-Selective Polymer Film Anal Chem, 83, 1766-1772.


32. Crosby, GA; Whan, RE; Freeman, JJ (1962) Spectroscopic Studies of Rare Earth Chelates J Phys Chem-Us, 66, 2493-&.

33. Crosby, GA; Alire, RM; Whan, RE (1961) Intramolecular Energy Transfer in Rare Earth Chelates - Role of Triplet State J Chem Phys, 34, 743-&.

34. Whan, RE; Crosby, GA (1962) Luminescence Studies of Rare Earth Complexes - Benzoylacetonate and Dibenzoylmethide Chelates J Mol Spectrosc, 8, 315-&.

35. Crosby, GA; Perkins, WG; Klassen, DM (1965) Luminescence from Transition-Metal Complexes - Tris(2,2'-Bipyridine)- and Tris(1,10-Phenanthroline)Ruthenium(2) J Chem Phys, 43, 1498.


38. Latva, M; Takalo, H; Mukkala, VM; Matachescu, C; RodriguezUbris, JC; Kankare, J (1997) Correlation between the lowest triplet state energy level of the ligand and lanthanide(III) luminescence quantum yield J Lumin, 75, 149-169.


43. Stegemiller, ML; Heineman, WR; Seliskar, CJ; Ridgway, TH; Bryan, SA; Hubler, T; Sell, RL (2003) Spectroelectrochemical sensing based on multimode selectivity simultaneously achievable in a single device. 11. Design and evaluation of a small portable sensor for the determination of ferrocyanide in hanford waste samples Environ Sci Technol, 37, 123-130.


47. Schroll, CA; Chatterjee, S; Heineman, WR; Bryan, SA (2012) Thin-Layer Spectroelectrochemistry on an Aqueous Microdrop Electroanal, 24, 1065-1070.


50. Dietzek, B; Cialla, D; Schmitt, M; Popp, J (2010) Introduction to the Fundamentals of Raman Spectroscopy; Springer.


52. De Beer, TRM; Wiggenhorn, M; Veillon, R; Debaaq, C; Mayeresse, Y; Moreau, B; Burggraeve, A; Quinten, T; Friess, W; Winter, G; Vervaet, C; Remon, JP; Baeyens, WRG (2009) Importance of Using Complementary Process Analyzers for the Process Monitoring, Analysis, and Understanding of Freeze Drying Anal Chem, 81, 7639-7649.
53. Gowen, AA; O'Donnell, CP; Cullen, PJ; Downey, G; Frias, JM (2007) Hyperspectral imaging - an emerging process analytical tool for food quality and safety control *Trends Food Sci Tech*, 18, 590-598.


56. Polkowska, Z; Astel, A; Walna, B; Malek, S; Medrzycka, K; Gorecki, T; Siepak, J; Namiesnik, J (2005) Chemometric analysis of rainwater and throughfall at several sites in Poland *Atmos Environ*, 39, 837-855.


CHAPTER TWO

ELECTROCHEMISTRY AND SPECTROELECTROCHEMISTRY OF LUMINESCENT EUROPIUM COMPLEXES

ABSTRACT

Fast, cost effective, and robust means of detecting and quantifying lanthanides are needed to support more efficient tracking within the nuclear, medicinal, and industrial fields. Furthermore, methods for isolating lanthanide signal from spectroscopic interferents are also needed. Applying spectroelectrochemistry to the detection of these species can meet those needs. However, application of this technique is limited by the low molar absorptivities and fluorescence quantum yields of the lanthanides. These limitations can be circumvented by complexing the lanthanides with sensitizing ligands that enhance fluorescence, thereby dropping the limits of detection. Complexation will also cause changes in the electrochemical behavior of the lanthanides. To demonstrate this concept, studies were completed using europium as a model lanthanide in complexes with four different sensitizing ligands, which included 2,2′-bipyridine and related derivatives. Results indicate that all four studied complexes demonstrate quasi-reversible redox couples and improvements in limits of detection where electrochemical and spectroscopic characteristics showed some dependence on attached ligand. All four complexes studied display the necessary characteristics for spectroelectrochemical analysis, which was successfully and reproducibly applied to all Eu complexes.
INTRODUCTION

Fast detection and quantification of the lanthanides is becoming increasingly important in numerous fields. In the nuclear chemistry field, monitoring lanthanide species is necessary for nuclear fuel reprocessing and nuclear forensics. Additionally, increasing numbers of lanthanide applications in industry and medicine help drive the need for new detection methods. An ideal example is europium (Eu), which has numerous applications due to its electronic and optical properties [1, 2]. Many current methods for detection of Eu and other lanthanides tend to involve expensive, stationary instruments and require time consuming sample preparation[1]. This work supports the development of a new method for identifying and quantifying lanthanides that can provide a fast, inexpensive, portable, and robust alternative. Specifically, applying spectroelectrochemistry to the analysis of lanthanides can meet those needs and provide a method where signal can be isolated using spectroelectrochemistry, and the lanthanide can be subsequently quantified spectroscopically.

By monitoring analytes with both electrochemistry and spectroscopy, spectroelectrochemistry demonstrates a powerful capability to offer multiple levels of selectivity for a desired analyte [3, 4]. The technique can isolate and identify analyte signatures by probing both select potential ranges and select wavelength ranges in either absorption or emission modes [4-6]. Spectroelectrochemistry therefore requires that analytes exhibit both an electrochemically active redox couple and a change in optical response due to conversion between oxidation states. These requirements give rise to difficulties in the application of spectroelectrochemistry to the lanthanides. Many lanthanides do not exhibit reversible redox couples in most aqueous and non-aqueous conditions. Only three lanthanides, Sm, Eu, and Yb, exhibit reversible II/III couples and only Ce exhibits a reversible III/IV couple in some aqueous and non-aqueous conditions [2, 7, 8].
Additionally, all lanthanides have low molar absorptivities, resulting in weak absorption bands and high limits of detection. Nearly all lanthanides are fluorescent, which is a useful characteristic to take advantage of in a sensor due to the selectivity of fluorescence measurements; however, due to the low molar absorptivities, emission signals are also weak [2, 7, 9]. Of the available electrochemically active lanthanides, Eu has the most Nernstian couple with a reduction potential that can easily fall within the potential window accessible for most solvent systems [2, 10]. Additionally, Eu has the strongest fluorescence response in the visible region relative to the other lanthanides [2, 9]. Eu is therefore a good starting point for designing a detection method for the lanthanides.

It is important to consider that while Eu may have one of the higher quantum yields of the lanthanides, the molar absorptivity of Eu is still low enough to lead to an undesirably high limit of detection. To overcome the poor light absorption and weak fluorescence signal, Eu can be complexed to a sensitizing ligand. Sensitizing, or antenna, ligands enhance the fluorescence of an ion by increasing the amount of energy delivered to the metal center. Mechanisms for this have been discussed previously [11-13]. Ligands must be appropriately selected for metal species to allow for strong binding, optimized energy transfer, and solubility of the resulting complex [9, 14]. Fortunately, many Eu complexes have already been studied in which ligands enhanced fluorescence of the Eu(III) species [9, 12, 15]. Other complexes have been used to stabilize the oxidation state of the Eu(II) species, which has useful MRI applications but lacks the same luminescence characteristics as the Eu(III) species [16, 17].

The ultimate goal of this work is to develop a spectroelectrochemistry sensor in which weakly luminescent lanthanides are complexed in situ to form strongly luminescent complexes.
**Figure 2.1:** Schematic indicating the in situ formation of the luminescent Eu(III) complex and the electrochemical modulation of that complex between the luminescent Eu(III) and non-luminescent Eu(II) species. Both Reaction A and B can be monitored spectrscopically.
This concept is demonstrated in Figure 2.1. This figure demonstrates that the sensor depends on two reactions. The first reaction involves the complexation of the metal ion by the sensitizing ligand. The second is an electrochemical reaction that involves applying a potential at a working electrode to reduce the complexed 3+ species to the 2+ species, and ideally this reaction should be reversible. In the case of Eu, the 3+ complex has a strong red emission while the 2+ species does not; the electrochemical reaction can be monitored by measuring the presence and intensity of the red emission.

Figure 2.1 also demonstrates the necessity of finding a ligand where thermodynamics will favor fast formation of a stable complex. This figure also subtly demonstrates the necessity of selecting ligands that not only have the appropriate energetics to sensitize Eu fluorescence but also are electrochemically inactive in the potential range of interest [6, 18, 19]. This avoids the addition of unnecessary complexity to the electrochemical behavior of the metal-ligand complex. To satisfy electrochemical requirements, 2,2’-bipyridine (bpy) and bpy derivatives were chosen as the focus of this work [18, 19]. Additionally, significant spectroscopic characterization has been completed on Eu complexes with bpy and many bpy derivatives, which have been shown to successfully sensitize Eu fluorescence [20-22]. It should be noted that under aqueous conditions, thermodynamics do not favor the formation of Eu-bpy complexes, however these species can form and persist under non-aqueous conditions [22-24].

The goal for the sensor design is to capture the Eu in complexes within the sensor environment; however, prior to testing the in situ formation and subsequent analysis of these complexes, it is necessary to first synthesize and characterize these lanthanide-sensitizing ligand complexes. While the spectroscopic behavior of the Eu(III) species is well known, limited
**Figure 2.2:** Structures of ligands of interest and the formulas of their complexes with Eu. Blue coloration indicates the atoms that bind to the metal center. Red coloration indicates the most electron-donating group.
information is available regarding the spectroscopy of the Eu(II) species of these complexes, the behavior of the redox couples, or the applicability of spectroelectrochemistry. This work focuses on addressing those knowledge deficiencies by fully characterizing these complexes and determining the applicability of spectroelectrochemistry to the analysis four different Eu-ligand complexes. Results from the characterization of these complexes will aid in the development of a sensor where these complexes are formed and subsequently analyzed in-situ.

The choice to study four different ligands in complexes with Eu was influenced by the fact that altering the bound ligand has been shown to have an impact on the quantum yield of emission of Eu (III), but also can affect electrochemical behavior [14, 19, 25]. The four ligands chosen were bpy, 4,4’-dimethyl-2,2’-bipyridine (diMeBpy), 4,4’-dimethoxy-2,2’-bipyridine (diMeObpy), and 1,10’-phenanthroline (phen), which are shown in Figure 2.2 along with the formulas of the complexes they form with Eu. These four ligands should provide an opportunity to explore effects from minor differences in ligand structure. This work has been summarized in the paper “electrochemistry and spectroelectrochemistry of luminescent Eu complexes” by Lines et al and accepted into Electroanalysis [26].

EXPERIMENTAL

Materials

All materials were obtained from Sigma-Aldrich and were used without further purification or recrystallization, including Eu(III) chloride hexahydrate (99.9%), 4,4’-dimethyl-2,2’-bipyridine (≥99%), 4,4’-dimethoxy-2,2’-bipyridine (≥99%), and 1,10’-phenanthroline (≥99%).
2,2′-bipyridine (≥99%), supporting electrolyte tetrabutylammonium hexafluorophosphate (TBAPF₆) (98%), and anhydrous acetonitrile (99.8%).

**Synthesis and characterization of complexes**

Complexes were synthesized according to previously published methods [22-24]. Complexes are of the form [Eu(ligand)₂(H₂O)₂]Cl₃ where the ligand is 2,2′-bipyridine for Complex I, 4,4′-dimethyl-2,2′-bipyridine for Complex II, 4,4′-dimethoxy-2,2′-bipyridine for Complex III, and 1,10′-phenanthroline for Complex IV. Note, even with excess ligand present, species maintain the 1:2 metal-to-ligand ratio.

Complex identity was verified by several methods, the first being Fourier transform infrared (FTIR) spectroscopy. Spectra indicated expected peak shifts between uncomplexed and complexed ligands and demonstrated good agreement with existing literature reports [23]. Spectra can be seen in Figure 2.3 below where the spectra of the free ligand and the respective complex are compared. Table 2.1 lists several of the significant peaks, their observed wavenumber position, and their assignments. Many peaks show a shift to higher wavenumbers between the bands associated with the free and bound ligands, indicating the ligands are bound and have higher frequency modes of vibration.
Table 2.1: Selected peaks demonstrating expected shifts and their assignments

<table>
<thead>
<tr>
<th>Free bpy</th>
<th>Complex I</th>
<th>Free diMebpy</th>
<th>Complex II</th>
<th>Free diMeObpy</th>
<th>Complex III</th>
<th>Free phen</th>
<th>Complex IV</th>
<th>assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1582</td>
<td>1595</td>
<td>1592</td>
<td>1597</td>
<td>1589</td>
<td>1592</td>
<td>1501</td>
<td>1518</td>
<td>H₂O</td>
</tr>
<tr>
<td>1560</td>
<td></td>
<td></td>
<td>1613</td>
<td>1562</td>
<td>1560</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1455</td>
<td>1455</td>
<td>1441</td>
<td>1441</td>
<td>1441</td>
<td>1437</td>
<td>1442</td>
<td>1447</td>
<td>Dipryidyl band</td>
</tr>
<tr>
<td>1420</td>
<td>1421</td>
<td>1375</td>
<td>1375</td>
<td>1375</td>
<td>1418</td>
<td>1418</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1250</td>
<td></td>
<td>1039</td>
<td>1039</td>
<td>1036</td>
<td>1039</td>
<td></td>
<td></td>
<td>Ortho-subs</td>
</tr>
<tr>
<td>1213</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>pyridine</td>
</tr>
<tr>
<td>1148</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>vibration</td>
</tr>
<tr>
<td>1089</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>991</td>
<td>1012</td>
<td>990</td>
<td>1010</td>
<td>985</td>
<td>1010</td>
<td>1035</td>
<td>1038</td>
<td>Pyridine</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>breathing</td>
</tr>
<tr>
<td>759</td>
<td>765</td>
<td>747</td>
<td>748</td>
<td>749</td>
<td>765</td>
<td>735</td>
<td>750</td>
<td>Out-plane</td>
</tr>
<tr>
<td></td>
<td>736</td>
<td></td>
<td></td>
<td></td>
<td>749</td>
<td>728</td>
<td></td>
<td>bending</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ring H’s</td>
</tr>
</tbody>
</table>
Figure 2.3: Normalized FTIR spectra of free ligands and complexes.
Complexes were also characterized using C, H, & N analysis. Samples were sent to Atlantic Microlab Inc. for C, H, & N analysis and duplicate measurements were collected. Reported values are the averages of the duplicate measurements and for all complexes except Complex III, duplicates showed roughly a 0.1 percent deviation. For Complex I calculated values are C:39.64, H:3.32, N:9.24 and observed results were C:38.25, H:3.40, N:8.81. This is consistent with Complex I retaining an extra H₂O molecule. Complex II calculated values are C:43.49, H:4.26, N:8.45 and observed results were C:44.83, H:4.49, N:8.88. This is consistent with Complex II holding only 1 H₂O molecule. Complex III calculated values are C:39.66, H:3.88, N:7.71 and observed results were C:47.90, H:4.57, N:9.36. In the case of Complex III duplicate measurements showed variations of up to 1%. Observed values and their variation is consistent with the sample of Complex III carrying up to 4 extra acetonitrile (MeCN) molecules.

Complexes were recrystallized in MeCN and Complex III did not dry completely. For Complex IV calculated values are C:44.02, H:3.08, N:8.56 and observed results were C:43.93, H:3.17, N:8.41.

Finally, fluorescence spectroscopy of synthesized complexes aligned well with literature for the bpy, diMebpy, and phen complexes [22, 23]. All characterization indicated the 1:2 metal-to-ligand species were successfully formed for all four complexes.

**Equipment:**

Potentiostats used included a PAR 273A (EG&G), a BASi Epsilon, and a CV27 (Bioanalytical Systems). The PAR 273A utilized Core Ware software v. 3.3b (Scribner Assoc.), the Epsilon utilized the BASi Epsilon software, and the CV27 used manual controls. Two types of electrochemical cells were used. The first cell was a cylindrical vial used in conjunction with a
platinum disk working electrode (area $1.84 \times 10^{-2} \pm 0.007 \times 10^{-2}$ cm$^2$), platinum wire auxiliary (Aux) electrode, and a silver wire reference electrode (RE). This cell was used primarily for electrochemical characterization of complexes. The second electrochemical cell was a BASi thin SEC cell with a 1 mm path length by 10 mm width quartz cell with a working electrode compartment volume of 0.09 mL and a total cell volume, including auxiliary compartment volume of 1 mL. This cell was used for spectroscopic and SEC characterization and utilized a Pt mesh WE along with a Pt wire Aux and an Ag wire RE. All solutions were purged and blanketed with N$_2$ prior to and then during experiments. The quasi reference electrode was inspected, cleaned, and occasionally sanded between experiments to ensure consistent surface.

Fluorescence spectra were collected using two fluorimeters. The first was a Horiba Jobin Yvon Fluorolog III fluorimeter equipped with a 450-W Xe lamp, double-emission monochromator blazed at 500 nm and a single-excitation monochromator blazed at 300 nm. Emission spectra were corrected for instrumental response. The second fluorimeter was an InSpectrum 150 spectrometer-CCD, using SpectraSense data acquisition software. A 404-nm laser source was used for excitation. Signal integration times were typically 999 ms using a 2-mm slit width for a 600 gr/mm grating blazed at 500 nm. FTIR spectra were collected using a Bruker Optics Alpha-P spectrometer equipped with a diamond attenuated total reflectance cell. All samples were analyzed as solids using 32 scans with a 4-cm-1 resolution.

Cyclic voltammograms were collected on the PAR273 using specified scan rates. Differential pulse voltammetry data were collected on the BASi Epsilon using a 2 mV step, a 50 ms pulse width with a 100 ms pulse period and a 10 mV pulse amplitude.
Figure 2.4: Top row: pictures of spectroelectrochemistry setup #1 with instruments components labeled. Bottom row: schematic and picture of the spectroelectrochemistry thin cell.
Two spectroelectrochemistry setups were utilized. Spectroelectrochemistry setup #1 combined the PAR273A and the InSpectrum detector with the 404-nm excitation source. Spectroelectrochemistry setup #2 combined the CV27 and the Horiba Jobin Yvon fluorimeter. The CV27 was also paired with the InSpectrum detector to verify that results between Potentiostats were comparable. Figure 2.4 presents a picture of spectroelectrochemistry setup #1 as well as a picture of the spectroelectrochemistry thin cell.

RESULTS AND DISCUSSION:

Spectroelectrochemistry allows for the isolation of desired analyte signal from an environment where spectroscopic interferents are present. Ultimately, the goal of this work is to develop a spectroelectrochemical sensor capable of isolating Eu signal in a complex system. However, we must first demonstrate that the Eu complexes meet the two criteria for spectroelectrochemistry to be applicable: they must exhibit an electrochemically active redox couple, and they must show a change in spectral signature with a change in oxidation state. Ideally the redox couples will be reversible or at least quasi-reversible, but a sensor designed for an irreversible couple is not impossible. The electrochemical and spectroscopic characteristics of the complexes were explored individually prior to running full spectroelectrochemical analysis. All characterizations of the complexes including spectroelectrochemical analysis were completed in acetonitrile (MeCN) utilizing 0.1 M TBAPF₆ as the supporting electrolyte.
Electrochemical characterization

From existing literature discussing similar Eu complexes, it is expected that all four complexes should demonstrate a quasi-reversible Eu II/III couple [16, 17, 27]. Cyclic voltammograms (CVs) of all four complexes suggested a quasi-reversible couple at a formal reduction potential ($E^\circ$) that that falls in the range of reported values for the Eu II/III couple. The top plot of Figure 2.5 presents CVs of all four complexes aligned to a ferrocene internal standard. Consecutive CVs could be overlaid, without indicating loss of either reduced or oxidized species to side reactions. This suggests a simple electrochemical mechanism is observed. For all four complexes, the ratio of the peak currents for the anodic and cathodic waves ($i_{p,a}/i_{p,c}$) decreased for increasing scan rates above 100-200 mV/s. Peak splitting, $\Delta E_p = E_{p,a} - E_{p,c}$, increased with increasing scan rate while the formal reduction potential, $E^{\circ'} = (E_{p,a} + E_{p,c})/2$, remained roughly constant. These results agree with what has previously been reported for quasi-reversible Eu II/III couples of other similar Eu complexes using comparable techniques and conditions [16, 17, 27]. Furthermore, all four complexes display asymmetric peaks with broader cathodic peaks, suggesting a cathodic charge transfer coefficient, $\alpha_c$, greater than 0.5 [16]. An $\alpha_c$ value greater than 0.5 indicates the thermodynamic $E^{\circ'}$ that is different than the observed $E^{\circ'}$ where the reduction peak is shifted negative from where it would ideally be if the system were truly reversible. The larger $\alpha$ value indicates the kinetics of reduction are slow, and a high overpotential is necessary to increase the speed at which the reduced species is produced [28, 29]. This, along with the large $\Delta E_p$’s observed, indicate relative difficulty in reducing the complexes.
**Figure 2.5:** Top: Overlay of CVs of complexes aligned to the $E^{\circ'}$ of the internal standard (ferrocene). CVs were collected at 100 mV/s using a Pt disk WE, Pt wire Aux, and an Ag wire quasi-RE. Bottom: Overlay of DPV responses of complexes. Black lines represent the background runs of the MeCN and 0.1 M TABF$_6$. All complexes were at approximately 1 mM for CVs and 0.01 mM for DPV.
A pattern can be seen where the four complexes display decreasing $\Delta E_p$ and negative shift of the $E^{\circ\prime}$ following the order I>II>IV>III. See Table 2.2 for values. The negative shift of the $E^{\circ\prime}$ has been interpreted to indicate an increasing favorability of Eu(III)-ligand interactions, suggesting stronger binding constants [30]. The decrease in $\Delta E_p$ indicates a trend towards increased reversibility of the redox couple, which is consistent with more thermodynamically favorable interaction between Eu and the respective ligand. This indicates that ligand choice can be used to tune the observed $E^{\circ\prime}$ as well as reversibility of the redox couple.

Complexes were also characterized using differential pulse voltammetry (DPV). Using Equation 2.1, the number of electrons transferred can be determined for each complex, where $W_{1/2}$ is the full width at half the peak maximum (V), $R$ is the ideal gas constant, $T$ is the temperature (K), $n$ is the number of electrons transferred, and $F$ is Faraday’s constant (C) [31].

$$W_{1/2} = \frac{RT}{nF}$$

Eq. 2.1

Results are reported in Table 2.2, with $n$ values ranging from 0.65 to 0.48 for the four complexes. Values less than one are not surprising due to the slow electron transfer kinetics expected and suggested by the behavior of their CVs. It is therefore possible to take the observed results as suggesting a 1-electron transfer.

The DPV results can also be used along with Equation 2.2 as another method for determining the formal reduction potential ($E^{\circ}$) of the complexes; where $E_{\text{max},i}$ is the potential at the maximum current (V) and $\Delta E$ is the pulse step (V) [31]. Ferrocene was again added as an internal standard and used to calculate the $E^{\circ\prime}$ of the complexes, which are reported in Table 2.2.
It should be noted that DPV results were likely affected by the quasi-reversibility of the redox couple and values obtained from DPV differed significantly from $E^{o'}$ values obtained from CVs. Additionally calculated $E^{o'}$ values from DPV results followed the same pattern as n values where decreasing n and decreasing $E^{o'}$ followed the order of I>III>II>IV for the complexes. The $E^{o'}$ values for Complexes II, III, and IV were the same within error. Interestingly, values for $E^{o'}$ (CV) and $E^{o'}$ (DPV) were the most similar for Complexes IV and III. This agreement in values again indicates a trend toward more Nernstian behavior of the redox couples as electron donating groups are added to the sensitizing ligands.

$$E_{max,i} = E^{o'} - \frac{\Delta E}{2} \quad \text{Eq. 2.2}$$

The CV behavior was also explored as a function of scan rate (ν). Results indicate the relationship between $i_p$ and $\nu^{1/2}$ is linear for both the anodic and cathodic peaks of all complexes. This indicates the electrochemical reaction is diffusion-controlled [3, 32]. Furthermore, scan rate studies did not indicate the loss of either reduced or oxidized species or presence of any side reactions, which suggests the system undergoes a simple electrochemical mechanism. This is ideal for future spectroelectrochemical analysis where side reactions resulting in a loss of complex will affect the reproducibility of measurements.

Because the relationship between $i_p$ and $\nu^{1/2}$ is linear, it is possible to apply Randles-Sevcik analysis to the system. It should be noted that applying this form of analysis to a quasi-reversible system is not ideal, however, it can still be used to roughly estimate diffusion coefficients of the complexes and look at trends in behavior. Equation 2.3 [3, 32] shows the Randles-Sevcik equation where $i_p$ is the peak height (A), n is the number of electrons transferred, A is the area of the electrode (cm²), D is the diffusion coefficient (cm²/s), $C^o$ is the bulk concentration of the
species (mol/cm$^3$), and $v$ is the scan rate (V/s). The area of the electrode was verified using the standard ferrocene, which has a well characterized diffusion coefficient in MeCN with 0.1 M TBAPF$_6$, 2.6·$10^{-5}$ cm$^2$/s [33]. Calculated values for the diffusion coefficients of both the reduced and oxidized species are reported in Table 2.2. Results are lower than anticipated, which is likely an effect of the quasi-reversibility of the couples. However, they generally show Complex III, with the polar methoxy groups, has the largest diffusion coefficient. Figure 2.6 presents the scan rate study CVs and the resulting Randles-Sevcik treatment for all four complexes. The best fit lines use for the Randles-Sevcik analysis utilize only the data collected at scan rates below 300 mV/s. At scan rates above 300 mV/s peak currents appears to deviate slightly from linear behavior. This deviation is a caused by the difficulty in properly identifying an accurate baseline within the CVs at higher scan rates.

$$i_p = (2.69 \cdot 10^5)n^{3/2}AD^{1/2}C^0v^{1/2}$$

Eq. 2.3

Behavior of the four complexes can be compared Eu-triflate, which was studied under the same conditions using the same techniques and observed behavior aligned with literature reports [34, 35]. Results can be seen in Table 2.2. It is interesting to note that the $E^\circ\prime$ obtained from CVs and the $E^\circ\prime$ obtained from DPV of Eu-triflate show the same deviation or differences in values as Complexes I and II, where DPV values are shifted negative as compared to CV values. Additionally, $E^\circ\prime$ values for Eu-triflate are significantly more positive than those of the Eu complexes of interest. This could possibly indicate the triflate ligand interacts in a more thermodynamically favorable way with Eu(II) than do the ligands of interest [30]. The $\Delta E_p$ of Eu-triflate is smaller than that of three of the complexes, indicating a trend toward more
Nernstian behavior as compared to those complexes. Calculated D values for Eu-triflate fell near reported literature values.

Experiments completed on complexes with excess ligand present had identical results to experiments on pure compounds. Experiments run using the blank solution, MeCN with 0.1 M TBAPF₆, and on solutions of free ligands, did not indicate any responses that would interfere with responses observed for the complexes. CV and DPV experiments completed with the blank solutions and free ligands had identical results, showing no electrochemical activity in the potential range of interest.

**Table 2.2: Results of electrochemical characterization**

<table>
<thead>
<tr>
<th>Eu complex</th>
<th>E°′ (V vs SHE): from CVs collected at 100 mV/s</th>
<th>∆Ep (V): from CVs collected at 100 mV/s</th>
<th>Number of e-transferred</th>
<th>E° (V vs SHE): from DPV</th>
<th>Anodic</th>
<th>Cathodic</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0.04 (± 0.03)</td>
<td>0.49 (± 0.07)</td>
<td>0.65 (± 0.04)</td>
<td>-0.17 (± 0.01)</td>
<td>3·10⁻⁸ (± 3·10⁻⁸)</td>
<td>6.6·10⁻⁸ (± 0.9·10⁻⁸)</td>
</tr>
<tr>
<td>II</td>
<td>-0.05 (± 0.01)</td>
<td>0.38 (± 0.03)</td>
<td>0.48 (± 0.07)</td>
<td>-0.22 (± 0.01)</td>
<td>4.4·10⁻⁸ (± 0.8·10⁻⁸)</td>
<td>2.8·10⁻⁷ (± 0.5·10⁻⁷)</td>
</tr>
<tr>
<td>III</td>
<td>-0.24 (± 0.01)</td>
<td>0.13 (± 0.08)</td>
<td>0.54 (± 0.05)</td>
<td>-0.224 (± 0.006)</td>
<td>1.6·10⁻⁷ (± 0.3·10⁻⁷)</td>
<td>3.9·10⁻⁷ (± 0.6·10⁻⁷)</td>
</tr>
<tr>
<td>IV</td>
<td>-0.21 (± 0.03)</td>
<td>0.22 (± 0.09)</td>
<td>0.49 (± 0.06)</td>
<td>-0.212 (± 0.001)</td>
<td>4.9·10⁻⁸ (± 0.5·10⁻⁸)</td>
<td>3.1·10⁻⁸ (± 0.6·10⁻⁸)</td>
</tr>
<tr>
<td>Eu-triflate</td>
<td>0.238 (± 0.008)</td>
<td>0.18 (± 0.01)</td>
<td>0.85 (± 0.02)</td>
<td>-0.02 (± 0.01)</td>
<td>4.3·10⁻⁶ (± 0.7·10⁻⁶)</td>
<td>5.5·10⁻⁷ (± 4·10⁻⁷)</td>
</tr>
</tbody>
</table>

Reported errors are 1 standard deviation
Figure 2.6: Data is organized into four boxes for each of the four complexes, where within a box the top plot presents the CVs collected at multiple scan rates (900-20 mV/s) which were collected in MeCN and 0.1 M TBAPF6 using Pt disk WE, a Pt wire Aux, and an Ag wire quasi-RE and the bottom plot presents the peak current vs the square root of the scan rate and the best fit lines used for Randles-Sevcik analysis.
Spectroscopic characterization:

Spectroscopic characterization of the Eu(III) species of the complexes aligned well with what has been reported in the literature [9, 22]. Specifically, an intense band at 613 nm and weaker band at 592 nm were observed, see the top plot of Figure 2.7. Complexing the Eu with bpy improved the emission intensity of the 613 nm peak by at least 3 orders of magnitude. A comparison of emission spectra of complexed and uncomplexed Eu can be seen in Figure 2.8. It should be noted that complexing the Eu altered the ratio of intensity between the 592 and 613 nm bands. The 613 band is hyper sensitive and enhanced more by a sensitizing ligand [9, 36].

To characterize the Eu(II) species, the complexes were reduced using spectroelectrochemistry setup #2, described in the experimental section. Spectra can be seen in the bottom plot of Figure 2.7. Notably, for the reduced species, the 613 and 592 nm peaks disappear while a very broad band at about 450 nm is present. The loss of the 613 nm peak suggests the Eu(III) was successfully reduced to Eu(II). The broad band at about 450 nm observed for the reduced species could have two possible explanations. These will be discussed in detail in a later portion of the chapter. It is important to note that when using a 330-nm excitation wavelength (λex), the intensity of the peaks of the Eu(II) species are very weak compared to the intensity of the Eu(III) species peaks. This is unsurprising given the excitation spectra shown in Figure 2.7.

The spectra did indicate that the complexes all showed some sensitivity to intense excitation light and showed evidence of photodecomposition. The emission spectra for the reduced forms of Complexes I and III in Figure 2.8 indicate a new peak at about 620 nm, which may be a product of photodecomposition. Given enough exposure time, all four complexes developed the
Figure 2.7: Top: Excitation spectra (dashed lines) and emission spectra (solid lines) of Eu (III)-based complexes, exciting at 330 nm for emission spectra. Bottom: Excitation spectra (dashed lines) and emission spectra (solid lines) of Eu(II) -based complexes.
peak at 620 nm that would not respond to applied potential. After this peak presented itself in the spectra, a black film could be observed on the Pt mesh working electrode. To determine whether both the Eu(III) and Eu(II) species were photosensitive, several spectra were collected over extended periods of time to monitor changes in intensity. Consistent exposure of the Eu(III) species to the excitation source indicated these species do not undergo photodecomposition. Therefore only the reduced, Eu(II), species is photosensitive and any sensor designed using these ligands should limit exposure of the reduced species to light.

Interestingly, the emission spectra of free ligands indicated phosphorescence at lower wavelengths (< 500 nm), very near the observed bands for the Eu(II) species. However, emission intensities of the free ligand solutions were very low compared to the emission observed from the Eu(II) species at equal ligand concentrations. This increase in intensity is a strong indication that the observed emission from the reduced species is not entirely produced by free ligand.

The 613 nm peak of the Eu(III) species had the strongest response and therefore provided the best limit of detection (LOD) for the Eu complexes. Emission intensity was measured as a function of concentration of the Eu(III) species of all four complexes and intensities of the 613-nm peak are presented in Figure 2.9. The LOD for the complexes could then be calculated using Equation 2.4, where $s_b$ is the standard deviation of the blank, and $m$ is the slope of the peak signal vs the concentration plot (M$^{-1}$). The LOD for the four complexes are reported in Table 2.3 and followed the pattern of I>II>III>IV for the four complexes. The addition of the electron-donating groups as well as rigidity to the sensitizing ligands is shown to generally reduce the LOD of complexes. This aligns with literature discussions suggesting that the addition of electron-donating groups to the sensitizing ligand improves absorption of light [21, 25, 37].
**Figure 2.8**: Comparison of the emission spectra of EuCl₃ in water and Complex I in acetonitrile. Different solvents were required due to solubility constraints and Complex I was measured at half the concentration of EuCl₃ due to detector limits. The right plot presents the same data as the left plot, but zoomed in to show the emission peaks from EuCl₃ in water.
While these reported LODs do not appear to be sufficiently low for trace analysis of Eu, it should be noted that typical spectroelectrochemical sensor designs utilize methods of pre-concentrating the analyte [38, 39]. Depending on the efficiency of pre-concentration in a detector setup, the reported LODs could be effective in trace analysis of Eu.

\[
LOD = \frac{3s_p}{m} \tag{Eq. 2.4}
\]

In a future detector setup, spectroelectrochemistry would be used to isolate the signal from the Eu species which could then be quantified by looking at the emission intensity from the isolated Eu (III) species.

<table>
<thead>
<tr>
<th>Complex</th>
<th>Maximum emission wavelength (nm)</th>
<th>LOD (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Oxidized species</td>
<td>Reduced species</td>
</tr>
<tr>
<td>I</td>
<td>613</td>
<td>450</td>
</tr>
<tr>
<td>II</td>
<td>613</td>
<td>460</td>
</tr>
<tr>
<td>III</td>
<td>613</td>
<td>440</td>
</tr>
<tr>
<td>IV</td>
<td>613</td>
<td>422</td>
</tr>
</tbody>
</table>

Reported errors are 1 standard deviation
Figure 2.9: Plots of Intensity of the 613 nm Peak versus Concentration of the Complex in Solution. Clockwise from top left: Complex I, Complex II, Complex III, and Complex IV
Spectroelectrochemical analysis

The experiments described in the electrochemical and spectroscopic sections verified that Complexes I-IV met the two criteria of spectroelectrochemistry. All four complexes demonstrate quasi-reversible redox couples and changes in spectral signature with a change in oxidation state. The next necessary step is to determine whether the spectroscopic signal for the respective oxidation states can be turned on and off reproducibly with applied potentials.

Spectroelectrochemical modulations involve switching between applying a fully oxidizing and a fully reducing potential while continuously monitoring spectroscopic signal. Potentials are held long enough to fully oxidize or reduce the species within the spectroscopic window in order to isolate all signal from the species of interest. In the case of Complexes I-IV, the red luminescence should change to indicate either ingrowth or disappearance of the oxidized species. Initial tests of the background solution, 0.1 M TBAPF6 in MeCN, and free ligands again demonstrated no interference with signal/behavior of the complexes. Spectroscopic signal did not vary with applied potential.

Spectroelectrochemical analysis was first attempted using spectroelectrochemistry setup #1 and Figure 2.10 presents the results for all four complexes. Solutions containing approximately 0.5 mM of the respective complex were exposed to applied potentials modulating between oxidizing (0.1 V) and reducing (-0.8 V) as spectra were collected. Modulation of the spectroscopic signal was successful, but results were not ideal and indicated a loss of complex over time. The first difficulty lies in the excitation source of setup #1, which is a 404-nm laser. If the plot of excitation spectra of the Eu(III) species in Figure 2.7 is consulted, it is clear that 404 nm is not ideal for exciting Eu(III). This leads to the 613 nm peak having a very low
intensity as compared to the 450 nm peak of the reduced species. The response of the 613 nm peak can be extracted from the shoulder of the 450 nm peak. This is accomplished by subtracting a suitable baseline from each spectrum. That baseline is estimated as a polynomial fit to the regions on either side of the 613 nm band of interest. Figure 2.10 presents the raw spectra from the modulations and overlays of the 450 nm peak and the extracted 613 nm peak behavior.

Results generally indicated both the 613 nm peak of Eu(III) and the 450 nm peak of Eu(II) species modulated appropriately with applied potential. Unfortunately in all four cases the intensity of the 613 nm band decreased after each reduction step, suggesting a non-negligible portion of the complex degraded when the reduced species was exposed to the excitation source. In the cases of Complexes I and III the intensity of the 450 nm bands increased after each oxidation step, again indicating the presence of a side reaction. The 450 nm band of Complex II did not modulate appropriately with applied potential and the band from the reduced species of Complex IV was not observable due to constraints on the detector wavelength range. While these results do not indicate an optimized application of SEC analysis, they do indicate successful modulation and SEC activity. These results also provide a starting point for optimizing the collection conditions to improve reproducibility of the modulated spectroscopic signal.

To optimize collection parameters and improve the reproducibility of the signal, the modulation experiments were repeated using spectroelectrochemistry setup #2. This setup allowed for the use of a better $\lambda_{ex}$ that could excite the Eu(III) species more effectively. Specifically the $\lambda_{ex}$ was set to 330 nm which falls within the maximum excitation profile for the Eu(III) species as demonstrated in the top plot of Figure 2.7. Additionally, the spectral collection parameters of setup #2 could be altered to significantly reduce the time the sample was exposed
Figure 2.10: Spectroelectrochemical modulation of complexes: Data is organizing into four boxes for each of the four complexes, within a box the top plot presents the applied potential over time, the middle plots the spectra over time for the duration of the modulation, and the bottom plots the intensity of the 613-nm peak (red) and the 450-nm peak (black) over time. Baseline subtraction methods were used to extract 613-nm peak from shoulder of 450-nm peak. Results were normalized to allow for ease of comparison.
Figure 2.11: Spectroelectrochemical modulation of complexes: Data is organizing into four boxes for each of the four complexes, where within a box the top plot presents applied potential over time and the bottom plots the intensity of 613 nm peak over time as applied potential was modulated. Red dots indicate points at which spectra were collected.
to the excitation source. Furthermore, spectra were only collected when oxidizing potentials were applied to further limit photo decomposition of the reduced species. Again 0.5 mM solutions of the complexes were exposed to modulating potentials while spectra were collected. Figure 2.11 presents the response of the four complexes in a format similar to that of Figure 2.10.

Using setup #2 three of the four complexes demonstrated successful spectroelectrochemical modulation where the intensity of the 613 nm band decreased when reducing potentials were applied and returned to the same intensity when oxidizing potentials were again applied. The exception was Complex IV which consistently displayed higher sensitivity to photodegradation of the reduced species. Overall, this suggests spectroelectrochemical analysis is generally applicable to these Eu complexes, and can be easily improved by optimizing spectroscopic collection parameters.

**Emission from the Eu(II) species**

As demonstrated by the successful modulation of the 613-nm peak associated with the Eu(III) species, SEC can be successfully applied to these Eu-ligand systems. However, it is still of interest to determine the source of the 450-nm signal observed from the reduced species. The 450 nm band could arise from two different sources: either emission from Eu(II), using a similar mechanism as the Eu(III) species, or it may arise from enhanced phosphorescence from the bound ligands.

Literature suggests that Eu(II) does fluoresce in a range of 390 nm to 580 nm, where the emission wavelength shows significant dependence on environment [40-42]. The observed emission for the reduced species of all four complexes fall within this range but do demonstrate shifting in their $\lambda_{\text{max}}$ positions. Again, these spectra can be viewed in Figure 2.7. It is uncertain if
the ligands can effectively sensitize the Eu(II) ion, given the fact that the accepting orbital energy of Eu(II) is different than that of Eu(III) which falls in the ideal range of the bpy donor orbitals [14].

It is also possible that the 450 nm band is due to enhanced ligand phosphorescence, which has been seen in some gadolinium complexes with bpy derivatives [43]. In that case, the accepting orbital of Gd was not energetically capable of accepting energy from bpy derivative, which forced increased emission from the triplet state of the bpy derivative. The observed emissions from the reduced species are consistent with the spectra of the free ligands, where the broad phosphorescence bands of the free ligands fell in the same range as observed peaks for the complexes, which is demonstrated in Figure 2.12. It is important to note that the shape and $\lambda_{\text{max}}$ of the emission bands from the complexes do not precisely fit the shape of the free ligand phosphorescence bands. However, the emission band from Complex I in the reduced form is very similar to what was reported by Yan et al. for the Gd-bpy-derivative emission [43]. This data was included in the bottom plot of Figure 2.12 for comparison purposes. It is also important to note that the top plot of Figure 2.12 presents the normalized spectra to allow for easier comparison. At equal concentrations of ligand, e.g. 0.5 mM Complex and 1 mM free ligand, maximum intensity from the $\lambda_{\text{max}}$ position of any of four reduced complexes was over an order of magnitude larger than for the free ligand.
Figure 2.12: Top) overlay of emission spectra from the reduced, Eu(II) species, of the complexes (solid lines) and the emission spectra of the free ligand (dashed lines) where $\lambda_{ex}$ was 330 nm. Bottom) Fig.7 from reference Yan et al. [42]
There are several methods available for determining the source of the emission bands from the reduced species. Lifetime studies could be used to differentiate between the two options. If the emission comes from enhanced phosphorescence of the ligand the lifetime will fall in the range of $10^{-4}$-$10^{-2}$ second, which is long compared to lifetimes of fluorescence [44]. The difference in lifetimes should be particularly apparent as Eu(II) emission is credited with a very short lifetime—on the order of 1 µs [41]. A lifetime study was attempted, but the photosensitivity of the reduced species rendered the results inconclusive. Temperature studies could also indicate the source of the emission where the intensity at $\lambda_{\text{max}}$ will decrease with an increase in temperature if it is a fluorescence based emission [45]. Temperature studies were also attempted but the range of temperatures studied was limited by the MeCN solvent which has a boiling point of 82 °C. A change in emission intensity was not observed when varying the temperature from 20-70 °C. Again this data was not sufficient enough to determine the source of emission from the reduced complexes.

CONCLUSIONS

Complexes I-IV which included Eu bound to four different bpy derivatives, were successfully synthesized and characterized using several techniques. All four complexes demonstrate quasi-reversible Eu II/III redox couples under non-aqueous conditions. Furthermore, their electrochemical characteristics, such as the $E^{\circ'}$ and $\Delta E_p$, are influenced by the bound ligand, where the addition of electron-donating groups or rigidity decrease $\Delta E_p$ and cause a negative shift in $E^{\circ'}$. These observed shifts indicate the addition of electron donating groups to the ligands resulted in a trend towards more Nernstian behavior of the Eu II/III redox couples. This indicates electrochemical characteristics can be tuned using bound ligand, and electrochemical reversibility of the Eu II/III couple could be improved by optimizing ligand choice.
Complexing Eu with the sensitizing ligands drastically improved emission from the Eu(III) species, where the emission intensity of the bound Eu was at least 3 orders of magnitude greater than that of unbound Eu. Additionally, differences between the spectroscopic signatures of the oxidized Eu(III), and reduced Eu(II), species were observed. Specifically the 592 nm and 613 nm emission peaks associated with Eu(III) disappear upon reduction to Eu(II), and a broad emission band at 450 nm concurrently grows in. Again, altering the bound ligand has a notable effect on the spectroscopic characteristics. The addition of electron-donating groups as well as rigidity to the ligand further increased emissions and thus lowered limits of detection. This indicates the limits of detection for Eu can be further dropped by optimizing ligand choice.

Spectroelectrochemical analysis of the four complexes was successful. The 613 nm and 592 nm peaks that distinguish Eu(III) modulated appropriately with applied potential, as did the 450 nm peak of the reduced species. The source of the emission from the reduced species could not be definitively identified, but likely arises from either fluorescence from Eu(II) or phosphorescence from the bound ligands. The emission from the reduced species may not be appropriate for uniquely identifying Eu, but emission from the oxidized species can uniquely identify Eu and has significantly better emission intensity. Overall, any difficulties identifying the spectral signature of the reduced species will not hamper spectroelectrochemical analysis of Eu complexes because signatures from the oxidized, Eu(III), species can be used. This work demonstrates that spectroelectrochemistry is applicable to the analysis of these Eu complexes and suggests a sensor based on complexing Eu to sensitizing ligands would be an effective way of detecting this lanthanide and can possibly be extended to other electroactive lanthanides.
REFERENCES


3. Chatterjee, S; Del Negro, AS; Edwards, MK; Bryan, SA; Kaval, N; Pantelic, N; Morris, LK; Heineman, WR; Seliskar, CJ (2011) Luminescence-Based Spectroelectrochemical Sensor for [Tc(dmpe)(3)](2+/+) (dmpe=1,2-bis(dimethylphosphino)ethane) within a Charge-Selective Polymer Film Anal Chem, 83, 1766-1772.


11. Crosby, GA; Alire, RM; Whan, RE (1961) Intramolecular Energy Transfer in Rare Earth Chelates - Role of Triplet State J Chem Phys, 34, 743-&.

12. Crosby, GA; Whan, RE; Freeman, JJ (1962) Spectroscopic Studies of Rare Earth Chelates J Phys Chem-Us, 66, 2493-&.

14. Latva, M; Takalo, H; Mukkala, VM; Matachescu, C; RodriguezUbis, JC; Kankare, J (1997) Correlation between the lowest triplet state energy level of the ligand and lanthanide(III) luminescence quantum yield J Lumin, 75, 149-169.

15. Whan, RE; Crosby, GA (1962) Luminescence Studies of Rare Earth Complexes - Benzoylacetonate and Dibenzoylmethide Chelates J Mol Spectrosc, 8, 315-318.


24. Sinha, SP (1965) 2,2'-Dipyridyl Complexes of Rare Earths .2. Reflection Spectra of Nd(3)-Bis-(2,2'-Dipyridyl) and Nd(3)-Bis-(4,4'-Dimethyl-2,2'-Dipyridyl) Chlorides J Inorg Nucl Chem, 27, 115-118.


26. Lines, AM; Wang, Z; Clark, SB; Bryan, SA (2016) Electrochemistry and spectroelectrochemistry of luminescent Eu complexes, accepted in Electroanalysis.


37. Watts, RJ; Crosby, GA (1971) Spectroscopic Characterization of Complexes of Ruthenium(I) and Iridium(III) with 4,4'-Diphenyl-2,2'-Bipyridine and 4,7-Diphenyl-1,10-Phenanthroline J Am Chem Soc, 93, 3184-&.


39. Stegemiller, ML; Heineman, WR; Seliskar, CJ; Ridgway, TH; Bryan, SA; Hubler, T; Sell, RL (2003) Spectroelectrochemical sensing based on multimode selectivity simultaneously achievable in a single device. 11. Design and evaluation of a small portable sensor for the determination of ferrocyanide in hanford waste samples Environ Sci Technol, 37, 123-130.


42. Kumar, ABVK; Jayasimhadri, M; Cha, H; Chen, K; Lim, JM; Lee, YI (2011) Synthesis and luminescence properties of cinnamidate based nanohybrid materials containing Eu (II) ions *J Cryst Growth*, 326, 128-134.


CHAPTER THREE

IN-SITU ELECTROCHEMICAL FORMATION AND SUBSEQUENT SPECTROELECTROCHEMICAL DETECTION OF LUMINESCENT RUTHENIUM COMPLEXES

ABSTRACT

Fast, robust, and cost-effective means of detecting spectroscopically inactive metal cations are necessary for field detection and applications within a variety of areas including industry and the nuclear safeguards fields. A sensor based on spectroelectrochemistry is an excellent candidate to meet these needs as it can specifically quantify metal ions by simultaneously monitoring at least two physico-chemical properties. This applicability of spectroelectrochemistry is demonstrated with ruthenium; which was chosen as an example of a transition metal fission product due to its spectroscopic and electrochemical characteristics as well as its relevance within the fuel cycle and industrial fields. Aqueous Ru displays multiple redox couples in which all available oxidation states have very low molar absorptivities. Ru can, however, form complexes with sensitizing ligands such as 2,2′-bipyridine, where the resulting \([\text{Ru(ligand)}_3]^{2+}\) species displays a red luminescence with a high quantum yield of emission. This significantly decreases detection limits of Ru and allows for the spectroscopic detection of the otherwise hard-to-detect metal ion. This work explores the in-situ generation of \(\text{Ru(ligand)}_3\) complexes and their subsequent spectroelectrochemical sensing using our sensor methodology.
INTRODUCTION

Spectroelectrochemistry is an analytical technique that combines electrochemistry and spectroscopy to provide multiple layers of selectivity for analytes of interest [1, 2]. The applicability of spectroelectrochemistry to the analysis of several metal species has been demonstrated [3-6], as has the ability of spectroelectrochemistry to isolate desired analyte signatures from interferents without completing prior separations [1, 7]. Furthermore, spectroelectrochemistry can be incorporated into field sensors that provide fast, cost-effective, and durable alternatives to collecting and sending samples to labs for time-consuming and expensive analysis [8, 9]. The spectroelectrochemistry technique has huge potential as a field sensor in many industrial, environmental, and nuclear chemistry safeguards applications.

Because spectroelectrochemistry primarily utilizes spectroscopy to detect and quantify analytes, it is limited by the spectral characteristics of the analytes of interest. This is particularly notable in cases where the metal species in solution exhibit very low molar absorptivities and/or extremely low quantum yields of emission in any of their available oxidation states. Examples include the lanthanides and many transition metals such as free Fe$^{2+/3+}$ and Ru$^{2+/3+}$, which tend to have fairly high limits of detection due to their lack of strong or unique spectroscopic signatures [10-13]. Sensors that could quickly and easily quantify these hard-to-detect species with the high selectivity inherent to the spectroelectrochemistry technique would be invaluable tools with numerous applications.

A method for detecting free Fe$^{2+}$ using spectroelectrochemistry has already been explored [12]. The technique involves complexing the Fe$^{2+}$ ions with 2,2’-bipyridine (bpy) to form [Fe(bpy)$_3$]$^{2+}$. This complex exhibits very strong absorbance at wavelengths near 500 nm, unlike
free Fe\(^{2+}\), which has little or no absorption in the visible range. Numerous metal complexes with absorption or luminescence signatures much stronger than those of the respective free metal cations have been characterized [10, 14-16]. This suggests that if a sensor could be made that allows for in situ formation of the appropriate complexes, these hard-to-detect species could be easily and quickly detected using spectroelectrochemistry. More importantly, a large amount of noise associated with absorption measurements could be eliminated by focusing sensor design on the use of luminescent complexes [4].

This work focuses on developing a method where a luminescent complex is electrochemically generated within a prototype sensor and then analyzed using spectroelectrochemistry. Ru was chosen as the analyte of interest as it provides a good model for metals which are more substitution-inert than Fe [17] and has been extensively studied in luminescent complexes [18-21]. The free Ru will be electrochemically encouraged to form luminescent \([\text{Ru(ligand)}_3]^{2+}\) complexes with two different ligands, bpy and 1,10′-phenanthroline (phen). Electrochemical generation of \([\text{Ru(bpy)}_3]^{2+}\) has been previously described, but required long periods of reduction (e.g. 24 hours) and solution conditions that are not ideal for fast and simple field detection [22]. This work demonstrates a fast and effective means for generating the luminescent \([\text{Ru(ligand)}_3]^{2+}\) complexes and the subsequent spectroelectrochemical detection and quantification of the resulting species. Figure 3.1 presents a schematic describing the sensor process and demonstrates the selectivity of fluorescence measurements, where only the generated \([\text{Ru(ligand)}_3]^{2+}\) species will be spectroscopically detected.

It should be noted that this work will focus on a solution based sensing of the Ru species. Several other spectroelectrochemistry based sensors have been described in the literature that
Figure 3.1: Schematic detailing the sensor mechanism. Free Ru$^{3+}$ will be reduced to Ru$^{2+}$ in the presence of excess ligand which will allow for the formation of the luminescent [Ru(ligand)$_3$]$^{2+}$ species which can be oxidized to the non-luminescent [Ru(ligand)$_3$]$^{3+}$ species. Note Ru$^{3+}$(aq), Ru$^{2+}$(aq), free ligand, and [Ru(ligand)$_3$]$^{3+}$ species all lack the strong luminescent characteristics of [Ru(ligand)$_3$]$^{2+}$. 

Reaction A

$$\text{Ru}^{3+} \rightarrow \text{Ru}^{2+} + e^-$$

Reaction B

$$\text{Ru}^{2+} + \text{L} \rightarrow [\text{Ru}^{2+}\text{L}_3]$$

Reaction C

$$[\text{Ru}^{2+}\text{L}_3] \rightarrow [\text{Ru}^{3+}\text{L}_3] + e^-$$

Red emission

Electrode surface
utilize a thin polymer ion exchange films instead of simple solution based sensors [1-3]. While the ion exchange films offer the benefit of concentrating analytes in the spectroscopically active window, thereby reducing limits of detection, they do alter mechanisms of complex formation and add other complications to the system. This solution work will allow for the optimizing of complexation conditions and will allow for a better informed approach to polymer film work in the future.

**EXPERIMENTAL**

**Materials:**

All materials were obtained from Aldrich and were used as received, including 1,10'-phenanthroline (≥ 99%), 2,2'-bipyridly (≥ 99%), RuCl₃ (Ru content 45-55%), [Ru(bpy)₃]Cl₂ (98%), and [Ru(phen)₃]Cl₂ (98%).

**Equipment:**

Potentiostats used included a PAR 273A (EG&G), and a CV27 (Bioanalytical Systems). The PAR 273A was controlled using CoreWare software v. 3.3b (Scribner Assoc.) and the CV27 was adjusted with manual controls. Two types of electrochemical cells were used. The first cell consisted of a cylindrical vial used in conjunction with a glass slide indium tin oxide (ITO) working electrode (WE), platinum wire auxiliary (Aux) electrode, and a Ag/AgCl, 3 M NaCl reference electrode (RE). This cell was used primarily for electrochemical characterization of complexes. The second electrochemical cell was a BASi thin layer cell with a 1-mm path length by 10-mm width quartz cell with a WE compartment volume of 0.09 mL and a total cell volume, including auxiliary compartment volume, of 1 mL. This cell was used for spectroscopic and
spectroelectrochemistry characterization and utilized a Pt mesh WE along with a Pt wire Aux and a Ag/AgCl, 3 M NaCl reference electrode (RE).

Fluorescence spectra were collected using two fluorimeters. The first was a Horiba Jobin Yvon Fluorolog III fluorimeter equipped with a 450 W xenon lamp, double-emission monochromator blazed at 500 nm and a single-excitation monochromator blazed at 300 nm. Emission spectra were corrected for instrumental response. The second fluorimeter was an InSpectrum 150 spectrometer-CCD, using SpectraSense data acquisition software. A 404 nm laser source was used for excitation. Signal integration times were typically 999 ms using a 2 mm slit width for a 600 gr/mm grating blazed at 500 nm.

Absorption spectra were collected using a Cary50Bio and associated software.

Spectroelectrochemistry experiments utilized the PAR273A and the InSpectrum detector with the 404 nm excitation source. The CV27 was used for verification experiments and paired with either the Horiba Jobin Yvon Fluorolog III fluorimeter or the Cary50Bio UV-vis.

RESULTS AND DISCUSSION

Ultimately the goal of this work is to develop a method by which weakly luminescent or non-luminescent metal ions can be quantitatively bound into a uniquely luminescent complex within a sensor environment. Following complexation, the metal centered complex can then be identified and quantified using spectroelectrochemistry. In this work, free Ru in solution was exposed to conditions that favor the formation of a luminescent Ru(ligand)3 species, where the ligand is either 2,2′-bipyridine (bpy) or 1,10′-phenanthroline (phen). In both cases the [Ru(ligand)3]2+ species has been well characterized in literature and is known to produce a strong red luminescence while the [Ru(ligand)3]3+ species is non-luminescent. Both Ru(ligand)3 species
also exhibit reversible II/III couples [18, 19, 23-27]. Reversible electrochemistry and a strong spectroscopic signal in one metal ion oxidation state, and a weak or different signal in the other oxidation state is important for optimal spectroelectrochemical sensing [1, 2].

**Optimizing solution conditions**

This paper focuses on a solution environment so solution conditions had to be optimized for both Ru and the chosen ligands. The solution chemistry of Ru is noted throughout literature to be complex and highly variable depending on pH and the presence of specific anions [28-30]. The ligands also put constraints on solution conditions where lowering the pH of the solution will lead to protonation of bpy and phen, thereby reducing chelation efficiency by blocking binding points of the ligands. Furthermore, the addition of anions or potential ligands that can interact more favorably with Ru than the bpy and phen ligands will reduce chelation efficiency. Hyperquad Simulation and Speciation (HySS) software was used to determine the ideal pH range and explore the various buffer solution options. Speciation of bpy and phen are demonstrated as a function of pH in top plots of Figure 3.2. As indicated by the plots, below a pH of 4.5, greater than 50% of the ligands will be mono-protonated. However lower pH values will limit chances of Ru hydrolysis and other truculent behavior of Ru [30]. This leads to choosing a compromise pH of about 4.5.

Given the desired pH, an appropriate buffer system of non-complexing anions was needed. One such buffer that will meet those needs is acetate. HySS was used to estimate speciation and check if acetate would meet needs of the system. Unfortunately, reliable thermodynamic data on Ru is not available in the literature, likely because Ru is extremely sensitive to solution conditions. The 6th column of the periodic table includes Fe, Ru, and Os. In terms of chemical
behavior, Os would give a better estimate of Ru, but again there is not extensive data available on Os [17]. The HySS exploration of the system was therefore limited to Fe, which at a minimum demonstrates the same change in lability between the 2+ and 3+ oxidation states. Results for the HySS speciation of Fe in a system of 0.1 M acetate buffer and 0.02 M bpy are shown in the bottom plots of Figure 3.2. The bottom left plot of 3.2 shows the speciation of Fe(III) and indicates the Fe(III) will predominantly interact with the acetate anion at a pH of 4.5. The bottom right plot of Figure 3.2 shows the speciation of the Fe(II) system and clearly demonstrates the Fe(II) will form the 1:3 M:L complex at a pH of 4.5. It should again be noted that Fe, like Ru, is significantly more labile in the 2+ state than in the 3+. This suggests and is confirmed by literature, that after the complex is formed in the 2+ state, it should persist upon electrochemical oxidation to the 3+ state [4, 12]. Overall, HySS analysis suggests an acetate buffer at a pH of roughly 4.5 will meet the needs of the Ru system.

The use of an acetate buffer system has some added benefits. Acetate is a well characterized supporting electrolyte and that will meet the electrochemical needs of the system without adding in any Cl\(^-\) or NO\(_3\)\(^-\) anions that tend to have complex interactions with Ru [30, 31]. Additionally utilizing a buffer at a pH of roughly 4.5 will also slightly increase the solubility of bpy and phen which under neutral conditions have reported solubilities of 38 mM and 14.9 mM respectively [32]. Furthermore, solutions of RuCl\(_3\) in the acetate buffer showed unexpected stability over time. Previously published studies of Ru indicate solutions of Ru will dimerize, and though kinetics are slow, fresh solutions of Ru must be used to avoid deleterious effects on the electrochemical and spectroscopic behavior of the Ru [28, 29, 33]. This dimerization can be seen where solutions will change from the brown color of RuCl\(_3\) to have red or blue tints that correspond to various metal-ligand charge transfer bands of different Ru dimers. Solutions of
RuCl₃ in the acetate buffer maintained appropriate coloration and appropriate speciation (as determined by spectroscopy and electrochemistry) for months while solutions in DI water changed color on the scale of weeks. Note, all samples were stored in the dark at room temperature. Figure 3.3 shows a picture of some of these solutions. The first two solutions pictured are 20 mM solutions of RuCl₃ in 0.1 M acetate buffer and in 0.1 M NaCl respectively. Originally both the acetate based solution and the NaCl based solution were originally a dark brown color which the acetate solution retained for several months while the NaCl based developed a red tint. The last two solutions pictured are 0.1 mM RuCl₃ solutions again in the acetate and NaCl solutions respectively. Again the acetate solution retained the original grey-brown color while the NaCl solution developed a blue tint. This is likely due to the formation of ruthenium red and ruthenium blue compounds.

As a result of this exploratory analysis, experiments were completed in a 0.1 M acetate buffer, which maintained a pH of roughly 4.4 and acted as the supporting electrolyte for electrochemical needs. Free ligand was added in excess and Ru was introduced to the system as Ru(III), from soluble RuCl₃. Again the 2+ state Ru is more labile than the 3+ state, which suggests the introduced Ru(III) will need to be reduced before any complexation is observed [17]. Therefore a reducing potential could be applied to reduce the Ru(III) to Ru(II) and encourage complex formation with the ligand present. The Ru(ligand)₃ complexes with either bpy or phen are known to be stable so complexes should persist and should allow for reproducible electrochemical modulation of the spectroscopic signal between the [Ru(ligand)₃]²⁺/³⁺ species with applied potential.
Figure 3.2: HySS speciation diagrams indicating speciation as a function of pH for 2,2′-bypridine (top left), 1,10′-phenanthroline (top right), an Fe(III)/acetate/bpy system (bottom left), and an Fe(II)/acetate/bpy system (bottom right). Data was taken from the NIST database.
Figure 3.3: Examples of RuCl₃ solutions from left to right 20 mM RuCl₃ in 0.1 M acetate buffer, 20 mM RuCl₃ in 0.1 M NaCl, 0.1 mM RuCl₃ in acetate buffer, 0.1 mM RuCl₃ in 0.1 M NaCl.
Ru solution chemistry and choice of Ru starting material

This paper focuses on a solution environment and experiments were completed in a 0.1 M acetate buffer, which maintained a pH of roughly 4.4 and acted as the supporting electrolyte for electrochemical needs. The pH of 4.4 was a necessary compromise to limit the protonation of the free ligands and hydrolysis of Ru.

Ru chemistry in solution can be extremely complex, where Ru speciation is highly dependent on available anions and pH [34]. Soluble RuCl₃ is the best option for introducing Ru to the system and is the starting material used in the synthesis of Ru(bpy)₃ as well as Ru(phen)₃ [35, 36]. However, RuCl₃ is a complex material that contains Ru(III) as well as Ru(IV) species as either oxo, nitrosyl, or chloride complexes [34, 37]. When pulled into a solution at pH 4.4, it is very likely that hydroxy species will form as well [34]. This complex speciation is unavoidable with Ru, but some techniques can be used to start with a slightly better characterized Ru species and test/prove the detector concept. Methods for isolating specific Ru(II) mono or di-chloride complexes have been discussed previously [38, 39], but will not be useful in this case as the pure chloride complex will begin to form hydroxy and possibly acetate species when introduced to the acetate buffer system.

In lieu of producing a pure Ru chloride species that will almost immediately exhibit complex speciation when introduced to solution, the RuCl₃ starting material can be treated according to the procedure used in the commercial synthesis of the [Ru(bpy)₃]Cl₂ complex. That procedure requires the heating of RuCl₃ at 120° for 4 hours [35, 36] which results in the formation of predominantly [Ru₂O]⁶⁺ species [37, 40].

Generation and analysis of spectroelectrochemical data of the Ru(ligand)₃ species can be tested with both the treated and untreated Ru starting material. The treated material will provide
a starting material that is more thoroughly described by the literature. Additionally, speciation concerns will be further limited when applied potentials are used to control the oxidation state of the Ru present. It is particularly beneficial to test detector design with the untreated material because its complex speciation more closely approximates conditions that would be expected in real samples.

Fortunately, the Ru(ligand)\textsubscript{3} complexes with either bpy or phen have been well characterized in literature, so there are excellent benchmarks available for identifying the species produced in the sensor environment. Despite the complexity of the Ru starting material (which will be minimized) it will still be possible to definitively determine if the Ru(ligand)\textsubscript{3} species was generated in the sensor environment. Additionally, the Ru(ligand)\textsubscript{3} complexes are known to be stable and should persist and allow for reproducible electrochemical modulation of the spectroscopic signal between the [Ru(ligand)\textsubscript{3}]\textsuperscript{2+/3+} species.

**Cyclic Voltammetry**

Electrochemical generation of the Ru(ligand)\textsubscript{3} species was first explored using cyclic voltammetry, and cyclic voltammograms (CVs) of these species are shown in Figure 3.4. Blank CVs of the 0.1 M acetate buffer both with and without free ligand lacked any electrochemical activity in the potential window of interest. CVs of solutions containing RuCl\textsubscript{3} without any ligand present are in good agreement with literature. It is important to note Ru speciation and electrochemistry varies greatly depending on the anions present, *e.g.* Cl\textsuperscript{−} versus NO\textsubscript{3}−. Ideally the introduced Ru(III) will only weakly interact with either the weakly complexing acetate ion or limited Cl\textsuperscript{−} present, and observed electrochemistry will more closely follow what has been
described for weakly complexed or the aquated Ru species. Within HClO₄ solutions, where ClO₄⁻ is a weak complexant similar to acetate, couples were observed at formal reduction potentials (E°) of 0.59, 0.39, and -0.11 V and were ascribed to

Ru(IV)→Ru(3.5)→Ru(III)→Ru(II) reduction steps [28]. This agrees with the II/III couple reported at -0.02 V for hexaaquoruthenium [13]. Other papers covering Ru in environments with Cl⁻ described a Ru(III)→Ru(II) couple anywhere in the range of -0.415 V to -0.11 V though the presence of Ru(IV)→Ru(3.5) or Ru(IV)→Ru(III) couples is less consistently described [11, 29, 30]. For this work, quasi-reversible couples were observed at E°'s of 0.531 V (with a peak splitting, ΔEₚ, of 0.136 V) and 0.114 V (with a ΔEₚ of 0.111 V). This more closely resembles the results in HClO₄ and for the aquated ion where the Ru is weakly complexed or totally aquated and couples can be assigned to Ru (III)/(IV) and the Ru (II)/(III) couples respectively. Evidence for the Ru(3.5) state was not observed under conditions used for this work.

Electrochemical generation experiments involved exposing a solution containing Ru(III) and excess ligand to reducing potentials of 0.05 V, which is a slightly more reducing potential than where the cathodic wave for the Ru II/III couple appears. CVs were collected between 5 min periods of applying a constant reduction potential to determine if waves corresponding the [Ru(ligand)₃]^{2+/3+} couples grew in. CVs are shown in Figure 3.4, and electrochemical characterizations have been tabulated in Table 3.1. In the bpy case, a new couple grows in at 1.08 V which aligns with the measured [Ru(bpy)₃]Cl₂ standard (light blue CV in Figure 3.4, top) and corresponds with the reported reduction potential of [Ru(bpy)₃]^{2+/3+} [23]. In the phen case, a new couple grows in at 1.108 V which again aligns with the measured [Ru(phen)₃]Cl₂ standard (pink CV in Figure 3.4, bottom) and is within the range reported for [Ru(phen)₃]Cl₂ [26, 41].
In the case of both ligands, applying reducing potentials for up to two hours did not fully convert all available Ru to the luminescent $[\text{Ru(ligand)}_3]^{2+}$ species. This is due to the slow kinetics of Ru chemistry. In order to approach 100% conversion the system needed to be heated to 50 °C and reducing potentials needed to be held in excess of 24 hours. This was done in an H cell to separate the working and auxiliary electrodes and an example of the result is shown in the top plot of Figure 3.4 as a teal line. Note that the $[\text{Ru(bpy)}_3]^{2+/3+}$ couple of the teal line is more defined and the current of the Ru $^{2+/3+}$ and Ru $^{3+/4+}$ couples is reduced.

In experiments involving electrochemical generation of Ru(ligand)$_3$ with either the bpy or phen ligand, successive CVs show a slight decrease in peak currents for the Ru II/III and Ru III/IV couples, while a new couple grows in at 1.08 V in the bpy case and 1.108 V in the phen case. This indicates there is a slight depletion in free Ru while the concentration of the complexed Ru species increases. An example of this can be seen in Figure 3.5, where the top plots shows successive CVs collected as electrochemical generation conditions are applied and the bottom plot presents the peak current as a function of time for multiple couples. Reported peak currents in the bottom plot of Figure 3.5 were calculated by subtracting the CV baseline from the measured peak current. It should also be noted that some shifts were observed for the Ru II/III and Ru III/IV couples after ligand was added and reducing potentials were applied. Figure 3.5 indicates the peak signal from the $[\text{Ru(ligand)}_3]^{2+/3+}$ system grows in faster than peak current for the aqueous Ru species decreases. This is observed because the aqueous Ru is in excess in the bulk phase of the solution and is only minimally depleted within the electrochemical window. Additionally, for either ligand it appears a new couple grows in slightly positive of the Ru III/IV couple. This could indicate the presence of a Ru(ligand)$_2$ species and is discussed in a later.
**Figure 3.4:** Top: CVs of solutions containing the bpy ligand. Bottom: CVs of solutions containing the phen ligand. CVs of both electrochemically generated complexes were collected after a total reduction time of 70 min (with exception of the teal line where potentials were applied in excess of 24 hrs). Data were collected using an ITO WE, Pt Aux, Ag/AgCl, 3 M NaCl RE in a 0.1 M acetate buffer. RuCl$_3$ was at 0.5 mM in all cases and ligands were at roughly 15 mM.
Figure 3.5: Top: CVs of experiments utilizing the bpy ligand collected between 10 min periods of applying a reducing potential of 0.05 V after a total reduction time of 70 min. Ru before the addition of ligand in grey, and successive CVs for the electrochemical generation are show from light to dark blue as total reduction time increases. Arrows indicate the direction of peak behavior as a function of time. Bottom: plot of peak current for the anodic (circles) and cathodic (triangles) waves of the Ru II/III (light blue), Ru III/IV (grey), and Ru(bpy)$_3$ II/III (blue) couples from CVs collected between periods of applying a reducing potential. Data were collected using an ITO WE, Pt Aux, Ag/AgCl RE in a 0.1 M acetate buffer. RuCl$_3$ was at 0.5 mM in all cases and ligands were at roughly 15 mM.
Further electrochemical characterization was completed to explore the scan rate dependent behavior of the electrochemically generated complex. The standards of [Ru(bpy)$_3$]Cl$_2$ and [Ru(phen)$_3$]Cl$_2$ were also characterized and measured values of these standards were again compared to those the electrochemically generated species. Results did indicate larger peak splitting ($\Delta E_p$) values for the electrochemically generated species, but otherwise results were in good agreement with values obtained for standard complexes and values reported in literature; see Table 3.1 for values. Furthermore, the relationship between peak currents and the square root of the scan rate was linear in all cases, indicating diffusion controlled mechanisms for both the standards and electrochemically generated species. It is also important to note that peaks for the electrochemically generated species are not as clearly defined as those of the standards. This is observed because the ratio of complexed Ru to free Ru is small, so the [Ru(ligand)$_3$]$^{2+/3+}$ couple is growing in on a larger free Ru background signal. Significantly longer periods of applying a reducing potential are needed to convert all free Ru to the complexed species. Time requirements for complete conversion are discussed in a later portion of the chapter. However, due to the high quantum yield of the [Ru(ligand)$_3$]$^{2+}$ species, complete conversion is not necessary for quantifiable spectroscopic detection of the Ru species.

Note, much of the CV characterization of the [Ru(ligand)$_3$]$^{2+/3+}$ species was carried out on samples that had been exposed to electrochemical generation potentials for about 2 hours. Therefore the samples did have other Ru species present, but those species were not observed to interfere with the electrochemistry of the Ru(ligand)$_3$ species. Characterization of the samples exposed to reducing potentials in excess of 24 hours (while solution temperature increased to 50° C) aligned well with results obtained from samples with incomplete conversion.
Table 3.1: Characterization of electrochemically generated species and standards

<table>
<thead>
<tr>
<th></th>
<th>[Ru(bpy)$_3$]$^{2+/3+}$</th>
<th></th>
<th>[Ru(phen)$_3$]$^{2+/3+}$</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Standard</td>
<td>Electrochemically generated complex</td>
<td>Standard</td>
<td>Electrochemically generated complex</td>
</tr>
<tr>
<td>E°′, V</td>
<td>Meas. (± 0.003)</td>
<td>1.08</td>
<td>1.08 (± 0.01)</td>
<td>Meas. (± 0.003)</td>
</tr>
<tr>
<td>ΔE_p, V</td>
<td>0.075 (± 0.004)</td>
<td>0.117 (± 0.003)</td>
<td>0.08 (± 0.02)</td>
<td>0.15 (± 0.01)</td>
</tr>
<tr>
<td>Emission λ$_{max}$, nm 2+species</td>
<td>605 [4]</td>
<td>605</td>
<td>591</td>
<td>591</td>
</tr>
<tr>
<td>Absorption λ$_{max}$, nm 2+ species</td>
<td>454 [23, 27]</td>
<td>458</td>
<td>446</td>
<td>463</td>
</tr>
</tbody>
</table>

*Value reported for propylene carbonate solvent and TBAPF6 supporting electrolyte

**Value reported for acetonitrile solvent and TBAPF6 supporting electrolyte

Reported errors are one standard deviation

**Absorption and Fluorescence Spectroscopy**

The electrochemical couples of the electrochemically generated complexes fall near reported values for the well characterized [Ru(bpy)$_3$]$^{2+/3+}$ and [Ru(phen)$_3$]$^{2+/3+}$ couples which supports the claim that these complexes were successfully generated. Further verification was provided by spectroscopic measurements including fluorescence and absorption spectroscopy. Fluorimetry measurements of the excitation and emission spectra of electrochemically generated species aligned with the measured standards and values agreed with what has been reported in literature [4, 26]. Results of these measurements are shown in the top plot of Figure 3.6. Spectra were normalized to allow for easier comparison of excitation and emission profiles. Given equal Ru concentrations, intensity of the electrochemically generated samples was significantly smaller than that of the standards. This again indicated that only a small fraction of the available Ru was converted to the [Ru(ligand)$_3$]$^{2+}$ species.
Figure 3.6: Top: Excitation (dotted lines) and emission (solid lines, excitation at 404 nm) profiles for electrochemically generated complexes and standards. Bottom: Absorption spectra (normalized for concentration differences) of electrochemically generated complexes and standards where [Ru] was constant at 0.01 mM and free ligand was at approximately 15 mM.
Absorption spectra of both the electrochemically generated species and the standards were also collected and are shown in the bottom plot of Figure 3.6. Absorption peaks for the electrochemically generated complexes fall near but do not perfectly overlap those of the standards. This can suggest that other species are being generated along with the Ru(ligand)$_3$ species and act as interferents in absorption based measurements. Observed values for emission maximum and absorption bands are reported in Table 3.1.

Much like what was observed with the electrochemistry, if the samples are exposed to a reducing potential in excess of 24 hours, (with heating to 50 °C) the absorption spectra of the generated samples look much more like the spectra of the standards. This indicated by the teal line in the bottom plot of Figure 3.6. This provides some indication that the source of the broadening of the absorbance band observed at shorter electrolysis times may actually a precursor to the Ru(ligand)$_3$ product.

It should be noted that Ru(ligand)$_2$ or Ru(ligand) species could form and interfere with either electrochemical or spectroscopic signatures of the Ru(ligand)$_3$ species. The 1:1 and 1:2 metal-to-ligand species for either ligand have been less thoroughly studied under conditions similar to those reported here. While many studies of bis-bpy or even bis-phen chelates have been reported, the remainder of the coordination sphere is often filled by other N-donor ligands which does not mimic the conditions most likely observed in this work [20, 25, 43-45]. These complexes generally absorb around 450 nm, but emission is often shifted to the deep red. Limited research has been completed on trans and cis isomers of [Ru(bpy)$_2$]$^{2+/3+}$ as aquo-complexes. The reported $E^\circ$s for the II/III couple of the [Ru(bpy)$_2$]$^{2+/3+}$ species are 0.63 V and 0.46 V for the cis- and trans- complexes, respectively [46]. The generation of the cis-Ru(ligand)$_2$ species would be consistent with the observed ingrowth of a couple at $E^\circ$s of about 0.76 V for the bpy species and

87
about 0.74 V for the phen species. In this case the difference between the reported 0.63 V and the observed 0.76 and 0.74 V for the bpy and phen species would be due to differences in solution conditions. Ru has consistently demonstrated sensitivity to solution conditions in both the literature and in this work, where the uncomplexed Ru (II/III) and (III/IV) couples shifted with the additions of free ligands [30]. Absorption bands for the 2+ species of the cis and trans complexes are reported to be 480 and 495 nm, respectively [47]. The formation of the cis-Ru(ligand)\textsubscript{2} species would again be consistent with the broadening and shifting of the observed absorption bands to higher wavelengths. Neither cis nor trans species were reported to be luminescent in water at room temperature, but the cis complex shows weak luminescence at 660 nm and the trans complex shows extremely weak luminescence at 700 nm in H\textsubscript{2}O-methanol glasses at 77 K [47]. This is again consistent with observed characteristics where the luminescence spectra of the electrochemically generated samples did not indicate the presence of any interferents. It should also be noted that the 3+ species of either the cis or trans complexes are not reported to absorb in the range of 400-700 nm or to be luminescent. Additionally, precipitates were not observed during electrochemical generation so it is unlikely that the highly insoluble trans species was formed [48].

Overall, the data suggests the cis-Ru(ligand)\textsubscript{2} species was generated along with the Ru(ligand)\textsubscript{3} complex. It is likely that other Ru-ligand species were formed, including the 1:1 species and any number of species where the bidentate bpy or phen ligand are bound only in a monodentate fashion. Literature discussing the characteristics of these species is not available and it is therefore difficult to determine electrochemically or spectroscopically if those species are present. Despite the confirmed presence of other Ru(ligand)\textsubscript{n} species, the observed data suggests that a sensor for the Ru(ligand)\textsubscript{3} complex will be very successful. This is because the
sensor will be based on fluorescence measurements, which appear to be completely unaffected by the presence of other Ru(ligand)ₙ species and only sensitive to the Ru(ligand)₃ species. This demonstrates the high selectivity of fluorescence spectroscopy for the analytes of interest, which are limited to the Ru(ligand)₃ species in this case.

**Spectroelectrochemistry**

With both the electrochemical and spectroscopic results supporting successful generation of Ru(ligand)₃ complexes with both bpy and phen, the next step was to determine if the electrochemical generation can be followed spectroscopically while reducing potentials were applied. Furthermore, it is necessary to determine if the luminescence from the generated [Ru(ligand)₃]²⁺ complexes could be reproducibly turned off and on as applied potential was cycled to oxidize to [Ru(ligand)₃]³⁺ (non-luminescent) and re-reduce to [Ru(ligand)₃]²⁺. The results of spectroscopically following the electrochemical generation of the Ru(bpy)₃ species using fluorescence measurements are shown in Figure 3.7.

As indicated in the figure, experiments started with applying a modulating potential to the acetate buffer blank, then free ligand was added, and finally RuCl₃ was added. There was no change in spectral signature when reducing (0.05 V) or oxidizing (1.3 V) potentials were applied to blank solutions (0.1 M acetate buffer) or to solutions of free ligand. Changes in luminescence were not observed until after 0.1 mM RuCl₃ was added and a reducing potential was applied to the system, which can be seen at about 22 minutes in Figure 3.7. The luminescence at 605 nm (for bpy experiments) or at 591 nm (for phen experiments) increased only after the three components of free ligand, Ru, and a reducing potential were all present. Additionally, signal
modulated appropriately with applied potential. Emission decreased when oxidizing potentials (1.3 V) were applied (to produce the non-luminescent \([\text{Ru(ligand)}_3]^{3+}\) species) and continued to increase as each successive wave of reducing potentials was applied, indicating that more \([\text{Ru(ligand)}_3]^{2+}\) was generated with each potential cycle. Figure 3.7 only presents data for an experiment where bpy was the free ligand. Experiments with phen produced similar results where emission only increased when the solution containing both free ligand and Ru were exposed to a reducing potentials. Experiments also produced the same results when the order of introduction to the system was flipped so that RuCl\(_3\) was added first, exposed to a modulating potential, and then free ligand was added. Again, none of the Ru species other than the \([\text{Ru(ligand)}_3]^{2+}\) species are luminescent in solution and an increase in emission was not observed until both Ru and the free ligand were present and exposed to a reducing potentials.

Evidence suggests the kinetics of the formation of the \([\text{Ru(ligand)}_3]\) species is very slow. This can be seen in the both the electrochemistry and the spectroscopy where electrolysis times in excess of 24 hours and the application of heat (50° C) are necessary to approach the quantitative conversion of all available Ru to the luminescent species. Despite the long time frames needed for quantitative conversion, the results presented in Figure 3.7 indicate excellent luminescence signal from the generated \([\text{Ru(ligand)}_3]\) species even in just the first few electrolysis steps. There are two primary reasons for this: 1) other Ru species in solution do not share the same luminescent signatures as the \([\text{Ru(ligand)}_3]\) species (meaning they do not interfere spectroscopically) and 2) the quantum yield of either \([\text{Ru(ligand)}_3]^{2+}\) species is very large. Overall this suggests that full conversion is not necessary to get excellent luminescence signal.
Figure 3.7: Top: emission spectra collected over the span of 40 min as free ligand (15 mM) and then RuCl₃ (0.1 mM) were added to the 0.1 M acetate solution and applied potential was stepped between 1.3 V and 0.05 V. Bottom: overlay of emission intensity at 605 nm for the spectra presented in the top panel (red, left axis) with the applied potential (black, right axis) versus time.
Generation and modulation of the Ru(ligand)$_3$ species and its spectroscopic signal was also attempted using absorption based measurements. Results can be seen in Figure 3.8 and experiments again demonstrated that the absorbance band associated with the [Ru(ligand)$_3$]$^{2+}$ species only appears after both the Ru and free ligand are present in solution and a reducing potential is applied. In the examples shown in Figure 3.7, emission from the [Ru(ligand)$_3$]$^{2+}$ species was observable after even the first reducing step. In the example presented in Figure 3.8, the absorption band of generated [Ru(ligand)$_3$]$^{2+}$ modulated appropriately but the $\Delta$ absorbance is on the order of 0.005. This is again another demonstration of the benefit of utilizing fluorescence based measurements in sensor design. The reduction of noise inherent to fluorescence spectroscopy allows for a significantly reduced limit of detection for the [Ru(ligand)$_3$]$^{2+}$ species. Figure 3.8 is also useful for pointing out the absorptions characteristics of the species other than [Ru(ligand)$_3$]$^{2+}$. The RuCl$_3$ solution does not have any defining bands. The absorbance spectra of the RuCl$_3$ solution in acetate buffer was consistent even in solutions that were allowed to sit (in the dark) for several months. Bands in either the red or blue regions that indicate Ru dimerization and other Ru side reactions were not observed. The [Ru(ligand)$_3$]$^{3+}$ species does not absorb around 460 nm and because the entire absorbance band around 460 nm disappears entirely upon oxidation, it is safe to assume the [Ru(ligand)$_2$]$^{3+}$ (where the cis-[Ru(ligand)$_2$]$^{2+}$ species contributes to this band) also does not absorb in this region. The free ligands have very large absorption bands in the UV region, and produce the large shoulder towards the lower wavelengths that is observed in Figure 3.8.
Figure 3.8: Top: absorption spectra collected over the span of 40 min as free ligand (15 mM) and then RuCl$_3$ (0.1 mM) were added to the 0.1 M acetate solution and applied potential was stepped between 1.3 V and 0.05 V. Bottom: overlay of absorption at 490 nm for the spectra presented in the top panel (red, left axis) with the applied potential (black, right axis) versus time.
Optimization of the conditions for the electrochemical generation of the Ru(ligand)$_3$ complexes was also considered. Electrochemical generation of the complexes was monitored using fluorescence spectroscopy while applied potential waveforms were altered. Three main variants of waveforms were used: 1) applying no potential, 2) applying constant reducing potential for the duration of the entire experiment, and 3) stepping the potential between oxidizing and reducing potentials (see example in Figure 3.7). A representative example of the results for solutions of constant [RuCl$_3$] and [phen] is shown in the top plot of Figure 3.9. When no potential was applied (waveform 1), there was no ingrowth of emission at 605 nm (bpy case) or 591 nm (phen case) to indicate the generation of Ru(ligand)$_3$. Duplicate samples of solutions where no potential was applied were kept in either dark or light (bench top) conditions for 48 h and again measured. Samples stored in the dark did not develop an increase in red luminescence while samples stored in the light did indicate very slight increases in emission. This increase in emission was only analytically significant with the phen samples and not the bpy samples. Experiments using a constant reducing potential (waveform 2) showed an increase in emission, indicating successful generation of the complexes. Interestingly, when a constant potential was applied, the system never appeared to reach equilibrium and level off in intensity. Emission intensity rose steadily even when reducing potentials were applied on excess of 70 minutes. This indicates the kinetics of the formation of the Ru(ligand)$_3$ complex from free Ru$^{2+}$ and bpy are very slow. This is unsurprising given the known slow kinetics of ligand exchange reactions of Ru [17]. Optimal results with regards to emission intensity and time to reach equilibrium (the point at which maximum emission intensity becomes constant for successive reducing steps) were obtained when the applied potential was modulated between reducing (0.05 V) and oxidizing (1.3 V) potentials. Time frames for applying oxidizing and reducing potentials were altered, e.g.
reducing for 2 minutes and oxidizing for 2 minutes vs reducing for 4 minutes and oxidizing for 2 minutes, but did not generally have significant effects on the ingrowth of emission. Despite increasing time frames for applying reducing potentials, the sharpest ingrowth was observed in the first 4 minutes and tapered off slightly thereafter. Emission intensity again did not level off in the fashion that is often observed in sensors where the analyte is not be generated in situ [2, 3]. Electrochemical generation was most effective when reducing potentials were applied for four minutes and oxidizing potentials were applied for two minute increments.

It should be noted that when using bpy instead of phen, there was less of a disparity between maximum emission when applying a constant potential and applying modulating potentials. In the bpy case consistent results were difficult to obtain when applying a constant reducing potential. Reasons for this are unclear but results were significantly more consistent when applying modulation potentials.

Results indicate the application of a reducing potential is necessary to quickly and reproducibly generate the Ru(ligand)$_3$ complexes. However it is unexpected that modulating the potential would encourage faster formation than simply applying a reducing potential for the same total amount of time. An explanation for this observation is that stepping between the two potentials causes a convection current that helps bring uncomplexed Ru to the surface of the electrode. In other words, modulation the applied potential results in micro-stirring and removes a diffusion based dependence from the rate of formation. This hypothesis was tested by applying different waveforms during the electrochemical generation of the Ru(ligand)$_3$ complex. Results can be seen in Figure 3.11. Applied potential was modulated between 0.05 V, 1.3 V, and 0.5 V
Figure 3.9: Top: emission from the 591 nm band when electrochemically generating Ru(phen)$_3$ species by applying waveform 3 (grey line, where red dotted line indicates maximum ingrowth behavior), applying waveform 2 constant potential (light pink line), and waveform 1 no potential (purple line, light purple solid line is same experiment measured 48 h after storing solution on the bench top, light purple dotted line is same experiment measured 48 h after storing solution in dark). Three bottom plots show applied wave forms for modulation experiment (3), constant potential experiment (2), and no potential experiment (1), respectively.
where different spectroscopic responses are expected at each potential. The reasoning behind these potential choices is most easily explained by Figure 3.10. The top schematic of Figure 3.10 again indicated the reactions leading to the formation of the Ru(ligand)$_3$ complex.

The bottom schematic of Figure 3.10 indicates what species are present at given potentials which dictates when complexation occurs and what is observed spectroscopically. When applying potentials more negative than 0.114 V (such as 0.05 V), uncomplexed or aquated Ru is being reduced to Ru(II) which can then form the luminescent [Ru(ligand)$_3$]$^{2+}$ species, and any previously generated [Ru(ligand)$_3$]$^{3+}$ is being reduced to the luminescent [Ru(ligand)$_3$]$^{2+}$; luminescence intensity is expected to increase. When applying potentials above 1.08 V (such as 1.3 V), Ru(ligand)$_3$ is no longer being generated (uncomplexed Ru(II) is not being generated at the electrode) and any previously generated [Ru(ligand)$_3$]$^{2+}$ is oxidized to the non-luminescent [Ru(ligand)$_3$]$^{3+}$; luminescence intensity is expected decrease. When applying 0.5 V, the potential is not low enough to reduce aquated Ru(III) to Ru(II) so new Ru(ligand)$_3$ is not generated. However, 0.5 V is capable of reducing any previously generated [Ru(ligand)$_3$]$^{3+}$ to the luminescent [Ru(ligand)$_3$]$^{2+}$; luminescence intensity should remain constant and proportional to the concentration of previously generated complex.

In these experiments the applied potential was modulated between 0.05 V and 1.3 V for 8 steps in order to generate the luminescent Ru(ligand)$_3$ species. Applied potentials were then switched to modulate between 0.5 V and 1.3 V for another 8 steps. When the potential is modulated between 0.05 V and 1.3 V for the entire experiment, maximum luminescence increases with each reducing step; which is indicated by the grey line in top plot of Figure 3.11. When switching between the 0.05 V and 1.3 V modulation to the 0.5 V and 1.3 V modulation, it is expected that maximum luminescence should remain constant for each subsequent reducing
**Figure 3.10:** Top: Reaction mechanism for the formation of $[\text{Ru(ligand)}_3]^{2+/3+}$ from Ru(III) at the surface of the electrode. Bottom: depiction of majority species present at the surface of the electrode when applying potentials in given ranges. The listed potentials are the $E^{\circ'}$ for the Ru (II)/(III), Ru (III)/(IV), and $[\text{Ru(ligand)}_3]^{2+/3+}$ couples from left to right. Note the printed 1.08 V will vary depending on ligand used.
step which is indicated by the dashed blue line in top plot of Figure 3.11. However, what is actually observed is a significant drop in luminescence intensity which is indicated by the solid blue line in the top plot of Figure 3.11. The observed data appears to suggest that when the applied potential is stepped from 0.05 V to 1.3 V, forced convection carries a portion of the generated \([\text{Ru(ligand)}_3]^{3+}\) out of the electrochemically active field. When the potential is then stepped back to 0.5 V, the previously generated \([\text{Ru(ligand)}_3]^{3+}\) is no longer within the electrochemical window and therefore cannot be reduced to \([\text{Ru(ligand)}_3]^{2+}\) and luminescence does not return to its previous maximum. When the waveform is switched to modulate between 0.5 V and 1.3 V maximum emission remains roughly constant because a bulk solution concentration equilibrium has roughly been reached.

To explain observed behavior, a proposed mechanism of formation is shown in the top box of Figure 3.10. Three reactions are depicted in the schematic where the first reaction, Reaction A, involves the reduction of solvated Ru(III) at the electrode surface to Ru(II). Reaction B can then take place where the Ru(II) is complexed by the ligand present to form \([\text{Ru(ligand)}_3]^{2+}\). Reaction C then involves the oxidation of luminescent \([\text{Ru(ligand)}_3]^{2+}\) to the non-luminescent \([\text{Ru(ligand)}_3]^{3+}\), which is electrochemically reversible. The bottom schematic of Figure 3.10 indicates which species are present in given potential ranges for the uncomplexed/aquated (shades of blue) and complexed (red and grey) Ru species. It should be noted that either schematic in Figure 3.10 does not include formation or behavior of the \(\text{Ru(ligand)}_2\) complex. Again the \(\text{Ru(ligand)}_2\) complex and any other undesired Ru products do not interfere with the fluorescence measurements of the sample and therefore are not of significant interest when looking at the general mechanism of sensing the species. The 1:2 complex may be of more interest in the actual mechanism of formation where the Ru might first have to go through a 1:2
Figure 3.11: Top: emission from the 605 nm band when electrochemically generating Ru(bpy)$_3$ species when applying potentials necessary to generate complex (grey), and what is observed when applying potentials capable of reducing complex but not capable of generating more complex (blue solid), the blue dashed line indicates the expected maximum emission intensity at 605 nm when applying potentials capable of reducing complex but not capable of generating more complex. Bottom: applied potential waveforms corresponding to top plot.
complex before re-arranging and making the 1:3 complex. This is a difficult system to test given the spectroscopic activity of the 1:2 complex.

The metal-to-ligand ratio was also optimized. Experiments were completed for a constant concentration of Ru in conditions with varying concentration of ligand and results can be seen in Figure 3.12. The solutions were exposed to applied potentials modulated between 0.05 V and 1.3 V (held for four and two minutes, respectively) for a total of 15 steps. The difference in emission intensity between the last oxidizing and reducing steps (Δ emission) was monitored as a function of amount of excess ligand and plots can be seen in the top plot of Figure 3.12. Results indicate that as the amount of excess ligand was increased, the Δ emission increases up to the solubility limit of the ligands. In the bpy case, for the set metal concentration of 0.02 mM Ru, the solubility limit of bpy was reached at about the 2000 times excess ligand (about 40 mM bpy) point. The data collected at the 3000 times excess point was not statistically different than the data collected at the 2000 times excess point. For the phen case, where the Ru concentration was again held at 0.02mM, the solubility limit of phen was reached at about the 1000 times excess point (about 20 mM phen). After the solubility limit was reached, the 2000 times excess point demonstrated leveling out of Δ emission but the 3000 times excess point indicated a drop in Δ emission. The drop in signal was unexpected but possibly arises from the higher incidence of phen particulates in solution scattering light. It should be noted that in studies where the solubility limit of the ligand was exceeded, care was taken to ensure any solid particles did not get trapped within the spectroscopic window of the spectroelectrochemistry cell. The bottom plot of Figure 3.12 presents the luminescence spectra from the last reduction step of the ligand dependence studies. Spectra do not show any shifts with changing excess ligand conditions.
Figure 3.12: Top) the $\Delta$ emission between the final oxidizing and reducing steps of several experiments where the solution ratio of Ru and the respective ligand was varied. Blue circles correspond to experiments completed with bpy and red circles correspond to experiments completed with phen. Bottom) Overlay of luminescence spectra from the final reduction step. Blue spectra correspond to the bpy experiments and red spectra correspond to the phen experiments. Color variation from dark (dark blue and red respectively) to lighter (light blue and light pink) colors corresponds to increasing ligand concentration.
Spectroelectrochemical Quantification

Using the optimized electrochemical parameters for modulation and a metal-to-ligand solution ratio of 1:1500 M:L, the limit of detection for the electrochemically generated species was determined. While the M:L ratio was kept constant, the concentration of initially added \(\text{RuCl}_3\) was varied from 0 mM to 0.1 mM. At 0 mM Ru various concentrations of the ligand were used but no red luminescence was observed. The applied potential was modulated for a total of 15 steps (a total of 47 min) which was the time necessary for the lowest concentrations of Ru to reach equilibrium, where the maximum emission at the end of a reducing step was constant for several reducing steps. Figure 3.14 presents the emission at 605 nm for the bpy experiments (top plot) and 591 nm for the phen experiments (middle plot) as a function of time while applied potential was modulated (bottom plot). Insets in the plots zoom in on the lowest concentration experiments and indicate when those experiments reached equilibrium and their maximum emission became consistent between subsequent reducing steps. It should be noted that for the higher concentrations of Ru, equilibrium was not reached and the maximum emission continues to increase for each subsequent reducing step. Interestingly in the case of the phen species, while the maximum emission continues to increase, the \(\Delta\) emission between the oxidizing and reducing steps remains relatively constant for the two highest concentrations of Ru. This is likely observed because the allotted 2 minutes of oxidation are not long enough to oxidize all of the \([\text{Ru(phen)}_3]^{2+}\) present to the non-luminescent \([\text{Ru(phen)}_3]^{3+}\) species. This would also indicate that the kinetics of formation of the phen complex are faster than those of the bpy complex.
Figure 3.13: Top: Emission at 605 nm as a function of time for electrochemical generation experiments at varying [Ru] and constant M:L with the bpy ligand. Middle: Emission at 591 nm as a function of time for electrochemical generation experiments at varying [Ru] and constant M:L with the bpy ligand. Middle: Emission at 591 nm as a function of time for electrochemical generation experiments at varying [Ru] and constant M:L with the phen ligand. Bottom: Applied potential as a function of time for the electrochemical generation experiments.
The Δ emission from experiments plotted in Figure 3.14 can then be plotted as a function of the initial concentration of RuCl₃ for both the bpy and the phen experiments. Results are shown in Figure 3.15. For both the bpy and phen experiments the Δ emission shows a linear trend between the total concentration of Ru, or the initial concentration of RuCl₃, from 0 mM to 0.02 mM. At concentrations above 0.02 mM, behavior deviates from linearity for two reasons: the system is nearing the saturation limits of the ligands and the allotted time is not long enough (would need to be in excess of 24 hours) to reach equilibrium. This is most apparent for the electrochemical generation experiments with phen where the Δ emission can be seen to level off between RuCl₃ concentration of 0.04 mM and 0.1 mM. Figure 3.15 also shows the calibration curves for the [Ru(bpy)₃]Cl₂ and [Ru(phen)₃]Cl₂ standards which were also measured in the thin layer cell. The limit of detection (LOD) calculated for the electrochemical generation method was 2⋅10⁻⁶ M for the electrochemically generated Ru(bpy)₃ species and 2⋅10⁻⁷ M for the electrochemically Ru(phen)₃ species. This can be compared to the LODs of the standards which are 9⋅10⁻⁸ M and 8⋅10⁻⁹ for the [Ru(bpy)₃]Cl₂ and [Ru(phen)₃]Cl₂ standards respectively. The LOD was calculated using Equation 3.1, where s_b is the standard deviation of emission for the 0 mM Ru experiments and m is the slope of the emission versus concentration plots (M⁻¹).

\[
LOD = \frac{3s_b}{m}
\]  
Eq. 3.1

Calibration curves of the standard indicate approximately 1% of the RuCl₃ is captured in complexes with bpy and approximately 5% with phen. The rate of complexation is affected by several factors, including the time necessary to electrolyze all Ru(III) to Ru(II) within the thin cell and the kinetics of complexing the generated Ru(II) to the free ligand to form the 1:3 species. As a lower limit bound to the amount of time necessary to convert 100% of the free Ru
Figure 3.14: Δ emission at 605 nm for Ru(bpy)$_3$ species (standard and electrochemically generated species) and at 591 nm for the Ru(phen)$_3$ species versus the total concentration of Ru in the system. Inset depicts linear range for the electrochemically generated species. Note this data corresponds to Figure 3.13 where electrochemical generation potentials were only held for 47 minutes.
to the luminescent complex, the time required to electrolyze the bulk solution can be estimated using Einstein equation, Eq. 3.2, which has been simplified to represent the time (t, s) needed for the average molecule to diffuse across a thin layer of solution (l, cm), given a known diffusion coefficient (D, cm$^3$/s) [31]. Data on the diffusion coefficients of aquated Ru(III) is not available but if a D of 1·10$^{-5}$ cm$^3$/s is assumed, it can be estimated that it should take the average molecule 8 min to diffuse to the electrode.

$$t = \frac{l^2}{2D} \quad \text{Eq. 3.2}$$

Experiments such as the example shown in Figure 3.9, where the reducing potential was held in excess of this diffusion time limit, indicate the complexation reaction is not complete. Therefore the observed low complexation is predominantly due to the slow kinetics of complexation of Ru(II). This is not surprising given the general tendency of Ru to have slow exchange kinetics [17]. In terms of sensor applications, if a long time scale is allowed it is possible that sensitivity could be increased by adjusting reducing time frames and allowing the slow kinetics to produce more luminescent complex. However, this work demonstrates this technique allows for µM sensitivity on the time scale of 47 min.

In fact, because that the experiments demonstrate reproducible Δ emission values even for the first several reduction and oxidation steps, it would be possible to reduce the time frame of analysis. As demonstrated in Figure 3.14, observed emission signal for the lowest studies Ru concentrations is well above the noise in the range of 20-30 min after applying electrochemical generation potentials. This system could be used to effectively quantify Ru concentrations in a solution in shorter time periods than the 47 min used for the above calculations. Depending on length of time chosen, the LOD may remain constant though the linear range may be reduced.
CONCLUSIONS

By applying appropriate potentials to solutions containing Ru and either bpy or phen, the luminescent [Ru(bpy)$_3$]$^{2+}$ or [Ru(phen)$_3$]$^{2+}$ species can be quickly and reproducibly generated. The electrochemical and spectroscopic characteristics of the electrochemically generated complexes align well with what was measured experimentally and what was reported in literature for the [Ru(bpy)$_3$]Cl$_2$ and [Ru(phen)$_3$]Cl$_2$ standards. This supports the claim that the Ru(ligand)$_3$ species were successfully electrochemically generated within solution and the sensor environment. Evidence of the generation of Ru(ligand)$_2$ species was also observed, but the 1:2 metal species did not interfere with the electrochemistry or the luminescence signals of the 1:3 species.

Furthermore, generation of the Ru(ligand)$_3$ complexes can be followed spectroscopically using luminescence measurements where the ingrowth of a red emission corresponds to the presence of the [Ru(ligand)$_3$]$^{2+}$ species in solution. Conditions for the electrochemical generation of the Ru(ligand)$_3$ were optimized. Species formed faster when applied potential was modulated between reducing and oxidizing potentials, which suggests the switching of potentials causes micro-stirring or perhaps heating within the spectroelectrochemistry cell. Finally, spectroelectrochemistry can be used to quantify the Ru in the system with a LOD of $2 \cdot 10^{-6}$ M for the bpy species and $2 \cdot 10^{-7}$ M for the phen species, though the linear range of quantification is limited by the amount of time allowed for the complexes to form. While the rate of complexation for these species is very slow, it is reproducible and allows for effective analysis of the species. This work suggests a solution environment sensor based on complexing the metal cation in a luminescent species is applicable to the analysis of Ru.
REFERENCES


3. Chatterjee, S; Del Negro, AS; Edwards, MK; Bryan, SA; Kaval, N; Pantelic, N; Morris, LK; Heineman, WR; Seliskar, CJ (2011) Luminescence-Based Spectroelectrochemical Sensor for [Tc(dmpe)(3)](2+/+) (dmpe=1,2-bis(dimethylphosphino)ethane) within a Charge-Selective Polymer Film Anal Chem, 83, 1766-1772.


9. Stegemiller, ML; Heineman, WR; Seliskar, CJ; Ridgway, TH; Bryan, SA; Hubler, T; Sell, RL (2003) Spectroelectrochemical sensing based on multimode selectivity simultaneously achievable in a single device. Design and evaluation of a small portable sensor for the determination of ferrocyanide in hanford waste samples Environ Sci Technol, 37, 123-130.


13. Mercer, EE; Buckley, RR (1965) Hexaaquoruthenium(2) Inorg Chem, 4, 1692-&.


18. Demas, JN; Crosby, GA (1968) On Multiplicity of Emitting State of Ruthenium(2) Complexes J Mol Spectrosc, 26, 72-&.


21. Watts, RJ; Crosby, GA (1971) Spectroscopic Characterization of Complexes of Ruthenium(Ii) and Iridium(Iii) with 4,4'-Diphenyl-2,2'-Bipyridine and 4,7-Diphenyl-1,10-Phenanthroline J Am Chem Soc, 93, 3184-&.

22. Sardarian, A; Coe, BJ; Douglas, KT (2003) Unusually facile syntheses of [Ru-II(bpy)(3)](2+) (bpy=2,2'-bipyridine) and [Ru-II(phen)(3)](2+) (phen=1,10-phenanthroline) Transit Metal Chem, 28, 905-907.


24. Crosby, GA; Perkins, WG; Klassen, DM (1965) Luminescence from Transition-Metal Complexes - Tris(2,2'-Bipyridine)- and Tris(1,10-Phenanthroline)Ruthenium(2) J Chem Phys, 43, 1498-&.


28. Atwood, DK; Devries, T (1962) Electrode Potential of Ruthenium (Iv) and Its Lower Oxidation States J Am Chem Soc, 84, 2659-.

29. Buckley, RR; Mercer, EE (1966) Potential of Ruthenium(2)-Ruthenium(3) Couple J Phys Chem-Ur, 70, 3103-.


35. Reagents for transition metal complex and organometallic synthesis; John Wiley & Sons.


37. Fletcher, JM; Hyde, KR; Woodhead, JL; Moore, FH; Hooper, EW; Gardner, WE (1963) Anhydrous Ruthenium Chlorides Nature, 199, 1089-.


45. Wu, JZ; Ye, BH; Wang, L; Ji, LN; Zhou, JY; Li, RH; Zhou, ZY (1997) Bis(2,2'-bipyridine)ruthenium(II) complexes with imidazo[4,5-f][1,10]phenanthroline or 2-phenylimidazo[4,5-f][1,10]phenanthroline *J Chem Soc Dalton*, 1395-1401.


47. Durham, B; Wilson, SR; Hodgson, DJ; Meyer, TJ (1980) Cis-Trans Photoisomerization in Ru(Bpy)2(Oh2)(Oh)2+ - Crystal-Structure of Trans-[Ru(Bpy)2(Oh2)(Oh)](Clo4)2 *J Am Chem Soc*, 102, 600-607.

CHAPTER FOUR

DEVELOPMENT AND TESTING OF A NOVEL MICROSCOPIC RAMAN PROBE AND APPLICATION OF CHEMOMETRIC ANALYSIS

ABSTRACT

The development and testing of a novel Raman probe capable of focusing excitation and light collection optics on the microscopic scale is discussed. This microscopic Raman probe response is tested on a variety simple and complex systems in long path length cells (4 to 1 cm) and also tested on a microfluidic device (100 µm path length). Under all conditions the probe performs well where the limit of detection of the species studied remains roughly constant despite the huge decrease in path length. Additionally, chemometric analysis was applied to the spectra and used to measure/predict the concentrations of analytes of interest. Chemometric models were built using ideal data sets (long path lengths, static solutions) and were successfully applied to less ideal data sets (shorter path lengths, flow conditions). This work demonstrates the microscopic Raman probe is capable of investigating solutions within a microfluidic device and that chemometric analysis of the resulting spectra can be used to identify and quantify species in solution.
INTRODUCTION

Methods for in-line real-time process monitoring are already successfully utilized in a variety of fields including the pharmaceutical and food industries [1-3]. Examples often utilize spectroscopy due to its non-destructive nature, versatility, and fast response. Spectroscopic methods are then often paired with sophisticated software analysis packages to quickly and accurately measure/predict data necessary for quality control.

There are several fields that could benefit from the application of in-line real-time process monitoring, but the appropriate analytical methods have not yet been developed. One important example is nuclear fuel reprocessing where there is no in-line monitoring technology currently available for determining the chemical composition within fuel reprocessing streams. Nuclear energy is a key component in the energy portfolios of many nations, including the United States where it accounts for approximately 20% of the power produced [4, 5], and France where it accounts for approximately 75% [6]. Additionally, countries such as China and India are planning to shift some of their energy needs to nuclear power as a method of meeting base load energy needs while limiting their greenhouse gas emissions [7-9]. Coupled with an increase of nuclear power, reprocessing of the used fuel is likely to increase as well, as it offers a pathway to recycle material and reduce hazardous lifetimes of wastes [5, 10-12].

Reprocessing currently is a controversial subject for many reasons, including the difficulties in safeguarding materials to ensure radioactive materials are not misused. Safeguards objectives cannot be met by material accountancy, containment, and surveillance alone [13]. Monitoring the processing of nuclear materials using grab samples is often too slow and expensive to be effective and is particularly difficult due to the high level of radiation present. In-line real-time process monitoring can help meet safeguards objectives while improving the safety of process
monitoring. Several reports have presented the effectiveness of utilizing in-line real-time process monitoring on systems similar to nuclear fuel reprocessing schemes [14-17]. Additionally, in-line monitoring based on spectroscopy is likely to be useful because many waste components are have well characterized spectroscopic responses [14, 15, 17-20].

Safety of sampling and the limitation of radiation damage to equipment can be improved by monitoring smaller streams of radioactive materials. This provides an opportunity for a unique application of microfluidic devices which could be plumbed in parallel to reprocessing streams to enable both a reduction in sampled volume and allow for the possibility of in-line monitoring simultaneously. An appropriately designed microfluidic device will be optically transparent and the flowing stream within the device could be monitored spectroscopically. Raman spectroscopy is one method that is particularly useful in these streams because of the well characterized responses to common reprocessing stream components such as NO$_3^-$ and UO$_2^{2+}$. In the pursuit of utilizing Raman spectroscopy on streams within microfluidic devices, we have designed and tested a Raman probe with a microscopic focus and on board camera to allow for effective excitation and spectral acquisition and imaging of the stream. Figure 1 presents the focal size of the Raman excitation laser as well as pictures of the probe itself.

This paper investigates the response of the microscopic Raman probe to complex solutions containing HNO$_3$ and UO$_2^{2+}$ in stationary samples and flowing systems. This work focuses on results obtained in cells with larger path lengths than what is expected in microfluidic devices to characterize response under ideal conditions. Additionally, this paper discusses the application of chemometric modeling to the identification and quantification of the species of interest. Overall this work provides the framework necessary for applying this unique instrumentation to the analysis of streams in microfluidic devices which have broad application potential.
**Figure 4.1:** A) Example of microfluidic chip B) schematic of microscopic Raman probe focused on chip C) picture of microscopic Raman probe focused on a flow cell D) Picture of focused excitation beam from microscopic Raman probe
EXPERIMENTAL

Materials:

All materials were used as received. Concentrated nitric acid, trace metal grade, was purchased from Fisher; NaNO$_3$ reagent grade($\geq 99\%$) was purchased from Aldrich; UO$_2$(NO$_3$)$_3$ was purchased from Spectrum Chemical Mfg. Corp, reagent grade, assay 98-102\%.

Equipment:

The instrument that was used to measure Raman from the micro-flow cell employs a fiber optically coupled Raman microscope probe, a 671 nm diode-pumped solids state (DPSS) laser, and a high-throughput volume phase holographic (VPH) grating Raman spectrograph. The Raman microscope probe employs a miniature fiber optic Raman probe with a backscattering optical design and a board level CCD video camera for live video imaging of the sample. A dichroic longpass filter that transmit the 671 nm Raman region and reflect the visible region for imaging is placed in between the CCD camera and the Raman probe. The dichroic filter overlaps the optical axis of the Raman and the video image so that both are focused on the same spot at the sample and thus have a common field of view. A 10x objective lens is used as the focusing lens for the microscope Raman probe, which focuses the laser beam to a very small spot, collects the Raman signal and also provides a magnified image of the sample. The Raman microscope was packaged in a small handheld probe head with fiber optic connections to the laser and the spectrograph. An Ethernet cable connects the CCD video camera to the computer for live display of the magnified sample image. A custom transmission VPH grating spectrograph with a TE-cooled CCD detector was used to record the Raman signal from the microscope Raman probe. It has a spectral range from 0 cm$^{-1}$ to 3500 cm$^{-1}$ and $ca.$ 6 cm$^{-1}$ spectral resolution.
Chemometric analysis:

The spectroscopic data were processed with the SIMPLS algorithm [21, 22] using the PLS_Toolbox (Eigenvector Research Inc.) in MATLAB (MathWorks R2015b). Data preprocessing included using a “salt and pepper” filter to removed cosmic rays (random noise that can add chemical rank to models) as well as smooth spectra to account for random noise resulting from digitalization of the data. Further model details regarding preprocessing, cross validation, and choices of calibration/prediction sets are included in the text and also referenced with more details in Table 4.3.

RESULTS AND DISCUSSION

General description

The microscopic Raman probe was designed to meet the goal of focusing an excitation beam within the dimensions of a microfluidic stream. As demonstrated in of Figure 1d, the probe has a focal point size of 69 µm, which can fit within the channel dimensions of most microfluidic devices. While ultimately this device could be used to detect any Raman active species within a solution, this work characterizes the system response to solutions containing HNO₃, UO₂(NO₃)₂, and NaNO₃, all of which are important components of nuclear material processing streams. This choice of system allows for the exploration of the probe response to complex systems containing multiple species, matrix effects, and large ionic strength ranges.

The probe response was tested on a series of cell types where the path length was sequentially decreased. This included cells with longer path lengths than what is typical of a
microfluidic device: 4 cm and 1 cm path lengths. It also included a typical microfluidic device with a solution path length of 250 µm. Testing the system response in longer path length cells provides an ideal starting point for characterizing probe response where Raman signal can be maximized. Additionally, building data sets of spectra collected on similar solutions in a series of cells with decreasing path lengths allows for a robust step-wise process of learning how to best apply chemometric modeling. Chemometric analysis software can be utilized to quantify species in solution, but this type of analysis requires building models based on calibration sets of spectra. Testing response and applying chemometric modeling in a series of cells where the path length is successively reduced allows for data analysis where true spectral variance associated with solution changes can be isolated from other variance due to cell type.

It should be noted that a reduction of Raman signal is observed with a reduction in path length. This is demonstrated by Figure 4.2. The top plot presents the Raman response to a solution of 3 M HNO₃ and 2 M UO₂(NO₃)₂ in a 4 cm path length (black) vial and a 1 cm path length (grey). Note, solutions containing UO₂(NO₃)₂ were not studied in the microfluidic device. The NO₃⁻ peak can be seen at 1048 cm⁻¹ and the UO₂²⁺ peak can be seen at 871 cm⁻¹ though the position of these peaks shift depending on ionic strength of solution. The intensity of the response clearly decreases with a decrease in path length. The inset of the top plot presents the Raman response of a 3 M HNO₃ solution in the 4 cm, 1 cm, and 250 µm path length cells. In this case, the signal from the 250 µm path length is equal to the response from the 4 cm path length cell. The data in the 4 and 1 cm path length cells was collected with an integration time of 3 sec, while the data in the 250 µm microfluidic device was collected with a 5 sec integration time. This demonstrates the decrease in path length can be accounted for by simply increasing the integration time for the spectra collections. The limits of detection (LOD) of the HNO₃ and
UO$_2$(NO$_3$)$_2$ species in the different cell types can be determined by utilizing the Raman intensity versus concentration profiles for single-component solutions and Equation 1.

\[
LOD = \frac{3s_b}{m} \quad \text{Eq. 4.1}
\]

Here $s_b$ is the noise of the 0 M solutions and $m$ is the slope of the Raman intensity vs concentration plot. Limits of detection for HNO$_3$ and UO$_2$(NO$_3$)$_2$ are listed in Table 1 and demonstrate the LOD for both the UO$_2$(NO$_3$)$_2$ and HNO$_3$ species remain roughly the same despite changes in cell path length. This is explained by the proportional decrease in noise between the different cell types/path lengths/integration times. The calculated LODs are on the range of 0.02 M for HNO$_3$ and 0.01 M for UO$_2$(NO$_3$)$_2$ which should be effective limits for most processing streams [23].

Solutions containing both HNO$_3$ and UO$_2$(NO$_3$)$_2$ in a large range of concentrations were used to explore system response against a range of matrix effects and ionic strengths. The solutions were comprised of 51 pure and multi-component solutions, and concentration ranges for the components are given in Table 4.1 and Table 4.2 lists the actual concentrations of samples. Examples of spectroscopic responses can be seen in Figures 4.2 and 4.3.

**Table 4.1:** Peaks of interest and limits of detection

<table>
<thead>
<tr>
<th></th>
<th>Raman $\lambda_{\text{max}}, \text{cm}^{-1}$</th>
<th>Limit of detection, M</th>
<th>Range of concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 cm path length</td>
<td>1 cm path length</td>
<td>250 $\mu$m path length</td>
</tr>
<tr>
<td>HNO$_3$</td>
<td>1048</td>
<td>$2.2 \cdot 10^{-2}$</td>
<td>$2.0 \cdot 10^{-2}$</td>
</tr>
<tr>
<td>UO$_2$(NO$_3$)$_2$</td>
<td>871</td>
<td>$9.8 \cdot 10^{-3}$</td>
<td>$1.2 \cdot 10^{-4}$</td>
</tr>
</tbody>
</table>
Figure 4.2: Top: comparison of Raman spectra of a sample of 3 M HNO\(_3\) and 2 M UO\(_2\)(NO\(_3\))\(_2\) collected in a 4 cm path length cell (black) and in a 1 cm path length cell (grey) where the inset presents the NO\(_3\) peak of a 3 M HNO\(_3\) solution in the three different cells; Bottom: Raman intensity versus concentration for UO\(_2\)(NO\(_3\))\(_2\) in the 4 cm path length cell (blue circles) the 1 cm path length cell (dark blue triangles), and the response for the HNO\(_3\) species in the 4 cm path length cell (red circles) and the 1 cm path length cell (dark red triangles) and the 250 µm path length (pink squares). Error bars are marked at 3σ.
## Table 4.2: Solution Sets 1 and 2

<table>
<thead>
<tr>
<th>Sample name</th>
<th>Solution Set 1</th>
<th>Solution Set 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HNO₃ M</td>
<td>UO₂(NO₃)₂ M</td>
</tr>
<tr>
<td>S1_1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S1_2</td>
<td>0</td>
<td>0.25</td>
</tr>
<tr>
<td>S1_3</td>
<td>0</td>
<td>0.5</td>
</tr>
<tr>
<td>S1_4</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>S1_5</td>
<td>0</td>
<td>1.25</td>
</tr>
<tr>
<td>S1_6</td>
<td>0</td>
<td>1.5</td>
</tr>
<tr>
<td>S1_7</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>S1_8</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>S1_9</td>
<td>0.5</td>
<td>0.25</td>
</tr>
<tr>
<td>S1_10</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>S1_11</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>S1_12</td>
<td>0.5</td>
<td>1.25</td>
</tr>
<tr>
<td>S1_13</td>
<td>0.5</td>
<td>1.5</td>
</tr>
<tr>
<td>S1_14</td>
<td>0.5</td>
<td>2</td>
</tr>
<tr>
<td>S1_15</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>S1_16</td>
<td>1</td>
<td>0.25</td>
</tr>
<tr>
<td>S1_17</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>S1_18</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>S1_19</td>
<td>1</td>
<td>1.25</td>
</tr>
<tr>
<td>S1_20</td>
<td>1</td>
<td>1.5</td>
</tr>
<tr>
<td>S1_21</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>S1_22</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>S1_23</td>
<td>2</td>
<td>0.25</td>
</tr>
<tr>
<td>S1_24</td>
<td>2</td>
<td>0.5</td>
</tr>
<tr>
<td>S1_25</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>S1_26</td>
<td>2</td>
<td>1.25</td>
</tr>
<tr>
<td>S1_27</td>
<td>2</td>
<td>1.5</td>
</tr>
<tr>
<td>S1_28</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>S1_29</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>S1_30</td>
<td>3</td>
<td>0.25</td>
</tr>
<tr>
<td>S1_31</td>
<td>3</td>
<td>0.5</td>
</tr>
<tr>
<td>S1_32</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>S1_33</td>
<td>3</td>
<td>1.25</td>
</tr>
<tr>
<td>S1_34</td>
<td>3</td>
<td>1.5</td>
</tr>
<tr>
<td>S1_35</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>S1_36</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Sample name</td>
<td>HNO₃ M</td>
<td>UO₂(NO₃)₂ M</td>
</tr>
<tr>
<td>-------------</td>
<td>--------</td>
<td>-------------</td>
</tr>
<tr>
<td>S1_38</td>
<td>4</td>
<td>0.5</td>
</tr>
<tr>
<td>S1_39</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>S1_40</td>
<td>4</td>
<td>1.25</td>
</tr>
<tr>
<td>S1_41</td>
<td>4</td>
<td>1.5</td>
</tr>
<tr>
<td>S1_42</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>S1_43</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>S1_44</td>
<td>6</td>
<td>0.25</td>
</tr>
<tr>
<td>S1_45</td>
<td>6</td>
<td>0.5</td>
</tr>
<tr>
<td>S1_46</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>S1_47</td>
<td>6</td>
<td>1.25</td>
</tr>
<tr>
<td>S1_48</td>
<td>6</td>
<td>1.5</td>
</tr>
<tr>
<td>S1_49</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>S1_50</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>S1_51</td>
<td>8</td>
<td>0.25</td>
</tr>
</tbody>
</table>

Confounding variables and chemometric modeling

Solutions containing both HNO₃ and UO₂(NO₃)₂ will demonstrate a confounding of variables where the NO₃⁻ peak will show a response to the presence of both species. This effect is demonstrated in Figure 4.3 where the top plot shows spectra from a series of solutions all at constant 2 M HNO₃ and varying UO₂(NO₃)₂ while the bottom plot shows a series of spectra at varying HNO₃ and constant 2 M UO₂(NO₃)₂. The intensity of the nitrate band is modulated by the addition of either HNO₃ or UO₂(NO₃)₂. Again, one of the goals of this work is to utilize chemometric modeling to quantify species in solution. Successful chemometric modeling will rely on building a mathematical model capable of correlating spectral variation to concentration variation. If a model puts too much weight on the confounded nitrate band, the model may have difficulty predicting the concentration of the species contributing to that band.
Figure 4.3: Top: Raman spectra as HNO₃ is held constant at 2 M and UO₂(NO₃)₂ is varied from 0 to 2 M. Bottom: Raman spectra as UO₂(NO₃)₂ is held constant at 2 M and HNO₃ is varied from 0 to 6 M.
To better isolate and understand the effects of having multiple sources of NO$_3^-$ on the Raman response and chemometric modeling, a second series of solutions containing only HNO$_3$ and NaNO$_3$ was studied and was designed to mimic the HNO$_3$ and NO$_3^-$ concentrations of solution set 1. This sample set allowed for the removal the complicating system effects of the UO$_2^{2+}$ species. Component concentrations in all 51 samples of Solution Set 2 are listed in Table 4.2 and can be easily compared to Solution Set 1 where HNO$_3$ and total NO$_3^-$ concentrations are roughly equal for the similarly numbered samples. These samples were measured under static conditions using the microscopic probe and the 4 cm path length vials. The response of the NO$_3^-$ peak again demonstrated the same confounding of variables as was observed with the HNO$_3$/UO$_2$(NO$_3$)$_2$ system.

For this work, partial least squares (PLS) regression was used to build a model correlating spectral variation to concentrations of analytes. The mathematics and theory behind PLS regression is described elsewhere [24-26] but the primary strengths of PLS are its ability to determine covariance between two datasets (in this case spectral and concentration data) in a situation where there are more variables (wavenumbers) than observations (samples measured). PLS models are typically built to predict the concentration of only one species; therefore in this work where there are multiple species of interest, a separate model will be needed for each species, i.e. HNO$_3$ and UO$_2$(NO$_3$)$_2$. The process of model building requires several steps. First a calibration set of data must be generated. The calibration set in this case will be a series of spectra collected on samples with known concentrations, i.e. Solution Sets 1 and 2. To allow for an appropriate working concentration range of the model, the model must be built using a large data set that covers the entire range of anticipated concentrations of the analytes of interest. Second, after collecting the spectra, data must be pre-processed. Essentially pre-processing
involves applying mathematical operations to the data to reduce noise, emphasize real areas of variance (as opposed to random noise), and center the data variance into a mathematical space where vectors can be used to describe spectral variation. The third step involves defining the mathematical model that then correlates spectra variation to known concentrations. The fourth and final step involves using the model to measure/predict on unknown samples, called the prediction set. For the purposes of this work, there were no true unknowns. Concentrations of samples used to collect data treated as the prediction set were known, and known values could be compared to model predictions to judge model performance.

A PLS model was built using a calibration set made up of the spectra from the 51 solutions in the 4 cm path length vials from Solution Set 2. The Raman data was preprocessed using a multiplicative scatter correction (MSC) with the mean as reference, a 1st derivative (2nd order polynomial), and mean centering. The multiplicative scatter correction was used to normalize data to account for the differences in the data collection conditions between the calibration set and the prediction set that will be described below. The 1st derivative acted as a form of baseline correction that emphasized the spectral variations of interest. The model robustness was tested by running cross validation, i.e. where models were iteratively built using subsets of the calibration set and used to predict on the removed subsets. In this case the “venetian blinds” method of cross validation was used with an appropriate split number to remove all spectra of a single sample. Figure 4.5 presents the performance of models for measurement/prediction of HNO₃ (top) and total NO₃⁻ (bottom) concentrations using Solution Set 2 as the calibration set. Figures of merit for model performance include the root mean square error of calibration (RMSEC) and cross validation (RMSECV) which provide an error in prediction. As indicated in Figure 4.5 and Table 4.3 RMSEC and RMSECV were approximately 0.13-0.14 M for either model. Because RMSEC
and RMSECV values are so similar, this indicates the model is fitting true variation instead of noise and the model can be considered robust. Furthermore, measurement errors on the order of 0.14 M in this system are acceptable as they are almost 2 order of magnitude lower than the entire studied concentration range. Table 4.3 provides more details on model parameters.

To truly test model performance, the model needs to successfully measure/predict the concentration of species within a new set of data (i.e. data not used in calibration set but containing same species as calibration set), which is referred to as the prediction set. In this work we measured the prediction set samples under the non-ideal conditions of a 1cm path length flow cell. Which means the prediction set was measured under conditions different from those of the calibration set. Since the cell type and cell path length changed between the calibration and prediction sets, it was important to normalize data to account for changes in spectral intensity (as demonstrated in Figure 4.2). The prediction set was generated by spectroscopically monitoring the 1 cm flow cell while a series of solutions of varying HNO₃ and NaNO₃ concentrations were pumped through the flow cell. Results can be seen in Figure 4.4, which focuses on the nitrate band response to changing HNO₃ and NaNO₃ concentrations and again demonstrates the confounding of the nitrate band variable. The results also indicate sharp increases in the band as solutions of different concentrations are introduced to the flow cell. This is likely due to the unavoidable presence of a bubble in the system when the new solution is hooked up to the solution pump. The data indicates the residence time of the bubble is short and does not interfere with signal longer than a few seconds.
Figure 4.4: Top: Raman spectra over the course of a flow experiment (8 µL flow cell with a 1 cm path length) where concentrations of HNO$_3$ and NaNO$_3$ were varied. Bottom: double axis plot, left axis corresponds to the concentration profile for HNO$_3$ (black) and NaNO$_3$ (grey dotted), right axis corresponds to the intensity of the NO$_3^-$ band at 1048 cm$^{-1}$ (red).
The model built using Solution Set 2 as the calibration set was then used to measure/predict the concentrations of HNO₃ and total NO₃⁻ within the solutions measured during the flow tests. Model predictions of HNO₃ and total NO₃⁻ in the flow set were then compared to the known values of HNO₃ and total NO₃⁻ concentration and are shown in Figure 4.5. For the HNO₃ model presented in the top plot Figure 4.5, error values for the prediction set, RMSEP: 0.17, compared well to error values for the calibration set, 0.14 M. Once again this indicated the model is robust and capable of accurately predicting HNO₃ concentration in a series of “unknown” samples. While the calibration set contains a small number of outliers that fall outside the 95% confidence limits of the model (red dashed lines), all predictions for the flow series (prediction set) fall within the limits. The calibration set contains a large number of measurements and most of the observed outliers fall at higher HNO₃ concentrations where matrix effects and the solubility limit of NO₃⁻ begin to interfere with the model. The insets of both Figure 5 plots present the predicted concentration of the target analyte over the course of the flow experiment. Measured/predicted concentrations are not only accurate over the constant concentration ranges but also closely match what is expected when the system adjusts to a new concentration of analyte. It is especially important to note the time period between 20 and 40 minutes within the insets. During this time period, HNO₃ was held constant while total NO₃⁻ was varied considerably; despite this, the HNO₃ model continues to perform very well. Overall, this is a convincing demonstration that chemometric modeling can be successfully applied to the Raman data despite a variety of difficulties. The models performed very well despite complex system response to matrix effects, confounding of variables, and even major differences in spectral collection parameters between the calibration and prediction sets.
Figure 4.5: PLS modeling results for HNO₃ (top) and total NO₃⁻ (bottom) where the black circles represent the calibration set (solution set 2 in 4 cm path length vials), the black lines indicate the best fit lines for the models, the red dashed lines indicate the 95% confidence limits of the models, and the red triangles indicate the prediction set (flow experiment in 1 cm path length cell, spectra presented in Figure 4.4). The insets show the model predictions (red triangles) as a function of time over the course of the flow test experiments (compare to Figure 4.4).
Incidentally, very similar results can be obtained using slightly different pre-processing steps. Models still perform well when spectra are normalized to the area of the water band instead of normalized using MSC. In the case of the data presented in Figure 4.5, a previous generation probe (with roughly the same focal point size) was used to collect the data from the flow experiment. In this case utilizing MSC more accurately accounted for all the differences between the training and prediction set including path length of the cells and optics of the different probes. This data suggests that a single model could be successfully used for spectra collected from cells of different path lengths and with slightly different optical equipment.

**Chemometric modeling of systems containing HNO$_3$ and UO$_2$(NO$_3$)$_2$**

The more complicated system containing both HNO$_3$ and UO$_2$(NO$_3$)$_2$ was then explored. A PLS model was built using the static measurements of Solution Set 1, again collected in the 4 cm path length vials, as the calibration set. For this data set, separate models were built to predict concentrations of HNO$_3$, UO$_2$(NO$_3$)$_2$, and total NO$_3^-$.

Again the goal was to use this model to predict the concentrations of analytes within a flow cell with a smaller path length. In this case, the same microscopic Raman probe/optics were used to collect both the calibration set spectra and the prediction set spectra. Using the consistent optics, the best modeling results were obtained by preprocessing the spectral data with a $1^{st}$ derivative ($2^{nd}$ order polynomial), normalization to the area of the water band, and mean centering. Spectra from the flow experiment are shown in the top plot of Figure 4.6. Results from the PLS modeling can be seen in the bottom plot of Figure 4.6, where the predicted values (open circles) of the species in solution successfully follow the known concentrations (lines). Much like the insets of Figure 4.5, the bottom plot of Figure 4.6 indicates excellent model performance. Predictions accurately follow changes in concentration but also accurately follow periods when one species is held
Figure 4.6: Top: Raman spectra from flow cell experiment where solutions of different HNO₃ and UO₂(NO₃)₂ concentrations were injected into a flow cell of 1 cm path length; Bottom: Plot of known concentrations of HNO₃, UO₂(NO₃)₂, and total NO₃⁻ along with predicted values from PLS model.
constant while others are varied. For example, between 7 and 22 minutes, HNO$_3$ is held constant while UO$_2$(NO$_3$)$_2$ is varied (which affects the NO$_3^-$ band) but the model still accurately predicts the concentration of HNO$_3$.

Figure 4.7 presents the models in the measured/predicted versus known concentration format. In all three cases the 95% confidence limits fit tightly to the model data, indicating high modeling accuracy. The red triangles represent the results from the flow test, all of which fall within the 95% confidence limits of the model. The RMSEC and RMSECV values also indicate low errors in predictions, on the order of 0.15 M for HNO$_3$, 0.05 M for UO$_2$(NO$_3$)$_2$, and 0.06 for total NO$_3^-$. Values for RMSEP are lower than RMSEC and RMSECV values for the HNO$_3$ and UO$_2$(NO$_3$)$_2$ models. This is mostly likely due to the reduced concentration range of the prediction set. The calibration set includes high concentrations of HNO$_3$ where ion pairing and the approaching saturation limit increase modeling error. In the case of total NO$_3^-$, the RMSEP is significantly higher than the RMSEC and RMSECV and indicates the model did not fully isolate the variances that indicate total NO$_3^-$ concentration. Overall, model predictions/measurements were accurate and figures of merit for the models indicated models were robust and applicable to “unknown” data sets.

PLS analysis was successfully applied to the measurement/prediction of the HNO$_3$, UO$_2$(NO$_3$)$_2$, and total NO$_3^-$ species. More importantly PLS modeling was successful despite two impressive difficulties: 1) the complex solutions exhibited matrix effects and limited linear response ranges and 2) the calibration set was collected in 4 cm path length cells (static) while the prediction set was collected in 1 cm path length cells (flowing).
Figure 4.7: Model performance data comparing the predicted versus the known concentrations for HNO₃ (top), UO₂(NO₃)₂ (middle) and total NO₃⁻ (bottom) solution components. Where the black circles represent the calibration set (solution set 1 in 4 cm path length vials), and the red triangles indicate the prediction set (flow experiment in 1 cm path length cell, spectra presented in Figure 6).
Table 4.3: Model details

<table>
<thead>
<tr>
<th>Model description</th>
<th>Figures depicting results</th>
<th>Calibration set</th>
<th>Preprocessing of spectra</th>
<th>Prediction set</th>
<th>Latent variables</th>
<th>RMSEC</th>
<th>RMSECV</th>
<th>RMSEP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modeling concentration of HNO₃ with NaNO₃ present</td>
<td>4.5 top</td>
<td>Solution Set 2, 4 cm path length static vials</td>
<td>1) MSC (mean center) 2) 1ˢᵗ derivative (2ⁿᵈ ord. polynomial) 3) Mean center</td>
<td>Figure 4.4 flow test, 1 cm path length cell</td>
<td>3</td>
<td>0.14</td>
<td>0.14</td>
<td>0.17</td>
</tr>
<tr>
<td>Modeling concentration of total NO₃ with HNO₃ and NaNO₃ present</td>
<td>4.5 bottom</td>
<td>Solution Set 2, 4 cm path length static vials</td>
<td>1) MSC (mean center) 2) 1ˢᵗ derivative (2ⁿᵈ ord. polynomial) 3) Mean center</td>
<td>Figure 4.4 flow test, 1 cm path length cell</td>
<td>4</td>
<td>0.13</td>
<td>0.13</td>
<td>0.18</td>
</tr>
<tr>
<td>Modeling concentration of HNO₃ with UO₂(NO₃)₂ present</td>
<td>4.6 bottom And 4.7 top</td>
<td>Solution Set 1, 4 cm path length static vials</td>
<td>1) 1ˢᵗ derivative (2ⁿᵈ ord. polynomial) 2) Normalize to area of the water band 3) Mean center</td>
<td>4.6 flow test, 1 cm path length cell</td>
<td>3</td>
<td>0.15</td>
<td>0.15</td>
<td>0.12</td>
</tr>
<tr>
<td>Modeling concentration of UO₂(NO₃)₂ with HNO₃ present</td>
<td>4.6 bottom And 4.7 middle</td>
<td>Solution Set 1, 4 cm path length static vials</td>
<td>1) 1ˢᵗ derivative (2ⁿᵈ ord. polynomial) 2) Normalize to area of the water band 3) Mean center</td>
<td>4.6 flow test, 1 cm path length cell</td>
<td>3</td>
<td>0.05</td>
<td>0.05</td>
<td>0.04</td>
</tr>
<tr>
<td>Modeling concentration of total NO₃ with HNO₃ and UO₂(NO₃)₂ present</td>
<td>4.6 bottom And 4.7 bottom</td>
<td>Solution Set 1, 4 cm path length static vials</td>
<td>1) 1ˢᵗ derivative (2ⁿᵈ ord. polynomial) 2) Normalize to area of the water band 3) Mean center</td>
<td>4.6 flow test, 1 cm path length cell</td>
<td>4</td>
<td>0.06</td>
<td>0.06</td>
<td>0.22</td>
</tr>
<tr>
<td>Modeling concentration of HNO₃ with NaNO₃ present</td>
<td>4.10 top</td>
<td>Solution Set 2, 4 cm path length static vials</td>
<td>1) Normalize to area of the water band 2) 1ˢᵗ derivative (2ⁿᵈ ord. polynomial) 3) Mean center</td>
<td>4.9 flow test, microfluidic device, 250 µm path length</td>
<td>3</td>
<td>0.19</td>
<td>0.19</td>
<td>0.30</td>
</tr>
<tr>
<td>Modeling concentration of total NO₃ with HNO₃ and NaNO₃ present</td>
<td>4.10 bottom</td>
<td>Solution Set 2, 4 cm path length static vials</td>
<td>1) Normalize to area of the water band 2) 1ˢᵗ derivative (2ⁿᵈ ord. polynomial) 3) Mean center</td>
<td>4.9 flow test, microfluidic device, 250 µm path length</td>
<td>3</td>
<td>0.12</td>
<td>0.12</td>
<td>0.20</td>
</tr>
</tbody>
</table>
The successful measurement/prediction of species concentration in the flow cell (1 cm path length) based on a model built from data collected under different conditions (static 4 cm path length vial) has significant implications for future work. It is ideal to collect a calibration set that has the best possible data under easily reproducible conditions. In this case, it is easier and cheaper to collect the calibration set in the static vials with the longest possible path length. This suggests that models built from this ideal and cost effective data could be applicable to data collected on solutions in microfluidic flow cells.

**Chemometric modeling on data collected within a microfluidic flow cell**

Probe response to solutions within a microfluidic device was also tested. Some results can be seen in Figure 4.2 where the Raman NO$_3^-$ band is shown in the inset of the top plot and the LOD plot for HNO$_3$ within the microfluidic device is presented in the bottom plot. Note, in those measurements the Raman integration (collection) time was increased from 2 seconds (with the 4 and 1 cm path lengths) to 5 seconds for the 250 µm path length microfluidic flow cell. By increasing collection time Raman response was at the same intensity as response observed within the 4 cm path length cell and a similar LOD was observed. The excellent Raman signal is due to the focal capabilities of the Raman probe. The excitation focal point is on the order of 69 µm which fits within the dimensions of the microfluidic channel. An image (taken using the onboard camera of the microscopic Raman probe) of the excitation beam focused within the channel of the microfluidic device can be seen in Figure 4.8.
Figure 4.8: The Raman exaction point (red dot) focused within the microfluidic channel. The edges of the channel can be seen along the top and bottom of the photo. Not the channel depth (path length) is 250 µm, while the channel width is 300 µm.
The microscopic Raman probe and microfluidic device system was initially tested with a series of simple HNO$_3$ solutions to determine general response and the LOD for the species (see Figure 4.2). It was then tested using a more complicated series of solutions during a flow test. Several solutions of varying concentrations of HNO$_3$ and NaNO$_3$ were pumped through the microfluidic device while the system was continuously monitored by the microscopic Raman probe. Results of the flow test can be seen in Figure 4.9 which has a set up very similar to Figure 4.4. The tested system contained two sources of NO$_3^-$ so the NO$_3^-$ band was confounded as demonstrated in Figure 4.9. It is important to note the peak response to the presence of bubbles in the system. Intensity drops considerably at these points, which is most obvious at times 10, 12 and 14 minutes. Bubble residence time is proportional to the size of the bubble, so these interferences can be limited by optimizing sampling to reduce bubble size. Furthermore, all bubbles were the result of pumping air into the system. Initial concerns regarding the microfluidic system included the possibility of boiling solution at the focal point of the laser. This was not observed even at very slow flow rates through the device when solution had more time to absorb heat energy from the focused excitation laser.

Again the goal is to use chemometric modeling to measure/predict the concentration of species present within the microfluidic stream. More importantly, the goal is to determine if a model built using a calibration set of ideal data (i.e. data collected in the 4 cm path length vials) can be used to measure/predict species concentration within the microfluidic device. To accomplish this, several chemometric approaches can be utilized. The approach described above for measuring/predicting concentrations in the 1 cm path length cells could be utilized: normalizing spectra to the water band to account for changes in overall intensity. Alternatively, a calibration transfer method could be implemented. This approach can be simply explained as
applying two different models to a prediction set. The first model essentially treats the data to make it look like it came from the same instrument/measurement conditions as the calibration set while the second model preforms the measurements/predictions of analyte concentrations. For the purposes of the work reported here only the first method was used. Future work will explore more sophisticated modeling approaches such as the applications of calibration transfers.

Results from modeling can be seen in Figure 4.10 and are presented in a fashion similar to data presented in figure 4.5. Solution set 2 data (collected in the static 4 cm path length vials) was used as the calibrations set. The data was preprocessed by normalizing the spectra to the area under the water band, applying a 1\textsuperscript{st} derivative (2\textsuperscript{nd} order polynomial), and mean centering the data. The model was cross validated using the venetian blinds method. The prediction set was the flow test through the microfluidic device presented in Figure 4.9. Further model details are presented in Table 4.3 It is important to note that the spectra collected on the microfluidic device were collected on a different spectrometer than the calibration set. Wavenumber axes of the two data sets had to be aligned, but this does not fully account for differences between the two detector registries. In this case using a calibration transfer could have a huge positive impact on the accuracy of the measurements/predictions. Given the difficulty of applying the simplistic model (no calibration transfer) to the microfluidic device, models preformed surprisingly well. Errors in measurements/predictions were on the order of 0.2-0.3 M for HNO\textsubscript{3} and total NO\textsubscript{3}\textsuperscript{-} and generally fell within the 95% confidence limits of the model. These results are encouraging and suggest calibration sets made under ideal conditions (cost effective and efficient) can be successfully used to measure/predict on spectra collected on the microfluidic device.
Figure 4.9: Top: Raman spectra over the course of a flow experiment within the microfluidic device where concentrations of HNO₃ and NaNO₃ were varied. Bottom: double y axis plot, left axis corresponds to the concentration profile for HNO₃ (black) and NaNO₃ (grey dotted), right axis corresponds to the intensity of the NO₃⁻ band at 1045 cm⁻¹ (red).
Figure 4.10: PLS modeling results for HNO₃ (top) and total NO₃⁻ (bottom) where the black circles represent the calibration set (solution set 2 in 4 cm path length vials) and the red triangles indicate the prediction set (flow experiment in microfluidic device, spectra presented in Figure 4.9). The insets show the model predictions (red triangles) as a function of time over the course of the flow test experiments (compare to Figure 4.9).
CONCLUSION:

A microscopic Raman probe was successfully utilized to measure a complex series of solutions in different environments. Limits of detection were on the order of 0.02 M for HNO$_3$ and 0.01 M for UO$_2$(NO$_3$)$_2$, and stayed roughly constant despite changes in cell path length from 4 cm to 1 cm which led to a considerable decrease in signal intensity. More importantly, initial testing completed on an actual microfluidic device, path length 250 µm, indicated Raman spectra could be successfully collected with the microscopic Raman probe with a limit of detection on the order of 0.02 M for HNO$_3$.

Additionally, chemometric modeling was successfully applied to the Raman data where models were built to measure/predict concentrations of analytes in solution. Models performed well where system complications such as confounding of variables and non-linear matrix/ionic strength effects did not hamper model accuracy. Models could also be used to accurately measure/predict species concentrations despite differences in cell path length between the calibration and prediction sets. With proper normalization, models built from data collected under ideal conditions (static solutions in 4 cm path length vial) could be accurately applied to data collected under less ideal conditions (flow cell with 1 cm path length and even data collected on the microfluidic device). Results suggest the application of the microscopic probe to the analysis of microfluidic streams is successful as is the application of chemometric modeling to measuring/predicting analyte concentration within the microfluidic device.
REFERENCES


2. De Beer, TRM; Wiggenhorn, M; Veillon, R; Debaq, C; Mayeresse, Y; Moreau, B; Burggraeve, A; Quinten, T; Friess, W; Winter, G; Vervaet, C; Remon, JP; Baeyens, WRG (2009) Importance of Using Complementary Process Analyzers for the Process Monitoring, Analysis, and Understanding of Freeze Drying Anal Chem, 81, 7639-7649.


10. Machiels, A; Sowder, A; Electric Power Research Institute, 2010.


22. de Jong, S; Wise, BM; Ricker, NL (2001) Canonical partial least squares and continuum power regression J Chemometr, 15, 85-100.


CHAPTER FIVE

CONCLUSIONS

Nuclear power provides a significant portion of the several nations’ energy portfolios worldwide and usage of nuclear power is likely to grow in the coming years [1, 2]. Reactor technology has made huge advancements since Enrico Fermi and his team made the first Chicago Pile-1 reactor, and analytical technology for monitoring reactor products needs to keep up. This is particularly important in the analysis of species within schemes for reprocessing used nuclear fuel. Available methods are often slow and expensive, and are complicated by the need to collect grab samples, which is difficult in the high radiation environments of these schemes. Overall, new methods are needed to provide fast and cost effective analysis of these systems.

SPECTROELECTROCHEMISTRY

A major difficulty in analyzing samples from used nuclear fuel is the complexity of the system. Within the used fuel matrix there are numerous metal ions present along with the various acids/other species used to dissolve the fuel. Many of the species present have well characterized traits that can be used in identification/quantification (emission, gamma ray, mass spectrometry, etc.) but the large number of interferents present makes simple detection impossible. Typically, samples must be prepared by separating target analytes from the systems containing the interferents. Performing these separations is time consuming and often expensive. Developing an analytical technique that could reduce the number of separations needed for effective analysis would drastically cut down on the time needed to provide vital information about solution
components. This suggests an analytical technique that can isolate desired analyte signature from an otherwise noisy environment is needed.

Spectroelectrochemistry is one such technique. By combining spectroscopy and electrochemistry, spectroelectrochemistry utilizes two physio-chemical characteristics to isolate target analyte signature from an environment with direct spectroscopic interferents. Several studies have demonstrated the ability of spectroelectrochemistry to identify and quantify species in multi-component solutions without preforming prior separations [3-6]. Some spectroelectrochemistry work has been completed on species of interest to the nuclear fuel cycle and suggest there is much area still be explored in terms of spectroelectrochemistry applications [6-9].

In particular some spectroelectrochemical work has focused on the lanthanides. However, in these cases the limitations of spectroelectrochemistry become very apparent. This technique relies on spectroscopic analysis to identify and quantify species. Therefore species with weak signatures (like the lanthanides) will have undesirably high limits of detection and species with practically no useful spectroscopic signatures (like many free transition metals such as Fe and Ru) will not be detected in spectroelectrochemistry based systems.

In this manuscript, methods for addressing the weak spectroscopic signatures of target analytes have been presented. Target analytes can be complexed to ligands that drastically improve their spectroscopic characteristics to improve detection. Chapter Two discusses the application of spectroelectrochemistry to a series of highly luminescent Eu complexes. Complexing the Eu not only improved luminescence yield, but also altered the electrochemical characteristics of Eu and improved the Nernstian behavior of the Eu (II/III) redox couple.
Overall, this work demonstrated spectroelectrochemistry applied to the analysis of lanthanides and can provide low limits of detection when the technique is paired with informed chemistry.

The methods utilized in Chapter Two did not necessarily provide an avenue for a fast and automatic characterization of Eu in solution. That work relied on the analysis of Eu complexes carefully synthesized \textit{ex situ} to the spectroelectrochemical cell. Ideally, a sensor for the characterization of these complexes would rely on \textit{in situ} formation of the luminescent complexes. This would reduce the time needed to analyze samples and provide a design for a field detector that could be easily utilized. Chapter Three addresses this by exploring a method of the \textit{in situ} formation and subsequent spectroelectrochemical analysis of luminescent Ru complexes. Ru is an excellent example of a metal that exhibits complex speciation within solution where few to none of the available species have strong or uniquely identifiable spectra in either absorption or emission modes. Fortunately (and much like many other metal species), Ru has been studied in numerous highly luminescent complexes [10-14]. Chapter Three successfully demonstrated a solution based sensor targeted at electrochemically generating and then analyzing two of those well know complexes from free Ru in solution. This provides the necessary proof of concept for the development of other sensors for the \textit{in situ} complexation and subsequent analysis of luminescent species.

Spectroelectrochemistry has the potential to provide a method of analysis for the nuclear fuel cycle where the needed number of prior separations can be reduced and thereby allow for faster analysis. Furthermore, the primary limitation of spectroelectrochemistry, its inability to provide information on species with poor spectral characteristics, has been addressed to a limited extent by this dissertation. The work presented in Chapters Two and Three lay the ground work for
sensors designed to capture target metal ions in complexes with enhanced spectral characteristics. Continued exploration is still needed where future work will include many areas such as designing sensors for species with irreversible redox couples, optimizing ligand choice for improved thermodynamics/kinetics/quantum yields of emission, and altering sensor environment from solution based to ion-exchange polymer film based.

**IN-LINE REAL-TIME PROCESS MONITORING**

While many industries take advantage of in-line real-time process monitoring for quality control and maintenance purposes [15, 16], there is currently no technology in use for the in-line real-time analysis of nuclear fuel reprocessing schemes. This form of process monitoring would be invaluable within the reprocessing schemes where it would immediately provide needed information for safeguards controls and limit or eliminate the costly and hazardous need for collecting grab samples for analysis.

Chapter Four discusses the development of technology and analytical methods that could fill the need for in-line real-time process monitoring within reprocessing schemes. Specifically it discusses the application of a novel Raman probe capable of focusing excitation and collection optics on the microscopic scale to the analysis of streams within microfluidic devices. This probe could provide the in-line measurements needed by probing streams within microfluidic devices plumbed in parallel to reprocessing streams. This chapter also discusses methods for providing the real-time analysis of the resulting spectral data through the application of chemometric analysis.

Chemometric analysis involves building a mathematical model that correlates spectral variation to concentration measurements. Generally speaking this form of analysis is more
powerful than using typical calibration curves or Beer’s Law type analysis because it is not necessarily limited to the linear response range of a particular peak and can also isolate complex spectral variations due to matrix and ionic strength effects.

The work presented in Chapter Four provides a convincing argument that the microscopic Raman probe could be used to successfully characterize species within a microfluidic device and chemometric modeling could be used to provide concentration data on the observed species. More importantly, it was demonstrated that chemometric models built from data collected under ideal conditions (where collection of data is cheap and efficient) could be used to quantify species under less ideal conditions (what is expected under typical short path length and flowing solution conditions).

Results were encouraging and suggest in-line real-time measurement of reprocessing streams is achievable. Much work is still needed in this area, aside from characterizing responses of more species within the microfluidic device and building the respective chemometric models, much work is still needed in integrating other types of spectroscopy into the in-line system. By utilizing several types of spectroscopic analysis, absorbance and emission measurements in addition to Raman, it will be possible to identify species that are not Raman active.

CONCLUSIONS AND COMMON THEMES

The connecting theme throughout this document is the application of spectroscopy to the analysis of species within solution. Spectroscopy is a powerful analytical method that can often quickly supply information using instruments that are cost effective. More importantly spectroscopy is incredibly versatile. Where spectroscopy alone cannot be easily utilized to isolate
target analyte signature in a complex matrix, it can be paired with other techniques such as electrochemistry to provide the needed signal isolation. Additionally, spectroscopy is useless in the analysis of species that are not spectroscopically active, but these limitations can be circumvented through the application of chemistry. Target analytes with weak or non-existent spectroscopic signals can be captured in complexes that drastically improve spectroscopic response. Furthermore, typical calibration curves based off of spectroscopic data have limited applicability. Quantification is limited to a linear response range and the presence of interferents or matrix effects will impede accurate concentration measurements. However, spectroscopic data can be treated with sophisticated algorithms such as those used in chemometric analysis to provide methods for quantifying target analytes even under very complex solution conditions.

There is a need for fast, cost effective detectors not only throughout the nuclear fuel cycle, but also in many other fields and industries as well. These detectors need to be capable of isolating target analyte signature despite the presence of interfering species. These detectors also need to have methods in place for overcoming high or unworkable limits of detection. Furthermore, these detectors need to be capable of quickly (ideally real-time) quantifying target analytes in complex systems. A promising avenue for meeting these needs is spectroscopy. Spectroscopy is versatile enough to provide characterization information in a variety of environments but can also easily be paired with other techniques and methods to overcome the limitations inherent to spectroscopy.
REFERENCES


9. Chatterjee, S; Del Negro, AS; Edwards, MK; Bryan, SA; Kaval, N; Pantelic, N; Morris, LK; Heineman, WR; Seliskar, CJ (2011) Luminescence-Based Spectroelectrochemical Sensor for [Tc(dmpe)(3)](2+/+) (dmpe=1,2-bis(dimethylphosphino)ethane) within a Charge-Selective Polymer Film Anal Chem, 83, 1766-1772.

10. Crosby, GA; Perkins, WG; Klassen, DM (1965) Luminescence from Transition-Metal Complexes - Tris(2,2'-Bipyridine)- and Tris(1,10-Phenanthroline)Ruthenium(2) J Chem Phys, 43, 1498-&.

11. Demas, JN; Crosby, GA (1968) On Multiplicity of Emitting State of Ruthenium(2) Complexes J Mol Spectrosc, 26, 72-&.


