



MSU Graduate Theses

Summer 2016

Electrogenerated Chemiluminescence Of Luminophores And Enhancement With Melatonin

Sarah Michelle Roughton

As with any intellectual project, the content and views expressed in this thesis may be considered objectionable by some readers. However, this student-scholar's work has been judged to have academic value by the student's thesis committee members trained in the discipline. The content and views expressed in this thesis are those of the student-scholar and are not endorsed by Missouri State University, its Graduate College, or its employees.

Follow this and additional works at: <https://bearworks.missouristate.edu/theses>

 Part of the [Chemistry Commons](#)

Recommended Citation

Roughton, Sarah Michelle, "Electrogenerated Chemiluminescence Of Luminophores And Enhancement With Melatonin" (2016). *MSU Graduate Theses*. 2975.
<https://bearworks.missouristate.edu/theses/2975>

This article or document was made available through BearWorks, the institutional repository of Missouri State University. The work contained in it may be protected by copyright and require permission of the copyright holder for reuse or redistribution.

For more information, please contact BearWorks@library.missouristate.edu.

**ELECTROGENERATED CHEMILUMINESCENCE OF LUMINOPHORES
AND ENHANCEMENT WITH MELATONIN**

A Masters Thesis

Presented to

The Graduate College of

Missouri State University

In Partial Fulfillment

Of the Requirements for the Degree

Master of Science, Chemistry

By

Sarah Michelle Roughton

July 2016

Copyright 2016 by Sarah Michelle Roughton

ELECTROGENERATED CHEMILUMINESCENCE OF LUMINOPHORES AND ENHANCEMENT WITH MELATONIN

Chemistry

Missouri State University, July 2016

Master of Science

Sarah Michelle Roughton

ABSTRACT

The photoluminescent (PL), electrochemical, and electrogenerated chemiluminescent (ECL) properties of 1,5-I-Aedans (*N*-(iodoacetylaminomethyl)-1-naphthylamine-5-sulfonic acid) in aqueous buffered (KH_2PO_4) and 50:50 (v/v) acetonitrile: KH_2PO_4 solutions were obtained. Tri-*n*-propylamine (TPrA) was used as the oxidative-reductive coreactant. The PL efficiencies (ϕ_{em}) were 1.83 in KH_2PO_4 and 4.81 in mixed solvent compared to $\text{Ru}(\text{bpy})_3^{2+}$ standard solutions ($\phi_{\text{em}} = 1.0$). 1,5-I-Aedans displayed quasi-reversible oxidative electrochemistry in aqueous solutions and irreversible to quasi-reversible oxidation in mixed solvent. ECL efficiencies (ϕ_{ecl}) were obtained by comparison to a $\text{Ru}(\text{bpy})_3^{2+}/\text{TPrA}$ ($\text{bpy} = 2,2'$ -bipyridine) standard ($\phi_{\text{ecl}} = 1$) and were higher in KH_2PO_4 ($\phi_{\text{ecl}} = 6.8 \times 10^{-4}$) than in the mixed solvent system ($\phi_{\text{ecl}} = 0.072 \times 10^{-4}$). A second project studied the ECL of the $\text{Ru}(\text{bpy})_3^{2+}/\text{TPrA}$ system when melatonin (*N*-acetyl-5-methoxytryptamine; MLT) is present in aqueous buffered solution. MLT displays weak ECL when TPrA is used as the oxidative-reductive coreactant, but when micromolar concentrations of MLT were added to the system, up to 2.5-fold enhancement was seen. PL efficiencies did not change in the presence of MLT unless the solution underwent electrochemical bulk oxidation. Spectroscopic, electrochemical and spectro-electrochemical studies indicate that the mechanism involves oxygen scavenging by MLT oxidation products. This scavenging prevents quenching of the $^*\text{Ru}(\text{bpy})_3^{2+}$ excited states.

KEYWORDS: enhanced electrogenerated chemiluminescence (ECL), coreactants, melatonin, 1,5-I-Aedans, $\text{Ru}(\text{bpy})_3^{2+}$

This abstract is approved as to form and content

Dr. Mark M. Richter
Chairperson, Advisory Committee
Missouri State University

**ELECTROGENERATED CHEMILUMINESCENCE OF LUMINOPHORES
AND ENHANCEMENT WITH MELATONIN**

By

Sarah Michelle Roughton

A Masters Thesis
Submitted to the Graduate College
Of Missouri State University
In Partial Fulfillment of the Requirements
For the Degree of Master of Science, Chemistry

July 2016

Approved:

Mark M. Richter, PhD: Committee Chairperson

Matthew R. Siebert, PhD, Committee Member

Adam Wanekaya, PhD, Committee Member

Kevin Evans, PhD, Committee Member

Julie Masterson, PhD: Dean, Graduate College

ACKNOWLEDGEMENTS

I would like to thank the following people for their support during the course of my graduate studies. Thanks to the Missouri State University Graduate College and the Department of Chemistry for funding this work. Thanks to Dr. Matthew Siebert, Dr. Kevin Evans, and Dr. Adam Wanekaya for being on my committee and for the guidance and advice. Thanks to my family and friends for encouragement, patience, and understanding during this journey. Finally, thanks to my mentor and friend, Dr. Mark Richter. He was the person who sparked my interest in chemistry and over the years his guidance, assistance, unending patience, encouragement, and somewhat snarky sense of humor have been invaluable. Words cannot express my gratitude.

TABLE OF CONTENTS

Chapter 1: Introduction	1
Chapter 2: Experimental Methods	7
Materials	7
Methods.....	8
Electrochemistry	8
ECL	8
Cyclic Voltammetry.....	9
UV/Vis and PL.....	9
Chapter 3: ECL of <i>N</i> -(iodoacetylaminoethyl)-1-naphthylamine-5-sulfonic acid (1,5-I-Aedans)	11
Introduction.....	11
Results and Discussion	12
Absorption and Photoluminescence.....	12
Electrochemistry	14
Electrogenerated Chemiluminescence	15
Conclusions.....	22
Chapter 4: Enhanced ECL via Oxygen Quenching Using Melatonin	23
Introduction.....	23
Results and Discussion	25
UV/Vis and PL.....	25
Electrochemistry	27
Electrogenerated Chemiluminescence	28
Mechanism of Enhancement.....	34
ECL in 2,2,2-trifluoroethanol and Triton X-100 Solutions	38
Conclusions.....	39
Chapter 5: Future Work	42
References.....	43

LIST OF TABLES

Table 1. Spectroscopic UV/Vis and PL data for 0.1 mM 1,5-I-Aedans	12
Table 2. Electrochemical data for 0.1mM 1,5-I-Aedans	15
Table 3. Spectroscopic UV/Vis, PL, and ECL data for MLT	26
Table 4. PL data with MLT in air or deoxygenated solution.	27
Table 5. Melatonin electrogenerated chemiluminescence data	41

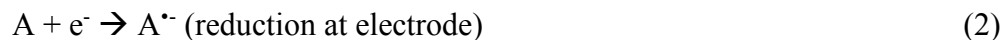
LIST OF FIGURES

Figure 1. ECL Reaction of $[\text{Ru}(\text{bpy})_3]^{2+}$ and TPrA	4
Figure 2. ECL Emission of $[\text{Ru}(\text{bpy})_3]^{2+}$ at a Platinum Electrode.....	5
Figure 3. Structures of Compounds	6
Figure 4. UV/Vis and PL of 1,5-I-Aedans	13
Figure 5. Cyclic Voltammograms for 1,5-I-Aedans	16
Figure 6. ECL Intensity vs Potential.....	18
Figure 7. ECL vs Time.....	19
Figure 8. Structure of Melatonin.....	23
Figure 9. PL Coupled with Bulk Electrolysis	28
Figure 10. ECL Spectra of $(\text{DC-bpy})\text{Ru}(\text{bpy})_2^{2+}$ with vs without Melatonin.....	29
Figure 11. ECL Intensity vs Time.....	31
Figure 12. Structures of AFMK, HO-MLT, and AMK	31
Figure 13. ECL Intensity vs Potential $\text{Ru}(\text{bpy})_3^{2+}$	32
Figure 14. ECL Intensity vs pH	33
Figure 15. ECL vs Time $\text{Ru}(\text{bpy})_3^{2+}$ with vs without Melatonin.....	34
Figure 16. ECL Coupled with Bulk Electrolysis	36
Figure 17. ECL vs Potential in Air vs Deoxygenated Atmosphere	38

CHAPTER 1: INTRODUCTION

Electrogenerated chemiluminescence (ECL) is a means of transforming electrical energy into radiative energy via electron transfer reactions. ECL systems can be used as markers to detect organic, inorganic, and bioactive molecules. Because of this, it has found use in the areas of analytical, inorganic, organic, environmental, and biochemistry.^[1-3]

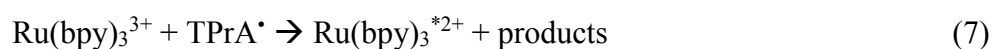
ECL can be initiated through several different routes, including annihilation and coreactant ECL. Annihilation ECL involves electron transfer reactions between two species (one oxidized and one reduced), generated at an electrode by alternate pulsing of the potential. The potential of the working electrode is quickly changed between two values to produce the oxidized and reduced radicals (Equations 1 and 2, below) that will then react (Equation 3) to emit light (Equation 4). Annihilation ECL has found uses in the rapid screening of potential organic light-emitting diodes (OLEDs), but it has some drawbacks. Cells, electrodes, and procedures have to be modified to allow for the generation of two reactants. Stray light must be blocked out and organic solvents must be extremely pure since water and oxygen tend to quench ECL in this method. A generic mechanism is presented in the following equations:^[3]



In coreactant ECL, the electrode either oxidizes or reduces the reagents in a single potential step (versus the double potential step of annihilation ECL). When a potential is applied to a solution through an electrode, an excited state is formed that is capable of light emission. Utilizing the commercially available $\text{Ru}(\text{bpy})_3^{2+}/\text{TPrA}$ ($\text{bpy} = 2,2'$ -bipyridine; $\text{TPrA} = \text{tri-}n\text{-propylamine}$) system as an example, an electric potential is applied to a solution containing both the luminophore ($\text{Ru}(\text{bpy})_3^{2+}$) and the coreactant (TPrA). This potential results in the oxidation of both species (Equations 5 and 6, below), with the TPrA forming a strong reducing agent (probably TPrA^\bullet , where $\text{TPrA}^\bullet = \text{TPrA}$ radical), which can then react with the oxidized $\text{Ru}(\text{bpy})_3^{3+}$ to produce an excited state molecule (i.e., $\text{Ru}(\text{bpy})_3^{*2+}$) (Equation 7). $\text{Ru}(\text{bpy})_3^{*2+}$ can then decay to the ground state, producing luminescence (Equation 8).^[4,5]

This system has found use in DNA quantification; clinical diagnostic immunoassays; in the measurement of biological and pharmacological compounds including opiates, antibiotics, and antihistamines; and in environmental applications, allowing detection of concentrations down to $\leq 10^{-18}$ M.^[1-3] In most clinical applications, $\text{Ru}(\text{bpy})_3^{2+}$ acts as an ECL marker. For example, in a study of human C-reactive protein (CRP), liposomes containing $\text{Ru}(\text{bpy})_3^{2+}$ were prepared as an ECL marker for a sandwich-type immunoassay. Human CRP antibodies were introduced onto liposomes and magnetic beads, allowing the antigen-antibody conjugates to be separated from unreacted species magnetically. Addition of 0.1M tri-*n*-propylamine and 0.1M phosphate buffer with Triton X-100 liberated the $\text{Ru}(\text{bpy})_3^{2+}$ from the liposome, allowing ECL detection down to 100ng/mL.^[6] $\text{Ru}(\text{bpy})_3^{2+}$ -containing microspheres have also been used to detect and quantify 2,4,6-trinitrotoluene (TNT) in soil and water down to ≤ 0.1 ppt or

0.1 pg. TNT is a toxic explosive chemical used in military shells, bombs, grenades, and underwater blasting. It is so dangerous, the United States Environmental Protection Agency has issued a lifetime health advisory level of 2 ppb in drinking water.^[7] The most commonly proposed mechanism for the Ru(bpy)₃²⁺/TPrA system is presented in the equations below and in Figure 1 with a representative photo in Figure 2:^[3]



While the Ru(bpy)₃²⁺/TPrA system is very useful, there is still a search for methods to improve detection limits. These methods may include the use of additives, utilizing different coreactants, synthesizing dual- or multi-core ruthenium complexes, and the study of complexes other than ruthenium to name a few.^[1] Structures of some of the compounds utilized in this study can be seen in Figure 3. In this work, two systems will be discussed. The first investigates the photoluminescent, electrochemical, and ECL properties of 1,5-I-Aedans (*N*-(iodoacetylaminioethyl)-1-naphthylamine-5-sulfonic acid) in aqueous buffered (KH₂PO₄) and 50:50 (v/v) acetonitrile:aqueous buffered (CH₃CN:KH₂PO₄) solutions. The second investigates the ECL of the Ru(bpy)₃²⁺/TPrA system when melatonin (*N*-acetyl-5-methoxytryptamine; MLT) is present in aqueous buffered solution.

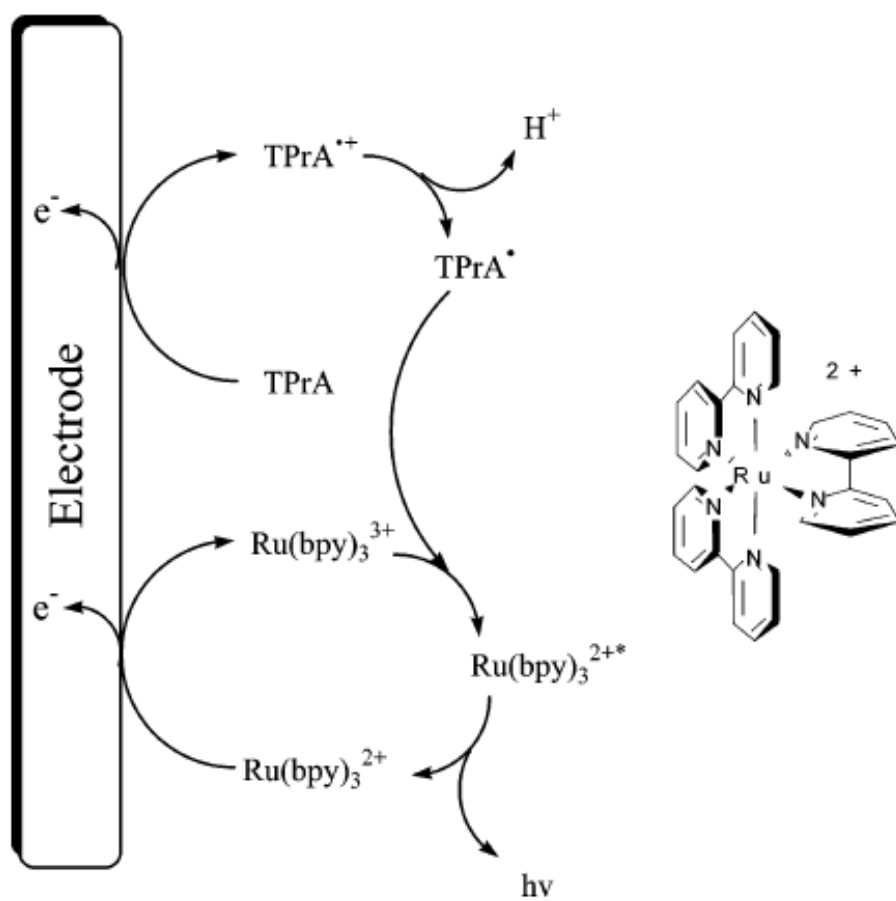
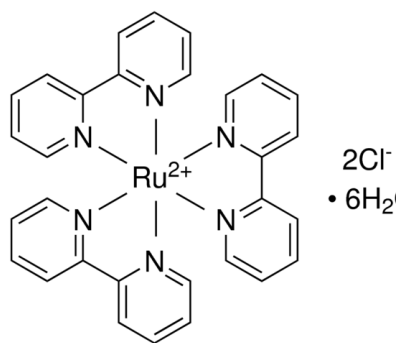


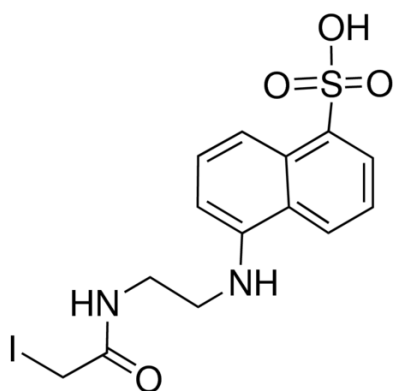
Figure 1. ECL reaction of $[\text{Ru}(\text{bpy})_3]^{2+}$ and TPrA. TPrA is tri-*n*-propylamine ($\text{Pr}_2\text{NCH}_2\text{CH}_2\text{CH}_3$)



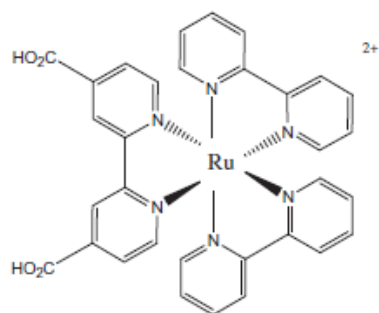
Figure 2. ECL emission of $[\text{Ru}(\text{bpy})_3]^{2+}$ at a platinum electrode. Stirred solution of 0.15 M $[\text{Ru}(\text{bpy})_3]^{2+}$ and 40 mM TPrA in 0.18 M potassium phosphate buffer (KPB) pH 8.0. Working electrode held at +2.0 V vs. Ag/AgCl. Photograph by D.J. Vinyard. 27 Jan. 2008.



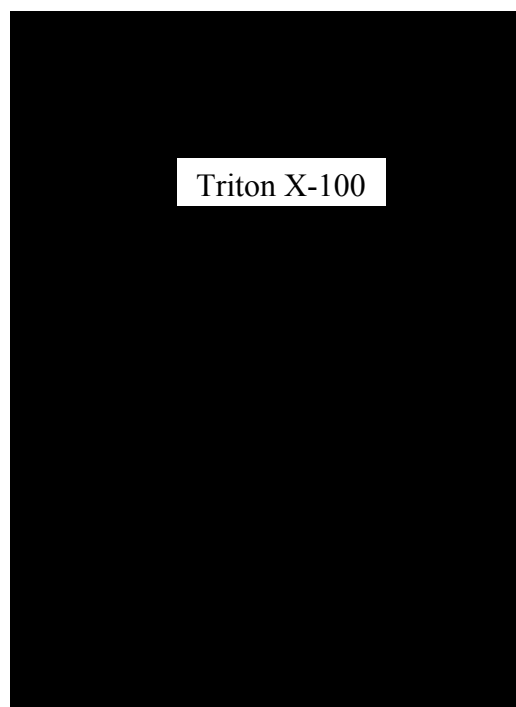
Ru(bpy)_3^{2+}



1,5-I-Aedans

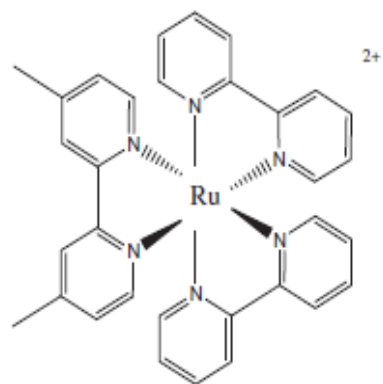


$(\text{bpy})_2\text{Ru(DC-bpy)}^{2+}$



Triton X-100

Ir(ppy)_3



$(\text{bpy})_2\text{Ru(DM-bpy)}^{2+}$

Figure 3. Structures of Compounds: Ru(bpy)_3^{2+} , 1,5-I-Aedans, $(\text{bpy})_2\text{Ru(DC-bpy)}^{2+}$, Triton X-100, Ir(ppy)_3 , and $(\text{bpy})_2\text{Ru(DM-bpy)}^{2+}$.

CHAPTER 2: EXPERIMENTAL METHODS

Materials

For Chapter 3. Potassium phosphate monobasic (KH_2PO_4 , Spectrum Chemical Mfg. Corp., Gardena, CA), tris(2,2'-bipyridyl)ruthenium (II) chloride hexahydrate ($\text{Ru}(\text{bpy})_3\text{Cl}_2 \cdot 6\text{H}_2\text{O}$; Strem Chemicals, Newburyport, MA), acetonitrile (CH_3CN ; Sigma-Aldrich Co., St. Louis, MO), tri-*n*-propylamine (TPrA; 98%, Sigma-Aldrich), were used as received. *N*-(iodoacetylaminoethyl)-1-naphthylamine-5-sulfonic acid (1,5-I-Aedans; Sigma-Aldrich) was purified by literature methods via recrystallization from hot deionized water.^[8] Purity was tested using thin layer chromatography with ethanol as the eluent following published procedures. Also, a molar absorptivity (A) of $5650 \text{ M}^{-1}\text{cm}^{-1}$ was obtained in deionized water, with a reported value^[8] of $5700 \text{ M}^{-1}\text{cm}^{-1}$ indicating greater than 99% purity after recrystallization. Aqueous experiments (including 50:50 (v/v) CH_3CN : KH_2PO_4) were performed in $\sim 0.18 \text{ M}$ potassium phosphate (Spectrum Chemical Manufacturing Corp., Gardena, CA) buffered solution. pH was adjusted using either sodium hydroxide or concentrated sulfuric acid. Deionized water was filtered using a Barnstead/Thermolyne triple filtration system.

For Chapter 4. $\text{Ru}(\text{bpy})_3\text{Cl}_2 \cdot 6\text{H}_2\text{O}$ (98%, Strem Chemical, Newburyport, MA), melatonin (Sigma-Aldrich, St. Louis, MO), serotonin (Sigma-Aldrich), 7-azatryptophan (Sigma-Aldrich), tryptophan (Sigma-Aldrich), and tri-*n*-propylamine (98%, Sigma-Aldrich) were used without further purification. $\text{Ir}(\text{ppy})_3$, and $(\text{DC-bpy})\text{Ru}(\text{bpy})_2^{2+}$ and $(\text{DM-bpy})\text{Ru}(\text{bpy})_2^{2+}$ ^[9] were available from previous studies. Aqueous experiments were performed in either 0.18 M potassium phosphate (Spectrum Chemical Manufacturing

Corp., Gardena, CA) buffered solution or Bioveris[®] assay buffer (approximate composition: 0.18M TPrA, 0.02M potassium phosphate buffer, 0.05 M TPrA, 0.1% by weight Triton X-100 at pH = 7.5). Deionized water was purified using a Barnstead E-Pure filtration system.

Methods

Electrochemistry. Electrochemical experiments with no photon detection used a CH Instruments electrochemical analyzer (Austin, TX) with a glassy carbon working electrode, a platinum auxiliary electrode and a Ag/AgCl electrode (0.20 V vs. the Normal Hydrogen Electrode (NHE)).^[10] The working electrode was cleaned after each run by polishing with 0.05 μm alumina, followed by rinsing with purified and filtered water.

ECL. ECL instrumentation and experimental methods incorporated a conventional three-electrode system. A CH Instruments electrochemical analyzer was paired with a Hamamatsu HC 135 Photomultiplier Tube (PMT) contained in a “light-tight” box. The working electrode was a platinum mesh electrode, with a platinum wire auxiliary electrode and a Ag/AgCl quasi-reference electrode (0.20 V vs. NHE).^[10] This same system was also used in conjunction with yellow, green and blue plastic absorption filters to obtain the wavelength ranges of ECL emission. The working electrode was cleaned before each run by repeated cycling (+2.0 to -2.0 V) in 6.0 M sulfuric acid followed by rinsing with deionized water. Luminophore concentrations were between $1 \times 10^{-3}\text{M}$ and $1 \times 10^{-7}\text{M}$ with 50mM TPrA in 0.18 M potassium phosphate at pH 8.0 ± 0.1 , and typically contained millimolar concentrations of analyte (e.g., MLT). ECL

efficiencies (ϕ_{ecl} = photons generated per redox event) were obtained using the following equations using $\text{Ru}(\text{bpy})_3^{2+}$ ($\phi_{\text{ecl}} = 1$) as the standard^[11]:

$$\phi_{\text{ecl}} = \phi_{\text{ecl}}^{\circ} \frac{I Q^{\circ}}{I^{\circ} Q} \quad (9)$$

where $\phi_{\text{ecl}}^{\circ}$ is the ECL efficiency of the standard, I and I° are the integrated intensities of the luminophore and the standard, and Q and Q° are the charges passed in Coulombs for 1,5-I-Aedans and $\text{Ru}(\text{bpy})_3^{2+}$, respectively. This equation is useful when the same electrode is used consistently, when luminophore and electrolyte concentrations are similar, and when electron transfers are equivalent between the target molecules. Reported values are the average of at least three scans with a relative standard deviation of $\pm 5\%$.

Cyclic Voltammetry. Cyclic voltammetry studies without photon detection utilized a CH instruments electrochemical analyzer (Austin, TX) with a glassy carbon working electrode (to prevent adsorption of compound on the surface of the electrode), a platinum wire auxiliary electrode and a Ag/AgCl quasi-reference electrode (0.20 V vs the Normal Hydrogen Electrode (NHE)).^[11] The glassy carbon electrode was cleaned after each run by polishing with 0.1 μm alumina, followed by rinsing with water.

UV-Vis and Photoluminescence. UV-Vis spectroscopy was performed using an Agilent Technologies Cary-60 UV-Visible Spectrophotometer. Photoluminescence spectra were obtained with a Shimadzu RF-5301 Spectrofluorophotometer (Shimadzu Corporation, Japan) with detection between 500 and 700 nm. Slit widths for PL spectroscopy were 4 nm for 1,5-I-Aedans. Slit widths for PL spectroscopy (for MLT) were 3nm and for ECL spectroscopy were 20nm. Excitation for photoluminescence was at the lowest energy absorption wavelength maximum. Photoluminescence efficiencies

(ϕ_{em} ; photons emitted per photons absorbed) were obtained relative to $\text{Ru}(\text{bpy})_3^{2+}$ in each solvent mixture using the following equation:

$$\phi_{\text{em}} = \phi_{\text{em}}^0 \frac{(I)}{I^0} \quad (10)$$

where ϕ_{em}^0 is the PL efficiency of the $\text{Ru}(\text{bpy})_3^{2+}$ while I and I^0 are the emission intensities of 1,5-I-Aedans and $\text{Ru}(\text{bpy})_3^{2+}$, respectively. The PL efficiency of $\text{Ru}(\text{bpy})_3^{2+}$ was set to 1.0 to allow direct comparisons between the different solvent mixtures. For the MLT studies, I represented the intensities of solutions containing MLT and I^0 represented the standard solution ($\text{Ru}(\text{bpy})_3^{2+}/\text{TPrA}/\text{KH}_2\text{PO}_4$). UV-Vis absorption and PL were run in these solvent systems to allow direct comparisons with ECL data.

CHAPTER 3: ECL OF *N*-(IODOACETYLAMINOETHYL)-1-NAPHTHYLAMINE-5-SULFONIC ACID (1,5-I-AEDANS)

Introduction

8-amino-1-naphthalenesulfonic acid (ANS) and its analogues such as *N*-(iodoacetylaminoethyl)-1-naphthylamine-5-sulfonic acid (1,5-I-Aedans; Figure 3) have been used as photoluminescent probes of biomolecular interactions.^[8,12,13,14] For example, they are in widespread use for the characterization of protein aggregates, fibrils, conformational changes, and to locate hydrophobic moieties in native proteins.^[15,16] 1,5-I-Aedans, in particular, has been used to determine conformational changes in skeletal muscle G-actin upon addition of Mg^{2+} and Ca^{2+} to aqueous solutions^[13] and to characterize the interactions between troponin units via fluorescence lifetime and quenching of the 1,5-I-Aedans excited state.^[14] In addition, they have been incorporated into high throughput screening assays to develop therapeutic protein formulations.^[17] The emission spectra of these compounds are also sensitive to the polarity of the environment, showing blue shifts in emission maxima and increased emission quantum efficiencies in less polar solvents due to decreased non-radiative decay.^[18,19] This makes them ideal probes for fundamental and applied luminescent studies such as electrogenerated chemiluminescence (ECL). In this study detailed spectroscopic, electrochemical and ECL properties of 1,5-I-Aedans are reported to better understand its properties and evaluate its potential usefulness as an ECL sensor.

Results and Discussion

Absorption and Photoluminescence. UV/Vis and PL data are presented in Table 1 and Figure 4. 1,5-I- Aedans displays an absorption maximum, λ_{max} , around 337 nm in aqueous buffered solution and 340 nm in mixed (50:50 v/v) $\text{KH}_2\text{PO}_4\text{:CH}_3\text{CN}$. The slight red shift of λ_{max} in the mixed solvent has been observed numerous times for naphthalene sulfonic acids as the hydrophobicity of the solvent increases.^[8,11,15] An absorption maximum is also observed at around 270 nm in both solvent systems, deriving from the aniline substructure of the molecules while the lower energy absorptions are from the fully delocalized naphthalene moiety.^[20] Absorption spectra were taken on a single sample in 10-minute intervals for a period of 120 minutes to test the stability of the complex to laboratory room light since 1,5-I-Aedans is sensitive to light exposure over extended periods of time. Consistent spectra (intensity, peak shape, etc.) were obtained in each experiment suggesting that no photodegradation was observed in the timeframe of normal spectroscopic, electrochemical or ECL experiments.

Table 1. Spectroscopic UV/Vis and PL data for 0.1 mM 1,5-I-Aedans.

Solvent	λ_{abs} (nm)	λ_{em} (nm)	$\phi_{\text{em}}^{\text{a}}$	$\phi_{\text{ecl}}^{\text{b}}$
KH_2PO_4	337	496	1.83	6.8×10^{-4}
50:50 $\text{KH}_2\text{PO}_4\text{:CH}_3\text{CN}$	340	500	4.81	0.072×10^{-4}

^a ϕ_{em} measured by comparison of 1,5-I-Aedans (0.01 mM) to 0.01 mM $\text{Ru}(\text{bpy})_3^{2+}$ ($\phi_{\text{em}}=1$) in each solvent.

^b ϕ_{ecl} measured relative to $\text{Ru}(\text{bpy})_3^{2+}$ ($\phi_{\text{ecl}}=1$) in each solvent.

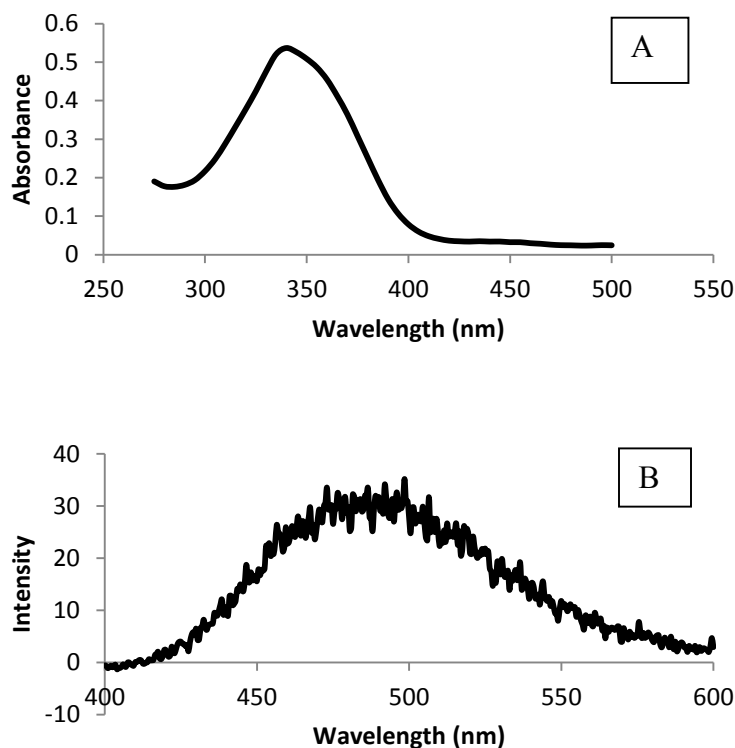


Figure 4: (A) UV-Vis, [1,5-I-Aedans = 1 mM], and (B) PL, [1,5-I-Aedans] = 0.1 mM, spectra of M 1,5-I-Aedans in 50:50 $\text{KH}_2\text{PO}_4\text{:CH}_3\text{CN}$.

Excitation into the broad visible absorbance band around 340 nm produces room temperature photoluminescence around 500nm for 1,5-I-Aedans in both solvents (Table 1) with a band width at half height of ~ 75 nm (Figure 4). The emission maxima are also solvent dependent, following the same trend observed in the absorbance studies.

Photoluminescence efficiencies (ϕ_{em} ; photons emitted per photons absorbed) for 1,5-I-Aedans are reported in Table 1, are solvent dependent and are higher than $\text{Ru}(\text{bpy})_3^{2+}$.

The photoluminescence efficiencies also increased with lower solvent polarity (the relative polarity of water is 1.000 and acetonitrile is 0.460).^[21] This was also observed for $\text{Ru}(\text{bpy})_3^{2+}$ in a series of alcohols^[22] and was traced to increased non-radiative decay as the polarity of the solvent increases.

Several studies have probed conformational changes in biomolecules using 1,5-I-Aedans. Metal cations such as Mg^{2+} , K^{+} and Ca^{2+} were shown to enhance or attenuate.^[13,14] 1,5-I-Aedans luminescence, presumably due to binding events occurring within the biomolecule itself. However, it is possible that the PL of 1,5-I-Aedans itself was impacted directly by the metal ions and since metal ions are often part of PL and ECL assays the effect of Fe^{+3} , Al^{+3} , Mg^{+2} , Zn^{+2} , and Cu^{+2} on 1,5-I-Aedans PL was included as part of this work. In summary, Al^{+3} had no effect upon 1,5-I-Aedans luminescence at $[\text{Al}^{+3}]$ between $1 \times 10^{-5} \text{ M}$ and $1 \times 10^{-3} \text{ M}$ and produced a slight attenuation ($\sim 20\%$) at $5 \times 10^{-3} \text{ M}$. Mg^{+2} , Zn^{+2} , and Cu^{+2} all behaved similarly with no effect at lower concentrations (between $1 \times 10^{-5} \text{ M}$ and $1 \times 10^{-4} \text{ M}$), $\sim 20\%$ attenuation at $1 \times 10^{-3} \text{ M}$ and $\sim 50\%$ attenuation of the PL signal at $5 \times 10^{-3} \text{ M}$. Fe^{+3} quenched at all concentrations, with $>90\%$ at $[\text{Fe}^{+3}] = 5 \times 10^{-3} \text{ M}$. Overall, the effect of metal ions on 1,5-I-Aedans is insignificant within experimental error at the micromolar concentrations routinely found in PL and ECL assays.

Electrochemistry. Representative cyclic voltammetric data is presented in Table 2 and cyclic voltammograms of 1,5-I-Aedans provided in Figure 5. The voltammograms were obtained for 1 mM 1,5-I-Aedans solutions by sweeping oxidatively starting at 0.0 V in both air saturated and deoxygenated solutions. No reductions were observed within the solvent windows ($\sim -1.6 \text{ V}$ vs Ag/AgCl).

In air saturated KH_2PO_4 an irreversible to quasi reversible peak occurs at $+0.42 \text{ V}$, with a smaller electrochemically irreversible peak at $\sim +0.72 \text{ V}$. In deoxygenated solution the peak at $+0.42 \text{ V}$ disappears, while the peak at $+0.72 \text{ V}$ becomes quasi reversible (i_a/i_c

= 2.1 where i_a is the anodic peak current, i_c is the cathodic peak current and a value of 1.0 is predicted for an electrochemically reversible system).

Table 2. Electrochemical data for 0.1mM 1,5-I-Aedans.

Solvent	E_a (V)	E_c (V)	$E_{1/2}$ (V)	i_a/i_c
KH ₂ PO ₄ – deoxygenated	0.71	0.61	0.66	2.1
KH ₂ PO ₄ – air saturated	0.42	---	---	---
50:50 (v/v) KH ₂ PO ₄ :CH ₃ CN – deoxygenated	0.49	---	---	---

However, in the deoxygenated solution a shoulder appears at slightly more positive potentials on the anodic wave suggesting that an electrochemically active species is being formed upon oxidation of 1,5-I-Aedans followed by a chemical reaction/decomposition or the system undergoes two closely spaced electrochemical oxidation events. This overlap of peaks might explain the less than ideal behavior of this system ($i_a/i_c = 2.1$). In mixed 50:50 KH₂PO₄:CH₃CN the complex displays an irreversible to quasi-reversible oxidation under both air saturated and deoxygenated conditions.

Electrogenerated Chemiluminescence. ECL was observed for 1,5-I-Aedans in aqueous buffered and 50:50 (v/v) KH₂PO₄:CH₃CN at a Pt interface by sweeping to positive potentials in the presence of 0.05M TPrA. Above pH 10 luminophores such as

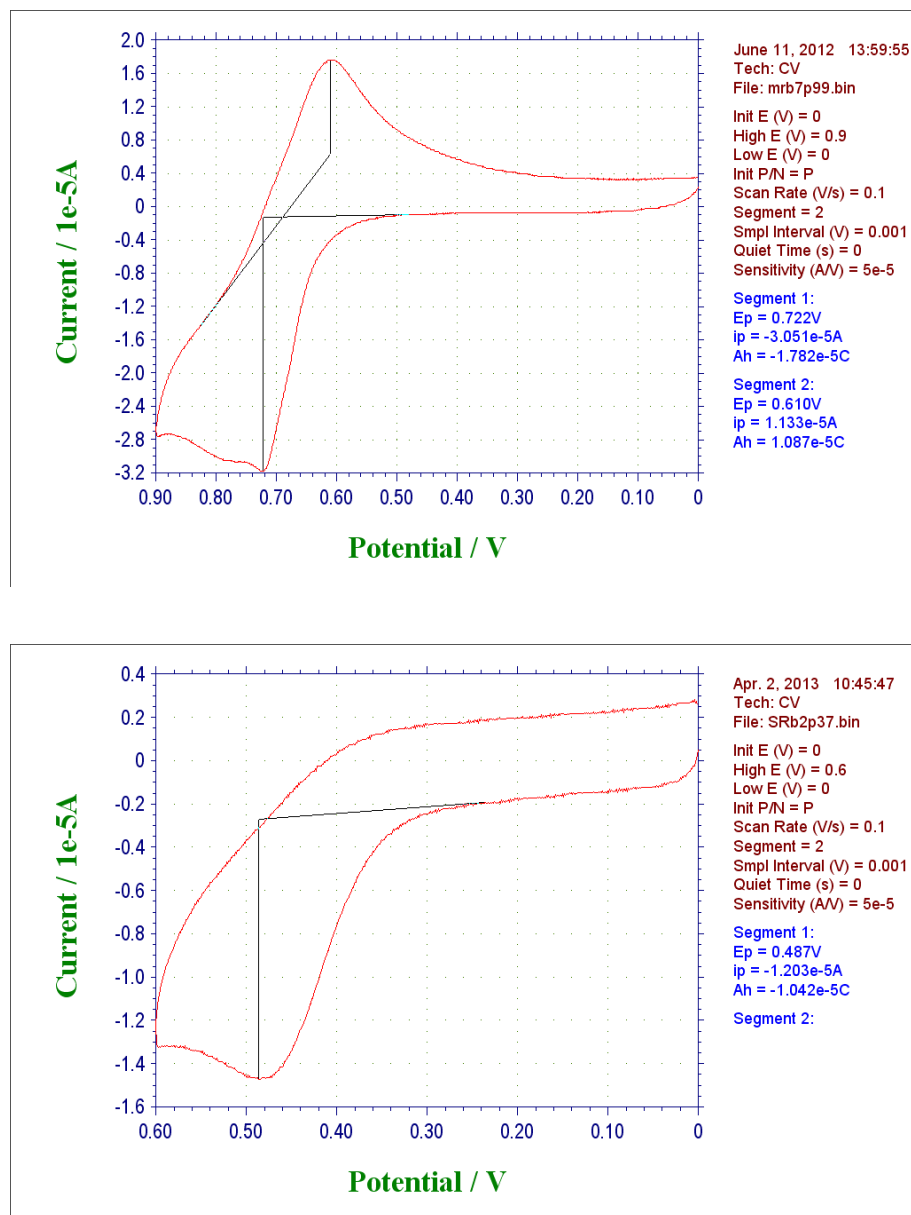


Figure 5. Electrochemical data for 1×10^{-3} M 1,5-I-Aedans in aqueous buffered solution (top) and 50:50 KH_2PO_4 : CH_3CN (bottom).

$\text{Ru}(\text{bpy})_3^{2+}$ can react with hydroxyl groups from solvent oxidation to produce ECL.^[23] To be certain that the ECL emission was from the reaction of 1,5-I-Aedans with TPrA all experiments were run at $\text{pH} = 8.0 \pm 0.2$ (this pH also resulted in very efficient ECL compared to lower pHs, *vide infra*). Background studies also confirmed that very

little ECL was emitted at this pH from 1,5-I-Aedans when TPrA was absent from solution and no ECL was observed above background in the absence of 1,5-I-Aedans.

Intensity vs. potential. The ECL intensity peaks at potentials around +1.0 V (Figure 6) in aqueous buffered solution and around +1.5 V in 50:50 (v/v) $\text{KH}_2\text{PO}_4\text{:CH}_3\text{CN}$, indicating oxidation of both TPrA ($E_a \sim +0.9$ V vs. Ag/AgCl) and 1,5-I-Aedans. The 500 mV difference in peak potential when acetonitrile is present in solution is surprising, and probably reflects the greater electrochemical irreversibility of the oxidative electrochemistry in 50:50 $\text{KH}_2\text{PO}_4\text{:CH}_3\text{CN}$.

Intensity vs. time. 1,5-I-Aedans ECL is diffusion controlled in KH_2PO_4 (Figure 7) and similar to that observed for other ECL luminophores such as $\text{Ru}(\text{bpy})_3^{2+}$. In the Intensity vs. time transient the potential of the working electrode was poised at +2.0 V for the duration of the experiment (typically 15 minutes), and the ECL intensity measured. As expected, there was a sharp increase in light intensity due to the higher concentrations of coreactant and luminophore near the electrode surface that then decreased over time due to the formation of a diffusion layer of electrochemical and ECL products near the electrode surface. Surprisingly, ECL in mixed solvent increases over time. This phenomenon has been observed before^[3] and suggests that either the reactants are being regenerated at the electrode surface (seen as unlikely due to the irreversible nature of both TPrA and 1,5-I-Aedans) or decomposition products are taking part in ECL.

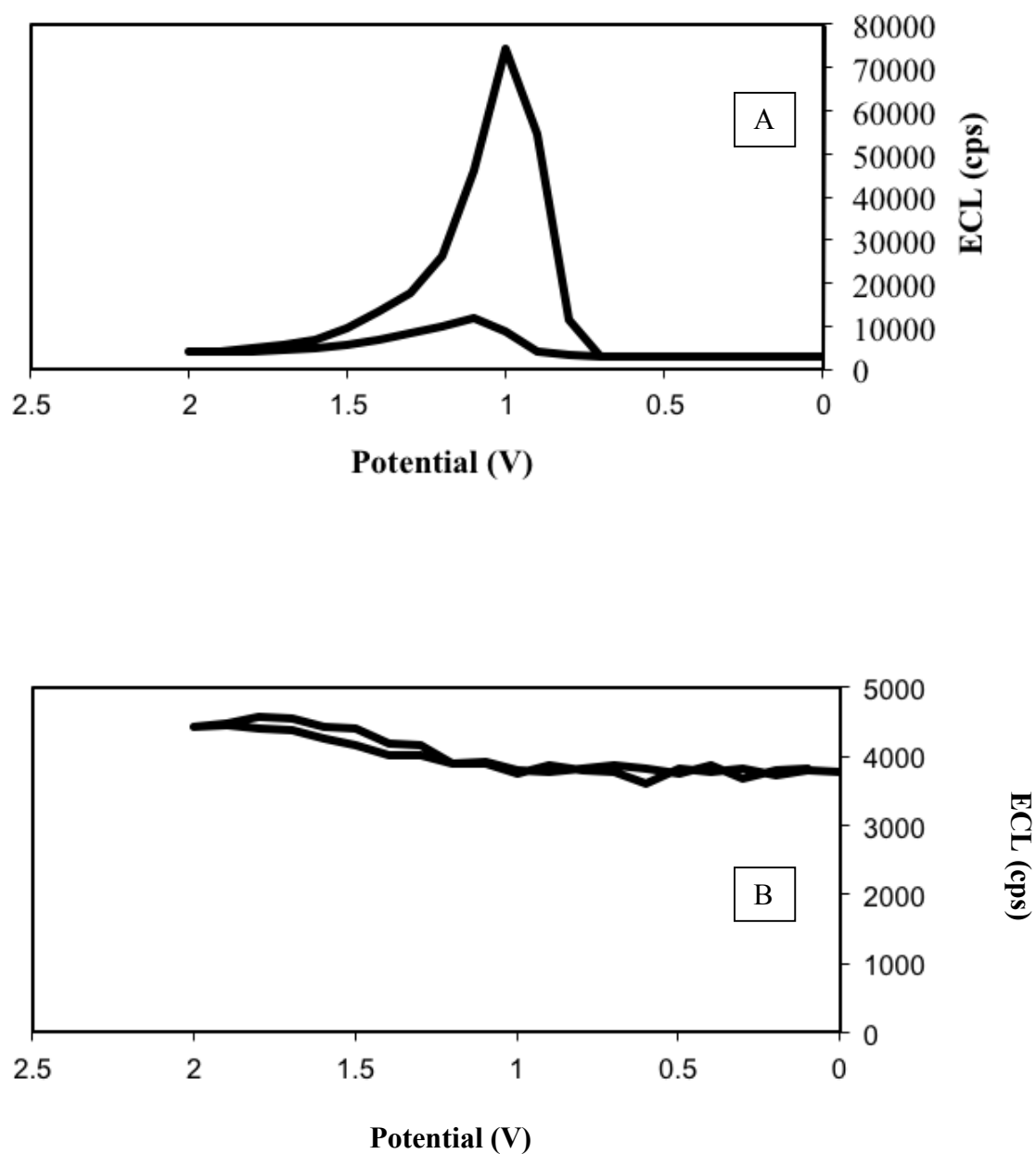


Figure 6: ECL Intensity versus potential for (A) 1×10^{-3} M 1,5-I-Aedans in aqueous buffered solution. Scan rate 0.1 mV/sec. (B) ECL Intensity versus potential for 1×10^{-3} M 1,5-I-Aedans in 50:50 (v/v) KH_2PO_4 : CH_3CN . Scan rate 0.1 mV/sec.

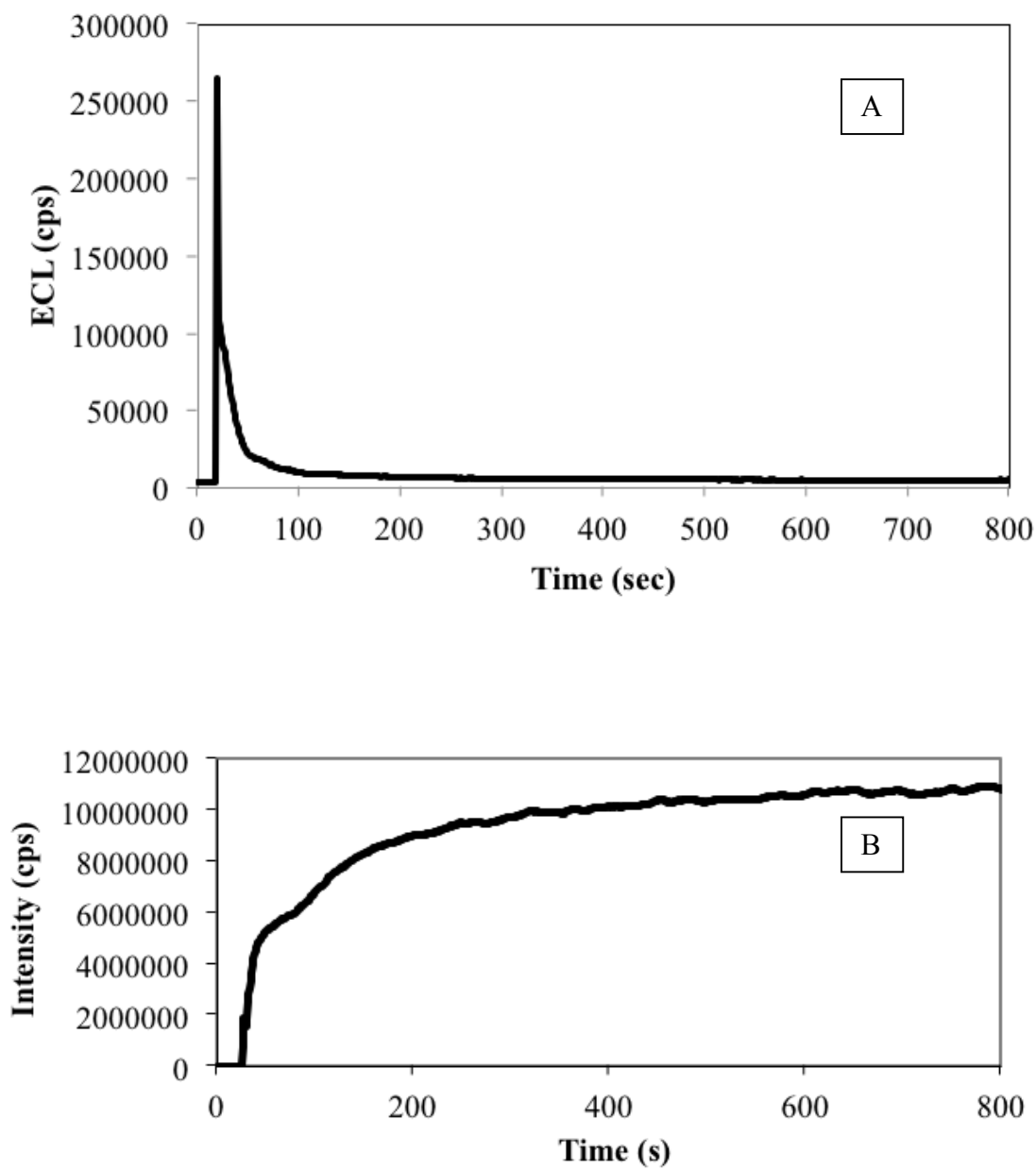


Figure 7: ECL Intensity versus time for 1×10^{-3} M 1,5-I-Aedans in (A) aqueous buffered solution and (B) 50:50 (v/v) KH_2PO_4 : CH_3CN ($[\text{TPrA}] = 50$ mM).

Intensity vs. pH. The ECL increases fairly linearly as a function of pH between pHs 3.0 and 8.0, mirroring other luminophores such as $\text{Ru}(\text{bpy})_3^{2+}$ when TPrA is the coreactant.⁵ This suggests that deprotonation of the TPrA radical cation is important to the generation of ECL in both 1,5-I-Aedans and ruthenium systems. The solubility of TPrA decreases at higher pHs with a clearly visible material suspended in solution above pH 9.3. Therefore, a pH of 8.0 was used in all experiments.

ECL. ECL efficiencies (photons emitted per redox event) of 1,5-I-Aedans were 6.8×10^{-4} and 0.072×10^{-4} in aqueous and 50:50, respectively (Table 1), much lower than the $\text{Ru}(\text{bpy})_3^{2+}$ standard ($\phi_{\text{ecl}} = 1$). Also, the efficiency is much lower in mixed solvent than aqueous buffer, opposite to the trend for other luminophores such as $\text{Ru}(\text{bpy})_3^{2+}$ and opposite to the trend in ϕ_{em} . This is not surprising, considering the irreversible to quasi-reversible oxidative electrochemistry.

Attempts to directly measure ECL spectra were unsuccessful, presumably due to the low ϕ_{ecl} . Therefore, yellow, green and blue plastic absorption filters were used to obtain the wavelength ranges of ECL in aqueous buffered solution containing TPrA. 1,5-I-Aedans displays blue-green emission with a maximum around 500 nm, indicating that the same excited states are formed via ECL and photoluminescence. However, the qualitative nature of this data makes definitive assignments impossible.

On the basis of photoluminescence emission wavelengths, the energy needed to generate the excited state of the complexes is in the range of 2.48 to 2.50 eV (e.g., if the wavelength is 496 nm (or 0.496 μm), the frequency is 6.04 GHz and the energy is 2.48 eV using $E = hc/\lambda$ where c is the speed of light and h is Planck's constant). Therefore, for direct generation of the excited state the energy of the electron or energy-transfer events

between the electrogenerated coreactant and luminophore molecules must exceed these values. However, studies on a series of polyaromatic hydrocarbons have indicated that while TPrA• is a good reductant, it does not have a sufficiently negative potential to generate excited states above 2.32 eV.^[24] Since qualitative ECL spectra suggest emission in the same region as photoluminescence emission, excited states in 1,5-I-Aedans are not populated by the direct reaction of oxidized 1,5-I-Aedans with TPrA• but by a more complicated mechanism. The exact mechanism of ECL in 1,5-I-Aedans is beyond the scope of the present work.

1,5-I-Aedans as a Ru(bpy)₃²⁺ Coreactant. It is well known that amines can act as ECL coreactants,^[1,5] with tertiary amines such as TPrA generating the most intense light emission.^[1-3] Since 1,5-I-Aedans contains two secondary amines it might be capable of acting as an ECL coreactant with Ru(bpy)₃²⁺ as the luminophore when no TPrA is present. These experiments used aqueous 0.18M KH₂PO₄ as the solvent and electrolyte, with 0.01 mM Ru(bpy)₃²⁺ and 1 mM 1,5-I-Aedans at pH = 8.0 ± 0.1. Blank solutions of Ru(bpy)₃²⁺ or 1,5-I-Aedans produced 1.4 x 10⁴ and 0.034 x 10⁴ counts per second (cps), respectively, while a solution containing both Ru(bpy)₃²⁺ and 1,5-I-Aedans produced 0.24 x 10⁴ cps. Clearly, 1,5-I-Aedans does not act as an ECL coreactant with Ru(bpy)₃²⁺ and, in fact appears to be quite efficient at attenuating the ECL signal of Ru(bpy)₃²⁺.

Mixed 1,5-I-Aedans and Ru(bpy)₃²⁺ with TPrA Coreactant. One goal of research in ECL is to discover new light emitting systems.^[1-3] Not only to find new molecules that emit light, but to find more efficient ECL luminophores (so that better detection limits and sensitivity than Ru(bpy)₃²⁺ might be obtained) and to find luminophores whose signal can be determined in the same sample solution as Ru(bpy)₃²⁺ (thus allowing so called

multianalyte detection). For example, Ir(ppy)₃ (ppy = 2-phenylpyridine) emits with an ECL maximum of approximately 500 nm and this allowed for the determination of both Ir(ppy)₃ and Ru(bpy)₃²⁺ in the same sample solution when TPrA was present.^[25]

Therefore, to test whether both 1,5-I-Aedans and Ru(bpy)₃²⁺ can emit ECL while in the same solution a series of experiments were conducted varying solution composition and using TPrA as the coreactant. A standard solution containing 0.1mM Ru(bpy)₃²⁺ and 0.40 mM TPrA in phosphate buffer produced 5.2×10^4 cps, a solution of 1mM 1,5-I-Aedans and 0.40 mM TPrA in phosphate buffer produced 0.46×10^4 cps, while a solution containing both 1,5-I-Aedans and Ru(bpy)₃²⁺ with TPrA produced 1.6×10^4 cps. Unfortunately, even with TPrA present in solution 1,5-I-Aedans attenuates the ECL signal of Ru(bpy)₃²⁺.

Conclusions

This study illustrates that 1,5-I-Aedans exhibits electrogenerated chemiluminescence in aqueous and 50:50 KH₂PO₄ solutions when TPrA is a coreactant. Although the ECL emission quantum efficiency is weaker than Ru(bpy)₃²⁺ under similar conditions, the excited state formed via ECL appears to be the same as that formed via PL suggesting the possibility of using 1,5-I-Aedans ECL as a probe of biomolecular interactions.

CHAPTER 4: ENHANCED ECL VIA OXYGEN QUENCHING USING MELATONIN

Introduction

Understanding the ECL properties of melatonin (*N*-acetyl-5-methoxytryptamine), Figure 8, is of interest for several reasons. Melatonin is a pineal hormone known for its role in numerous physiological processes such as metabolism, reproduction, circadian sleep-wake cycles, appetite, and muscular coordination. It also plays a role in psychiatric processes (such as anxiety and seasonal depression), in the immune system, has anti-oxidant properties^[26,27] and is a free radical scavenger.^[26,28,29]

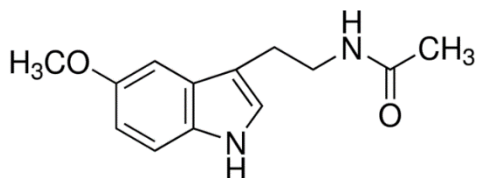


Figure 8. Structure of melatonin.

Analytical methods for the detection of melatonin include voltammetric,^[30] photoluminescence^[31] (PL) and chemiluminescence^[32] spectroscopy, liquid^[33] and gas^[34] chromatography, capillary electrophoresis,^[35] liquid chromatography coupled with mass spectroscopy,^[36] and electrogenerated chemiluminescence.^[37-40] ECL offers some advantages over other methods of analyses. For example, compared to fluorescent methods those involving ECL do not require an excitation source and tend to be less sensitive to interferences from luminescent impurities and scattered light.^[29] Several

reports have appeared on the ECL properties of structurally similar compounds.^[37-39] Although $\text{Ru}(\text{bpy})_3^{2+}$ is the most widely used luminophore in both fundamental and applied studies, a number of other transition metal chelates also display ECL. For example, ortho-metalated complexes of Ir(III) display ECL that, like their Ru(II) counterparts, make them of interest in fundamental and applied studies.^[25,41-49] Also, the ruthenium chelates $(\text{bpy})_2\text{Ru}(\text{DC-bpy})^{2+}$ ($\text{bpy} = 2,2'$ -bipyridine and $\text{DC-bpy} = (4,4'$ -dicarboxy- $2,2'$ -bipyridine) and $(\text{bpy})_2\text{Ru}(\text{DM-bpy})^{2+}$ ($\text{DM-bpy} = 4,4'$ -dimethyl- $2,2'$ -bipyridine), much like $\text{Ru}(\text{bpy})_3^{2+}$, show enhanced ECL in the presence of the nonionic surfactant Triton X-100 making it possible that they, too, will show sensitivity to the presence or absence of oxygen in solution.^[9] In the case of MLT, ECL has focused on MLT quenching of the luminol/ H_2O_2 system. However, to our knowledge, the ECL of melatonin itself or its effect on the ECL luminophore $\text{Ru}(\text{bpy})_3^{2+}$ have not been reported.

Therefore, this work focuses on the ECL of Ruthenium^[9] and Iridium^[47] complexes in the presence and absence of melatonin. In particular, a method for the enhancement of the commercially important $\text{Ru}(\text{bpy})_3^{2+}/\text{TPrA}$ reaction system via a unique mechanism is presented. The effect on the coreactant ECL of $(\text{bpy})_2\text{Ru}(\text{DC-bpy})^{2+}$ ($\text{bpy} = 2,2'$ -bipyridine and $\text{DC-bpy} = 4,4'$ -dicarboxy- $2,2'$ -bipyridine), $(\text{bpy})_2\text{Ru}(\text{DM-bpy})^{2+}$ ($\text{DM-bpy} = 4,4'$ -dimethyl- $2,2'$ -bipyridine), and $\text{Ir}(\text{ppy})_3$ ($\text{ppy} = 2$ -phenylpyridine) when MLT is present in aqueous buffered solution with tri-*n*-propylamine as the coreactant is also reported.

Results and Discussion

UV-Vis and PL. MLT displays strong absorptions between 250 and 325 nm characteristic of $\pi\text{-}\pi^*$ and $n\text{-}\pi^*$ events while $\text{Ru}(\text{bpy})_3^{2+}$ displays a series of ligand based transitions in the UV and Metal-To-Ligand Charge Transfer (MLCT) bands in the visible ($\lambda_{\text{abs}} \sim 450 \text{ nm}$). The presence of MLT does not affect the wavelength or absorbance of the MLCT transitions in any of the luminophores within experimental error.

Excitation into the lowest energy absorption band for each complex (λ_{abs}) produces room temperature photoluminescence (PL) with emission bands (λ_{em}) centered at 612 nm for $\text{Ru}(\text{bpy})_3^{2+}$, 507 nm for $\text{Ir}(\text{ppy})_3$, 605 nm for $((\text{bpy})_2\text{Ru}(\text{DM-bpy}))^{2+}$, and 629 nm for $((\text{bpy})_2\text{Ru}(\text{DC-bpy}))^{2+}$ as shown in Table 3. Since MLT enhanced $\text{Ru}(\text{bpy})_3^{2+}/\text{TPrA}$ ECL, the PL of MLT was studied in detail. Excitation into the ultraviolet band of MLT ($\lambda_{\text{exc}} = 300\text{nm}$) produces PL between 325 and 450 nm, with a wavelength maximum of 375 nm. The photoluminescence efficiency of MLT (ϕ_{em} ; photons emitted per photons absorbed) is 0.002 (or 0.2%) relative to $\text{Ru}(\text{bpy})_3^{2+}$ at 0.042 (or 4.2%). The presence of melatonin does not affect the shape, intensity or ϕ_{em} of the MLCT emission bands when excited at their lowest energy absorption. This suggests that a product of melatonin oxidation, and not the MLT itself, is responsible for the observed increases in ECL.

Table 3. Spectroscopic UV/Vis, PL, and ECL data for MLT.^a

Luminophore	$\lambda_{\text{abs}}(\text{nm})$	$\lambda_{\text{em}}(\text{nm})$	$\phi_{\text{em}}^{\text{b}}$	$\phi_{\text{ECL}}^{\text{c}}$	$\phi_{\text{ECL-MLT}}^{\text{d}}$
$\text{Ru}(\text{bpy})_3^{2+}$	454	596	0.042	1.0	2.5
$(\text{bpy})_2\text{Ru}(\text{DC-bpy})^{2+}$	458	629	0.020	0.73	0.99
$(\text{bpy})_2\text{Ru}(\text{DM-bpy})^{2+}$	455	605	0.030	0.84	1.2
$\text{Ir}(\text{ppy})_3$	380	507,532(sh)	0.050	0.0044	0.0073

^a $[\text{KH}_2\text{PO}_4] = 0.18 \text{ M}$ (aqueous buffered solution) at $\text{pH } 8.0 \pm 0.1$. $[\text{Luminophore}] = 0.01 \text{ mM}$. $[\text{TPrA}] = 0.05 \text{ M}$.

^bRelative photoluminescence emission efficiencies using $\text{Ru}(\text{bpy})_3^{2+} (\text{H}_2\text{O})$ as a standard.

^cRelative ECL efficiencies (photons emitted per ejected) using $\text{Ru}(\text{bpy})_3^{2+}/\text{TPrA}/\text{KH}_2\text{PO}_4$ as a standard ($\phi_{\text{ECL}} = 1$). Values are the averages of at least 3-replicates with a standard deviation of $\pm 5\%$.

^d $[\text{MLT}] = 0.05 \text{ M}$. Relative efficiencies using the luminophore/ $\text{TPrA}/\text{KH}_2\text{PO}_4$ solution with no MLT as a standard. Values are the averages of at least 3-replicates with a standard deviation of $\pm 5\%$.

Several PL experiments were run in both air saturated and deoxygenated solution. The results for $\text{Ru}(\text{bpy})_3^{2+}$ are reported in Table 4 and Figure 9. Simply deoxygenating a solution results in an approximately 20% increase in PL intensity, consistent with ECL experiments. Bulk electrolyzing the MLT prior to mixing with the other reagents generates results consistent with deoxygenating with Argon. Also, when the MLT solution is degassed prior to bulk electrolysis the increase in PL is approximately the same as the air saturated bulk-electrolyzed solution. The results for all three compounds are consistent and also correlate well with the $\text{Ru}(\text{bpy})_3^{2+}/\text{TPrA}$ system suggesting that oxygen scavenging is an integral part of the enhancement mechanism.

Table 4. PL data with MLT in air or deoxygenated solution.

Solution Composition	Air Saturated (Air) or Deoxygenated (Deox) ^a	PL Intensity (au) ^b
KH ₂ PO ₄ , TPrA, Ru(bpy) ₃ ²⁺	Air	1.795
KH ₂ PO ₄ , TPrA, Ru(bpy) ₃ ²⁺	Deox	2.216
KH ₂ PO ₄ , TPrA, Ru(bpy) ₃ ²⁺ , MLT	Air	1.630
KH ₂ PO ₄ , TPrA, Ru(bpy) ₃ ²⁺ , MLT	Deox	2.222
KH ₂ PO ₄ , TPrA, Ru(bpy) ₃ ²⁺ , MLT Oxidation products ^c	Air	2.102
KH ₂ PO ₄ , TPrA, (DC)Ru(bpy) ₂ ²⁺	Air	15.775
KH ₂ PO ₄ , TPrA, (DC)Ru(bpy) ₂ ²⁺	Deox	20.476
KH ₂ PO ₄ , TPrA, (DC)Ru(bpy) ₂ ²⁺ , MLT	Air	13.549
KH ₂ PO ₄ , TPrA, (DC)Ru(bpy) ₂ ²⁺ , MLT	Deox	19.333
KH ₂ PO ₄ , TPrA, (DC)Ru(bpy) ₂ ²⁺ , MLT Oxidation products ^c	Air	20.391
KH ₂ PO ₄ , TPrA, (DC)Ru(bpy) ₂ ²⁺ , MLT Oxidation products ^c	Deox	20.495

^aDeoxygenated with Argon for ~ 5 minutes prior to each run.

^b λ_{exc} = 450 nm, Slits 5 nm, value is the average of three runs with freshly mixed solutions.

^cThe MLT was bulk electrolyzed for 40 minutes prior to mixing with the other reagents.

Electrochemistry. The electrochemistry of the luminophores has been extensively studied.^[10] They display electrochemically reversible oxidation around +1.2 V for Ru(bpy)₃²⁺, +0.452 V for Ir(ppy)₃, +0.982 V for (DM-bpy)Ru(bpy)₂²⁺ and +1.08 V for (DC-bpy)Ru(bpy)₂²⁺ and a series of three sequential ligand based reductions. MLT displays one oxidation with a peak potential (E_a) of 0.58 V vs Ag/AgCl in aqueous buffered solution.

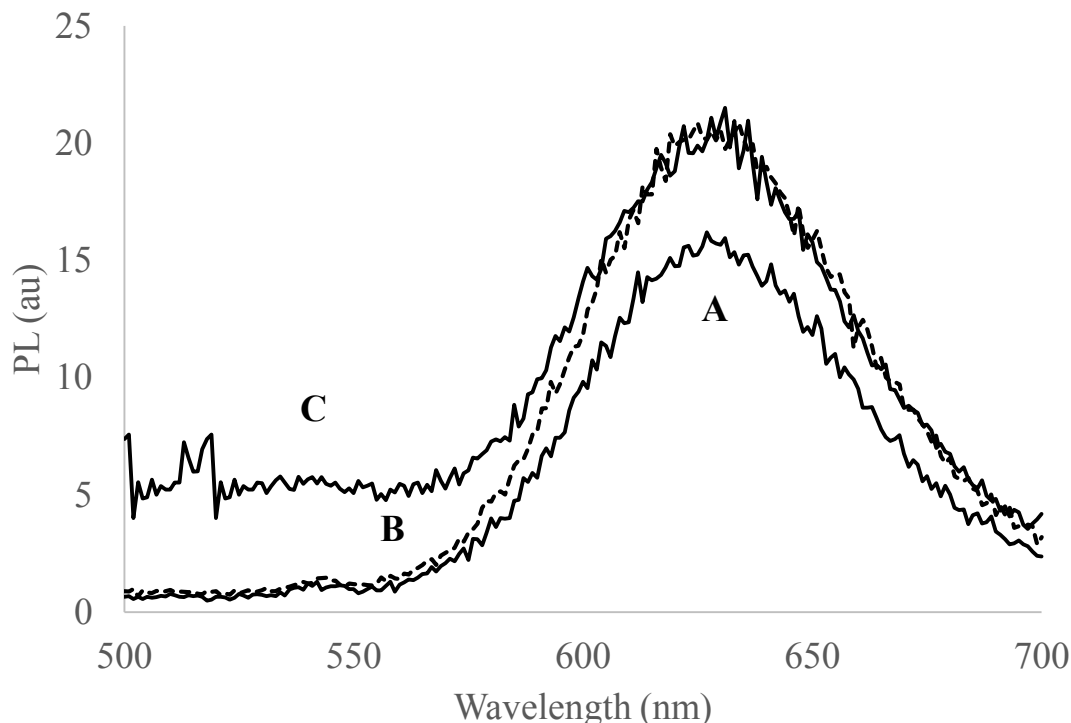


Figure 9. Photoluminescence coupled with bulk electrolysis scans of solutions containing different combinations of $1 \times 10^{-5} \text{M}$ (DC-bpy)Ru(bpy) $_2^{2+}$, 0.05M TPrA, and 5mM MLT in aqueous 0.18M KH $_2$ PO $_4$ (pH = 8.0). (A, solid line) (DC-bpy)Ru(bpy) $_2^{2+}$ /TPrA in air saturated solution with no bulk electrolysis, [MLT] = 0; (B, dashed line) (DC-bpy)Ru(bpy) $_2^{2+}$ /TPrA in deoxygenated solution with no bulk electrolysis, [MLT] = 0; and (C, solid line) MLT/TPrA bulk electrolyzed for 40 minutes at a potential of +2.0 V and added to an air saturated solution containing (DC-bpy)Ru(bpy) $_2^{2+}$ /TPrA.

The process is electrochemically irreversible on the time scale of the experiment (i.e., 0.1 V/sec) and at scan rates up to 5 V/s. Dissolved oxygen in solution does not affect the MLT oxidation potential since identical results are observed in air saturated solution and solutions degassed for 10 minutes with Argon.

Electrogenerated Chemiluminescence. ECL data is shown in Table 3 (above).

MLT is a poor coreactant compared to TPrA when Ru(bpy) $_3^{2+}$ is the luminophore (ϕ_{ECL} (Ru(bpy) $_3^{2+}$ /TPrA) = 1) with an ECL efficiency of 0.0053 when TPrA is absent from

solution. This is not surprising since MLT contains secondary amines, which are weaker ECL coreactants than tertiary amines like TPrA.^[1-3] A representative example of ECL for a solution of (DC-bpy)Ru(bpy)₂²⁺ containing MLT and TPrA is also shown in Figure 10. All luminophores display ECL at potentials corresponding to oxidation of both luminophore and coreactant ($E_a = 0.90\text{V}$) with ECL efficiencies between 4.4×10^{-3} and 0.84 when MLT is absent from solution. In all four luminophore/TPrA systems, the shape of the emission and the potentials at which emission occurs are similar to solutions without MLT. This suggests that the presence of MLT or MLT oxidation products do not interfere with excited state formation or emission.^[29,49]

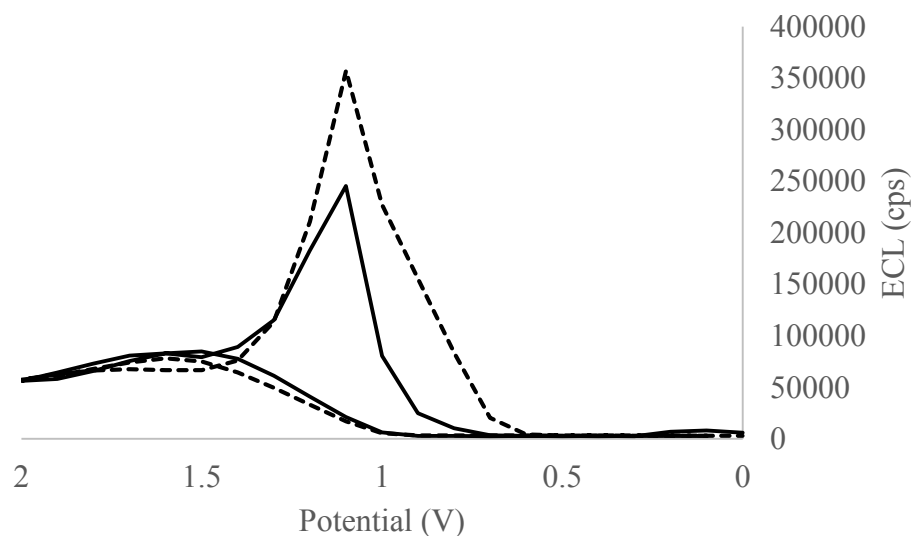


Figure 10. ECL Intensity versus potential for $1 \times 10^{-6}\text{M}$ (DC-bpy)Ru(bpy)₂²⁺ and 0.05M TPrA in 0.18M KH₂PO₄ with (—) no MLT and (- - -) $1 \times 10^{-5}\text{ M}$ MLT. Scan rate 0.1 mV/sec.

A representative ECL emission intensity versus time profile is shown in Figure 11. In these experiments, the potential of the working electrode was stepped to +2.0V in a quiescent solution while light emission was measured. All systems showed the characteristic sharp increase in light intensity upon application of the electrochemical potential due to the high concentrations of co-reactant and luminophore near the electrode surface.^[29,49] Within approximately 1 second, the ECL emission becomes diffusion controlled in the presence or absence of MLT. As in the Ru(bpy)₃²⁺/TPrA system, the presence of melatonin does not appear to interact directly with the ECL reaction sequence to enhance ECL efficiencies.^[49] Furthermore, the formation of two possible oxidation products,^[27] *N*¹-acetyl-*N*²-formyl-5-methoxykynurenine (AFMK), and hydroxymelatonin (HO-MLT), depend on the presence of molecular oxygen in solution (Figure 12).^[29,49] The most likely mechanism is electrochemical production of ortho- and benzo-quinones followed by energy or electron transfer to nonemissive states.^[50] The 2.5-fold enhancement with melatonin was unexpected given the structural similarities to other complexes and the experimental observation that melatonin is an efficient free radical scavenger that might interact with TPrA•.^[29]

The ECL of MLT/Ru(bpy)₃²⁺/TPrA is linear between 1x10⁻⁸ and 1x10⁻⁴ M with an r² value of 0.9851 (experimental range tested was from 1 x 10⁻³ to 1 x 10⁻¹² M) for six data points with a theoretical detection limit (blank signal plus three times the standard deviation of the blank) of 1x10⁻¹² M and a practical detection limit of 1x10⁻⁸ M using our experimental system. At mM concentrations of MLT the ECL intensity plateaus, indicating that the enhanced ECL is dependent on melatonin concentration. The observation that the presence of melatonin does not affect the PL of Ru(bpy)₃²⁺/TPrA

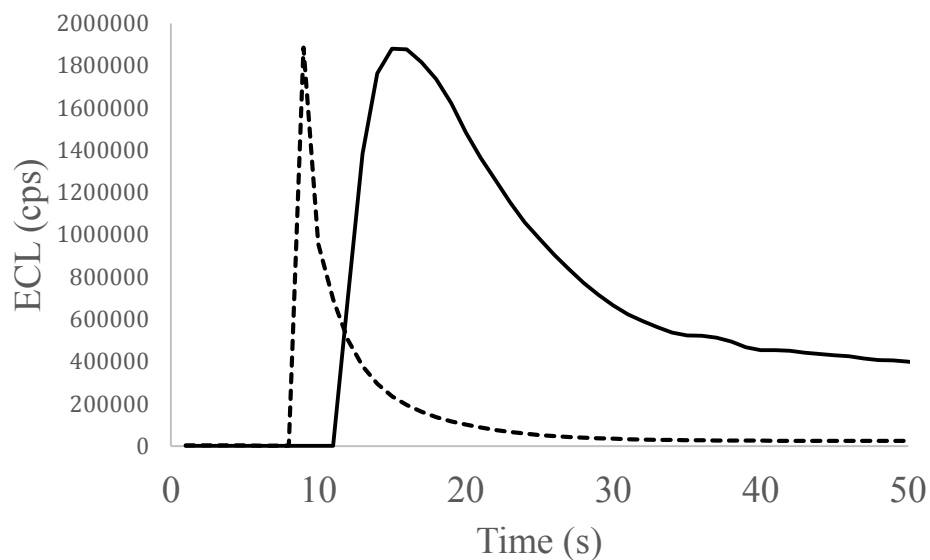


Figure 11. ECL Intensity versus time for $1 \times 10^{-6} \text{M}$ (DC-bpy)Ru(bpy) $_2^{2+}$ and 0.05M TPrA in 0.18M KH_2PO_4 with (—) no MLT and (- - -) $1 \times 10^{-5} \text{M}$ MLT. The scans have been normalized to the same scale and offset for clarity. Potential was poised at +2.0V for the duration of the experiment.

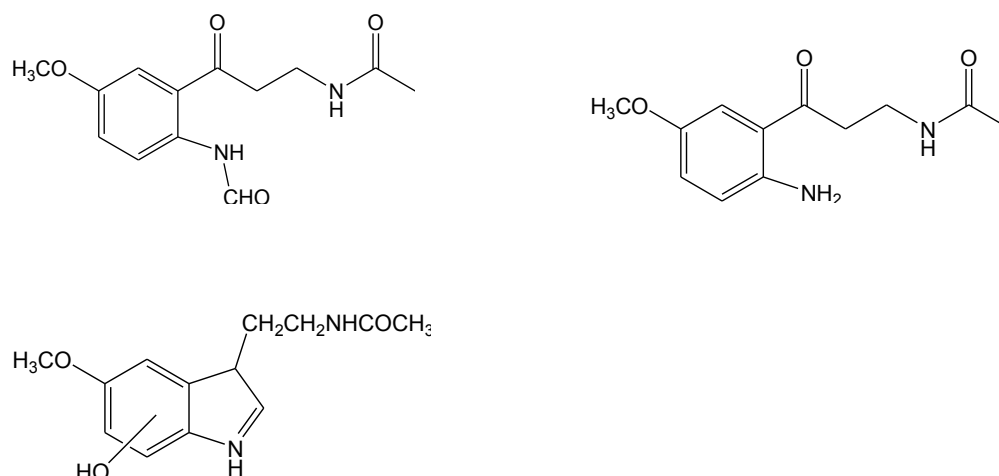


Figure 12. Structures of AFMK (top), HO-MLT (bottom), and AMK (right).

also suggests that a product of melatonin oxidation is responsible for the observed ECL increases.^[29]

A representative example of ECL for a solution of $\text{Ru}(\text{bpy})_3^{2+}$ containing MLT and TPrA is shown in Figure 13. Emission occurs at potentials corresponding to oxidation of both $\text{Ru}(\text{bpy})_3^{2+}$ ($E_a = 1.20 \text{ V}$) and TPrA ($E_a = 0.90 \text{ V}$) and are similar in shape and potential of emission to solutions without MLT present. ECL emission spectra (Figure 11) are nearly identical to photoluminescence spectra, indicating the same MLCT excited state is formed in both ECL and PL and that the presence of MLT or oxidation products does not interfere with excited state formation or emission.^[29]

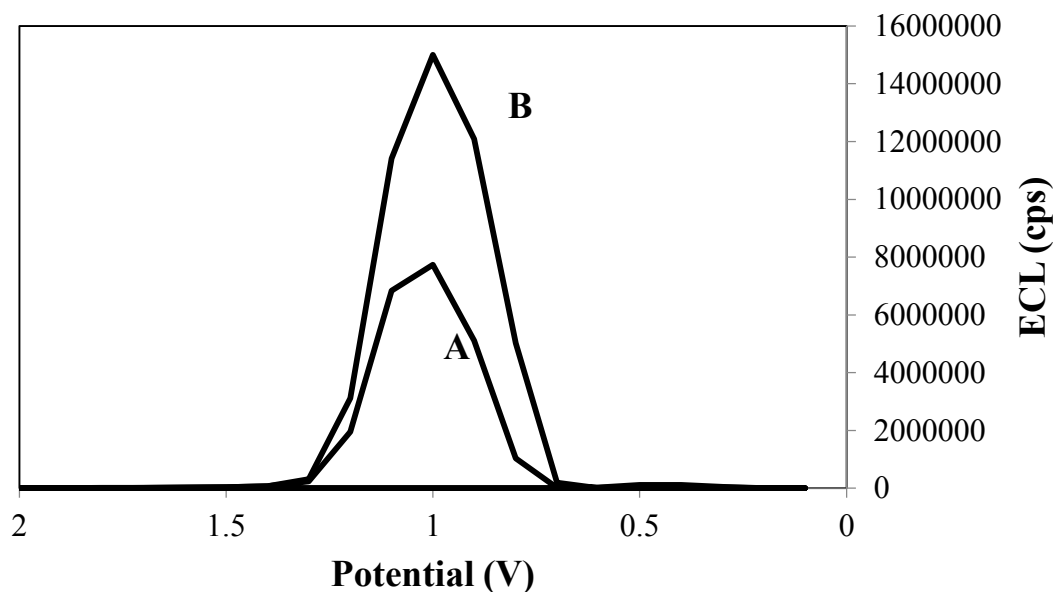


Figure 13. ECL Intensity Versus Potential plots of $1 \times 10^{-5} \text{ M Ru}(\text{bpy})_3^{2+}$ and 0.05 M TPrA in aqueous $0.18 \text{ M KH}_2\text{PO}_4$ ($\text{pH} = 8.0$) with (A) $[\text{MLT}] = 0$ and (B) $[\text{MLT}] = 1 \times 10^{-5} \text{ M}$.

The pH was varied from 4 to 9 with maximum ECL intensities observed between pHs 8 and 9 (Figure 14). Similar trends are observed for $\text{Ru}(\text{bpy})_3^{2+}$ under a variety of

conditions using TPrA as a coreactant. This indicates that the proposed mechanism of TPrA oxidation/deprotonation to form a highly reducing intermediate is operating when MLT is present in solution.^[29]

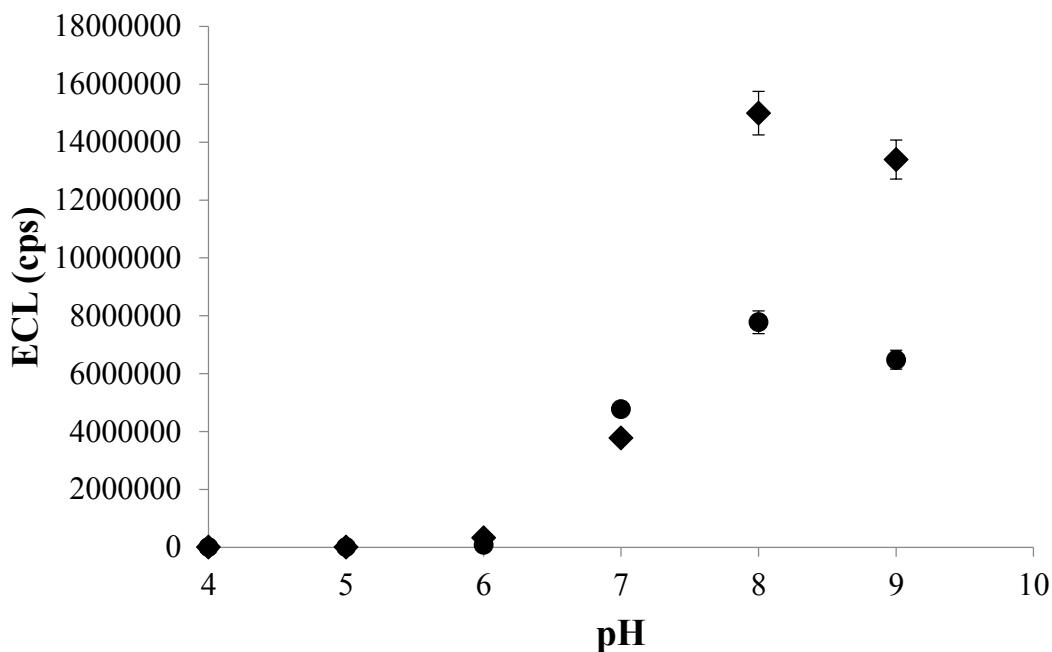


Figure 14. ECL intensity versus pH of 1×10^{-5} M $\text{Ru}(\text{bpy})_3^{2+}$ and 0.05 M TPrA in aqueous 0.18 M KH_2PO_4 solution with (●●●) $[\text{MLT}] = 0$ and (◆◆◆) $[\text{MLT}] = 1 \times 10^{-5}$ M. Each point is the average of at least three scans with error bars at $\pm 5\%$.

An ECL emission intensity versus time profile displays diffusion controlled characteristics (Figure 15). The potential of the working electrode was stepped to +2.0 V, and the ECL intensity measured. As expected, there was a sharp increase in light intensity due to the high concentrations of co-reactant and $\text{Ru}(\text{bpy})_3^{2+}$ near the electrode surface. Under these conditions, the time taken to reach peak intensity was approximately 1 s. At longer times, the ECL emission becomes diffusion controlled. Again, the presence of

melatonin does not appear to interact directly with the $\text{Ru}(\text{bpy})_3^{2+}/\text{TPrA}$ system to change ECL intensities.^[29]

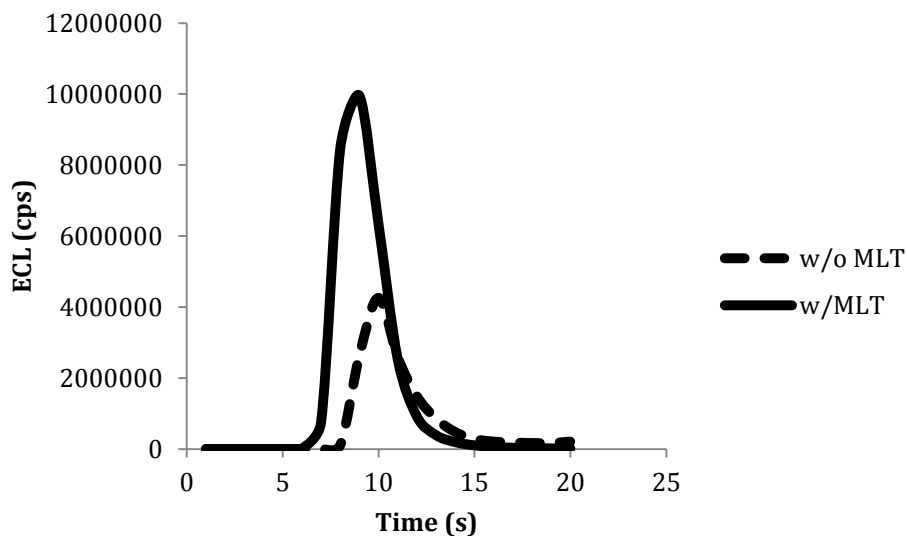


Figure 15. ECL intensity versus time for $1 \times 10^{-5}\text{M}$ $\text{Ru}(\text{bpy})_3^{2+}$ and 0.05M TPrA in aqueous 0.18M KH_2PO_4 solution with (- -) $[\text{MLT}] = 0$ and (-) $[\text{MLT}] = 1 \times 10^{-5}\text{M}$. Each point is the average of at least three scans with a standard deviation of $\pm 5\%$.

Mechanism of Enhancement. The experimental observation that PL is not affected by the presence of MLT while ECL is enhanced suggests that a product or products of melatonin oxidation are responsible for the increases. To test this, bulk electrolysis was coupled with PL spectral analysis. During bulk electrolysis the potential of the working electrode was poised at $+2.0\text{ V}$ vs Ag/AgCl while the solution was stirred, and aliquots tested every 10 minutes for a period of approximately 40-minutes ($\lambda_{\text{exc}} = 450\text{ nm}$).^[29]

A background solution containing TPrA and MLT displayed no PL between 400 and 700 nm. Also, a solution containing TPrA and $\text{Ru}(\text{bpy})_3^{2+}$ with no MLT displayed no

increases in PL intensity during bulk electrolysis. With a MLT/TPrA/Ru(bpy)₃²⁺ solution, however, a 17% increase in PL was observed at 10 minutes that gradually changed to 23% at 40 minutes. No further increases were observed beyond 40 minutes. Another BE/PL experiment was conducted where only MLT in buffer underwent BE. Aliquots were then added to a cuvette containing Ru(bpy)₃²⁺ and TPrA and PL measured, with nearly identical results (i.e., ~23% increase in PL after 40 minutes).^[29]

To probe the phenomenon further a solution of MLT in phosphate buffer was bulk electrolyzed for 40 minutes and added to a solution of Ru(bpy)₃²⁺/TPrA. The ECL of this solution was measured, with a 2.3-fold enhancement relative to a Ru(bpy)₃²⁺/TPrA solution containing no MLT (Figure 16). For reference (Table 4), a Ru(bpy)₃²⁺/TPrA/MLT solution that has not undergone BE prior to mixing displays a 2.5-fold enhancement. This clearly shows that the products of MLT oxidation are leading to increased ECL intensities and efficiencies.^[29]

Several oxidation products have been identified in the literature,^[27,29,49] including N¹-acetyl-N²-formyl-5-methoxykynurenine (AFMK), N¹-acetyl-5-methoxykynurenine (AMK), and hydroxymelatonin (HO-MLT).^[27,29,49] However, the exact nature of the products depends upon solution conditions (e.g., oxygenated versus deoxygenated solutions) and the means by which oxidation was induced (e.g., radiolysis of melatonin solutions, metabolism, or electrochemical). Numerous electrochemical studies^[29,51] show that MLT undergoes a two-electron oxidation followed by a proton transfer with the formation of a cation at the 5-position of the indole ring.^[29]

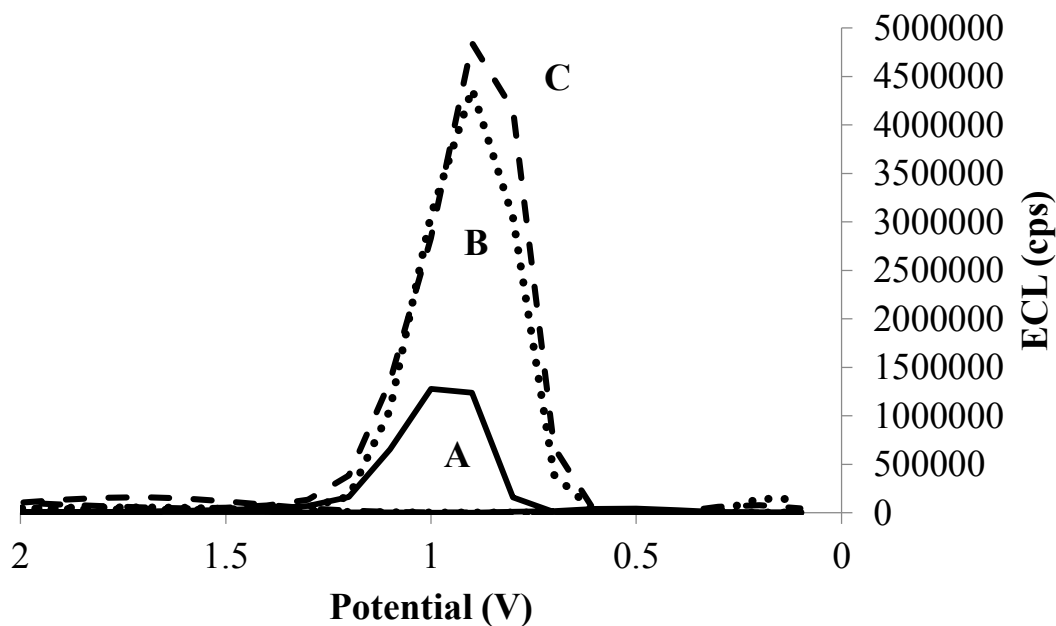


Figure 16. ECL coupled with bulk electrolysis scans of solutions containing different combinations of 1×10^{-7} M $\text{Ru}(\text{bpy})_3^{2+}$, 0.05M TPrA, and 1×10^{-5} M MLT in aqueous 0.18M KH_2PO_4 (pH = 8.0). (A, solid line) $\text{Ru}(\text{bpy})_3^{2+}$ /TPrA in air saturated solution with no bulk electrolysis, $[\text{MLT}] = 0$; (B, dotted line) $\text{Ru}(\text{bpy})_3^{2+}$ /TPrA/MLT in air saturated solution with no bulk electrolysis; (C, dashed line) MLT/TPrA bulk electrolyzed for 40 minutes at a potential of +2.0 V and added to an air saturated solution containing $\text{Ru}(\text{bpy})_3^{2+}$ /TPrA.

Of particular interest to this study is that melatonin quenched H_2O_2 /luminol ECL.^[36] The quenching was traced to the annihilation of the oxygen free radicals by MLT or an oxidation product. These free radicals are an integral part of the light generating mechanism in H_2O_2 /luminol. Also, the generation of AFMK and HO-MLT strongly depended on the presence of molecular oxygen in solution,^[27] showing that MLT interacts directly with dissolved oxygen.^[29]

Ruthenium polypyridyl excited states are sensitive to microenvironmental effects of the solvent media,^[52] such as interactions of the excited state species with solvent,

electrolyte and dissolved oxygen in solution. In particular, the $\text{Ru}(\text{bpy})_3^{2+}$ excited state can be quenched by dissolved oxygen in solution. If a melatonin oxidation product is intercepting dissolved O_2 this might explain why the ECL increases when MLT undergoes electrochemical oxidation, leading to enhanced luminescence. To test this hypothesis, several PL experiments were run in both air saturated and deoxygenated solution. The results are reported in Table 4. Simply deoxygenating a solution results in an approximately 20% increase in PL intensity, consistent with the increases observed in the ECL experiments. Bulk electrolyzing the MLT prior to mixing with the other reagents generates results consistent with deoxygenating with Argon. Also, when the MLT solution is degassed prior to bulk electrolysis the increase in PL is approximately 2% compared to the air saturated bulk electrolyzed solution suggesting that oxygen scavenging is an integral part of the enhancement mechanism. Also, several ECL experiments were run in air saturated and deoxygenated solutions and compared to the ECL of a luminophore/TPrA/MLT solution (Figure 17). To summarize, an air saturated solution containing $(\text{DC-bpy})\text{Ru}(\text{bpy})_2^{2+}$ and TPrA with no MLT displayed ECL of approximately 1,100,000 counts per second (cps). A degassed solution containing $(\text{DC-bpy})\text{Ru}(\text{bpy})_2^{2+}$ and TPrA with no MLT displayed ECL of approximately 2,200,000 cps while an air saturated solution containing $(\text{DC-bpy})\text{Ru}(\text{bpy})_2^{2+}$ and TPrA with 5mM MLT displayed ECL of approximately 1,800,000 counts per second (cps). Clearly, the presence of dissolved oxygen influences the ECL in these systems.^[29,49]

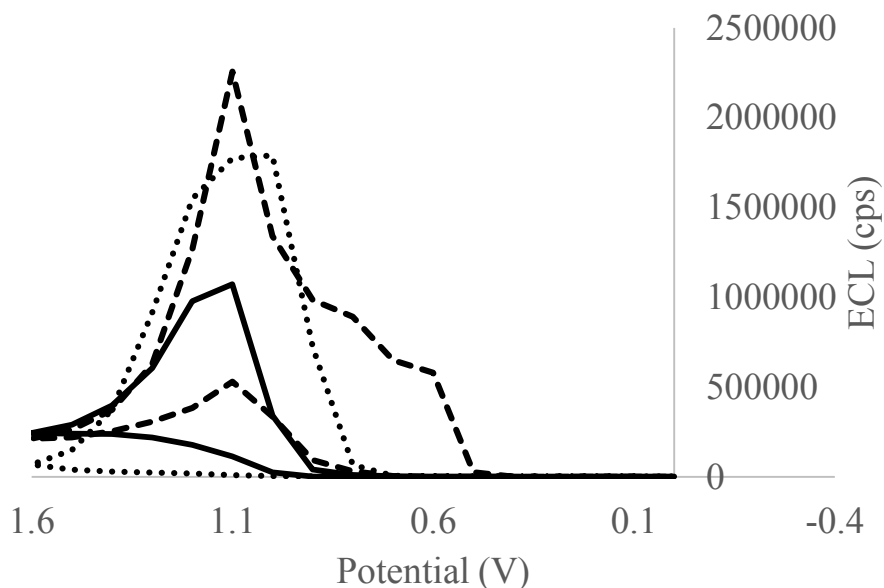


Figure 17. ECL intensity versus potential scans of solutions containing different combinations of 1×10^{-6} M (DC-bpy)Ru(bpy) $_2^{2+}$, 0.05M TPrA, and 5mM MLT in aqueous 0.18M KH $_2$ PO $_4$ (pH = 8.0). (Solid line) (DC-bpy)Ru(bpy) $_2^{2+}$ /TPrA in air saturated solution with [MLT] = 0; (Dotted line) (DC-bpy)Ru(bpy) $_2^{2+}$ /TPrA/MLT in air saturated solution; (Dashed line) (DC-bpy)Ru(bpy) $_2^{2+}$ /TPrA in a solution degassed for a minimum of 10 minutes with argon.

ECL in 2,2,2-trifluoroethanol (TFE) and Triton X-100 (TX100) Solutions.

Numerous methods have been reported for increasing ECL intensities, including the addition of surfactants to solutions, changing the nature of luminophore and coreactants and changing solution composition.^[1] However, none to our knowledge have observed ECL enhancement in the presence of oxygen scavengers. For example, dramatic increases in ϕ_{ecl} ranging from 6- to 270-fold were reported when fluorinated alcohol:water mixtures were combined with the Ru(bpy) $_3^{2+}$ /TPrA system.^[53] In particular, 30:70 (v/v) 2,2,2-trifluoroethanol (TFE):H $_2$ O results in a 270-fold increase in

$\text{Ru}(\text{bpy})_3^{2+}/\text{TPrA}$ ECL and potentially much lower detection limits compared to $\text{Ru}(\text{bpy})_3^{2+}/\text{TPrA}$ in water.^[29]

Therefore, the effect of melatonin in $\text{Ru}(\text{bpy})_3^{2+}/\text{TPrA}$ ECL in 30:70 (v/v) TFE:H₂O was investigated. An approximately 273-fold increase in ECL was obtained in a solution containing $\text{Ru}(\text{bpy})_3^{2+}/\text{TPrA}/\text{MLT}$ 30:70 (v/v) TFE:H₂O, and an approximately 270-fold increase in a solution containing $\text{Ru}(\text{bpy})_3^{2+}/\text{TPrA}$ in 30:70 (v/v) TFE:H₂O compared to a $\text{Ru}(\text{bpy})_3^{2+}/\text{TPrA}$ standard in aqueous buffered solution ($\phi_{\text{ECL}} = 1$). Clearly, the ECL enhancement of MLT and TFE is additive in the $\text{Ru}(\text{bpy})_3^{2+}/\text{TPrA}$ system.^[29]

Similarly, the effect of MLT on the surfactant effect with $\text{Ru}(\text{bpy})_3^{2+}/\text{TPrA}$ was investigated. This effect is well established^[54] and has been traced to adsorption of a surfactant layer on the surface of an electrode. In the case of TX100 this renders gold and platinum surfaces more hydrophobic, facilitating pre-concentration of coreactant and $\text{Ru}(\text{bpy})_3^{2+}$ near the surface of the electrode and more light emitting events. $\text{Ru}(\text{bpy})_3^{2+}/\text{TPrA}$ in potassium phosphate buffer produced an ECL signal of 9.9×10^5 cps (counts per second); $\text{Ru}(\text{bpy})_3^{2+}/\text{TPrA}$ with MLT 2.6×10^6 cps, $\text{Ru}(\text{bpy})_3^{2+}/\text{TPrA}$ with TX100 7.2×10^6 cps and $\text{Ru}(\text{bpy})_3^{2+}/\text{TPrA}$ with both MTL and TX100 1.3×10^7 cps showing that a combination of methods can be used to enhance $\text{Ru}(\text{bpy})_3^{2+}/\text{TPrA}$ ECL.^[29]

Conclusions

Weak ECL has been observed from aqueous solutions of MLT using TPrA as an oxidative-reductive coreactant. When $\text{Ru}(\text{bpy})_3^{2+}$ is present in solution, MLT enhances the ECL emission by approximately 2.5-fold (Table 5). The enhancement by MLT is

linear over a wide concentration range leading to its possible use in ECL applications and assays. Detailed spectroscopic, electrochemical and spectroelectrochemical studies indicate that products of MLT oxidation are responsible for the enhancement. Also, the mechanism appears to be solution phase oxygen “scavenging”. These scavenging reactions prevent the quenching of the $^*\text{Ru}(\text{bpy})_3^{2+}$ excited states by dissolved oxygen in solution opening up a new method for enhancing ECL in fundamental and applied studies. Melatonin can be coupled with 30% by volume 2,2,2-trifluorethanol (TFE) or with 3% (v/v) Triton X-100 for even greater enhancement of $\text{Ru}(\text{bpy})_3^{2+}/\text{TPrA}$ ECL. Furthermore, ECL enhancement has been observed in ruthenium chelates and iridium complexes when melatonin is present in solution.

Table 5. Melatonin electrogenerated chemiluminescence data.^a

Solution Composition	Analyte/Coreactant ^b	ECL (cps) ^c	ϕ_{ECL} ^d
KH ₂ PO ₄	—————	2.3 x 10 ³	3.4 x 10 ⁻⁴
KH ₂ PO ₄ , TPrA	—————	3.8 x 10 ³	5.6 x 10 ⁻⁴
KH ₂ PO ₄ , Ru(bpy) ₃ ²⁺	—————	3.9 x 10 ³	5.7 x 10 ⁻⁴
KH ₂ PO ₄ , TPrA, Ru(bpy) ₃ ²⁺	—————	6.8 x 10 ⁶	1.0
KH ₂ PO ₄ , Ru(bpy) ₃ ²⁺	MLT	3.6 x 10 ⁴	0.0053
KH ₂ PO ₄ , TPrA, Ru(bpy) ₃ ²⁺	MLT	1.7 x 10 ⁷	2.5
KH ₂ PO ₄ , TPrA, (bpy) ₂ Ru(DC-bpy)	—————	1.3 x 10 ⁷	1.0
KH ₂ PO ₄ , TPrA, (bpy) ₂ Ru(DC-bpy)	MLT	1.8 x 10 ⁷	1.4
KH ₂ PO ₄ , TPrA, (bpy) ₂ Ru(DM-bpy)	—————	6.5 x 10 ⁶	1.0
KH ₂ PO ₄ , TPrA, (bpy) ₂ Ru(DM-bpy)	MLT	8.9 x 10 ⁶	1.4
KH ₂ PO ₄ , TPrA, Ir(ppy) ₃	—————	9.5 x 10 ³	1.0
KH ₂ PO ₄ , TPrA, Ir(ppy) ₃	MLT	1.5 x 10 ⁴	1.7

^a[KH₂PO₄] = 0.18 M (aqueous buffered solution) at pH 8.0 ± 0.1. [Ru(bpy)₃²⁺] = 0.01 mM. [TPrA] = 0.05 M.

^bWhen TPrA is absent in the solution composition, then the species listed acts as a coreactant. If TPrA is present the species is an analyte. All species were present in solution at 0.005 M.

^cCPS = counts per second.

^dRelative ECL efficiency (photons emitted per ejected) using Ru(bpy)₃²⁺/TPrA/KH₂PO₄ as a standard (ϕ_{ECL} = 1). All values are the averages of at least 2-replicates with a standard deviation of ±5% (or 0.05).

CHAPTER 5: FUTURE WORK

This project can be expanded in several ways. There are many other luminophore/coreactant combinations that could be studied with MLT to see if enhancement occurs (e.g., $\text{Ru}(\text{bpy})_3^{2+}/\text{S}_2\text{O}_8^{2-}$) ECL in those as well. So far, the ECL enhancement MLT offers has only been studied in inorganic systems, but there are numerous inorganic and organic systems that could be utilized. Another possibility for further study is to bind the luminophore to a biological molecule and test its physical applicability in a bioassay with MLT in the buffer/assay solution. Finally, it would be interesting to test other molecules similar to melatonin that form products that are oxygen scavengers that might also prevent quenching.

REFERENCES

- [1] (a) A.J. Bard, J.D. Debad, J.K. Leland, G.B. Sigal, J.L. Wilbur, J.N. Wohlstadter, Chemiluminescence Electrogenated, in: R.A. Meyers (Ed), Encyclopedia of Analytical Chemistry, Wiley, Chichester, 2000, pp. 9842-9849.
 (b) L.R. Faulkner, A.J. Bard, Electrogenated Chemiluminescence, in: A.J. Bard (Ed), Electroanalytical Chemistry, Volume 10, Marcel Dekker, New York, 1977, pp. 1-95.
 (c) L.R. Faulkner, R.S. Glass, Electrochemiluminescence, in: A. Waldemer, C. Giuseppe (Eds), Chemical and Biological Generation of Excited States, Academic Press, New York, 1982, pp. 197-227.
 (d) A.J. Bard, L.R. Faulkner, Electrochemical Methods, Wiley, New York, 1980.
 (e) A.J. Bard, L.R. Faulkner, Electrochemical Methods Fundamentals and Applications, second ed, Wiley, New York, 2001.
 (f) A.W. Knight, G.W. Greenway, Analyst 119 (1994) 879 - 890.
 (g) R.D. Gerardi, N.W. Barnett, A.W. Lewis, Anal. Chim. Acta 378 (1999) 1 - 10.
 (h) A.V. Kukoba, A.I. Bykh, I.B. Svir, J. Anal. Chem. 368 (2000) 439-442.
 (i) U. Mitschke, P. Bauerle, J. Mater. Chem. 10 (2000) 1471-1507.
 (j) H. Aboul-Enein, R.I. Stefan, J.F. van Staden, X.R. Zhang, A.M. Garcia-Campana, W.R.G. Baeyens, Critical Rev. Anal. Chem. 30 (2000) 271-289.
 (k) A.W. Knight, Electrogenated Chemiluminescence, in: A.M. Garcia-Campagna, W.R.G. Baeyens (Eds.), Chemiluminescence in Analytical Chemistry, Marcel Dekker, New York, 2001, pp. 211 - 247.
 (l) A.M. Andersson, R.H. Schmehl, Mol. Supramol. Photochem. 7 (2001) 153-187.
 (m) K.A. Fahrnich, M. Pravda, G.G. Guilbault, Talanta 54 (2001) 531 - 559.
 (n) M.M. Richter, Electrochemiluminescence, in: F.S. Ligler, C.A. Rowe-Taitt (Eds.), Optical Biosensors: Present and Future, Elsevier, New York, 2002, pp. 173-205.
 (o) S. Kulmala, J. Suomi, Anal. Chim. Acta 500 (2003) 21-69.
 (p) A.W. Knight, Trends in Anal. Chem. 18 (1999) 47-62.
 (q) B.A. Gorman, P.S. Francis, N.W. Barnett, Analyst 131 (2006) 616-639.
 (r) R. Pyati, M.M. Richter, Ann. Rep. C 103 (2007) 12-78
 (s) W. Miao. Chem. Rev. 108 (2008) 2506-2553.
- [2] A.J. Bard. Electrogenated Chemiluminescence, Marcel Dekker, New York, 2004.
- [3] M.M. Richter. Chem. Rev. 104 (2004) 3003 - 3036.
- [4] (a) M.M. Chang, T. Saji, A.J. Bard. J. Am. Chem. Soc. 99 (1977) 5399-5403.
 (b) H.S. White, A.J. Bard. J. Am. Chem. Soc. 104 (1982) 6891-6895.
 (c) M.M. Richter, A.J. Bard, W.K. Kim, R.H. Schmehl. Anal. Chem. 70 (1998) 310 - 318.
- [5] J.K. Leland, M.J. Powell. Electrochem. Soc. 137 (1990) 3127-3131.
- [6] W. Miao, A.J. Bard. Anal. Chem. (2003) 75, 5825.

- [7] J.G. Bruno, J.C. Cornette. *Microchem. J.* (1997) 56, 305.
- [8] E.N. Hudson, G. Weber. Synthesis and characterization of two fluorescent sulfhydryl reagents. *Biochem* 1973;12:4154-4161.
- [9] D. Bruce, J. McCall, M.M. Richter. *Analyst*. 127 (2002) 125-128.
- [10] A.J. Bard, L.R. Faulkner. *Electrochemical Methods Fundamentals and Applications*. 2nd ed. New York: Wiley, 2001.
- [11] (a) M.M. Chang, T. Saji, A.J. Bard. Electrogenated chemiluminescence 30. Electrochemical oxidation of oxalate ion in the presence of luminescers in acetonitrile solutions. *J. Am. Chem. Soc.* 1977;99:5399-5403.
(b) H.S. White, A.J. Bard. Electrogenated chemiluminescence. 41. Electrogenated Chemiluminescence and chemiluminescence of the tris(2,2'-bipyridine)ruthenium(2+)-peroxydisulfate(2-) system in acetonitrile-water solutions. *J. Am. Chem. Soc.* 1982;104:6891-6895.
(c) M.M. Richter, A.J. Bard, W.K. Kim, R.H. Schmehl. Electrogenated chemiluminescence. 62. Enhanced ECL in bimetallic assemblies with ligands that bridge isolated chromophores. *Anal. Chem.* 1998;70:310-318.
- [12] (a) Z. Grabarek, J. Grabarek, P.C. Leavis, J. Gergely. Cooperative binding to the calcium-specific sites of troponin C in regulated actin and actomyosin. *J. Biol. Chem.* 1983;258:14098-14102.
(b) A.J. Bennett, N. Patel, C. Wells, C.R. Bagshaw. 8-Anilino-1-naphthalenesulfonate, a fluorescent probe for the regulatory light chain binding site of scallop myosin. *J. Muscle Research and Cell Motility*. 1984;5:165-182.
(c) G. Loving, B. Imperiali. Thiol-reactive derivatives of the solvatochromic 4-N,N-dimethylamino-1,8-naphthalimide fluorophore: A highly sensitive toolset for the detection of biomolecular interactions. *Bioconj. Chem.* 2009;20:2133-2141.
- [13] C. Frieden, D. Lieberman, H.R. Gilbert. A fluorescent probe for conformation changes in skeletal muscle G-actin. *J. Biol. Chem.* 1980;255:8991-8993.
- [14] P.C. Leavis, E. Gowell, T. Tao. Fluorescence lifetime and acrylamide quenching studies of the interactions between troponin subunits. *Biochem.* 1984;23: 4156-4161.
- [15] A. Hawe, M. Sutter, W. Jiskoot. Extrinsic Fluorescent Dyes as Tools for Protein Characterization. *Pharm. Res.* 2008;25:1487-1499.
- [16] (a) L. Stryer. The interaction of a naphthalene dye with apomyoglobin and apohemoglobin. A fluorescent probe of nonpolar binding sites. *J. Mol. Biol.* 1965;13:482-495.
(b) M. Cardamone, K. Puri. Spectrofluorimetric assessment of the surface hydrophobicity of proteins. *Biochem. J.* 1992;282:589-593.

- (c) J.J. Cocco, J.T. Lecomte. The native state of apomyoglobin described by proton NMR spectroscopy: interaction with the paramagnetic probe HyTEMPO and the fluorescent dye ANS. *Protein Sci.* 1994;3:267–281.
- (d) D.M. Togashi, A.G. Ryder. A fluorescence analysis of ANS bound to bovine serum albumin: Binding properties revisited by using energy transfer. *J. Fluoresc.* 2008;18:519–526.
- [17] M.A. Capelle, R. Gurny, T. Arvinte. High throughput screening of protein formulation stability: Practical considerations. *Eur. J. Pharm. Biopharm.* 2007;65:131–148.
- [18] (a) T.L. Chang, H.C. Cheung. A model for molecules with twisted intramolecular charge transfer characteristics: solvent polarity effect on the nonradiative rates by dyes in a series of water-ethanol mixed solvents. *Chem. Phys. Lett.* 1990;173:343–348.
- (b) K. Das, N. Sarker, D. Nath, K. Bhattacharyya. Nonradiative pathways of anilidonaphthalenesulfonates: twisted intramolecular charge transfer versus intersystem crossing. *Spectrochim. Acta.* 1992;48:1701–1705.
- [19] A. Hawe, R. Poole, W. Jiskoot. Misconceptions over Foerster resonance energy transfer between proteins and ANS/bix-ANS: Direct excitation dominates dye fluorescence. *Anal. Biochem.* 2010;401:99–106.
- [20] (a) C.W.N. Cumper, A. Singleto. The ultraviolet spectra of aniline, 2-,3-,and4-aminopyridines, and some of their derivatives in n-hexane,1,4-dioxane,ethanol, and water solutions. *J. Chem. Soc. B.* 1968;6:649–651.
- (b) E. Klimtchuk, S. Venyaminov, E. Kurian, W. Wessels, W. Kirk, F.G. Predergast. Photophysics of ANS. I. Protein-ANS complexes: Intestinal fatty acid binding protein and single-trp mutans. *Biophys. Chem.* 12007;25:1–12.
- [21] R. Christian. *Solvents and Solvent Effects in Organic Chemistry*. 3rd ed. New York: Wiley-VCH, 2003.
- [22] P. Hartmann, M.J.P. Leiner, S. Draxler, M.E. Lippitsch. Specific solvent effects of linear alcohols on the emission spectrum and the excited state decay of tris(2,2'-bipyridyl)ruthenium(II). *Chem. Phys.* 1996;207:137-146.
- [23] (a) H. Karatani, M. Kojima, H. Minakuchi, N. Soga, T. Shizuki. Development and charaterization of anodically initiated luminescent detection for alcohols and carbohydrates. *Anal. Chim. Acta.* 1997;337:207-215.
- (b) H. Karatani. An electrochemically triggered chemiluminescence from polyhydric alcohols. *J. Photochem. Photobiol.* 1994;79:71-80.
- (c) H. Karatani, T. Shizuki, Luminescent electrooxidation of methanol in aqueous alkaline media. *Electrochim. Acta.* 1996;41:1667-1676.
- (d) X. Chen, L. Jia, X. Wang, G. Hu. Study of the electrochemiluminescence based on the reaction of hydroxyl compounds with ruthenium complex. *Anal. Sci.* 1997;13:71-75.
- (e) X. Chen, M. Sato, Y. Lin. Study of the electrochemiluminescence based on tris(2,2'-

- bipyridine)ruthenium(II) and alcohols in a flow injection system. *Microchem. J.* 1998;58:13-20.
- (f) X. Chen, L. Jia, M. Sato, Study on the electrochemiluminescence based on tris(2,2'-bipyridine)ruthenium and methanol. *Huaxue Xuebao*. 1998;56:238-243.
- [24] R.Y. Lai, A.J. Bard. Electrogenated chemiluminescence. 70. The application of ECL to determine electrode potentials of Tri-n-propylamine, its radical cation, and intermediate free radical in MeCN/Benzene solutions. *J. Phys. Chem. A*. 2003;107:3335–3340.
- [25] D. Bruce, M.M. Richter. Green electrochemiluminescence from ortho-metalated Tris(2-phenylpyridine)iridium(III). *Anal. Chem.* 2002;74:1340-1342.
- [26] D. Bonnefont-Rousselot, F. Collin, Melatonin: Action as antioxidant and potential applications in human disease and aging. *Toxicology*. 278 (2010) 55-67.
- [27] D. Bonnefont-Rousselot, F. Collin, D. Jore, M. Gardes-Albert, Reaction mechanisms of melatonin oxidation by reactive oxygen species in vitro. *J. Pineal Res.* 50 (2011) 328 – 335.
- [28] (a) R.J. Reiter, D. Melchiorri, E. Sewerynek, B. Poeggeler, B. Poeggeler, L. Barlow-Walden, J. Chuang, G.G. Ortiz, D.A. Castroviejo, A review of the evidence supporting melatonin's role as an antioxidant, *J. Pineal Res.* 18 (1995) 1-11.
 (b) A. Imran, H.Y. Aboul-Enein, V.K. Gupta, Analysis of melatonin in dosage formulation by capillary electrophoresis. *J. Liq. Chromatogr. Relat. Technol.* 30 (2007) 545-556.
- [29] M.D. Witt, S. Roughton, T.J. Isakson, M.M. Richter *J. Luminescence* 171 (2016) 118-123.
- [30] A. Levent, Electrochemical determination of melatonin hormone using a boron-doped diamond electrode. *Diamond & Related Materials*. 21 (2012) 114–119.
- [31] (a) E. Chanut, J. Nguyen-Legros, C. Versaux-Botteri, J.H. Trouvin, J.M. Launay, Determination of melatonin in rat pineal, plasma and retina by high-performance liquid chromatography with electrochemical detection, *J Chromatogr. B Biomed. Sci. Appl.* 709 (1998) 11-18.
 (b) R.E. Galian, A.V. Veglia, R.H. de Rossi, Hydroxypropyl- β -cyclodextrin enhanced fluorimetric method for the determination of melatonin and 5-methoxytryptamine, *Analyst*. 125 (2000) 1465-1470.
- [32] (a) J. Lu, C. Lau, M. Koo Lee, M. Kai, Simple and convenient chemiluminescence method for the determination of melatonin, *Anal. Chim. Acta.* 455 (2002) 193-198.
 (b) N. Sugiyama, M. Akutagawa, T. Gasha, Y. Saiga, H. Yamamoto, Chemiluminescence of indole derivatives, I, *Bull. Chem. Soc. Jpn.* 40 (1967) 347-350.

(c) G. Chen, X. Xu, J. Duan, M. He, F. Zhang, Chemiluminescent characteristics of some indole derivatives, *Analyst*. 20 (1995) 1699-1704.

(d) A.A. Alwarthan, Chemiluminescent determination of tryptophan in a flow injection system, *Anal. Chim. Acta*. 317 (1995) 233-237.

[33] (a) R. Viera, J.M. Miguez, M. Lema, M. Aldegunde, Pineal and plasma melatonin as determined by high-performance liquid chromatography with electrochemical detection, *Anal. Biochem.* 205 (1992) 300 – 305.

(b) T. Harumi, H.G. Akutsu, S. Matsushima, Simultaneous determination of serotonin, N-acetylserotonin and melatonin in the pineal gland of the juvenile golden hamster by high-performance liquid chromatography with electrochemical detection, *J. Chromatogr. B*. 675 (1996) 152-156.

(c) L.P. Jose Munoz, M. Rosa Ceinos, L. Jose Soengas, M. Jesus Miguez, A simple and sensitive method for determination of melatonin in plasma, bile and intestinal tissue by high performance liquid chromatography with fluorescence detection, *J. Chromatogr. B*. 877 (2009) 2173–2177.

[34] (a) J. B. Fourtillan, P. Gobin, B. Faye, J. Girault, A highly sensitive assay of melatonin at the femtogram level in human plasma by gas chromatography/negative ion chemical ionization mass spectrometry, *Biol. Mass. Spectrom.* 23 (1994) 499–509.

(b) H.Y. Aboul-Enein, C. Doneau, A. Covaci, Capillary GC-MS determination of melatonin in several pharmaceutical tablet formulations, *Biomed. Chromatogr.* 13 (1999) 24-26.

(c) L.J. Nuenz-Vergara, J.A. Squella, J.C. Sturm, H. Baez, C. Camargo, Simultaneous determination of melatonin and pyridoxine in tables by gas chromatography-mass spectrometry, *J. Pharm. Biomed. Anal.* 26 (2001) 929-938.

[35] (a) G.P. Cartoni, F. Coccioli, R. Jasionowska, M. Masci, Rapid analysis of melatonin in pharmaceutical tablets by capillary electrophoresis with UV detection, *Chromatographica*. 52 (2000) 603-606.

(b) G. Chen, X. Ding, Z. Cao, J. Ye, An optical pH sensor with a linear response over a broad range, *Anal. Chim. Acta*. 48 (2000) 249 – 256.

[36] A. Numan, N.D. Danielson, On-line photo-derivation with flow injection and liquid chromatography atmospheric pressure electrospray mass spectrometry for the identification of indoles, *Anal. Chim. Acta*. 460 (2002) 49 – 60.

[37] (a) H-h. Chu, Y. Wu, J-w. Di, Y-f. Tu, Study on sensitization of electrochemiluminescence of luminol in microemulsion in neutral medium, *Fenxi Siyanshi*. 25 (2006) 6-9.

(b) H-h. Chu, H-Y. Xu, Y. Wu, Y-W. Hu, Y-F. Tu, Determination of melatonin in capsules by quenching electrochemiluminescence analysis. *Fenxi Siyanshi*. 25 (2006) 17-19.

- [38] H. Jiang, X. Wang, Blocking effect on adsorption-controlled electrochemiluminescence of CdSe nanoparticles for detection of the neurotransmitter serotonin. *Chem. Asian J.* 6 (2011) 1533-1538.
- [39] K. Uchikura, M. Kirisawa, Chemiluminescence of tryptophan with electrogenerated tris(2,2'-bipyridine)ruthenium(III), *Chem. Lett.* 8. (1991) 1373 – 1376.
- [40] M. Xiang, R. Lei, N. Li, K. Li, Electrogenerated chemiluminescence of ruthenium(II)bipyridyl complex directly immobilized on glassy carbon electrodes, *J. Appl. Electrochem.* 39 (2009) 921 – 925.
- [41] K.A. King, P.J. Spellane, R.J. Watts, *J. Am. Chem. Soc.* 107 (1985) 1431.
- [42] A. Vogler, H. Kunkely, *Electrochemiluminescence of Organometallics and Other Transition Metal Complexes in ACS Symposium Series No. 333 High Energy Processes in Organometallic Chemistry*; Ed K.S. Suslick; Washington D.C.: American Chemical Society, 1987, p 155.
- [43] (a) M.A. Baldo, S. Lamansky, P.E. Burrows, M.E. Thompson, S.R. Forrest, *Appl. Phys. Lett.* 75 (1999) 4.
 (b) V.V. Grushin, N. Herron, D.D. LeCloux, W.J. Marshall, V.A. Petrov, Y. Wang, *Chem. Comm.* (2001) 1494.
- [44] K. Nishimura, Y. Hamada, T. Tsujioka, K. Shibata, T. Fuyuki, *Jpn. J. Appl. Phys.* 40 (2001) L945.
- [45] E.M. Gross, N.R. Armstrong, R.M. Wightman, *J. Electrochem. Soc.* 149 (2002) E137.
- [46] E. Palecek, in *Topics in Bioelectrochemistry and Bioenergetics*, Vol. 5; G. Milazzo (Ed.); Wiley, London, 1983; p. 65.
- [47] A. Kapturkiewicz, G. Angulo, *Dalton Trans.* 2003, 3907.
- [48] B.D. Muegge, M.M. Richter, *Anal. Chem.*, 76 (2004) 73.
- [49] Sarah Roughton, Mark M. Richter. "Enhanced Electrogenerated Chemiluminescence of Ruthenium and Iridium Coordination Compounds Using Melatonin." *Inorganica Chimica Acta* (2016). Web.
- [50] (a) J. McCall, C. Alexander, M.M. Richter, Quenching of electrogenerated chemiluminescence by phenols, hydroquinones, catechols and benzoquinones, *Anal. Chem.* 71 (1999) 2523 - 2527.
 (b) J. McCall, M.M. Richter, Phenol substituent effects on electrogenerated chemiluminescence quenching, *Analyst.* 125 (2000) 545-548.

- (c) H. Cui, F. Li, M-J. Shi, Y-Q. Pang, X-Q. Lin, Inhibition of Ru complex electrochemiluminescence by phenols and anilines, *Electroanal.* 17 (2005) 589-598.
- (d) Y-Q. Pang, H. Cui, H-S. Zheng, G-H. Wan, L-J. Liu, X-F. Yu, Flow injection analysis of tetracyclines using inhibited Ru(bpy)₃/tripropylamine electrochemiluminescence system, *Luminescence.* 20 (2005) 8-15.
- (e) F. Li, Y-Q. Pang, X-Q. Lin, H. Cui, Determination of noradrenaline and dopamine in pharmaceutical injection samples by inhibition flow injection electrochemiluminescence of ruthenium complexes, *Talanta.* 59 (2003) 627–636
- (f) F. Li, H. Cui, X-Q. Lin, Determination of adrenaline by using inhibited Ru(bpy)₃²⁺ electrochemiluminescence, *Anal. Chem.* 471 (2002) 187–194
- (g) X-Q. Lin, F. Li, Y.Q. Pang, H. Cui, Flow injection analysis of gallic acid with inhibited electrochemiluminescence data, *Anal. Bioanal. Chem.* 378 (2004) 2028–2033.

- [51] (a) A. Radi, G.E. Bekhiet, Voltammetry of melatonin at carbon electrodes and determination in capsules. *Bioelectrochem. Bioenerg.* 45 (1998) 275-279.
- (b) J.L. Corujo-Antuna, S. Martinez-Montequin, M.T. Fernandez-Abedul, A. Costa-Garcia, Sensitive adsorptive stripping voltammetric methodologies for the determination of melatonin in biological fluids, *Electroanalysis.* 15 (2003) 773-778.

- [52] (a) J.V. Caspar, T.J. Meyer, Photochemistry of tris(2,2'-bipyridine)ruthenium(2+) ion (Ru(bpy)₃²⁺). Solvent effects, *J. Am. Chem. Soc.* 105 (1983) 5583-5590.
- (b) J. Van Houten, R.J. Watts, Temperature dependence of the photophysical and photochemical properties of the tris(2,2'-bipyridyl)ruthenium(II) ion in aqueous solution, *J. Am. Chem. Soc.* 98 (1976) 4853-4858.

- [53] D.J. Vinyard, M.M. Richter. Enhanced electrogenerated chemiluminescence in the presence of fluorinated alcohols, *Anal. Chem.* 79 (2007) 6404 – 6409.

- [54] (a) P.M. McCord, A.J. Bard. Electrogenerated chemiluminescence. Part 54. Electrogenerated chemiluminescence of ruthenium(II)4,4'-diphenyl-2,2'-bipyridine and ruthenium(II) 4,7-diphenyl-1,10-phenanthroline systems in aqueous and acetonitrile solutions. *J. Electroanal. Chem.* 31 (1991) 91–99.
- (b) Y. Zu, A.J. Bard. Electrogenerated chemiluminescence. 67. Dependence of light emission of the tris(2,2'-bipyridyl)ruthenium(II)/tripropylamine system on electrode surface hydrophobicity. *Anal. Chem.* 73 (2001) 3960-3964.
- (c) S. Workman, M.M. Richter. The effects of nonionic surfactants on the tris(2,2'-bipyridyl)ruthenium(II)-tripropylamine electrochemiluminescence system. *Anal. Chem.* 72 (2000) 5556-5561.
- (d) B. Factor, B. Muegge, S. Workman, E. Bolton, J. Bos, M.M. Richter. Surfactant chain length effects on the light emission of tris(2,2'-bipyridyl)ruthenium(II)/tripropylamine electrogenerated chemiluminescence. *Anal. Chem.* 73 (2001) 4621-4624.