Evaluation of Selected Platinum Group Metal Complexes as Chemiluminescence Reagents

by

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Date  
*November 12th, 2012*
Patience, Determination, Perseverance
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Abstract

Described herein is a series of projects, all designed to extend the scientific knowledge on the chemiluminescence of platinum group metal complexes. This is a broad aim, and the work consolidates five distinct lines of research.

The chiral nature of platinum group metal complexes such as tris(2,2′-bipyridyl)ruthenium(II) (\([\text{Ru(bipy)}_3]^{2+}\)) presents an intriguing possibility: its use as a chirally selective reagent. The enantiomers were successfully isolated from a racemic mixture. The selectivity of this isolation was confirmed by both capillary electrophoresis and chiral stationary phase high performance liquid chromatography. While both separation techniques are suitable for this application, the use of capillary electrophoresis is advantageous as it provided better resolution in a shorter separation time. The enantiomers were then reacted with the enantiomers of tartaric acid, benzoyletartrate, proline and 4-hydroxyproline (using a range of oxidants) to determine if any differences in the reaction kinetics could be observed for the different combinations of isomers. The larger, more chiral analytes showed some small differences in their kinetic profiles with the \([\text{Ru(bipy)}_3]^{2+}\) enantiomers. Further work is required to confirm this finding.

The immobilisation of simple \([\text{Ru(bipy)}_3]^{2+}\) analogues has been explored. A small suite of 2,2′-bipyridine ligands have been bonded to silica via a siloxane bond, then co-ordinated to Ru(bipy)₂Cl₂. These functionalised silicas differ in the length and rigidity of the linker between the 2,2′-bipyridine moiety and the silica, as well as the protecting groups on the siloxane. The analytical tests of these silicas revealed that, contrary to some literature reports [1, 2], a single siloxane bond between the linking 2,2′-bipyridine unit and the silica is sufficiently robust to prevent leaching. The compound containing a methyl linker to the silica and tertiary butyl protecting groups on the silane showed both the greatest sensitivity and the greatest stability of the compounds studied. Perhaps the most intriguing discovery to come from this work is the observation that the each of the functionalised silicas experienced a decay over time, but if they were given a rest period then tested again, their chemiluminescence intensity would have regenerated. When the same cell was tested over three consecutive days (with an overnight regeneration period), the initial intensity and decay profile would be identical on each day.

The chemiluminescence chemistry of ruthenium(II) complexes has been extended to other platinum group metal complexes. The use of iridium(III) and osmium(II) complexes as
analytical reagents has mostly been restricted to electrochemiluminescence techniques, although the chemiluminescence of iridium(III) complexes has been demonstrated [3]. The most promising candidate, (4,7-diphenyl-1,10-phenanthroline)bis(2-phenylpyridine)iridium(III) ([Ir(ppy)$_2$BPS$^-$]), and its fluorinated analogue ([Ir(dfppy)$_2$BPS$^-$]) have been examined in an extension of this study. By substituting 2-phenylpyridine for 2-(4,6-difluorophenyl)pyridine, the emission energy is hypsochromically shifted, and so this is the first example of colour tuning the emission of such a chemiluminescence reagent. This complex has a lower optimal concentration than [Ru(bipy)$_3$]$^{2+}$, and at this concentration can be the more sensitive reagent.

The chemiluminescence of osmium(II) complexes was also explored. This is the first time the chemiluminescence of such complexes has been documented. Reagents Os 1, Os 2, Os 4, Os 5 and Os 6 were all coordinated to two 1,10-phenanthroline derivatives and either a diphosphine or a diarsine ligand; Os 3 was coordinated to two diarsine ligands and a single 1,10-phenanthroline analogue. The presence of two diarsine ligands induces a hypsochromic shift in the emission energy and also impacts negatively on the chemiluminescence and electrochemiluminescence intensities. Os 1 and Os 5 showed greater potential as ECL reagents than [Ru(bipy)$_3$]$^{2+}$, while Os 6 showed a good chemiluminescence performance.

The diverse spectral and electrochemical properties of the iridium(III) and osmium(II) complexes studied have been further exploited in the development of a dual emission system. In this system, two complexes with spectrally distinct properties are combined in a single solution and detected by ECL. Various combinations of [Ir(ppy)$_3$], [Ir(dfppy)$_2$BPS$^-$] (as the high energy emitters) and [Ru(bipy)$_2$(Me-ALA-bipy-dc)]$^{2+}$, Os5 and Os6 (as the low energy emitters) were tested, and the emission of each complex was successfully resolved in each case. The control afforded by electrochemical oxidation also allowed the selective excitation of one of the chemiluminophores in the mixture; in the case of the [Ir(ppy)$_3$] combinations, both complexes could be oxidised and their emission detected at the exclusion of the other.
Acknowledgements

It's been a long and exhausting ride, but here I am! Thesis actually in hand. Well, electronically, anyway. No PhD is completed by one person in isolation, and therefore no thesis is complete without a long list of people to thank. I have drawn upon the help of many people, too many to mention, but here I go.

To everyone who has helped me throughout the years: Ms Gail Dyson, Ms Donna Squire, Dr Stephen Moran, Dr Michelle Moran (née Gange), Prof. Mark Richter, Assoc. Prof. Michael Breadmore, Dr Conor Hogan and his research group, Dr Jessica Terry - a sincere and heart-felt thank-you. To Dr Tiffany Goodie, for being awesome and for the mass spec help, and to Dr Gregory Barbante and (soon to be) Dr Egan Doeven, thanks for the crash course in ECL and for making my work fun. Your help and support have been invaluable and I couldn't have done this without you. Greg, you are worth your weight in gold, and Egan, we made the dual emitter stuff work when no-one else could because we are just that damn good 😊 An especial thank-you to Tif, Neil, Paul, Greg, Xav, Luke, Jacqui, Darlene, Paul and Joanne for proofing this manuscript for me.

To my supervisors: Dr Paul Francis, Prof Neil Barnett and Dr Luke Henderson, thanks for inspiring me to be the scientist I am. Thank-you for your guidance and (above all!!) patience; for challenging me; for allowing me enough rope to run around and research what I wanted and for always being there to rescue me when that rope got tangled around my neck.

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A huge thank-you to my parents for their unwavering support, encouragement and belief in me. You have kept me strong, and it has been greatly appreciated. To my brother, for setting the bar high for me, yet never being too busy to give me a leg-up. There is no doubt in my mind that I would be stark, raving mad if not for your cool logic and insane attempts to make me laugh.

I think I have been babbling for too long now… I'd better wrap this up. I'd like to conclude with some simple words I heard from a very wise man:

"We do many things, to benefit ourselves, our families and our friends, but ultimately:

'if it is to be, it is up to me!' "
List of Publications


### Glossary and List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAS</td>
<td>Atomic Absorption Spectroscopy</td>
</tr>
<tr>
<td>Ancillary ligand</td>
<td>In heteroleptic complexes, the ancillary ligand is different to the main two ligands present. For example, in [Ir(ppy)$_2$BPS]$^-$, the 2-phenylpyridine is the main ligand and the 4,7-diphenyl-1,10-phenanthroline disulphonate is the ancillary ligand.</td>
</tr>
<tr>
<td>Bathochromic shift</td>
<td>Altering the emission energy to a longer wavelength, also known as ‘red shift’.</td>
</tr>
<tr>
<td>Bipy or bpy</td>
<td>2,2′-bipyridine</td>
</tr>
<tr>
<td>BPS</td>
<td>Bathophenanthroline disulphonate, also known as 4,7-diphenyl-1,10-phenanthroline disulphonate</td>
</tr>
<tr>
<td>C^N</td>
<td>A cyclometallating ligand</td>
</tr>
<tr>
<td>CE</td>
<td>Capillary Electrophoresis</td>
</tr>
<tr>
<td>CSP</td>
<td>Chiral Stationary Phase</td>
</tr>
<tr>
<td>CV</td>
<td>Cyclic Voltammetry</td>
</tr>
<tr>
<td>Cyclometallating</td>
<td>A ring formed by a carbon-metal bond. In the context used here, it refers to a ligand that forms a five-membered ring with the transition metal centre through a nitrogen-metal coordination bond and a carbon-metal ionic bond.</td>
</tr>
<tr>
<td>dfppy</td>
<td>2-(2,4-difluorophenyl)pyridine</td>
</tr>
<tr>
<td>dppene</td>
<td>1,2-bis(diphenylphosphino)ethene</td>
</tr>
<tr>
<td>ECL</td>
<td>Electrochemiluminescence</td>
</tr>
<tr>
<td>Energy Gap Law</td>
<td>As the emission energy decreases, there is a concurrent increase in the rate of radiationless decay.</td>
</tr>
<tr>
<td>EOF</td>
<td>Electroosmotic Flow</td>
</tr>
<tr>
<td>FIA</td>
<td>Flow Injection Analysis</td>
</tr>
<tr>
<td>Heteroleptic Complex</td>
<td>Containing at least two different molecules as ligands. In iridium(III) complexes, there are generally two cyclometallating ligands and one ancillary ligand that is either a diketonate or polypyridine.</td>
</tr>
<tr>
<td>HOMO</td>
<td>Highest Occupied Molecular Orbital</td>
</tr>
<tr>
<td>Homoleptic Complex</td>
<td>Containing three identical ligands</td>
</tr>
<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
</tr>
<tr>
<td>Hypsochromic shift</td>
<td>Altering the emission energy to a shorter wavelength, also known as ‘blue shift’.</td>
</tr>
<tr>
<td>ILCT</td>
<td>Intra-Ligand Charge Transfer</td>
</tr>
<tr>
<td>ILET</td>
<td>Inter-Ligand Energy Transfer</td>
</tr>
<tr>
<td>[Ir(dfppy)$_2$BPS]$^-$</td>
<td>Bis[2-(4,6-difluorophenyl)pyridinato-C$_2$N](4,7-diphenyl-1,10-phenanthroline disulphonate)iridium(III)</td>
</tr>
<tr>
<td>[Ir(dfppy)$_3$]</td>
<td>Tris(2-(4,6-difluorophenyl)pyridinato-C$_2$N)iridium(III)</td>
</tr>
<tr>
<td>[Ir(ppy)$_2$BPS]$^-$</td>
<td>Bis[2-phenylpyridinato-C$_2$N](4,7-diphenyl-1,10-phenanthroline disulphonate)iridium(III)</td>
</tr>
<tr>
<td>[Ir(ppy)$_3$]</td>
<td>Tris(2-phenylpyridine)iridium(III)</td>
</tr>
<tr>
<td>LEC or LECC</td>
<td>Light-Emitting Electrochemical Device</td>
</tr>
<tr>
<td>LED</td>
<td>Light Emitting Diode</td>
</tr>
<tr>
<td>LUMO</td>
<td>Lowest Unoccupied Molecular Orbital</td>
</tr>
<tr>
<td>LX</td>
<td>A diketonate ligand</td>
</tr>
<tr>
<td>MLCT</td>
<td>Metal-to-Ligand Charge Transfer</td>
</tr>
<tr>
<td>N^N</td>
<td>A diimine ligand</td>
</tr>
<tr>
<td><strong>NMR</strong></td>
<td>Nuclear Magnetic Resonance</td>
</tr>
<tr>
<td><strong>OLED</strong></td>
<td>Organic Light Emitting Diode</td>
</tr>
<tr>
<td><strong>Os 1</strong></td>
<td>(1,2-bis(dicyclohexylphosphino)ethane)bis(3,4,7,8-tetramethyl-1,10-phenanthroline)osmium(II)</td>
</tr>
<tr>
<td><strong>Os 2</strong></td>
<td>(1,2-bis(dimethylphosphino)ethane)bis(4,7-diphenyl-1,10-phenanthroline)osmium(II)</td>
</tr>
<tr>
<td><strong>Os 3</strong></td>
<td>Bis(1,2-bis(dimethylarseno)benzene)(4,7-diphenyl-1,10-phenanthroline)osmium(II)</td>
</tr>
<tr>
<td><strong>Os 4</strong></td>
<td>(1,2-bis(dimethylphosphino)ethane)bis(3,4,7,8-tetramethyl-1,10-phenanthroline)osmium(II)</td>
</tr>
<tr>
<td><strong>Os 5</strong></td>
<td>(1,2-bis(dimethylarseno)benzene)bis(3,4,7,8-tetramethyl-1,10-phenanthroline)osmium(II)</td>
</tr>
<tr>
<td><strong>Os 6 or [Os(dppene)(phen)2]</strong></td>
<td>(1,2-bis(diphenylphosphino)ethene)bis(1,10-phenanthroline)osmium(II)</td>
</tr>
<tr>
<td><strong>[Os(phen)3]</strong></td>
<td>Tris(1,10-phenanthroline)osmium(II)</td>
</tr>
<tr>
<td><strong>PBS</strong></td>
<td>Phosphate buffer solution</td>
</tr>
<tr>
<td><strong>phen</strong></td>
<td>1,10-phenanthroline</td>
</tr>
<tr>
<td><strong>ppy</strong></td>
<td>2-phenylpyridine</td>
</tr>
<tr>
<td><strong>[Ru(bipy)2(Me-ALA-bipy-dc)]</strong></td>
<td>Bis(2,2'-bipyridine)(N^4,N^4'-Bis((2S)-1-methoxy-1-oxopropan-2-yl)(2,2'-bipyridyl-4,4'-dicarboxamide)ruthenium(II)</td>
</tr>
<tr>
<td><strong>[Ru(bipy)3]</strong></td>
<td>Tris(2,2'-bipyridyl)ruthenium(II)</td>
</tr>
<tr>
<td><strong>[Ru(phen)3]</strong></td>
<td>Tris(1,10-phenanthroline)ruthenium(II)</td>
</tr>
<tr>
<td><strong>SMAxx</strong></td>
<td>A series of [Ru(bipy)2(N^N)] complexes immobilised to silica beads via a siloxane bond on the ancillary diimine ligand</td>
</tr>
<tr>
<td><strong>TBAPF6</strong></td>
<td>Tetrabutylammonium hexafluorophosphate</td>
</tr>
<tr>
<td><strong>Triplet-Triplet (T-T) Annihilation</strong></td>
<td>The interaction of two excited state molecules upon collision, which results in one molecule in an excited state and the other in the ground state</td>
</tr>
<tr>
<td><strong>UV-vis</strong></td>
<td>The ultraviolet and visible region of the electromagnetic spectrum</td>
</tr>
</tbody>
</table>
Legend

- Olive: Racemic [Ru(bipy)$_3$]$^{2+}$
- Green: (+)-[Ru(bipy)$_3$]$^{2+}$
- Dark yellow: (-)[Ru(bipy)$_3$]$^{2+}$
- Orange: [Ru(phen)$_3$]$^{2+}$
- Grass green: [Ir(ppy)$_3$]
- Purple: [Ir(ppy)$_2$BPS]$^-$
- Dark cyan: [Ir(dfppy)$_2$BPS]$^-$
- Cyan: [Ir(phen)$_3$]$^{3+}$
- Wine: [Ru(bipy)$_2$(Me-ALA-bipy-dc)]$^{2+}$
- Black: Os 1
- Navy: Os 2
- Red: Os 3
- Blue: Os 4
- Violet: Os 5
- Pink: Os 6 - [Os(dppene)(phen)$_2$]$^{2+}$
- Lime green: SMA61B
- Royal blue: SMA69
- Sky blue: SMA80
- Yellow: SMA81
- Burnt orange: SMA82
- Crimson: SMA90
Chapter One: Introduction
1.1 Chemiluminescence

Since the beginning of time, people have been fascinated by the power to generate light. The sun and moon have been worshipped as gods, he who could create fire held power, and the generation of ‘cold light’ – luminescence – was a phenomenon of awe and mystery. Aristotle was the first to recognise the difference between luminescence and incandescence [4], and since then great minds such as Sir Francis Bacon, Hennig Brand, Robert Boyle and Sir George Gabriel Stokes have pondered on these emissions [4]. It was not until the nineteenth century, however, that the term ‘luminescence’ was conceived [5]. In the pivotal publication *Ueber Fluorescenz und Phosphorescenz I. Abhandlung*, Eilhardt Wiedemann defined luminescence and outlined six categories, dependant on the mode of excitation: photoluminescence (fluorescence and phosphorescence), chemiluminescence, thermoluminescence, cristalloluminescence, triboluminescence and electroluminescence [5].

Chemiluminescence is a marvel of chemistry that has found widespread use in a variety of different applications [4, 6-8]. It is analytically useful, as the generation of light is indicative of the concentration of emitting species present [6]. The instrumentation required is simple, inexpensive and robust, making chemiluminescence an attractive detection methodology [6]. Additionally, these reactions are more sensitive and selective than fluorescence detection [9]. The famed reaction between luminol and the iron in blood produces a bright bluish glow and is an example of direct chemiluminescence: the reaction of two molecules to form an excited state product, which releases energy in the form of a photon [6].

Once excited, a molecule has various options to return to the ground state. The majority of luminescent molecules undergo fluorescence, relaxing to ground state from an excited state of the same spin multiplicity (generally a singlet-to-singlet transition) [10]. Some molecules, however, can undergo intersystem crossing to enter an excited state of different spin multiplicity prior to emission (phosphorescence) [10]. The latter process is spin forbidden and less common than fluorescence, although it can be promoted by the presence of a heavy metal [11]. Spin statistics show that the processes of electron transfer and energy transfer produce 25% singlet and 75% triplet states [12-15]. Theoretically, molecules such as transition metal complexes that can emit from both the singlet and triplet states can attain 100% internal quantum efficiency [12-15]. They are consequently highly prized as efficient luminophores [12-15].
1.2 Ruthenium(II)

1.2.1 Tris(2,2′-bipyridyl)ruthenium(II)

Ruthenium, located in the middle of the \(d\)-block of the periodic table, is a metal that exemplifies many of the properties of transition metals [16]. It forms highly coloured complexes, is inert to many chemicals and has a wide range of formal oxidation states, from VIII to \(-II\) [16, 17]. Discovered in 1827 by Osann and later verified by Karl Klaus (1884), ruthenium (Latin, Ruthenia: Russia) [17, 18] is a rare metal that has been shown to be a versatile catalyst [19] and has been used extensively in the chemical industry [18]. In addition, this \(4d^6\) metal forms a variety of complexes, particularly with bipyridine derivatives. The foremost of these, tris(2,2′-bipyridyl)ruthenium(II) ([Ru(bipy)\(_3\)]^{2+}\)), displays such exemplary excited state properties that it has secured its reign as the prototypical chemiluminescence reagent.

1.2.2 The chemiluminescence of tris(2,2′-bipyridyl)ruthenium(II)

The chemiluminescence of this complex is the result of a redox reaction [9]. [Ru(bipy)\(_3\)]^{2+}\) is oxidised to its ruthenium(III) state, and the subsequent reduction by an appropriate analyte generates the excited state complex [9]. The cycle is completed by the emission of a photon to regenerate the ground state [Ru(bipy)\(_3\)]^{2+}\) (Scheme 1.01) [9].

![Scheme 1.01: The chemiluminescent redox cycle of [Ru(bipy)\(_3\)]^{2+}\) [9].](image-url)
The oxidation of \([\text{Ru(bipy)}_3]^2^+\) is commonly achieved by chemical reaction with lead dioxide \([20, 21]\) or cerium(IV) sulphate \([3, 22-24]\), although permanganate \([25, 26]\), peroxydisulphate \([27, 28]\) and bromate \([29]\) have also been used. This can be performed offline \([20, 21]\), or both reagent and analyte can be oxidised online \([3, 22-24]\). In many cases, greater temporal and spatial control of the reaction is desired, in which case electrogenerated chemiluminescence (ECL) is employed \([30-35]\). There are three classical ECL experiments: the first involves sweeping the applied potential to create both oxidised and reduced forms of the chemiluminophore, which then react together in an annihilation reaction to produce a ground state and an excited state complex \([36]\). The other two methods require the addition of another molecule, known as a co-reactant. The complex can either be reduced in the presence of a strong oxidant (reductive-oxidation) to produce ECL, or oxidised in the presence of a strong reductant (oxidative-reduction) \([36]\). Like the chemiluminescence reactions, the co-reagent pathways are analytically useful, and are routinely used to determine the presence of co-reactants such as tripropylamine (TPA) \([37-39]\), oxalate \([3, 7, 22, 39-42]\), peroxydisulphate \([28, 38]\) and codeine \([7, 43]\), and (unlike the annihilation reaction, which requires organic solvents with a large potential window \([36]\)) can be performed in an aqueous environment \([9, 34, 36]\). As a result, both chemiluminescence and ECL have been invaluable tools in clinical/diagnostic, environmental, biological and analytical sciences \([7, 36]\).

The same \([\text{Ru(bipy)}_3]^2^+\) excited state can also be attained via the absorption of a photon of appropriate energy \([22, 44, 45]\). This has been used in sensing applications such as the detection of molecular oxygen \([46-49]\) and various anions \([50-52]\), and in the design of live cell stains \([53-55]\).

\([\text{Ru(bipy)}_3]^3^+\) will react with tertiary amines and organic acids to return to \([\text{Ru(bipy)}_3]^2^+\), however not all analytes will create the excited state complex \([9]\). There are therefore only a limited number of compounds that will produce the intense emission, and these include analytes of medicinal, pharmaceutical and forensic importance \([7, 8]\). There have been many reviews illustrating the variety of uses of ruthenium(II) complexes in environmental analyses, food and water testing, military/defence applications and clinical diagnostics \([7, 8, 30, 56, 57]\). The desirable photochemical and photophysical properties of ruthenium(II) polypyridine complexes have caused them to play an important role, both currently and historically, in the development and understanding of chemi- and electrochemiluminescence, photochemistry, photoelectrochemistry, photophysics, photocatalysis, electrochemistry, and electron and energy transfer \([57]\). Consequently, they have been propelled to the forefront of many
important areas of science, such as green chemistry (the harnessing of solar energy [58-62], light emitting devices [63, 64] and light mediated catalysis [65, 66]), chemical sensors [46, 47, 67, 68], protein staining [69, 70], cell imaging [55, 71], and in the cleavage, imaging, and intercalation of DNA [55, 72-77]. The detection of DNA in particular has become of great importance in areas of clinical testing, pathogen detection, forensic chemistry and the diagnosis of genetic diseases due to gene mutations [32].

1.2.3 Tuning the properties of ruthenium(II) complexes

Due to the resounding success of [Ru(bipy)₃]²⁺ in a variety of different fields, many researchers have investigated the spectral, physical and chemical properties of [Ru(bipy)₃]²⁺ and its analogues [22, 44, 45, 60, 61, 78]. By altering one or more of the ligands, researchers have been able to change many properties, including the sensitivity and selectivity of the reagents [22, 45]; solubility [22]; photoluminescence quantum yields [61, 78]; chemiluminescence and electrochemiluminescence efficiencies [22, 44, 45]; oxidation and reduction potentials [44]; excited state lifetimes [61, 78, 79]; and wavelengths of absorption [61, 78, 79]. One important property that has proved resistant to change is the emission wavelength. Most luminescent polypyridine ruthenium(II) complexes studied have a red or orange emission [7, 21, 22, 44, 45, 60, 61, 78-83]. Extending the conjugation of the π-system of diimine ligands causes a shift to a longer emission wavelength in these complexes [84]. Efforts to alter the wavelength further have been futile, generally resulting in a non-emissive complex. The reason for this lies in the energy levels of the molecule. The ‘emitting Metal to Ligand Charge Transfer (MLCT) excited state’ is actually a composite of closely lying electronic states that are almost degenerate (at ambient temperatures), and the photophysical properties of the complex can be approximated as arising from an average of the contributing states [85] (for further explanation, see Section 1.3). Another state (e₉) is available at a slightly higher energy level and decays very quickly through radiationless pathways [85]. The e₉ is thermally accessible at ambient temperatures, and its population directly influences the properties of the complex [85]. The easier it is to populate (the closer in energy it is to the Lowest Unoccupied Molecular Orbital or LUMO), the less emissive the complex becomes, until the complex becomes completely non-emissive [85].

The biggest rival to the use of [Ru(bipy)₃]²⁺ is tris(1,10-phenanthroline)ruthenium(II), or [Ru(phen)₃]²⁺. Various researchers have claimed that [Ru(phen)₃]²⁺ provides superior sensitivities than [Ru(bipy)₃]²⁺ as a chemiluminescence reagent [86, 87], whereas others have
refuted this claim [88, 89]. Due to these contradictory assertions, a comprehensive study of [Ru(bipy)$_3$$_n$(phen)$_n$]$^{2+}$ (where $n = 0, 1, 2$ or $3$) has been conducted [45]. The spectroscopic and electrochemical properties of the complexes displayed clear trends – increasing the number of 1,10-phenanthroline ligands both increases the emission energy and decreases the stability of the excited state complex [45]. The latter effect reduces the reversibility of the redox reaction and makes the complexes more reactive with water than [Ru(bipy)$_3$]$^{2+}$ [45]. The sensitivity of the reagent is thus lowered by the larger blank (electro)chemiluminescence signals produced [45]. The relative intensity of the complexes in both chemiluminescence and ECL with a range of analytes/co-reactants is more complicated, and no clear trend was observed [45]. It was concluded that there is no real advantage to using one reagent over the other, and the generalised notion that [Ru(phen)$_3$]$^{2+}$ is a more sensitive reagent than [Ru(bipy)$_3$]$^{2+}$ for all analytes is unfounded [45].

In a quest to design a complex with intense absorption of visible light, a large Stokes shift and efficient emissions for use as a solar energy harnessing dye, Alford et al. systematically evaluated a series of ruthenium(II) and osmium(II) complexes containing substituted 2,2'-bipyridine and 1,10-phenanthroline ligands [61, 78]. The majority of complexes exhibited a longer emission wavelength than the parent complex [61, 78]. This study highlighted the influence of substituent position as well as nature: the 4,4'-bisethoxycarbonyl-2,2'-bipyridine complex did not have as large a bathochromic shift compared to the complex substituted in the 5,5'-position ($\lambda_{\text{max}} = 655$ nm and 720 nm, respectively) [61, 78]. Amongst these complexes, those containing two phenyl groups (4,4'-diphenyl-2,2'-bipyridine and 4,7-diphenyl-1,10-phenanthroline) exhibited the highest quantum yields [61, 78]. These complexes, however, are so hydrophobic that difficulties were encountered during ECL analysis [90]. The addition of sulphate groups to create tris(4,7-diphenyl-1,10-phenanthroline disulphate)ruthenium(II) ([Ru(BPS)$_3$]$^{4-}$) has no effect on the spectrochemical properties, yet it creates a water soluble, analytically useful complex [22, 91, 92]. The complex has subsequently been utilised in many diverse applications, such as an analytical chemiluminescence reagent [22, 91, 92], a protein stain [70, 93] and a DNA label [72, 74]. The intense (electro)chemiluminescence emission of [Ru(BPS)$_3$]$^{4-}$ has been successfully utilised to detect various analytes, including codeine [22], tripropylamine [92, 94], furosemide and hydrochlorothiazide [22, 91], oxalate [22, 95, 96] and piroxicam [97]. It has been demonstrated that [Ru(BPS)$_3$]$^{4-}$ was twice as sensitive as [Ru(bipy)$_3$]$^{2+}$ in the ECL detection of oxalate under the same conditions [96], and a six-fold greater ECL intensity can
be seen in the reaction between $[\text{Ru(BPS)}_3]^{4-}$ and tripropylamine over $[\text{Ru(bipy)}_3]^{2+}$ [92]. The selectivity of $[\text{Ru(BPS)}_3]^{4-}$, in comparison to $[\text{Ru(bipy)}_3]^{2+}$ and $[\text{Ru(phen)}_3]^{2+}$, is analyte dependent; superior detection limits were observed with $[\text{Ru(bipy)}_3]^{2+}$, $[\text{Ru(phen)}_3]^{2+}$ and $[\text{Ru(BPS)}_3]^{4-}$ in their respective reactions with codeine, oxalate and hydrochlorothiazide [22].

A comprehensive study on the chemiluminescence of the $[\text{Ru(bipy)}_{3-n} (\text{BPS})_n (2n-2)]^{-}$ series has found that the presence of BPS ligands in the complex increases the photoluminescence quantum yields and decreases the stability of the oxidised form in aqueous solution [42]. The maximum wavelength of absorbance and the photoluminescence intensity, on the other hand, do not follow a simple trend [42]. It was noted that the BPS-containing complexes produced larger chemiluminescence blank signals than $[\text{Ru(bipy)}_3]^{2+}$ due to the decreased stability of the oxidised state of complexes, and therefore superior signal-to-blank ratios were obtained with $[\text{Ru(bipy)}_3]^{2+}$ [42]. This investigation revealed that there was no significant improvement in either signal intensity or signal-to-blank ratios of the heteroleptic complexes over the homoleptic $[\text{Ru(bipy)}_3]^{2+}$ and $[\text{Ru(BPS)}_2]^{4-}$ [42]. An analogic ECL study, on the other hand, revealed a dramatic improvement in the ECL intensity from the reactions of $[\text{Ru(bipy)}_2 (\text{BPS})]^{2-}$ and $[\text{Ru(bipy)} (\text{BPS})_2]$ with tripropylamine (compared to $[\text{Ru(bipy)}_3]^{2+}$) [94]. The neutral $[\text{Ru(bipy)} (\text{BPS})_2]$ in particular showed up to 26-fold greater intensity signals than $[\text{Ru(bipy)}_3]^{2+}$ [94]. Whilst the chemiluminescence study seems to contradict this, it confirms the conclusion of Della Ciana et al. [94] that the differences in ECL intensity were mainly due to interactions of the charged complexes and the electrode surface, not the reaction between complex and analyte.

The substitution of 4,7-diphenyl-1,10-phenanthroline for 4,7-diphenyl-1,10-phenanthroline disulphonate is an example of changing the ligand structure of a complex in order to alter its solubility [22]. Alternatively, a simple exchange of the counter ion can sometimes achieve the same effect. For example, the chloride salt of $[\text{Ru(bipy)}_3]^{2+}$ is soluble in aqueous solution, whereas the perchlorate salt is more soluble in organic solvents such as acetonitrile [21]. In cases where increased hydrophobicity is desired, long aliphatic chains can be added to the base ligand to create a complex such as tris(4,4′-dinonyl-2,2′-bipyridine)ruthenium(II) [98]. Once immobilised within a poly(tetrafluoroethylene) membrane, the hydrophobicity of the complex discourages leaching [98]. Another application in which the complex’s solubility is critical is in fluorescence cell staining [71]. The complex is required to be lipophilic enough to cross cell membranes, yet hydrophilic enough to be used in aqueous solutions [71, 99].
Many other ruthenium(II) complexes have been investigated, as researchers attempt to design the perfect complex for their particular application. By 1988, approximately 300 \([\text{Ru(bipy)}_3]^{2+}\) analogues had been synthesised and evaluated, as summarised in the review by Juris and collaborators [57], and the research output has not slowed since then. Despite this effort in producing a myriad of analogues over four decades, \([\text{Ru(bipy)}_3]^{2+}\) (and simple derivatives for labelling in immunoassays) has remained the most commonly used reagent.

### 1.3 Crystal-Field Theory and Metal-Ligand Complex Emissions

Crystal Field Theory is a model that describes the electronic structure of transition metal coordination complexes [100]. The interaction between a transition metal and its ligands is a result of a strong attraction between the positive metal ion and the negative charge of the ligand electrons [100]. When in a coordination complex, the metal centre is surrounded by ligands and their electrons, whose close proximity affects the \(d\)-orbitals of the metal [100]. As the ligands draw near to the metal, repulsion occurs between the electrons of the metal and the ligands [11, 100]. The ligands approach the metal along the \(x\), \(y\) and \(z\) planes, causing the electrons to be closer to the \(d\)-orbitals along the planes than those between the planes [11, 100]. The orbitals consequently experience slightly different levels of repulsion and there is an alteration of the energies [11, 100]. Thus, the orbitals are no longer degenerate – they have had their energy levels split, with the orbitals close to the ligands having a higher energy level than those further away [100]. In octahedral complexes, the \(d\)-orbitals are split into two sets, the energy difference of which is defined by the crystal-field splitting parameter [100]. These sets are known as the \(t_{2g}\) and the \(e_g\) orbitals [100]. The \(t_{2g}\) orbitals comprise \(d_{xy}\), \(d_{xz}\) and \(d_{yz}\), and are lower in energy than the \(e_g\) orbitals, \(d_{xz}^2\) and \(d_{yz}^2\) [100]. The extent of this energy difference is affected by the nature of the ligands (for example, cyclometallated ligands are stronger field ligands than diimine ligands and induce greater splitting [101]), the oxidation state of the metal (high oxidation states allows the ligands to approach closer, giving rise to a greater splitting of the orbitals), the position of the metal in the periodic table (the magnitude of the crystal field splitting parameter increases down a group for a given oxidation state and ligand) and the geometry of the complex (octahedral, tetrahedral, etc.) [102]. When the crystal field of the metal-ligand complex is strong enough, the \(e_g\) state is raised above that of the emitting state, causing the complex to exhibit luminescence [11]. The degree of the crystal field splitting parameter determines the sensitivity of the complex to temperature: the larger the crystal field splitting parameter, the less thermally accessible the \(e_g\) state is and
therefore the less likely the complex is rendered non-emissive with increasing temperature (i.e. the more photostable the complex) [11].

The excitation of transition metal complexes is typically a MLCT [85]. In the case of \([\text{Ru(bipy)}_3]^{2+}\), an electron is promoted from the t\textsubscript{2g} orbital (often simplified as the metal orbital [103]) to the t\textsubscript{1u} [85, 89]. This orbital is comprised primarily of the ligand \(\pi^*\) orbital and is of slightly lower energy than the non-emissive e\textsubscript{g} [85, 89]. A singlet state is initially populated after optical excitation, followed by a very rapid intersystem crossing (the efficiency of which is quantitative due to high spin paramagnetism) to the triplet state (Figure 1.01) [85].

![Jablonski diagram of transition metal complexes](image)

Figure 1.01: Jablonski diagram of transition metal complexes, depicting the absorption (solid line) to both the ligand-centred (LC) and metal-to-ligand charge transfer (MLCT) singlet states, intersystem crossing (isc) to the triplet states and their combined emissive state (T\textsubscript{1}), as well as the radiative (wavy line) and non-radiative (dashed line) decay to the ground state. \(k_{isc}\) is the equilibrium constant of intersystem crossing, \(k_{nr}\) the non-radiative decay equilibrium constant and \(k_r\), the radiative decay equilibrium constant [103].

### 1.4 Other Platinum-Group Metals - Iridium(III) and Osmium(II)

Ruthenium is a member of a family of rare metals known as the platinum group [16]. This group includes osmium, rhodium, iridium, palladium and of course, platinum, and they are found in conjunction with each other in platinum ores [16]. Several sources describe how iridium and osmium were discovered by Smithson Tennant, after dissolution of the ore in \textit{aqua regia} resulted in the formation of a black precipitate [104, 105]. After alternate
treatment with alkalis and acids, this residue yielded two previously unknown elements [104-107]. One of these proved extraordinarily resistant to heat, wear, and most chemicals, had considerable hardness and strength, and formed salts of diverse colours [104-107]. This last property prompted Tennant to name the element ‘iridium’, after the Greek messenger goddess and goddess of the rainbow, Iris [105, 106]. The other exhibited similar properties, but was found to be susceptible to the formation of oxides at room temperature, one of which (OsO4) is so ‘pungent and penetrating’ that the element earned the name 'osmium' (Greek, osme: smell) [105, 108, 109]. These elements are often used to impart strength, hardness and durability to various alloys in applications ranging from deep-water pipes, to electrical contacts and even to jewellery [17, 109]. The extraordinary chemical and thermal resistance of iridium has been exploited in long-life engine parts of the aerospace industry [104, 107], and osmium has also been found to exhibit astonishing thermal stability [110]. Osmium is also the most dense of the elements, only slightly more so than iridium (22,590 kg m$^{-3}$ and 22,560 kg m$^{-3}$, respectively, at 20°C) [111].

The organometallic complexes formed from iridium and osmium (like those from ruthenium) possess stable and accessible oxidation and reduction states, and exhibit high triplet quantum yields [80]. The quality that makes them particularly attractive, especially in light emitting diode (LED) and light emitting electrochemical cell (LEC) research, is the large crystal field splitting parameter [12, 80, 101, 112, 113] which causes a greater separation between the LUMO and the higher energy, non-emissive states. Consequently, extraordinary variation is seen in emission wavelengths [14, 80, 101, 114-120], from the near-UV [121] to the near-IR [122-124] and even up to wavelengths over 1000 nm long [123]. Although it has been acknowledged that osmium(II) complexes may have greater potential as blue emitters [80], research into the colour tuning of these reagents has not been as thorough as that of iridium(III) complexes. Additionally, these complexes can exhibit extraordinary photoluminescence quantum yields which can approach unity (97% [101]).

The favourable properties of bipyridine-based complexes of ruthenium(II), iridium(III) and osmium(II) have been applied to various purposes. Applications range from energy conversion [125-127], light emitting devices [63, 64, 80, 101, 116, 128, 129] and visible light mediated catalysts [65, 130, 131], to analytical reagents [3, 132-143], biological probes [72, 144, 145] and as a pressure sensitive paint [49, 110, 146]. The high absorptivities of long wavelength light shown by some osmium complexes has even allowed their use as transcutaneous oxygen sensors [133]. This application allows the non-invasive measurement
of oxygen under physiological conditions in tissue reactors, and from this, it is easy to envision the production of transcutaneous chemical sensors for pH, carbon dioxide and glucose [133].

1.5 Aims and Objectives

There is a bewildering array of organometallic chemiluminescence reagents described in the literature and summarised in reviews such as those by Evans et al., Gorman et al., Richter, Baranoff et al. and Knight [7, 30, 80, 116, 147]. Each complex exhibits properties specifically tailored to its application, applications which are extraordinarily diverse, and hence require a variety of physical and spectroscopic properties. There has been a vast amount of research into the ECL and EL of iridium(III) complexes, and a lesser amount into osmium(II) complexes and other platinum group metal complexes [115, 116, 142, 148, 149]. Despite this research indicating that they all have similar properties, and proving that iridium(III) complexes are also capable of chemiluminescence [3, 136], ruthenium(II) complexes remain the undisputed chemiluminescence reagent of choice [7, 32]. Using this fount of knowledge, the aim of this work is to investigate the potential of iridium(III) and osmium(II) complexes as analytical chemiluminescence and ECL reagents, particularly those exhibiting diverse emission wavelengths. The following chapters will explore the chemiluminescence and ECL of this class of compounds, and demonstrate their utility through the exploitation of their unique spectrochemical properties.
Chapter Two: Chemiluminescence and Chirality - Tris(2,2′-bipyridyl)ruthenium(II)
2.1 Introduction

In the mid 19th century, the renowned Louis Pasteur was captivated by the asymmetry of natural organic substances [150, 151]. After separating ammonium tartrate salts into right-hemihedral (hemihedral: adj; crystallography; having half the number of planes required for symmetry of the holohedral form [152]) and left-hemihedral shapes, he noted that the direction of the rotation of linearly polarised light was different for the salts [150, 151]. He further noted that only one form underwent yeast fermentation [153]. The concept, subsequently dubbed 'chirality' (from the Greek cheir or 'hand') by Lord Kelvin [154], is a geometric one; the molecules (enantiomers) are non-superimposable mirror images of each other [155]. They are identical to each other in every respect save the direction in which they rotate linearly polarised light: levorotatory (−) isomers rotate light anticlockwise, whereas dextrorotatory (+) isomers cause a clockwise rotation [155].

Since Pasteur's initial recognition and separation of the enantiomers of ammonium tartrate, it has been found that chirality is ubiquitous in nature and some classes of molecules are actually homochiral [155-157]. The difference in stereochemistry causes variation in the chemical interactions of enantiomers in chiral environments, resulting in differences in their pharmacological, metabolic and/or toxicologic activities [155-157]. This can be harmless, as with (+)-glucose and (−)-alanine, where the unwanted enantiomer produces either no activity or another, innocuous activity [155, 156]. Unfortunately, there are also numerous instances where the other enantiomer can cause antagonistic, adverse and/or toxic effects [155, 156]. This clearly has important implications in the pharmaceutical industry and food and environmental analyses [156, 157]. The notorious fiasco of thalidomide, a racemic mixture of a drug that was used to combat morning sickness (the enantiomer containing an R stereocentre is a sedative while the S stereocentre causes the enantiomer to be a potent teratogen), brought this sharply to the attention of the world [156]. Since then, the separation and characterisation of biochemically active molecules such as drugs, herbicides and pesticides has been rigorously controlled [156, 157]. This has resulted in the establishment of a multitude of techniques for the detection, quantification and isolation of enantiomers, at analytical, preparative and industrial scales [156].

A common method for the separation of optically pure substances is the use of Chiral Stationary Phase High Performance Liquid Chromatography (CSP HPLC) [156]. These CSPs take
advantage of the homochirality extant in nature [156]. They are derived from such easily accessible and naturally abundant polysaccharides as amylose and cellulose, the latter of which is the most abundant biomacromolecule on Earth [156]. Both are polymers of d-(+)-glucose, differing by the linkage between the units (cellulose has β-(1,4) and amylose α-(1,4) linkages) [156]. The chiral analytes are separated both by intermolecular interactions with the stationary phase (H-bonding, π-π interactions, dipole-dipole interactions, etc.) and by their physical fit within the chiral cavities of the stationary phase [156].

When HPLC proves insufficient for the separation of charged species, Capillary Electrophoresis (CE) comes to the fore [10]. According to the van Deemter equation, the resolution obtained in column chromatography is affected by the multiple flow paths through the stationary phase, longitudinal diffusion and the finite rate of mass transfer between the stationary and mobile phases [10]. CE, performed in open-tubular columns (fused silica capillaries, typically between 25 and 100 μm internal diameter [156]), has effectively removed two of these three restrictions, leaving the separation affected only by longitudinal diffusion [10]. This allows for exceptional resolution of the analytes [10].

The concept of CE is very simple: an electric field is applied to a solution of ions connected via a capillary, and the said ions migrate under this influence [10, 156]. In normal application, the anode is positively charged, and so attracts anions, and the cathode is negative, attracting cations [10]. This migration, termed the electrophoretic mobility, is affected by both the charge of the ion in question and its hydrodynamic radius, resulting in the separation of the analytes [10, 156]. The capillary itself has negative silanol groups along the interior wall that are neutralised by an electrical double layer of electrolyte [10, 156]. The layer closest to the capillary is immobile and only partially negates the negative charge; full electrical neutrality is established by the diffuse, more mobile layer of solvated cations [10, 156]. The excess of solvated cations in the diffuse layer causes a net movement of the entire solution, creating a uniform Electroosmotic Flow (EOF) towards the negative cathode [10, 156]. There are thus two forces acting on the charged species present in a separation: the electrophoretic mobility and the EOF [10]. So cations, which are already attracted to the cathode, migrate faster than the EOF, whereas anions, which are attracted to the anode yet are swept along by the EOF, travel slower [10, 156]. In this way, both anions and cations can be separated and detected at the same electrode.
As enantiomers do not differ in their chemical and physical properties, they also do not differ in their electrophoretic mobility under normal conditions [156]. This, however, is easily remedied by the introduction of a chiral selector to the electrolyte, to allow the formation of transient diastereomeric complexes between the chiral selector and the analyte [156]. The chiral selector interacts slightly differently with and therefore has differing binding constants for each enantiomer, and this results in their separation [156].

Like a myriad of other complexes, \([\text{Ru}(\text{bipy})_3]^{2+}\) and its analogues exhibit chirality, and this has been exploited in the creation of DNA intercalators [73, 76, 158-162]. There are many cases detailing the preferential interaction of one enantiomer over the other with optically pure substances like DNA [159, 160] and tris(tetrachlorobenzenediolato)phosphate(V) [163]. Such interactions are employed in the separation and detection of enantiomers; a range of ruthenium(II) complex enantiomers have been successfully isolated through the use of Sephadex column chromatography and a chiral eluant [163, 164], and the chiral agent tris(tetrachlorobenzenediolato)phosphate(V) has been introduced to racemic and enantiomeric samples in order to distinguish between the enantiomers though \(^1\text{H}\) NMR spectroscopy [163, 165-168]. The optical purity of ruthenium(II) complexes has been achieved through the isolation of the enantiomers from a racemic solution [159, 169] or by exploiting the high thermal stereochemical stability of optically pure \([\text{Ru}(\text{bipy})_2(\text{py})_2]^{2+}\) (where py is pyridine) and substituting the pyridine ligands for another ligand [159, 163, 164]. Chiral ligands have also been used to enhance the luminescence of Eu\(^{3+}\) and Tb\(^{3+}\) ions, however the complexes themselves existed in a racemic mixture [170].

It has already been proven that the enantiomers of ruthenium(II) complexes exhibit different behaviour when interacting with DNA [73, 76, 158-162]; theoretically, they should also interact differently as chemiluminescence reagents with enantiomerically pure analytes, and the difference in the reaction kinetics of each enantiomer should be measurable in the intensity \textit{versus} time plots of the reactions. This is the premise of this chapter: the investigation of the chemiluminescence of a chirally selective reagent. The isolation of the enantiomers of \([\text{Ru}(\text{bipy})_3]^{2+}\) and their use as a probe of various reagent-analyte interactions is described herein. If the differences in stereochemistry do indeed affect the kinetics, then a distinct profile ought to be obtained from the different combinations of enantiomers (\(+/+, +/−, −/+\) and \(−/−\)).
Despite the use found for chiral ruthenium complexes, to date the only attempt to quantify the chemiluminescence of chiral molecules has been briefly examined in this research group by Pappin [169]. This preliminary study established a method for the isolation and characterisation of the enantiomers [169], and it was decided that the time was ripe to reinvestigate this intriguing theory.
2.2 Experimental

2.2.1 Materials

HPLC grade acetonitrile and ammonium acetate were obtained from Ajax FineChem (Tarren Point, New South Wales, Australia) and HPLC grade methanol from Scharlau Chemie (Sentmenat, Spain). Deionised water (Continental Water Systems, VIC, Australia) was used throughout. \([\text{Ru(bipy)}_3\text{Cl}_2•6\text{H}_2\text{O}\) was purchased from Strem Chemicals (Newbury, Minnesota, USA). Potassium hydroxide, tris(hydroxymethyl)aminomethane, polyvinylpyrrolidone, potassium antimony D-tartrate (dextrorotatory), (+)-tartaric acid, (−)-tartaric acid, (+)-proline, (−)-proline, trifluoroacetic acid, Dowex 1x8 strongly basic anion exchange resin (chloride form), antimony oxide, ammonium hexafluorophosphate, (2S,4S)-4-hydroxyproline and (2R,4S)-4-hydroxyproline and antimony oxide were obtained from Sigma-Aldrich (Castle Hill, New South Wales, Australia). Orthophosphoric acid and (2S,4R)-4-hydroxyproline was purchased from British Drug House Ltd. (London, UK) and (2R,4R)-4-hydroxyproline from BioScientific Pty. Ltd. (Sydney, Australia). Tetrahydrofuran and isopropanol were obtained from ChemSupply and oxalic acid from Ajax Finechem. Deuterated methanol and deuterated acetonitrile were obtained from Cambridge Isotope Laboratories, Inc. (Andover, USA).

2.2.2 General instrumentation

Melting points were determined using Stuart Scientific melting point apparatus SMP3.

NMR characterisation was performed on a Jeol Eclipse Plus 400 MHz ft-NMR spectrometer. The chloride salts of the complexes were analysed in deuterated methanol, and the hexafluorophosphate salts in deuterated acetonitrile.

Absorbance spectra were collected using a Cary 300 Bio UV-visible Spectrophotometer (Varian Australia, Mulgrave, Victoria, Australia) in a quartz cell of 1 cm path length.

The \([\alpha]_{D}^{22.8}\) values of the enantiomers were determined using a JASCO DIP-1000 Digital Polarimeter, containing a sodium lamp (Na 589 nm) and in a 100.0 mm sample cell at 22.8°C.

A Varian AA140 Atomic Absorption Spectrometer, equipped with ruthenium (Photron Lamps, Australia), potassium and antimony (Varian Techtron Pty. Ltd. Australia) hollow cathode lamps,
was used to determine the amount of each metal present in the enantiomerically pure samples. The operational parameters can be found in Table 2.01.

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<td>Air-acetylene</td>
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<td>Potassium antimony tartrate</td>
<td>Potassium chloride</td>
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</tbody>
</table>

Table 2.01: Operational parameters for the detection of ruthenium, antimony and potassium via AAS.

2.2.3 Synthesis of potassium antimony tartrate (levorotatory)

Equimolar amounts of (−)-tartaric acid (15.1 g, 0.100 mol) and potassium hydroxide (5.60 g, 0.0998 mol) were mixed at room temperature overnight. The resultant white crystals were collected by vacuum filtration and dried (15.6 g, 83% yield). This potassium (−)-tartrate (15.5 g, 0.082 mol) was subsequently dissolved in approximately 100 mL water, with 0.620 g (6.89 mmol) oxalic acid and 7.68 g (0.0264 mol) antimony oxide. The mixture was refluxed for two hours under atmospheric conditions, after which time the solid was filtered off. The filtrate was collected and the solvent removed under vacuum, to produce a white product. Yield: 18.5 g, 61%. Melting point: 313.7 - 321.3°C.

2.2.4 Isolation of the enantiomers of [Ru(bipy)₃]²⁺

As per Pappin [169], 453 mg (0.604 mmol) of [Ru(bipy)₃]Cl₂·6H₂O was dissolved in 20 mL of water and heated, with stirring. The desired chiral selector - either synthesised potassium antimony tartrate (levorotatory) or commercially obtained potassium antimony tartrate (dextrorotatory) - was then added (391 mg, 0.585 mmol). The mixture was cooled on ice overnight, to yield an orange precipitate which was collected by vacuum filtration and dried under vacuum. Enantiomeric purity was established by CE or CSP HPLC. The chloride salt of the enantiomers was obtained by anion exchange chromatography using a Dowex 1×8 200 - 400 mesh resin in the chloride form (strongly basic). Each sample was passed twice over a freshly
made column approximately 15 cm in length (2.0 cm i.d.) using water as eluant. The column was regenerated prior to the second exchange with 2.0 M HCl. Yield: 130 mg, 57%. The hexafluorophosphate salt was obtained by metathesis of the antimonyl tartrate salt with ammonium hexafluorophosphate in aqueous solution. Yield: 127 mg, 74%. AAS was used to confirm the absence of potassium and antimony; $^{13}$C NMR confirmed the absence of tartrate.

### 2.2.5 Chiral stationary phase HPLC

The Lux 5u Cellulose-1 column, 150 x 4.60 mm, and guard column were obtained from Phenomenex (Lane Cove, NSW, Australia). The chromatographic experiments were performed using a 1260 Infinity Series HPLC system from Agilent Technologies, equipped with a quaternary pump, solvent degasser system, autosampler, and diode array and UV-visible absorbance detectors (Agilent Technologies, Victoria, Australia). The instrument was controlled by Hewlett-Packard ChemStation software (Agilent Technologies). An isocratic elution method was employed for the resolution of the enantiomers of $[\text{Ru(bipy)}_3]^2^+\text{,}$ consisting of 90:10 20 mM ammonium acetate:acetonitrile, with both solutions containing 0.1% tetrahydrofuran, at a flow rate of 1.5 mL/min. The column temperature was kept at a constant 20°C. HPLC grade methanol and acetonitrile were employed. Aqueous solvents and isopropanol were filtered prior to use. An injection volume of 1 μL was used to introduce the 1 mg/mL samples onto the column. The column was stored in 100% methanol, as per the manufacturer's instructions.

### 2.2.6 Capillary electrophoresis

A Hewlett Packard 3D CE system was used (G1600AX). The electrolytic buffer employed was 100 mM tris(hydroxymethyl)methylamine, adjusted to pH 2.50 - 2.66 with orthophosphoric acid, containing 60 mM potassium antimonyl tartrate (dextrorotatory). The capillary was 65 cm long with a 50 μm internal diameter. Samples (1 mM) were injected by application of 50 mBar of pressure for 5 seconds.

### 2.2.7 Stopped-flow injection analysis with chemiluminescence detection

The stopped-flow manifold consisted of a programmable dual-syringe pump (Model sp210iw, World Precision Instruments, Glen Waverly, Victoria, Australia Reagent concentration: 0.01 mM, 1 mM), Valco six-port injection valve and a GloCell chemiluminescence detector (Global
FIA) equipped with a dual inlet serpentine flow cell [136, 171]. Luer lock syringes (10 mL, Terumo) were primed and loaded with the appropriate solution, as was the 70 μL injection loop. The pump was programmed to dispense 0.1 mL of solution at 10 mL min\(^{-1}\) line\(^{-1}\).

2.2.8 Electrochemiluminescence evaluation

An Autolab PGSTAT12 potentiostat was used to control the collection of electrochemiluminescence data, which were obtained in a three-electrode electrochemical cell consisting of 3 mm diameter glassy carbon working electrode, Ag/AgNO\(_3\) (0.02 M) reference electrode and Pt wire counter electrode. Autolab GPES software was used to record the electrochemical data. The ECL detector employed was a Electron Tubes model 9828SB, PMT (ETP, Ermington, Australia). Solution mixtures contained 0.5 mM [Ru(bipy)_3]^{2+} and 0.5 mM tartaric acid or proline co-reactant in 0.1 M phosphate buffer solution (PBS) at pH 7.
2.3 Results and Discussion

2.3.1 Synthesis of potassium antimonyl tartrate (levorotatory)

Initial attempts to create this complex followed the procedure outlined by Pappin [169]: oxalic acid, (−)-tartaric acid and antimony oxide were dissolved in water and refluxed for an hour, after which the white precipitate was collected by vacuum filtration and dried. Pappin [169] characterised the product by melting point (298 - 332°C); when this was attempted however, the solid did not decompose even after the maximum temperature of the melting point apparatus (350°C) was achieved. Additionally, the solid was not soluble in water, although the commercially sourced enantiomer was. It was therefore thought that the desired product was lost in the filtrate, and the solid collected was unreacted antimonyl oxide. Furthermore, even though Pappin described this method as the synthesis of the potassium salt of antimonyl tartrate, there was no potassium source in the mixture. To rectify this, the potassium (−)-tartrate was used instead of (−)-tartaric acid. This was obtained from reaction of equimolar amounts of (−)-tartaric acid with potassium hydroxide, with stirring overnight at room temperature. The resultant white crystals were collected by vacuum filtration and dried. This was subsequently dissolved in water along with oxalic acid and antimony oxide and the mixture refluxed for two hours under atmospheric conditions. The resultant clear solution was separated from the white precipitate by filtration, then the solvent was removed under vacuum. The melting point of this product was 313.7 - 321.3°C, well within the range specified by Pappin [169], and so it was concluded that this was the desired chiral selector.

2.3.2 The isolation of the enantiomers of [Ru(bipy)_3]^{2+} as the antimonyl tartrate salt

In an effort to improve upon Pappin's relatively low yield of 40% [169], the amount of chiral selector was increased to excess (two molar equivalents). This resulted in a moderate yield (59%), and the addition of a further 0.5 g of chiral selector to the collected filtrate did not have a significant impact on the yield. Once the enantiomeric excess of the isomer was established, the antimony tartrate was substituted for a more appropriate counterion (see Section 2.3.4).
Pure samples of the [Ru(bipy)_3]Cl_2 enantiomers and the potassium antimonyl tartrate enantiomers were analysed by polarimetry. The specific rotation values are given in Table 2.02. Sample A was isolated using the synthesised potassium antimonyl tartrate, whereas Sample B was resolved using the commercial potassium antimonyl tartrate. It is apparent that the levorotatory enantiomer of one species preferentially forms an adduct with the dextrorotatory enantiomer of the other, in accordance with Pappin's findings [169].

<table>
<thead>
<tr>
<th></th>
<th>(\alpha^{22.8}_D)</th>
<th>RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercial KSBTartrate</td>
<td>+0.1701(^\circ)</td>
<td>5.6%</td>
</tr>
<tr>
<td>Synthesised KSBTartrate</td>
<td>-0.1917(^\circ)</td>
<td>6.4%</td>
</tr>
<tr>
<td>[Ru(bipy)_3]^{2+} Sample A</td>
<td>+1.2060(^\circ)</td>
<td>3.3%</td>
</tr>
<tr>
<td>[Ru(bipy)_3]^{3+} Sample B</td>
<td>-1.3055(^\circ)</td>
<td>2.2%</td>
</tr>
</tbody>
</table>

Table 2.02: Specific rotation of the enantiomers of [Ru(bipy)_3]^{2+} and potassium antimonyl tartrate. Values determined at 22.8\(^\circ\)C and 589 nm.

### 2.3.3 Choice of counterion

The physical properties of a complex can be subtly tuned through the use of specific counterions. In this way, the complex's solubility can be controlled. ECL experiments require the chloride salt of the chosen complex for aqueous analyses [45] and the hexafluorophosphate salt for non-aqueous analyses [44]. Chemiluminescence experiments are generally performed in aqueous solution with Ce(SO_4)_2 and PbO_2 oxidation, and so the chloride salts are commonly used [3, 7, 22, 45]. Recent investigations into the stability of the ruthenium(III) state after oxidation with PbO_2 have indicated that the presence of water is detrimental, as it quickly reacts with the ruthenium(III) complex [21]. If the solvent is acetonitrile containing 0.05 M perchloric acid, however, the stability is greatly improved; the perchlorate salt of [Ru(bipy)_3]^{3+} dissolved in acetonitrile containing 0.05 M perchloric acid was stable over 48 hours compared to less than five hours for [Ru(bipy)_3]Cl_2 in 0.05 M sulphuric acid [21].

In order to test all three methods of oxidation, appropriate counterions must be chosen. Due to the chemiluminescence reaction between tartrate and [Ru(bipy)_3]^{2+} [172], antimonyl tartrate is clearly not suitable. The method outlined by Pappin [169] involved conversion to the chloride salt by an anion exchange resin, and [Ru(bipy)_3](ClO_4)_2 and [Ru(bipy)_3](PF_6)_2 are easily
obtained by an aqueous metathesis reaction [21]. To ensure consistency between experiments, it is desirable to have the same counterion present in both the ECL and the non-aqueous chemiluminescence experiments. As the counterion itself is inert in the chemiluminescence reactions, it was hypothesised that the hexafluorophosphate salt would exhibit the same stability in acidified acetonitrile as the perchlorate salt. Consequently, the stability of the ruthenium(III) state of \([\text{Ru(bipy)}_3\text{Cl}_2]\) in 0.05 M H\(_2\)SO\(_4\) and solutions of \([\text{Ru(bipy)}_3](\text{ClO}_4)_2\) and \([\text{Ru(bipy)}_3](\text{PF}_6)_2\) in acetonitrile containing 0.05 M HClO\(_4\) was monitored over 24 hours using UV-visible absorbance. Immediately after oxidation by lead dioxide (which was subsequently removed from the solution), a small peak attributable to the ruthenium (III) state appears at 660 nm and there is a subsequent loss of the ruthenium(II) peak at 453 nm. Over time, as the ruthenium(III) returns to ruthenium(II), the absorbance of these peaks alters accordingly - that is, there is an increase in the absorbance at 453 nm and a loss of absorbance at 660 nm. The profiles obtained from monitoring these peaks (Figure 2.01) confirms the observation of McDermott et al. [21] that the use of acidified acetonitrile greatly increases the stability of the ruthenium(III) state compared to aqueous solution. The concentration of the ruthenium(III) state of the perchlorate and hexafluorophosphate salts stay quite constant over the experimental time period (25 hours), whereas the chloride salt shows a gradual return to the ruthenium(II) state. Furthermore, no appreciable difference between the perchlorate and hexafluorophosphate salts can be seen; the inert ion only effects the complex's overall solubility. Based on this, it was decided that the hexafluorophosphate salt would be suitable for all organic analyses, and these should be conducted in acidified acetonitrile to ensure stability of the ruthenium(III) reagent.
Figure 2.01: The blank-subtracted absorbance intensities of the hexafluorophosphate (blank), perchlorate (sky blue) and chloride (olive) salts of [Ru(bipy)$_3$]$^{2+}$ over time after oxidation by PbO$_2$. (a) the absorbance intensities at 453 nm. (b) Absorbance intensities at 660 nm. The hexafluorophosphate and perchlorate salts are dissolved in 0.05 M HClO$_4$ acetonitrile solution; the chloride salt was prepared in 0.05 M H$_2$SO$_4$. 
2.3.4 Conversion to the chloride and hexafluorophosphate salts

The efficiency of the counterion exchanges (that is, the presence or absence of potassium antimonyl tartrate) was evaluated by AAS and $^{13}$C NMR. In the case of the anion exchange to form the chloride salt, a single pass over the anion exchange column resulted in no tartrate being detected in either the (+) or (−) sample by $^{13}$C NMR. Both, however, contained some potassium and antimony as determined by AAS. The (−) sample was found to contain 0.45% of potassium and 3.5% of antimony (relative to the concentration of ruthenium), and the (+) sample contained 0.39% and 4.1% potassium and antimony, respectively. These samples were found to exhibit a chemiluminescence signal approximately 5.5 times that of the commercial [Ru(bipy)$_3$]$^{2+}$ under the same conditions. A second anion exchange column reduced the amounts of antimony to 1.1% (for the dextrorotatory sample) and 1.0% (for the levorotatory sample). This provided an acceptable comparison to the commercial product in chemiluminescence intensity. After aqueous metathesis to the hexafluorophosphate salt, the samples were found to contain no tartrate, but approximately 0.40% potassium and 1.7% antimony.

2.3.5 Chiral stationary phase HPLC

In order to evaluate the suitability of chiral HPLC as a method of evaluating the purity of the resolved enantiomers, the chiral screening service offered by Phenomenex was used. Through this, it was found that (despite a lack of success in the separation of similar metal complexes in the past) the commercial [Ru(bipy)$_3$]Cl$_2$ sample could be successfully resolved into its enantiomers, and a Lux-1 cellulose column was therefore purchased. Using this column, a method was developed using isocratic conditions of 90:10 20 mM ammonium acetate:acetonitrile, both containing 0.1% trifluoroacetic acid, at 20°C. Detection of the enantiomers was achieved by UV-visible absorbance at 450 nm and 280 nm, both with and without a reference at 550 nm. Absorbance at 450 nm with a reference at 550 nm provided superior results, and these are henceforth reported. Although baseline resolution between the enantiomers was not realised, an analytically useful separation was achieved with good reproducibility (Figure 2.02). This separation was achieved within 30 minutes. While this is a relatively long separation, attempts to improve the run time resulted in an unacceptable loss of resolution.
A study was undertaken to determine the reproducibility of the separation over an extended period of time. This showed that, while the separation was initially achieved in 30 minutes, the enantiomers eluted slightly earlier on each successive day of analysis (see Figure 2.03 for a comparison of two consecutive days), despite the fact that the column was temperature controlled (20°C). The separation could ultimately be performed within 20 minutes, with the isomers eluting at 13.2 and 16.0 minutes. This change in retention times is attributed to a 'settling down' period of the column. While less than ideal, the difference in day-to-day performance can be borne as the separation is intended to be a method of characterisation, merely to ascertain the presence or absence of the enantiomers.
Figure 2.03: A comparison between the average separations of racemic [Ru(bipy)$_3$]Cl$_2$ using a Lux-1 cellulose chiral column.

The elution order of the enantiomers was determined by the injection of a pure sample of the antimonyl tartrate salts (Figure 2.04). As can be seen, the levorotatory enantiomer has the least affinity for the cellulose stationary phase, while the dextrorotatory enantiomer is retained longer.
2.3.6 Capillary electrophoresis

In the forerunning study [169], enantiomeric purity was confirmed by CE, and so this method was also trialled here. Again, the absorbance of the eluant was monitored at 453 and 280 nm. As the molar absorptivity of \([\text{Ru(bipy)}_3]^{2+}\) at 453 nm is lower than at 280 nm, the sensitivity of detection was decreased when using this wavelength. As such, the more sensitive detection wavelength of 280 nm was subsequently used. A replication of Pappin's CE method [169] was initially attempted. This gave baseline resolution of the peaks at approximately 8.5 minutes (Figure 2.05). Various electrolyte and chiral selector concentrations were trialled, and it was concluded that the optimal current and resolution were achieved using 100 mM Tris buffer (tris(hydroxymethyl)methylamine) and 60 mM potassium antimonyl tartrate.
Using these conditions, the purity of the antimony tartrate salts of the enantiomers was determined (Figure 2.06). From this, it was confirmed that the (+) and (−) samples prepared by Pappin had not degraded or racemised over time, and exhibited the same purity as the freshly prepared (+)-[Ru(bipy)₃]²⁺ sample.
These studies revealed that the migration times of the enantiomers were not constant, varying between approximately 6 and 10 minutes. Differences in migration times is not unprecedented in CE [169, 173, 174]: in Pappin's work [169], the enantiomers eluted between eight and twelve minutes. In order to improve the reproducibility, a preconditioning wash sequence was introduced. The capillary was flushed with 1.0 M sodium hydroxide, water and then 1.0 M hydrochloric acid (one minute of each), followed by 5 minutes of flushing with the buffer solution. This improved the reproducibility somewhat, although variation could still be seen (Figure 2.07).
Figure 2.07: Four consecutive separations of commercially sourced racemic [Ru(bipy)$_3$]$_2^+$. Conditions: 100 mM Tris buffer and 60 mM potassium antimonyl tartrate, with a pre-conditioning wash sequence.

To further this enhancement, 1% polyvinylpyrrolidine (PVP) (w/v) was introduced. This polymer forms hydrogen bonds to the silica of the capillary, impeding the EOF and causing the separation to occur by the electrophoretic mobility of the ions alone. A vastly improved reproducibility of the separation was achieved (Figure 2.08), although at the cost of sensitivity (the peak area decreased by 41%). This is probably due to the increase in the viscosity of the buffer solution causing a reduction of the sample load. The loss of EOF caused the enantiomers to migrate more slowly, which has the added benefit of improving the resolution of the enantiomers.
Figure 2.08: Five consecutive separations of racemic [Ru(bipy)₃]²⁺ using 100 mM Tris buffer, 60 mM potassium antimonyl tartrate and 1% PVP with a preconditioning wash sequence.

2.3.7 Enantiomeric excess of the reagents as determined by CE and CSP

HPLC

The purity of the racemic [Ru(bipy)₃]²⁺ and the isolated enantiomers was estimated using both CSP HPLC and CE, by establishing the ratio of the integrated peak areas of each peak. From the CSP HPLC studies, it was determined that the racemic sample contained 52% and 48% (−)- and (+)-[Ru(bipy)₃]²⁺, respectively. This is in good agreement with the estimate obtained from the electropherograms of 49% (−)-[Ru(bipy)₃]²⁺ and 51% (+)-[Ru(bipy)₃]²⁺. Enantiomeric excesses of between 95% and 99% were obtained for the purified enantiomers by CSP HPLC. As baseline resolution between the peaks was not quite achieved in this method, the purity of the least retained enantiomer is artificially lowered; a visual inspection of the chromatograms reveals that the samples of both enantiomers have a similar purity. This is supported by the enantiomeric
excess values obtained via CE, in which the baseline resolution allows a value of 98% to be calculated for each isomer.

2.3.8 Optimisation of the analytical chemiluminescence trials

Pappin [169] experienced some difficulties regarding the reproducibility of the chemiluminescence responses. Minimising this variation is critical: any differences in the kinetic profiles that arise as a result of the interactions of the chiral molecules may be minor, so good reproducibility of the reactions is essential to measure these differences. Both batch and stopped-flow instrumentation were trialled in the previous investigation [169]. Stopped-flow has the advantage of introducing a precise volume of solutions into a flow cell in a reproducible manner, and so was chosen for this study. The coiled-tube flow cell used by Pappin was replaced by a dual inlet cell, which allows the solutions to mix directly in front of the PMT. Furthermore, the cell's channels follow a serpentine design rather than a coil, enhancing the mixing of the solutions [136].

Initial experiments involved optimising the stopped-flow system with commercially sourced racemic \([\text{Ru(bipy)}_3]^{2+}\) and either (+)-tartaric acid or (−)-proline. These particular analytes were chosen as they are simple and represent the organic acid and amine groups that are known to elicit chemiluminescence from \([\text{Ru(bipy)}_3]^{2+}\) [7]. As different sensitivities can be obtained when using different oxidation methods, the reagent was oxidised off-line (with PbO\(_2\)) and online in the presence of analyte (with Ce(SO\(_4\))\(_2\)) [20, 175]. It has further been noted that organic acids require oxidation prior to reaction with the reagent, as reaction with the organic acid radical is required for chemiluminescence [20]. Consequently, the Ce(SO\(_4\))\(_2\) system was optimised using (+)-tartaric acid and the PbO\(_2\) system with (−)-proline. The effect of pH on the peak shapes was investigated by testing acidic and neutral solutions of both the reagent and the analyte (carrier). The concentration of these reactants was also examined. In both systems, the manifold was designed to mimic FIA conditions.

Cerium(IV) sulphate system

Initially, the reagent was injected directly into a carrier stream (containing analyte), which was merged with the oxidant stream within a dual-inlet serpentine cell. Greater signals were obtained when acid was omitted from the reagent solution, and to avoid pH effects upon mixing, the
analyte was also dissolved in the same solvent. Hence, the only source of acid in this system is in the oxidant solution. The optimal volume of solution introduced to the cell by the syringe pump was found to be 0.2 mL. It was also found that better peak shapes were obtained with increasing concentrations of reagent and analyte concentration, up until the limit of the concentration study: 1 mM [Ru(bipy)₃]²⁺ and 0.01 mM (+)-tartaric acid.

When this system was subsequently used in the reactions between the enantiomers of [Ru(bipy)₃]²⁺ and tartaric acid or benzoyltartrate, no differences in the kinetic profiles could be observed and poor signal-to-blank ratios were obtained. This could indicate that there was insufficient time for the reagent to disperse into the analyte prior to reaction with Ce(SO₄)₂. The manifold was therefore modified such that a solution of reagent and analyte, prepared offline, was injected into a carrier stream of water, which then merged with the oxidant within the cell.

Previous reports of the simultaneous determination of tartaric acid and either ascorbic [176] or oxalic acid [172] have discriminated between the analytes by the differences in their reaction kinetics, using very low concentrations of all species. In light of this, the concentration of [Ru(bipy)₃]²⁺ was optimised between 5 × 10⁻⁴ M and 5 × 10⁻⁶ M, and (+)-tartaric acid was tested at 5 × 10⁻⁶ M and 5 × 10⁻⁷ M. The signal intensities were found to increase as the reagent concentration increased (Figure 2.09). With regards to the analyte concentration, no significant difference in the peak shapes can be seen in the reactions between 5 × 10⁻⁷ M and 5 × 10⁻⁶ M L-tartaric acid with 5 × 10⁻⁶ M and 5 × 10⁻⁵ M [Ru(bipy)₃]²⁺. At the optimal reagent concentration (5 × 10⁻⁴ M), however, the lower analyte concentration produced superior chemiluminescence intensity. The oxidant concentration was also investigated, and a concentration of 1 × 10⁻³ M was found to be optimal. Furthermore, the volume of solution introduced to the cell by the syringe pump was re-optimised for this system, and in this case 0.1 mL was found to be most favourable.
In order to minimise matrix effects, the \([\text{Ru(bipy)}_3]^{2+}\) used in the analytical evaluations is a 1:1 mixture of the isolated enantiomers. It is, however, desirable to identify any differences between this mixture and the racemic substance obtained commercially. A quick comparison was therefore conducted using the Ce(SO₄)₂ system. It was found that, after only a single pass over the anion exchange resin, a signal five and a half times that of the commercial reagent was obtained from the reaction with water. This increase in chemiluminescence intensity was attributed to residual antimony tartrate in the sample. If the enantiomer samples were passed over the anion exchange resin a second time, then the signal decreased to one and a half times that of the commercially sourced reagent.

**Lead(IV) dioxide system**

As the reagent is oxidised off-line in this system, a slightly modified manifold was used. This entailed the injection of filtered \([\text{Ru(bipy)}_3]^{3+}\) into a carrier stream (0.05 M perchloric acid),
which then merged with the analyte within the dual-inlet serpentine cell. The pump was programmed to dispense 0.1 mL of solution at 10 mL min\(^{-1}\). Due to the solvent incompatibility of the syringe pump fittings, acetonitrile was only present in the reagent solution. The presence of acid in this system caused a decrease in the peak intensities, as well as an increase in signal-to-blank ratios. It was therefore concluded that the presence of 0.05 M HClO\(_4\) is preferable to using neutral solutions. An investigation into reagent concentration revealed that 1 mM [Ru(bipy)\(_3\)]\(^{2+}\) solutions produced signals an order of magnitude greater than the 0.1 mM solutions, while insufficient chemiluminescence was obtained using 0.01 mM [Ru(bipy)\(_3\)]\(^{2+}\). Consequently, a reagent concentration of 1 mM was utilised in the analytical studies. A further study indicated the optimal analyte concentration to be \(1 \times 10^{-5}\) M.

### 2.3.9 Chiral discrimination

As previously mentioned, [Ru(bipy)\(_3\)]\(^{2+}\) is known to produce chemiluminescence from reaction with tartarate and with proline [7]. These are both simple molecules, and it was thought that differences in the reaction kinetics may be encouraged by larger chiral analytes. To investigate this, the enantiomers of benzoyltartrate were studied alongside those of tartaric acid. Additionally, 4-hydroxyproline was included in the study as it is not only a larger molecule than proline, it also has two stereocentres. No significant differences in chemiluminescence intensities were observed for these analytes, and so the normalised kinetic profiles are reported for ease of comparison.

**Cerium(IV) sulphate system**

When Ce(SO\(_4\))\(_2\) was used as the oxidant, some small differences were seen in the kinetic profiles of the enantiomers. The profiles obtained from these reactions reveal an initial sharp increase in peak intensity, followed by a decay that is generally slower for (+)-[Ru(bipy)\(_3\)]\(^{2+}\) than for (−)-[Ru(bipy)\(_3\)]\(^{2+}\). The enantiomers of tartaric acid yield identical kinetic profiles when reacted with (+)-[Ru(bipy)\(_3\)]\(^{2+}\), but not with (−)-[Ru(bipy)\(_3\)]\(^{2+}\) (Figure 2.10). A sharper decay rate is seen in the reaction of (−)-[Ru(bipy)\(_3\)]\(^{2+}\) and (−)-tartaric acid than with (−)-[Ru(bipy)\(_3\)]\(^{2+}\) and (+)-tartaric acid, and both of these profiles are sharper than those obtained for (+)-[Ru(bipy)\(_3\)]\(^{2+}\) (Figure 2.10).
The larger benzoyltartrate, on the other hand, produces different profiles for each combination of enantiomers (Figure 2.11). A similar profile was obtained for each [Ru(bipy)_3]^{2+} enantiomer with (-)-benzoyltartrate and with (+)-benzoyltartrate. That is, the kinetic profile obtained from the reaction of (+)[Ru(bipy)_3]^{2+} and (-)-benzoyltartrate paralleled that of (-)[Ru(bipy)_3]^{2+} and (-)-benzoyltartrate, and both these profiles show a quicker decrease in light intensity than the combinations of (+)[Ru(bipy)_3]^{2+} and (-)[Ru(bipy)_3]^{2+} with (+)-benzoyltartrate. The sharpest decay is seen with (-)[Ru(bipy)_3]^{2+} and (-)-benzoyltartrate, while the slowest decay was obtained from (+)[Ru(bipy)_3]^{2+} and (+)-benzoyltartrate.
In the reaction kinetics of the enantiomers of \([\text{Ru(bipy)}_3]^{2+}\) and proline (Figure 2.12) or 4-hydroxyproline, very little differences can be seen. Proline, for instance, produced essentially the same kinetic profile for \((+)-[\text{Ru(bipy)}_3]^{2+} - (+)-\text{proline}\), \((+)-[\text{Ru(bipy)}_3]^{2+} - (-)-\text{proline}\) and \((-)-[\text{Ru(bipy)}_3]^{2+} - (+)-\text{proline}\). The kinetic profiles obtained for the 4-hydroxyproline enantiomers were also all identical.
Figure 2.12: Averaged and normalised kinetic profiles of the reaction of the enantiomers of [Ru(bipy)3]2+ and proline with 1 × 10⁻³ M Ce(SO₄)₂ in 0.05 M H₂SO₄.

**Lead(IV) dioxide system**

When using PbO₂ as oxidant, the signal-to-blank ratios obtained from tartaric acid and benzoyltartrate were inferior to those from the Ce(IV) system. The profiles obtained for the reaction with (+)-tartaric acid were essentially the same for both enantiomers of [Ru(bipy)₃]²⁺, although somewhat different to those obtained with (−)-tartaric acid (Figure 2.13).
Figure 2.13: Averaged and normalised kinetic profiles of the reaction of the enantiomers of \([\text{Ru(bipy)}_3^{2+}]\) and tartaric acid in 0.05 M HClO₄ after oxidation by PbO₂.

A greater difference in the kinetics can be seen in the reactions with benzoyltartrate (Figure 2.14). When the dextrorotatory enantiomers of analyte and reagent are reacted together, the resultant emission profile shows the slowest rate of decay. The sharpest emission decay obtained from this combination of enantiomers was obtained from the reaction of \((+)-[\text{Ru(bipy)}_3^{2+}]\) and \((-)-\text{benzoyltartrate}\). The profile of \((-)-[\text{Ru(bipy)}_3^{2+}]\) and either benzoyltartrate roughly parallels those profiles obtained with dextrorotatory \([\text{Ru(bipy)}_3^{2+}]\), with (+)-benzoyltartrate producing the longer emission lifetime. Despite the sharp decay in the profile of \((-)-[\text{Ru(bipy)}_3^{2+}]\) and \((-)-\text{benzoyltartrate}\), this combination has an emission that holds at maximum intensity for the longest period of time. Conversely, \((-)-[\text{Ru(bipy)}_3^{2+}]\) and (+)-benzoyltartrate has a slow decay yet a very narrow maximum emission.
The kinetic profiles obtained from the reaction with proline and 4-hydroxyproline are interesting, as two distinct peaks can be observed (Figure 2.15). The first is a short, sharp peak, and the second a more long-lived emission. The second peak may have some contribution from the blank peak, however it was more intense and showed different rates of decay. In the reactions between the [Ru(bipy)3]2+ and proline, the enantiomer combinations all show essentially the same emission profile. On the other hand, very different kinetic profiles are observed in the reactions of the [Ru(bipy)3]2+ and 4-hydroxyproline enantiomers. When comparing the series containing a 2S stereocentre (Figure 2.15), for example, a greater intensity second peak was obtained from (+)-[Ru(bipy)3]2+. Similar profiles can be seen for (−)-[Ru(bipy)3]2+ with both (2S,4R) and (2S,4S)-4-hydroxyproline. The sharpest rate of decay was given by (+)-[Ru(bipy)3]2+ and (2S,4R)-4-hydroxyproline, while the slowest was from (+)-[Ru(bipy)3]2+ and (2S,4S)-4-hydroxyproline. The (2R)-4-hydroxyproline series also exhibits considerable variation in the kinetic profiles: the sharpest rate of decay was observed with (2R,4S)-4-hydroxyproline and (−)-[Ru(bipy)3]2+, and the slowest was found for (+)-[Ru(bipy)3]2+ and (2R,4R)-4-hydroxyproline.
Despite the attempts at optimising the systems, the reproducibility with both methods of oxidation was very poor, ranging from 5.5% RSD to 50.9% RSD. The reproducibility of the Ce(SO₄)₂ reactions was much better than that of the PbO₂ reactions, however it is still not acceptable. In some of the reactions studied, the differences in the kinetic profiles were minor (for example, Figure 2.12). Unfortunately though, due to the reproducibility issues, it cannot be said with confidence that the slight differences seen are the result of chiral interactions of the molecules. The signal-to-blank ratios obtained in these methods were also quite poor. If these were improved, perhaps the differences in kinetics may be more obvious.

**Electrochemiluminescence**

To compliment the chemical methods of oxidation, ECL studies were also undertaken. These were performed using the enantiomers of [Ru(bipy)₃](PF₆)₂ in dry acetonitrile. Tartaric acid and proline were chosen as representative analytes, however proline is not soluble in dry acetonitrile. Consequently, only the enantiomers of tartaric acid were examined. In an effort to keep
conditions as constant as possible across the different oxidation methods, the reagent concentration was 0.5 mM and the analyte was initially 0.01 mM. This, however, did not produce a signal above the blank at 1.27 V (vs. ferrocene) and so the analyte concentration was increased to 0.1 mM. While this did produce a signal, it was poor and no differences could be observed between the different combinations of the enantiomers.

The experiment was repeated in aqueous PBS (pH 7.5) using the chloride salt of the ruthenium(II) complex, with both the reagent and the analyte tested at 0.5 mM. The reproducibility of these reactions was vastly improved compared to the chemiluminescence experiments. Although a signal was not obtained under these conditions from the reaction with tartaric acid, proline produced a good signal with both [Ru(bipy)₃]²⁺ enantiomers at 1.27 V (Figure 2.16). Interestingly, the intensity produced from the reaction of dextrorotatory enantiomer of [Ru(bipy)₃]²⁺ and both enantiomers of proline was more than threefold that obtained with the levorotatory enantiomer of [Ru(bipy)₃]²⁺.
If the kinetic profiles of the reactions of \([\text{Ru(bipy)}_3]^{2+}\) and proline are examined, it appears that \((-\)-proline \(\text{when reacted with both enantiomers of } [\text{Ru(bipy)}_3]^{2+}\)) results in a slightly gentler rise to maximum intensity, coupled with a somewhat quicker decay than the reaction with \((+)-\)proline. These differences, however, are insignificant when the profiles are normalised (Figure 2.17).

![Figure 2.17: Normalised ECL kinetic profiles of the reaction of the enantiomers of \([\text{Ru(bipy)}_3]^{2+}\) and proline in 0.1 M PBS (pH 7.5) at 1.27 V vs. ferrocene.](image)
2.4 Conclusions

The method developed by Pappin [169] has been successfully utilised to separate a racemic mixture of \([\text{Ru(bipy)}_3]\text{Cl}_2\) into its enantiomers. This exploited the selective manner in which potassium antimony tartrate interacts with \([\text{Ru(bipy)}_3]^{2+}\): dextrorotatory antimony tartrate complexes with levorotatory \([\text{Ru(bipy)}_3]^{2+}\) and vice versa, causing the desired enantiomer to precipitate out of solution. The purity of the isomers was established not only by Pappin's CE method [169], but also by Chiral Stationary Phase HPLC. Each of these techniques has its own advantages: CSP HPLC is inherently more reproducible than CE, however the resolution and run time is inferior. Furthermore, the reproducibility of the CE method was vastly improved by the addition of 1% PVP. It was consequently concluded that, although CSP HPLC was sufficient to establish the purity of the enantiomer samples, CE is the preferred method.

In the analytical evaluation of the possibility of chiral selection through chemiluminescence, some differences can be seen in the kinetic profiles. Larger differences seem to be produced from the reaction of larger molecules (compare benzoyltartrate to tartaric acid) and by extending the chirality of the molecule (the two stereocentres of 4-hydroxyproline compared to proline's single stereocentre). These differences seem to be enhanced when using \(\text{PbO}_2\) as the oxidant. \(\text{Ce(SO}_4\text{)}_2\) is a very powerful oxidant that can also react with the analyte; its presence in the reaction mixture complicates the system, and this may be reflected in the small differences seen in the kinetic profiles. As \(\text{PbO}_2\) is isolated from the analyte, it may be that this simplification in the reaction results in a more measurable difference in the kinetics. However, the reproducibility of these systems is insufficient to draw definite conclusions about the dependence of the kinetic profiles on the chirality of the reagent and analyte. The electrochemical oxidation of the reagent enantiomers resulted in vastly improved reproducibility of the reaction, but no real differences can be observed in the kinetic profiles with \((-)\) and \((+)\)-proline. These findings warrant further investigation into this phenomenon.
Chapter Three: The Immobilisation of Ruthenium(II) Complexes
3.1 Introduction

The emission of light from transition metal complexes such as tris(2,2′-bipyridyl)ruthenium(II) is a result of a redox reaction [9]. The complex is oxidised, then reduced to its excited state, from where it releases a photon [9]. In commonly used flow analysis techniques, the reaction occurs in solution and the regenerated complex is lost to the waste. In order to reduce this unnecessary and expensive wastage, many researchers have investigated the concept of immobilizing the complex to create a cost effective, regenerable sensor that can be used in analytical techniques such as HPLC [177] and CE [178] or as a chemical sensor [98]. When coupled with magnetic beads, immobilised complexes can be very effective tools in immunoassay applications, for example in the detection of Burkitt's lymphoma [179].

Sensors can be made simply through the physical entrapment of a complex within a solid matrix [2, 180] or by utilizing the charge on [Ru(bipy)₃]²⁺ to entrap it within cation-exchange polymers such as Nafion [177, 178, 181, 182]. This allows analytes or co-reactants to flow through the porous substrate to reach the immobilised [Ru(bipy)₃]²⁺ and takes full advantage of the benefits of [Ru(bipy)₃]²⁺ compared to its analogues [181]. Unfortunately, this immobilization is not robust and the films exhibit leaching over time [181]. The electrochemiluminophore can also migrate into the hydrophobic, electro-inactive regions of the polymer [178]. In order to improve the reactivity and long-term stability of the films, Nafion-based composites have been introduced [178, 182]. Other ion exchange polymers have also been used; the perfluorinated ion exchange solution Nepem-105D has been utilised to immobilise a [Ru(bipy)₃]²⁺ derivative onto the surface of a platinum wire in order to develop an ECL sensor for the detection of fluoroquinolones in milk samples [177]. The use of nanoparticles has not been neglected in this field: [Ru(bipy)₃]²⁺ has been incorporated into silica doped nanoparticles [183], Nafion-stabilised magnetic nanoparticles [181], silica nanoparticles [145, 184] and silica nanoparticles conjugated with a biopolymer chitosan membrane [185]. Sensors have also been fabricated by encapsulation of the complex into a sol gel [67, 68, 186] and by self-assembly techniques [187]. In addition, light emitting devices can be manufactured using the Langmuir-Blodgett technique [188, 189].

A more permanent method of immobilization is the covalent bonding of a [Ru(bipy)₃]²⁺ analogue to a solid support such as silica. Greenway et al. have reported a simple and efficient technique for the covalent immobilisation of a [Ru(bipy)₃]²⁺ derivative, creating a device that is suitable for
chemiluminescence and electrochemiluminescence detection [2]. They describe both the physical encapsulation of the complex in silicate sol-gels and the covalent attachment onto silica (vía six siloxane bonds per complex) [2]. The covalent attachment was found to be advantageous, as it ensured a homogeneous distribution of the reagent within the matrix and less leaching was observed [2]. These sensors exhibited reduced analysis costs, reproducible analyte responses and extended sensor lifetimes [2]. Barnett and co-workers published a description of the synthesis of a ruthenium(II) reagent covalently attached to silica particles vía only two siloxane bonds per complex [1]. They found this immobilised complex performed well (under unoptimised conditions) in both flow injection analysis (FIA) and sequential injection analysis (SIA) [1]. The detection limits of codeine and sodium oxalate with this sensor rivalled those obtained from solution phase reaction with $[\text{Ru(bipy)}_3]^{2+}$ [1]. Various pyrrolizidine alkaloids have also been sensitively detected using this functionalised silica cell [190].

The studies discussed above, while achieving a regenerable sensor, highlight a couple of common failings. The first is the inferiority of solid phase (electro)chemiluminescence to solution phase reactions [191]. Studies have shown that the ECL reaction kinetics limit the emission intensity obtained from immobilised complexes, resulting in lower intensity signals compared to the solution phase reaction [186]. The mobility of both the reagent and the analyte within the solid host matrix has a large influence on the ECL emission; the authors demonstrate [186] that the more mobile oxalate molecules react more efficiently than some simple alkylamines (a 25 - 35% decrease in intensity compared to a 94 - 97% decrease). It can therefore be concluded that the physical immobilisation of a complex can actually hinder the interaction between analyte and reagent, or worse, prevent the reaction occurring by physically separating the two. The other issue regarding these sensors is one that is addressed in almost every paper: operational and temporal stability. Complicating this issue is the differing opinions as to the extent of stability required, with tests over hours [192, 193], weeks [145, 181] and months [26, 177, 184, 185] all being reported. This investigation aims to produce a variety of immobilised $[\text{Ru(bipy)}_3]^{2+}$ derivatives and evaluate their stability and analytical utility, with the hope of addressing these issues.
3.2 Experimental

3.2.1 Materials

Flame-dried glassware and standard Schlenk and vacuum-line techniques were employed for moisture sensitive manipulations. Unless otherwise stated, deionised water (Continental Water Systems, Australia) and a dry nitrogen atmosphere were used in all syntheses, and solvents were dried over the appropriate desiccants and distilled immediately prior to use. Starting materials were used without further purification. Sigma-Aldrich (Castle Hill, New South Wales, Australia) supplied 1-bromoprop-2-ene, bis(phenyl)chlorosilane, bis(tertbutyl)chlorosilane, n-butyllithium, 1-chloro-3-iodopentane, 4,4′-dibromobiphenyl, 1,4-dibromophenyl, 4,4′-dimethyl-2,2′-bipyridine, dimethylchlorosilane, hexachloroplatinic acid, imidazole, lithium granules, methyl dichlorosilane, methyllithium, 35-70 mesh silica, trichlorosilane, cerium(IV) sulphate and silver permanganate. Diethyl ether, ethanol, sodium sulphate and toluene were obtained from Merck (Kilsyth, Victoria, Australia). Chloroform, isopropanol and tetrahydrofuran were purchased from ChemSupply (Gillman, South Australia, Australia) and hexane from Scharlau Chemie (Sentmenat, Spain). Sulfuryl chloride, sodium chlorate and cerium(IV) ammonium nitrate were bought from Ajax Finechem Inc. (Sydney, Australia). Sodium metal and potassium persulphate were obtained from BDH (Poole, England) and cis-dichlorobis(2-(4,6-difluorophenyl)pyridin)eiridium(III) from Rubipy Scientific Inc. (Toronto, Canada). The sodium hypochlorite (Hy-Clor supershock granular pool chlorine) is available for domestic use from Hy-Clor Australia Pty. Ltd. (Sydney, Australia), and the lead nitrate is of unknown origin. Cis-dichlorobis(2,2′-bipyridyl)ruthenium(II) was synthesised by Pappin [169]. Lithium diisopropylamine was prepared from n-butyllithium and diisopropylamine in tetrahydrofuran or diethyl ether. The mesophase and bis(2,2′-bipyridine)(4,4′-dinonyl-2,2′-bipyridine)ruthenium(II) was kindly donated by Egan Doeven from La Trobe University, who synthesised and characterised the complex. Deuterated solvents were obtained from Cambridge Isotope Laboratories, Inc. (Andover USA). In all synthetic methods, the silica was dried under a static vacuum overnight at 100°C prior to functionalisation.
3.2.2 Synthesis of silica-bound 4-methyl-4'-\((\text{C}_4\text{H}_8\text{Si(CH}_3\text{)}_2\text{Cl})\)-2,2'-bipyridine

(Method 1)

As per Barnett et al. [1], n-butyllithium (21 mL; 33 mmol) was added to diisopropylamine (5.8 mL; 39 mmol) in 30 mL of diethyl ether. This was added drop-wise to 4,4'-dimethyl-2,2'-bipyridine (5.03 g; 27.3 mmol) in 100 mL of diethyl ether at -80°C. After one hour 1-bromoprop-2-ene (2.7 mL; 33 mmol) was added to the cooled mixture, which was then allowed to return to room temperature overnight. The product was hydrolysed with water, extracted using diethyl ether and dried over Na$_2$SO$_4$ before the volatiles were removed under reduced pressure.

$^{13}$C NMR (CDCl$_3$, 75 MHz) $\delta$ (ppm): 21.1, 34.1, 34.5, 115.3, 120.9, 121.7, 123.7, 124.5, 137.1, 147.2, 148.8, 148.9, 156.0, 156.1. This was then dissolved in dry toluene (25 mL), added to 0.94 mL (8.6 mmol) methyldichlorosilane and 50 mg H$_2$PtCl$_6$ in 3 mL isopropanol and heated overnight at 100°C. After cooling to room temperature, the solvent was removed by evaporation and the product ($^{13}$C NMR (CDCl$_3$, 75 MHz) $\delta$ (ppm): 0.837, 21.9, 33.4, 34.8, 116.4, 124.4, 125.1, 126.4, 126.9, 135.9, 145.0, 147.9, 148.0, 155.1, 157.7) redissolved in ethanol and added via syringe to 2.00 g (wet mass) silica and imidazole (590 mg; 8.71 mmol) and gently agitated for at least 16 hours on a platform stirrer. The functionalised silica was filtered then washed with distilled water, tetrahydrofuran and benzene, starting with water and becoming successively less polar, then reversing the process and allowed to air-dry.

3.2.3 Synthesis of silica-bound 4-methyl-4'-\((\text{C}_4\text{H}_8\text{Si(CH}_3\text{)}_2\text{Cl})\)-2,2'-bipyridine

(Method 2)

This method was performed under argon, due to the use of lithium metal. 1 molar equivalent of lithium diisopropylamine was added drop-wise to 4,4'-dimethyl-2,2'-bipyridine (1.00 g; 5.43 mmol) dissolved in 40 mL diethyl ether at -80°C. The colourless solution immediately turned dark brown. 1-chloro-3-iodopentane (0.69 mL; 6.5 mmol) was added after an hour to the cooled mixture and left stirring overnight. The mixture was then hydrolysed with water, extracted using dichloromethane and brine and dried over Na$_2$SO$_4$. The solvent was removed under reduced pressure to yield a dark brown product. $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ (ppm): 19.8, 26.1, 30.8, 33.2, 43.6, 119.7, 120.6, 122.5, 123.4, 146.5, 147.6, 147.7, 150.3, 154.5.
This dark brown product was dissolved in 50 mL tetrahydrofuran and added drop-wise to lithium (250 mg; 36.0 mmol) and sodium (100 mg; 4.35 mmol) in tetrahydrofuran. After 5 hours, the mixture was cooled to -80°C and dichloromethylsilane (1 mL; 9.2 mmol) was added. This mixture was stirred and allowed to warm to room temperature overnight, then hydrolysed with water, extracted with diethyl ether and dried over Na₂SO₄. A red oil was formed upon removal of the solvent under reduced pressure. ¹³C NMR (CDCl₃, 100 MHz) δ (ppm): 14.0, 19.6, 24.4, 26.4, 31.1, 33.4, 43.1, 119.7, 120.5, 122.5, 123.3, 146.2, 147.7, 147.9, 150.2, 154.9.

Sulfuryl chloride was added to the above compound in chloroform (10 mL), which was then added to 2.00 g (wet mass) silica and imidazole (576 mg; 8.46 mmol) in THF (20 mL). This mixture was left at room temperature on a platform stirrer for 48 hours. The functionalised silica was filtered then washed with distilled water, tetrahydrofuran and benzene, starting with water, becoming successively less polar then reversing the process, and air dried.

### 3.2.4 Synthesis of silica-bound 4-methyl-4’-(CH₂SiR₂H)-2,2’-bipyridine (R = CH₃, C(CH₃)₃ or C₆H₅) (Method 2)

4,4'-dimethyl-2,2'-bipyridine (1.00 g; 5.43 mmol) was dissolved in 50 mL diethyl ether and, once cooled to -80°C, 1 molar equivalent of lithium diisopropylamine was added drop-wise and the mixture allowed to stand for an hour. This was cooled to -80°C, 7 mmol of the appropriate silane was added and the reaction was stirred overnight. The room temperature mixture was then hydrolysed with water, extracted with diethyl ether, dried over Na₂SO₄ and the solvent removed under reduced pressure.

¹³C NMR (CDCl₃, 75 MHz) δ (ppm):

- 4-methyl-4’-(CH₂Si(CH₃)₂H)-2,2’-bipyridine: -5.37, 20.4, 24.0, 120.2, 121.3, 122.9, 123.9, 124.0, 147.1, 148.2, 154.0
- 4-methyl-4’-(CH₂Si(C(CH₃)₃)₂H)-2,2’-bipyridine: 21.7, 23.5, 29.9, 31.3, 124.3, 124.5, 126.9, 149.7, 151.0, 151.2, 154.2, 158.5
- 4-methyl-4’-(CH₂Si(C₆H₅)₂H)-2,2’-bipyridine: 23.2, 25.1, 124.3, 126.8, 130.2, 130.4, 132.1, 136.6, 137.4, 149.9, 151.0, 151.5, 158.2
To a 5 mL solution of the product in chloroform, 0.60 mL (7.3 mmol) SO₂Cl₂ was added via syringe. This was subsequently added to dry silica (2.00 g wet mass) and imidazole (590 mg, 8.71 mmol) in 50 mL tetrahydrofuran and left on a platform stirrer overnight. The functionalised silica was filtered then washed with distilled water, tetrahydrofuran and benzene, starting with water and becoming successively less polar, then reversing the process and air dried.

3.2.5 Synthesis of silica-bound 4-methyl-4'-(CH₂-C₆H₄-Si(CH₃)₂H)-2,2'-bipyridine (Method 2)

To a cooled (-80°C) mixture of 1,4-dibromobenzene in diethyl ether (50 mL), 5 mL (7.75 mmol) of n-butyllithium was added by syringe. After an hour, dimethylchlorosilane (0.92 mL, 8.5 mmol) was added to the cooled mixture, which was subsequently allowed to stir overnight before being hydrolysed with water. The product was extracted using diethyl ether and dried over Na₂SO₄; the solvent was removed under reduced pressure. ⁱ³C NMR (CDCl₃, 100 MHz) δ (ppm): -5.16, 122.7, 129.7, 134.1, 134.5.

One molar equivalent of lithium diisopropylamine in diethyl ether (40 mL) was added to 4,4'-dimethyl-2,2'-bipyridine (1.50 g; 8.14 mmol) in diethyl ether (150 mL) at -80°C. After 10 minutes the previously prepared silane was added and the mixture was left to warm slowly overnight, with stirring. This was then hydrolysed with water, extracted with diethyl ether and dried over Na₂SO₄; the solvent was then removed under reduced pressure. ⁱ³C NMR (CDCl₃, 100 MHz) δ (ppm): -4.2, 20.7, 20.9, 121.6, 121.8, 123.6, 124.2, 130.6, 130.8, 135.1, 135.3, 135.7, 147.5, 148.5, 155.6.

The product was dissolved in chloroform (30 mL) and sulfuryl chloride (0.67 mL; 8.3 mmol) was slowly added via syringe. Once cool, this was added to 2.50 g (wet mass) silica and 665 mg (9.77 mmol) imidazole and left on a platform stirrer overnight at room temperature. The functionalised silica was filtered then washed with distilled water, tetrahydrofuran and benzene, starting with water and becoming successively less polar, then reversing the process and air dried.
3.2.6 Synthesis of Ru(BPS)₂Cl₂

As per Della Ciana et al. [94], 500 mg (0.850 mmol) of 4,7-diphenyl-1,10-phenanthroline disulphonic acid (bathophenanthrolinedisulphonic acid), 110 mg (0.420 mmol) of ruthenium trichloride hydrate and 107 mg (2.53 mmol) of lithium chloride were dissolved in 2.5 mL of dimethylformamide (not distilled, stored under N₂). This was heated in a microwave for 10 minutes at 160°C. The resultant solution was added drop-wise to rapidly stirring dichloromethane (300 mL). The product was filtered and washed with dichloromethane and acetone. 1H NMR (DMSO-d₆, 270 MHz) δ (ppm): 8.41-8.00 (m, 7H), 7.93-7.55 (m, 21H). NMR was consistent with literature [94].

3.2.7 Synthesis of immobilised complexes

To a suspension of the appropriate silica-bound ligand in wet ethanol (20 mL) was added 10 mg Ru(bipy)₂Cl₂ (0.021 mmol), 24 mg Ru(BPS)₂Cl₂ (0.021 mmol) or 25 mg [Ir(dfppy)₂Cl]₂ (0.024 mmol) per 1 g silica. This was heated at 50°C overnight (without stirring) in the exclusion of light. The functionalised silica was filtered then washed with distilled water, tetrahydrofuran and benzene, starting with water and becoming successively less polar, then reversing the process, and air dried.

3.2.8 General instrumentation

¹H, ¹³C and ²⁹Si NMR spectra were measured in deuterated chloroform using a JEOL Eclipse Plus 400 MHz NMR spectrometer (JEOL, Tokyo, Japan), JEOL Eclipse Plus 270 MHz NMR spectrometer (JEOL, Tokyo, Japan) or Varian Unity Plus 300 MHz NMR spectrometer (Varian Inc., Palo Alto, USA). The spectra are referenced externally against tetramethylsilane; chemical shifts are reported in ppm.

Photoluminescence spectra were collected using a Cary Eclipse Spectrofluorimeter (Varian Analytical Instruments, Australia) with a R928 photomultiplier tube (Hamamatsu, Iwata-gun, Shizuoka-ken, Japan). The correction factors for the emission spectra were acquired using an Optronic Laboratories model OL 65A constant current source, as previously described [194].
3.2.9 Construction of flow cells

The functionalised silica (11 mg) was packed between small (approximately 10 mm) plugs of glass wool in a glass tube (approximately 40 mm length, 1.5 mm internal diameter, 3 mm external diameter).
3.3 Results and Discussion

3.3.1 Synthesis of the functionalised silicas

Method 1 was successfully employed to synthesise an immobilised complex. During the formation of the siloxane bond between the ligand and the silica, it was found that stirring caused the silica to be crushed into very fine particles. These particles proved to be too fine for use in the flow cells, as they could not be contained by the glass wool. Subsequent reactions were therefore performed either without stirring or on a platform stirrer, to ensure the correct bead size. SMA61 was synthesised in this manner. The product was divided into samples A and B, where A incorporated an additional 0.5 M sodium hydroxide/acetonitrile wash after the functionalisation of the silica. This caused SMA61A to retain the dark colour of Ru(bipy)$_2$Cl$_2$, and characterisation via photoluminescence indicated that the complex was not coordinated to the immobilised ligand. The sample was therefore disregarded, and analytical tests were performed on SMA61B.

The hydrosilation reaction employed in Method 1 was previously developed [1] to produce a compound containing two siloxane bonds to the silica in order to ensure a strong attachment. In this work, it was postulated that due to its inherent strength, a single siloxane bond per ligand was sufficient, and this change may improve the loading density. Despite all efforts, Method 1 was not robust enough to substitute the dichloromethylsilane with chlorodimethylsilane. A search of the literature revealed that the reactivity of silanes has been noted [195] to follow the trend:

\[
\text{SiHCl}_3 > \text{RSiHCl}_2 > \text{R}_2\text{SiHCl} > \text{R}_3\text{SiH}
\]

As a result, Method 1 could not be used to synthesise the desired compound, containing only one siloxane bond to the silica. At this time, Method 2 was developed in order to allow a degree of flexibility in the synthesis. Using this method, complexes SMA69, SMA81 and SMA82 were synthesised. These contain a methyl linker between the bipyridine and the siloxane group, as well as a single siloxane bond. They differ in the protecting groups present on the molecule: SMA69 has a dimethyl siloxane, SMA81 a di-tert-butyl siloxane and SMA82 a diphenyl siloxane. Method 2 therefore allowed a comparison of the differences caused by reducing the
number of siloxane bonds as well as the effect of protecting groups on the performance of the functionalised silica.

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Compound Attached to Silica</th>
<th>Ligand Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMA61B</td>
<td>4-[4-(dichloromethylsilanyl)butyl]-4’-methyl-2,2’-bipyridyl]bis(2,2’-bipyridyl)ruthenium(II)</td>
<td><img src="image" alt="Structure" /></td>
</tr>
<tr>
<td>SMA69</td>
<td>4-[(chlorodimethylsilanyl)methyl]-4’-methyl-2,2’-bipyridyl]bis(2,2’-bipyridyl)ruthenium(II)</td>
<td><img src="image" alt="Structure" /></td>
</tr>
<tr>
<td>SMA80</td>
<td>4-[(chlorodimethylsilanyl)tolyl]-4’-methyl-2,2’-bipyridyl]bis(2,2’-bipyridyl)ruthenium(II)</td>
<td><img src="image" alt="Structure" /></td>
</tr>
<tr>
<td>SMA81</td>
<td>4-[(di-tert-butylchlorosilanyl)methyl]-4’-methyl-2,2’-bipyridyl]bis(2,2’-bipyridyl)ruthenium(II)</td>
<td><img src="image" alt="Structure" /></td>
</tr>
<tr>
<td>SMA82</td>
<td>4-[(chlorodiphenylsilanyl)methyl]-4’-methyl-2,2’-bipyridyl]bis(2,2’-bipyridyl)ruthenium(II)</td>
<td><img src="image" alt="Structure" /></td>
</tr>
<tr>
<td>SMA90</td>
<td>4-[4-(chlorodimethylsilanyl)butyl]-4’-methyl-2,2’-bipyridyl]bis(2,2’-bipyridyl)ruthenium(II)</td>
<td><img src="image" alt="Structure" /></td>
</tr>
<tr>
<td>SMA91</td>
<td>4-[(chlorodimethylsilanyl)methyl]-4’-methyl-2,2’-bipyridyl]bis(2,2’-bipyridyl)ruthenium(II)</td>
<td><img src="image" alt="Structure" /></td>
</tr>
</tbody>
</table>

Table 3.01: The full name and abbreviation of the synthesised complexes, and the structure of the ligand prior to the formation of the siloxane bond to silica.
In order to have a more direct comparison between a single and double siloxane bond, a butyl linker between the bipyridine and this functional group was required. This synthesis was more sensitive to the quenching effects of water, and so reactions were performed under argon. Initial trials involved attaching the butyl group to the silane, then forming the bond to the 2,2′-bipyridine unit. This pathway was, however, prohibited by the volatility of the silane produced. The synthesis was then approached from the opposite direction - first forming the bond between the 2,2′-bipyridine and the linker, then attaching the silane. The desired complex was thus obtained in SMA90. Method 2 was also used to create immobilised complexes containing a rigid linking group. The synthesis of a complex containing a diphenyl linking group was attempted, however it was unfortunately not successful. This was attributed to the substitution of one bromine from 4,4′-dibromo-1,1′-diphenyl for the silane making the following reaction - the formation of the bond to the bipyridine - unfavourable. Attempts at bonding the linker to the bipyridine first then to the silane were also ineffective. The synthesis of a complex containing a single phenyl linkage group (SMA80), on the other hand, proceeded without difficulties. A small library of complexes immobilised to silica was thus ultimately synthesised, exploring the effect of linker length (butyl vs. methyl linkers) and rigidity (tolyl vs. butyl linkers) and the effect of methyl, tert-butyl and phenyl protecting groups.

When examined visually, the silicas functionalised via Method 2 were much brighter in colour (and similar to the colour of 1 mM [Ru(bipy)₃]²⁺) than those obtained through Method 1, indicating they may contain more ruthenium(II) complex. These were packed into glass tubing and their chemiluminescence reactions examined by SIA. Through this, it was shown that all the cells exhibited a decay in light intensity over time, and each complex has a unique rate of decay (Figure 3.01). SMA82, with the diphenyl protecting groups, shows the greatest initial peak area and the sharpest decay rate. As a result, it has the lowest projected maximum lifetime. SMA61B, with its two siloxane bonds to the silica, and SMA81, boasting di-tert-butyl protecting groups, both show an initial increase in peak area, followed by a decrease. This is consistent with that seen by Barnett et al. [1], although the cause is still unidentified. SMA90, which differs from SMA61B by the substitution of one siloxane bond to the silica for an additional methyl protecting group, shows an interesting pattern. Its initial peak area is just short of that of SMA61B, and an immediate decay is observed. This complex exhibited the lowest peak heights and peak areas. The most stable of the complexes over time is SMA81.
Figure 3.01: Stability study of the functionalised silicas. Chemiluminescence reactions of the functionalised silica cell with $1 \times 10^{-4}$ M codeine in 0.05 M H$_2$SO$_4$ and 0.1 M cerium(IV) ammonium nitrate in 1 M H$_2$SO$_4$ were obtained using SIA.

The three complexes differing by their protecting groups, SMA69, SMA81 and SMA82, do not show similar trends. SMA82, as previously mentioned, has high signal intensities and a rapid decay. In contrast, SMA81 is the most stable of all the complexes and exhibits a slight initial increase in chemiluminescence intensity, although this is still markedly lower than the signal intensity of SMA69 and SMA82. SMA69 shows the second highest peak intensities and a decay that is intermediate between SMA81 and SMA82. In light of this data, it was concluded that tertiary-butyl protecting groups offer superior stability.

When a cell was re-tested the following day, a regeneration of the response was observed. This regenerated emission was also found to decrease over time. A stability study was performed in which the same cell was analysed repeatedly over three consecutive days (see Figure 3.02 for the data for SMA69). On each day, the stability profiles were practically the same: an initial peak intensity of almost 2000 a.u. decreasing over time to approximately 1500 a.u. A similar trend was observed for the complexes attached to silica via one siloxane bond and by two. The decrease seen in emission intensity can be ascribed to the number of ruthenium(II) centres
available to react. As a regeneration of the response was observed, the physical loss of complex through leaching can be discounted as a possible cause. It seems probable that there is something inhibiting either the repeated oxidation of the ruthenium(II) centres or their reaction with the analyte. The volume of oxidant used and the length of time in which it is in contact with the functionalised silica should be further optimised. Alternatively, there remains the fact that the silica surface has not been endcapped. The silanol groups present may be encouraging polar-polar interactions with either the analyte or by-products of the reaction, and a build up of these molecules may shield the ruthenium(II) centres from further reaction. Although the cause of this decay and regeneration is still unknown, the fact that it was seen in all the functionalised silicas indicates that the postulation was correct: a single siloxane bond is strong enough to bind the complex to the silica without leaching.

As seen in Figure 3.03, SMA61B exhibited signal-to-blank ratios almost nine times that of its analogue, SMA90. This could indicate a greater loading on the part of SMA61B, which is
unexpected considering that the complex in SMA90 has double the amount of Si-OH groups on the silica available for bonding. SMA80 and SMA82, both containing phenyl groups in their structure, also show low signal-to-blank ratios, indicating that the majority of their large signal intensities are due to a blank signal. SMA81 resulted in the greatest signal-to-blank ratios of all the functionalised silicas, in addition to the best stability. Despite this obvious superiority, it must be noted that the detection limit of $1 \times 10^{-7}$ M codeine under these conditions was far inferior to the solution phase detection limit of $5 \times 10^{-9}$ M [196].

Figure 3.03: Signal-to-blank ratios of the peak area of the functionalised silicas. Chemiluminescence reactions of the functionalised silica cell with $1 \times 10^{-4}$ M codeine in 0.05 M H$_2$SO$_4$ and 0.1 M cerium(IV) ammonium nitrate in 1 M H$_2$SO$_4$ were obtained using SIA.

In addition to these functionalised silicas, the endcapping of SMA69 was attempted. Trimethylsilane was added to the silica functionalised with 4-[(chlorodimethylsilanyl)methyl]-4′-methyl-2,2′-bipyridyl) and imidazole in dry tetrahydrofuran and the mixture stirred on a platform stirrer for 48 hours. The product was washed with a series of solvents of decreasing polarity and then the order of washings was reversed. The resultant silica was a pale creamy colour, as though
some of its original colour had been removed. The immobilised ligand was then coordinated to cis-dichlorobis(2,2′-bipyridyl)ruthenium(II) and analysed using SIA. The suspicion that the ‘endcapping’ had actually stripped off the desired ligand was confirmed by the lack of response. Further attempts at endcapping were abandoned.

In order to improve the low signal intensities and sensitivities of the functionalised silicas, the use of parent complexes other than cis-dichlorobis(2,2′-bipyridyl)ruthenium(II) (Ru(bipy)₂Cl₂) was trialled. Tris(4,7-diphenyl-1,10-phenanthroline disulphonate)ruthenium(II) has previously been found to give large signal intensities [22], and so Ru(bipy)₂Cl₂ was substituted by cis-dichlorobis(4,7-diphenyl-1,10-phenanthroline disulphonate)ruthenium(II). The ligands of SMA69 and SMA81 were chosen for this experiment, as they had shown a great amount of potential when coordinated to Ru(bipy)₂Cl₂. Although these complexes showed a large average peak intensity, they also exhibited a very sharp decay and poor signal-to-blank ratios. [Ir(dfppy)₂]-based complexes were also investigated, however these were unstable and did not last more than 50 replicates.

3.3.2 Entrapment of [Ru(bipy)₂(dnbipy)]²⁺

It has been found that the entrapment of [Ru(bipy)₃]²⁺ in a solid phase allows the complex to retain many of its favourable properties, although the stability of such an immobilisation is generally inferior to the formation of covalent bonds [2]. The stability can be improved by the use of a heteroleptic complex that exhibits similar properties, yet has an extended hydrophobic region on the ancilliary ligand to anchor it to the stationary phase and prevent leaching. A small amount of bis(2,2′-bipyridine)(4,4′-dinyonyl-2,2′-bipyridine) ruthenium(II) ([Ru(bipy)₂(dnbipy)]²⁺) and some mesophase were obtained from colleagues at LaTrobe University. This had shown promising results in ECL experiments, and a distinct colour change was observed after the electrochemical oxidation of the complex [197]. When in contact with aqueous solution, the gel solidified, yet regained its gel-like fluidity in the absence of water. It was thought that the gel may be able to coat the interior of a glass flow cell when in the oil-form, then solidify to prevent loss of complex during the aqueous experiments.

The functionalised gel was prepared by dissolving 1 g of gel in dichloromethane and adding 10 mg of [Ru(bipy)₂(dnbipy)]²⁺, resulting in a bright orange solution. The solvent was gently
evaporated off to leave an oil that was slightly less viscous than the gel starting material. Upon addition of water, the oil formed a solid gel. This solid was separated into two, with one half being left under aqueous conditions and the other was allowed to dry in air. The following day, the gel under aqueous conditions was still solid, and the dried sample had returned to its oily form. Having confirmed that the physical properties could be manipulated by choice of solvent, the effect of chemical oxidants was examined. Unlike the ECL experiments, no visible change could be observed upon addition of Ce(IV)(SO₄)₂, acidic (pH 2.5) potassium permanganate, nitric acid, cerium(IV) ammonium nitrate, silver permanganate, potassium persulphate, sodium chlorate, lead nitrate or sodium hypochlorite. These solutions were all in deionised water, so it was theorised that the aqueous solution could not penetrate into the hydrophobic gel and therefore could not oxidise the [Ru(bipy)₂(dnbipy)]²⁺. Cerium(IV) ammonium nitrate in acetonitrile that had been acidified with HNO₃ was therefore added to the oil and stirred. No change could be seen, so ethanol was added to ensure that the gel had fully dissolved. This still had no effect, and as a result it was concluded that the chemical oxidation of this complex was not practicable.
3.4 Conclusions

In conclusion, a small library of ligands covalently bonded to silica has been synthesised, coordinated to a transition metal complex and evaluated as regenerable chemiluminescence cells. The synthetic methods described may also be of use in the design of bioconjugates and solar energy conversion cells. Although the sensitivities of solution phase [Ru(bipy)$_3$]$_{2^{+}}$ chemiluminescence reactions cannot be rivalled by these solid phase cells, a robust, flexible and versatile synthetic method was developed. Using this method, it was demonstrated that a single covalent bond is all that is necessary to bind the ligand to the silica. An intriguing trend has also been observed, in that despite a decrease in light intensity with repeated use, the cell can regenerate over time. From these tests, SMA81 - containing a single methyl group between the bipyridine and the di-tert-butyl protected siloxane group - showed the greatest stability and sensitivities of the immobilised complexes. Additionally, the chemical oxidation of [Ru(bipy)$_2$(dnbipy)]$_{2^{+}}$ entrapped within mesophase is not feasible, so this immobilised complex is more suitable for ECL analysis.
Chapter Four: The Chemiluminescence of Iridium(III) Complexes
4.1 Introduction

In recent years, much interest has been shown in the photophysical and photochemical properties of iridium(III) complexes \([12, 30, 80, 101]\). It has been found that the emission efficiencies can be improved by using complexes containing two different ligands (heteroleptic complexes) instead of a single type of ligand (homoleptic complexes) \([198-200]\). The ancillary ligand can be chosen to affect properties such as emission energy and efficiency \([53, 122, 201-204]\), or they can be more passive and allow the complex to exhibit the same properties as its homoleptic parent \([115, 205-207]\). The choice of ligand is not limited to the diimines like with ruthenium(II) chemiluminescence reagents; complexes have been described with diimine \([208]\), cyclometalating \([116]\) and monoanionic \([207]\) ligands such as acetylacetonate or picolinate. A vast amount of research, spurred by LED fabrication \([115, 116]\), has been invested in the synthesis, characterisation and evaluation of neutral iridium complexes, containing cyclometalated ligands and/or monoanionic ancillary ligands \([80, 116]\). More recently, single-layer LECs have been developed using ionic transition metal complexes as the chromophoric material \([209]\). These complexes generally include diimine \([208, 210]\) and pseudohalogen \([101, 209]\) ligands, but the substitution of the ligands with ionic functional groups has also been described \([3, 210]\).

The tuning of the emission efficiency of complexes is closely tied to the tuning of the emission wavelength. Certain ligands and substituents are chosen to either stabilise or destabilise the HOMO and LUMO energies. According to the Energy Gap Law \([11]\), the non-radiative decay rate of a metal-ligand complex increases exponentially as the emission energy decreases. Consequently, the emission efficiency is expected to increase with energy \([11, 211]\) and the introduction of electron-withdrawing fluorine or trifluoromethane groups (on the phenyl moiety of 2-phenylpyridine) has been utilised for this purpose \([201]\). While effective in inducing a hypsochromic shift of the emission, this demonstrates a problem encountered by such a destabilization: as the LUMO energy increases, a decrease in quantum yield can be seen due to the increased accessibility of other, non-radiative electronic states \([116, 201, 212]\). It is therefore difficult to predict the efficiency of an emission based solely upon its wavelength.

There have been several studies indicating that despite boasting high photoluminescence quantum yields, the ECL performance of iridium(III) complexes is generally inferior to that of \([\text{Ru(bipy)}_3]^{2+}\) \([213, 214]\). Clearly, there are other factors that influence the efficiency of the
light emitting reaction, including the efficiency of the formation of the excited state [215-217]. For instance, the photoluminescence quantum yield of [Ir(ppy)₃] is greater than that of [Ru(bipy)₃]²⁺ (0.069 and 0.042, respectively), yet its ECL quantum yield is 0.33 with respect to [Ru(bipy)₃]²⁺ (Φₑcl = 1) [214]. These differences in the ECL and photoluminescence quantum yields may be attributable to interactions of the excited state [Ir(ppy)₃]* with solvent molecules or decomposition upon electrolysis and interaction with excited state co-reactant [214]. Subsequent studies have found that ECL quantum yields can actually be improved by performing the analysis in organic solvents rather than in aqueous, due to the complexes’ superior solubility in organic solvents [214, 218]. Other researchers have shown that ECL efficiencies greater than that of the standard [Ru(bipy)₃]²⁺ can be obtained by selecting a reagent that has a lower reduction potential than the analyte (tripropylamine, TPA) and a more positive oxidation potential than the chosen reference ([Ir(ppy)₃]) [219]. The complexes [Ir(ppy)₂(bipy)]⁺, [Ir(ppy)₂(phen)]⁺, [Ir(pq)₂(acac)] and [Ir(pq)₂(tmd)] (where ppy is the 2-phenylpyridine anion, bipy is 2,2'-bipyridine, phen is 1,10-phenanthroline, pq is the 2-phenylquinoline anion, acac is acetylacetonate and tmd is 2,2',6,6'-tetramethylhepta-3,5-dione anion) all exhibited more intense electrochemiluminescence than the benchmark [Ru(bipy)₃]²⁺ upon reaction with TPA [219]. Similarly, high ECL efficiencies can be gained purely by using a co-reactant other than TPA. There are records of ECL efficiencies of up to 0.67 for the reactions between [Ir(ppy)₃] and 2-cyanofluorene in acetonitrile:dioxane (1:1) solutions, which is higher than that obtained from either the annihilation reaction or reaction with 1-cyanonaphthalene or 1,2-dicyanobenzene [220]. More recently, it has been discovered that the green-emitting (picolinate)bis(2-(p-tolyl)pyridinato-C²,N)iridium(III) [Ir(tpy)₂(pico)] is capable of very intense ECL [38]. ECL efficiencies relative to [Ru(bipy)₃]²⁺ were determined to be 0.6, 0.18 and 8 for the annihilation reaction, the oxidation-reduction reaction with TPA and the reduction-oxidation reaction with peroxodisulphate, respectively [38]. Clearly, the intensity of the ECL emission is dependent on not merely the reagent, but the entire reaction system.

In ECL experiments, there exists an inherent disadvantage: the electrode surface is prone to fouling, particularly in the analysis of complex media such as biological samples and under the widely variable conditions of HPLC [21, 221]. Consequently, the chemical oxidation of the reagents may sometimes be preferable. Recently, it has been proven that not only do these iridium(III) analogues behave similarly to ruthenium(II) electrochemically, they can also exhibit chemiluminescence [3, 136]. The water soluble complexes bis[2-phenylpyridinato-
C\textsubscript{2},N\textsubscript{(2,2\texttextsuperscript{	extprime}-bipyridine)}iridium(III) ([Ir(ppy)\textsubscript{2}(bipy)]\textsuperscript{+}), bis[2-phenylpyridinato-C\textsubscript{2},N\textsubscript{(1,10-phenanthroline)}iridium(III) ([Ir(ppy)\textsubscript{2}(phen)]\textsuperscript{+}) and bis[2-phenylpyridinato-C\textsubscript{2},N\textsubscript{(4,7-diphenyl-1,10-phenanthroline disulphonate)}iridium(III) ([Ir(ppy)\textsubscript{2}BPS]\textsuperscript{−}) were reacted with cerium(IV) sulphate and a range of organic reducing agents in acidic media [3]. The complexes can produce chemiluminescence comparable to that of [Ru(bipy)\textsubscript{3}]\textsuperscript{2+} or [Ru(phen)\textsubscript{3}]\textsuperscript{2+}, and [Ir(ppy)\textsubscript{2}BPS]\textsuperscript{−} in particular produced very intense signals [3]. Similar to [Ru(BPS)\textsubscript{3}]\textsuperscript{4−} [22], however, much of this was attributable to a blank signal. This large blank signal allowed the detection of the reagent at a concentration two orders of magnitude below that of [Ru(bipy)\textsubscript{3}]\textsuperscript{2+} under the same conditions [3]. Despite the report of [Ir(ppy)\textsubscript{2}(bipy)]\textsuperscript{+} and [Ir(ppy)\textsubscript{2}(phen)]\textsuperscript{+} producing greater ECL than [Ru(bipy)\textsubscript{3}]\textsuperscript{2+} [219], this was not found to be the case for the analogous chemiluminescence reactions [3]. This study was the first account of chemiluminescence from iridium(III) complexes, and it has since sparked several investigations into the chemiluminescence of such complexes [136, 222-225].

This chapter describes a continuation of the research exploring the chemiluminescence of iridium complexes. The most promising of the three iridium complexes, [Ir(ppy)\textsubscript{2}BPS]\textsuperscript{−}, is tested alongside its fluorinated derivative, bis[2-(3,5-difluorophenyl)pyridinato-C2,N](4,7-diphenyl-1,10-phenanthroline disulphonate)iridium(III) ([Ir(dfppy)\textsubscript{2}BPS]\textsuperscript{−}). In particular, the possibility of producing an emission in a more sensitive region of the photodetector whilst still retaining water solubility is explored through an evaluation of the latter complex.
4.2 Experimental

4.2.1 Materials

The complexes bis[2-phenylpyridinato-C2,N](4,7-diphenyl-1,10-phenanthroline disulphonate)iridium(III), bis[2-(4,6-difluorophenyl)pyridinato-C2,N](4,7-diphenyl-1,10-phenanthroline disulphonate)iridium(III) and tetrakis(2,4-difluorophenyl)pyridine-C2,N′(μ-dichloro)diiridium(III) were obtained from Rubipy Scientific Inc. (Toronto, Canada), tris(2-phenylpyridine)iridium(III) from Sigma-Aldrich (Castle Hill, New South Wales, Australia) and tris(2,2′-bipyridine)ruthenium(II) was obtained from Strem Chemicals (Newbury, Minnesota, USA). Iridium(III) chloride hydrate and 2-phenylpyridine were obtained from AlfaAesar (Johnson Matthey, London, UK). Sulphuric acid was purchased from Merck (Kilsyth, Victoria, Australia) and ethanol and diethyl ether from ChemSupply (Gilman, South Australia, Australia), respectively. Cerium(IV) sulphate, 4,7-diphenyl-1,10-phenanthroline disulphonate sodium salt, 1,10-phenanthroline, ethylene glycol, Sephadex LH-20, sodium perchlorate, hydrochlorothiazide, ofloxacin, enrofloxacin, difloxacin hydrochloride, danofloxacin, fleroxacin, pipemidic acid, marbofloxacin, piroxicam, ciprofloxacin, acetazolamide, sulfadiazine sodium salt and sulphanilamide were also obtained from Sigma-Aldrich. Pazufloxacin and hydroflu methazide were supplied by Riedel-De Haen (Germany) and Alltech Associates (Deerfield, Illinois, USA) respectively. Codeine was kindly donated by GlaxoSmithKline (Port Fairy, Victoria, Australia). Potassium oxalate and sodium dodecyl sulphate were purchased from BDH Chemicals (Poole, England). Triton X-100 was from Ajax Finechem (Sydney, New South Wales, Australia). Deuterated solvents were obtained from Cambridge Isotope Laboratories, Inc. (Andover USA).

4.2.2 Tetrakis(2-phenylpyridine-C2,N′)(μ-dichloro)diiridium(III)

\([\text{Ir}(\text{ppy})_2\text{Cl}_2]\) synthesis

This synthetic method was based on one described by Sprouse et al. [226]. 746 mg (2.50 mmol) of IrCl3 and 0.73 mL (5.0 mmol) of 2-phenylpyridine were dissolved in 5 mL of 75:25 ethoxyethanol:water and heated in a microwave at 135°C for 10 minutes. The precipitated product was washed with water, 1:1 ethoxyethanol:water and 1:1 ethanol:acetone, then purified by flash chromatography (silica column, dichloromethane eluent). The first band was collected and dried to give the final compound. Yield: 624 mg, 47%. 1H NMR (400 MHz, CDCl3) δ (ppm): 9.22 (d, \(J_{HH} = 4.94\), 1H), 7.86 (d, \(J_{HH} = 7.91\), 1H), 7.72 (t, \(J_{HH} = 6.91\), 1H),
7.47 (d, $J_{HH} = 6.43$, 1H), 6.75 (q, $J_{HH} = 6.76$, 2H), 6.56 (t, $J_{HH} = 6.67$, 1H), 5.92 (d, $J_{HH} = 7.91$, 1H).

4.2.3 [Ir(C\(^N\))\(_2\)BPS\(^-\)] \(^-\) synthesis

The appropriate iridium(III) dimer ([Ir(ppy)\(_2\)Cl\(_2\)]\(_2\), 100 mg, 0.0930 mmol or [Ir(dfppy)\(_2\)Cl\(_2\)], 100 mg, 0.0820 mmol) and 2.2 equivalents of 4,7-diphenyl-1,10-phenanthroline disulphonate sodium salt (128 mg, 0.220 mmol) were dissolved in 100 mL of a 90:10 ethanol:water mixture and refluxed at 80°C for 6.5 hours under a nitrogenous atmosphere, with stirring. This resulted in a bright orange solution of [Ir(ppy)\(_2\)BPS\(^-\)], or a bright yellow-green solution of [Ir(dfppy)\(_2\)BPS\(^-\)]. The solvent was removed under vacuum, and the resultant solid purified on a Sephadex LH-20 column (20 cm length, 1 cm i.d.) using 20:80 methanol:ethanol eluent. The first fractions were collected, the solvent removed and the product dried under vacuum ([Ir(ppy)\(_2\)BPS\(^-\)]: 160 mg, 85%; [Ir(dfppy)\(_2\)BPS\(^-\)]: 121 mg, 70%).

Figure 4.01: Structure of [Ir(C\(^N\))\(_2\)BPS\(^-\)] where X = H ([Ir(ppy)\(_2\)BPS\(^-\)] or F ([Ir(dfppy)\(_2\)BPS\(^-\)]). The numbers correspond to the NMR assignments in Table 4.01.
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Table 4.01: $^{13}$C NMR (100 MHz) and $^1$H NMR (400 MHz) peak assignments of [Ir(ppy)$_2$BPS]$^-$ and [Ir(dfppy)$_2$BPS]$^-$ in CD$_3$OD.
4.2.4 [Ir(phen)$_3$]$^{3+}$ synthesis

IrCl$_3$ (100 mg, 0.335 mmol) and 1,10-phenanthroline (362 mg, 2.01 mmol) were dissolved in 5 mL ethylene glycol and heated in a microwave to 200°C for 30 minutes. This resulted in a dark red solution that was added drop-wise to rapidly stirring diethyl ether. A dark red oil was isolated from the diethyl ether and the desired complex was precipitated out of solution using finely ground sodium perchlorate. The brick-red precipitate was collected, washed with water and allowed to air-dry. Yield: 50 mg, 18%. $^{13}$C NMR (75 MHz, CD$_3$OD) δ (ppm): 149.16, 144.944, 136.665, 128.927, 126.485, 123.357.

4.2.5 Spectroscopic characterisation

Absorbance spectra were collected using a Cary 300 Bio UV-visible Spectrophotometer (Varian Australia, Mulgrave, Victoria, Australia) in quartz cuvettes of 1 cm path length. A Cary Eclipse Spectrofluorimeter (Varian Analytical Instruments, Australia) with a R928 photomultiplier tube (Hamamatsu, Iwata-gun, Shizuoka-ken, Japan) was used to collect photoluminescence spectra of the reagents in 1 cm quartz cuvettes (5 nm band pass, 1 nm data interval, PMT voltage: 600 V). The emission spectra were corrected as previously described [194]. The same instrument was used to obtain chemiluminescence spectra of the reagents. A solution of 1 mM reagent and 5 × 10$^{-5}$ M ofloxacin in 50:50 acetonitrile:water was merged with 1 mM Ce(SO$_4$)$_2$ in 0.05 M H$_2$SO$_4$ within a mirror-backed spiral flow cell fabricated from clear PTFE tubing. NMR characterisation was performed on a Varian Unity Plus 300 MHz ft-NMR spectrometer or a Jeol Eclipse Plus 400 MHz ft-NMR spectrometer, in deuterated methanol.

To obtain the photoluminescence quantum yields, the integrated emission spectra (500 - 850 nm, $\lambda_{ex} = 450$ nm) for each complex at concentrations of 1 × 10$^{-5}$ M, 7.5 × 10$^{-6}$ M, 5 × 10$^{-6}$ M and 2.5 × 10$^{-6}$ M were plotted against their absorbance at 450 nm. The quantum yields are relative proportionality constants based on the [Ru(bipy)$_3$]$^{2+}$ literature value of 0.028 in air-saturated aqueous solution [227].

4.2.6 Flow injection analysis with chemiluminescence detection

Relative chemiluminescence intensities were evaluated using flow analysis methodology. The detector consisted of a coiled piece of 0.8 mm i.d. PTFE tubing (DKSH) mounted flush against the PMT (Electron Tubes model 9124B40, ETP, Ermington, New South Wales, Australia) and both the flow cell and the PMT were kept in custom-built light-tight housing.
The PMT was kept at a steady voltage of 1 kV by a stable power supply (Electron Tubes model PM28B, ETP) and voltage divider (Electron Tubes model C611, ETP). The reagent was manually loaded onto the sample loop (70 μL) using a syringe, injected into the analyte stream and merged with the oxidant stream via a T-piece immediately prior to entering the flow cell. The flow rate was analyte dependent; the solutions were propelled at either 1 mL min$^{-1}$ or 3.5 mL min$^{-1}$. Detection limits were determined using a constant concentration of reagent and oxidant, with the analyte concentration ranging from $1 \times 10^{-10}$ M to $1 \times 10^{-5}$ M.

4.2.7 Stopped-flow injection analysis with chemiluminescence detection

The stopped-flow manifold was constructed as previously described [136], consisting of a programmable dual-syringe pump (Model sp210iw, World Precision Instruments, Glen Waverly, Victoria, Australia Reagent concentration: 0.01 mM, 1 mM), Valco two position six-port injection valve and a GloCell chemiluminescence detector (Global FIA) equipped with a dual inlet serpentine flow cell. 10 mL Luer lock syringes (Terumo) were loaded with 1 mM Ce(SO$_4$)$_2$ in 0.05 M H$_2$SO$_4$ and carrier solution of 50:50 acetonitrile:water. The 70 μL injection loop was filled with a reagent mixture before the pump was activated, dispensing 120 μL of carrier and oxidant solutions (10 mL min$^{-1}$). The reagent mixtures were: $5 \times 10^{-4}$ M [Ru(bipy)$_3$]$^{2+}$ or [Ir(dfppy)$_2$BPS]$_-$; $5 \times 10^{-6}$ M [Ru(bipy)$_3$]$^{2+}$ or [Ir(dfppy)$_2$BPS]$_-$; $5 \times 10^{-4}$ M [Ru(bipy)$_3$]$^{2+}$ or [Ir(dfppy)$_2$BPS]$_-$ containing $5 \times 10^{-6}$ M ofloxacin; and $5 \times 10^{-6}$ M [Ru(bipy)$_3$]$^{2+}$ or [Ir(dfppy)$_2$BPS]$_-$ containing $5 \times 10^{-7}$ M ofloxacin, mixed off-line in a 1:1 ratio from stock solutions.
4.3 Results and Discussion

4.3.1 Synthesis of $[\text{Ir(ppy)}_2\text{Cl}]_2$

The title compound was synthesised using a method that has its origins in Nonoyama’s method [228]. Initial attempts followed a method that called for an extended period (24 hours) of refluxing. This, however, resulted in the tris-chelated $[\text{Ir(ppy)}_3]$ instead of the desired dimer. The method was therefore modified to use microwave irradiation instead of thermal heating, and a short reaction time. This resulted in a satisfactory yield of the dimer (66.34%) in a very short time frame (10 minutes).

4.3.2 Homoleptic iridium(III) reagents

The literature abounds with cases of heteroleptic iridium(III) complexes being superior to homoleptic complexes in both EL and ECL applications, and this has been attributed to a reduction of Triplet-Triplet (T-T) annihilation (the collision and deactivation of excited state molecules) [198-200]. There have also been reports of heteroleptic ruthenium(II) complexes being superior to homoleptic complexes in ECL [94], however they do not show any improvement in emission intensity when excited chemically [42, 45]. In particular, it has been suggested that the differences seen in ECL are due to interactions of the charged complexes on the electrode surface, not the complexes themselves [94]. As chemiluminescence does not utilise electrodes, no great difference was seen between the heteroleptic and homoleptic ruthenium(II) complexes [42]. The field of iridium(III) chemiluminescence is novel, so any difference in the performance of heteroleptic and homoleptic complexes is unknown. In light of this, a brief investigation was undertaken, initially utilising the commercially available $[\text{Ir(ppy)}_3]$. In this test, 1 mM reagent in 100% acetonitrile was oxidised by 1 mM cerium(IV) sulphate in 0.05 M $\text{H}_2\text{SO}_4$. Some light was produced, however it was faint and the reaction with $1 \times 10^{-5}$ M codeine did not enhance the signal above the blank. It was concluded that the energy of the reaction was not sufficient to create the excited state $[\text{Ir(ppy)}_3]^+$. To remedy this, $[\text{Ir(phen)}_3]^{3+}$ was chosen. The diimine ligands increase the oxidation potential of the complex [219] and impart a degree of water solubility. The diimine itself, 1,10-phenanthroline, was chosen as it is a rigid molecule. Use of the more common 2,2’-bipyridine, which has free rotation around the bond between C1 and C1’, could allow the iridium(III) to preferentially form a bond to C3’ over N [229] (Figure 4.02).
Figure 4.02: Coordination of iridium(III) to 2,2′-bipyridine through N,N′ (left) and N,C3′ (right) coordination.

In this synthesis, the reactants (IrCl$_3$ and 1,10-phenanthroline) were dissolved in ethylene glycol and heated to 200°C in a microwave for 30 minutes. The resultant transparent, red-brown solution exhibited a very faint yellow luminescence and was added drop-wise to rapidly stirring diethyl ether to produce an oil. An excess of finely ground sodium perchlorate was added to this, causing the precipitation of a brick-red solid, which was collected by gravity filtration and washed with water before being air-dried.

The photoluminescence quantum yield ($\Phi_{PL}$) of [Ir(phen)$_3$]$_3^+$ was 94% less than that of [Ru(bipy)$_3$]$_2^+$ (Table 4.02). The broad emission (Figure 4.03) spanned over 300 nm, and exhibited dual peaks at 418 nm and 455 nm, with a minor shoulder apparent at 600 nm. Despite the maximum emission being in the 'true blue' region of the electromagnetic spectrum (at or below 460 nm [128]), the broadness of the spectrum and the shoulder in the red region results in a yellow apparent emission.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$\lambda_{abs}$ (nm)</th>
<th>$\lambda_{em}$ (nm)</th>
<th>$\Phi_{PL}$ *</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Ru(bipy)$_3$]$_2^+$</td>
<td>286, 453</td>
<td>627</td>
<td>0.028</td>
</tr>
<tr>
<td>[Ir(phen)$_3$]$_3^+$</td>
<td>275</td>
<td>418, 455</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Table 4.02: Spectroscopic properties of [Ru(bipy)$_3$]$_2^+$ and [Ir(phen)$_3$]$_3^+$ in 0.05 M H$_2$SO$_4$. *Data derived from integrated emission spectra of four concentrations at an excitation wavelength of 275 nm and relative to the literature value of [Ru(bipy)$_3$]$_2^+$ in aqueous solution, 0.028 [227].
The chemiluminescence potential of the complex was then ascertained, using Ce(SO₄)₂ as the oxidant and a small suite of structurally varied analytes. As can be seen in Figure 4.04, [Ir(phen)₃]³⁺ only barely produced chemiluminescence under the conditions tested. The raw signal intensity is orders of magnitude inferior to [Ru(bipy)₃]²⁺, and the presence of analyte barely enhanced the blank signal.
Based on the data obtained from both [Ir(ppy)$_3$] and [Ir(phen)$_3$]$^{3+}$, it was concluded that homoleptic iridium(III) complexes are not viable chemiluminescence reagents.

4.3.3 [Ir(ppy)$_2$BPS]$^-$ and [Ir(dfppy)$_2$BPS]$^-$

*Synthesis of [Ir(ppy)$_2$BPS]$^- and [Ir(dfppy)$_2$BPS]$^-$*

The title complexes were obtained both through synthetic procedures and from a commercial source. Initially, [Ir(ppy)$_2$BPS]$^-$ was synthesised in refluxing ethylene glycol. The product, however, proved very difficult to isolate from the solvent; attempts at distillation were fruitless, and the solvent level was only reduced to a level appropriate for chromatography using a rotary evaporator equipped with an ultra-high vacuum pump. The synthesis was then repeated in dichloromethane, which proved much more practical. The product purity from both reaction solvents was comparable, therefore dichloromethane was used for the synthesis of [Ir(dfppy)$_2$BPS]$^-$. The complexes were characterised by $^1$H and $^{13}$C NMR, and compared to the purchased complexes. This comparison confirmed the identity and purity of the complexes from both sources.
It was expected that the inclusion of 4,7-diphenyl-1,10-phenanthroline disulphonate in [Ir(dfppy)$_2$BPS]$^-$ would make the overall complex water soluble and result in a green-emitting transition metal chemiluminescence reagent. Unfortunately, the water solubility of the complex was not as good as anticipated. The addition of fluorine substituents has previously been noted to cause an increase in volatility and solubility in organic solvents, along with an increase in emission energy [15]. This effect clearly counters the high water solubility of the 4,7-diphenyl-1,10-phenanthroline disulphonate ligand, necessitating the use of a mixed organic-aqueous solvent system. In light of this, it was decided that the synthesis of other, higher emission energy complexes containing 2-(2,4-difluorophenyl)pyridine such as [Ir(dfppy)$_2$(X)$_2$] (where X = CN or pyridine) was inadvisable.

**Spectroscopic properties**

The absorbance spectra of [Ru(bipy)$_3$]$^{2+}$, [Ir(dfppy)$_2$BPS]$^-$ and [Ir(ppy)$_2$BPS]$^-$ can be seen in Figure 4.05a. All three show absorption peaks in the ultraviolet region of the spectrum. The [Ru(bipy)$_3$]$^{2+}$ spectrum has one small peak located at 240 nm and an intense peak at 286 nm. The spectra of [Ir(ppy)$_2$BPS]$^-$ and [Ir(dfppy)$_2$BPS]$^-$, on the other hand, have multiple overlapping peaks from 250 – 400 nm, with the maximum absorbance occurring at 268 nm. [Ru(bipy)$_3$]$^{2+}$ also exhibits a 2,2'-bipyridine absorption peak at 453 nm, whereas the iridium(III) complexes lack a ligand peak in this area. The spectra of the iridium(III) complexes was consistent with that previously reported for [Ir(ppy)$_2$(dpp)]$^+$ and [Ir(dfppy)$_2$(dpp)]$^+$ (dpp is 4,7-diphenyl-1,10-phenanthroline) [112]. The strong bands below 300 nm were attributed to intra-ligand $\pi-\pi^*$ transitions and the weaker, poorly defined bands above 300 nm were ascribed to MLCT transitions. The spectra are also similar to that of the homoleptic [Ir(ppy)$_3$] and [Ir(dfppy)$_3$] in dichloromethane reported by Dedeian et al. [230], indicating that the cyclometallated ligands (not 4,7-diphenyl-1,10-phenanthroline disulphonate) are involved in the absorption process.
Figure 4.05: (a) The absorbance spectra of $1 \times 10^{-5}$ M [Ru(bipy)$_3$]$^{2+}$ (olive), [Ir(ppy)$_2$BPS]$^-$ (purple) and [Ir(dfppy)$_2$BPS]$^-$ (dark cyan) in 1% ethanol. (b) The absorbance spectra of $3.3 \times 10^{-5}$ M [Ru(bipy)$_3$]$^{2+}$ (olive), [Ir(ppy)$_2$BPS]$^-$ (purple) and [Ir(dfppy)$_2$BPS]$^-$ (dark cyan) in 1% ethanol, after oxidation with $5 \times 10^{-5}$ M Ce(SO$_4$)$_2$ in 0.05 M H$_2$SO$_4$.

The oxidation of [Ru(bipy)$_3$]$^{2+}$ can be observed through the emergence of a peak at about 660 nm and a decrease in the intensity of the 450 nm MLCT absorbance, indicating the
conversion of ruthenium(II) to the ruthenium(III) state. If this peak is monitored over time, the gradual return of ruthenium(III) to ruthenium(II) can be seen [45]. The stability of the iridium(III) complexes was investigated in a similar manner, but very little change in the spectrum could be observed upon oxidation (Figure 4.05b). This may indicate that the reversion to the iridium(III) state is too fast to monitor. Consequently, it was decided that online oxidation of the iridium(III) complexes is required for reproducible analysis.

As can be seen in Figure 4.06 and Table 4.03, the inclusion of a diimine ligand causes the emission of \([\text{Ir}(\text{ppy})_2\text{BPS}]^−\) to undergo a significant bathochromic shift compared to \([\text{Ir}(\text{ppy})_3]\) (530 nm in dry acetonitrile). This is countered in \([\text{Ir}(\text{dfppy})_2\text{BPS}]^−\) by the electron-withdrawing fluorine groups, a common strategy for increasing the emission energy [14, 15, 199]. Whilst this does decrease the emission wavelength of the complex, the emission is still 70 nm longer than the homoleptic \([\text{Ir}(\text{dfppy})_3]\) (469 nm [200]). The photoluminescence quantum yield of \([\text{Ir}(\text{ppy})_2\text{BPS}]^−\) and \([\text{Ir}(\text{dfppy})_2\text{BPS}]^−\) is more than double that of \([\text{Ru}(\text{bipy})_3]^{2+}\) in the same solvent (50:50 acetonitrile:water), relative to the literature value of \([\text{Ru}(\text{bipy})_3]^{2+}\) in aqueous solution [227].

Figure 4.06: Normalised and corrected room temperature photoluminescence spectra of \([\text{Ru}(\text{bipy})_3]^{2+}\) (olive), \([\text{Ir}(\text{ppy})_2\text{BPS}]^−\) (purple) and \([\text{Ir}(\text{dfppy})_2\text{BPS}]^−\) (dark cyan) in 50:50 acetonitrile:water solution; excitation wavelength of 280nm. Inset: Photoluminescence of \([\text{Ru}(\text{bipy})_3]^{2+}\), \([\text{Ir}(\text{dfppy})_2\text{BPS}]^−\) and \([\text{Ir}(\text{ppy})_2\text{BPS}]^−\) (from left to right), excitation wavelength 365 nm. Photographs were taken using a Nikon D700 digital camera (photography courtesy of Ms. Donna Squire).
The chemiluminescence spectra of these complexes were also obtained and are in good agreement with their photoluminescence maxima (Table 4.03 and Figure 4.07). Consequently, it can be concluded that the chemiluminescence emission arises from the same excited state as in photoluminescence – that is, the $^3\text{MLCT}$ state.

![Figure 4.07: Chemiluminescence of $5 \times 10^{-4} \text{ M } [\text{Ir(ppy)}_2\text{BPS}]$ (left) and $5 \times 10^{-4} \text{ M } [\text{Ir(dfppy)}_2\text{BPS}]$ (right) in 50:50 acetonitrile:water after reaction with $1 \times 10^{-3} \text{ M Ce(SO}_4)_2$ and $5 \times 10^{-6} \text{ M ofloxacin. Reaction occurs within a dual inlet serpentine flow cell. Photographs were taken using a Nikon D700 digital camera, with 42 s and 30 s exposure time, respectively (photography courtesy of Ms. Donna Squire).}
Chemiluminescence intensity comparison

As the optimal concentration of [Ru(bipy)$_3$]$^{2+}$ is approximately 1 mM [22], [Ir(ppy)$_2$BPS]$^-$ and [Ir(dfppy)$_2$BPS]$^-$ were initially evaluated at this concentration in the analytically useful 50:50 acetonitrile:water solvent system. Preliminary optimization studies indicated that the optimum cerium(IV) sulphate concentration is 1 mM, in 0.05 M sulphuric acid. Cerium(IV) ammonium nitrate in nitric acid was briefly trialed, as it has previously been found to be superior to cerium(IV) trifluoromethanesulphonate in the oxidation of water due to the absence of sulphonate [231]. This was found to cause precipitation of the iridium(III) complexes and Ce(SO$_4$)$_2$ was consequently selected as the oxidant. The use of surfactants was also trialed, since their use in ECL and chemiluminescence experiments has been found to improve the emission intensity of the reaction without affecting the emissive properties [37, 218, 232, 233]. Overall, it was concluded that whilst having no real effect on the signal intensity or signal to blank ratios, somewhat of an improvement in reproducibility and peak shape could be seen when the neutral Triton X-100 was included in the $1 \times 10^{-5}$ M reagent solutions. This was enhanced when Triton X-100 was also included in the analyte stream, although not enough to warrant the use of surfactant in further studies.

Having established this, a brief comparative study was conducted to compare the complexes’ responses after reaction with $1 \times 10^{-5}$ M of codeine, ofloxacin, enrofloxacin and oxalate (Figure 4.08). They represent the organic carboxylate and tertiary amine groups that are known to produce intense chemiluminescence upon reaction with [Ru(bipy)$_3$]$^{2+}$. Large signal intensities could be obtained from the iridium(III) complexes, although [Ru(bipy)$_3$]$^{2+}$ was by far the more sensitive analytical reagent. The greatest signal-to-blank ratios across all analytes were obtained by [Ru(bipy)$_3$]$^{2+}$, despite its raw signal intensity being eclipsed by [Ir(ppy)$_2$BPS]$^-$ and [Ir(dfppy)$_2$BPS]$^-$. The results are in good agreement with what was previously discovered [3]: [Ir(ppy)$_2$BPS]$^-$ can produce a greater chemiluminescence intensity than [Ru(bipy)$_3$]$^{2+}$ but with inferior signal-to-blank ratios. [Ir(dfppy)$_2$BPS]$^-$, which was examined for the first time, follows the same trend.
Figure 4.08: Signal intensities (a) and signal-to-blank ratios (b) of 1 mM [Ru(bipy)$_3$]$^{2+}$ (olive), [Ir(ppy)$_2$BPS]$^-$ (purple) and [Ir(dfppy)$_2$BPS]$^-$ (dark cyan), in 50:50 acetonitrile:water, when reacted with 1 mM Ce(SO$_4$)$_2$ and $1 \times 10^{-5}$ M analytes.
An improvement in the sensitivities of the iridium(III) complexes was found when the reagent concentration was lowered to $1 \times 10^{-5}$ M (Figure 4.09).

Figure 4.09: Signal intensities (a) and signal-to-blank ratios (b) 0.01 mM [Ru(bipy)$_3$]$_2^{2+}$ (olive), [Ir(ppy)$_2$BPS]$^-$ (purple) and [Ir(dfppy)$_2$BPS]$^-$ (dark cyan), in 50:50 acetonitrile:water, when reacted with 1 mM Ce(SO$_4$)$_2$ and $1 \times 10^{-5}$ M analytes.
Following this, a more extensive study of eleven fluoroquinolones (Figure 4.10) was conducted at a reagent concentration of 0.01 mM. In this experiment, the organic portion of the solvent was reduced to 1% ethanol as an aqueous environment was desired. The FIA system used was optimised for ofloxacin and utilised $1 \times 10^{-4}$ M Ce(SO$_4$)$_2$ at a flow rate of 3.5 mL min$^{-1}$ line$^{-1}$. It was revealed that the iridium complexes, and [Ir(dfppy)$_2$BPS]$^-\,$ in particular, produced greater chemiluminescence intensity than the conventional [Ru(bipy)$_3$]$^{2+}\,$. Additionally, the signal-to-blank ratios of the iridium complexes were greater than those of [Ru(bipy)$_3$]$^{2+}\,$ for eight of the eleven analytes. This indicates that, under these conditions, the iridium complexes may be more sensitive and have lower detection limits than the conventional [Ru(bipy)$_3$]$^{2+}\,$. There is no pattern evident concerning which particular reagent produces the best signal-to-blank ratios; in most cases, [Ir(ppy)$_2$BPS]$^-\,$ yielded signal-to-blank ratios either comparable to or double that of [Ir(dfppy)$_2$BPS]$^-\,$, which only gave significantly greater signal-to-blank ratios when reacted with ciprofloxacin. The analytes ofloxacin and danofloxacin produced a signal upon reaction with [Ru(bipy)$_3$]$^{2+}\,$ that completely eclipsed those of the iridium(III) complexes, and they were the only two for which [Ru(bipy)$_3$]$^{2+}\,$ gave a superior response. For ease of comparison, these analytes were omitted from Figure 4.10.
Figure 4.10: Chemiluminescence signal intensity (a) and signal-to-blank ratios (b) of [Ru(bipy)$_3$]$^{2+}$ (olive), [Ir(ppy)$_2$BPS]$^{-}$ (purple) and [Ir(dfppy)$_2$BPS]$^{-}$ (dark cyan), all at $1 \times 10^{-5}$ M in 1% ethanol, when reacted with $1 \times 10^{-6}$ M fluoroquinolones and $1 \times 10^{-3}$ M Ce(SO$_4$)$_2$. 
Six sulphonamides were studied in a similar fashion (Figure 4.11). As with the fluoroquinolone screen, [Ir(dfppy)₂BPS]⁻ gave the largest signal intensities, except in the reaction with furosemide. Both iridium complexes produced much greater signals than [Ru(bipy)₃]²⁺ with all the analytes. Unlike the fluoroquinolones, however, the signal-to-blank ratios were not improved over [Ru(bipy)₃]²⁺ by using either of the iridium complexes. Furosemide yielded large signal-to-blank ratios when reacted with [Ru(bipy)₃]²⁺ and [Ir(ppy)₂BPS]⁻; [Ir(dfppy)₂BPS]⁻ on the other hand, gave relatively low signal-to-blank ratios. Hydrochlorothiazide and hydroflumethazide, structurally similar complexes that differ by the substituent on carbon 6 (chloro or trifluoromethyl), gave similar results for [Ir(dfppy)₂BPS]⁻. Strangely though, [Ir(ppy)₂BPS]⁻ and [Ru(bipy)₃]²⁺ yielded signal-to-blank ratios more than double for hydroflumethazide than for hydrochlorothiazide.
Figure 4.11: Chemiluminescence signal intensity (a) and signal-to-blank ratios (b) of [Ru(bipy)$_3^{2+}$] (olive), [Ir(ppy)$_2$BPS]$^-$ (purple) and [Ir(dfppy)$_2$BPS]$^-$ (dark cyan), all at $1 \times 10^{-5}$ M in 1% ethanol, when reacted with $1 \times 10^{-6}$ M sulphonamides.
To further investigate the effect of reagent concentration, the detection limits of \([\text{Ir(dfppy)}_2\text{BPS}]^-\) and \([\text{Ru(bipy)}_3]^{2+}\) for ofloxacin and furosemide were determined in 50:50 acetonitrile:water, at a concentration of both 1 mM and 0.01 mM (Table 4.04). This comparison revealed greater sensitivity in the detection of ofloxacin with \([\text{Ir(dfppy)}_2\text{BPS}]^-\) than with \([\text{Ru(bipy)}_3]^{2+}\) at a concentration of 0.01 mM. The greater sensitivities that can be achieved by using a lower concentration of reagent have promising implications in the commercial use of such reagents.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Ofloxacin (M)</th>
<th>Furosemide (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>([\text{Ru(bipy)}_3]^{2+})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 mM</td>
<td>(1.1 \times 10^{-9})</td>
<td>(4.3 \times 10^{-7})</td>
</tr>
<tr>
<td>0.01 mM</td>
<td>(3.0 \times 10^{-8})</td>
<td>(1.1 \times 10^{-7})</td>
</tr>
<tr>
<td>([\text{Ir(dfppy)}_2\text{BPS}]^-)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 mM</td>
<td>(1.9 \times 10^{-8})</td>
<td>(2.5 \times 10^{-8})</td>
</tr>
<tr>
<td>0.01 mM</td>
<td>(5.2 \times 10^{-9})</td>
<td>(1.1 \times 10^{-8})</td>
</tr>
</tbody>
</table>

Table 4.04: Detection limits of furosemide and ofloxacin with \([\text{Ir(dfppy)}_2\text{BPS}]^-\) and \([\text{Ru(bipy)}_3]^{2+}\) at concentrations of 0.01 mM and 1 mM.

The iridium(III) complexes seem to produce a large blank signal that competes with the desired reagent-analyte reaction and compromises sensitivity. These two competing chemiluminescence reactions were further explored using stopped-flow analysis. The reagents \([\text{Ru(bipy)}_3]^{2+}\) and \([\text{Ir(dfppy)}_2\text{BPS}]^-\) were examined at concentrations of \(5 \times 10^{-4}\) M and \(5 \times 10^{-6}\) M, while the oxidant and analyte concentration remained constant. A reagent concentration of 1 mM resulted in similar reaction profiles for both reagents with the blank and analyte. The signal peaked at 6.9 s and 2.2 s, respectively, for \([\text{Ru(bipy)}_3]^{2+}\) (Figure 4.12b) and \([\text{Ir(dfppy)}_2\text{BPS}]^-\) (Figure 4.12a) before showing a typical decay pattern. At 0.01 mM, there was little effect observed on the shape of either the blank or analyte profiles of \([\text{Ru(bipy)}_3]^{2+}\) (Figure 4.12d) but the blank profile of \([\text{Ir(dfppy)}_2\text{BPS}]^-\) was drastically altered (Figure 4.12c). Instead of an sharp increase in signal followed by a gradual tailing off, the blank profile of \([\text{Ir(dfppy)}_2\text{BPS}]^-\) showed an initial small, sharp peak, followed by a relatively slow rise and decay in intensity over the next 80 seconds. In FIA, only the first few seconds of the emission is detected before the mixture is flushed out of the cell. The majority of the \([\text{Ir(dfppy)}_2\text{BPS}]^-\) blank signal is thus not detected at 0.01 mM, and the sensitivity of the reagent can be improved by lowering the reagent concentration.
Figure 4.12: Intensity versus time profiles for the chemiluminescence reactions of: (a) $5 \times 10^{-4}$ M $[\text{Ir(dfppy)}_2\text{BPS}]^-$ or (b) $5 \times 10^{-4}$ M $[\text{Ru(bipy)}_3]^2^+$, and $1 \times 10^{-3}$ M Ce(SO$_4$)$_2$, with (black traces) or without (red traces) $5 \times 10^{-5}$ M ofloxacin; and (c) $5 \times 10^{-6}$ M $[\text{Ir(dfppy)}_2\text{BPS}]^-$ or (d) $5 \times 10^{-6}$ M $[\text{Ru(bipy)}_3]^2^+$, and $1 \times 10^{-3}$ M cerium(IV), with (black traces) or without (red traces) $5 \times 10^{-7}$ M ofloxacin. The intensity of the red trace in Figure 3.10b was multiplied by 5 for comparison purposes. Discrepancies in the signal/blank ratios obtained in these experiments and the flow-injection analysis procedure described in the paper arise due to differences in the final reactant concentrations, the mode of mixing and the portion of the emission profile measured.
4.4 Conclusion

An extensive study into the chemiluminescence of \([\text{Ir}(ppy)_2\text{BPS}]^-\) and \([\text{Ir}(dfppy)_2\text{BPS}]^-\) has been performed. This is the first demonstration of tuning the emission colour of chemiluminescence reactions of metal complexes, and has resulted in a chemiluminescence reagent with an emission in a more sensitive region of conventional photomultiplier tubes [234]. These complexes have been reacted with Ce(SO₄)₂ and various analytes, and compared to the archetypal \([\text{Ru}(bipy)_3]^{2+}\). This study has revealed that, at low reagent concentrations, the iridium(III) complexes can produce greater signal-to-blank ratios than \([\text{Ru}(bipy)_3]^{2+}\) with some analytes. These results have been summarised in:

Chapter Five: The Chemiluminescence of Osmium(II) Complexes
5.1 Introduction

As mentioned in the previous chapter, there is an emerging market for \([\text{Ru(bipy)}_3]^{2+}\) analogues exhibiting properties that cannot be achieved in ruthenium(II)-based complexes. In the design of photoluminescence sensors, for example, photodegradation and the temperature dependent emissions of ruthenium(II) complexes have been noted to lead to difficulties in their real-world application [110]. This problem can be surmounted through the use of osmium(II) complexes, as the large crystal field splitting parameter of osmium raises the energy of the non-emissive states [102, 235]. The complexes are thus more photostable, and (like iridium(III) complexes) extensive colour tuning is possible [102, 235]. Furthermore, osmium(III) complexes have been found to be less aggressive oxidants than \([\text{Ru(bipy)}_3]^{2+}\) [30, 135, 138, 236]. This could have important implications, such as the design of ECL active DNA-labelling reagents that are not in danger of causing irreversible oxidative damage to the oligonucleotides [138, 229] or OLED candidates that better match the work function of the ITO electrode [142].

Like the ruthenium(II) and iridium(III) analogues, osmium(II) complexes have been used in various applications, from energy conversion [63, 110, 125-127, 129] to chemical sensors [132, 133]. The early work on osmium(II) reagents focused on tris-diimine complexes; these exhibited short-lived excited states and very long emission wavelengths (greater than 700 nm) [30, 85, 237]. The absorption of red light [85, 132] makes the complexes attractive transcutaneous oxygen sensors despite their short excited state lifetimes, as the skin is translucent to long wavelength light [133]. These complexes, however, can have poor quantum yields [30, 85, 237], so research has focused on improving the emission efficiencies. The introduction of a strong-field diphosphine or diarsine ligand raises the energy level of the non-radiative \(e_g\) orbitals, improving the ability to alter the emission energy and efficiency, as well as increasing the excited state lifetime and inducing a hypsochromic shift of the absorption spectra [11, 30, 110]. Longer excited state lifetimes and increased quantum yields can be induced by including two such ligands, due to an increase in the mixing between the LC and MLCT states [110]. This is elegantly illustrated by a series of Os(L^L)_n(N^N)_{3-n}^{2+} complexes, where L^L is a diphosphine or diarsine ligand and N^N is a derivative of 1,10-phenanthroline. While the complexes containing only a single diphosphine or diarsine ligand all exhibited photoluminescence quantum yields of approximately 10% - 20%, bis(1,2-bis(dimethylarseno)benzene)(4,7-diphenyl-1,10-phenanthroline)osmium(II) was found to have a quantum yield of 35% [110]. This complex also had the greatest emission energy...
Using a variety of ligands, emissions have been reported from the blue [114, 117], through the visible region [118-120], and well into the IR (emission wavelengths over 1000 nm) [123, 124]. Although the short excited state lifetimes and low quantum yields have somewhat limited the extension of [Ru(bipy)$_3$]$^{2+}$ ECL chemistry to these osmium counterparts, some investigations have been documented [135, 137-142, 238]. A study of tris-diimine (2,2′-bipyridine, 1,10-phenanthroline and 2,2′-bipyrazine) osmium(II) complexes has found that the 2,2′-bipyrazine complex exhibited greater ECL than the other two complexes, although the extent of this improvement was not elaborated upon and neither was there any comparison to [Ru(bipy)$_3$]$^{2+}$ [238]. The ECL performance can be improved by substituting one of the diimine ligands for diphosphine or diarsine ligands [138, 141, 142, 239]. Richter et al. have found that ECL efficiencies double that of [Ru(bipy)$_3$]$^{2+}$ were obtained from the reaction of TPA and (bis(diphenylphosphino)ethene)bis(1,10-phenanthroline)osmium(II) in aqueous solution [138]. This is further enhanced by the presence of Triton X-100 [137] or ionic liquids [148]. ECL efficiencies of up to five times greater than [Ru(bipy)$_3$]$^{2+}$ have been observed in a similar series of complexes, from both the annihilation reaction and the reductive-oxidative reaction with 1-cyanonaphthalene [142]. This revealed that the π-acidity of the phosphine ligand affects the efficiency of the ECL reaction, and the oxidation potential is lowered by strong electron withdrawing groups on the diimine ligand [142]. The emission energies of the complexes were kept constant, indicating similar changes were caused in the oxidation and reduction potential by the ligands [142]. In another study, ECL efficiencies of up to eight times that of [Ru(bipy)$_3$]$^{2+}$ were reported (in acetonitrile or 50:50 acetonitrile:water and reacted with TPA) [141]. Four of the five complexes in the study showed photoluminescence and ECL efficiencies either comparable to or greater than that of [Ru(bipy)$_3$]$^{2+}$ in both solvent systems [141]. It was found that greater conjugation of the ligands stabilises the osmium(II) centre and increases the oxidation potential of the complex, and that the arsine complexes are more difficult to oxidise than the phosphine complexes [141]. Osmium(II) carbonyl complexes have also been studied, however they showed poor ECL efficiencies (between 1.1% and 13% that of [Ru(bipy)$_3$]$^{2+}$) [239].

Despite the promising ECL results, the chemiluminescence of osmium(II) complexes has previously been unexplored. Consequently, this chapter describes the examination of a suite of structurally related complexes (Figure 5.01) and their assessment for use as analytical chemiluminescence reagents.
Figure 5.01: Structures of the osmium(II) complexes.
5.2 Experimental

5.2.1 Materials

The hexafluorophosphate salts of the osmium(II) complexes (1,2-bis(dicyclohexylphosphino)ethane)bis(3,4,7,8-tetramethyl-1,10-phenanthroline)osmium(II) (Os 1), (1,2-bis(dimethylphosphino)ethane)bis(4,7-diphenyl-1,10-phenanthroline)osmium(II) (Os 2), bis(1,2-bis(dimethylarseno)benzene)(4,7-diphenyl-1,10-phenanthroline)osmium(II) (Os 3), (1,2-bis(dimethylphosphino)ethane)bis(3,4,7,8-tetramethyl-1,10-phenanthroline)osmium(II) (Os 4), (1,2-bis(dimethylarseno)benzene)bis(3,4,7,8-tetramethyl-1,10-phenanthroline)osmium(II) (Os 5) and (1,2-bis(diphenylphosphino)ethene)bis(1,10-phenanthroline)osmium(II) (Os 6), and also the chloride salt of Os 6, were kindly donated by Prof. Mark M. Richter of Missouri State University. Tris(2,2′-bipyridine)ruthenium(II) chloride hexahydrate was obtained from Strem Chemicals (Newbury, Minnesota, USA) and tris(1,10-phenanthroline)ruthenium(II) hexahydrate from Sigma-Aldrich (Castle Hill, New South Wales, Australia). Osmium trichloride was bought through BioScientific Pty. Ltd. (Alfa Aesar, London, UK). Sulphuric acid was purchased from Merck (Kilsyth, Victoria, Australia). Cerium(IV) sulphate, 1,10-phenanthroline, ethylene glycol, Sephadex LH-20, furosemide, hydrochlorothiazide and ofloxacin were obtained from Sigma-Aldrich (Castle Hill, New South Wales, Australia). Codeine was kindly donated by GlaxoSmithKline (Port Fairy, Victoria, Australia). Potassium oxalate was purchased from BDH Chemicals (Poole, England) and lead dioxide from Ajax Finechem (Sydney, Australia).

5.2.2 [Os(phen)₃]²⁺ synthesis

This synthesis is based on the method of Guadiello et al. [240]. 108 mg (0.309 mmol) OsCl₃ and 187 mg (0.9983 mmol) 1,10-phenanthroline was allowed to reflux at 200°C in 50 mL ethylene glycol for 72 hours under N₂, using wet glassware and solvents. After 3 days, the mixture was allowed to cool slowly to room temperature. The majority of the solvent was removed under vacuum and the resultant viscous oil dissolved in hot water and filtered hot. The filtrate was collected and heated, and KCl was added to make a saturated solution (approximately 3 M). The mixture was then vacuum filtered, leaving a dark purple solid. The product was extracted from the KCl-saturated aqueous solution using dichloromethane and the solvent removed by evaporation. The remaining solid was dissolved in acetonitrile and filtered to remove excess salt. The resultant dark brown product was dried under vacuum. Yield: 10%. ¹H NMR indicated the presence of [Os(phen)₃]: ¹H NMR (400 MHz, D₂O) δ
(ppm): 9.03 (d, $J_{HH} = 4.00$, 2H), d, 8.44 (d, $J_{HH} = 8.04$, 2H), 7.90 (s, 2H), 7.77 (q, $J_{HH} = 4.15$, 2H). Mass spectrometry confirmed the presence of the tris-substituted complex (HRMS-ESI m/z $[C_{36}H_{24}N_{6}Os_{2}]^+$ calc 366.08368, found 366.11250).

5.2.3 Spectroscopic characterisation

Absorbance spectra were collected using a Cary 300 Bio UV-visible Spectrophotometer (Varian Australia, Mulgrave, Victoria, Australia) using $1 \times 10^{-5}$ M reagent in 0.05 M H$_2$SO$_4$, in a quartz cell of 1 cm path length. This instrument was also used to monitor the stability of the oxidised complexes. When oxidised by PbO$_2$, approximately 20 mg of PbO$_2$ was mixed with 10 mL of solution, then allowed to settle. The supernatant was filtered off and the absorbance was immediately monitored at 660 nm. In the case of Ce(SO$_4$)$_2$ oxidation, the solutions were mixed within the instrument, resulting in a solution of $3.3 \times 10^{-4}$ M reagent and $6.6 \times 10^{-5}$ M Ce(SO$_4$)$_2$.

A Cary Eclipse Spectrofluorimeter (Varian Analytical Instruments, Australia) with a R928 photomultiplier tube (Hamamatsu, Iwata-gun, Shizuoka-ken, Japan) was used to collect photoluminescence spectra of the reagents ($1 \times 10^{-5}$ M in either 0.05 M H$_2$SO$_4$ or 50:50 acetonitrile:water) in standard 1 cm quartz cuvettes (5 nm band pass, 1 nm data interval, PMT voltage: 600 V). The emission spectra were corrected as previously described [194].

To obtain the photoluminescence quantum yields, the integrated emission spectra (500 - 850 nm, $\lambda_{ex} = 450$ nm) for each complex at $1 \times 10^{-5}$ M, $7.5 \times 10^{-6}$ M, $5 \times 10^{-6}$ M, $2.5 \times 10^{-6}$ M and $1 \times 10^{-6}$ M were plotted against their absorbance at 450 nm. The quantum yields are relative proportionality constants based on the $[\text{Ru(bipy)}_3]^{2+}$ literature value of 0.028 in air-saturated aqueous solution [227].

5.2.4 Flow injection analysis with chemiluminescence detection

Flow analysis methodology was used to evaluate the relative chemiluminescence intensities of the reactions between the reagents and various analytes. The detector consisted of a coiled piece of 0.8 mm i.d. PTFE tubing (DKSH) mounted flush against the PMT and contained within a custom-built light-tight housing. The PMT was kept at a steady voltage by a stable power supply (Electron Tubes model PM28B, ETP) and voltage divider (Electron Tubes model C611, ETP). The reagent was manually loaded onto the sample loop (70 $\mu$L) via a syringe, injected into the analyte stream and merged with the oxidant stream via a T-piece immediately prior to entering the flow cell. The flow rate was analyte dependent; the
solutions were propelled at either 1 mL min\(^{-1}\) or 3.5 mL min\(^{-1}\). In experiments comparing the chemiluminescence of all six osmium(II) complexes, solutions of 1 mM reagent (hexafluorophosphate salts) in 50:50 acetonitrile were injected into an analyte stream, also in 50:50 acetonitrile:water. This was then merged with 1 mM Ce(SO\(_4\))\(_2\) in 0.05 M H\(_2\)SO\(_4\). The complexes were compared using both a red-sensitive PMT (Electron Tubes model 9828SB, ETP, Ermington, New South Wales, Australia at 0.9 kV) and a green-sensitive PMT (Electron Tubes model 9124B40, ETP, Ermington, New South Wales, Australia at 1.3 kV). Aqueous experiments were carried out in front of the green sensitive PMT in 0.05 M H\(_2\)SO\(_4\).

**5.2.5 Electrochemical and electrochemiluminescence studies**

A µ-AUTOLAB electrochemical station potentiostat (MEP Instruments, North Ryde, NSW, Australia) with General Purpose Electrochemical Systems (GPES) software (version 4.9) was used to perform electrochemical experiments. A three-electrode glass electrochemical cell with quartz window base and Teflon cover with spill tray was held within a custom-built light-tight faraday cage. A 3 mm diameter glassy carbon working electrode, shrouded in Teflon (CH Instruments, Austin, TX, USA), a 1 cm\(^2\) platinum gauze auxiliary electrode and a silver wire quasi reference electrode were used. All co-reactant ECL experiments were performed in a 50:50 mixture of acetonitrile and 0.1 M phosphate buffer solution (PBS, pH 7). The working electrode was polished using 0.3 mm and then 0.05 mm alumina with Milli-Q water on a felt pad, rinsed in freshly distilled acetonitrile and dried with a stream of nitrogen prior to each experiment. The electrode was positioned approximately 2 mm from the bottom of the cell and the solution purged with argon for 5 minutes. The ECL detector employed was a Electron Tubes model 9828SB, PMT (ETP, Ermington, Australia, operated at 0.3 kV). ECL spectra were were collected using an Ocean Optics QE65000 CCD spectrometer with UV-visible fibre optic (length 1.00 m) and the trigger was a HR 4000 Break-Out box in conjunction with the P.G stat 12 AUTOLAB. All ECL emission spectra are autocorrected.

Solution phase ECL intensities were obtained by electrochemically cycling the complexes to their respective oxidative potentials to generate the 3+ forms of the osmium and ruthenium complexes in the presence of \(1 \times 10^{-5}\) M ofloxacin or potassium oxalate. All intensities were compared to [Ru(bipy)\(_3\)]Cl\(_2\) (100%).
5.3 Results and Discussion

5.3.1 Reagent screen

A set of structurally related osmium(II) reagents (Figure 5.01) were obtained as the hexafluorophosphate salts. Figure 5.02 shows the emissions of the osmium complexes spanning from 607 nm (Os 3) to 703 nm (Os 2). The high energy emission of Os 3 is to be expected, as the complex contains two diarsine ligands. This ligand has a large field strength, increasing the crystal field splitting of the $d$ orbitals [110]. Two such ligands have an increased effect, although the presence of the highly conjugated diimine ligand (4,7-diphenyl-1,10-phenanthroline) protects the emission energy from a further hypsochromic shift compared to the other complexes. The effect of the conjugated ligand is more apparent in the very long wavelength emission of Os 2. The complexes coordinated to 3,4,7,8-tetramethyl-1,10-phenanthroline (Os 1, Os 4 and Os 5) have been reported to have a LUMO centred on this ligand and the HOMO on a $d$ orbital of osmium(II) [110]. Interestingly, the maximum emissions of Os 4 and Os 5 are both at 645 nm, despite one having a diphosphine ligand and the other a diarsine ligand. This similarity indicates that 1,2-bis(dimethylarseno)benzene and 1,2-bis(dimethylphosphino)ethane cause similar effects to the electron density of the osmium(II) centre, allowing the HOMO-LUMO energy gap to remain comparable. Os 1 also has a similar emission energy, slightly lower than Os 4 and Os 5.

Figure 5.02: Normalised and corrected emission spectra of the osmium(II) complexes, $\lambda_{\text{excitation}} = 450$ nm.
A comparison of the ECL of the complexes was undertaken in 50:50 acetonitrile:0.1 M PBS (pH 7) and the results can be seen in Figure 5.03. The analytes oxalate and ofloxacin were chosen as they are structurally diverse (an organic acid and a tertiary amine, respectively) and are known to produce intense signals upon reaction with [Ru(bipy)₃]²⁺ [22, 42, 45]. When reacted with ofloxacin, Os 1, Os 2, Os 5 and Os 6 all gave greater signal intensities than [Ru(bipy)₃]²⁺. In the reaction with oxalate, Os 2 gave a comparable signal intensity to [Ru(bipy)₃]²⁺, while the other three reagents retained their excellent emissions. With both analytes, Os 4 gave approximately half the signal intensity of [Ru(bipy)₃]²⁺, and the poorest ECL reagent was Os 3.

![Figure 5.03: Electrochemiluminescence of 0.1 mM [Ru(phen)₃]²⁺, Os 1, Os 2, Os 3, Os 4, Os 5 and Os 6, compared to 0.1 mM [Ru(bipy)₃]²⁺ when reacted with 500 µM analyte in 50:50 acetonitrile:0.1 M PBS at pH 7.](image)

A plot of the ECL emission wavelength of the complexes versus their oxidation potential can be seen in Figure 5.04. From this, it is evident that most of the osmium(II) complexes do have a lower oxidation potential than the ruthenium(II) complexes, as previously noted [30, 135, 138, 236]. The potentials of the osmium(II) complexes range from 0.94 V (Os 4) to 1.46 V (Os 3). The high oxidation potential of Os 3 indicates a significant stabilisation of the HOMO. This complex also has the highest energy emission of the osmium(II) complexes. Os 2 has a relatively high oxidation potential as well as the lowest energy emission, implying a
stabilisation of both the HOMO and the LUMO. In addition to having the same emission energy, Os 4 and Os 5 also have very similar oxidation potentials. This confirms that the 1,2-bis(dimethylarseno)benzene and 1,2-bis(dimethylphosphino)ethane ligands induce similar alterations in the energy levels of the complexes. Os 6 has an emission that is near identical to \([\text{Ru(bipy)}_3]^{2+}\), but it is harder to oxidise.

![Figure 5.04](image.png)

Figure 5.04: Plot of the ECL emission wavelength of 0.1 mM \([\text{Ru(bipy)}_3]^{2+}\), \([\text{Ru(phen)}_3]^{2+}\), Os 1, Os 2, Os 3, Os 4, Os 5 and Os 6, against the oxidation potential of the complexes (M\(^{2+/3+}\)) vs. Ag/AgCl. The ruthenium(II) complexes are depicted as red circles, while the osmium(II) complexes are blue squares.

No correlation could be seen between the ECL emission wavelength of the osmium(II) reagents and the intensity of their ECL reaction with ofloxacin. When taken in conjunction with the report that there is a decrease in photoluminescence quantum yield with increasing emission wavelength [110], it is obvious that there are other factors that also influence the ECL quantum yield, and one property cannot be used to predict the other. In fact, in this series of reagents, there was a decrease in ECL intensity as the photoluminescence quantum yield [110] increased. A decrease in ECL intensity could generally be seen with an increase in oxidation potential (Figure 5.05), with the greatest intensity signals being observed for complexes having oxidation potentials between 0.9 V and 1.1 V.
Figure 5.05: The oxidation potential (M$^{2+/3+}$) vs. Ag/AgCl of 0.1 mM [Ru(bipy)$_3$]$^{2+}$, [Ru(phen)$_3$]$^{2+}$, Os 1, Os 2, Os 3, Os 4, Os 5 and Os 6, against the oxidation potential of the complexes plotted against the intensity of the ECL reaction with $1 \times 10^{-5}$ M ofloxacin. The ruthenium(II) complexes are depicted as red circles, while the osmium(II) complexes are blue squares.

The chemiluminescence of the six osmium complexes was evaluated in 50:50 acetonitrile:water with oxalate and ofloxacin. The signal intensities and signal-to-blank ratios for these reactions can be seen in Figure 5.06. There is a distinct difference in the performance of the reagents with either oxalate or ofloxacin. For example, when reacted with oxalate, Os 4 and Os 5 exhibit greater signals than [Ru(bipy)$_3$]$^{2+}$ and [Ru(phen)$_3$]$^{2+}$, yet relatively small signals are obtained upon reaction with ofloxacin. The opposite is true of Os 6: small signals are produced when reacted with oxalate, and large signals that are comparable to [Ru(bipy)$_3$]$^{2+}$ when reacted with ofloxacin. Unlike in the ECL screen, [Ru(phen)$_3$]$^{2+}$ showed superior chemiluminescence to [Ru(bipy)$_3$]$^{2+}$; in fact, it has the greatest signal-to-blank ratios of all the complexes. This was followed by Os 2, [Ru(bipy)$_3$]$^{2+}$ and Os 4 for oxalate, and Os 6 and [Ru(bipy)$_3$]$^{2+}$ for ofloxacin. Clearly, the method of oxidation - chemical or electrochemical - plays an important role in the generation of light, although the sensitivity of the complex cannot always be manipulated in this manner. For instance, the complex containing two diarsine ligands, Os 3, is a poor reagent in both ECL and chemiluminescence. As with the ECL screen, no correlation between emission wavelength...
and chemiluminescence performance was observed; that is, there was no evidence that the Energy Gap Law held true in either ECL or chemiluminescence.

Figure 5.06: The chemiluminescence screen of the osmium reagents compared to \([\text{Ru(bipy)}_3]^{2+}\) and \([\text{Ru(phen)}_3]^{2+}\), showing the signal intensities (a) and signal-to-blank ratios (b). The reagents (0.1 mM) and analytes \((1 \times 10^{-5} \text{ M})\) are dissolved in 50:50 acetonitrile:water and react with 1.25 M \(\text{Ce(SO}_4)_2\) in 0.05 M \(\text{H}_2\text{SO}_4\), in front of a green sensitive PMT.
The previous screening of osmium(II) reagents was performed using a green-sensitive PMT detector. When this was replaced by a red-sensitive device and the experiment repeated, some differences could be seen (compare Figure 5.06 to Figure 5.07). The most prominent difference is the improvement in the signal (relative to the other reagents) obtained from the reactions of Os 2 with both analytes. This complex exhibits the longest wavelength emission (703 nm), and it is clear that much of the emission was lost when the green-sensitive PMT was used. The improvement in signal intensities, however, does not translate to greater signal-to-blank ratios; the sensitivity of Os 2 when reacted with oxalate decreased substantially (relative to the other reagents). [Ru(bipy)₃]²⁺ showed a relative improvement over [Ru(phen)₃]²⁺ when reacted with oxalate, as expected considering the lower emission energy of [Ru(bipy)₃]²⁺. The poor signals and sensitivities of Os 3 are not improved by the change in PMT.
Figure 5.07: The chemiluminescence screen of the osmium reagents compared to [Ru(bipy)]$_3^{2+}$ and [Ru(phen)]$_3^{2+}$, showing the signal intensities (a) and signal-to-blank ratios (b). The reagents (0.1 mM) and analytes (1 × 10$^{-5}$ M) are dissolved in 50:50 acetonitrile:water and react with 1.25 M Ce(SO$_4$)$_2$ in 0.05 M H$_2$SO$_4$ in front of a red-sensitive PMT.
5.3.2 [Os(dppene)(phen)]_{2}^{2+} and [Os(phen)]_{3}^{2+} comparison

Great promise was shown by Os 6 in the chemiluminescence and ECL screens; consequently, this reagent was chosen as a representative reagent for further investigation (from here onwards, Os 6 will be referred to as [Os(dppene)(phen)]_{2}^{2+}). The hexafluorophosphate salt previously examined is poorly soluble in aqueous solution; thus, in order to examine the potential of the complex in a more analytically useful aqueous solvent, the chloride salt was obtained. Also included in the evaluation is tris(1,10-phenanthroline)osmium(II), [Os(phen)]_{3}^{2+}. Unlike in the preceding set of complexes, which displayed a broad array of structural differences, a direct comparison can be made between these two complexes as the only difference is the substitution of 1,2-bis(diphenylphosphino)ethene for another 1,10-phenanthroline in [Os(phen)]_{3}^{2+} (Figure 5.08). The result of this substitution is a lower energy emission; the emission energy of the [Os(dppene)(phen)]_{n}(phen)]_{3-n}^{2+} family, where n = 1 or 2, has been shown to increase with the addition of the diphosphine ligands [235].

![Figure 5.08: The structure of [Os(phen)]_{3}^{2+}.

Initially, the synthesis of [Os(phen)]_{3}^{2+} was performed in dimethylformamide at relatively low temperatures (140°C), as per Carlson et al. [241]. This resulted in the bis-substituted complex Os(phen)_{2}Cl_{2}, and it was decided that harsher reaction conditions were required in order to coordinate the third 1,10-phenanthroline. The synthesis was subsequently repeated in ethylene glycol and at higher temperatures. Some difficulties were encountered during purification of the product; a Sephadex LH-20 column was trialled, however it proved unsuccessful. The pure product was ultimately obtained through extraction into acetonitrile, although in low yield. As sufficient product was obtained for the experiments, the synthesis
was not optimised. The mass spectrum showed a peak at m/z 366.11250 with characteristic osmium isotope splitting pattern, confirming the presence of the desired product.

<table>
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<td>263</td>
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Table 5.01: Spectroscopic properties of the complexes in 0.05 M \( \text{H}_2\text{SO}_4 \). Photoluminescence quantum yields were obtained at an excitation wavelength of 450 nm and are relative to the literature [227] value of \([\text{Ru(bipy)}_3]^{2+}\) in aqueous solution, 0.028.

The absorbance spectra of \([\text{Os(phen)}_3]^{2+}\) and \([\text{Os(dppene)(phen)}_2]^{2+}\) are characterised by a series of peaks below 300 nm assigned to spin-allowed \( \pi-\pi^* \) transitions of the 1,10-phenanthroline ligand, and MLCT transitions at longer wavelengths. \([\text{Os(phen)}_3]^{2+}\) shows substantial overlap between the singlet (433 nm) and triplet (478 nm) MCLT transitions [11, 30]. These peaks are better resolved in the \([\text{Os(dppene)(phen)}_2]^{2+}\) spectrum due to the hypsochromic shift of the singlet peak (to 369 nm). This is attributed to the presence of the diphosphine ligand, as a hypsochromic shift in absorbance spectra has previously been observed in similar complexes [11, 30]. The \(^3\text{MLCT}\) transition observed at 478 nm in \([\text{Os(phen)}_3]^{2+}\) and 476 nm in \([\text{Os(dppene)(phen)}_2]^{2+}\) is shifted bathochromically relative to the analogous peak in \([\text{Ru(phen)}_3]^{2+}\), which is centred at 450 nm. The absorbance of longer wavelength visible light by osmium complexes has previously been noted and exploited in the design of oxygen sensors [132, 133]. Overall, the spectra are in good agreement with previously published data on these [138, 235] and other, similar complexes [110].

Upon excitation in aqueous solution, the complexes exhibit a broad and featureless photoluminescence that is independent of excitation wavelength and characteristic of a MLCT emission (Table 5.01). This is concordant with previous data [235], although the spectra are hypsochromically shifted due to the difference in solvent. The quantum yield of \([\text{Os(dppene)(phen)}_2]^{2+}\) is more than double that of the ruthenium(II) complexes (Table 5.01),
and as such the complex shows promise in a variety of fundamental and applied studies. The quantum yield of \([\text{Os(phen)}_3]^{2+}\) is very low, as predicted by the Energy Gap Law and previously seen by Kober et al. [235].

The oxidation of osmium(II) to osmium(III) causes a colour change in the solution, similar to that seen in ruthenium(II) complexes [22, 45]. The complex then gradually returns to the 2+ state, with an associated colour change. This reversion can be monitored over time to determine the stability of the 3+ state. Figure 5.09 shows the stability of \([\text{Os(dppene)(phen)}_2]^{3+}\) and \([\text{Ru(bipy)}_3]^{3+}\) over 15 minutes after oxidation by either Ce(SO₄)₂ or PbO₂, which are routinely used to oxidise ruthenium complexes [7, 20, 21, 45]. It is clear that, while \([\text{Ru(bipy)}_3]^{3+}\) is relatively stable over the time period, \([\text{Os(dppene)(phen)}_2]^{3+}\) quickly returns to the 2+ state. It can therefore be concluded that online oxidation using Ce(SO₄)₂ is the more appropriate method for the reproducible generation of the 3+ state of \([\text{Os(dppene)(phen)}_2]^{2+}\). As the temporal instability of \([\text{Os(dppene)(phen)}_2]^{2+}\) has also been seen in various ruthenium(II) complexes containing 1,10-phenanthroline-type ligands [22, 45], it was considered appropriate to extend this conclusion to \([\text{Os(phen)}_3]^{2+}\).
Figure 5.09: Temporal stability of [Os(dppene)(phen)$_2$]$^{2+}$ (pink) and [Ru(bipy)$_3$]$^{2+}$ (olive) in 0.05 M H$_2$SO$_4$, monitoring the decay of the peak attributable to the 3$^+$ state at 660 nm for 15 minutes after oxidation with (a) Ce(SO$_4$)$_2$ and (b) PbO$_2$. 
In order to optimise the chemiluminescence emission, the concentration of reagent (in 0.05 M H₂SO₄) was tested at 0.1 mM, 0.5 mM and 1 mM with a range of analytes and 1 mM Ce(SO₄)₂. It was found that while 1 mM reagents produced the greatest signal intensities, 0.1 mM tended to give superior signal-to-blank ratios with little loss of signal intensity. Subsequent experiments were performed at a reagent concentration of 0.1 mM. A brief optimisation of flow rates was also undertaken; as with [Ru(bipy)₃]²⁺ and [Ru(phen)₃]²⁺, the most favourable flow rate is analyte dependent and was therefore varied between 1 and 3.5 mL min⁻¹.

When the performance of the reagents with a small range of structurally diverse analytes was compared (Figure 5.10), [Os(dppene)(phen)₂]²⁺ gave comparable results to the standard ruthenium(II) reagents, and double the intensity of [Ru(bipy)₃]²⁺ with furosemide. Unlike the iridium complexes described in previous chapters, the blank signal was comparable to that of the ruthenium(II) complexes, and so there was no deleterious effect on the sensitivity of the complex. This is further supported by the detection limit obtained for the reaction with ofloxacin. Similar detection limits were obtained for all three reagents, with [Ru(bipy)₁]²⁺, [Ru(phen)₃]²⁺ and [Os(dppene)(phen)₂]²⁺ being able to detect ofloxacin at concentrations of 4.5 × 10⁻⁹ M, 1.7 × 10⁻⁹ M and 1.8 × 10⁻⁹ M, respectively.

[Os(phen)₃]²⁺, on the other hand, did not perform as well. While [Os(dppene)(phen)₂]²⁺ had impressive sensitivities comparable to [Ru(bipy)₁]²⁺ and [Ru(phen)₃]²⁺, [Os(phen)₁]²⁺ did not produce a signal more than five times that of the blank. The greatest response for [Os(phen)₃]²⁺ (4.58 mV) was obtained from the reaction with oxalate, and even this was completely eclipsed by [Ru(bipy)₃]²⁺ (which, at 479.11 mV, had the next lowest intensity signal with this analyte).
Figure 5.10: (a) signal intensities and (b) signal-to-blank ratios of the reagent screen of 0.1 mM [Ru(bipy)$_3$]$^{2+}$, [Ru(phen)$_3$]$^{3+}$ and [Os(dppene)(phen)$_2$]$^{2+}$ in 0.05 M H$_2$SO$_4$, when reacted with 1 mM Ce(SO$_4$)$_2$ and $1 \times 10^{-5}$ M furosemide, codeine and oxalate and $1 \times 10^{-6}$ M ofloxacin.
5.4 Conclusions

The work described herein demonstrates that osmium(II) complexes are not only capable of chemiluminescence, but some produce comparable signals to that of [Ru(bipy)$_3$]$^{2+}$ and [Ru(phen)$_3$]$^{2+}$. The reagents contained at least two 1,10-phenanthroline-based ligands (with the other being a diarsine or diphosphine ligand) except Os 3, which was coordinated to two diarsine ligands. The presence of this second ligand causes the complex to have the highest energy emission, and may also contribute to its poor chemiluminescence and electrochemiluminescence performance. Os 1 and Os 5 showed greater capability as ECL reagents than as chemiluminescence reagents. The chemiluminescence intensities and signal-to-blank ratios of Os 6 ([Os(dppene)(phen)$_2$]$^{2+}$) compare favourably to [Ru(bipy)$_3$]$^{2+}$, as did its detection limit for ofloxacin. This extension of the (electro)chemiluminescence chemistry of [Ru(bipy)$_3$]$^{2+}$ and analogues has thus provided novel alternative reagents that possess valuable properties. These findings have been summarised in:

Chapter Six: The Selective Oxidation and Simultaneous Detection of Spectrally Distinct Luminophores
6.1 Introduction

For the last four decades, the chemiluminescence detection of a variety of compounds of medical, forensic and pharmaceutical importance has been predominantly performed using [Ru(bipy)₃]²⁺ [7, 8, 30]. This incredible success has spurred considerable interest in the development of ruthenium(II) complex analogues [22, 44, 45, 61, 78, 80, 83, 242]. Over the past decade, iridium(III) complexes have captured researchers’ attention, as they exhibit relatively high quantum yields and fairly stable redox characteristics [30, 80, 200, 220, 226, 243, 244]. The ECL of these complexes has been performed in both aqueous and non-aqueous solutions [15, 205, 214-216, 218-220, 245], and significant attention has been paid to the manipulation of their spectroscopic properties, particularly the oxidation potential [103, 116, 128, 200, 226, 246-248] and emission wavelength [14, 53, 80, 101, 121, 122, 202-204, 206, 208, 244, 249-252]. The last property is important, as the emission maxima of ruthenium(II) complexes are predominantly restricted to the red-orange region of the electromagnetic spectrum [22, 44, 45, 61, 78, 80, 83, 242], while iridium(III) complexes have been reported to have emissions spanning the entire visible spectrum [14, 53, 80, 101, 121, 122, 202-204, 206, 208, 244, 249-252].

Cationic iridium complexes containing a diimine ancillary ligand have been extensively utilised as protein and cell dyes, as they are more water soluble than their neutral counterparts [53, 54, 210, 253, 254]. The inclusion of a diimine ligand induces a bathochromic shift of the emission of the complex relative to the cyclometallated parent [204]. While substitutions on the diimine ligand strongly influence the quantum yields and excited state lifetimes, the emission wavelength is left relatively unchanged [204]. Exchange for another type of diimine – for example, replacing 1,10-phenanthroline with 2,2’-bipyridine – can effectively tune the emission energies [53, 244]. This was elegantly demonstrated by Zhao and co-workers, who synthesised bis(2-(3,5-difluorophenyl)pyridine)bis(pyridine)iridium(III) (λ_max = 486 nm), then substituted the pyridine ligands for diimines of increasing conjugation length to create a small family of complexes that emit across a broad spectral range (457 - 634 nm) [53].

The HOMO and LUMO energies (and therefore the emission wavelength) of a complex can be approximated from its oxidation and reduction potentials. The oxidation potential is thought to correlate with the iridium centre, with some contribution from the ligands, as is the HOMO [128, 226]. It has been demonstrated that a destabilisation of the HOMO by the introduction of electron-donating substituents on the phenyl ring of 2-phenylpyridine results
in a less positive oxidation potential [246, 247]. Conversely, electron-withdrawing groups decrease the electron density on the metal, thereby stabilising the HOMO and shifting the oxidation potential to a more anodic potential [246, 247]. Cyclic voltammetry studies performed on heteroleptic \( \text{Ir(C^N)2(N^N)}^+ \) complexes (C^N is 2-phenylpyridine or benzo[h]quinoline and N^N is 2,2'-bipyridine or 1,10-phenanthroline) [248] have revealed that cyclometallated iridium complexes are more easily oxidised than the diimine complexes due to the strong \( \sigma \)-donor ability of these ligands [248]. The reductions, on the other hand, are ligand-centred [226]. The number of reductive processes correspond to the number of pyridine rings present in the ligands, with the pyridine rings of the diimine ligands being more easily reduced than those of ortho-metallating ligands [248].

In addition to altering the oxidation potential of the complex, manipulation of the HOMO and/or LUMO energy levels inevitably induces a change in the emission wavelength. The effect of particular ligands on the emission wavelength is predictable, and entire libraries of complexes have been designed using Density Functional Theory calculations [122, 249, 255, 256]. It has been found that an extended aromatic system causes a bathochromic shift of the emission wavelength, while groups such as pyrazolyl and carbine can result in a hypsochromic shift [101, 116, 206]. The design of blue-emitting complexes has been fraught with complications: too large a destabilization of the LUMO generally increases the non-radiative deactivation of the complexes, and therefore decreases the quantum yield [204, 251, 257]. True blue emissions have been difficult to achieve; many complexes that exhibit a maximum emission in the ‘true blue’ region – approximately 460 nm – also have a vibronic shoulder near 490 nm that leads to an apparent sky blue emission [128]. A common approach to induce a hypsochromic shift in the emission wavelength is the introduction of fluorine and/or trifluoromethane substituents [116]. This can also increase the complex's volatility and solubility in organic solvents, reduce concentration quenching of the luminescence and increase oxidation potentials [258]. This strategy has been employed to develop the commercially available tris(2-(4,6-difluorophenyl)pyridinato-C^2,N)iridium(III), which is widely used as the standard for blue-emitting iridium complexes (emission wavelength 480 nm in chloroform [128]).

The vast range of luminescence complexes described in the literature [14, 80, 101, 115, 116, 121, 122] provides the exciting possibility of combining two chemiluminophores with distinct spectral distributions in a single solution and detecting them simultaneously. This would be beneficial in multi-analyte determinations, in studies that require an internal
standard, and in studies using probes and labels [218]. Reviews have been published illustrating the many uses of ruthenium(II) labels [7, 30, 32], and it has been noted that major advances in the future of ECL detection may rely upon breakthroughs in multiplexing analysis of ECL emitters, with the aim of high-throughput analysis [34]. Many researchers have appreciated the need for a simultaneous detection of compounds: Marin-Suarez del Toro et al. [112] and Kapturkiewicz [215, 216, 220] have acknowledged that this is ‘important from a practical point of view’. Yu and co-workers have also recognised the usefulness of detecting cytoplasm and nucleus stains under the same conditions [54]; the second stain in that study was an organic dye rather than another platinum group metal complex, but this simultaneous detection was only feasible due to the distinct emission wavelengths of the stains [54]. Similarly, Gill et al. have noted that the red emission of ruthenium(II) complexes is complementary to other commonly used luminescent labels such as green fluorescent protein [55]. Other researchers have recognised that colour-tunable OLEDs can be created from complexes with different emission energies [188]. In light of this, it is evident that the restricted range of emission wavelengths of ruthenium(II) complexes precludes their use in such multiplex analyses, unless combined with other complexes that exhibit a broader range of emission wavelengths.

Despite the fact that many studies aimed at tuning the emission wavelength of iridium complexes have produced potential candidates for simultaneous detection, very few researchers have explored its practical implementation [214, 218, 259]. Proof-of-concept ECL experiments have been performed by Richter et al. using [Ru(bipy)$_3$]$^{2+}$ and [Ir(ppy)$_3$] [214]. This highlighted the fundamental limitation of this approach: the emission bandwidths, which can span hundreds of nanometres in these type of complexes [3, 23, 214, 218, 252]. The authors determined that despite the lack of baseline resolution, the spectral separation of approximately 100 nm between the emission maxima of these complexes is sufficient to be analytically useful. The complexes bis(2-(3,5-difluorophenyl)pyridine)(2-carboxypyridyl)iridium(III) ([Ir(dfppy)$_2$(pic)]) and bis(2-(2′-benzothienyl)pyridine)(acetylacetonate)iridium(III) ([Ir(btp)$_2$(acac)]) were subsequently examined and it was found that the peak resolution obtained in the initial study was improved by substituting [Ir(ppy)$_3$] for [Ir(dfppy)$_2$(pic)] [218]. The same concept, using photoluminescence detection instead of ECL, has been patented [259]. The detection of at least two 'physically matched yet optically distinct' luminescent labels is described in 'Cyclometallated transition metal complexes for multiplex analyte detection', primarily for
use in microarray and immunodetection applications [259]. These complexes offer the advantage of a common excitation wavelength in the UV, which is easily filtered from the visible emission [259]. This means that only simple instrumentation is required, with no need for the multiple light sources generally used by organic fluorophores [259]. The patent is inclusive of the simultaneous detection of these luminophores, and their prior chromatographic separation [259].

These studies stop just short of the enticing prospect of choosing two chemiluminophores of differing oxidation potentials, and selectively oxidising one in the presence of the other. This is possible, and such multiple emissions can also be seen in multinuclear complexes [260]. A non-Kekulé trinuclear Ir(III)-Ru(II)-Ir(III) structure has been designed exhibiting an emission that is dependent on the applied potential [260]. The emission originates from a number of distinct excited states, located on either the ruthenium(II) or the iridium(III) centre [260]. The concentrations of the different excited state species change with increasing anodic potential, resulting in a maximum emission that is dependent on the applied potential [260]. The net spectrum is a broad one that consists of the overlapping emissions of each of the different species generated, at all applied potential [260].

The simultaneous detection of multiple, spectrally distinct luminophores is clearly desirable, and it takes little imagination to realise the great potential of selectively oxidising the luminophores. This is the goal that forms the basis of this chapter. Reagents are chosen based on their oxidation potential and emission wavelength, and are evaluated through photoluminescence, electrochemiluminescence and chemiluminescence.
6.2 Methods

6.2.1 Materials

Tetrakis(2,4-difluorophenyl)pyridine-C$_2$N$'$-dichloro)diiridium(III) was obtained from Rubipy Scientific Inc. (Toronto, Canada), tris(2-phenylpyridine)iridium(III) and tris(2-(4,6-difluorophenyl)pyridine)iridium(III) from Sigma-Aldrich (Castle Hill, New South Wales, Australia) and tris(2,2'-bipyridine)ruthenium(II) was obtained from Strem Chemicals (Newbury, Minnesota, USA). Bis(2,2'-bipyridine)(N4,N4'-bis((2S)-1-methoxy-1-oxopropan-2-yl)(2,2'-bipyridyl-4,4'-dicarboxamide)ruthenium(II) was available from a previous study and its synthesis has been described [44]. The complexes Os 5 and Os 6 were kindly donated by Prof. Mark M. Richter of Missouri State University. Sulphuric acid and methanol were purchased from Merck (Kilsyth, Victoria, Australia) and ethanol from ChemSupply (Gilman, South Australia, Australia). Cerium(IV) sulphate, 4,7-diphenyl-1,10-phenanthroline disulphonate sodium salt, Sephadex LH-20, tetrabutylammonium hexafluorophosphate, phosphate buffer, ofloxacin and tripropylamine were also obtained from Sigma-Aldrich. Acetonitrile was purchased from Ajax Finechem (Sydney, Australia).

6.2.2 [Ir(dfppy)$_2$BPS]$^{-}$ synthesis

For the synthesis and characterisation of this complex, please refer to Section 4.2.3.

6.2.3 Spectroscopic characterisation

Absorbance spectra were collected using a Cary 300 Bio UV-visible Spectrophotometer (Varian Australia, Mulgrave, Victoria, Australia) using 1 × 10$^{-5}$ M reagent in 50:50 acetonitrile:water, in a quartz cell of 1 cm path length.

A Cary Eclipse Spectrofluorimeter (Varian Analytical Instruments, Australia) with a R928 photomultiplier tube (Hamamatsu, Iwata-gun, Shizuoka-ken, Japan) was used to collect photoluminescence spectra of the reagents (50:50 acetonitrile:water solvent mixture) in standard 1 cm quartz cuvettes (5 nm band pass, 1 nm data interval, PMT voltage: 600 V). The emission spectra were corrected as previously described [194]. The same instrument was used to obtain chemiluminescence spectra of the reagents. A solution containing both reagent and analyte (in 50:50 acetonitrile:water) was continuously merged with 1 mM Ce(SO$_4$)$_2$ in 0.05 M H$_2$SO$_4$ within a mirror-backed spiral flow cell fabricated from clear PTFE tubing.
NMR characterisation was performed on a Jeol Eclipse Plus 400 MHz ft-NMR spectrometer, in deuterated methanol.

### 6.2.4 Electrochemical and electrochemiluminescence studies

An Autolab PGSTAT12 potentiostat (MEP Instruments, North Ryde, NSW, Australia) was used to control the collection of electrochemiluminescence spectra, which were obtained in a three-electrode electrochemical cell consisting of 3 mm diameter glassy carbon working electrode, Ag|AgCl (3 M) or Ag|AgNO₃ (0.02 M) quasi reference electrode and Pt wire counter electrode. The electrode was positioned approximately 2 mm from the bottom of the cell. The electrochemical cell was held within a custom-built light-tight faraday cage. Autolab General Purpose Electrochemical Systems (GPES) software (version 41.9) was used to record the electrochemical data. Spectra were collected by an Ocean Optics QE65000 CCD spectrometer with UV-visible fibre optic (length 1.00 m) and the trigger was a HR 4000 Break-Out box in conjunction with the P.G stat 12 AUTOLAB. The integration time depended on the experiment being performed: 30 seconds in chronoamperometry experiments, or matched to the cyclic voltammogram during the collection of ECL emission whilst performing a cyclic voltammetry (CV) experiment. The [Ir(ppy)₃] series was studied in dry acetonitrile containing 0.1 M tetrabutylammonium hexafluorophosphate (TBAPF₆) and the [Ir(dfppy)₂BPS]⁻ series in 50:50 acetonitrile:0.1 M PBS (pH 7). Solutions were degassed for 5 - 10 minutes prior to and whilst taking measurements. All ECL emission spectra were autocorrected and all potentials are referenced against ferrocene.

Square wave voltammetry (amplitude 0.025 V, frequency 15 Hz) was utilised to determine the \( E^{0'} \) of each complex, using a CH Instruments 660B potentiostat and a three-electrode electrochemical cell consisting of 3 mm glassy carbon or boron-doped diamond electrode, Ag|AgCl or Ag|AgNO₃ reference electrode and platinum auxiliary electrode. The concentration of the complex was 0.25 mM in all cases.

This chapter describes a collaborative project between Deakin University and La Trobe University. Acknowledgement is given to Egan Doeven and Dr Gregory Barbante for their assistance in the acquisition of the electrochemical and electrochemiluminescence data.
6.3 Results and Discussion

6.3.1 [Ir(ppy)$_3$] systems

Initially, a similar system to that of Richter et al. [214, 218] was utilised. These experiments were performed under standard conditions (in dry acetonitrile containing 0.1 M TBAPF$_6$ and 10 mM TPA) using [Ir(ppy)$_3$] and a [Ru(bipy)$_3$]$^{2+}$ derivative. Based on the experience of Richter et al. [214, 218], it was decided that at least 100 nm separation between the emission maxima of the complexes was required. The desired spectral resolution was provided by the complex bis(2,2'-bipyridine)(N4,N4'-bis((2S)-1-methoxy-1-oxopropan-2-yl)(2,2'-bipyridyl-4,4'-dicarboxamide)ruthenium(II) ([Ru(bipy)$_2$(Me-ALA-bipy-dc)]$^{2+}$), which has been shown to have a lower energy emission than [Ru(bipy)$_3$]$^{2+}$ [44]. Preliminary experiments found that a ten-fold greater concentration of [Ir(ppy)$_3$] provided peaks of similar intensity to [Ru(bipy)$_2$(Me-ALA-bipy-dc)]$^{2+}$, and the limited solubility of [Ir(ppy)$_3$] necessitated the use of this complex at 0.25 mM. When an appropriate potential was applied, emission from both reagents was observed (Figure 6.01). Figure 6.01 also displays a possibility unexplored by Richter: the selective oxidation of the complexes. Due to the distinct oxidation potentials of the complexes (0.33 V and 0.96 V vs. ferrocene for [Ir(ppy)$_3$] and [Ru(bipy)$_2$(Me-ALA-bipy-dc)]$^{2+}$, respectively), it is feasible to selectively oxidise [Ir(ppy)$_3$] in the presence of [Ru(bipy)$_2$(Me-ALA-bipy-dc)]$^{2+}$, and then increase the potential to oxidise both complexes.

![ECL spectra](image_url)

Figure 6.01: Corrected ECL spectra generated using chronoamperometrey pulses at 1.04 V (purple spectrum) or 0.44 V (red spectrum) vs. ferrocene for 0.25 mM [Ir(ppy)$_3$] and 0.025 mM Ru(bipy)$_2$(Me-ALA-bipy-dc)$^{2+}$ in acetonitrile containing 0.1 M TBAPF$_6$ with 10 mM TPA as co-reactant.
In general, the application of high overpotentials to an electrochemiluminophore either causes the ECL emission to increase or plateau [261, 262]. Curiously, this is not seen here with [Ir(ppy)$_3$]. The 3D ECL plot (ECL intensity as a function of applied potential and emission wavelength) of [Ir(ppy)$_3$] and [Ru(bipy)$_2$(Me-ALA-bipy-dc)]$^{2+}$ (Figure 6.03) clearly shows a decay in the emission of [Ir(ppy)$_3$] with increasing voltages. This results in distinct potentials at which each electrochemiluminophore is emissive at the exclusion of the other.
The loss of the \([\text{Ir}(ppy)_3]\) emission at anodic potentials is more obvious when observed visually (Figure 6.04). A green emission can be seen at low potentials, with a very short transition to a pure red emission (System A). If the red emitting \([\text{Ru}(bipy)_2(\text{Me-ALA-bipy-dc})]^2^+\) is replaced with the blue emitting \([\text{Ir}(dfppy)_3]\) (System B in Figure 6.04), then the ‘switch off’ behaviour is even more noticeable; the faint transitional emissions from green to blue photographed between 0.53 and 0.73 V were not actually visible to the naked eye. In both cases, the switching of the emission colour was immediate and reversible, depending only on the applied potential.
It is generally accepted that ECL is generated as a result of the following set of reactions [263-266]:

\[
\begin{align*}
M & \rightarrow M^* + e^- \\
TPA & \rightarrow TPA^{**} + e^- \\
TPA^{**} & \rightarrow TPA^* + H^+ \\
M^+ + TPA^* & \rightarrow M^* + P \\
M^* & \rightarrow M + h\nu
\end{align*}
\]

Where M is the electrochemiluminophore and P represents degradation products of TPA. In some cases, the TPA radical may also be generated by:

\[
M^+ + TPA \rightarrow TPA^{**}
\]

There is, however, another mechanism occurring in both Systems A and B that induces the 'switching off' of \([Ir(ppy)_3]\). The fact that this occurs in both systems indicates that the phenomenon occurs independently of the second emitting complex in the solution. If this is omitted from the reaction mixture, leaving only \([Ir(ppy)_3]\), co-reactant and electrolyte, the same 'switching off' behaviour is observed - but only at high TPA concentrations. When the co-reactant is present in the same concentration as \([Ir(ppy)_3]\), then no appreciable suppression of the emission is seen. With this in mind, and considering the redox potentials of all species, the following oxidative quenching mechanism is proposed:

\[
\begin{align*}
[Ir(ppy)_3] & \rightarrow [Ir(ppy)_3]^+ + e^- \\
TPA & \rightarrow TPA^{**} + e^- \\
TPA^{**} & \rightarrow TPA^* + H^+ \\
[Ir(ppy)_3]^+ + TPA^* & \rightarrow [Ir(ppy)_3]^* + P \\
[Ir(ppy)_3]^* & \rightarrow [Ir(ppy)_3] + h\nu \\
[Ir(ppy)_3]^* + TPA^{**} & \rightarrow [Ir(ppy)_3]^+ + P
\end{align*}
\]
While Equation 11 prevails at low potentials, increasing the applied potential raises the concentrations of the TPA radical cation and allows for Equation 12 to dominate and quench the [Ir(ppy)3] emission. This explains the 'switch off' behaviour of [Ir(ppy)3], but not why the same is not seen with other complexes. A more complete explanation takes into account the excited state properties of the complexes. The excited state redox potential (E^*) may be estimated by using [121]:

\[ E^* = E_{ox}^{0'} + E_{MLCT} \]  

(13)

An approximation of E^* can be obtained using the redox potential (E_{ox}^{0'}) and spectroscopic energy of the excited state (E_{MLCT}) obtained at room temperature. From this, it is shown that [Ir(ppy)3]^* (-2.1 V vs. ferrocene) is a significantly stronger reductant than either [Ir(dfppy)3]^* (-1.82 V vs. ferrocene) or [Ru(bipy)2(Me-ALA-bipy-dc)]^{2+*} (-0.90 V vs. ferrocene), and is therefore able to react with TPA^{**}. The fact that [Ir(ppy)3] is an exceptional reductant in the excited state is demonstrated by its rapid quenching by oxygen [267]. It is clear that the 'switch off' behaviour is related to both the nature of the metal complex and the concentration of the co-reactant present in the reaction mixture.

6.3.2 [Ir(dfppy)2BPS]^- systems

In the previously discussed systems, the green emission from [Ir(ppy)3] was elicited at low potentials. If this green emission was desired to occur at the more anodic potentials, [Ir(ppy)3] can be replaced by another complex. The substitution of a single 2-phenylpyridine ligand for a diimine such as 4,7-diphenyl-1,10-phenanthroline disulphonate increases the oxidation potential of the complex [243, 248, 268]; however it also induces a bathochromic shift of the emission [3]. This can be countered by fluorinating the remaining 2-phenylpyridine ligands [116, 204, 244, 269, 270], resulting in the green emitting [Ir(dfppy)2BPS]^- . The fluorine groups also increase the oxidation potential [271], and the complex is consequently oxidised at more anodic potentials than [Ru(bipy)2(Me-ALA-bipy-dc)]^{2+}. Additionally, an improvement in ECL performance can be expected, as it has previously been established that complexes with a higher oxidation potential than [Ir(ppy)3] (and lower reduction potential than TPA) undergo more efficient ECL reactions [219]. Another advantage is the presence of the highly water soluble sulphonate substituents causes the complex to be soluble in the analytically useful 50:50 acetonitrile:water solvent system, unlike [Ir(ppy)3].
During tests under typical ECL conditions (10 mM TPA and 0.1 M PBS), [Ir(dfppy)$_2$BPS]$^-$ was found to exhibit greater ECL efficiency than [Ir(ppy)$_3$]. Various concentrations of the complexes were trialed, and it was found that 0.1 mM [Ir(dfppy)$_2$BPS]$^-$ and 0.01 mM [Ru(bipy)$_2$(Me-ALA-bipy-dc)]$^{2+}$ was optimal. The ECL spectra of the individual solutions (Figure 6.05) clearly show that [Ru(bipy)$_2$(Me-ALA-bipy-dc)]$^{2+}$ is emissive at lower potentials than [Ir(dfppy)$_2$BPS]$, confirming the potential of this system for selective oxidation and simultaneous detection.

![ECL spectra](image)

**Figure 6.05:** Corrected ECL spectra of (a) [Ru(bipy)$_2$(Me-ALA-bipy-dc)]$^{2+}$ and (b) [Ir(dfppy)$_2$BPS]$^-$ over a range of potentials (vs. ferrocene).
When a solution containing both reagents is electrochemically oxidised (Figure 6.06), ECL peaks characteristic of both complexes can be seen at a potential of 1.30 V (purple trace). Lowering the potential results in considerable selectivity towards $[\text{Ru(bipy)}_2(\text{Me-ALA-bipy-dc})]^2^+$ (red trace).

![Figure 6.06: Corrected ECL spectra generated using chronoaamperometry pulses at 1.30 V (purple spectrum) or 1.04 V (red spectrum) vs. ferrocene for 0.1 mM $[\text{Ir(dfppy)}_2\text{BPS}]^-$ and 0.01 mM $[\text{Ru(bipy)}_2(\text{Me-ALA-bipy-dc})]^2^+$ in 50:50 acetonitrile:0.1 M PBS with 10 mM TPA co-reactant.](image)

If the emission of these chemiluminophores is monitored over a wide range of both electrode potentials and emission wavelength, it can be seen that the red emission of $[\text{Ru(bipy)}_2(\text{Me-ALA-bipy-dc})]^2^+$ is generated first. Then as the potential becomes more and more anodic, the green $[\text{Ir(dfppy)}_2\text{BPS}]^-$ emission appears and gradually increases in intensity. Photographs depicting the observed colour change can be seen in Figure 6.07, highlighting the initial red emission and the transition through orange and yellow. Ultimately, both complexes are simultaneously emissive, although the intense emission of $\text{Ir(dfppy)}_2\text{BPS}^-$ allows the green colour to dominate at anodic potentials (under these conditions).
The selective chemical oxidation of one chemiluminophore in the presence of another is also theoretically possible, however the oxidation potentials are limited to that of the available oxidants rather than controlled by a potentiostat. The oxidants Ce(SO$_4$)$_2$ and PbO$_2$ are most commonly used in the chemiluminescence reactions of ruthenium(II) [7] and iridium(III) [3] complexes, however the use of permanganate [25, 225, 272] and bromate [163] has also been documented. The feasibility of this concept was explored using [Ru(bipy)$_3$]$^{2+}$. Solutions of 1 mM potassium bromate in acidic (0.05 M H$_2$SO$_4$) and neutral media, as well as 1 mM acidic potassium permanganate (pH 2.5, containing 1% w/v sodium polyphosphates to stabilise the reactive intermediates) were trialed. PbO$_2$ was discounted as a possible oxidant as it was previously found unsuitable for the chemical oxidation of iridium(III) complexes (Chapter Four). The chosen oxidants were merged with either 1 mM [Ru(bipy)$_3$]$^{2+}$ and 0.001 mM ofloxacin or 1 mM [Ru(bipy)$_3$]$^{2+}$ and 0.01 mM codeine, in 50:50 acetonitrile:water, 50:50 methanol:water or 0.05 M H$_2$SO$_4$. The oxidation of [Ru(bipy)$_3$]$^{2+}$ by Ce(SO$_4$)$_2$ resulted in typical spectra with both analytes and for all solvent systems (see, for example, the spectra of the reactions with 0.001 mM ofloxacin in 0.05 M H$_2$SO$_4$ in Figure 6.08). The use of other oxidants, however, all proved unsuccessful. No signal could be detected within the noise. As [Ir(dfppy)$_2$BPS]$^-$ has a more anodic oxidation potential than [Ru(bipy)$_3$]$^{2+}$, an oxidant that is unable to elicit chemiluminescence would also be unable to do so with [Ir(dfppy)$_2$BPS]$^-$. It was therefore concluded that the only oxidants suitable to elicit chemiluminescence under these conditions are Ce(SO$_4$)$_2$ and PbO$_2$, and of these two, Ce(SO$_4$)$_2$ is more appropriate for the oxidation of iridium complexes (Chapter Four). When Ce(SO$_4$)$_2$ was used to oxidise a solution containing [Ru(bipy)$_3$(Me-ALA-bipy-dc)]$^{2+}$ and [Ir(dfppy)$_2$BPS]$^-$, an emission was
observed from both complexes. While Ce(SO₄)₂ is sufficient to generate the dual emission from the complexes, discrimination on the basis of oxidation potential was not achieved in chemiluminescence experiments.

![Chemiluminescence spectra](image)

**Figure 6.08:** Corrected chemiluminescence spectra of the reaction between 0.001 mM ofloxacin and 1 mM [Ru(bipy)₃]²⁺ with 1 mM Ce(SO₄)₂ in 0.05 M H₂SO₄, KMnO₄ containing 1% w/v sodium polyphosphates and KBrO₃ (in 0.05 M H₂SO₄ and neutral solution).

### 6.3.3 Energy transfer

The inclusion of two fluorophores in a solution presents the possibility of energy transfer [11], and so it was thought that perhaps it was also true of two chemiluminophores in solution. There is precedence for this phenomenon: in their study of metallosurfactants, Guerrero-Martinez *et al.* found that despite the absorbance spectrum of a 0.025 mM solution of both [Ir(dfppy)_2(C₁₇H₃₅-bipy)]⁺ and [Ru(bipy)_2(C₁₇H₃₅-bipy)]²⁺ resembling the sum of the individual spectra, upon excitation (350 nm) the only observable emission is that of the ruthenium(II) complex [246]. This energy transfer was not observed with any other combination of the control complexes ([Ru(bipy)_3]²⁺ and [Ir(dfppy)_2(bipy)]⁺) or the control complexes and the metallosurfactants, leading the authors to conclude that the amphiphilic
nature of the molecules was essential to the energy transfer process [246]. Although the concentration used was below the critical micellar concentration of the complexes, it is possible that there was some physical interaction between the hydrophobic moiety of the metallosurfactants. This indicates that a close association of the molecules may be required for the energy transfer. There is an energy transfer mechanism that relies on the orbital overlap (and therefore close proximity) of the donor and acceptor molecules [103, 273]. Known as the Exchange (or Dexter) Mechanism, in essence it involves a double electron transfer, with the excited state donor molecule transferring the electron in its LUMO to the LUMO of the acceptor molecule and simultaneously receiving into its HOMO an electron from the acceptor molecule's HOMO [103, 273]. Considering the lack of amphiphilicity in the complexes of this study, it seems unlikely that the required spatial overlap of the orbitals will occur in these dilute solutions.

A more plausible source of energy transfer is the Förster (or Resonance) Mechanism, which allows the transfer of energy through dipole-dipole interactions without the requirement of physical contact [11, 103, 273]. An oscillating dipole is able to exchange energy with another dipole that has a similar resonance frequency [11]. Essentially, the excited state donor molecule relaxes to the ground state and this transition simultaneously excites the acceptor complex [11, 103, 273]. The greater the overlap between the absorption and emission spectra of the donor and acceptor molecules, the more likely this mechanism will occur [11]. In order to investigate this, the emission and excitation spectra of \([\text{Ir(dfppy)}_2\text{BPS}]^\text{−}\) and \([\text{Ru(bipy)}_2(\text{Me-ALA-bipy-dc})]^{2+}\) were obtained (Figure 6.09). Examination of the excitation spectra revealed that both complexes have an intense ligand excitation peak at 260 nm, and so the emission spectra of the complexes were obtained at this wavelength. From this, it is evident that there is some overlap between the emission spectrum of \([\text{Ir(dfppy)}_2\text{BPS}]^\text{−}\) and the excitation spectrum of \([\text{Ru(bipy)}_2(\text{Me-ALA-bipy-dc})]^{2+}\). This may facilitate energy transfer, however the overlap is quite small, so the extent of the transfer is expected to be minimal. Furthermore, if Förster energy transfers were a major contributor to the systems, then the emission of \([\text{Ru(bipy)}_2(\text{Me-ALA-bipy-dc})]^{2+}\) would be expected to occur concurrently with that from \([\text{Ir(ppy)}_3]\) when the two electrochemiluminophores are combined in solution. The 3D ECL plot in Figure 6.03 shows that, while the emission of \([\text{Ru(bipy)}_2(\text{Me-ALA-bipy-dc})]^{2+}\) does occur prior to the formal oxidation potential of the complex, this is when the \([\text{Ir(ppy)}_3]\) emission has almost completely ‘switched off’ and there is little or no opportunity
for energy transfer. Hence, it was concluded that Förster energy transfers are unlikely to be occurring in these systems.

![Figure 6.09: The normalised excitation (solid lines) and emission (crosses) spectra of 0.1 mM \([\text{Ir}(dfppy)_2\text{BPS}]^-\) (dark cyan) and 0.01 mM \([\text{Ru}(bipy)_2(\text{Me-ALA-bipy-dc})]^{2+}\) (wine) in 50:50 acetonitrile:water (excitation wavelength 260 nm).](image)

To further explore this, the interactions of the oxidised states need to be considered. The oxidation potential of \([\text{Ir}(dfppy)_2\text{BPS}]^-\) was found to be just on the cusp of the potential window of the solvent (50:50 acetonitrile:0.1 M PBS). Potentials outside of this range lead to the oxidation or reduction of the solvent. To avoid this potentially confounding variable, chemiluminescence experiments with various concentrations of the luminophores were performed. Figure 6.10 shows the chemiluminescence intensity of \([\text{Ir}(dfppy)_2\text{BPS}]^-\) both in the presence and in the absence of \([\text{Ru}(bipy)_2(\text{Me-ALA-bipy-dc})]^{2+}\). This plainly shows that the presence of \([\text{Ru}(bipy)_2(\text{Me-ALA-bipy-dc})]^{2+}\) causes a decrease in the intensity of the \([\text{Ir}(dfppy)_2\text{BPS}]^-\) emission. A corresponding increase in the emission intensity of a constant concentration of \([\text{Ru}(bipy)_2(\text{Me-ALA-bipy-dc})]^{2+}\) is seen with increasing concentrations of \([\text{Ir}(dfppy)_2\text{BPS}]^-\).
Figure 6.10: The corrected chemiluminescence spectra of the reaction between 1 mM ofloxacin, 1 mM Ce(SO$_4$)$_2$ and (a) 0.01 mM; (b) 0.1 mM; and (c) 1 mM $[\text{Ir(dfppy)}_2\text{BPS}]^-$ in both the presence (wine) and absence (dark cyan) of 1 mM $[\text{Ru(bipy)}_2\text{(Me-ALA-bipy-dc)}]^2^+$. For simplicity, $[\text{Ir(dfppy)}_2\text{BPS}]^-$ has been abbreviated to 'Ir' and $[\text{Ru(bipy)}_2\text{(Me-ALA-bipy-dc)}]^2^+$ to 'Ru'. The reagent and ofloxacin were in 50:50 acetonitrile:water solution, while Ce(SO$_4$)$_2$ was dissolved in 0.05 M H$_2$SO$_4$. 
The quenching of the \([\text{Ir(dfppy)}_2\text{BPS}]^-\) emission increases with increasing concentrations of \([\text{Ru(bipy)}_2(\text{Me-ALA-bipy-dc})]^2^+\), as seen in the Stern-Volmer plot in Figure 6.11. The linear relationship that exists between the concentration of \([\text{Ru(bipy)}_2(\text{Me-ALA-bipy-dc})]^2^+\) and the emission intensity of \([\text{Ir(dfppy)}_2\text{BPS}]^-\) is a clear indication that some form of dynamic quenching is taking place. The fact that the same wasn't observed in photoluminescence indicates that the process involves some interaction of the Ir(III), Ir(IV), Ir(III)*, Ru(II), Ru(III), and/or the Ru(II)* species of the complexes present after oxidation.

Figure 6.11: Stern-Volmer plot of the chemiluminescence intensity of 1 mM \([\text{Ir(dfppy)}_2\text{BPS}]^-\) in the presence of increasing amounts of quencher (i.e., \([\text{Ru(bipy)}_2(\text{Me-ALA-bipy-dc})]^2^+\)) at 536 nm. The chemiluminescence was obtained from the reaction of the chemiluminophores with 1 mM Ce(SO$_4$)$_2$ and 0.01 mM ofloxacin. The reagent and analyte were in 50:50 acetonitrile:water solution, while the oxidant was dissolved in 0.05 M H$_2$SO$_4$. The Stern-Volmer equation thus obtained is $I_0/I = 1.00 + 1.65[\text{Ru(bipy)}_2(\text{Me-ALA-bipy-dc})]^2^+$; $R^2 = 0.992$.

6.3.4 Other luminophore combinations

It is apparent that there is some interaction between the chemiluminophores during chemiluminescence reactions, and the same is true in ECL: \([\text{Ru(bipy)}_2(\text{Me-ALA-bipy-dc})]^2^+\) is emissive prior to its formal oxidation potential when combined with \([\text{Ir(ppy)}_3]\) (see Figure 6.03). Furthermore, this only seems to occur with certain complexes: in Figure 6.04, \([\text{Ru(bipy)}_2(\text{Me-ALA-bipy-dc})]^2^+\) (in system A) emits at lower potentials than \([\text{Ir(dfppy)}_3]\) (system B), despite their respective formal oxidation potentials of 0.96 V and 0.69 V (vs.
ferrocene). It is possible that this is due to differences in the ECL mechanisms of the complexes. Bard et al. [264] proposed that, at low reagent concentrations and a large co-reactant excess, the following could occur at lower voltages than the $E^{0'}$ of $[\text{Ru(bipy)}_3]^{2+}$:

$$\text{TPA} \rightarrow \text{TPA}^{++} + e^- \quad (14)$$

$$\text{TPA}^{++} \rightarrow \text{TPA}^- + H^+ \quad (15)$$

$$[\text{Ru(bipy)}_3]^{2+} + \text{TPA}^- \rightarrow [\text{Ru(bipy)}_3]^+ + P \quad (16)$$

$$[\text{Ru(bipy)}_3]^+ + \text{TPA}^{++} \rightarrow [\text{Ru(bipy)}_3]^{2+*} + P \quad (17)$$

$$[\text{Ru(bipy)}_3]^{2+*} \rightarrow [\text{Ru(bipy)}_3]^{2+} + h\nu \quad (18)$$

It seems reasonable to extend this to system A; the TPA radical has an $E^{0'}$ of -2.09 V vs. ferrocene [264], which is more than sufficient to reduce $[\text{Ru(bipy)}_2(\text{Me-ALA-bipy-dc})]^{2+}$ ($E^{0'} = -1.47$ V vs. ferrocene [44]). The same early emission is not seen in system B, as TPA$^-$ is not powerful enough to reduce $[\text{Ir(dfppy)}_3]$ ($E^{0'} = -2.51$ V vs. ferrocene [274]). In support of this postulation is the fact that the emission from the reaction of $[\text{Ru(bipy)}_2(\text{Me-ALA-bipy-dc})]^{2+}$ and high concentrations of TPA is apparent at potentials approximately 200 mV less oxidative than the potential required to produce ECL emission with low concentrations of TPA. This, however, does not explain why the onset of emission is even earlier (0.20 V compared to 0.90 V vs. ferrocene) when $[\text{Ir(ppy)}_3]$ is also present in the solution. In order to investigate this, other dual emission systems were studied. The complexes Os 5 and Os 6 from Chapter Five were chosen to complement $[\text{Ru(bipy)}_2(\text{Me-ALA-bipy-dc})]^{2+}$, as they have a similar emission wavelength yet a lower and higher oxidation potential, respectively (Table 6.01). These were used in conjunction with the same green emitters, $[\text{Ir(dfppy)}_2\text{BPS}^-]$ and $[\text{Ir(ppy)}_3]$. As before, the colour of the initial emission is controlled through the applied potential: green appears at low voltages when $[\text{Ir(ppy)}_3]$ is present, and at higher voltages in the $[\text{Ir(dfppy)}_2\text{BPS}^-]$ series.
Table 6.01: Electrochemical data for the complexes studied. (a) determined in acetonitrile solution containing 0.1 M TBAPF₆, glassy carbon electrode. (b) determined in 50:50 acetonitrile:0.1 mM PBS, boron-doped diamond electrode.

<table>
<thead>
<tr>
<th>Complex</th>
<th>E°, vs. ferrocene (1 mM)</th>
<th>λₑcl (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Ir(ppy)₃]</td>
<td>0.33</td>
<td>530</td>
</tr>
<tr>
<td>[Ir(dfppy)₃]</td>
<td>0.69</td>
<td>495</td>
</tr>
<tr>
<td>[Ir(dfppy)₂BPS]⁻</td>
<td>1.46</td>
<td>541</td>
</tr>
<tr>
<td>[Ru(bipy)₂(Me-ALA-bipy-dc)]²⁺</td>
<td>0.96</td>
<td>660</td>
</tr>
<tr>
<td>Os 5</td>
<td>0.87</td>
<td>630</td>
</tr>
<tr>
<td>Os 6</td>
<td>1.25</td>
<td>620</td>
</tr>
</tbody>
</table>

Figure 6.12 shows the applied potential vs. emission intensity at 530 nm for the [Ir(ppy)₃] series; these traces are extracted from the 3D ECL plots in Figure 6.13. These figures show that the maximum emission of [Ir(ppy)₃] always occurs at 0.22 - 0.27 V. The emission also spans the same range in each case, between 0.12 V and 0.52 V. As was noted previously, a 'switch off' of emission intensity is seen at high overpotentials. Interestingly, the inclusion of the red emitter causes a change in the emission intensity of the [Ir(ppy)₃] peak: [Ru(bipy)₂(Me-ALA-bipy-dc)]²⁺ drastically reduces the emission (by 68%), as does Os 6 (by 35%), while Os 5 induces a marked improvement (to 196% of the individual complex's emission intensity).
Figure 6.13: Surface plots of 0.25 mM [Ir(ppy)$_3$] in isolation and in combination with various concentrations of either Os 5, Os 6 or [Ru(bipy)$_3$(Me-ALA-bipy-dc)]$^{2+}$ in dry acetonitrile containing 0.1 M TBAPF$_6$. The intensity plots are a function of both wavelength and applied potential (vs. ferrocene).
The emissive range of [Ir(dfppy)$_2$BPS]$^-$ is slightly different in each combination. (Figure 6.14 shows the applied potential vs. intensity traces of this series at 541 nm, extracted from the 3D ECL plots in Figure 6.15.) The largest range is obtained from the [Ir(dfppy)$_2$BPS]$^-$ - Os 5 combination, spanning 0.56 - 1.26 V. This is followed by the individual complex (0.71 - 1.26 V) and its admixture with [Ru(bipy)$_2$(Me-ALA-bipy-dc)]$^{2+}$ (0.86 - 1.26 V). Most of the emission, however, is between 0.92 V and 1.26 V. The only exception was the combination with Os 6, in which [Ir(dfppy)$_2$BPS]$^-$ was almost completely quenched. The emission intensity of [Ir(dfppy)$_2$BPS]$^-$ was quenched to differing degrees by the presence of the red emitters: the least quenching is seen with Os 5 and the most with Os 6. Despite these differences, a fairly similar intensity profile vs. applied voltage is obtained for all mixtures except the [Ir(dfppy)$_2$BPS]$^-$ - Os 6 combination.

Figure 6.14: The ECL intensity vs. applied potential (vs. ferrocene) cross sections extracted from the 3D plots of the [Ir(dfppy)$_2$BPS]$^-\text{ series (in isolation and in combination with various concentrations of either Os 5, Os 6 or [Ru(bipy)$_2$(Me-ALA-bipy-dc)]$^{2+}$, in 50:50 acetonitrile:0.1 M PBS, pH 7) at 541 nm.}
Figure 6.15: Surface plots of 0.05 mM [Ir(dfppy)₂BPS]⁺ in isolation and in combination with various concentrations of either Os 5, Os 6 or [Ru(bipy)₂(Me-ALA-bipy-dc)]²⁺, in 50:50 acetonitrile:0.1 M PBS (pH 7). The intensity plots are a function of both wavelength and applied potential (vs. ferrocene).
The ECL emission intensity as a function of applied potential of the [Ru(bipy)$_2$(Me-ALA-bipy-dc)]$^{2+}$ series at 660 nm can be seen in Figure 6.16; the 3D plots of this series is in Figure 6.17. When the spectra of [Ru(bipy)$_2$(Me-ALA-bipy-dc)]$^{2+}$ in dry acetonitrile are examined, it can be seen that the complex is emissive from 0.52 V to 1.37 V. A small shoulder is apparent from 0.52 - 0.92 V, before the formal oxidation potential of the complex. The emission is all but extinguished upon addition of [Ir(ppy)$_3$], yet an almost negligible trace of light can be detected from 0.32 V - 1.37 V. Despite the weakness of emission, it is apparent that something is exciting the complex at potentials less oxidative than its formal oxidation potential. When tested in 50:50 acetonitrile:0.1 M PBS, the emission intensity of [Ru(bipy)$_2$(Me-ALA-bipy-dc)]$^{2+}$ was found to increase with the applied potential, and the addition of [Ir(dfppy)$_2$BPS]$^-$ increases this by 391%. An early onset of the [Ru(bipy)$_2$(Me-ALA-bipy-dc)]$^{2+}$ emission is not seen in this combination, but a decrease in emission intensity can be seen after 1.16 V. It appears that the presence of [Ir(dfppy)$_2$BPS]$^-$ causes a 'switch off' of the emission at high overpotentials, similar to that seen with [Ir(ppy)$_3$].
Figure 6.16: The ECL intensity vs. applied potential (vs. ferrocene) cross sections extracted from the 3D plots of the \([\text{Ru(bipy)}_2(\text{Me-ALA-bipy-dc})]^2^+\) series at 660 nm. (a): \([\text{Ru(bipy)}_2(\text{Me-ALA-bipy-dc})]^2^+\) alone and in combination with \([\text{Ir(ppy)}_3]\) in dry acetonitrile containing 0.1 M TBAPF\(_6\). (b): \([\text{Ru(bipy)}_2(\text{Me-ALA-bipy-dc})]^2^+\) alone and in combination with \([\text{Ir(dfppy)}_2\text{BPS}]^-\) in 50:50 acetonitrile:0.1 M PBS.
Figure 6.17: Surface plots of 0.05 mM [Ru(bipy)$_2$(Me-ALA-bipy-dc)]$^{2+}$ in dry acetonitrile (upper left) and in 50:50 acetonitrile:0.1 M PBS (upper right), and 0.05 mM [Ru(bipy)$_2$(Me-ALA-bipy-dc)]$^{2+}$ with 0.25 mM [Ir(ppy)$_3$] (in dry acetonitrile, lower left) or 0.05 mM [Ir(dfppy)$_2$BPS$^-$] (in 50:50 acetonitrile:0.1 M PBS, lower right). The intensity plots are a function of both wavelength and applied potential (vs. ferrocene).
A similar pattern to that described for \([\text{Ru(bipy)}_2(\text{Me-ALA-bipy-dc})^2^+\] can be seen in the Os 5 series. (Figure 6.18 shows the ECL intensity of the Os 5 series vs. applied potential extracted from the 3D plots at 630 nm; the 3D ECL plots themselves are depicted in Figure 6.19.) The inclusion of \([\text{Ir(ppy)}_3]\) both reduces the maximum emission of Os 5 by half, and advances the onset of the emission by 450 mV. \([\text{Ir(dfppy)}_2\text{BPS}]^-\) does not affect the onset of emission, although its inclusion in the solution causes the emission to build to an intensity 300% greater than the independent Os 5. Again, a rapid decline can be seen in emission intensity after this maximum; \([\text{Ir(dfppy)}_2\text{BPS}]^-\) also induces a 'switch off' of the Os 5 emission. The emission intensity of Os 5 also decreases slightly at high potentials when \([\text{Ir(dfppy)}_2\text{BPS}]^-\) is not present in the solution. This decay, however, is not as dramatic as that seen in the dual emission system and may be attributable to the approach of the solvent limit.
Figure 6.18: The ECL intensity vs. applied potential (vs. ferrocene) cross sections extracted from the 3D plots of the Os 5 series at 630 nm. (a) Os 5 alone and in combination with [Ir(ppy)$_3$] in dry acetonitrile containing 0.1 M TBAPF$_6$.
(b) Os 5 alone and in combination with [Ir(dfppy)$_2$BPS]$^-$ in 50:50 acetonitrile:0.1 M PBS.
Figure 6.19: Surface plots of 0.003 mM Os 5 in dry acetonitrile (upper left) and 50:50 acetonitrile:0.1 M PBS (upper right), and 0.003 mM Os 5 with 0.25 mM [Ir(ppy)$_3$] (in dry acetonitrile, lower left) or 0.05 mM [Ir(dfppy)$_2$BPS]$^-$ (in 50:50 acetonitrile:0.1 M PBS, lower right). The intensity plots are a function of both wavelength and applied potential (vs. ferrocene).
Figure 6.20 shows the applied potential vs. ECL intensity cross sections of the Os 6 series at 620 nm; the 3D ECL plots can be found in Figure 6.21. An almost negligible shoulder is apparent at approximately 0.80 V in the emission of Os 6 in both solvents. A more intense emission then begins at 0.90 V and increases with the overpotential. As seen with [Ru(bipy)$_2$(Me-ALA-bipy-dc)]$^{2+}$ and Os 5, the inclusion of [Ir(ppy)$_3$] both reduces the emission intensity (by 93%) and causes this to occur at a potential less positive (by 600 mV). The intensity then holds comparatively steady as the potential increases. [Ir(dfppy)$_2$BPS]$^-$ increases the emission intensity by 264%, yet does not induce the 'switch off' behaviour seen with Os 5 and [Ru(bipy)$_2$(Me-ALA-bipy-dc)]$^{2+}$. What looks like the beginning of a 'switch off' can be seen after 1.21 V vs. ferrocene, however this could not be explored as this is the limit of the potential window of this solvent.
Figure 6.20: The ECL intensity vs. applied potential (vs. ferrocene) cross sections extracted from the 3D plots of the Os 6 series at 620 nm. (a) Os 6 alone and in combination with [Ir(ppy)$_2$] in dry acetonitrile containing 0.1 M TBAPF$_6$. (b) Os 6 alone and in combination with [Ir(dfppy)$_2$BPS]$^-$ in 50:50 acetonitrile:0.1 M PBS.
Figure 6.21: Surface plots of 0.02 mM Os 6 in dry acetonitrile (upper left) and 50:50 acetonitrile:0.1 M PBS (upper right), and 0.02 mM Os 6 with 0.25 mM [Ir(ppy)$_3$] (in dry acetonitrile, lower left) or 0.05 mM [Ir(dfppy)$_2$BPS]$^-$ (in 50:50 acetonitrile:0.1 M PBS, lower right). The intensity plots are a function of both wavelength and applied potential (vs. ferrocene).
In summation, the addition of \([\text{Ir(ppy)}_3]\) to \([\text{Ru(bipy)}_2(\text{Me-ALA-bipy-dc})]^2^+\), Os 5 or Os 6 quenches their emission by up to 93%, and causes an early onset of the red emission. All the red emitters can in this way be manipulated to be emissive from 0.17 V vs. ferrocene, the same potential at which \([\text{Ir(ppy)}_3]\) itself reacts. This early onset is not caused by \([\text{Ir(dfppy)}_2\text{BPS}]^-\), although it seems as though this complex induces a 'switch off' of the red emission of Os 5 and \([\text{Ru(bipy)}_2(\text{Me-ALA-bipy-dc})]^2^+\). It is possible that this 'switch off' is due to the instability of aqueous solvents at high potentials [36], but this seems unlikely as the same effect is not seen in every aqueous system studied. \([\text{Ir(dfppy)}_2\text{BPS}]^-\) also triples the emission intensity of all three complexes. The emission of \([\text{Ir(ppy)}_3]\) and \([\text{Ir(dfppy)}_2\text{BPS}]^-\) is reduced by the inclusion of the red emitters by up to 91% (\([\text{Ir(dfppy)}_2\text{BPS}]^- - \text{Os 6}\)). The only exception is the combination of \([\text{Ir(ppy)}_3]\) and Os 5, in which case the green emission is enhanced. No significant change in the emissive range of \([\text{Ir(ppy)}_3]\) or \([\text{Ir(dfppy)}_2\text{BPS}]^-\) can be seen in any of the combinations of electrochemiluminophores. A slight shoulder is apparent before the maximum emission of \([\text{Ru(bipy)}_2(\text{Me-ALA-bipy-dc})]^2^+, \text{Os 5, Os 6 and } [\text{Ir(dfppy)}_2\text{BPS}]^-\) in their respective ECL intensity vs. applied potential cross sections. This can be seen in both the individual and the dual emission systems and it is apparent in both solvents. It is likely that this is due to the reaction of the chemiluminophore with excess TPA$^+$ (equations 14 - 18), followed by the classical ECL route of oxidation at the electrode prior to reaction with the co-reactant (equations 1 - 5). Although this shoulder is apparent in the dual emission systems, it is more obvious in solutions of the individual chemiluminophores. This can be attributed to the competition of the chemiluminophores in the composite ECL systems for the reaction with TPA$^+$, the concentration of which is constant (10 mM TPA) in all experiments. The same reaction does not occur with \([\text{Ir(ppy)}_3]\), because TPA$^+$ (0.43 V vs. ferrocene) is not a powerful enough oxidant to react with this reagent (0.33 V vs. ferrocene).

The increase in emission intensity of \([\text{Ru(bipy)}_2(\text{Me-ALA-bipy-dc})]^2^+, \text{Os 5 and Os 6 when in combination with } [\text{Ir(dfppy)}_2\text{BPS}]^-\), coupled with the decrease in the emission intensity of the latter, is in good agreement with the photoluminescence and chemiluminescence data in Section 6.3.3: the apparent energy transfer causes a decrease in the chemiluminescence peak height of \([\text{Ir(dfppy)}_2\text{BPS}]^-\) and an increase in \([\text{Ru(bipy)}_2(\text{Me-ALA-bipy-dc})]^2^+\) (Figure 6.10). Furthermore, this was found to involve some interaction of the reactive species present after the oxidation of both complexes (Figure 6.11). It appears that there is a similar interaction of the reactive species of the complexes in ECL. The effects of combining \([\text{Ir(ppy)}_3]\) with the
red emitters, however, suggests that the interaction may not be a simple energy transfer. This complex decreases the emission intensity of all the red emitters, and is itself enhanced by Os 5 and decreased by Os 6 and \([\text{Ru(bipy)}_2(\text{Me-ALA-bipy-dc})]^2+\). There is clearly no simple trend followed by each of these combinations. The enhancements (or reductions) seen in the ECL emission may be due to the presence of one chemiluminophore facilitating (or inhibiting) the ECL pathway of the other, that is, acting as either a positive or negative interferent.

The differences in the oxidation potentials of \([\text{Ru(bipy)}_2(\text{Me-ALA-bipy-dc})]^2+\), Os 5 and Os 6 do not seem to have an effect on the interference mechanism occurring in these the composite ECL systems. The greatest difference in oxidation potential is seen in the combination of \([\text{Ir}(\text{ppy})_3]\) - Os 6, and lowest in \([\text{Ir(dfppy)}_2\text{BPS}]^-\) - Os 6. These differences can result in spectra that produce two distinct emissions separated on the potential axis, for example \([\text{Ir}(\text{ppy})_3]\) and \([\text{Ru(bipy)}_2(\text{Me-ALA-bipy-dc})]^2+\) or Os 6, however the resolution is less than expected due to the early onset of the emission of the red emitting complexes. Resolution between the chemiluminophores was not achieved in the \([\text{Ir(dfppy)}_2\text{BPS}]^-\) series due to the similarity of the complexes' oxidation potentials.
6.4 Conclusions

This work has significantly built upon the proof-of-concept experiments by Richter et al. [214, 218]. It has been proven that two spectrally distinct luminophores can not only be detected via ECL, but also by chemiluminescence. This can be important in many applications, from cell staining [54] to analytical chemistry [112, 215, 216, 220], with the ultimate aim of multiplexing for high-throughput analysis [34]. In addition to the implications for multi-analyte detection, the exciting possibility of controlling the ECL emission through the manipulation of the complexes’ oxidation potentials has opened up new avenues for the creation of colour-tunable LEDs. This can involve colours that are the combination of light from both luminophores, or the pure emission from each complex. It has also been established that interactions occur between the chemiluminophores. In the [Ir(dfppy)$_2$BPS]$^{-}$-[Ru(bipy)$_2$(Me-ALA-bipy-dc)]$^{2+}$ system, this looks likely to be an energy transfer process involving the ground, excited and oxidised states of the complexes. In the other combinations, this explanation is too simple and does not account for the differences (either synergistic or antagonistic) seen in the emission intensities. A further finding of this work is the fascinating influence that the iridium(III) complexes have on emissive range of all three red emitters, particularly the early onset of the emission.

Some of the contents of this chapter have been published:


Chapter Seven: Summary and Future Work
This work has significantly expanded upon the body of knowledge on the chemiluminescence of platinum group metal complexes. The use of $[\text{Ru(bipy)}_3]^+$ as a chirally selective chemiluminescence reagent was investigated. The enantiomers were successfully isolated then reacted with the enantiomers of various analytes, and some differences in the kinetic profiles were apparent. The chemiluminescence chemistry of $[\text{Ru(bipy)}_3]^{2+}$ was extended to a variety of iridium(III) and osmium(II) complexes. This allowed extensive manipulation of properties that are insensitive to alterations in ruthenium(II) complexes, such as tuning the emission wavelength to green and altering the oxidation potential of the complexes. The great sensitivities obtained from these complexes confirms their potential as viable chemiluminescence reagents. A dual emission system has been created including these complexes; multiple combinations of ruthenium(II), osmium(II) and iridium(III) complexes exhibiting distinct emissive properties have been selectively oxidised and simultaneously detected in a single solution. Discrimination between the chemiluminophores was achieved by their emission energy or oxidation potential. Some very interesting effects were observed when the dual emission ECL experiments were performed, including the enhancement of the ECL intensity and an alteration in the emissive range of the complexes.

Much work remains to be performed in these areas. Some possible future directions are listed below:

Chapter Two - Chemiluminescence and Chirality - Tris(2,2′-bipyridyl)ruthenium(II)

First and foremost, the chemiluminescence detection method requires considerable optimisation to improve the reproducibility. This may involve the use of ECL, or a batch chemiluminescence system, as Pappin found it to be preferable to the stopped-flow instrumentation available at the time [169]. The stopped-flow apparatus itself can be optimised: replacing the syringe pump and injection valves with more precise and reliable apparatus is an option, as is investigation into other manifolds. Secondly, it seems as though the differences in kinetics observed for the enantiomers where enhanced through the use of larger, more chiral molecules. If a broad library of chiral analytes of different sizes were obtained, for example the various drugs that exhibit chirality or enantiomerically pure metal complexes, the differences may be even more obvious. Conversely, the chirality of the reagent itself can be extended. In the study of the interactions of ruthenium(II) complexes and DNA, complexes containing large planar ligands such as 1,10-phenanthroline were found to bind more closely to the DNA than $[\text{Ru(bipy)}_3]^{2+}$ [160]. If large ligand-bearing complexes are
studied, then perhaps the chiral interactions will be exaggerated enough for to clearly distinguish their chemiluminescence profiles. Likewise, the inclusion of enantiomerically pure chiral ligands may also enhance these differences.

Chapter Three: The Immobilisation of Ruthenium(II) Complexes

The mysterious nature of the regeneration of the signal needs to be investigated. The length of time required for this regeneration needs to be determined, as well as whether there is a finite lifespan of each cell. The chemiluminescence intensity of the functionalised silica needs to be improved. This can either be through alterations made to the linking group between the silica and the complex itself - e.g., optimise the length, rigidity and polarity of the linker - or through immobilising other metal complexes. Additionally, the sensitivity may be enhanced by the presence of more reactive ruthenium(II) centres. As there is a limit to the loading of the complexes, it may be interesting to investigate the effect of creating multinuclear immobilised complexes. Such complexes have been shown to exhibit potential as solution-phase ECL reagents [275]. In this case, the extent of communication between the metal centres must be kept to a minimum, allowing independent action of the ruthenium(II) centres [275]. This was attempted, via the cross metathesis of 4-methyl-4′-propen-1-yl-2,2′-bipyridine, however the desired linker could not be achieved by either Grubb’s catalyst 1st generation or 2nd generation and so no multinuclear immobilised complexes were obtained. Further attempts were abandoned due to time constraints.

Chapter Four - The Chemiluminescence of Iridium(III) Complexes

While an extensive study into the chemiluminescence of a select few iridium(III) complexes has been performed, more data is needed before generalised conclusions can be drawn. The chemiluminescence potential of other water-soluble complexes should investigated. As mentioned in Chapter Two, the stability of \([\text{Ru(bipy)}_3]^{3+}\) is greatly increased merely by substituting the solvent for acidified acetonitrile. This could be trialled for iridium(III) complexes, as even if the stability is not improved to the same extent, the use of acetonitrile would allow new, organic-soluble complexes to be tested. A large analyte screen could also be useful, to determine any differences in the selectivity of iridium(III) complexes to ruthenium(II) complexes.
Chapter Five - The Chemiluminescence of Osmium(II) Complexes

The preliminary work described in Chapter Four indicates that there may be a disadvantage to using osmium(II) (electro)chemiluminescence reagents containing more than one diarsine ligand. Further exploration of such complexes is required, to determine if it is indeed detrimental to the chemiluminescence performance and if this trend can be extended to bis-diphosphine complexes. Similarly to the iridium(III) complexes, a large analyte screen should be performed to determine the selectivity of these reagents. The effect of acidified acetonitrile on the stability of the oxidised state should also be determined.

Chapter Six - The Selective Oxidation and Simultaneous Detection of Distinct Chemiluminophores

This chapter has emphasised just how immature this field is. In order to fully understand all that is happening, more complexes need to be tested. Clearly, from Chapters Four and Five, there are many, many different iridium(III) - ruthenium(II) - osmium(II) combinations that can be trialled. Complexes containing other metal centres such as platinum or copper may also provide interesting results. The complexes must to be fully soluble in a solvent like acetonitrile in order to extend the potential window. One possibility is [Ir(dfppy)2(phen)]+, as it is similar to the already characterised [Ir(dfppy)2BPS]− but lacks hydrophilic sulphonate groups. It may also be worthwhile to investigate the effect of ionic liquids on the system, as they may increase the potential window and/or increase the solubility of the complexes. The resolution between the emissions should be improved by choosing complexes with increased differences in emission wavelengths or narrower emission profiles. Concentrating on the effect of the oxidation potential of the complexes may also provide some insight into the mechanism of energy transfer/interference. The nature of this interaction (or interactions) does need to be determined, and if possible, avoided or strictly controlled through judicious choice of reagents or experimental conditions. The extent of the interactions ought to be verified over a range of concentrations. The system described is an oxidative-reductive ECL reaction, using TPA as the co-reactant. It is possible that different selectivities can be obtained for the reagents with a range of co-reactants. If other compounds are used, for example amines with different redox potentials, or the reductive-oxidative co-reactant S2O8, the system may be further manipulated.
I am still learning

-Michelangelo

Learning is a treasure that will follow its owner everywhere

- Chinese proverb
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