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Short communication

Chemiluminescence reactions with cationic, neutral, and anionic ruthenium(II) complexes containing 2,2′-bipyridine and bathophenanthroline disulfonate ligands

Paul S. Francis [a,b,*], Dimitra Papettas [a], Elizabeth M. Zammit [a], Neil W. Barnett [a]

[a] School of Life and Environmental Sciences, Deakin University, Geelong, Victoria 3217, Australia
[b] Institute for Technology Research and Innovation, Deakin University, Geelong, Victoria 3217, Australia

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ABSTRACT

Ruthenium complexes containing 4,7-diphenyl-1,10-phenanthroline disulfonate (bathophenanthroline disulfonate; BPS) ligands, $Ru(BPS)_3^{4-}$, $Ru(BPS)_2(bipy)^{2-}$ and $Ru(BPS)(bipy)_2$, were compared to tris(2,2′-bipyridine)ruthenium(II) ($Ru(bipy)_3^{2+}$), including examination of the wavelengths of maximum absorption and corrected emission intensity, photoluminescence quantum yield, stability of their oxidised ruthenium(III) form, and relative chemiluminescence intensities and signal-to-blank ratios with cerium(IV) sulfate and six analytes (codeine, morphine cocaine, potassium oxalate, furosemide and hydrochlorothiazide) in acidic aqueous solution. The presence of BPS ligands in the complex increased the photoluminescence quantum yield, but decreased the stability of the oxidised form of the reagent. In contrast to previous evidence showing much greater electrochemiluminescence intensities using $Ru(BPS)_2(bipy)^{2-}$ and $Ru(BPS)(bipy)_2$, these complexes did not provide superior chemiluminescence signals than their homoleptic analogues.

1. Introduction

The extraordinary success of tris(2,2′-bipyridine)ruthenium(II) ($Ru(bipy)_3^{2+}$) as a chemiluminescence and electrochemiluminescence reagent has spurred exploration of analogues to develop reagents with superior properties or extend this highly sensitive mode of detection to new analytical applications [1–5]. The relationship between ligand structure and the photoluminescence characteristics of ruthenium complexes has been studied extensively [6–9], but the influence of different ligands on (electro)chemiluminescence reactions with various analytes/co-reactants is yet to be fully understood.

One promising line of investigation has involved complexes with 4,7-diphenyl-1,10-phenanthroline-disulfonate (bathophenanthrolinedisulfonate; BPS) [10–14]. Blanchard and co-workers found that $Ru(BPS)_3^{4-}$ (Fig. 1) was twice as sensitive as $Ru(bipy)_3^{2+}$ for the electrochemiluminescence determination of oxalate using flow injection analysis methodology [10]. Della Ciana and co-workers reported that the electrochemiluminescence intensity for $Ru(BPS)_3^{4-}$ was six times greater than for $Ru(bipy)_3^{2+}$, using tripropylamine as a co-reactant [12]. Moreover, they obtained much greater electrochemiluminescence intensities (up to 26-fold compared to $Ru(bipy)_3^{2+}$) with the zwitterionic $Ru(BPS)(bipy)_2$, which they attributed to the absence of fast adsorption/crystallisation at the electrode surface that was thought to occur with $Ru(BPS)_3^{4-}$ [14].

$Ru(BPS)_3^{4-}$ has been used as a chemiluminescence reagent (with cerium(IV)) for the detection of several pharmaceuticals, including hydrochlorothiazide [11], furosemide [11], piroxicam [15], mitoxantrone [16], prulifloxacin [17] and codeine [13], but very few direct comparisons with $Ru(bipy)_3^{2+}$ have been made. Xi and co-workers noted that the limit of detection for hydrochlorothiazide using a batch luminometer was an order of magnitude lower using $Ru(BPS)_3^{4-}$ compared to $Ru(bipy)_3^{2+}$, and the limits of detection for furosemide using the two reagents were similar [11]. A re-evaluation of these reactions using flow injection analysis methodology [13] revealed that in general, $Ru(BPS)_3^{4-}$ produces a more intense emission, but the oxidised $Ru(BPS)_3^{3-}$ is less stable in aqueous solution than $Ru(bipy)_3^{3+}$, resulting in higher blank signals. In that study, the greatest signal-to-blank ratios for codeine, oxalate and hydrochlorothiazide were obtained with $Ru(bipy)_3^{2+}$, $Ru(phen)_3^{2+}$ and $Ru(BPS)_3^{4-}$, respectively, highlighting the influence of ligand structure on the selectivity of the chemiluminescence reagent [13]. Herein we describe an evaluation of ruthenium complexes containing bathophenanthroline disulfonate ligands ($Ru(BPS)_3^{4-}$, $Ru(BPS)_2(bipy)^{2-}$, $Ru(BPS)(bipy)_2$) as reagents for chemiluminescence detection, in direct com-

* Corresponding author at: School of Life and Environmental Sciences, Deakin University, Geelong, Victoria 3217, Australia. Tel.: +61 3 5227 1294.
E-mail address: psf@deakin.edu.au (P.S. Francis).

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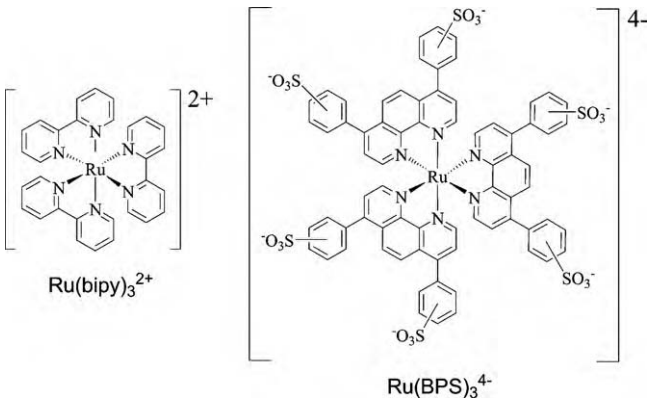

Fig. 1. Tris(2,2′-bipyridine)ruthenium(II) ($Ru(bipy)_3^{2+}$) and tris(4,7-diphenyl-1,10-phenanthrolinedisulfonate)ruthenium(II) ($Ru(BPS)_3^{4-}$). Although the sulfonate groups of the BPS ligand are often drawn in the *para* position, they are at least a mixture of both *para*- and *meta*-substituted [27]. We previously found that one commercial batch contained sulfonate groups predominantly in the *meta* position [13].

parison with the conventional $Ru(bipy)_3^{2+}$ reagent. This first examination of chemiluminescence reactions with the heteroleptic ($Ru(BPS)_2(bipy)^{2-}$ and $Ru(BPS)(bipy)_2$) complexes (with cerium(IV) and a range of analytes) provides insight into the mechanism of enhancement in the previous electrochemiluminescence experiments, and a greater understanding of the relationship between ligand structure and chemiluminescence intensity, which is important for the development of new solution phase and immobilised chemiluminescence reagents systems.

2. Experimental

2.1. Spectroscopic characterisation

Absorbance spectra were collected with a Cary 300 Bio UV-Vis Spectrophotometer (Varian Australia, Mulgrave, Victoria, Australia) using 5×10^{-6} M ruthenium complex in 0.05 M sulfuric acid, in a 1 cm path-length quartz cuvette. Photoluminescence and chemiluminescence spectra were collected with a Cary Eclipse Spectrofluorimeter (Varian Australia) with a red-sensitive photomultiplier tube. When required, emission spectra were corrected, as previously described [18]. Photoluminescence spectra were collected using 1×10^{-5} M ruthenium complex in 0.05 M sulfuric acid in a standard 1 cm quartz cuvette (5 nm band pass, 1 nm data interval, PMT voltage: 800 V).

Photoluminescence quantum yields: for each complex, the integrated corrected emission spectrum (500–850 nm, λ_{ex} = 450 nm) was plotted against the absorbance at 450, using four standard solutions (concentrations between 2×10^{-6} and 8×10^{-6} M for $Ru(bipy)_3^{2+}$, $Ru(BPS)(bipy)_2$ and $Ru(BPS)_2(bipy)^{2-}$; between 1.5×10^{-6} and 6×10^{-6} M for $Ru(BPS)_3^{4-}$). All solutions contained 0.05 M sulfuric acid. Quantum yields were derived from the relative proportionality constants, based on the literature value of 2.8% for $Ru(bipy)_3^{2+}$ (in air-equilibrated aqueous solution) [19].

2.2. Stability of oxidised reagents

The relative stability of the ruthenium(III) state of the complexes after oxidation with cerium(IV) was examined by combining solutions of cerium(IV) sulfate in 0.05 M H_2SO_4 and the ruthenium complex in 0.05 M H_2SO_4 in a cuvette placed in the spectrophotometer, and monitoring the absorbance over time. The stability of the complexes after oxidation with lead dioxide was examined by adding 3 mg of the solid oxidant to 5 mL of the ruthenium complex (in 0.05 M H_2SO_4); the oxidised complex was injected through an Acrodisc filter into a cuvette in the spectrophotometer.

2.3. Flow injection analysis with chemiluminescence detection

The relative chemiluminescence intensities for the reactions between the ruthenium complexes ($Ru(BPS)_3^{4-}$, $Ru(BPS)_2(bipy)^{2-}$, $Ru(BPS)(bipy)_2$, $Ru(bipy)_3^{2+}$) and the analytes were established using flow injection analysis (FIA) methodology [13]. The chemiluminescence detector consisted of a T-piece and coiled flow cell (PTFE tubing, 0.8 mm i.d.; DKSH) positioned against a photomultiplier tube (Electron Tubes model 9828SB, ETP, Ermington, New South Wales, Australia) encased in a light-tight housing. The photomultiplier tube was operated at 900 V provided by a stable power supply (electron Tubes model PM20D, ETP) and voltage divider (Electron Tubes model C611, ETP). Unless otherwise stated, the ruthenium complex was injected (70 μL) into an analyte stream, which merged with a cerium(IV) sulfate solution at the T-piece immediately prior to entering the flow cell.

2.4. Reagents

Tris(2,2′-bipyridine)ruthenium(II) chloride hexahydrate ($[Ru(bipy)_3]Cl_2 \cdot 6H_2O$) was obtained from Strem Chemicals (Newbury, Minnesota, USA). [(Bathophenanthrolinedisulfonate)-bis(2,2′-bipyridine)ruthenium(II)] tetrahydrate ($[Ru(BPS)(bipy)_2] \cdot 4H_2O$), [bis(bathophenanthrolinedisulfonate)(2,2′-bipyridine)ruthenium (II)] disodium salt nonahydrate ($[Ru(BPS)_2(bipy)]Na_2 \cdot 9H_2O$), and [tris(bathophenanthroline disulfonate)ruthenium(II)]tetrasodium salt hexahydrate ($[Ru(BPS)_3]Na_4 \cdot 6H_2O$) were from Cyanagen (Bologna, Italy). Furosemide, hydrochlorothiazide and cerium(IV) sulfate were from Sigma-Aldrich (Castle Hill, New South Wales, Australia). Lead dioxide was from Ajax (Auburn, New South Wales, Australia). Potassium oxalate was from BDH Chemicals (Poole, England). Sulfuric acid was from Merck (Kilsyth, Victoria, Australia). Codeine, morphine and cocaine were donated by GlaxoSmithKline (Port Fairy, Victoria, Australia).

3. Results and discussion

3.1. Spectroscopic characterisation

The wavelengths of maximum absorbance of the metal to ligand charge transfer (MLCT) $d \rightarrow \pi^*$ transition and maximum photoluminescence emission are shown in Table 1. The values for absorbance and uncorrected photoluminescence were in good agreement with those reported by Della Ciana and co-workers [14]. Correction of the emission spectra for the wavelength dependence of the detector response and monochromator transmission shifted the maximum emission by 15–18 nm. Corrected values for $Ru(bipy)_3^{2+}$ and $Ru(BPS)_3^{4-}$ were in close agreement with previous reports [19,20]. Corrected values for $Ru(BPS)_2(bipy)^{2-}$ and $Ru(BPS)(bipy)_2$ have not previously been published. Unlike $Ru(bipy)_n(phen)_{3-n}^{2+}$ (where n = 0–3) [21], the $Ru(BPS)_n(bipy)_{3-n}^{2-2n}$ series does not show a simple trend for the wavelength of maximum absorbance or photoluminescence intensity. However, the photoluminescence quantum yield clearly increased with the number of BPS ligands.

3.2. Stability of the oxidised ruthenium(III) species in acidic aqueous solution

Although other pathways are known [1], the application of ruthenium(II) complexes as chemiluminescence reagents normally involves oxidation to the corresponding ruthenium(III)

Table 1
Photophysical properties of the ruthenium complexes in acidic aqueous solution.

Complex	Abs.	Photoluminescence		
	MLCT, λ_{max} (nm)	Uncorr. λ_{max} (nm)	Corrected λ_{max} (nm)	ϕ_{PL} (%)
$Ru(bipy)_3^{2+}$	453	610	628	2.8[a]
$Ru(BPS)(bipy)_2$	453	622	637	3.5
$Ru(BPS)_2(bipy)^{2-}$	434	618	633	3.9
$Ru(BPS)_3^{4-}$	464	615	630	4.3

[a] Reference value (air-equilibrated aqueous solution) [19].

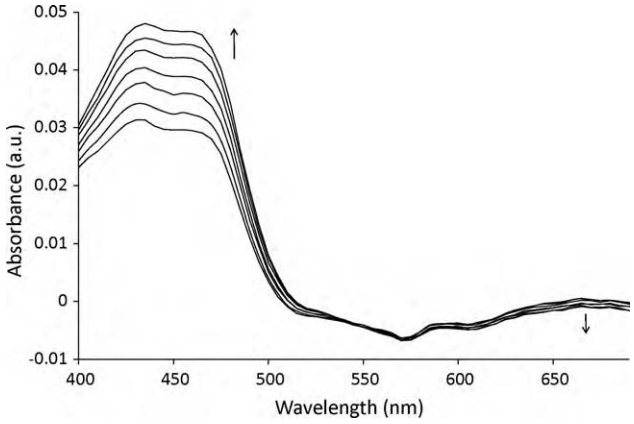

Fig. 2. Absorption spectra for 1:1 (v/v) mixture of 1×10^{-5} M $Ru(BPS)_2(bipy)^{2-}$ and 2×10^{-5} M cerium(IV) sulfate, both in 0.05 M sulfuric acid (data for time = 3, 4, 5, 6, 7, 8 and 9 min shown).

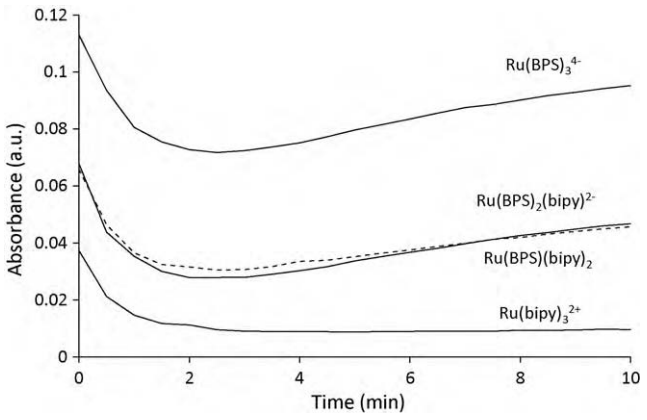

Fig. 3. Change in concentration of 1×10^{-5} M ruthenium(II) species after mixing 1:1 (v/v) with 2×10^{-5} M cerium(IV) sulfate, monitored by absorbance at 465 nm. $Ru(BPS)_2(bipy)^{2-}$ data shown as a dashed line.

species before reaction with an appropriate analyte (reducing agent). The ruthenium(III) states of these complexes, however, have limited temporal stability in aqueous solution [13,21,22]. Relative stabilities of the oxidised $Ru(BPS)_3^{3-}$, $Ru(BPS)_2(bipy)^{1-}$, $Ru(BPS)(bipy)_2^{1+}$, $Ru(bipy)_3^{3+}$ complexes in acidic aqueous solution were examined by monitoring the absorbance spectrum over time. A sulfuric acid concentration of 0.05 M was selected because it was found in a previous study to be appropriate for several chemiluminescence reactions with $Ru(BPS)_3^{4-}$ [13]. As shown in Fig. 2, the ruthenium(II) complexes ($\lambda \sim 460$ nm) have higher molar absorptivities than the corresponding oxidised species ($\lambda \sim 670$ nm), and therefore provided a clearer indication of change in these systems.

When 1×10^{-5} M of each ruthenium(II) complex was combined (1:1) with 2×10^{-5} M cerium(IV) sulfate, only the $Ru(bipy)_3^{3+}$ appeared stable over the 10-min time period of the experiment (Fig. 3). When 2×10^{-4} M cerium(IV) sulfate was used, no complex returned to the ruthenium(II) state over the period of the experiment, presumably due to the continuous re-oxidation by the large excess of cerium(IV). Nevertheless, the time-scale for on-line oxidation of the reagent within a flow injection analysis manifold is very short compared to these experiments and as shown in Fig. 3, low concentrations of cerium(IV) can generate appreciable quantities of the oxidised reagent for a period of time that is suitable for flow analysis. Stability studies were also conducted using solid lead dioxide as the oxidant and it was found that the ruthenium(III) reagents that contained one or more BPS ligands were not sufficiently stable for flow injection analysis with preliminary off-line oxidation.

3.3. Comparison of chemiluminescence intensities

Using flow injection analysis methodology, the chemiluminescence intensities from reactions of $Ru(BPS)_3^{4-}$, $Ru(BPS)_2(bipy)^{2-}$ and $Ru(bipy)_3^{2+}$ (2×10^{-4} M in 0.05 M H_2SO_4) with cerium(IV) sulfate (1×10^{-3} M in 0.05 M H_2SO_4) and analytes (1×10^{-5} M in water), including morphine, codeine, cocaine, potassium oxalate, hydrochlorothiazide and furosemide, over a range of flow rates (1–3.5 mL min^{-1} per line). The $Ru(BPS)(bipy)_2$ complex, with overall neutral charge, was not soluble at 2×10^{-4} M and was therefore excluded from this comparison. The optimum flow rate for each combination was found to be much more dependent on the analyte than the reagent (see Supplementary Data for the results for furosemide and hydrochlorothiazide).

The signals for analytes that elicited a relatively intense response are shown in Table 2. In each of these cases, the largest signal was obtained with the $Ru(BPS)_3^{4-}$ complex. However, the complexes containing BPS ligands produced larger blank signals, and the greatest signal-to-blank ratio was obtained using $Ru(bipy)_3^{2+}$, except in the case of hydrochlorothiazide, for which the largest signal-to-blank ratio was obtained with $Ru(BPS)_3^{4-}$.

In spite of the fact that compounds containing aliphatic tertiary amines often elicit an intense response with $Ru(bipy)_3^{2+}$ [2], morphine and cocaine produced a signal/blank ratio of less than 1.6 with all three ruthenium complexes. The relatively

Table 2
Chemiluminescence intensity and signal-to-blank ratios for the reaction of ruthenium complexes (2×10^{-4} M) with cerium(IV) sulfate (1×10^{-3} M) and various analytes (1×10^{-5} M). The optimum flow rate (between 1 and 3.5 mL min^{-1} per line) was selected for each combination of analyte and reagent (HCT = hydrochlorothiazide).

	Oxalate	Codeine	Furosemide	HCT
Chemiluminescence signal (V)				
$Ru(bipy)_3^{2+}$	3.9	1.2	2.6	0.4
$Ru(BPS)_2(bipy)^{2-}$	5.3	1.1	1.2	3.3
$Ru(BPS)_3^{4-}$	6.9	1.7	2.8	14.0
Signal-to-blank ratio				
$Ru(bipy)_3^{2+}$	52.6	37.9	30.5	13.6
$Ru(BPS)_2(bipy)^{2-}$	42.3	14.0	9.5	41.4
$Ru(BPS)_3^{4-}$	39.7	13.2	14.8	110.2

weak response from morphine (compared to codeine) in acidic aqueous solution with $Ru(bipy)_3^{2+}$ is well known [23], and has been attributed to quenching from its phenolic functionality [24], but the lack of chemiluminescence response from cocaine is yet to be understood. It should be noted, however, that reasonable detection limits have been reported for cocaine using the electrochemiluminescence reaction with $Ru(bipy)_3^{2+}$ in a phosphate-acetate buffer at pH 7.2 [25] and a borate buffer at pH 10 [26].

The comparison was repeated using all four ruthenium complexes at a concentration of 1×10^{-5} M (in 0.05 M H_2SO_4). The oxidant and analyte concentrations were lowered to 2×10^{-4} and 1×10^{-6} M, respectively. Signal intensities were reduced by more than two orders of magnitude, but the blank responses were also lowered. Most importantly, as for the conditions described above, the heteroleptic complexes did not produce significantly greater signals or signal-to-blank ratios than the largest signal from $Ru(bipy)_3^{2+}$ or $Ru(BPS)_3^{4-}$.

4. Conclusions

Although the ruthenium complexes containing BPS ligands had higher photoluminescence quantum yields than $Ru(bipy)_3^{2+}$, and in some cases much greater chemiluminescence intensities were obtained using $Ru(BPS)_3^{4-}$ than using $Ru(bipy)_3^{2+}$, heteroleptic complexes containing bipy and BPS ligands did not produce superior chemiluminescence signals than their homoleptic analogues, which supports Della Ciana and co-workers' assertion that the dramatic increase in electrochemiluminescence intensity for the heteroleptic complexes (compared to $Ru(BPS)_3^{4-}$) resulted from changes in electrode surface effects [14]. Furthermore, the differences in chemiluminescence response from the four complexes with these analytes cannot be predominantly attributed to the overall charge of the complex as previous studies have demonstrated that even minor changes to ligand structure within ruthenium complexes of the same overall charge can have a significant effect on chemiluminescence intensity [21]. The $Ru(BPS)(bipy)_2$ complex is less soluble in aqueous solution which restricts its use in that solvent. However, this characteristic may be advantageous for immobilised reagent systems, which often suffer from reduced performance due to leaching of the $Ru(bipy)_3^{2+}$ complex into the surrounding aqueous solution [2].

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.talanta.2010.05.047.

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