

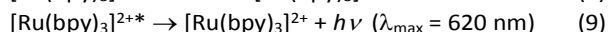
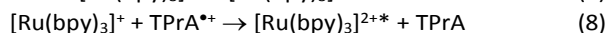
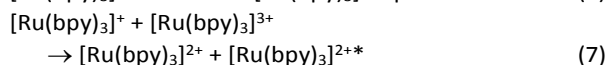
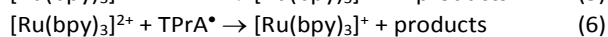
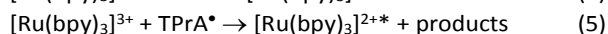
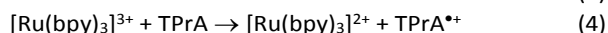
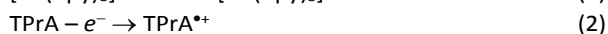
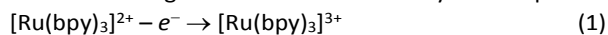
'Cathodic' electrochemiluminescence of [Ru(bpy)₃]²⁺ and tri-*n*-propylamine confirmed as emission at the counter electrode

Ashton G. Theakstone,^a Egan H. Doeven,^b Xavier A. Conlan,^a Lynn Dennany^{*c}, Paul S. Francis^{*a}

A new approach to examine electrochemiluminescence (ECL), in which the potentials at both the working and counter electrodes are measured and the emitted light is detected by photomultiplier tube, camera and then a charge-coupled device (CCD) spectrometer, provides unequivocal evidence that the purported cathodic ECL of [Ru(bpy)₃]²⁺ and tri-*n*-propylamine actually arises from anodic reactions at the counter electrode.

Electrochemiluminescence (ECL) continues to be exploited in a diverse range of analytical applications,¹⁻⁷ as the emission of light from the excited products of electrogenerated species against a dark background, and the ability to repeatedly excite electrochemiluminophores in the presence of 'co-reactants', provides an exceedingly sensitive mode of detection. Moreover, the temporal and spatial control of the electrochemical excitation process creates opportunities for multiplexing and imaging approaches.⁸⁻¹¹

The majority of analytical applications of ECL have been based on tris(2,2'-bipyridine)ruthenium(II) ([Ru(bpy)₃]²⁺) with various secondary or tertiary alkylamine co-reactants such as tri-*n*-propylamine (TPrA). The mechanisms of these 'oxidative-reduction' (or anodic) co-reactant systems have been widely explored.¹²⁻¹⁴ The ECL of [Ru(bpy)₃]²⁺ with TPrA, for example, proceeds *via* Eqn 1 to 9, where one or both species are oxidised at the electrode surface followed by homogeneous electron transfer reactions involving the aminium cation (TPrA^{•+}) and α -amino alkyl (TPrA[•]) radicals.¹² In applications in which this ECL system is used to detect the amine co-reactant with relatively high concentrations of [Ru(bpy)₃]²⁺, the predominant reaction pathways are depicted by Eqn 1-5 and 9. ECL can also be generated through analogous 'reductive-oxidation' (or cathodic) pathways, using co-reactants such as persulfate¹⁵⁻¹⁸ and benzoyl peroxide,^{19, 20} where the reactants are reduced at the working electrode before homogeneous electron transfers generate the electronically excited product.



Cathodic ECL has relatively few analytical applications, but Cao *et al.*²¹ reported that many compounds that traditionally served as oxidative-reduction co-reactants (including TPrA) could also be sensitively detected with [Ru(bpy)₃]²⁺ in aqueous phosphate buffer solution when applying a low cathodic potential (between -0.4 V and -0.8 V vs Ag/AgCl). The ECL was attributed to the reduction of dissolved oxygen, leading to the

formation of [•]OH, capable of oxidising [Ru(bpy)₃]²⁺. Choi and Bard²² subsequently reported the ECL of [Ru(bpy)₃]²⁺ with hydrogen peroxide as a co-reactant in phosphate buffer solution when applying cathodic potentials (between -1.3 V and -1.5 V vs Ag/AgCl) and proposed a mechanism involving the [•]OH radical (Eqn 10-13, 7 and 9).

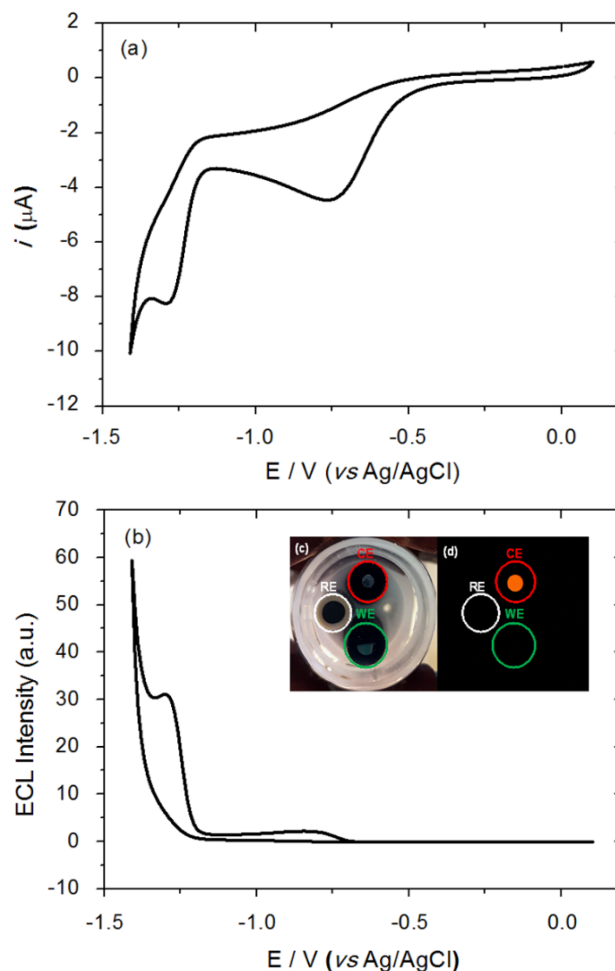


Fig. 1. (a) Cyclic voltammogram of 1 mM [Ru(bpy)₃]²⁺ with 50 mM TPrA in 0.1 M phosphate buffer (pH 6.8) at a scan rate of 100 mV s⁻¹ over the potential range -1.60 V to 0.20 V vs Ag/AgCl. (b) ECL signals recorded simultaneously with the traces from (a). (c) Inset shows the three-electrode configuration of GC working electrode (WE, green line), Pt disk counter electrode (CE, red line) and Ag/AgCl reference electrode (RE, white line). (d) ECL emission recorded for electrode set up in (c) recorded on a Canon DSLR camera.











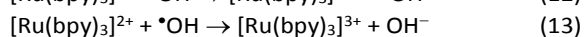
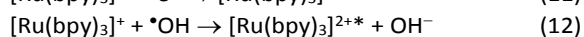
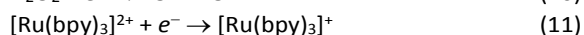
Electrode	Potential (E) / V vs Ag/AgCl											
	-0.40	-0.50	-0.60	-0.70	-0.80	-0.90	-1.00	-1.10	-1.20	-1.30	-1.40	-1.50
Pt wire counter	0.62	0.69	0.76	0.76	0.77	0.77	0.75	0.75	0.83*	0.85*	0.88*	0.93*
Pt disk counter	0.72	0.85	0.90*	0.91*	0.91*	0.90*	0.90*	0.89*	0.91*	0.92*	0.93*	0.94*
Photographs of ECL at the Pt disk counter electrode ^a												

Table 1. Potentials applied at the GC working electrode and measured at the Pt wire or Pt disk counter electrode, and photographs of the ECL at the Pt disk counter electrode upon application of each working electrode potential.

^aPotentials at which ECL was observed at the counter electrode. ^aPhotographs of ECL at the Pt disk counter electrode recorded on a Canon EOS 6D DSLR camera (Canon, Japan) fitted with a Tonika AT-X PRO MACRO 100 mm f/2.8 D lens (Kenko Tonika Co., Japan). A 40 s exposure time was used for each image with an ISO value of 8000 and an aperture of F2.8. See ESI for photographs of ECL at the Pt wire counter electrode. Conditions: 0.1 M phosphate buffer solution (pH = 6.8) containing 1 mM [Ru(bpy)₃]²⁺ and 50 mM TPrA.



Choi and Bard measured a weak background ECL in the absence of hydrogen peroxide, when scanning the electrode potential in the negative direction.²² In contrast to Cao and co-workers' observations,²¹ Choi and Bard reported that the intensity of the background emission increased upon the removal of oxygen. Moreover, the emission was no longer detected when the counter electrode was isolated from the catholyte using a glass capillary with porous frit, suggesting the background emission that they observed, and by extension the ECL reported by Cao *et al.*,²¹ may actually arise from anodic processes at the counter electrode, but Choi and Bard²² did not examine the reaction with TPrA. In a related study, Yuan *et al.*²³ reported 'cathodic' ECL from [Ru(bpy)₃]²⁺ in acetonitrile containing acetic acid, sodium acetate and tetrabutylammonium perchlorate. The emission was enhanced by TPrA and inhibited by H₂O₂. These authors also stated that no emission was seen when the counter electrode was isolated in a glass capillary with porous frit.

In conventional ECL experiments, the light is generally assumed to emanate from solution near the working electrode surface, but contributions from simultaneous reactions at the counter electrode may be more common than previously recognised.^{24, 25} Furthermore, the advancement towards multi-luminophore and potential-resolved ECL approaches,^{4, 26-28} which require wider ranges of electrode potentials, has placed new importance in understanding ECL reactions at the counter electrode. With this in mind, we sought to elucidate the nature of the ECL reaction reported by Cao *et al.*²¹ by monitoring the potential at both the working and the counter electrode, and visualising the source of light using a digital camera positioned under the electrochemical cell and synchronised with the potentiostat. As in the previous studies^{21, 22} we used a glassy carbon working, platinum counter and Ag/AgCl reference electrode. As Cao *et al.* did not give details of the Pt counter

electrode, we conducted experiments using a Pt wire, which is more commonly employed, and then with a Pt disk electrode, which provided clearer visualisation of its entire active surface.

We first interrogated the electrochemical behaviour using cyclic voltammetry while monitoring the ECL emission with an extended-range trialkali S20 photomultiplier tube (PMT; ET Enterprises model 9828B) positioned under the cell. Fig. 1 shows the cathodic cyclic voltammogram (CV) and associated ECL response for 1 mM [Ru(bpy)₃]²⁺ and 50 mM TPrA in 0.1 M phosphate buffer solution (pH 6.8). The CV exhibits two reduction waves. The first starts at approximately -0.50 V, which we attribute to the reduction of dissolved oxygen. The second begins at -1.20 V. In agreement with Cao *et al.*,²¹ ECL was observed when these negative potentials were applied to the working electrode, after the onset of the O₂ reduction.

We then replaced the PMT with a DSLR camera (Canon, Japan) fitted with a Tonika AT-X PRO MACRO 100 mm f/2.8 D lens (Kenko Tonika, Japan), and added to the potentiostat a pX1000 module (Metrohm Autolab B.V, Netherlands) configured to measure the potential occurring at the counter electrode. Using a camera as the photodetector enables direct visualisation of the location of the emission.^{27, 29} As shown in Fig. 1d, under these conditions, the ECL was observed at the Pt disk counter electrode, while no emission was seen at the working electrode.

Using a series of chronoamperometric experiments in which potentials from -0.40 V to -1.50 V vs Ag/AgCl were applied at the working electrode (Table 1), the corresponding potentials measured at the counter electrode ranged from 0.72 V to 0.94 V for the Pt disk and 0.62 V to 0.93 V for the Pt wire. Images obtained for the Pt wire electrode are shown in Supplementary Material (Fig. S1). The greater potentials at the disk electrode were ascribed to its lower effective surface area, resulting in higher current densities at the electrode surface.³⁰ Consequently, ECL was observed at the Pt disk counter electrode when lower cathodic potentials were applied at the working electrode (-0.60 V, compared to -1.20 V for the Pt wire).

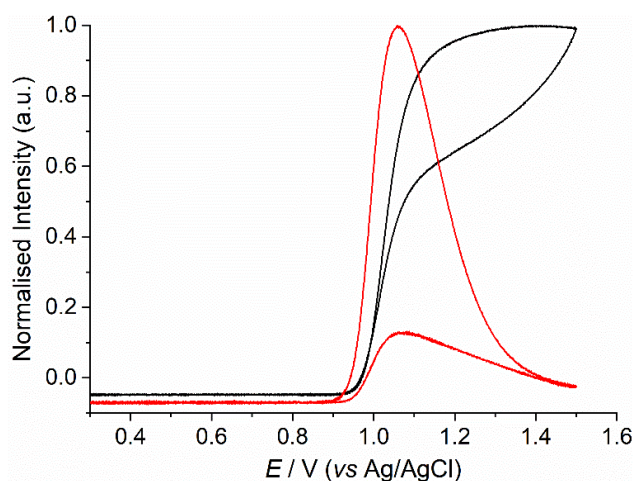


Fig. 2. ECL response for 1 mM $[\text{Ru}(\text{bpy})_3]^{2+}$ with 50 mM TPrA in 0.1 M phosphate buffer (pH = 6.8) when scanning from 0 V to 1.5 V vs Ag/AgCl and then back to 0 V, at a scan rate of 100 mV s^{-1} , when using a GC working electrode and Pt counter electrode (black line) or a Pt disk working electrode and GC counter electrode (red line). The dissimilarity in the shape of the ECL response arises from differences in the rates of electron transfer and/or changes in surface properties during the oxidation scan.³¹

As shown in Table 1, ECL was observed at the counter electrode when the potential at that electrode was approximately 0.85 V (vs Ag/AgCl) or above, which is consistent with the oxidative-reduction (anodic) co-reactant pathway of $[\text{Ru}(\text{bpy})_3]^{2+}$ with TPrA (Fig. 2). The onset of anodic ECL is similar for GC and Pt working electrodes. To confirm that the orange emission observed at the counter electrode (Table 1) was the characteristic luminescence from the $^3\text{MLCT}$ excited state of $[\text{Ru}(\text{bpy})_3]^{2+}$,^{32, 33} we replaced the camera with an Ocean Optics QEPro CCD spectrometer interfaced with the cell *via* optical fibre and collimating lens. The maximum ECL emission was $\sim 620 \text{ nm}$, matching that of the ECL observed at the working electrode when anodic potentials were applied, and the photoluminescence of the same complex (Fig. 3).

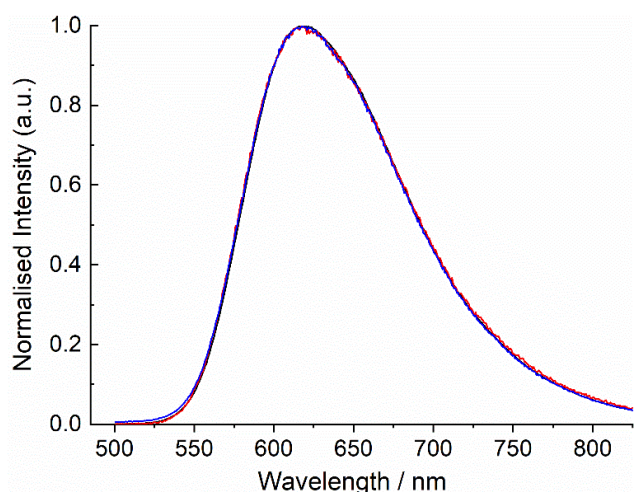


Fig. 3. Emission spectra for ECL at the counter electrode (blue line) upon application of cathodic potentials at the working electrode, as shown in Fig. 1; and ECL at the working electrode (red line) upon application of anodic potentials, as shown in Fig. 2, for 1 mM $[\text{Ru}(\text{bpy})_3]^{2+}$ with 50 mM TPrA in 0.1 M phosphate buffer (pH = 6.8) solution; and the photoluminescence (black line) for the same solution upon excitation with UV light (370 nm LED). See ESI for individual emission spectra.

Contrary to the previous proposal that the ECL of $[\text{Ru}(\text{bpy})_3]^{2+}$ and TPrA when applying cathodic potentials arises from the generation of a reactive oxygen species capable of oxidising the metal complex, the data presented here shows that the emission from the $^3\text{MLCT}$ excited state of $[\text{Ru}(\text{bpy})_3]^{2+}$ occurs predominantly at the counter electrode through an oxidative-reduction co-reactant pathways (Eqn 1-5 and 9).¹² This finding is congruent with Choi and Bard's account²² of removing the weak background ECL from $[\text{Ru}(\text{bpy})_3]^{2+}$ under cathodic potentials by isolating the counter electrode in a capillary with a porous frit.

The operation of both ECL pathways proposed in the previous studies was conceivable, if the light from the counter electrode was unintentionally obscured from the photodetector in the work of Cao *et al.*,²¹ and the absence of TPrA in Choi and Bard's study rendered the intensity of the cathodic ECL far lower than the weak emission attributed to electron transfers at the counter electrode. The conflicting observations of the two groups on the influence of dissolved oxygen on their respective reactions provides some support for this notion. In our examination of the conditions described by Cao *et al.*, however, we did not observe a decrease in ECL intensity when deaerating the solution. Moreover, we found that the onset of ECL when applying cathodic potentials (as shown in Table 1) was dependent on the configuration of the counter electrode (Pt wire or disk), which was ascribed to the difference in their effective surface area. The on-set occurred at more negative potentials than that required to reduce oxygen ($E_p = -0.4\text{-}0.5 \text{ V vs Ag/AgCl}$ ^{21,34}). Most convincingly, the absence of any observable emission from the working electrode in our study when cathodic potentials were applied, considering Cao and co-workers' claim that the cathodic ECL was similar in intensity to conventional anodic ECL of $[\text{Ru}(\text{bpy})_3]^{2+}$ and TPrA, leads us to rule out any analytically useful ECL from this purported cathodic ECL pathway

This work highlights the importance of considering electrochemical processes occurring at the counter electrode in the elucidation of ECL reaction mechanisms, and we outline a convenient instrumental approach to ascertain the source of emission, both the emitting species and its spatial distribution within the electrochemical cell. Not surprisingly, the processes that we observed to occur at the counter electrode were dependent on the configuration of the electrochemical cell, as (i) the voltage at the counter electrode is adjusted by the potentiostat to account for any change within the system to maintain the desired potential at the working electrode, and (ii) the position and geometry of the counter electrode will determine the portion of emitted light that is measured by the photodetector. Beyond the implications for investigation into ECL reaction mechanism, the recognition of processes at the counter electrode resulting in intense ECL and the development of our instrumental approach to distinguish the source of the emission creates exciting possibilities for analytical applications involving a dual ECL response at the working and counter electrode.

Conflicts of interest

There are no conflicts to declare.

Acknowledgments

PSF thanks Deakin University and the Australian Research Council (DP160103046) for funding. LD acknowledges The Royal Society (IES\R3\170367) for funding this work.

Notes and references

1. Y. N. Khonsari and S. Sun, *Chem. Commun.*, 2017, **53**, 9042-9054.
2. L. Li, Y. Chen and J.-J. Zhu, *Anal. Chem.*, 2017, **89**, 358-371.
3. N. Sojic, S. Arbault, L. Bouffier and A. Kuhn, in *Luminescence in Electrochemistry*, eds. F. Misomandre and P. Audebert, Springer, Gewerbestrasse, Switzerland, 2017, pp. 257-291.
4. E. H. Doeven, G. J. Barbante, C. F. Hogan and P. S. Francis, *ChemPlusChem*, 2015, **80**, 456-470.
5. A. J. Stewart, J. Hendry and L. Dennany, *Anal. Chem.*, 2015, **87**, 11847-11853.
6. Q. Zhai, J. Li and E. Wang, *ChemElectroChem*, 2017, **4**, 1639-1650.
7. W. Gao, M. Saqib, L. Qi, W. Zhang and G. Xu, *Curr. Opin. Electrochem.*, 2017, **3**, 4-10.
8. W. Guo, H. Ding, C. Gu, Y. Liu, X. Jiang, B. Su and Y. Shao, *J. Am. Chem. Soc.*, 2018, **140**, 15904-15915.
9. S. Voci, B. Goudeau, G. Valenti, A. Lesch, M. Jovic, S. Rapino, F. Paolucci, S. Arbault and N. Sojic, *J. Am. Chem. Soc.*, 2018, **140**, 14753-14760.
10. G. Valenti, S. Scarabino, B. Goudeau, A. Lesch, M. Jović, E. Villani, M. Sentic, S. Rapino, S. p. Arbault, F. Paolucci and N. Sojic, *J. Am. Chem. Soc.*, 2017, **139**, 16830-16837.
11. E. H. Doeven, G. J. Barbante, E. Kerr, C. F. Hogan, J. A. Endler and P. S. Francis, *Anal. Chem.*, 2014, **86**, 2727-2732.
12. W. Miao, J.-P. Choi and A. J. Bard, *J. Am. Chem. Soc.*, 2002, **124**, 14478-14485.
13. I. Svir, A. Oleinick, O. V. Klymenko and C. Amatore, *ChemElectroChem*, 2015, **2**, 811-818.
14. M. Sentic, M. Milutinovic, F. Kanoufi, D. Manojlovic, S. Arbault and N. Sojic, *Chem. Sci.*, 2014, **5**, 2568-2572.
15. D. Ege, W. G. Becker and A. J. Bard, *Anal. Chem.*, 1984, **56**, 2413-2417.
16. R. Russell, A. J. Stewart and L. Dennany, *Anal. Bioanal. Chem.*, 2016, **408**, 7129-7136.
17. A. J. Stewart, K. Brown and L. Dennany, *Anal. Chem.*, 2018, **90**, 12944-12950.
18. Y. N. Khonsari and S. Sun, *Microchim. Acta*, 2018, **185**, 430.
19. T. D. Santa Cruz, D. L. Akins and R. L. Birke, *J. Am. Chem. Soc.*, 1976, **98**, 1677-1682.
20. C.-W. Hsu, E. Longhi, S. Sinn, C. S. Hawes, D. C. Young, P. E. Kruger and L. De Cola, *Chem. - Asian J.*, 2017, **12**, 1649-1658.
21. W. Cao, G. Xu, Z. Zhang and S. Dong, *Chem. Commun.*, 2002, 1540-1541.
22. J.-P. Choi and A. J. Bard, *Anal. Chim. Acta*, 2005, **541**, 143-150.
23. B. Yuan, H. Du and T. You, *Talanta*, 2009, **79**, 730-733.
24. L. C. Soulsby, D. J. Hayne, E. H. Doeven, L. Chen, C. F. Hogan, E. Kerr, J. L. Adcock and P. S. Francis, *ChemElectroChem*, 2018, **5**, 1543-1547.
25. E. Kerr, E. H. Doeven, G. J. Barbante, C. F. Hogan, D. J. Hayne, P. S. Donnelly and P. S. Francis, *Chem. Sci.*, 2016, **7**, 5271-5279.
26. L. Dennany, T. E. Keyes and R. J. Forster, *Analyst*, 2008, **133**, 753-759.
27. E. H. Doeven, E. M. Zammit, G. J. Barbante, C. F. Hogan, N. W. Barnett and P. S. Francis, *Angew. Chem., Int. Ed.*, 2012, **51**, 4354-4357.
28. A. S. Danis, J. B. Gordon, K. P. Potts, L. I. Stephens, S. C. Perry and J. Mauzeroll, *Anal. Chem.*, 2019, **91**, 2312-2318.
29. G. J. Barbante, N. Kebede, C. M. Hindson, E. H. Doeven, E. M. Zammit, G. R. Hanson, C. F. Hogan and P. S. Francis, *Chem. Eur. J.*, 2014, **20**, 14026-14031.
30. D. Laser and A. J. Bard, *J. Electrochem. Soc.*, 1975, **122**, 632-40.
31. G. Valenti, A. Fiorani, H. Li, N. Sojic and F. Paolucci, *ChemElectroChem*, 2016, **3**, 1990-1997.
32. D. W. Thompson, A. Ito and T. J. Meyer, *Pure Appl. Chem.*, 2013, **85**, 1257-1305.
33. K. Suzuki, A. Kobayashi, S. Kaneko, K. Takehira, T. Yoshihara, H. Ishida, Y. Shiina, S. Oishi and S. Tobita, *Phys. Chem. Chem. Phys.*, 2009, **11**, 9850-9860.
34. H.-H. Yang and R. L. McCreery, *J. Electrochem. Soc.*, 2000, **147**, 3420-3428.