

## Phenol substituent effects on electrogenerated chemiluminescence quenching

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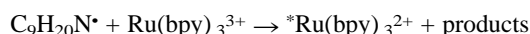
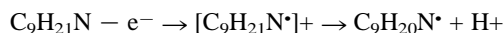
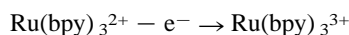
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Efficient quenching of  $\text{Ru}(\text{bpy})_3^{2+}$  (bpy = 2,2'-bipyridine) electrogenerated chemiluminescence (ECL) was observed in the presence of phenol and substituted phenols (*e.g.*, 4-fluorophenol). Spectroscopic and electrochemical studies indicated that the mechanism of quenching involves energy transfer from the excited state luminophore to a benzoquinone derivative formed at the electrode. The efficiency of ECL quenching is directly related to the position of the substituent on the aromatic ring, with *meta* derivatives displaying the greatest magnitude of quenching. The degree of quenching does not appear to be related to the electron-donating or -withdrawing ability of the phenol substituent.

## Introduction

The photochemical, photophysical and electrogenerated chemiluminescence (ECL) properties of  $\text{Ru}(\text{bpy})_3^{2+}$  (bpy = 2,2'-bipyridine) are very well known.<sup>1–4</sup> ECL involves the electrochemical formation of excited states and as such is a sensitive probe of electrochemical, energy and electron transfer at electrified interfaces. It is also being commercially developed for the clinical diagnostic market<sup>5</sup> and many of its known applications have been reviewed.<sup>3</sup>

ECL can be generated in a single electrochemical step using a species able to form strong oxidants or reductants upon bond cleavage (*i.e.*, a co-reactant).<sup>4,6,7</sup> For example, ECL is produced in the  $\text{Ru}(\text{bpy})_3^{2+}$ – $\text{C}_9\text{H}_{21}\text{N}$  ( $\text{C}_9\text{H}_{21}\text{N}$  = tripropylamine) system upon oxidation of both  $\text{Ru}(\text{bpy})_3^{2+}$  and  $\text{C}_9\text{H}_{21}\text{N}$ :<sup>6</sup>



where  ${}^*\text{Ru}(\text{bpy})_3^{2+}$  is the emitting species. Upon oxidation, the co-reactant  $\text{C}_9\text{H}_{21}\text{N}$  is believed to form a strong reducing agent (*e.g.*,  $\text{C}_9\text{H}_{20}\text{N}^{\bullet}$ ) that can then reduce  $\text{Ru}(\text{bpy})_3^{3+}$  to  ${}^*\text{Ru}(\text{bpy})_3^{2+}$ .<sup>6,7</sup>

We have shown that the ECL emission of  $\text{Ru}(\text{bpy})_3^{2+}$  is quenched in the presence of phenol, catechol, hydroquinone and benzoquinone (Scheme 1) using both  $\text{C}_9\text{H}_{21}\text{N}$  and oxalate ( $\text{C}_2\text{O}_4^{2-}$ ) as co-reactants.<sup>8</sup> Quenching was observed with a 100-fold excess of quencher with complete quenching observed

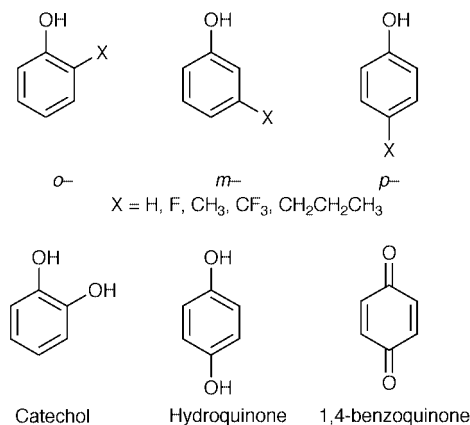
between a 1000- and 2000-fold excess. Photoluminescence and UV-Vis experiments coupled with controlled potential coulometry supported the formation of benzoquinone products upon electrochemical oxidation (Scheme 2). Therefore, quenching is believed to occur *via* energy transfer from  ${}^*\text{Ru}(\text{bpy})_3^{2+}$  to benzoquinone or a benzoquinone derivative formed at the electrode.

Owing to the pharmaceutical and environmental importance of phenolic compounds, we were interested in testing other derivatives for their effect on the ECL excited state. Here we report on ECL quenching with phenols substituted in the 2-, 3- and 4-positions (*e.g.*, *o*-, *m*- and *p*-fluorophenol), Scheme 1.

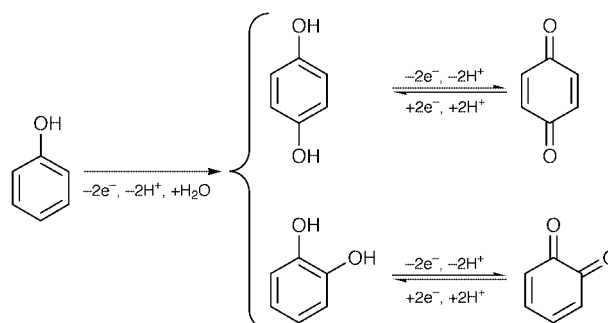
## Experimental

## Materials

$\text{Ru}(\text{bpy})_3\text{Cl}_2$  (98%) (Strem Chemical, Newbury Port, MA, USA), potassium phosphate monobasic hydrate (99.0%) (EM Science, Gibbstown, NJ, USA), benzoquinone (99.5%) (Fluka, Milwaukee, WI, USA) and tripropylamine ( $\text{C}_9\text{H}_{21}\text{N}$ , 98%) (Avocado Research Chemicals, Ward Hill, MA, USA) were used as received. Phenol (99.99%), catechol (99%), hydroquinone (99+%), *o*-cresol (99%), *m*-cresol (99%), *p*-cresol (99%), 2-fluorophenol (98%), 3-fluorophenol (98%), 4-fluorophenol (99%),  $\alpha\alpha\alpha$ -trifluoro-*o*-cresol (97%),  $\alpha\alpha\alpha$ -trifluoro-*m*-cresol (99%),  $\alpha\alpha\alpha$ -trifluoro-*p*-cresol (99%), 4-phenylphenol (99%), 2-propylphenol (98%), 4-propylphenol (99%) and 2,4,6-trimethylphenol (97%) were obtained from Aldrich Chemical (Milwaukee, WI, USA) and used as received.



Scheme 1 Structures of phenolic compounds.

Scheme 2 Electrochemical oxidation of phenol to form hydroquinone, catechol and *o*- and *p*-benzoquinone.<sup>8</sup>

Potassium phosphate buffer solutions, 0.20 M  $\text{KH}_2\text{PO}_4$ , were prepared with doubly de-ionized water. Buffer solutions containing  $\text{C}_9\text{H}_{21}\text{N}$  (0.05 M) were prepared similarly except that it was necessary to stir vigorously to dissolve the amine completely. The pH of these solutions was adjusted to 7.5 ( $\pm 0.1$ ) with either concentrated HCl or 6 M NaOH.

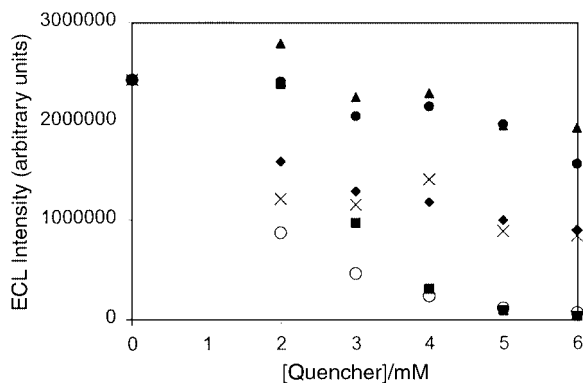
## Methods

ECL intensity *versus* potential and controlled potential coulometry (*i.e.*, bulk electrolysis) experiments were performed as described previously.<sup>8</sup> Solutions used to obtain intensity *vs.* potential profiles were 0.3–30  $\mu\text{M}$   $\text{Ru}(\text{bpy})_3^{2+}$ , 0.05 M  $\text{C}_9\text{H}_{21}\text{N}$ , 0.2 M potassium phosphate buffer and 0–1 mM phenolic compound (*i.e.*, quencher). Fluorescence experiments were run in potassium phosphate buffer using a Shimadzu (Tokyo, Japan) RF-5301 spectrofluorimeter. Excitation was at 452 nm, the lowest energy absorption for the  $\text{Ru}(\text{bpy})_3^{2+}$  luminophore, with detection between 550 and 650 nm ( $\lambda_{\text{em}} \approx 620$  nm).

Absorption spectra were recorded with a Hitachi (Tokyo, Japan) U-2001 spectrophotometer. All solutions were composed of micromolar concentrations of  $\text{Ru}(\text{bpy})_3\text{Cl}_2$  and 0.05 M  $\text{C}_9\text{H}_{21}\text{N}$  in water (0.2 M potassium phosphate). Solutions of 10 mM quencher (*e.g.*, phenol) were prepared by dissolving an appropriate amount of compound in the  $\text{Ru}(\text{bpy})_3^{2+}$ – $\text{C}_9\text{H}_{21}\text{N}$  solution. Working standard solutions were then prepared by diluting aliquots of these stock standard solutions.

## Results and discussion

Phenol quenches solution ECL in the  $\text{Ru}(\text{bpy})_3^{2+}$ – $\text{C}_9\text{H}_{21}\text{N}$  system<sup>8</sup> as does its derivatives. Fig. 1 shows the quenching efficiency of *o*-, *m*- and *p*-cresol compared with *o*-, *m*- and *p*-fluorophenol. Interestingly, phenol itself quenches to a greater degree despite the electron-withdrawing or –donating character of the substituents. Similar trends were seen with the trifluoromethyl phenols (*i.e.*, X =  $\text{CF}_3$  in Scheme 1) and phenols containing electron-donating groups (*i.e.*, 2- and 4-propylphenol). It is possible that substituents hinder the formation of the oxidation products that are responsible for quenching the  $^*\text{Ru}(\text{bpy})_3^{2+}$  excited state. The position of the substituent on the aromatic ring affects the efficiency of quenching. *Meta* derivatives display the greatest magnitude of quenching (Fig. 1), and *para* derivatives the least. This supports previous experiments<sup>8</sup> indicating that *o*- and *p*-benzoquinone or structurally related analogs are the primary oxidation products responsible for quenching the ECL excited state (Scheme 2).



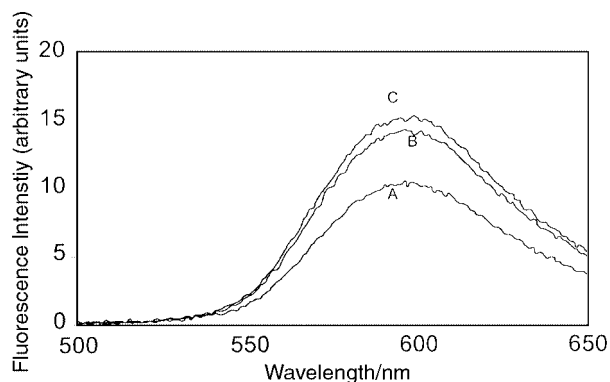
**Fig. 1** ECL intensity *vs.* concentration of quencher for (◆) *o*-fluorophenol, (■) *m*-fluorophenol, (▲) *p*-fluorophenol, (×) *o*-cresol, (○) *m*-cresol and (●) *p*-cresol.  $[\text{Ru}(\text{bpy})_3^{2+}] = 1 \mu\text{M}$ ,  $[\text{C}_9\text{H}_{21}\text{N}] = 0.05$  M in 0.2 M potassium phosphate buffer (pH  $\approx 7.5$ ). Error bars removed for clarity (error of each measurement  $\approx \pm 10\%$ ).

Also, the electron-donating or -withdrawing nature of the substituent appears to play little role in the efficiency of product formation and subsequent ECL quenching (Fig. 1).

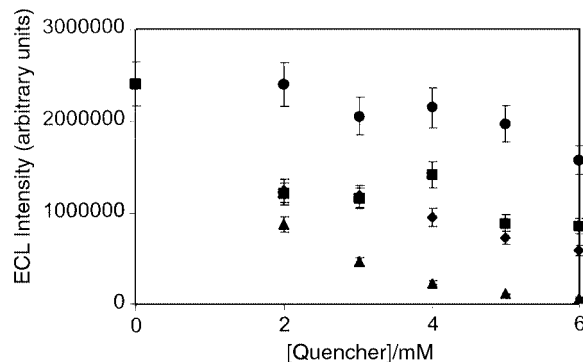
Phenol does not quench  $\text{Ru}(\text{bpy})_3^{2+}$  photoluminescence owing to unfavorable energetics.<sup>9</sup> Concentrations of phenol greater than 0.1 M in aqueous solution result in a slight increase in  $\text{Ru}(\text{bpy})_3^{2+}$  photoluminescence intensity, with a blue shift ( $< 5$  nm) in the emission spectrum. The photoluminescence emission of  $^*\text{Ru}(\text{bpy})_3^{2+}$  when substituted phenol derivatives are present at concentrations  $> 0.1$  M display similar trends (Fig. 2). Detailed studies by Li *et al.*<sup>9</sup> on the phenol– $\text{Ru}(\text{bpy})_3^{2+}$  system indicate the existence of ground-state interactions between phenol and the bipyridyl rings of  $\text{Ru}(\text{bpy})_3^{2+}$  that are hydrophobic or  $\pi$ -stacking in nature. These interactions undoubtedly play a role in the efficiency of quencher formation and their interaction with the  $^*\text{Ru}(\text{bpy})_3^{2+}$  ECL excited states.

Fig. 3 compares ECL intensity *versus* concentration for 2,4,6-trimethylphenol with *o*-, *m*- and *p*-cresol. Interestingly, trimethylphenol quenches to about the same degree as *o*-cresol. It is possible that electrochemical oxidation results in the loss of the methyl substituent *para* to the hydroxy group, with subsequent formation of benzoquinone. Experiments are under way to identify the products of phenol oxidation to understand the observed trends better. Unfortunately, it was not possible to test trisubstituted derivatives containing electron-withdrawing groups owing to their limited solubility in aqueous media.

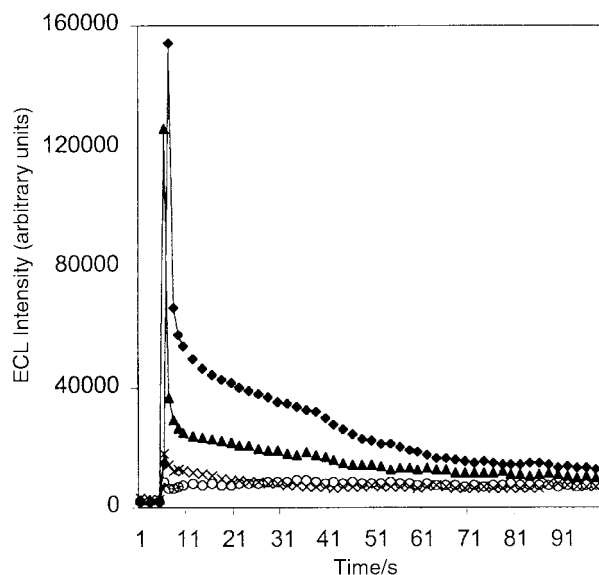
The stability of the ECL signal was studied by stepping the potential to +2.0 V (*vs.* Ag/AgCl) and measuring the ECL intensity under diffusion controlled conditions. Fig. 4 compares the ECL intensity *vs.* time profiles for a blank solution [*i.e.*,  $\text{Ru}(\text{bpy})_3^{2+}$  and  $\text{C}_9\text{H}_{21}\text{N}$  with no phenol present] with solutions containing *o*-, *m*- and *p*-cresol. As expected, there was a sharp increase in light intensity due to the high concentrations of electroactive species near the electrode surface. Under our



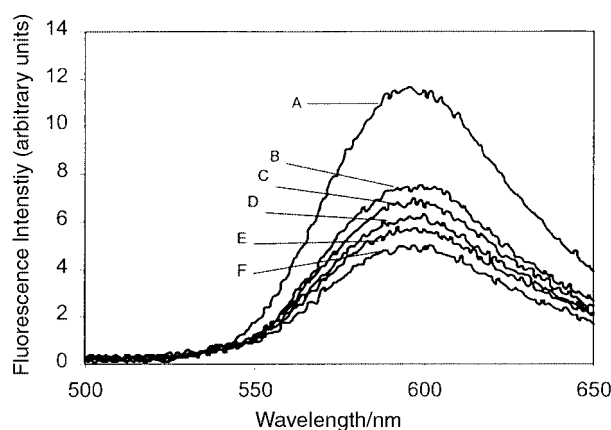
**Fig. 2** Fluorescence spectra of  $\text{Ru}(\text{bpy})_3^{2+}$  (10  $\mu\text{M}$ ) in aqueous buffer media containing (A) 0, (B) 0.02 and (C) 0.06 M *o*-cresol ( $\lambda_{\text{exc}} = 452$  nm).



**Fig. 3** ECL intensity *vs.* concentration for (◆) 2,4,6-trimethylphenol, (■) *o*-cresol, (▲) *m*-cresol and (●) *p*-cresol.  $[\text{Ru}(\text{bpy})_3^{2+}] = 1 \mu\text{M}$ ,  $[\text{C}_9\text{H}_{21}\text{N}] = 0.05$  M in 0.2 M potassium phosphate buffer (pH  $\approx 7.5$ ).



**Fig. 4** ECL intensity as a function of time for (◆) no quencher, (○) *o*-cresol, (▲) *m*-cresol and (×) *p*-cresol. [Quencher] = 0.3 mM, [Ru(bpy)<sub>3</sub><sup>2+</sup>] = 1 μM, [C<sub>9</sub>H<sub>21</sub>N] = 0.05 M in 0.2 M potassium phosphate buffer (pH ≈ 7.5). The PMT was set to record for approximately 5 s prior to stepping the potential from 0.0 to +2.0 V vs. Ag/AgCl to measure background intensities.



**Fig. 5** Fluorescence spectra of Ru(bpy)<sub>3</sub><sup>2+</sup> (10 μM) in aqueous buffer media containing 0.05 M C<sub>9</sub>H<sub>21</sub>N and 0.02 M *m*-cresol ( $\lambda_{\text{exc}}$  = 452 nm). The potential was stepped to +2.0 V vs. Ag/AgCl to oxidize effectively Ru(bpy)<sub>3</sub><sup>2+</sup>, C<sub>9</sub>H<sub>21</sub>N, and *m*-cresol. Bulk electrolysis was performed for 2.5 h with samples taken at 30 min intervals. The spectra are for the following times: (A) 0 min, no electrolysis; (B) 30 min; (C) 60 min; (D) 90 min; (E) 120 min; and (F) 150 min.

conditions, the time to reach peak intensity for all systems was approximately 3 s (there is a 4–6 second delay before the potential was stepped to allow for baseline values). Clearly, the presence of quencher results in lower ECL intensities. At longer times, the ECL emission becomes diffusion controlled and the blank solution decays at a noticeably slower rate than its counterparts containing phenols. This was expected, owing to the quenching of the ECL emission in the presence of the phenols. Slight differences were also observed among the cresol, fluoro-, phenyl- and trifluoromethylphenols and may reflect the efficiency of quencher formation upon oxidation.

Controlled potential coulometry (bulk electrolysis) coupled with fluorescence and UV-Vis detection was used to probe the nature of the products formed during electrolysis. The potential was stepped to +2.0 V to oxidize Ru(bpy)<sub>3</sub><sup>2+</sup> effectively ( $E^\circ$  = 1.4 V vs. Ag/AgCl), C<sub>9</sub>H<sub>21</sub>N ( $E_{\text{anodic}}$  ≈ 0.5 V)<sup>6</sup> and the phenolic compound ( $E^\circ$  ≈ 0.6 V). Fig. 5 shows the fluorescence spectrum for Ru(bpy)<sub>3</sub><sup>2+</sup> in the presence of *m*-cresol. Approximately half of the signal was lost within 3 h of electrolysis

[charge passed during electrolysis ( $Q$ ) =  $9.83 \times 10^2$  C]. These results are similar to those observed when phenol<sup>8</sup> or other substituted derivatives are used in place of *m*-cresol and indicate that a product of phenol oxidation is directly responsible for photo- and electrochemiluminescence quenching. The position of the substituent within the aromatic ring also affects the degree of fluorescence quenching in the bulk electrolysis experiment with substituents in the *meta* position quenching more efficiently than substituents *ortho* and *para*.

Changes in the UV-Vis absorption spectra of the substituted phenol derivatives show a clear resemblance to benzoquinone after bulk electrolysis, similar to that observed with phenol.<sup>8</sup> A shift in the band maximum from ~270 nm to ~295 nm and a broadening of the absorption bands are observed. Therefore, UV-Vis and fluorescence experiments coupled with bulk electrolysis further support the mechanism shown in Scheme 2, where analogues of *o*- and *p*-benzoquinone are formed upon electrochemical oxidation.

## Conclusions

ECL quenching has been observed for a variety of phenol complexes containing substituents in the 2-, 3- and 4-positions. The degree of quenching depends on the position of the substituent within the aromatic ring, and less on its electron-withdrawing or -donating character. Owing to the ECL activity of a variety of phenols, it is difficult to discriminate between different structures based solely on ECL quenching data. However, the reported method may be appropriate for semi-quantitative analysis and determining the total phenol content of a solution. Experiments are under way to determine the detection limits and sensitivity of Ru(bpy)<sub>3</sub><sup>2+</sup>-C<sub>9</sub>H<sub>21</sub>N ECL for detecting phenolic compounds in pharmaceutical and environmental assays.

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