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Novel tris(2,2′-bipyridine)ruthenium(II) cathodic electrochemiluminescence in aqueous solution at a glassy carbon electrode

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A novel approach of generating cathodic electrochemiluminescence of $Ru(bpy)_3^{2+}$ at −0.4 V triggered by reactive oxygen species is reported for detecting alkylamines and some organic acids.

Following the discovery of applying a suitable potential (positive or negative enough) to generate electrochemiluminescence (ECL) of $Ru(bpy)_3^{2+}$ (bpy = 2,2′-bipyridine),[1] a large amount of fundamental and applied studies have centred on $Ru(bpy)_3^{2+}$ as a powerful analytical reagent due to its superior intrinsic properties such as excellent stability and high efficiency. Generally, $Ru(bpy)_3^{2+}$ ECL reaction is initiated by three main modes. The first ECL reaction sequence is based on the annihilation of $Ru(bpy)_3^{+}$ and $Ru(bpy)_3^{3+}$, which are produced by applying positive and negative potential in turn.[2,3] The annihilation reaction sequence has been widely used in mechanistic investigations and light-emitting device researches.[4–6] Another ECL reaction sequence is an oxidative–reduction type.[7,8] The excited species $Ru(bpy)_3^{2+*}$ is formed by reduction of $Ru(bpy)_3^{3+}$ in the presence of a strong reducing agent. This type of reaction has been well documented and led to the recent development of highly sensitive detection schemes for immunoassays,[9,10] DNA probes,[11–13] and numerous analytes, such as oxalate[14,15] and amine-containing compounds.[8,16,17] The third ECL reaction sequence is a reductive–oxidation type. So far, only one system, the $Ru(bpy)_3^{2+}$–$S_2O_8^{2-}$ pair, shows this bahavior.[18–20] The above three modes all involve in the direct electron transfer between $Ru(bpy)_3^{2+}$ and an electrode. The applied potential on the working electrode is either very positive (1.2 V *vs.* Ag/AgCl),[7,8,14,15] where oxygen evolution occurs on the electrode, or very negative (−2.0 V *vs.* Ag/AgCl) applied in an organic phase.[18,19]

In this work an entirely different approach has been adopted to generate cathodic ECL of $Ru(bpy)_3^{2+}$ triggered by reactive oxygen species (ROS). Electrochemical measurements were performed in a conventional three-electrode cell.[21] A model 600 electrochemical workstation (CH Instruments, USA) was used for linear sweep and constant potential. A BPCL ultra-weak luminescence analyzer (Beijing, China) was used to detect any photons. Fig. 1(a) shows cyclic voltammograms (CVs) of oxygen reduction at a 4 mm radius glass carbon electrode in an air-saturated phosphate buffer containing 1.0 mM $Ru(bpy)_3^{2+}$. Two reduction waves were observed (Fig. 1(a)). The first wave starting at −0.4 V was attributed to the reduction of dissolved oxygen. The latter wave between −1.13 and −1.18 V, just before the onset of water reduction, was attributed to the reduction of $Ru(bpy)_3^{2+}$.[3] An enduring light emission phenomenon accompanying the reduction of oxygen was observed (Fig. 1(b)). Light emission appeared at the onset of oxygen reduction (−0.4 V). The emission intensity increased sharply from −0.5 to −0.7 V with the highest point reached at −0.8 V. Then it decreased slightly beyond −0.8 V. In the presence of TPA, the reduction current of oxygen did not apparently change (Fig. 1(a)). However, the light emission increased dramatically (Fig. 1(b)). When the dissolved oxygen was eliminated by argon, light emission was not observed again. When the solution was saturated with the oxygen, the light emission started with higher intensity. Altering the scan rate from 10 to 400 mV s^{-1}, the ECL intensity at −0.8 V increased from 3234 to 18675 counts in a gradual manner. However, the ECL starting potential did not shift with the scan rate. At low sweep rates the light intensity was proportional to the square root of the scan rate. At higher sweep rates of ⩾400 mV s^{-1}, a noticeable deviation from linearity was noted. This indicates that the ECL reaction rate is dependent on the diffusion of reactant to the electrode surface.

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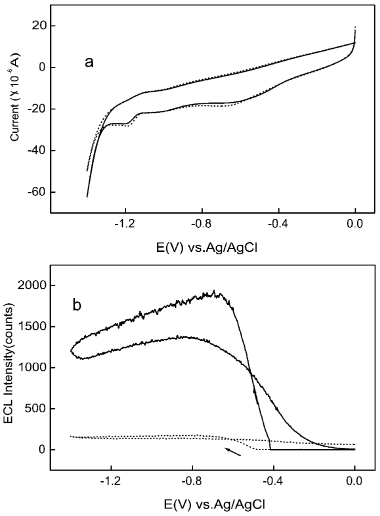

Fig. 1 Comparison of the cyclic voltammogram (solid line) and ECL intensity (solid line) for the $Ru(bpy)_3^{2+}$–TPA system (PMT is biased at 600 V) with the cyclic voltammogram (dotted line) and ECL intensity (dotted line) for the $Ru(bpy)_3^{2+}$ system (PMT is biased at 700 V). The concentration of TPA is 50 mM in 0.1 M buffer solution (pH = 6.8). Scan rate 10 mV s^{-1}.

To identify that the light emission in cathodic ECL was generated from $Ru(bpy)_3^{2+*}$ decaying to the ground state, the emission spectrum (Fig. 2) was recorded in 0.1 M pH 7.5 phosphate buffer with 5.0 mM $Ru(bpy)_3^{2+}$ and 50 mM TPA. The maximum emission wavelength was at 620 nm, which is identical to that obtained with photoexcitation, indicating a population of the same emitting states by the cathodic ECL process as formed photochemically. In the process of ECL, only

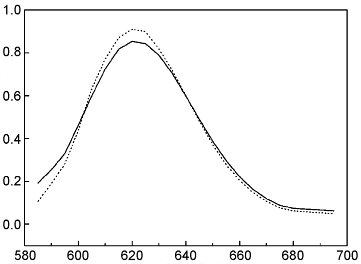

Fig. 2 Cathodic ECL emission spectrum (solid line) and photoluminescence spectrum (dotted line) of 0.1 M phosphate solution (pH 7.4) containing 50 mM TPA and 5 mM $Ru(bpy)_3^{2+}$. Applied potential of cathodic ECL is −0.6 V. The excited wavelength of photoluminescence is 450 nm.

oxygen was reduced on the electrode. Some types of reactive oxygen species such as $O_2^{\cdot-}$, H_2O_2 and $^{\cdot}OH$ were generated when dissolved oxygen was reduced.[22–24] Considering the strong oxidizability of ROS, we propose that the cathodic ECL is initated by ROS. The standard potentials of $^{\cdot}OH/OH^-$, H_2O_2/H_2O and $HO_2^{\cdot}/H_2O_2$ are 2.02, 1.77 and 1.5 V, respectively.[25] By comparing the redox potential of these species, we infer that $^{\cdot}OH$ oxidizes $Ru(bpy)_3^{2+}$. This speculation was demonstrated by the experiment of the Fenton reaction in the presence of $Ru(bpy)_3^{2+}$. In the Fenton reaction, H_2O_2 reacts with Fe^{2+} to produce $^{\cdot}OH$. In the absence of $Ru(bpy)_3^{2+}$, reaction of 0.03% H_2O_2 with 0.1 M Fe^{2+} did not generate light. When 1.0 mM $Ru(bpy)_3^{2+}$ was added, a strong light emission occurred immediately, while for $Ru(bpy)_3^{2+}$ with H_2O_2 or Fe^{2+} alone, no light emission occurred. Thus, we infer that $^{\cdot}OH$ oxidizes $Ru(bpy)_3^{2+}$ to form $Ru(bpy)_3^{3+}$. Although H_2O_2 can not directly oxidize $Ru(bpy)_3^{2+*}$, it can react with $O_2^{\cdot-}$ to generate $^{\cdot}OH$.[26] In the absence of TPA, $Ru(bpy)_3^{3+}$ reacts with OH^- to generate $Ru(bpy)_3^{2+*}$.[27] In the presence of TPA, $Ru(bpy)_3^{3+}$ oxidizes TPA to form a reducing radical.[28] The latter reacts with another $Ru(bpy)_3^{3+}$ to produce $Ru(bpy)_3^{2+*}$, which decays *via* an orange emission centered at 620 nm. In contrast to previous modes, the ECL potential was reduced to -0.4 V where only dissolved oxygen was reduced on the electrode.

In a preliminary comparison of the anodic ECL (initiated at 1.2 V) with the cathodic ECL (initiated at -0.8 V), the intensity of cathodic ECL for 10 μM TPA in neutral phosphate solution was 0.94 times that of the anodic ECL, indicating that the sensitivity of the cathodic ECL for TPA was high enough to be used for analytical determination. Using the cathodic ECL method, a linear calibration for TPA for three orders of magnitude with a correlation coefficient of 0.9994 was obtained, which has the same wide linear range as that obtained by anodic ECL.[29] The detection limit (S/N = 3) was estimated to be 7.5×10^{-8} M, which is higher than that obtained by anodic ECL (2.5×10^{-8} M).[29] The RSD ($n = 10$) for 50 μM TPA was 6.08%.

A survey of other organic compounds was carried out to see which could enhance cathodic ECL. Compounds selected contain $-NH_x$, $-CH(OH)(CO_2H)$ or $-CO_2H$. The results are shown in Table 1. All the alkyl amines enhanced cathodic ECL. The intensity for alkyl amines decreased in the order tertiary > secondary > primary, which was also similar to that of anodic ECL. Some organic acidic salts enhanced cathodic ECL markedly, especially citrate. Due to its high redox potential, citrate can not be oxidized by $Ru(bpy)_3^{3+}$ directly. To obtain an anodic ECL response for citrate, cerium(III) was added in the solution.[30] However, in cathodic ECL, citrate can enhance ECL intensity directly. We propose that citrate can be oxidized by ROS to form a reducing radical, which reacts with $Ru(bpy)_3^{3+}$ to form $Ru(bpy)_3^{2+*}$. The plot of peak intensity *vs.* concentration of citrate was linear with a correlation coefficient of 0.999 over a range from 1×10^{-5} to 1×10^{-4} M. The detection limit was 1.2×10^{-6} M. The RSD for citrate at 40 μmol L^{-1} ($n = 8$) was 2.3%. We found that amygdalic acid, with the same –CH(OH)(COOH) group in its structure as citrate, also enhanced cathodic ECL. We did not observe cathodic ECL for lactic acid and acetic acid. Comparing the molecular structure of citrate and lactic acid, we propose that the presence of –OH, an electron acceptor group, at an α-carbon is advantageous for the generation of the reducing radical.

Table 1 Cathodic ECL of some organic compounds in 0.1 M phosphate buffer solution (pH = 6.8) containing 1.0 mM $Ru(bpy)_3^{2+}$

Compound	Concentration/μM	ECL Intensity/ counts
TPA	5	1600
Proline	100	7890
Arginine	100	1320
Methionine	100	1510
Valine	100	570
Tryptophan	100	425
Serine	100	302
Leucine	100	127
L-Isoleucine	100	305
Threonine	100	173
Glutamic acid	100	356
EDTA	100	1142
Oxalate	100	2320
Sodium citrate	100	1380
Itaconic acid	100	252
D-Gluconic acid	100	256
Amygdalic acid	100	999
Lactic acid	100	—
Acetic acid	100	—
Creatine	100	346

In conclusion, cathodic ECL, triggered by ROS, reveals a unique approach for analysis. It provides a suitable ECL potential and oxygen evolution on the electrode can be avoided. Compared with the anodic ECL method, the sensitivity of cathodic ECL is not decreased. Moreover, the method also extends ECL detecting species. Cathodic ECL can also directly detect some organic substances.

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