VIP Electrochemiluminescence

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Environmentally Friendly and Highly Sensitive Ruthenium(II) Tris(2,2′-bipyridyl) Electrochemiluminescent System Using 2-(Dibutylamino)ethanol as Co-Reactant**

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Ruthenium(II) tris(2,2′-bipyridyl) ($[Ru(bpy)_3]^{2+}$) has received increasing attention in clinical diagnosis and scientific research because of its high sensitivity, wide dynamic range, stability, simplicity, and versatility as an electrochemiluminescent probe.[1] As the electrochemiluminescence (ECL) results from electrochemical reactions between $[Ru(bpy)_3]^{2+}$ and co-reactants, extensive research has been focused on exploring effective co-reactants for the sensitive determination of $[Ru(bpy)_3]^{2+}$, which has important bioanalytical applications.[2] In 1984, Bard and co-workers first reported the determination of $[Ru(bpy)_3]^{2+}$ ECL using either oxalate or peroxydisulfate as co-reactants.[3] Later, Leland and Powell suggested tripropylamine (TPA) as a co-reactant,[4] and Blackburn et al. soon developed $[Ru(bpy)_3]^{2+}$ ECL immunoassays and DNA probe assays using TPA as co-reactant and $[Ru(bpy)_3]^{2+}$ as the label.[5] $[Ru(bpy)_3]^{2+}$/TPA ECL assays have been widely used for over fifteen years now.[2,6]

Despite its exclusive popularity, TPA has several important disadvantages.[2] First, TPA is toxic and volatile, but it is used in high concentrations (usually up to 100 mM) to attain good sensitivity. Second, its slow electrochemical oxidation rate limits ECL efficiency. Third, high concentrations of acidic phosphate solutions are needed to prepare concentrated neutral solutions of TPA because TPA is basic. Finally, the ECL intensity of the $[Ru(bpy)_3]^{2+}$/TPA system depends strongly on electrode materials. For example, the ECL intensity at Pt electrodes is only about 10% of that at Au electrodes. Thus, it is desirable to find alternatives to TPA.

Several groups have studied the ECL from $[Ru(bpy)_3]^{2+}$/amine systems under the condition that the concentrations of $[Ru(bpy)_3]^{2+}$ are higher than that of amines.[2,7] Generally; tertiary amines are more effective than secondary amines, primary amines, and other kinds of co-reactants. Also, electron-donating groups tend to increase ECL. It seemed impossible to find better co-reactants. Recent studies showed that ECL efficiencies can be improved through increasing the electrochemical oxidation rate of amines when the concentration of $[Ru(bpy)_3]^{2+}$ is much lower than that of the amines, as in the cases of ECL immunoassays and DNA probe assays. To our knowledge, all methods reported to date use additives to increase the oxidation rate of amines and, thus, ECL efficiencies.[8] Also, there have been only a few reports that attempt to find better co-reactants from easily oxidizable tertiary amines.

Herein, we report an investigation of the ECL of a series of tertiary amines with various substituents whilst keeping the concentration of $[Ru(bpy)_3]^{2+}$ lower than that of the amine. The purpose of the present study is to provide a new way to enhance ECL efficiencies by using easily oxidizable tertiary amines, and develop more efficient and environmentally friendly co-reactants for ECL immunoassays and DNA probe assays.

Figure 1 shows the dependence of ECL intensities on the concentrations of nine co-reactants. The comparison of triethylamine with other amines shows that hydroxyethyl groups tend to substantially increase ECL intensities, hydroxypropyl groups affect ECL intensities slightly, while

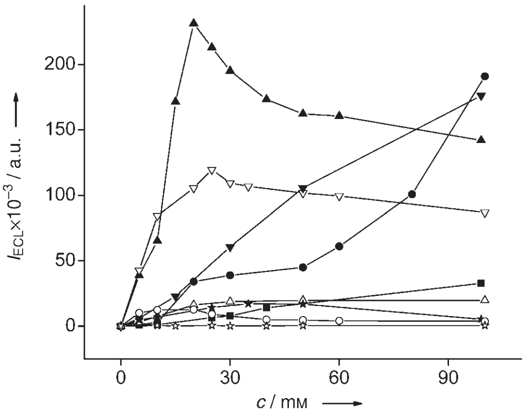

Figure 1. Dependence of the ECL peak intensity on the concentrations of 2-(dibutylamino)ethanol (DBAE, ▲), triethanolamine (▽), *N*,*N*-diethylethanolamine (▼), TPA (●), triethylamine (■), *N*,*N*-diethyl-*N*′-methylethylenediamine (△), 3-diethylamino-1-propanol (filled stars), nitrilotriacetic acid (○), and ethylenediaminetetraacetic acid (empty stars), measured at the Au electrode in 0.1 M phosphate buffer solution (pH 7.5) containing 1 μM $[Ru(bpy)_3]^{2+}$. The potential was stepped from 0 to 1.35 V.

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carboxyl and secondary amine groups tend to decrease ECL intensities. Carboxylic groups decrease ECL intensities because they are electron-withdrawing groups, while secondary amine groups decrease ECL intensities because they are less effective co-reactants than tertiary amines and waste part of the electrochemically generated ruthenium(III) tris(2,2′-bipyridyl). It is surprising that electron-withdrawing hydroxyethyl groups increase ECL intensities.[7]

To explain the positive effect of the hydroxyethyl group on ECL intensities, the electrochemistry of triethylamine, TPA, 2-(dibutylamino)ethanol (DBAE), *N*,*N*-diethylethanolamine, and 3-diethylamino-1-propanol was studied. As shown in Figure 2, the oxidation currents of all amines containing hydroxy groups are higher than that of triethylamine, indicating that the hydroxy group catalyzes the oxidation of amines. The anodic current of *N*,*N*-diethylethanolamine is higher than that of 3-diethylamino-1-propanol, suggesting that the hydroxyethyl group may catalyze the oxidation of amines more effectively than the hydroxypropyl group. The anodic current decreases in the order DBAE > TPA > *N*,*N*-diethylethanolamine > 3-diethylamino-1-propanol > triethylamine, nearly consistent with the sequence of ECL efficiency for these amines. These results suggest that the hydroxyethyl group may increase ECL efficiencies through catalyzing the oxidation of amines.[9]

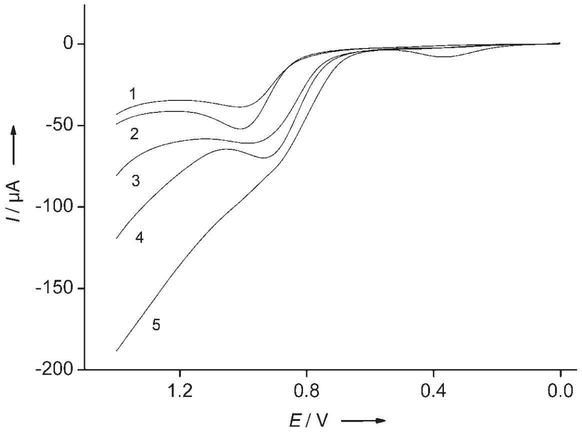

Figure 2. Linear sweep voltammograms of 1 µM $[Ru(bpy)_3]^{2+}$ and either 1) triethylamine, 2) 3-diethylamino-1-propanol, 3) *N*,*N*-diethylethanolamine, 4) TPA, or 5) DBAE (25 mM each) measured at the Au electrode in 0.1 M phosphate buffer solution (pH 7.5). Scan rate: 0.1 V s^{-1}.

The importance of the oxidation of DBAE was further confirmed by the following two experimental results. First, the ECL intensity and the anodic current both vary linearly with the square root of the scan rate ($\nu^{1/2}$), demonstrating that the progress of ECL is directly related to the oxidation of DBAE. Second, the lower the concentration of $[Ru(bpy)_3]^{2+}$, the larger the ratio of the ECL intensity of the $[Ru(bpy)_3]^{2+}$/DBAE system to that of the $[Ru(bpy)_3]^{2+}$/TPA system. For example, the ECL intensity for the $[Ru(bpy)_3]^{2+}$/DBAE system is close to that of the $[Ru(bpy)_3]^{2+}$/TPA system when the concentration of $[Ru(bpy)_3]^{2+}$ is 1 mM; however, the intensity of the DBAE system is about ten times that of the TPA system if the concentration of $[Ru(bpy)_3]^{2+}$ is 1 µM. These results are consistent with recent findings that direct oxidation of the co-reactant plays an important role at low concentrations of $[Ru(bpy)_3]^{2+}$ and that electrocatalytic oxidation of the co-reactant by $[Ru(bpy)_3]^{2+}$ plays an important role at high concentrations of $[Ru(bpy)_3]^{2+}$.[9]

Electrochemiluminescence of the $[Ru(bpy)_3]^{2+}$/DBAE system at Pt and glassy carbon electrodes was also studied. The ECL of the $[Ru(bpy)_3]^{2+}$/DBAE system at Pt electrodes is comparable to that at Au electrodes, and is about 100-times stronger than that of the $[Ru(bpy)_3]^{2+}$/TPA system at a Pt electrode (Figure 3). The striking difference in ECL intensity can be attributed to the different oxidation rate of DBAE and TPA at platinum electrodes. An earlier report by Zu and Bard showed that the ECL intensity of the $[Ru(bpy)_3]^{2+}$/TPA system at Pt electrodes is only about 10% of that at Au electrodes because the growth of anodic oxide films at platinum electrodes significantly inhibited the direct oxidation of TPA.[8a] In contrast, the direct oxidation of DBAE at Pt electrodes is much faster, indicating that hydroxyethyl also catalyzes the direct oxidation of amines at Pt electrodes, and thus dramatically enhance ECL intensity. At the glassy carbon electrode, the intensity of ECL first increased with the concentration of DBAE up to 3 mM and then leveled off as a result of side reactions at higher concentrations.[8a,9f,g] The ECL intensity of the 3 mM DBAE system at glassy carbon electrodes is about three times that of the 3 mM TPA system, as a result of easier oxidation of DBAE (Figure 4).

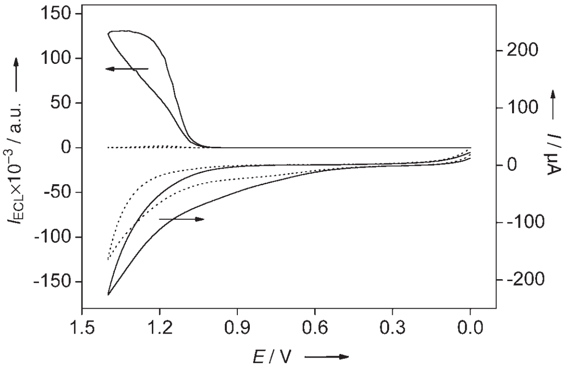

Figure 3. Cyclic voltammograms and corresponding ECL signals at the Pt electrode in 0.1 m phosphate buffer solution (pH 7.5) containing 1 µM $[Ru(bpy)_3]^{2+}$ and either 25 mM DBAE (——) or 25 mM TPA (•••••). Scan rate: 0.1 V s^{-1}.

As shown in Figure 1, the ECL of DBAE increases rapidly initially with increasing concentration up to 20 mM, and then it decreases slowly upon further increasing its concentration. The decrease in ECL intensity at high concentrations of DBAE may be attributed to side reactions.[8a,9f,g] In comparison, 20 mM DBAE is more effective than 100 mM *N*,*N*-diethylethanolamine and TPA, despite increases in the ECL

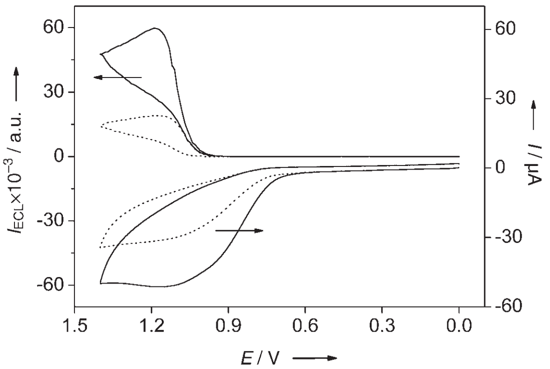

Figure 4. Cyclic voltammograms and corresponding ECL signals at the glassy carbon electrode in 0.1 M phosphate buffer solution (pH 7.5) containing 1 μM $[Ru(bpy)_3]^{2+}$ and either 3 mM DBAE (——) or 3 mM TPA (•••••). Scan rate: 0.1 V s^{-1}.

of *N*,*N*-diethylethanolamine and TPA upon increasing their concentrations. Moreover, DBAE is much less toxic, more soluble, and less volatile than TPA. The low optimum concentration of DBAE allowed the concentration of the supporting electrolyte to be decreased to between one-third and two-thirds the concentration of TPA used in the TPA system reported. As DBAE exhibits better ECL behavior than other co-reactants, it was chosen as the co-reactant for the determination of $[Ru(bpy)_3]^{2+}$.

The logarithmic plot of ECL versus the concentration of $[Ru(bpy)_3]^{2+}$ is linear over a concentration range 5.0×10^{-14}–1.0×10^{-3} M (slope = 0.619; intercept = 9.044; correlation coefficient = 0.993; $n = 12$). The relative standard deviation is 2.4 % for six determinations at a $[Ru(bpy)_3]^{2+}$ concentration of 1.0 pM, and the detection limit is 2.0×10^{-14} M. In comparison with the $[Ru(bpy)_3]^{2+}$/TPA system, the $[Ru(bpy)_3]^{2+}$/DBAE system exhibits the following advantages (Table 1).[2] First, the dynamic range of the $[Ru(bpy)_3]^{2+}$/DBAE system is about four orders of magnitude wider than that of the $[Ru(bpy)_3]^{2+}$/TPA system, while the sensitivity of the former is about an order of magnitude better than that of the latter system.[4,5] Second, DBAE is much less toxic and less volatile than TPA. Third, the concentration of co-reactant in the $[Ru(bpy)_3]^{2+}$/DBAE system is only one-fifth of that in the $[Ru(bpy)_3]^{2+}$/TPA system. Fourth, the $[Ru(bpy)_3]^{2+}$/DBAE system does not require high concentrations of buffer. The concentration of neutral phosphate buffer needed in the $[Ru(bpy)_3]^{2+}$/DBAE system is about one-third to two-thirds that used in the $[Ru(bpy)_3]^{2+}$/TPA system. Finally, the $[Ru(bpy)_3]^{2+}$/TPA system reveals poor ECL efficiency at the platinum working electrode, while the $[Ru(bpy)_3]^{2+}$/DBAE system exhibits intense ECL intensity at both Au and Pt working electrodes.

Table 1: Comparison of the $[Ru(bpy)_3]^{2+}$/DBAE system with the $[Ru(bpy)_3]^{2+}$/TPA system.

	$[Ru(bpy)_3]^{2+}$/DBAE	$[Ru(bpy)_3]^{2+}$/TPA[5]
Co-reactant	DBAE	TPA
Co-reactant concentration	20 mM	100 mM
Dynamic range	5.0×10^{-14} – 1.0×10^{-3} M	1.0×10^{-12} – 1.0×10^{-6} M
Detection limit	20 fM	200 fM
Hazard warning for co-reactant	corrosive	toxic, corrosive
PBS concentration[a]	0.05–0.1 M	≥0.15 M
Working electrode	Au or Pt	Au

[a] Phosphate buffer solution.

In conclusion, hydroxyethyl promotes the oxidation of amines and substantially increases electrochemiluminescence intensities, thus providing a means to enhance ECL efficiencies by using suitable substituents to catalyze the oxidation of amines. Among the various co-reactants investigated, DBAE was found to be the most effective co-reactant reported so far.[2] As DBAE contains the electron-withdrawing hydroxyethyl group, the outstanding ECL efficiency of DBAE suggests that electron-withdrawing groups can enhance ECL efficiencies besides electron-donating groups. In view of its important properties, DBAE is a very promising co-reactant for $[Ru(bpy)_3]^{2+}$ ECL immunoassays and DNA probe assays.

Experimental Section

Electrochemical and electrochemiluminescence (ECL) measurements were performed with a 800B potentiostat (CHI Inc., USA) and a BPCL Ultra-Weak luminescence analyzer using an Ag/AgCl reference electrode (saturated KCl) and a Pt wire counter electrode.[8d] The values obtained for the ECL intensity were normalized to those measured at a photomultiplier tube voltage of 900 V. The working electrodes (3-mm diameter) were polished with a slurry of 0.05-μm alumina, then sonicated, and rinsed with deionized water. The calibration curve was measured with an applied potential of 1.35 V and at pH 7.5.

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