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ORIGINAL PAPER

# Electrogenerated chemiluminescence of ruthenium (II) bipyridyl complex directly immobilized on glassy carbon electrodes

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**Abstract** $Ru(bpy)_3Cl_2$ was used to modify the glass carbon electrodes (GCE) by oxidation and co-deposition on the electrode surface. The modified electrodes were characterized by atomic force microscopy (AFM) and X-ray photoelectron spectroscopy (XPS). About $2.2 \times 10^{-9}$ mol $Ru(bpy)_3^{2+}$ was immobilized on the GCE surface ($\phi = 4$ mm). The modified GC electrodes showed stable electrochemiluminescence with tripropylamine (TPrA) as the co-reactant with a linear range from 10 to 500 μM ($R^2 = 0.999$). Among the 10 amino acids tested, the modified electrode system showed selective response to arginine and lysine, indicating that the molecular structure played an important role as co-reactant. This simple and sensitive electrode modifying method when combined with flow-injection or liquid chromatography systems has the potential for amino acid analyses.

**Keywords** Ruthenium (II) bipyridyl complex · Glassy carbon electrode · Electrogenerated chemiluminescence · Tripropylamine · Amino acids

## 1 Introduction

Ruthenium polypyridyl complexes, particularly tris (2, 2′-bipyridine) ruthenium (II) ($Ru(bpy)_3^{2+}$), have been among the most widely studied organometallic molecules in recent years [1, 2] due to their attractive properties, e.g., high photoluminescence quantum yields, long excited-state lifetime, high thermal/chemical stability, moderate reactivity of the excited state in electron/energy transfer reactions, and reversible reduction/oxidation reactivity [2, 3]. Due to the metal-to-ligand charge transfer (MLCT) state, the luminescent excited state of $Ru(bpy)_3^{2+}$ is very sensitive to the polarity and viscosity of the environment. These properties of $Ru(bpy)_3^{2+}$ and derivatives have been useful in analytical applications such as optical sensors as well as electrogenerated chemiluminescence(ECL) measurements [4–8].

For practical purpose, it is desirable that $Ru(bpy)_3^{2+}$ molecules be immobilized in a solid matrix. Immobilization provides mechanical stability and enables the subtle modifications of dopant properties specific to host–guest interactions [3]. Sol–gel materials [3, 9] including xerogels [10] have been used to immobilized ruthenium complexes to produce gel support matrices with good stability, optical permeability and biocompatibility. Nanoparticles (NPs) and nanotubes (NTs), for example, AuNPs [11, 12], SiNPs [13], and CNTs have been used as dopants to form composite systems to prepare modified electrodes for ECL determinations [11–16].The ECL systems have shown a wide linear range, high sensitivity and good stability in the detection of DNA, proteins and amines. Polymer films formed in a layer-by-layer (LBL) assembly have been used to immobilize $Ru(bpy)_3^{2+}$ to fabricate ECL sensors. Ruthenium polypyridyl complexes were immobilized with polymer films [17–22], e.g., ion exchange film, Nafion film and PVP film, as well as multilayer films [23, 24]. Nanoparticles, gels and polymer films form composite films to immobilize the ruthenium complexes, e.g., carbon nanotube/Nafion [25, 26], $V_2O_5$/Nafion [27], and $TiO_2$/Nafion

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[27, 28]. Metallopolymer [29, 30], monolayer [31] and other methods [32] have also been used to fabricate ECL sensors.

The diversified methods of $Ru(bpy)_3^{2+}$ immobilization have facilitated and enhanced the study of ECL sensors. Recently Premkumar [33] immobilized $Ru(bpy)_3^{2+}$ on highly oxidized GC electrodes(GCE) without using additional host materials. The modified electrodes showed good cyclic voltammetric response and stability. Because there were no host matrices involved, this approach facilitated electron transfer and avoided problems related to the lack of permeability, conductivity, and uniformity of the host membrane. When $Ru(bpy)_3^{2+}$ has been immobilized in this way, a reasonably fast response may be anticipated. The use of such electrodes with such characteristics will be optimized when used with separation techniques such as capillary electrophoresis (CE) and liquid chromatography (LC).

Up to now, there have been few published ECL studies using electrodes with directly deposited $Ru(bpy)_3^{2+}$. In this work, we investigated the ECL property of $Ru(bpy)_3^{2+}$ immobilized by direct deposition for applications in analytical chemistry.

## 2 Experimental

### 2.1 Reagents

Tris (2, 2′-bipyridine) ruthenium (II) dichloride (99.99%) and tripropylamine (TPrA) (98%) from Aldrich Chemical Co. were used without further purification. L-arginine was obtained from Institute of Microbiology (Chinese Academic of Science, China) and L-lysine was from Beijing Chemical Reagents Company (China). $NaH_2PO_4 \cdot 2H_2O$, $Na_2HPO_4 \cdot 2H_2O$ and $KNO_3$ were obtained from Beijing Beihua Fine Chemicals Co. Ltd. (China).

### 2.2 Instrumentation

ECL experiments were performed on a MPI-B multichannel data-analysis system (MPI-B, Xi'an Remax Electronic Science Tech. Co. Ltd., China) with a potentiostat, a data-collecting system and a photomultiplier tube (PMT). A three-electrode set-up was employed for electrochemical study. A GC electrode was used as working electrode and a platinum electrode was used as the counter electrode. An Ag/AgCl/KCl (saturation) reference electrode was used for all measurements. Atomic force microscopy (SPI 3800N, SIINT, Japan) and X-ray photoelectron spectroscopy (Axis Ultra, Kratos, UK) were also used for characterization of electrode surface.

### 2.3 Preparation of immobilized $Ru(bpy)_3^{2+}$

In the preparation of $Ru(bpy)_3^{2+}$ immobilized electrodes, the GC electrodes were polished sequentially with an aqueous slurry of 0.5 and 0.05 micron alumina on polishing cloth, sonicated to remove the particulates and rinsed with deionized water. The $Ru(bpy)_3^{2+}$ molecules were co-deposited [31] on GC electrode surface by cyclic voltammetric scans from 0 to 2.0 V for 100 cycles at 100 mV $s^{-1}$ in a solution composed of 0.1 mmol $L^{-1}$ $Ru(bpy)_3^{2+}$ and 0.1 mol $L^{-1}$ $KNO_3$. The ECL was collected by a photomultiplier tube (PMT) at 800 V with intermittent cyclic voltammetry (ICV) applied in the range from −1.50 to +1.50 V at 500 mV $s^{-1}$ with 30 s intermittence between two cycles. Before electrochemistry scanning, all of the solutions were deoxygenized with nitrogen purging for 15 min. After deposition of $Ru(bpy)_3^{2+}$, the electrode was removed, rinsed with deionized water and stored in deionized water until use.

## 3 Results and discussion

### 3.1 Characterization of the modified electrode

The electrochemical behavior of the modified electrodes was investigated in 0.01 M PBS buffer (pH = 7.0). Figure 1 shows the cyclic voltammograms of bare GCE and $Ru(bpy)_3^{2+}$ modified electrode. The immobilized $Ru(bpy)_3^{2+}$ displayed an oxidation peak at 0.608 V, indicating its electrochemical activity after immobilization on the GC electrode. At scan rates from 10 to 50 mV $s^{-1}$, the peak current increased linearly with increasing scan rate for both oxidation and reduction processes (inset in Fig. 1). The amount of $Ru(bpy)_3^{2+}$ immobilized on the surface obtained from the slope [34] was about $2.2 \times 10^{-9}$ mol.

Figure 2 shows the three-dimensional atomic force microscope (AFM) images of the bare GC and oxide covered GC electrode surfaces. The oxidation of carbon and the deposition of the $Ru(bpy)_3^{2+}$ at the electrode surface increased the roughness of the GC electrode surface. There was an increased peak-to-valley height of the surface structure from 30 to 80 nm after oxidation. The major type of oxygen functional group on the electrochemically oxidized surface was –C=O as verified by FTIR ($\nu_s$, 1625 $cm^{-1}$), thus, electrons could tunnel more easily across the electrode.

Table 1 shows the X-ray photoelectron spectroscopy (XPS) data of the bare and modified GC electrode surfaces. The intensity of the O (1 s) peak increased from 11.38 to 20.66%. The oxidation of carbon on the electrode surface produced C=O groups which increased the content of the oxygen. The Ru (3d) peaks were found at the modified GC

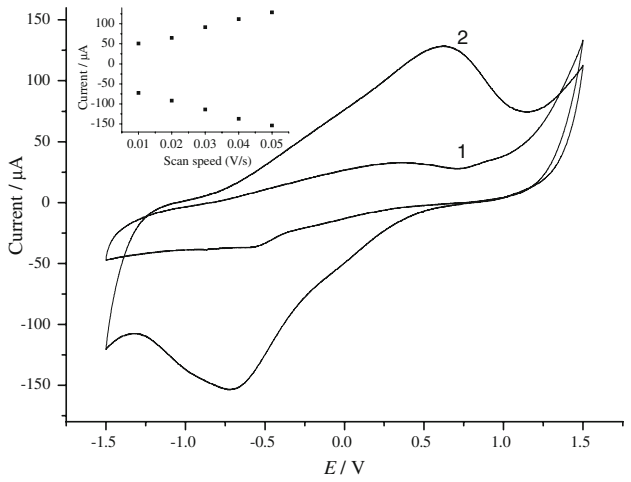


**Fig. 1** Cyclic voltammograms of bare GCE (1) and $Ru(bpy)_3^{2+}$ modified electrode (2) at 0.05 V $s^{-1}$ from −1.5 to 1.5 V in pH 7.0, 0.01 M PBS buffer. Inset graph: dependence of peak current on scan rate

electrode surface, demonstrating that $Ru(bpy)_3^{2+}$ was deposited on the electrode surface.

### 3.2 Electrochemiluminescence of $Ru(bpy)_3^{2+}$ modified electrodes with TPrA as the co-reactant

It is well known that $Ru(bpy)_3^{2+}$ ECL can be generated by alternate pulsing of an electrode potential to form oxidized $Ru(bpy)_3^{3+}$ and reduced $Ru(bpy)_3^{+}$. Then $Ru(bpy)_3^{3+}$ and $Ru(bpy)_3^{+}$ undergo annihilation to form $Ru(bpy)_3{}^{*2+}$, which was produced to emit light [33–35]. However, when $Ru(bpy)_3^{2+}$ was immobilized on the electrode surface, there was no luminescence observed without a co-reactant such as TPrA. In the presence of TPrA, the following reaction occurred [36, 37]:

**Table 1** XPS data for the bare GC and the modified GC electrode surfaces

| | Bare GC electrode | Modified GC electrode |
|---|---|---|
| O (1s) Peak intensity (%) | 11.38 | 20.66 |
| C (1s) Peak intensity (%) | 84.53 | 73.12 |
| Ru (3d) Peak intensity (%) | – | 0.12 |

$$Ru(bpy)_3^{2+} - e^- \rightarrow Ru(bpy)_3^{3+}$$

$$TPrA - e^- \rightarrow [TPrA^{\bullet}]^+ \rightarrow TPrA^{\bullet} + H^+$$

$$Ru(bpy)_3^{3+} + TPrA^{\bullet} \rightarrow Ru(bpy)_3{}^{*2+} + \text{products}$$

$$Ru(bpy)_3{}^{*2+} \rightarrow Ru(bpy)_3^{2+} + h\nu$$

Other reaction mechanisms have also been proposed for the production of the excited state [38]. Overall, a stable ECL response has been observed when TPrA was added to the solution with the modified electrode as a working electrode.

The $H^+$ affected the production of the free radical of $TPrA^{\bullet}$, so the pH of the solution was an important factor for ECL emission. For $Ru(bpy)_3^{2+}$ immobilized GCE, the ECL intensity was more consistent between pH 6 and 9 with a maximum at about 7 with TPrA as the co-reactant (Fig. 3).

Figure 4 presents a typical calibration curve for TPrA concentration using $Ru(bpy)_3^{2+}$ modified electrode as the working electrode. The linear range was 10–500 μM ($R^2 = 0.999$), which showed that the $Ru(bpy)_3^{2+}$ modified GC electrodes had very high luminescent activity with TPrA as the co-reactant. This electrode with separation techniques such as liquid chromatography and capillary electrophoresis could be a simple, reagent saving ECL sensor.

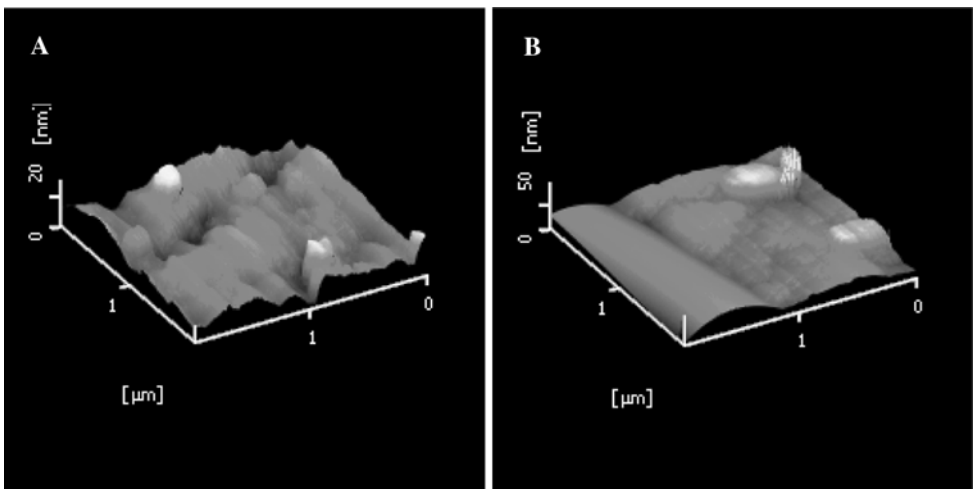


**Fig. 2** AFM images of the bare (**a**) and oxide covered (**b**) GC electrode surfaces

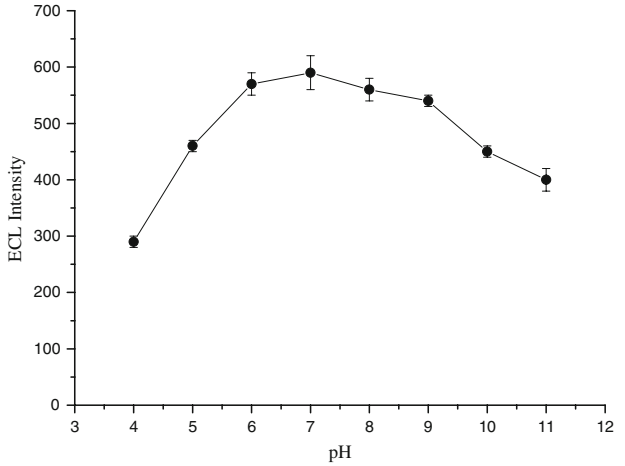


**Fig. 3** The pH dependence of ECL emission of immobilized $Ru(bpy)_3^{2+}$ in the presence of 1 mM TPrA with ICV scans from −1.5 to 1.5 V at 0.5 V $s^{-1}$

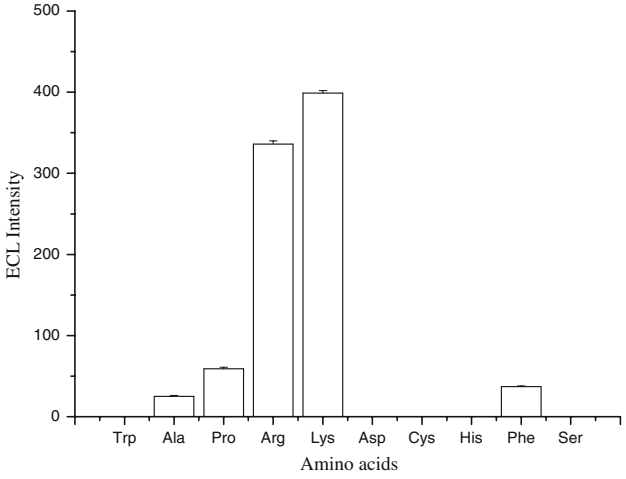


**Fig. 5** The ECL response of amino acids (10 mM) with the modified GC electrode in pH 7.0, 0.01 M PBS buffer with ICV scans from −1.5 to 1.5 V at 0.5 V $s^{-1}$

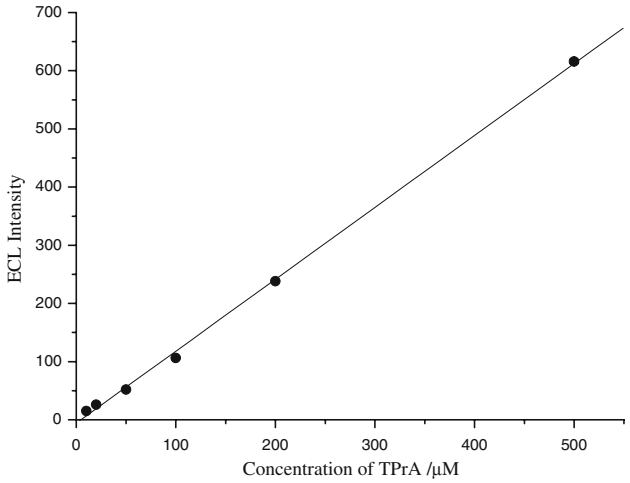


**Fig. 4** Typical calibration curve for TPrA in pH 7.0, 0.01 M PBS buffer with ICV scans from −1.5 to 1.5 V at 0.5 V $s^{-1}$

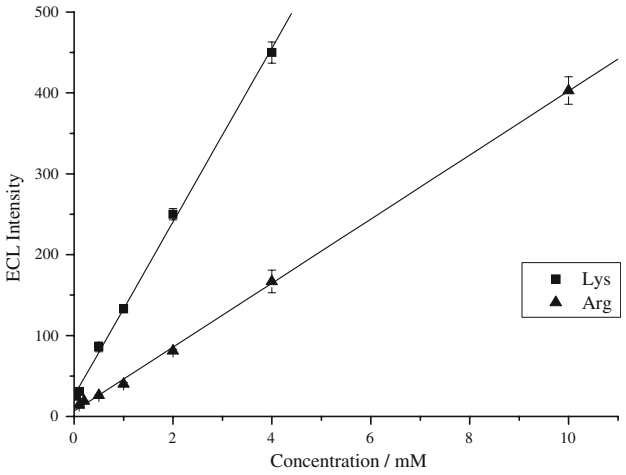


**Fig. 6** Calibration curves for arginine and lysine with the modified GC electrode in pH 7.0, 0.01 M PBS buffer with ICV scans from −1.5 to 1.5 V at 0.5 V $s^{-1}$

### 3.3 Electrochemiluminescence with amino acids

To demonstrate the application potential of GC electrode modified with $Ru(bpy)_3^{2+}$, the ECL with amino acids as co-reactants were tested. Figure 5 shows the ECL response of some amino acids with the $Ru(bpy)_3^{2+}$ modified GC electrode. Enhanced ECL was observed for alanine, proline, arginine, lysine and phenylalanine solution. Arginine and the lysine solutions had greater enhancements probably due to the additional amino groups in these amino acids that acted as co-reactants.

### 3.4 Calibration curves for arginine and lysine

ECL experiments revealed that the intensity increased linearly with concentration of the analytes. Figure 6 shows typical calibration plots of ECL intensity versus arginine and lysine concentration. The ECL intensity increased linearly from 0.05 to 4 mM ($R^2 = 0.998$) for lysine and from 0.10 to 10 mM ($R^2 = 0.999$) for arginine. The detection limits were 0.02 mM for lysine and 0.06 mM for arginine based on S/N = 3.

### 3.5 Electrochemical stability of immobilized $Ru(bpy)_3^{2+}$

The relative stability of the modified electrode was demonstrated when only 3.1% loss in the ECL intensity was observed over 3 h, when ICV scanning was performed in phosphate buffer in the potential range from −1.5 to 1.5 V at 500 mV $s^{-1}$.

## 4 Conclusions

The $Ru(bpy)_3^{2+}$ complex was immobilized on a GC electrode by cyclic voltammetric from 0 to 2.0 V in 0.1 mM $Ru(bpy)_3^{2+}$ and 0.1 M $KNO_3$ solution and confirmed by AFM and XPS. The modified electrodes showed stable ECL response in the presence of TPrA and selective response toward individual amino acids. Arginine and lysine enhanced the $Ru(bpy)_3^{2+}$ ECL substantially. Other amino acids showed very limited enhancement.

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