Anal. Chem. **2008**, *80,* 5635–5639

Technical Notes

Bis(2,2′-bipyridine)(5,6-epoxy-5,6-dihydro-[1,10]phenanthroline)ruthenium: Synthesis and Electrochemical and Electrochemiluminescence Characterization

Hui Wei, Jianyuan Yin, and Erkang Wang*

State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, Jilin 130022, P. R. China; Graduate School of the Chinese Academy of Sciences, Beijing 100039, P. R. China

In this work, an electrochemiluminescence (ECL) reagent bis(2,2′-bipyridine)(5,6-epoxy-5,6-dihydro-[1,10]phenanthroline)ruthenium complex (Ru-1) was synthesized, and its electrochemical and ECL properties were characterized. The synthesis of Ru-1 was confirmed by IR spectra, element analysis, and ^{1}H NMR spectra. For further study, its UV−vis absorption and fluorescence emission spectra were investigated. Ru-1 also exhibited quasi-reversible Ru^{II}/Ru^{III} redox waves in acetonitrile solution. The aqueous ECL behaviors of Ru-1 were also studied in the absence and in the presence of tripropylamine. The complex was fabricated on a γ-(aminopropyl) triethoxysilane (APTES) pretreated indium tin oxide (ITO) substrate via aminolysis reaction between the 5,6-epoxy-5,6-dihydro-[1,10]phenanthroline ligand and APTES. The resulting Ru-1 modified ITO substrate exhibited a broad absorption band in the visible region (350−600 nm) and its fluorescence emission spectrum was centered at 622 nm. The Ru-1 modified ITO electrode showed relative low ECL response. To improve the solid-state ECL response, a gold nanoparticles (GNP)/Ru-1 modified ITO electrode was constructed. The mixing of GNP and Ru-1 could produce the aggregates, which were further immobilized onto a 3-mercaptopropyltrimethoxy-silane (3-MPTMS) pretreated ITO substrate via Au−S interactions to construct the GNP/Ru-1 modified electrode.

Electrochemiluminescence[1–7] (ECL; also called electrogenerated chemiluminescence) of tris(2,2′-bipyridine)ruthenium(II) [$Ru(bpy)_3^{2+}$] first reported by Tokel and Bard[8] in 1972 has played an important role in the development of ECL and its applications, as manifested in, for example, the growing interest in clinical tests and biomolecule detection.[9–20] The ECL of $Ru(bpy)_3^{2+}$ involves the production of light at the electrode surface by generating species that can undergo highly energetic electron transfer reactions to form excited states.[1]

Although the most thoroughly studied ECL system has been the $Ru(bpy)_3^{2+}$/TPA system (where TPA = tripropylamine) due to its commercial and clinical importance,[1–7,9–20] many other polypyridine ruthenium complexes have also been investigated as $Ru(bpy)_3^{2+}$ analogues. $Ru(bpy)_3^{2+}$-*N*-hydroxysuccinimide (NHS) ester and phosphoramidite conjugate, for example, were used as nonradioactive ECL labels in clinical diagnostic assays.[1,2,9] Research has also extended to ruthenium coordination complexes containing phenanthroline and other related ligands.

Phenanthroline as a particular ligand for coordination chemistry has been intensively studied since the late nineteenth century,

---

* To whom correspondence should be addressed. State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, Jilin, P. R. China. Fax: (+86) 431-85689711. Phone: (+86) 431-85262003. E-mail: ekwang@ciac.jl.cn.

(1) Bard, A. J. *Electrogenerated Chemiluminescence*; Marcel Dekker: New York, 2004, Ch. 1.
(2) Richter, M. M. *Chem. Rev.* **2004**, *104*, 3003.
(3) Knight, A. W. *TrAC, Trends Anal. Chem.* **1999**, *18*, 47.
(4) Lee, W. Y. *Mikrochim. Acta* **1997**, *127*, 19.
(5) Pyati, R.; Richter, M. M. *Annu. Rep. Prog. Chem., Sect. C: Phys. Chem.* **2007**, *103*, 12.
(6) Xu, G. B.; Pang, H. L.; Xu, B.; Dong, S. J.; Wong, K. Y. *Analyst* **2005**, *130*, 541.
(7) Wei, H.; Wang, E. *TrAC, Trends Anal. Chem.* **2008**, *27*, 447.
(8) Tokel, N. E.; Bard, A. J. *J. Am. Chem. Soc.* **1972**, *94*, 2862.
(9) Blackburn, G. F.; Shah, H. P.; Kenten, J. H.; Leland, J.; Kamin, R. A.; Link, J.; Peterman, J.; Powell, M. J.; Shah, A.; Talley, D. B.; Tyagi, S. K.; Wilkins, E.; Wu, T. G.; Massey, R. J. *Clin. Chem.* **1991**, *37*, 1534.
(10) Leland, J. K.; Powell, M. J. *J. Electrochem. Soc.* **1990**, *137*, 3127.
(11) Yin, X. B.; Dong, S. J.; Wang, E. K. *TrAC, Trends Anal. Chem.* **2004**, *23*, 432.
(12) Du, Y.; Wei, H.; Kang, J. Z.; Yan, J. L.; Yin, X. B.; Yang, X. R.; Wang, E. K. *Anal. Chem.* **2005**, *77*, 7993.
(13) Wei, H.; Du, Y.; Kang, J. Z.; Wang, E. K. *Electrochem. Commun.* **2007**, *9*, 1474.
(14) Dennany, L.; Forster, R. J.; Rusling, J. F. *J. Am. Chem. Soc.* **2003**, *125*, 5213.
(15) Sun, X. P.; Du, Y.; Dong, S. J.; Wang, E. K. *Anal. Chem.* **2005**, *77*, 8166.
(16) Martin, A. F.; Nieman, T. A. *Biosens. Bioelectron.* **1997**, *12*, 479.
(17) Li, J. G.; Yan, Q. Y.; Gao, Y. L.; Ju, H. X. *Anal. Chem.* **2006**, *78*, 2694.
(18) Wei, H.; Wang, E. K. *Chem. Lett.* **2007**, *36*, 210.
(19) Miao, W. J.; Bard, A. J. *Anal. Chem.* **2003**, *75*, 5825.
(20) Miao, W. J.; Bard, A. J. *Anal. Chem.* **2004**, *76*, 5379.

10.1021/ac8001462 CCC: $40.75

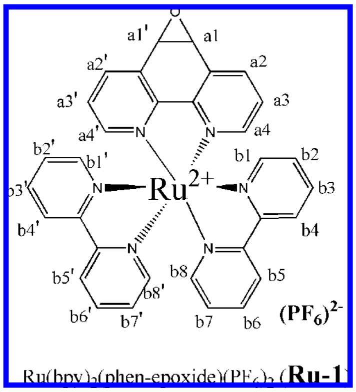
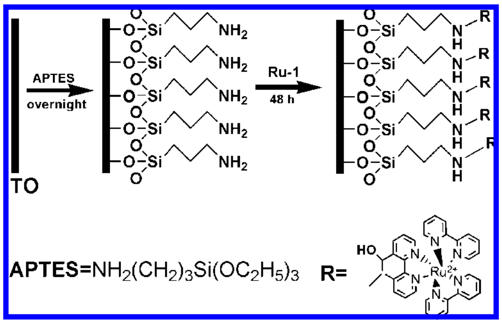


Figure 1. Structure of **Ru-1** complex included in this study.

Scheme 1. Schematic Routine for the Rabrication of Ru-I Modified ITO Electrode



and much progress has been made in this field.[21] Some ruthenium coordination complexes containing phenanthroline ligands have been synthesized and studied as ECL chromophores.[22,23] Oxidation of 1,10-phenanthroline gives the ligand 5,6-epoxy-5,6-dihydro-[1,10] phenanthroline (**Phen-epoxide**), which can be converted into several 5-substitution products such as 5-cyano-[1,10]phenanthroline.[24]

In this paper, a ruthenium complex containing **Phen-epoxide** ligand (**Ru-1**, as shown in Figure 1) was synthesized. Its spectroscopic, electrochemical, and electrochemiluminescent characteristics were also extensively evaluated. Because of the chemical reactivity of the **Phen-epoxide** ligand, **Ru-1** could be fabricated on a γ-(aminopropyl) triethoxysilane (APTES) pretreated indium tin oxide (ITO) substrate via aminolysis reaction of the ligand **Phen-epoxide** and the amino group of APTES (Scheme 1).[25,26] The absorption, emission, and ECL properties of the **Ru-1** modified ITO substrate was characterized. Furthermore, by mixing gold nanoparticles (GNP) and **Ru-1**, one could obtain the aggregates containing **Ru-1**. An improved solid-state ECL electrode could be fabricated by assembling these GNP/**Ru-1** aggregates on a 3-mercaptopropyltrimethoxysilane (3-MPTMS) pretreated ITO substrate via gold−thiol interactions.[15]

EXPERIMENTAL SECTION

Chemicals and Materials. Ruthenium(III) chloride hydrate was obtained from Kunming Institute of Precious Metals (Kuming, China). 5,6-Epoxy-5,6-dihydro-[1,10] phenanthroline (**Phen-epoxide**), sodium hexafluoro-phosphate, APTES, 3-MPTMS, and ferrocene were purchased from Aldrich Chemical Co. (Milwaukee, WI) and used without further purification. Tripropylamine (TPA) was obtained from Acros (NJ). Chloroauric acid ($HAuCl_4$) was purchased from Shanghai Chemical Reagent Company (Shanghai, China). Sodium citrate and sodium chloride were purchased from Beijing Chemical Reagent Company (Beijing, China). Acetonitrile was dried over CaH_2 and freshly distilled under nitrogen prior to electrochemical measurement and characterization. Other reagents and chemicals were at least analytical reagent grade. The water used throughout all experiments was purified by a Milli-Q system (Millipore, Bedford, MA).

Synthesis. The ligand cis-bis(2,2′-bipyridine) dichlororuthenium [$Ru(bpy)_2Cl_2$] was prepared according to a previously published method.[27] Ruthenium complex **Ru-1** was then synthesized according to a previous literature procedure.[27] Generally, the synthesis was carried out as follows giving a good yield (70−80%): A mixed solution of 10 mL of ethanol, 40 mL of water, 180 mg of the ligand **Phen-epoxide**, and 500 mg of $Ru(bpy)_2Cl_2$ were refluxed for 4 h under nitrogen atmosphere. After refluxing, the solution was cooled to room temperature and 5 mL of saturated sodium hexafluoro-phosphate was added to precipitate the product **Ru-1**. The red precipitate was collected by filtration and washing.

Ru-1 was certified using elemental analysis and ^{1}H NMR and IR spectra. The data are shown as follows. ^{1}H NMR (400 MHz, DMSO) δ_H (ppm): 5.120 (s, 2H, a1 and a1′), 7.677 (12H, a2 and a2′, a3 and a3′, b2 and b2′, b3 and b3′, b6 and b6′, b7 and b7′), 8.170 (4H, b4 and b4′, b5 and b5′), 8.540 (2H, a4 and a4′), 8.851 (4H, b1 and b1′, b8 and b8′). IR ν_{max}/cm^{-1}: 840 (P−F), 802 (C−O−C). Anal. Calcd for $C_{32}H_{24}N_6OF_{12}P_2Ru$: C, 42.67; H, 2.69; N, 9.34; Ru, 11.3. Found: C, 42.38; H, 3.02; N, 9.217; Ru, 11.

Preparation of Ru-1 **Modified ITO Electrode.** For the **Ru-1** assembly experiment, the transparent ITO glass was pretreated following a reported method.[28] The monolayer of **Ru-1** on the ITO substrate was fabricated as follows (Scheme 1). Before the assembly of the monolayer of **Ru-1**, a precursor film of APTES was deposited on a clean ITO surface by dipping the ITO substrate in 5% APTES (v/v) ethanol solution overnight. After thoroughly rinsing with ethanol and water, the ITO/APTES substrate was then immersed into the 0.25 mM **Ru-1** aqueous solution containing 1 mM $NaHCO_3$ for 48 h to assemble **Ru-1** through aminolysis of the ligand **Phen-epoxide**.[25,26] The resulting ITO substrate was thoroughly rinsed with water, dried with nitrogen, and used for the electrochemical and spectroscopic measurements.

Solid-State ECL of GNP/Ru-1 **Modified ITO Electrode.** GNP (13 nm) was prepared via a citrate reduction approach.[29] According to our previously reported approach,[15] 13 nm GNP and **Ru-1** aggregates were assembled on a 3-MPTMS pretreated ITO substrate[28] and used for solid-state ECL characterization.

Instrumentation. Infrared (IR) spectra were recorded within the 4000−400 cm^{-1} region on a Perkin-Elmer model 580B infrared spectrophotometer with the KBr pellet technique. Absorption spectra were recorded on a Cary 500 scan UV−vis-NIR spectro-

---

(21) Luman, C. R.; Castellano, F. N. In *Comprehensive Coordination Chemsitry II*; McCleverty, J. A., Meyer, T. J., Eds.; Elsevier: Oxford, U.K., 2004; Vol. 1, p 25.

(22) McCord, P.; Bard, A. J. *J. Electroanal. Chem.* **1991**, *318*, 91.

(23) Wang, P.; Zhu, G. Y. *Synth. Commun.* **2000**, *30*, 4057.

(24) Shen, Y. B.; Sullivan, B. P. *Inorg. Chem.* **1995**, *34*, 6235.

(25) Azizi, N.; Saidi, M. R. *Org. Lett.* **2005**, *7*, 3649.

(26) Lee, M.; Shin, I. *Angew. Chem., Int. Ed.* **2005**, *44*, 2881.

(27) Sullivan, B. P.; Salmon, D. J.; Meyer, T. J. *Inorg. Chem.* **1978**, *17*, 3334.

(28) Wei, H.; Li, J.; Wang, Y. L.; Wang, E. K. *Nanotechnology* **2007**, *18*, 17561.

(29) Grabar, K. C.; Freeman, R. G.; Hommer, M. B.; Natan, M. J. *Anal. Chem.* **1995**, *67*, 735.

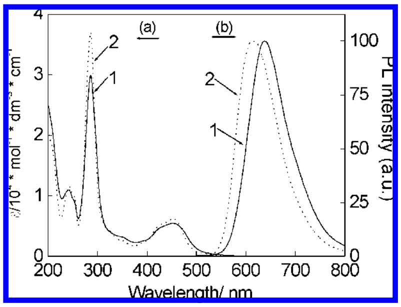


Figure 2. UV−vis absorption (a) and fluorescence emission (b) spectra of 0.02 mM **Ru-1** (line 1) and Ru(bpy)$_3$Cl$_2$ (line 2) in 100 mM phosphate buffer (pH = 7.4) at room temperature. The excitation wavelength is 460 nm.

photometer (Varian, Harbor City, CA) at room temperature. Fluorescence emission spectra were recorded on a Perkin-Elmer LS55 luminescence spectrometer at room temperature. Element analysis was performed using Elementar Analysensystem GmbH VarioEL. For ruthenium element determination, inductively coupled plasma mass spectrometry (POEMS, TJA Corporation) was applied.

The electrochemical and electrochemiluminescent measurements were performed at room temperature with a CHI 800 workstation (CH Instruments, Austin, TX) with an ECL detector using a three-electrode system. A KCl-saturated Ag/AgCl electrode and a platinum wire electrode were used as the reference and the auxiliary electrode, respectively. To inspect the ECL response of **Ru-1** on the electrode, a **Ru-1** modified ITO electrode (or GNP/**Ru-1** modified ITO electrode) was used as the working electrode. To investigate the ECL behaviors of **Ru-1** in solution, an unmodified platinum plate electrode was used as the working electrode. The ECL emission was detected with a model MCFL-A chemiluminescence analyzer system (Xi'An Remax Science & Technology Co. Ltd., Xi'An, China).

RESULTS AND DISCUSSION

UV−Visible Absorption and Fluorescence Emission Spectra of Ru-1. Electronic spectrum of Ru-1 was acquired in phosphate buffer solution. As shown in figure 2, the absorbance spectrum of **Ru-1** was similar to that of Ru(bpy)$_3$Cl$_2$. The spectrum was dominated by the ligand-centered (LC) and intraligand (IL) bands in the UV region and the spin allowed metal-to-ligand charge-transfer (MLCT) transitions in the visible region.[30,31]

The room temperature fluorescence emission spectrum in phosphate buffer solution is shown in Figure 2. Compared to Ru(bpy)$_3$Cl$_2$, the complex **Ru-1** exhibited a red shift. The results suggested that the emitting MLCT excited-state energy of **Ru-1** was lower than its corresponding complex Ru(bpy)$_3$Cl$_2$. The red shift effect might due to the epoxide structure of the **Phen-oxide** ligand.[32]

Electrochemical and ECL Characterization of Ru-1. To investigate the electrochemical properties of the **Ru-1** complex,

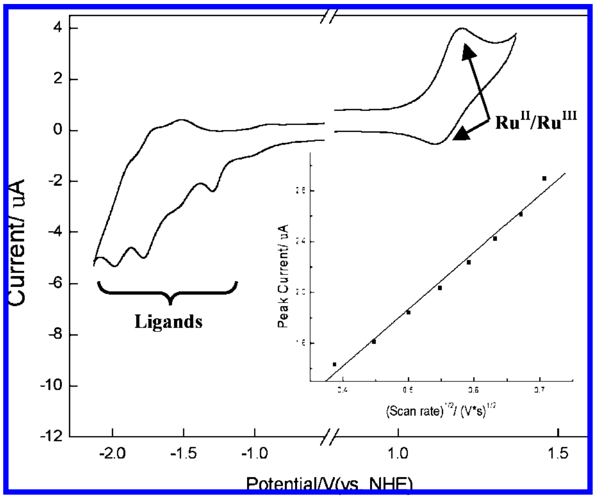


Figure 3. Cyclic voltammogram of 1 mM **Ru-1** in acetonitrile at room temperature (scan rate = 0.15 V/s). Inset: the relationship between the anodic peak current of Ru^{II}/Ru^{III} and the square root of the scan rate.

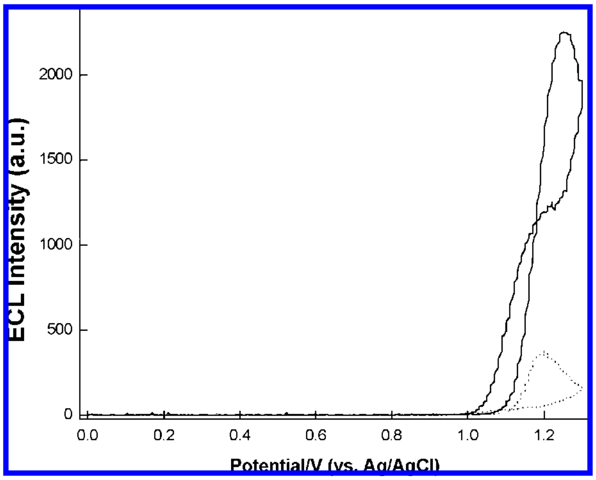


Figure 4. ECL intensity-potential curve of 0.2 mM **Ru-1** in 100 mM buffer (pH = 7.4) in the absence (- - -) and presence of 0.45 mM TPA (—) at room temperature (scan rate = 0.1 V/s).

cyclic voltammograms (CVs) were obtained in acetonitrile solution. Quasi-reversible Ru^{II}/Ru^{III} redox pairs were observed (as indicated by the arrows in Figure 3). Under the experimental conditions and over the range of scan rates from 20 m to 300 mV/s, the dependence of anodic peak current of **Ru^{II}-1/Ru^{III}-1** redox pair on the scan rates was investigated to identify the type of current. As shown in the inset of Figure 3, the peak current was approximately proportional to the square root of scanning rate, $v^{1/2}$, which indicated that the electro-oxidation of the **Ru-1** complex on the platinum electrode underwent a diffusion process.[33] It is well documented that the ligands 2,2′-bipyridine and [1,10] phenanthroline could be successively reduced in the cathodic region and featured a series of quasi-reversible one-electron reduction waves.[34,35] The complex **Ru-1** here exhibited

---

(30) Castellano, F. N.; Heimer, T. A.; Tandhasetti, M. T.; Meyer, G. J. *Chem. Mater.* **1994**, *6*, 1041.

(31) Staffilani, M.; Ho1ss, U.; Giesen, U.; Schneider, E.; Hartl, F.; Josel, H. P.; Cola, L. D. *Inorg. Chem.* **2003**, *42*, 7789.

(32) Durham, B.; Caspar, J. V.; Nagle, J. K.; Meyer, T. J. *J. Am. Chem. Soc.* **1982**, *104*, 4803.

(33) Bard, A. J.; Faulkner, L. R. *Electrochemical Methods: Fundamentals and Applications*, 2nd ed.; John Wiley and Sons: New York, 2000; Chapter 6.

(34) Draper, S. M.; Gregg, D. J.; Schofield, E. R.; Browne, W. R.; Duati, M.; Vos, J. G.; Passaniti, P. *J. Am. Chem. Soc.* **2004**, *126*, 8694.

(35) Wong, C. Y.; Chan, M. C. W.; Zhu, N.; Che, C. M. *Organometallics* **2004**, *23*, 2263.

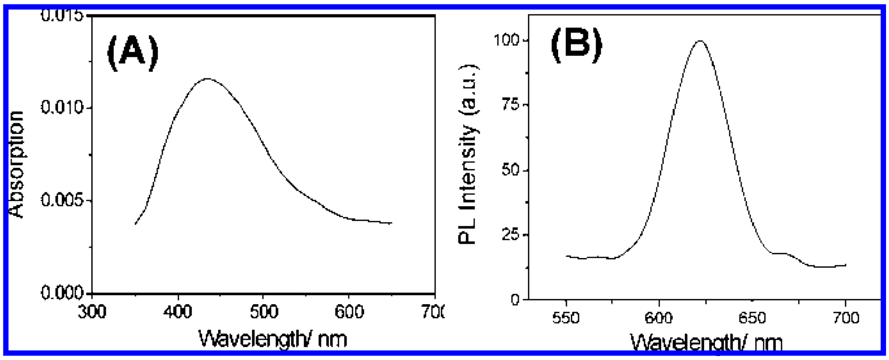


Figure 5. Absorption (A) and fluorescence emission (B) spectra of **Ru-1** immobilized on an ITO substrate at room temperature. The excitation wavelength is 460 nm.

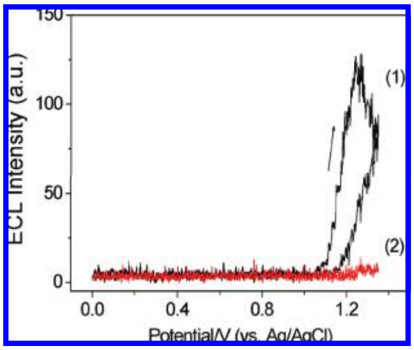


Figure 6. ECL intensity−potential curve of **Ru-1** modified ITO electrode (line 1) and unmodified ITO electrode (line 2) in 18 μM TPA at room temperature (scan rate = 0.05 V/s).

similar one-electron reduction behavior in the cathodic region; however, the reduction waves were not well-resolved (see Figure 3). These reduction waves might be assigned to the 5,6-epoxy-5,6-dihydro substitution of the [1,10] phenanthroline.

To check the ECL behavior of **Ru-1** in 100 mM phosphate buffer (pH = 7.4) in the absence and presence of TPA as coreactant, the CVs were recorded as shown in Figure 4. The onset of luminescence occurred at approximately 1.0 V, at which Ru^{II} was oxidized to Ru^{III}, and then the ECL intensity rose steeply due to the catalytic oxidation of coreactant TPA.[1,36]

Characterization of Ru-1 **Modified ITO Electrode. Ru-1** immobilized on the ITO electrode through aminolysis of the ligand **Phen-epoxide** was investigated by absorption and fluorescence emission spectra. Figure 5A shows the absorption spectrum of the **Ru-1** modified ITO electrode. It exhibits a broad absorption band in the visible region (350−600 nm) due to the spin allowed dπ(Ru)−π(ligand)* metal-to-ligand charge transfer (MLCT) transitions.[29] The room temperature fluorescence emission spectrum of **Ru-1** immobilized on the ITO electrode is presented in Figure 5B showing a maximum intensity centered at 622 nm.[30,31]These results confirm the successful immobilization of **Ru-1** on the ITO substrate.

Figure 6 shows the ECL behaviors of the **Ru-1** modified ITO electrode and the unmodified ITO electrode in 18 μM TPA. As shown in Figure 6, the **Ru-1** modified ITO electrode shows similar but lower ECL intensity compared to **Ru-1** in solution (Figure 4). The lower ECL signals could be a consequence of limited loading of **Ru-1** because of the monolayer nature.[37]

Solid-State ECL of GNP/Ru-1 **Modified ITO Electrode.** According to our previous study, because of the electrostatic interactions between the positive charges of cationic molecules (i.e., **Ru-1** in this work) and the negatively charged citrate coating on the GNP, the addition of **Ru-1** into citrate-stabilized GNP resulted in a black-red colloidal solution. Such colloidal solution would further aggregate to form a black precipitate. The resulting GNP/**Ru-1** aggregates could be used to fabricate solid state ECL sensor on a 3-MPTMS pretreated ITO substrate via strong Au−S interactions.[15]

Figure 7 shows the CVs and corresponding ECL intensity−potential curves of GNP/**Ru-1** modified ITO in the absence and presence of 167 μM TPA at scan rate of 50 mV/s at room temperature. A pair of characteristic reversible redox peaks of **Ru-1** near 1.1 V could be observed in the absence of TPA (Figure 7A, dotted line), which could be attributed to the successful assembly of GNP/**Ru-1** on the ITO substrate. The other two kinds of peaks in Figure 7A could be assigned to the oxidation peak and reduction peak of the GNP contained in the aggregated structures, respectively.[15] Specifically, the oxidation peak P_1 could be assigned to the formation of an oxidation layer (layer P_1) on the surface of the GNPs during the forward scan while the reduction peak P_2 should be the reduction wave of the oxidized layer P_1 during the backward scan. In the presence of 167 μM TPA (Figure 7A, solid line), typical catalytic oxidation behaviors were observed near 1.1 V where TPA was catalytically oxidized by immobilized **Ru-1**. A negative shift was observed for the oxidation and reduction peaks of the GNP in the presence of TPA in this case (Figure 7A). The reason for this negative-shift is not clear at the current stage and needs further investigation. To check the ECL properties of **Ru-1** immobilized on the modified ITO electrode, the ECL intensity−potential curves were recorded in a detection reservoir with and without 167 μM TPA. As shown in Figure 7B, the onset of luminescence occurred at approximately 1.1 V, at which Ru^{2+} was oxidized to Ru^{3+}. The ECL signal rose quite steeply when 167 μM TPA was added into the reaction buffer.

Figure 8 shows the ECL intensity−potential curves of the GNP/**Ru-1** modified ITO electrode in 100 mmol/L phosphate (pH = 8.5) buffer solution with and without 167 μM TPA under

---

[36] Zu, Y. B.; Bard, A. J. *Anal. Chem.* **2000**, *72*, 3223.

[37] Tang, Z. Y.; Wang, Y.; Podsiadlo, P.; Kotov, N. A. *Adv. Mater.* **2006**, *18*, 3203.

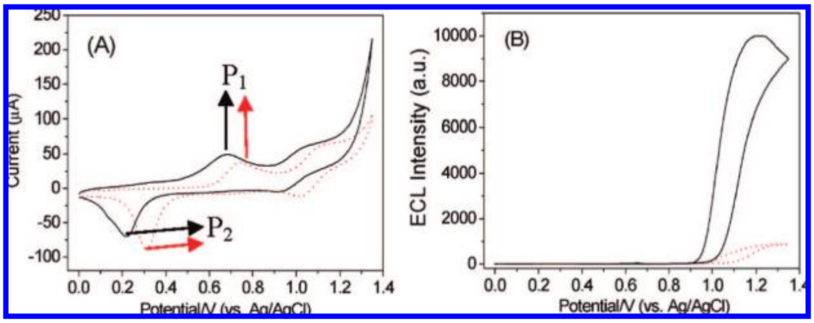


Figure 7. Cyclic voltammograms (A) and corresponding ECL intensity−potential curves (B) of GNP/**Ru-1** modified ITO in the absence (- - -) and presence (−) of 167 μM TPA at room temperature (scan rate = 0.05 V/s).

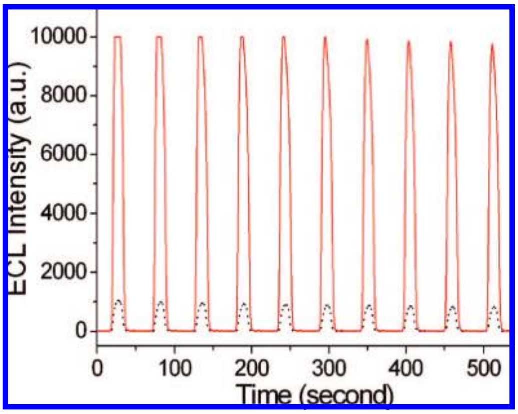


Figure 8. ECL intensity−time curves of the GNP/**Ru-1** modified ITO electrode in the absence (- - -) and presence (−) of 167 μM TPA at room temperature (scan rate = 0.05 V/s).

continuous CV for 10 cycles. The relative standard deviation of the ECL peak height of the GNP/**Ru-1** modified ITO electrode in buffer solution was 0.95%, which reflects the good stability of the GNP/**Ru-1** modified ITO electrode.

CONCLUSION

In summary, we have synthesized a ruthenium(II) complex containing **Phen-epoxide** ligand. Its spectroscopic, redox, and ECL properties were carefully examined. Because of the chemical reactivity of the **Phen-epoxide** ligand, the complex was successfully fabricated on an APTES pretreated ITO substrate via aminolysis reaction. The fabrication, emission, and ECL properties of the **Ru-1** modified ITO substrate were characterized. Solid-state ECL was further realized through a GNP/**Ru-1** aggregates based assembly approach on a 3-MPTMS pretreated ITO substrate. Since the complex prepared could be converted into other 5-substitution products due to the chemical reactivity of the **Phen-epoxide** ligand, the **Ru-1** complex could be even used to label materials with an ECL tag in the future. Moreover, the GNP/**Ru-1** aggregates modified ITO substrate exhibits excellent ECL behaviors and therefore holds great promise as new luminescent materials for solid-state ECL detection in CE or a CE microchip.

ACKNOWLEDGMENT

This work is supported by the National Natural Science Foundation of China with Grants 20575063 and 20675078, 863 Project No. 2006AA020701, 973 Project No. 2007CB714500 and the Chinese Academy of Sciences Grant KJCX2-YW-H09, H11. Prof. Guobao Xu is acknowledged for his helpful discussions. We especially appreciate our two anonymous referees for their valuable comments and suggestions.

Received for review January 21, 2008. Accepted May 19, 2008.

AC8001462