

Electrochemiluminescence sensor using gold-nanoparticle modification combining mercaptoacetic acid-assembly

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In this paper, an electrochemiluminescence (ECL) sensor is fabricated using a covalent bond immobilization approach. A glassy carbon electrode (GCE) modified with gold nanoparticles (AuNPs@GCE) was prepared using a wet chemical method which acts as a support for the ECL reagent. A monolayer of mercaptoacetic acid (MA) was then assembled onto the AuNPs@GCE surface. Finally, an ECL reagent, bis(2,2'-bipyridyl) (5-amino-1,10-phen) dichlororuthenium [Ru(bpy)₂(phen-NH₂)₂]²⁺, was attached onto the AuNPs@GCE by condensation of the amino group and carboxylic group of MA. Because [Ru(bpy)₂(phen-NH₂)₂]²⁺ is covalently immobilized on the electrode surface without any polymaterials and directly contacts to the electrolyte, in contrast to traditional ECL sensors fabricated using a polymer technique, mass transportation and electron transfer hindrance is negligible and ECL stability is increased obviously. The sensor is applied in cathodic ECL analysis and presents good performance in a linear range from 1.0 × 10⁻⁶ mol L⁻¹ to 0.1 mol L⁻¹ and a detection limit of 5 × 10⁻⁷ mol L⁻¹ for potassium persulfate.

1. Introduction

Electrochemiluminescence (ECL) is a phenomenon whereby light is generated by electrochemical and successive chemical reactions between an ECL reagent and coreactants on an electrode surface. Since the ECL reaction is carried out in a light-tight dark box without any exciting light source, ECL is characterized by its high sensitivity and low detection limit. As a result, it has received great attention during the last 40 years.¹⁻³

In traditional ECL modes of flow injection or batch methods, ECL reagent, such as tris(2,2'-bipyridyl) ruthenium(II) (Ru(bpy)₃²⁺), is generally dissolved in a mobile phase or solution together with analytes. Although it is electroregenerable, in this case, Ru(bpy)₃²⁺ could not be reused after the reaction since it is polluted with the analytes from the solution. In order to avoid wasting Ru(bpy)₃²⁺ and to reduce the analysis cost, solid-state ECL techniques have been developed to immobilize the ECL reagent onto the working electrode surface. There are several kinds of main approaches to achieve this purpose, including Langmuir-Blodgett (LB),^{4,5} self-assembly,^{6,7} ion-exchange,⁸⁻¹⁰ sol-gel preparation,¹¹ Nafion compositing chitosan,¹² and as in recent reports,¹³ gold nanoparticle (AuNP)-modified materials. Zhang and Bard⁴ first reported the fabrication of an ECL monolayer on an electrode surface using surfactant-like

derivatives of Ru(bpy)₃²⁺ and the LB technique. Obeng and Bard⁶ immobilized Ru(bpy)₃²⁺ on a gold electrode using its mercaptolized derivatives and self-assembly, and the ECL was bright enough to be captured by a CCD camera. Although there have been several application reports,¹⁴ these two approaches suffer from some disadvantages. For example, the LB layer is easily destroyed by some organic solvents and is sensitive to surfactants.¹⁵ The self-assembly approach always requires expensive gold electrodes and is difficult to apply in a higher-potential anodic process (e.g. over 0.8 V, vs. SCE), since the mercapto group is easily oxidized at the potential applied. In addition to the LB and self-assembly approaches, ion-exchange and sol-gel polymers have been applied to immobilize Ru(bpy)₃²⁺ directly onto an electrode surface. Nafion ion exchanging polymer^{8,10} and silica sol-gel¹¹ are the commonly used polymers. In order to improve the conductivity of the polymer layer, composite polymers are sometimes synthesized, such as silica-sol-gel-Nafion composites¹⁶ and titanium-sol-gel-Nafion composites.¹⁷ Compared to the LB technique, the polymer technique can fabricate a relatively stable modified electrode. However, several problems have still to be overcome:^{18,19} (1) Ru(bpy)₃²⁺ is incorporated into the hydrophobic areas of the ion-exchanging polymers and so they lose their ECL activity; (2) the existence of a polymer membrane greatly affects mass transportation and electron transfer during the ECL process; and (3) Ru(bpy)₃²⁺ leakage occurs due to its high solubility in aqueous media. Since mass transportation and electron transfer would be obviously increased in a monolayer, where the ECL reagent would directly contact with the analytes in the solution, the fabrication of an ECL monolayer using a

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covalent bond approach may be a good way to resolve the problems described above, and to provide stable ECL signals in aqueous media.

Several studies on ECL using gold-based materials have been reported. Yao *et al.*²⁰ reported the ECL determination of tris(2,3-dibromopropyl) isocyanurate using a gold nanoparticles-modified gold electrode. In their study, Ru(bpy)₃²⁺ dissolved in the test solution was applied. Actually, self-assembly or covalent techniques should be good approaches to immobilize the ECL reagents onto the gold-based materials due to the strong interaction between the mercapto group and gold. Although the mercapto group is not stable during an anodic process, gold based mercapto group self-assembly layers are still available using a cathodic process. In recent years, gold nanoparticle (AuNP)-modified materials have been developed and widely used because of their excellent properties and low cost.^{21–23} Kang *et al.*²⁴ reported an ECL sensor based on a self-assembled monolayer of Ru(II)-bis(2,2'-bipyridyl)(aminopropyl imidazole) on a gold-deposited screen printed electrode, which reveals that it is an applicable route based on the adherence of AuNPs on an electrode surface. In order to obtain higher ECL sensitivity and reduce the expense of analysis, in this work, we prepared glassy carbon electrode modified AuNPs (AuNPs@GCE) as a support for mercaptoacetic acid (MA), and then [Ru(bpy)₂(phen-NH₂)₂]²⁺ was immobilized on the electrode by the condensation reaction of amino and carboxylic groups. The electrochemical and ECL characteristics of the AuNPs@GCE were studied, and it was then applied in a cathodic ECL process.

2. Materials and methods

2.1. Materials

1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC), hydrogen tetrachloroaurate (HAuCl₄·3H₂O, 99%), 3-mercaptopropyl trimethoxysilane (MPTMS) and MA were purchased from Sigma Aldrich (USA). Bi(2,2'-bipyridyl) (5-amino-1,10-phen) dichlororuthenium chlorate ([Ru(bpy)₂(phen-NH₂)₂](ClO₄)₂, Fig. 1) was synthesized and supplied by the University of Hong Kong. All chemicals were used without further purification. All solutions in the experiments were prepared with ultrapure water obtained using a Millipore (USA) purification system.

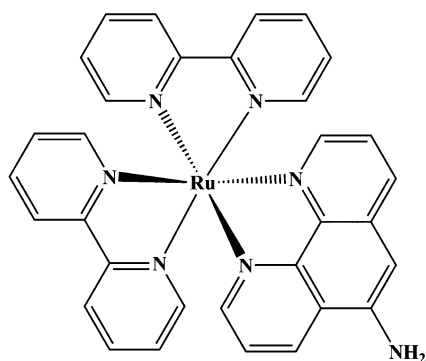


Fig. 1 Molecular structure of [Ru(bpy)₂(phen-NH₂)₂]²⁺.

2.2. Preparation of AuNPs@GCE

AuNPs@GCE was prepared with a little modification from the report of Lin *et al.*²⁵ The seed solution was prepared by adding 0.5 mL of 0.01 mol L⁻¹ HAuCl₄, 0.5 mL of 0.01 M trisodium citrate, and 0.5 mL of 0.1 mol L⁻¹ NaBH₄ (in sequence) into 18 mL of water, while stirring. The resulting solution was aged for 2 h. The growth solution was prepared by adding 0.1 mL of 0.1 mol L⁻¹ ascorbic acid and 0.025 mL of 0.1 mol L⁻¹ NaOH to a solution containing 4.5 mL of 0.1 mol L⁻¹ CTAB and 0.125 mL of 0.01 mol L⁻¹ HAuCl₄. In order to increase the density of AuNPs grown on a GCE, the GCE surface was mercaptolized by immersing the electrode in an ethanol solution containing 5% (v/v) MPTMS for 24 h. The GCE was then placed in a AuNP seed solution for 2 h, and in the growth solution for 6 h. Finally, the AuNPs@GCE was rinsed several times with ultrapure water.

2.3. Preparation of [Ru(bpy)₂(phen-NH₂)₂]²⁺-modified GCE

The AuNPs@GCE was immersed into the MA solution for 24 h to make sure that it was functionalized with the carboxylic groups. Then, the AuNPs@GCE was immersed in a solution containing 0.1 mol L⁻¹ EDC and 1 mg mL⁻¹ [Ru(bpy)₂(phen-NH₂)₂]²⁺ for 24 h. Finally, the [Ru(bpy)₂(phen-NH₂)₂]²⁺-modified GCE (Ru-AuNPs@GCE) was rinsed several times with ultrapure water.

2.4. Apparatus

A three-electrode-system with a home-made electrochemical cell was used in all the electrochemical experiments. In this system, a Ru-AuNPs@GCE was taken as the working electrode, while a saturated calomel electrode and a platinum electrode were used as the reference electrode and the counter electrode. Cyclic voltammetry (CV) measurements were performed on a CHI 660B electrochemical analyzer (CHI Co., China). ECL measurements were made using a flow injection chemiluminescence analyzer (Xi'an Remax Electronic Science Tech. Co. Ltd., China). The Ru-AuNPs@GCE morphology was examined using an LEO1530 scanning electron microscope (SEM) (Oxford Co. UK).

3. Results and discussion

The homogeneous and suitably higher density immobilization of [Ru(bpy)₂(phen-NH₂)₂]²⁺ on a GCE are the fundamental points to assure its ECL characteristics. Although a gold electrode could be used for the fabrication of the mercapto group self-assembly layers, the position and density of the immobilized [Ru(bpy)₂(phen-NH₂)₂]²⁺ on the entire electrode surface is difficult to control. Even in a lower density of the Ru complex immobilization, heteropical immobilization will still cause a partially higher Ru complex on part of the electrode surface, generating ECL self-quenching (the same as fluorescence self-quenching) and resulting in a lower ECL sensitivity. AuNPs, attaching to the GCE surface separately, provide isolated island working faces for the Ru complex immobilization. The concentration and distribution of the immobilized [Ru(bpy)₂(phen-NH₂)₂]²⁺ could be controlled by the density of AuNPs on the AuNPs@GCE: since AuNPs were the carriers of

$[\text{Ru}(\text{bpy})_2(\text{phen-NH}_2)_2]^{2+}$, the growth density of AuNPs would greatly affect the modification amount of $[\text{Ru}(\text{bpy})_2(\text{phen-NH}_2)_2]^{2+}$, and hence, the sensitivity of the ECL method. In previous work, we successfully developed a wet chemical method to directly attach modified AuNPs onto an electrode surface and realize shape control,²⁵ however, the density of AuNPs was not high enough in this experiment (Fig. 2a). Considering the strong interaction between the mercapto group and gold, the GCE surface was first mercaptolized with MPTMS, and then the MPTMS modified GCE was taken as a support to grow AuNPs. As shown in Fig. 2b, the density of AuNPs was greatly increased after mercaptolization and the AuNPs attached to the GCE were relatively monodispersed and homogeneous.

The modification of MPTMS would need to be considerable in order to greatly affect the electrode conductivity in phosphate buffer solution (PBS, pH 7.0). Our experimental results (as shown in Fig. 3) confirmed this: after mercaptolization, the resistivity of the GCE was greatly increased (Fig. 3 line 3). The same phenomenon could be found when the GCE was modified with Nafion ion-exchanging film (Fig. 3 line 2). It is interesting that the resistivity of the Ru-AuNPs@GCE (Fig. 3 line 4) was dramatically decreased, compared to that of the mercaptolized GCE, and was even smaller than the bare GCE resistivity (Fig. 3 line 1). These results indicated that the modification of AuNPs could facilitate the electron transfer, and that the Ru-AuNPs@GCE contributed a lower resistivity than that of the mercaptolized GCE.

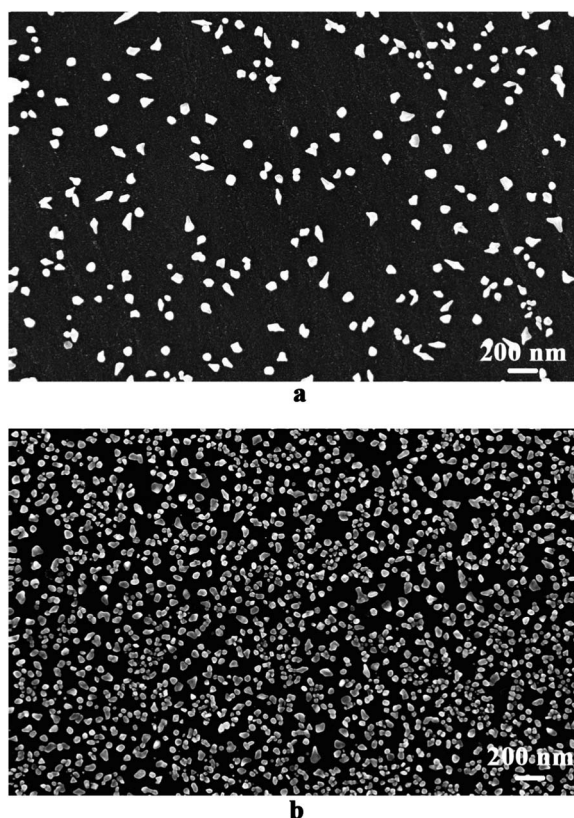


Fig. 2 SEM images of a AuNPs@GCE. (a) Without mercaptolization; (b) with mercaptolization.

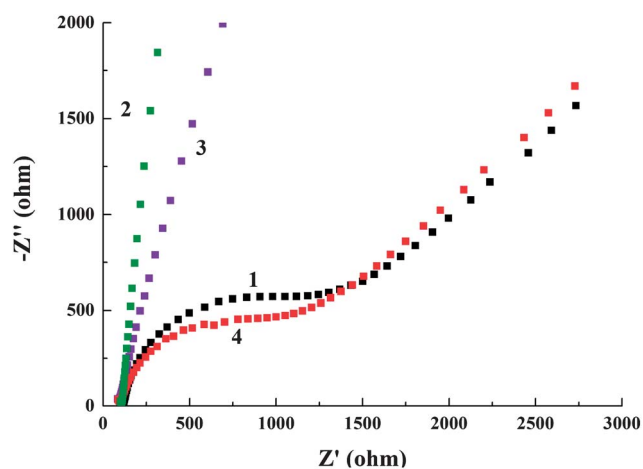


Fig. 3 AC-impedance spectra of different electrodes. (1) Bare GCE; (2) Nafion modified GCE; (3) mercaptolized GCE; (4) Ru-AuNPs@GCE. Electrolyte: 0.1 mol L⁻¹ PBS (pH 7.0) containing 1.0×10^{-3} mol L⁻¹ $\text{K}_3\text{Fe}(\text{CN})_6/\text{K}_4\text{Fe}(\text{CN})_6$.

To evaluate the ECL ability of the Ru-AuNPs@GCE, the modified electrode was first applied in an anodic ECL process from 0 to 1.30 V giving consideration to the oxidation potential of the Ru complex. As shown in Fig. 4, in the first CV cycle, the ECL intensity of the Ru-AuNPs@GCE in PBS was 122 mV, indicating that $[\text{Ru}(\text{bpy})_2(\text{phen-NH}_2)_2]^{2+}$ was successfully modified on the electrode surface. However, the ECL intensity was dramatically decreased with the increase of CV cycle numbers. Although the formation of a diffusion layer would cause the concentration decrease of tripropylamine (TPA) around the electrode surface and hence, the decrease of ECL intensity during CV scanning, it was impossible to decrease it to such an extent. Further experiments on the CV voltogram of differently modified GCE electrodes, as shown in Fig. 5, confirmed that the ECL intensity decrease was caused by the oxidation of the AuNPs and mercapto groups. There was no obvious oxidation peak for mercaptolized GCE (Fig. 5a), but a cathodic and anodic peak at 0.43 V and 0.9 V were found after

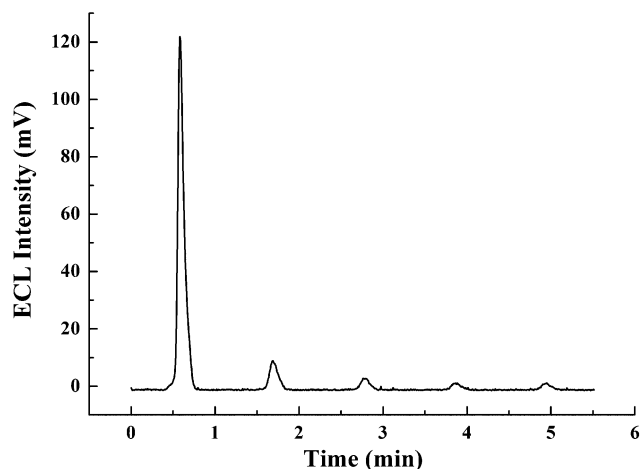


Fig. 4 ECL responses of a Ru-AuNPs@GCE. Electrolyte: 0.1 mol L⁻¹ PBS (pH 7.0) containing 1.0×10^{-3} mol L⁻¹ TPA.

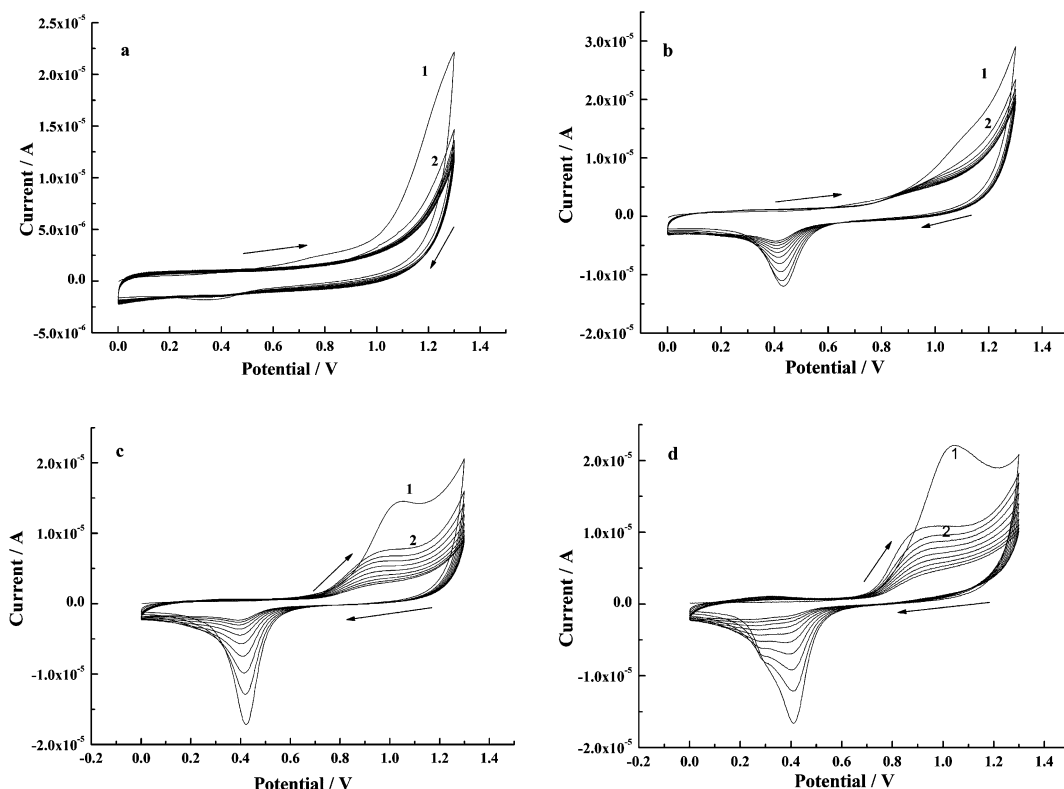


Fig. 5 CV voltammogram of different electrodes. (a) Mercaptolized GCE, (b) AuNPs@GCE, (c) AuNPs@GCE further modified with mercapto acid, (d) Ru-AuNPs@GCE.

the GCE was modified with AuNPs (Fig. 5b), which showed the same potentials as those of gold. Because there was no other electroactive component in the electrolyte, these redox peaks could be attributed to the reduction and oxidation of AuNPs. Further modification of MA on AuNPs greatly increased the oxidation peak (Fig. 5c and d), which could be attributed to the oxidation of the mercapto group of MA.²⁶ The oxidation current gradually decreased with the increase of the CV cycle numbers, corresponding to the ECL intensity decrease. Because the mercapto group was the connector of $[\text{Ru}(\text{bpy})_2(\text{phen-NH}_2)_2]^{2+}$, the oxidation of MA would lead to the disconnection of $[\text{Ru}(\text{bpy})_2(\text{phen-NH}_2)_2]^{2+}$, and hence the decrease of ECL intensity.

In order to avoid the oxidation of MA and the decrease of ECL intensity, the Ru-AuNPs@GCE was then used in a cathodic ECL process. As shown in Fig. 6a, in a blank PBS, no Ru-AuNPs@GCE ECL peak was found when the applied potential was from -1.5 V to 0 V (Fig. 6a). However, a strong ECL could be detected when the PBS contained potassium persulfate (Fig. 6b), and the ECL intensity remained very stable during the CV cycles. These results indicated that the mercapto group of MA was chemically stable during the cathodic process. Since the ECL intensity was related to the potassium persulfate concentration, a calibration curve was plotted on logarithmic axes for the ECL intensity and potassium persulfate concentration on the Ru-AuNPs@GCE. The ECL intensity presented good linearity with the potassium persulfate concentration ranging from 1.0×10^{-6} to 2.0×10^{-3} mol L⁻¹ with a correlation coefficient of 0.9953. The detection limit ($S/N = 3$) for potassium persulfate in the present approach was 5×10^{-7} mol L⁻¹.

To evaluate the long term stability of Ru-AuNPs@GCE, a Ru-Nafion@GCE electrode was prepared and used for ECL comparison. Under the same experimental conditions, it was found that the ECL intensity did not reach a stable state during the potential scan (Fig. 7a solid line), indicating Ru(bpy)₃²⁺ leakage from the electrode. Another experimental result showed that the ECL intensity of Ru-Nafion@GCE was dramatically decreased

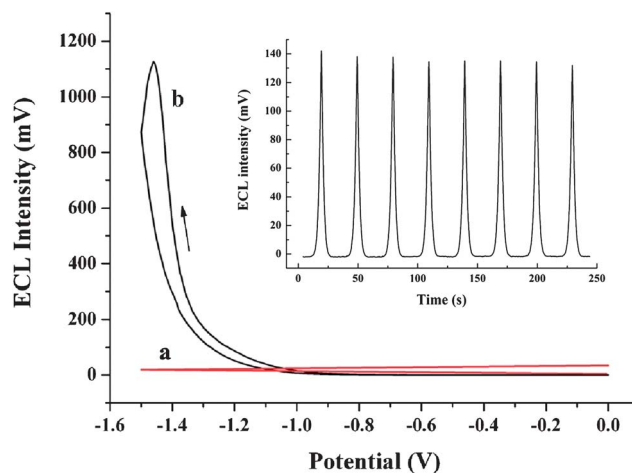


Fig. 6 ECL responses during a cathodic process. Electrolyte: (a) 0.1 mol L⁻¹ Na₂SO₄, (b) 0.1 mol L⁻¹ Na₂SO₄ containing 0.1 mol L⁻¹ K₂S₂O₈, and the working stability of the Ru-AuNPs@GCE during continuous CV scanning (inset figure).

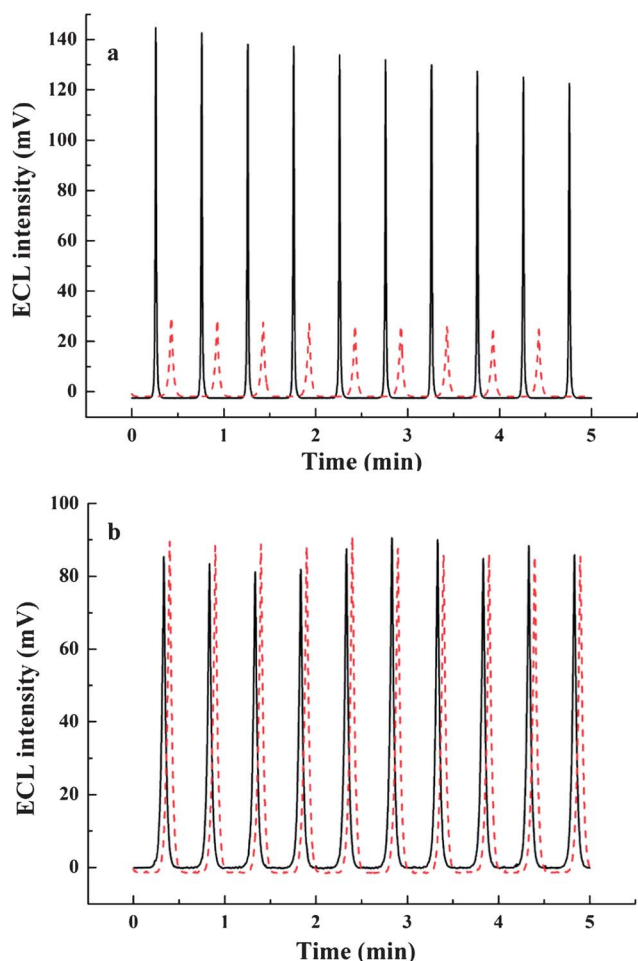


Fig. 7 Long term stability of different electrodes. (a) Ru-Nafion@GCE; (b) Ru-AuNPs@GCE. Dashed line is the ECL response of different electrodes after storage for one week.

after one week of storage (Fig. 7a dashed line). It is reported that $\text{Ru}(\text{bpy})_3^{2+}$ migrates into the hydrophobic area of the Nafion film and becomes electroinactive.²¹ As a result, the ECL intensity of Ru-Nafion@GCE is decreased. In contrast, the ECL intensity of Ru-AuNPs@GCE remained very stable during CV scanning (Fig. 7b solid line) and remained at almost the same intensity after one week's storage (Fig. 7b dashed line). These results indicated that the long term stability of the Ru-AuNPs@GCE was much better than that of the Ru-Nafion@GCE. This approach fabricated a ECL sensor with a chemically stable monolayer of $[\text{Ru}(\text{bpy})_2(\text{phen-NH}_2)_2]^{2+}$ and a sensitive ECL response, since the $[\text{Ru}(\text{bpy})_2(\text{phen-NH}_2)_2]^{2+}$ is lower solubility than that of $\text{Ru}(\text{bpy})_3^{2+}$ in aqueous solution and it is in the monolayer made direct contact with the analytes and electrolyte.

4. Conclusions

In conclusion, an ECL sensor was fabricated based on a covalent bond immobilization approach. $[\text{Ru}(\text{bpy})_2(\text{phen-NH}_2)_2]^{2+}$ was attached to a AuNPs@GCE by condensation of the amino and carboxylic groups of MA, after mercaptolization of the GCE. Because the $[\text{Ru}(\text{bpy})_2(\text{phen-NH}_2)_2]^{2+}$ monolayer was

directly immobilized on the electrode surface without any other immobilization material, the mass transportation and electron transfer hindrance was negligible and the stability was obviously increased, compared to the traditional ECL sensor fabricated using the polymer technique. This sensor was applied in cathodic ECL analysis and presented its performance with longer usage time due to the lower solubility of $[\text{Ru}(\text{bpy})_2(\text{phen-NH}_2)_2]^{2+}(\text{ClO}_4)_2$ in aqueous solution. The sensor presented a good linear range from $1.0 \times 10^{-6} \text{ mol L}^{-1}$ to 0.1 mol L^{-1} and a detection limit of $5 \times 10^{-7} \text{ mol L}^{-1}$ for potassium persulfate.

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