Short communication

A novel electrochemiluminescence sensor based on bis(2,2′-bipyridine)-5-amino-1,10-phenanthroline ruthenium(II) covalently combined with graphite oxide

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ABSTRACT

This communication reports a novel electrochemiluminescence (ECL) sensor based on covalently linking bis(2,2′-bipyridine)-5-amino-1,10-phenanthroline ruthenium(II) (Ru(II)-NH₂) with graphite oxide (GO) on a glassy carbon electrode. 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride and N-hydroxy-succinimide were applied to activate the carboxyl groups on the GO surface and catalyze the formation of amido link between Ru(II)-NH₂ and carboxyl groups on GO. The composite film was characterized using atomic force microscopy, transmission electron microscopy and Fourier transform infrared absorption spectroscopy. Based on ECL experimental results, the composite film modified electrode displayed high electrochemical activity towards the oxidation of 2-(dibutylamino) ethanol (DBAE). Under optimized conditions, the linear response of ECL intensity to DBAE concentration was valid in the range $6.0 \times 10^{-7} – 2.0 \times 10^{-4}$ mol L^{-1} ($r^2 = 0.9948$) with a detection limit (S/N = 3) of 5.0×10^{-8} mol L^{-1}. Furthermore, the ECL sensor presented good characteristics in terms of stability and reproducibility, promising the development of ECL sensors for biologically important compounds.

1. Introduction

Graphite oxide (GO), an exfoliated product from graphite powder, has attracted tremendous attention recently (Chen et al., 2009a,b; Cote et al., 2009; Wang et al., 2009). This novel material usually consists of graphene-like sheets, functional groups such as carboxyl, hydroxyl and epoxy groups (Kosynkin et al., 2009; Lv et al., 2009). The generation of such functional groups enhances the reactivity and specificity and provides an avenue for further chemical modification of GO such as ion adsorption (Xu et al., 2008), metal deposition (Scheuermann et al., 2009), and the grafting reaction (Lu et al., 2009). GO has rapidly become a promising material in chemical communities including electrochemiluminescence (ECL) (Fan et al., 2009; Li et al., 2009a,b).

Recently, ruthenium(II) compounds (expensive ECL reagents) have usually acted as immobilizers in solid state ECL due to their high ECL efficiency, good electrochemical stability, and wide linear range (Wei et al., 2007). Various methods have been attempted in ECL studies, including physical incorporation of ruthenium(II) compounds into various sol–gel composites (Choi et al., 2003), nanomaterial-based films (Zhou et al., 2008), polymer films (Guo and Dong, 2004), or using the Langmuir–Blodgget technique (Zhang and Bard, 1988). However, such ECL approaches have several drawbacks, such as lacking long-term stability due to the leakage of the luminophore, or low sensitivity because of the poor conductivity of silica materials. To overcome these problems, covalent bonding of ruthenium(II) compounds onto an electrode surface is advocated in other reports (Tao et al., 2008; Li et al., 2009a,b).

On the other hand, 2-(dibutylamino) ethanol (DBAE) has been taken as a new ECL co-reactant substrate in recent reports. Owing to its high ECL efficiency, lower toxicity, greater solubility and less volatility than the other commonly co-reactant such as tripropylamine (TPrA), DBAE excites more and more interests in ECL studies (Liu et al., 2007). Zhang et al. (2008) extended the application field of QDs ECL from anhydrous solution to aqueous solution by introducing DBAE into CdTe NCs ECL. Xue et al. (2009) discovered dopamine could inhibit Ru(bpy)₃²⁺/DBAE system and developed a sensitive ECL approach for the determination of dopamine. DBAE

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has been proved to be an excellent ECL co-reactant substrate, which expands it to be applied in bio- or colorimetric ECL sensors (Yin et al., 2009; Lin et al., 2009), and ECL mechanism study (Xu et al., 2007). In this report, bis(2,2′-bipyridine)-5-amino-1,10-phenanthroline ruthenium(II) (Ru(II)-NH$_2$) was covalently linked to GO on a glassy carbon electrode (GCE) surface as a novel ECL sensor. DBAE was taken as a representative analyte to investigate the performances of the ECL sensor. To further verify its extensive applications and reliability, the ECL sensor was applied in the determination of TPrA, proline (Pro), as well as the detection recovery of methamphetamine (METH) in urine samples. In addition, the effect of Ru(II)-NH$_2$ adsorbed on GO surface in electrochemical and ECL curves was also discussed.

2. Experimental

2.1. Materials

Ru(II)-NH$_2$ was synthesized and supplied by The University of Hong Kong; DBAE, TPrA, Pro, EDC and NHS were purchased from Aldrich Chem. Co. (Milwaukee, USA); METH was provided by Institute of Criminal Science and Technology (Xiamen, China); and the graphite powder was from Lvyin Co. (Xiamen, China). All other reagents were of analytical grade and were used without further purification. The pure water for solution preparation was from a Millipore autopure WR600A system (USA) and was used throughout.

2.2. Instrumentation

ECL measurements were performed using a system fabricated in our laboratory (Chen et al., 2009a,b). A three-electrode system was assembled using a GCE (diameter 3.0 mm, BAS Co. Ltd., Japan) coated with GO-Ru(II) composite film, a platinum auxiliary electrode and a silver reference electrode. In addition, all the potentials reported in this paper are referred to the SCE scale. Other instruments are presented in the supporting information in detail.

2.3. Procedures

Graphite oxide was prepared from graphite spheres (GS) following the modified Hummers' method (Hummers and Offeman, 1958). To coat GO-Ru(II) composite film onto a GCE, several microlitres of 10 mg mL^{-1} GO suspension were dropped on the GCE, and dried at room temperature. A versatile method using EDC and NHS linking reaction for covalently combined Ru(II)-NH$_2$ to GO was applied (Nanjo et al., 2005). The GO modified GCE (GO-GCE) was first dipped into phosphate buffer solution (PBS, pH 5.45) containing 2 mmol L^{-1} EDC and 5 mmol L^{-1} NHS for 2 h. In this section, the carboxylic acid groups at the edges of GO were activated for the condensation reactions. Then, the activated electrode was immersed in 5 mmol L^{-1} Ru(II)-NH$_2$ solution (pH 7.6) for 18 h at room temperature. After the generation of GO-Ru(II) composite film, the electrode was washed carefully to remove excess reagents (see the details in Scheme S1). Ru(II)-NH$_{2\text{ads}}$ could not be washed clearly due to the strong adsorbability of GO. In order to study this effect, another GO-Ru(II) composite without the activated procedure was prepared. In the following description, the two electrodes are named GO-EDC/NHS-Ru(II)-GCE and GO-Ru(II)-GCE, respectively.

3. Results and discussion

3.1. Surface state of the GO-EDC/NHS-Ru(II) composite film

Fig. 1A shows the atomic force microscopy (AFM) image of GO. The thickness of GO sheets was around 1.0–1.1 nm, indicating that the GO sheets are generally single layered. The length and width

dimensions were in the range of several micrometers in agreement with previous reports (Chen et al., 2009a,b). Fig. 1B is the transmission electron microscopy (TEM) image of GO dispersed in ethanol. Obviously, the GO sheet is a very flat thin layer. To provide further evidence that the oxygen-containing groups were generated and functionalized with Ru(II)-NH$_2$ on the GO surface, we performed Fourier transform infrared spectrometry (FTIR) analysis. As shown in Fig. 1C (line b), two new peaks at 1620 cm^{-1} (υ_s of C=C) and 1735 cm^{-1} (υ_s of >C=O) indicate that GO were terminated with carboxylic acid group (Kosynkin et al., 2009). After reaction with Ru(II)-NH$_2$ (line c), two vibration modes corresponding to stretching of acylamino present at 1299 cm^{-1} (C–N–H) and 927 cm^{-1} (C–N–C) implicated the formation of amide bond (Tao et al., 2008). These results are also supported by energy dispersive X-ray spectroscopy (EDX) analysis (Fig. S1). An obvious Ru peak was found, indicating that Ru(II)-NH$_2$ had been successfully attached to GO.

3.2. ECL behavior

The cyclic voltammogram (CV) method was used to compare and investigate the electrochemical behavior of the prepared GO-EDC/NHS-Ru(II)-GCE. As can be seen from Fig. 2A, in the potential scan region from 0 to 1.5 V, no visible electrochemical response was obtained on the bare or modified electrodes, except on the GO-EDC/NHS-Ru(II)-GCE. The CV curves of the novel electrode showed a reversible redox wave with an oxidation peak at 1.06 V and a reduction peak at 0.94 V due to the electrochemical transition between Ru(III) and Ru(II), which was analogous with the CV behavior of Ru(II)-NH$_2$ under the same conditions (the inset of Fig. 2A). This result demonstrated that Ru(II)-NH$_2$ had been successfully modified on GCE and the composite film showed excellent electrochemical activity in 0.05 mol L^{-1} PBS (pH 7.6).

To verify the feasibility of this novel composite film for determination of DBAE, we further studied the I_{ECL}/E curves with and without DBAE in 0.05 mol L^{-1} PBS (pH 7.6) using the GO-EDC/NHS-Ru (II)-GCE (Fig. 2B). As expected, both solutions showed ECL signals, the onset luminescence occurred at 0.98 V, and then rose steeply until it reached a maximum at +1.10 V, which was coincident with the oxidation potential of Ru(II)-NH$_2$. Furthermore, the ECL peak intensity was much brighter (ca. 20 folds) with the addition of DBAE, suggesting that the ECL signal at 1.10 V is applicable for the determination of DBAE.

However, in consideration of the strong adsorption ability of GO, the above-mentioned discussion was not sufficient to confirm that Ru(II)-NH$_2$ was covalently linked rather than absorbed on the GO surface. Therefore, another electrode, a GO-Ru(II)-GCE one, was prepared to study the adsorption effect. In addition, a fluorescence experiment was performed to ensure that adsorption onto the surfaces of the two electrodes was achieved to a saturation state. The detection model was set up as shown in Scheme S2. After the electrodes were immersed for 3 h, the fluorescence intensity of Ru(II)-NH$_2$ solution remained constant, illustrating that saturation adsorption was achieved on the electrodes (Fig. S2A). Moreover, the decrease of fluorescence intensity on the GO-EDC/NHS-GCE was larger than that on the GO-GCE, demonstrating that the covalent linking could increase the loading density of Ru(II)-NH$_2$. This result was also supported by the CV results (Fig. S2B). In this case, the redox peak currents on the GO-Ru(II)-GCE were smaller than those obtained from the GO-EDC/NHS-Ru(II)-GCE. In addition, it should be noted that the adsorption effect of Ru(II)-NH$_2$ on the GO surface provided an oxidation current of about 18 μA on the GO-Ru(II)-GCE. This was only half the value obtained from the GO-EDC/NHS-Ru(II)-GCE. To further study the adsorption effect on ECL intensity, we observed the ECL intensities using the two electrodes in PBS containing 3 × 10^{-5} mol L^{-1} DBAE. Interestingly, the ECL intensity from
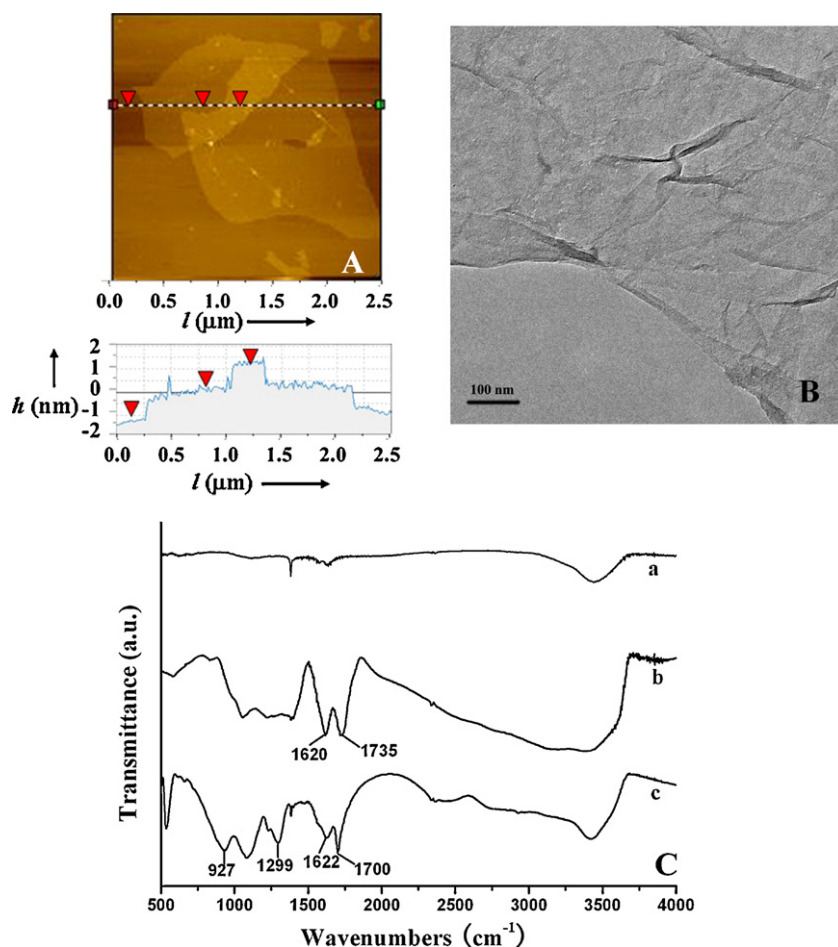
Fig. 1. (A) AFM height image of GO sheets deposited on mica substrate. (B) TEM image of GO sheets dispersed in ethanol. (C) FTIR spectra of GS (a), GO (b) and GO-Ru(II) composite (c).

the GO-EDC/NHS-Ru(II)-GCE was around 25 folds larger than that from the GO-Ru(II)-GCE. The conceivable (and likely to be acceptable) explanations were as follows: (1) the residual $\pi-\pi$ conjugated domains in the GO sheets contributed to the attraction of Ru(II)-NH$_2$, which caused a serious absorption influence on the CV curves and (2) the large $\pi-\pi$ conjugation effectively quenched the ECL intensity of Ru(II)-NH$_2$ absorbed by energy transfer.

3.3. Performance of the ECL biosensor for DBAE detection

The amount of GO coated on the electrode greatly affected the immobilization of Ru(II)-NH$_2$ as well as the corresponding ECL intensity (Fig. S3). Based on the experimental results, if the amount of GO was less than 5 μg, the composite film cracked easily from the electrode. Although more GO coated on the electrode meant more Ru(II)-NH$_2$ attached onto the GO surface, leading to higher ECL intensity, on the contrary, the ECL intensity was decreased when the GO amount was more than 30 μg, since the GO might have absorbed and scattered the ECL emission within the film. Moreover, pH values ranging from 6.8 to 8.0 were also selected to investigate the effect of pH on ECL emission. The strong pH dependence of ECL intensity indicated that deprotonation of DBAE was required for an efficient ECL emission from the modified electrode. As shown in Fig. S3, the ECL peak intensity reached a maximum value at pH 7.6, and so in the following detection, pH was kept at this level.

Under optimal experimental conditions, 30 μg GO were selected to provide high sensitivity and a stable ECL signal in 0.05 mol L^{-1} PBS (pH 7.6). A calibration curve was plotted for the ECL intensity and DBAE concentration using the GO-EDC/NHS-Ru(II)-GCE

(Fig. 3). The logarithm of ECL intensity presented a good linearity with the logarithm of DBAE concentration ranging from 6.0×10^{-7} to 2.0×10^{-4} mol L^{-1} with a correlation coefficient of 0.9948. The detection limit (S/N = 3) was 5.0×10^{-8} mol L^{-1}. As shown in the Fig. 3 inset, the relative standard deviation of the ECL response to 2.0×10^{-5} mol L^{-1} DBAE was 4.2% for ten successive measurements. The long-term storage stability of the present ECL sensor was studied over a month by monitoring its ECL response to 2.0×10^{-5} mol L^{-1} DBAE in 0.05 mol L^{-1} PBS (pH 7.6) with an intermittent usage (at 5-day intervals), and storage in the air at room temperature when not in use. It was found that the response of the ECL sensor gradually decreased to approximately 75% of its initial value within a month.

To further verify the extensive application and reliability of the ECL sensor, it was applied in the determination of TPrA, proline (Table S1), as well as the recovery of METH in urine samples. In the recovery test, urine samples were diluted 1000 folds with 0.1 mol L^{-1} phosphate buffer solution (PBS, pH 8.0) before adding various amounts of METH. As shown in Table S2, the results indicated that our ECL sensor could be used for the determination of METH, with satisfactory recoveries of 96–104% (see the details in supplementary materials).

3.4. ECL mechanism of Ru(II)-NH$_2$/DBAE

Recent study results reveal that the ECL of ruthenium(II) compounds can be generated through a route involving reaction between ruthenium(II) compounds and amine cation radicals. Liu et al. investigated Ru(bpy)$_3^{2+}$ ECL containing several co-reactant
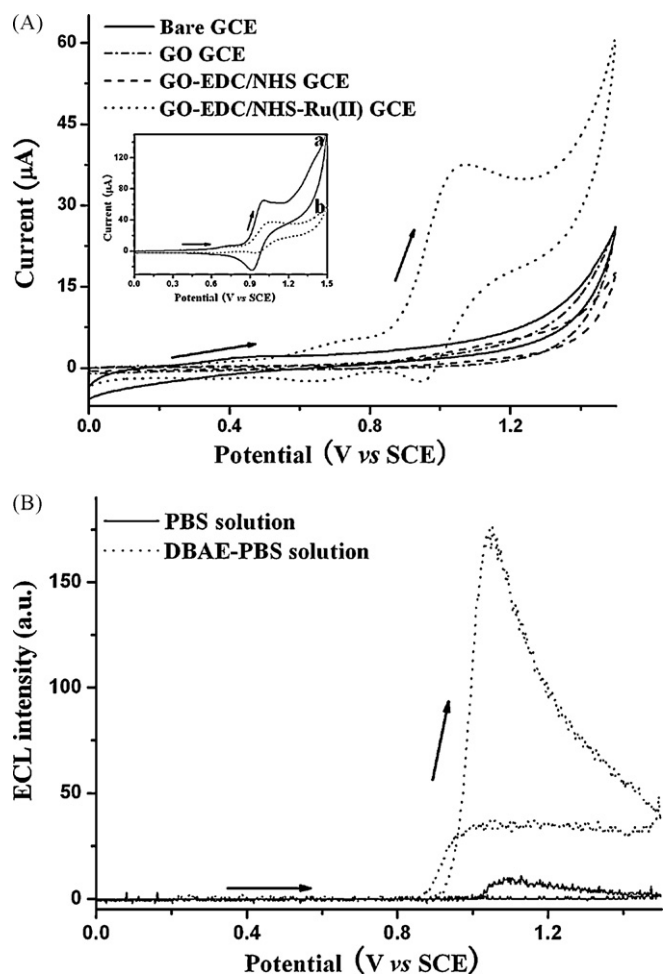
Fig. 2. (A) CV curves of PBS solution on different modified GCEs. The inset shows the CV curve of 1×10^{-3} mol L^{-1} Ru(II)-NH$_2$ on a bare GCE (a) and the CV curve of PBS solution on a GO-EDC/NHS-Ru(II)-GCE (b). (B) I_{ECL}/E curves in the solution with and without 3×10^{-5} mol L^{-1} DBAE on a GO-EDC/NHS-Ru(II)-GCE. Scan rate: 50 mV s^{-1}; pH 7.6; PBS: 0.05 mol L^{-1}; the amount of GO: 30 μg; negative high voltage of PMT: −800 V.
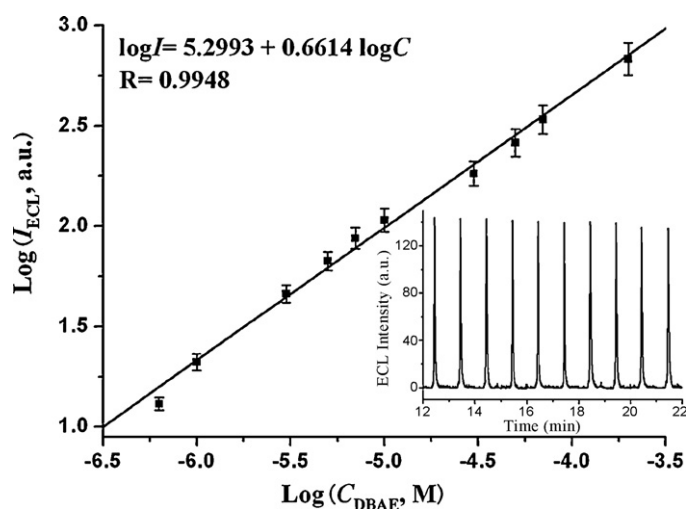

Fig. 3. Calibration curve of DBAE concentration and ECL intensity. The inset shows ECL emission of 2×10^{-5} mol L^{-1} DBAE on a GO-EDC/NHS-Ru(II)-GCE under continuous cyclic scans. Scan rate: 50 mV s^{-1}; pH 7.6; PBS: 0.05 mol L^{-1}; the amount of GO: 30 μg; negative high voltage of PMT: −800 V.

amines with different numbers of hydroxyl and amino groups and pointed out that electro-withdrawing hydroxyethyl groups could increase ECL intensities due to the fact that hydroxyethyl catalyzes the oxidation of amines (Liu et al., 2007). Since DBAE is a kind of aliphatic tertiary amine, and its molecular structure is similar to TPrA, the mechanism on Ru(II)-NH$_2$/DBAE was similar to that on Ru(II)-NH$_2$/TPrA (Richter, 2004). Generally, upon oxidation, the short-lived DBAE radical cation (DBAE$^{\bullet+}$) is supposed to lose a proton from an α-carbon to form the strongly reducing intermediate DBAE$^{\bullet}$ (Scheme S3). This radical then reduces Ru(III) to Ru(II)*, which causes luminescence occurrence when Ru(II)* decays to its ground state. Therefore, DBAE strongly increases the ECL emission of Ru(II)-NH$_2$.

4. Conclusions

In summary, this study proposed a novel ECL sensor for DBAE based on the covalent linking between GO and Ru(II)-NH$_2$. As a novel material, GO provides sufficient carboxyl groups for the formation of amido link with Ru(II)-NH$_2$, which makes the composite film displays high electrochemical activity towards the oxidation of DBAE. Moreover, owing to the excellent stability of GO and the activation effect of EDC and NHS, the composite film presented good characteristics in stability. It is the first time to introduce GO into ECL for high sensitive DBAE sensing. This approach provides a new facile route to construct effective ECL sensors, and may provide a promising way to detect biologically important compounds. Further experiments, such as the practical application of this novel ECL sensor and the construction of ECL labels for ultrasensitive bioanalysis, are underway.

Acknowledgements

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2010.07.083.

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