Full Paper

Study on Electrochemiluminesce of Ru(bpy$_3$)$^{2+}$ Immobilized in a Titania Sol-Gel Membrane

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Abstract
The immobilization of tris(2,2′-bipyridyl)ruthenium(II), Ru(bpy)$_3^{2+}$, at a glassy carbon electrode was achieved by entrapping the Ru(bpy)$_3^{2+}$ in a vapor deposited titania sol-gel membrane. The electrogenerated chemiluminescence (ECL) of the immobilized Ru(bpy)$_3^{2+}$ was studied. The Ru(bpy)$_3^{2+}$ modified electrode showed a fast ECL response to both oxalate and proline. The ECL intensity was linearly related to concentrations of oxalate and proline over the ranges from 20 to 700 μmol L^{-1} and 20 to 600 μmol L^{-1}, respectively. The detection limits for oxalate and proline at 3σ were 5.0 μmol L^{-1} and 4.0 μmol L^{-1}, respectively. This electrode possessed good precision and stability for oxalate and proline determinations. The electrogenerated chemiluminescence mechanism of proline system was discussed. This work provided a new way for the immobilization of Ru(bpy)$_3^{2+}$ and the application of titania sol-gel membrane in electrogenerated chemiluminescence.

Keywords: Electrochemiluminescence, Ru(bpy)$_3^{2+}$, Chemically modified electrodes, Titania sol-gel

1. Introduction

Electrogenerated chemiluminescence or electrochemiluminescence (ECL), described as chemiluminescence (CL) produced directly or indirectly due to electrochemical reactions [1], has been extensively exploited in analytical chemistry. It not only retains the advantages of sensitivity and selectivity inherent to conventional CL methodology, but also has several advantages over CL in that fewer reagents are needed, the reactions can be easily controlled and monitored [2]. Many compounds such as ruthenium, osmium, platinum and palladium complexes have been used to produce ECL [3]. Among these compounds, tris(2,2′-bipyridyl)ruthenium(III) (Ru(bpy)$_3^{3+}$), whose visible light generated upon reduction was first reported by Hercules and Lytle in 1966 [4], is the most studied ECL compound. It is of particular advantages such as excellent stability, convenient immobilization and electrochemical generation for ECL detection [2, 5–7], thus has been used for determinations of organic acid [8, 9] and a variety of amines containing analytes [10–12].

The immobilization of reaction reagents can avoid external addition of reagents in analytical procedure and is a comfortable way to overcome the limitation of reagent consumed and to achieve a totally "solid-state" scheme, which makes the miniaturization of the configuration easier [6, 13]. Up to now, several methods have been developed to immobilize Ru(bpy)$_3^{2+}$ on electrode surfaces for ECL detection [5, 6, 14–17]. Nafion film was the first used matrix for this purpose [5]. It has been used for ECL measurements of NADH, oxalate or antibiotics [6] and for the development of enzymatic biosensors [14]. However, this film has the drawback of the slow mass transfer through the film and the partition of Ru(bpy)$_3^{2+}$ into the more hydrophobic regions of Nafion [5, 15]. An improved method is to incorporate Ru(bpy)$_3^{2+}$ into the Nafion-silica nanocomposites [16] and Eastman-AQ55D-silica composite thin-film [15]. These modified films exhibit faster response, higher sensitivity and better stability. Ru(bpy)$_3^{2+}$ has also been immobilized on electrode surfaces by Langmuir–Blogett technique [17, 18], self-assembly technique [19, 20] and other polymers such as chitosan [21] and polyvinyl pyrrolidone [22].

As is well known, sol-gel materials are frequently used membranes for the noncovalent immobilization of molecules due to their tunable porosity, high photochemical and thermal stability, chemical inertness and negligible swelling in aqueous and non-aqueous solutions [23]. Few works on the application of sol-gel membranes in ECL detection have been reported, though silica sol-gel has been used for fabrication of a type of optical fiber biosensor based on luminol electrochemiluminescence by immobilization of glucose oxidase [24]. Titania sol-gel is one kind of nonsilica materials and is of great interest for potential applications in photovoltaic cell [25], electrochemical photolysis of water and semiconductor [26]. This material can be easily obtained with a simple vapor deposition process [27]. This work tries to use titania sol-gel as a matrix for immobilization of Ru(bpy)$_3^{2+}$ on electrode surface. This modified electrode shows good stability and fast response for ECL detection of oxalate, which was usually used to examine the ECL characteristic of immobilized Ru(bpy)$_3^{2+}$ such as those

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immobilized in Nafion [6], Nafion-Silica composite films [28] and Eastman-AQ55D-silica composite thin-film [15]. This work also examines the response of this modified electrode to proline. This is the first application of titania sol-gel membrane in electrogenerated chemiluminescence.

2. Experimental

2.1. Chemicals

Tris(2,2′-bipyridyl)dichlororuthenium hexahydrate was obtained from Aldrich and used without further purification. Titanium(IV) isopropoxide ($Ti(i\text{-}PrO)_4$, 98 + %) was from Acros Organics (New Jersey, USA). Sodium oxalate and L-proline were purchased from Shanghai Chemical Reagent Factory and Shanghai Biotechnology Co. Ltd. (Shanghai), respectively. All other reagents were of analytical grade, and twice-distilled water was used throughout.

2.2. Electrode Preparation

Prior to modification, glassy carbon electrode (diameter of 3 mm) was polished with 1.0, 0.3 and 0.05 μm alumina slurry (Beuhler), respectively, and rinsed thoroughly with doubly distilled water between each polishing step. The electrode was then successively sonicated in 1 : 1 nitric acid and doubly distilled water, and then allowed to dry at room temperature.

Titania sol-gel and $Ru(bpy)_3^{2+}$/titania sol-gel membranes were prepared as described previously [27]. Briefly, 2.5 μL twice-distilled water or 50 mmol L^{-1} $Ru(bpy)_3^{2+}$ solution was dropped onto the surface of a pretreated glassy carbon electrode. The electrode was then suspended vertically above titanium isopropoxide in a sealed flask kept at 35 °C for 2 h. This resulted in absorption of saturated titanium isopropoxide vapor by the solution and slow formation of a titania sol-gel or $Ru(bpy)_3^{2+}$/titania sol-gel membrane through hydrolysis of titanium isopropoxide on the surface. Before each experiment the modified electrode was rinsed thoroughly with doubly distilled water and was cyclically swept in the potential range from + 0.6 to + 1.4 V in 0.1 mol L^{-1} pH 6.3 phosphate buffer solution until a reproducible voltammogram was obtained.

2.3. Instruments

ECL experiments were carried out on a CHI 812 electrochemical analyzer (Shanghai, China) at room temperature with a three-electrode cell consisting of a modified electrode, a Ag/AgCl electrode (sat. KCl) and a platinum electrode as working, reference and counter electrodes. The ECL reaction cell was set in front of the detection window of photomultiplier tube (PMT) that was biased at 800 V, the ECL emission was recorded with a CHI 812 electrochemical analyzer (Shanghai, China) and a flow injection chemiluminescence analyzer (IFFL-D) (Xi'an Remax Electronic Science-Tech Co. Ltd.) controlled by a personal computer.

3. Results and Discussion

3.1. Electrochemistry of $Ru(bpy)_3^{2+}$/Titania Sol-Gel Modified Electrode

Figure 1 shows the cyclic voltammograms of $Ru(bpy)_3^{2+}$/titania sol-gel modified electrode at various scan rates. The immobilized $Ru(bpy)_3^{2+}$ displayed a couple of redox peaks at + 1.116 and + 1.040 V at 0.1 V s^{-1}, indicating its electroactivity after entrapped in the titania sol-gel membrane. This diagram also demonstrated that changing the applied potential could control the form of the immobilized ECL reagent. That was, if the potential was at the values more positive than + 1.116 V, the reagent would be in the oxidized form, $Ru(bpy)_3^{3+}$, and capable of producing CL when it reacted with the analyte; if the potential was then taken at more negative values than + 1.040 V, the reagent was reduced to its original form, $Ru(bpy)_3^{2+}$, which did not yield CL when in contact with the analyte. Moreover, as shown in inset of Figure 1, the reduction peak current was proportional to the scan rate in the range from 0.05 to 0.4 V s^{-1}, indicating that the electrode reaction of the immobilized $Ru(bpy)_3^{2+}$ was a surface-controlled process.

3.2. Electrochemiluminescence of Oxalate-Immobilized $Ru(bpy)_3^{2+}$ System

After successful immobilization of $Ru(bpy)_3^{2+}$ in titania sol-gel membrane, we first chose oxalate as the analyte to investigate its analytical application. Upon addition of

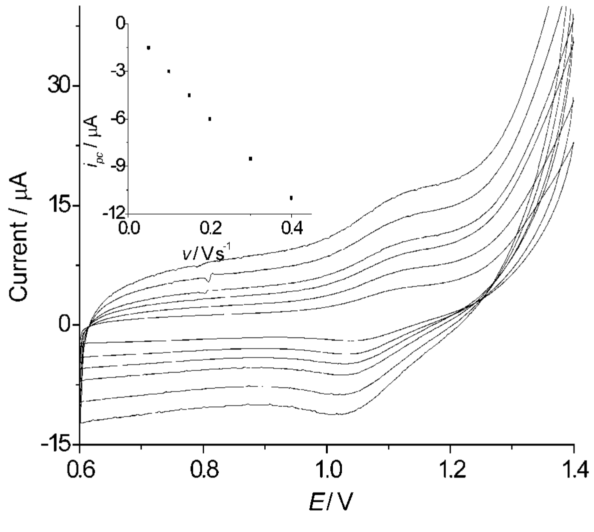

Fig. 1. Cyclic voltammograms of $Ru(bpy)_3^{2+}$/titania sol-gel modified electrode at 0.05, 0.1, 0.15, 0.2, 0.3 and 0.4 V s^{-1} (from inner to outer) in 0.1 mol L^{-1} pH 6.3 phosphate buffer. Inset: dependence of cathodic peak current on scan rate.

oxalate into pH 6.3 phosphate buffer the anodic current of the cyclic voltammogram of Ru(bpy)$_3^{2+}$/titania sol-gel modified electrode showed an obviously increase, while the cathodic current decreased (curves a and b in Fig. 2A). The changes in the cathodic and anodic currents at Ru(bpy)$_3^{2+}$/ titania sol-gel modified electrode were much greater than those at titania sol-gel modified electrode (curves c and d in Fig. 2A), indicating a chemical reaction occurring between oxalate and the oxidized form of Ru(bpy)$_3^{2+}$, Ru(bpy)$_3^{3+}$, which led to the electrogeneration of Ru(bpy)$_3^{2+}$ during the cyclic sweep, i.e., composing an EC electrocatalytic cycle [29]. At the same time, a corresponding ECL signal was observed in the anodic process (Fig. 2B). The ECL intensity-potential curve displayed a steep increase around the anodic peak potential of the immobilized Ru(bpy)$_3^{2+}$. No ECL signal was observed before the potential reached the oxidation peak potential.

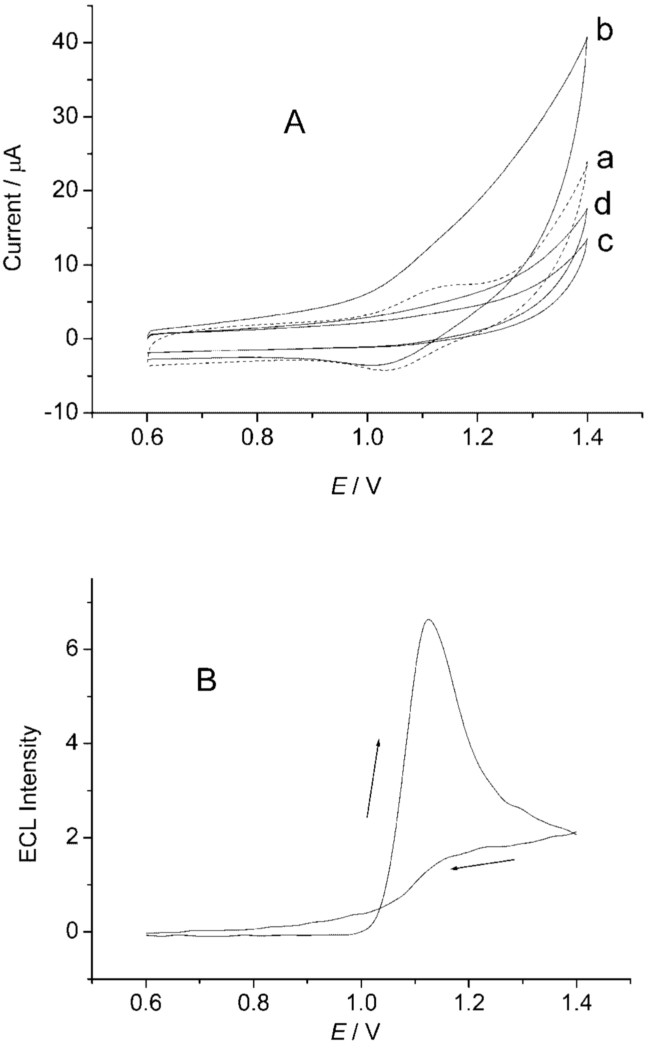

Fig. 2. Cyclic voltammograms (A) of Ru(bpy)$_3^{2+}$/titania sol-gel (a, b) and titania sol-gel modified electrodes (c, d) in pH 6.3 phosphate buffer (a, c) and pH 6.3 phosphate buffer containing 0.4 mmol L^{-1} Na$_2$C$_2$O$_4$ (b, d) at 0.1 V s^{-1} and corresponding ECL intensity-potential curve for curve b (B).

With an increasing pH value the ECL intensity increased and reached a maximum value at pH 6.3. At pH 6.3 the ECL intensity increased linearly with the increasing C$_2$O$_4^{2-}$ concentration over the range from 20 to 700 μmol L^{-1} with a correlation coefficient of 0.9948. From the slope of 0.0146 μmol^{-1} L and the value of three times noise, the detection limit was obtained to be 5.0 μmol L^{-1}. The relative standard deviation (RSD) for 7 repetitive determinations of ECL intensity at 100 μmol L^{-1} C$_2$O$_4^{2-}$ was 2.8%, indicating a good precision.

3.3. Electrochemiluminescence of Proline-Immobilized Ru(bpy)$_3^{2+}$ System

The Ru(bpy)$_3^{2+}$ ECL system has been used for highly sensitive detection of amino acids [30, 31]. The reaction between Ru(bpy)$_3^{3+}$ and proline can produce strong CL response [30]. Nieman et al. [32] used proline to examine the effect of hydrodynamics on Ru(bpy)$_3^{2+}$ ECL system in which Ru(bpy)$_3^{2+}$ was immobilized in Nafion at a glassy carbon electrode. However, this film showed instability and the lack of reproducibility among different film preparations. In this work the ECL of proline-Ru(bpy)$_3^{2+}$ system was also used to check the validity of the immobilization method of Ru(bpy)$_3^{2+}$ in a vapor deposited titania sol-gel membrane. The cyclic voltammograms of the immobilized Ru(bpy)$_3^{2+}$ in absence and presence of proline are shown in Figure 3A. Upon addition of proline into the buffer the shape of the cyclic voltammogram of Ru(bpy)$_3^{2+}$/titania sol-gel modified electrode showed an obvious change. The anodic current increased, while the cathodic current decreased. At titania sol-gel modified electrode the increase in anodic current was also observed. The increased anodic current was slightly smaller than that at the Ru(bpy)$_3^{2+}$/ titania sol-gel modified electrode, which was different from that of the oxalate – immobilized Ru(bpy)$_3^{2+}$ system. Obviously, proline could be oxidized at the modified electrodes. On the other hand, the corresponding ECL intensity–potential curve (Fig. 3B) also showed a difference from that of oxalate-immobilized Ru(bpy)$_3^{2+}$ system. According to the results reported for the ECL of tripropylamine-Ru(bpy)$_3^{2+}$ system [33], the ECL mechanism could be expressed as follows:

$$\mathrm{Ru(bpy)_3^{2+}} \rightarrow \mathrm{Ru(bpy)_3^{3+}} + \mathrm{e^-} \tag{1}$$

$$\text{proline} \rightarrow \text{proline}^{\bullet+} + \mathrm{e^-} \tag{2}$$

$$\text{proline}^{\bullet+} \rightarrow \text{proline}^{\bullet} + \mathrm{H^+} \tag{3}$$

$$\text{proline}^{\bullet} + \mathrm{Ru(bpy)_3^{3+}} \rightarrow \text{proline fragments} + \mathrm{Ru(bpy)_3^{2+*}} \tag{4}$$

$$\mathrm{Ru(bpy)_3^{2+*}} \rightarrow \mathrm{Ru(bpy)_3^{2+}} + h\nu \tag{5}$$

At electrode surface the immobilized Ru(bpy)$_3^{2+}$ was oxidized to Ru(bpy)$_3^{3+}$, while proline was also oxidized to

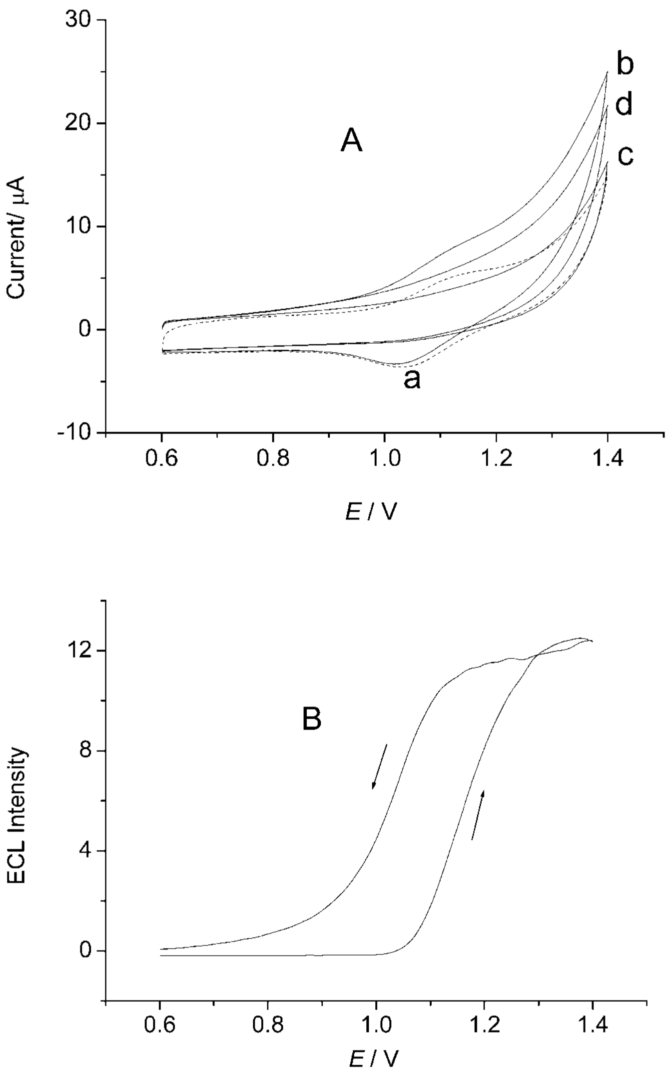

Fig. 3. Cyclic voltammograms (A) of $Ru(bpy)_3^{2+}$/titania sol-gel (a, b) and titania sol-gel modified electrodes (c, d) in pH 6.3 phosphate buffer (a, c) and pH 6.3 phosphate buffer containing 0.3 mmol L^{-1} proline (b, d) at 0.1 V s^{-1} and corresponding ECL intensity-potential curve for curve b (B).

produce a cation radical, proline$^{\bullet +}$. After deprotonation the uncharged radical, proline$^{\bullet}$, was further oxidized to form an energetically exited state of $Ru(bpy)_3^{2+}$, which produced light emission.

Data obtained for analyte determinations at various pH values showed the maximum ECL intensity occurred at pH 6.3, which was close to the isoelectric point of 6.1 for proline [34]. To examine the effect of proline concentration on the ECL intensity, the ECL intensity was tested at the concentration range from 20 to 1100 μmol L^{-1}. The ECL intensity displayed a linear increase over the concentration range of proline from 20 to 600 μmol L^{-1} with a correlation coefficient of 0.9955. The linear regression equation was $y = 0.0288 + 0.0351x$ (x: μmol L^{-1}). From the slope and the value of three times the noise, the detection limit for proline was 4.0 μmol L^{-1}. The RSD for 7 repetitive determinations of ECL intensity at 400 μmol L^{-1} proline was 5.2%.

3.4. Electrochemical Stability of Immobilized $Ru(bpy)_3^{2+}$

The good stability of the vapor deposited titania and protein/titania sol-gel membranes was demonstrated in our previous work [27]. When the $Ru(bpy)_3^{2+}$/titania sol-gel modified electrode was continuously cycled in phosphate buffer in the potential range from 0.6 to 1.4 V at 0.1 V s^{-1} for 15 min and 30 min, only 3.69% and less than 9.01% losses in Faradaic peak current were observed. The stability was better than that immobilized in a silica film fabricated from the acid-catalyzed hydrolysis of TMOS [35, 36], in which the cyclic sweep resulted in a loss of the immobilized $Ru(bpy)_3^{2+}$ in a relatively short period of time, and similar to the results observed at Nafion-silica composite films [28]. Thus, the electrochemical stability of $Ru(bpy)_3^{2+}$ immobilized in titania sol-gel membrane was also good. The good stability could be attributed to the strong interaction between $Ru(bpy)_3^{2+}$ and the negative-charged group in titania sol-gel film.

4. Conclusions

The electrogenerable chemiluminescent reagent, $Ru(bpy_3)^{2+}$, has been successfully immobilized at a glassy carbon electrode by a vapor deposited titania sol-gel membrane. Its analytical application has been demonstrated by the ECL detections of oxalate and proline. One of the major advantages offered by this modified electrode is the drastic decrease in the amount of the expensive $Ru(bpy_3)^{2+}$ needed for analyses. This work provided a new way for the immobilization of $Ru(bpy)_3^{2+}$ and the application of titania sol-gel membrane in electrogenerated chemiluminescence. Our previous work [27] has demonstrated the titania sol-gel material was a suitable matrix for enzyme immobilization on an electrode surface, so it is possible to coimmobilize $Ru(bpy_3)^{2+}$ and dehydrogenase enzymes or other biomolecules on the electrode surface for its further application.

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