Preparation of Nafion-Ru(bpy)₃²⁺-Chitosan/Gold Nanoparticles Composite Film and Its Electrochemiluminescence Application

Yingjuan QU, Xiaoli LIU, Xingwang ZHENG,[†] and Zhihui GUO

School of Chemistry & Chemical Engineering, Shaanxi Normal University, Xi'an 710062, P. R. China

In this research, it was found that a composite film could be formed by mixing Nafion with chitosan on the graphite electrode surface. Then, based on the strong absorption of both chitosan for AuCl₄⁻ and Nafion for Ru(bpy)₃²⁺, respectively, the gold nanoparticles and electrochemiluminescence (ECL) active molecules, Ru(bpy)₃²⁺, were effectively incorporated into the composite film of chitosan with Nafion. Lastly, based on the interaction of Ru(bpy)₃²⁺ with Nafion inside the Nafion-chitosan/gold nanoparticles film, Ru(bpy)₃²⁺ was successfully immobilized within this composite film. In this case, a new composite film for fabricating Ru(bpy)₃²⁺-based ECL sensors was developed. The performances of this composite film were characterized by transmission electron microscopy (TEM), electrochemistry and electrochemiluminescence methods. Our results showed that: firstly, the gold nanoparticles in the resulting composite film could act as conducting pathways to connect Ru(bpy)₃²⁺ sites; the electrode surface accelerated the charge transport through the composite film, and the diffusion coefficient of Ru(bpy)₃²⁺ within this composite film modified electrode was 65 times higher than that of the pure Nafion film modified electrode. Secondly, due to its unique polymeric cationic character and better film-forming properties, chitosan could improve the compact structure of pure Nafion and greatly enhance the mass-transfer speed of Ru(bpy)₃²⁺. Then, the co-reactant tripropylamine (TPA) inside the composite film could offer better ECL performances such as more rapid ECL response speed, longer-term stability and higher sensitivity compared with the performances of pure Nafion film.

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Introduction

Ru(bpy)₃²⁺ electrogenerated chemiluminescence (ECL) has attracted much attention as a powerful analytical tool due to its inherent sensitivity, selectivity, and wide linear dynamic range for amine containing analytes such as amino acids, NADH, and a variety of pharmaceutical compounds.¹ In order to develop cost-effective, simple and regenerable ECL-based chemical sensors, biosensors, and detection devices in flowing streams, much effort had been focused on the immobilization of Ru(bpy)₃²⁺ on different electrode surfaces.² To date, many approaches had been developed to immobilize Ru(bpy)₃²⁺ and its derivatives onto a variety of different electrode surfaces. For example, the immobilization of Ru(bpy)₃²⁺ in polymer layers on electrode surfaces,³⁻⁶ the direct attachment to an electrode as a monolayer *via* Langmuir-Blodgett techniques,⁷⁻¹⁰ self-assembly techniques¹¹⁻¹³ and layer-by-layer (LBL) technique^{14,15} had been achieved. However, because of the instability at positively biased potentials and possible desorption in organic solvent, these techniques cannot be applied widely. Moreover, the incorporation of Ru(bpy)₃²⁺ within perfluorosulfonated ionomer Nafion films^{16,17} had received considerable attention. Such an ECL sensor was used not only for the determination of oxalate, amines, NADH, and antibiotics, but also for the enzymatic biosensors to determine glucose or ethanol.¹⁸ Although the Ru(bpy)₃²⁺ ECL sensor based on the Nafion matrix can be easily

fabricated, it suffered the drawbacks of slow mass-transfer and unstability because the immobilized Ru(bpy)₃²⁺ migrates into the electroinactive hydrophobic region with time.^{4,17} So researchers had committed themselves to look for new materials and immobilization methods to fabricate ECL sensors in order to increase the sensitivity and stability. Sensors such as carbon nanotube/Nafion,¹⁴ Nafion-silica,¹⁹ Nafion-titania,²⁰ and Nafion-zirconia composite film²¹ had been reported. Several recent papers concerned with the use of nanomaterials to fabricate ECL sensors were reported.²²⁻²⁴ Dong's group developed an advanced immobilization method involving doping Ru(bpy)₃²⁺ into silica nanoparticles based on the microemulsion method,²⁵ and Ru(bpy)₃²⁺-doped SiO₂@MWNTs coaxial nanocable.²⁶ The Ru(bpy)₃²⁺-doped new materials and the composite containing carbon nanotube exhibited outstanding advantages of both good stability and high sensitivity compared with the previous reported ECL sensors. Although all reported ECL sensors exhibited improved ECL characteristics compared to pure Nafion films, new materials and immobilization methods for the higher sensitivity and stability of ECL sensors are still needed.

Nafion contains a hydrophobic domain or phase composed of fluorocarbon skeleton and anionized sulfonate group,²⁷ it has been known for its good performance as a cation exchange membrane and for presenting better thermal stability.²⁸ It possesses large ion-exchange selective coefficient for cation metal complexes, especially the large ones such as Ru(bpy)₃²⁺, Fe(bpy)₃²⁺, Os(bpy)₃²⁺, Co(bpy)₃²⁺, Co(terpy)₃²⁺. The ECL behaviors of Ru(bpy)₃²⁺ in pure Nafion films and some other composite films containing Nafion had been widely

[†] To whom correspondence should be addressed.
E-mail: zhengxw@snnu.edu.cn

investigated.^{29,30} The previous results showed that Nafion was an effective ion exchanger for $\text{Ru}(\text{bpy})_3^{2+}$. On the other hand, chitosan is a polysaccharide biopolymer, which displays excellent film-forming ability, high water permeability, and good adhesion to the electrode surfaces.^{31,32} If the chitosan was dissolved in aqueous acidic medium ($\text{pH} < 6.3$), it took the positive charge based on the $-\text{NH}_3^+$ groups. However, up to now, no research work has reported if chitosan could be incorporated into Nafion and could form the composite film.

In this paper, we report a new and simple method for the fabrication of $\text{Ru}(\text{bpy})_3^{2+}$ -ECL sensor using Nafion-chitosan (CHIT)/gold nanoparticles (GNPs) composite film. Cation-exchanger Nafion with CHIT (electropositive in acidity) could form a composite film with an appropriate volume ratio. $\text{Ru}(\text{bpy})_3^{2+}$ can be immobilized through the ion exchange function of Nafion, and AuCl_4^- can be absorbed within the composite film *via* electrostatic interaction with CHIT. After being reduced by NaBH_4 , GNPs can be *in-situ* produced and stably distributed in the composite films. The electrochemical behavior and ECL characteristics of the proposed ECL sensor based on $\text{Ru}(\text{bpy})_3^{2+}$ immobilized in Nafion-CHIT/GNPs composite film-modified graphite electrode were evaluated. Compared with pure Nafion-modified electrode, the composite films-modified electrode had fast mass transfer, charge transport speed and long-term stability. In the proposed composite films, CHIT acted as a channel for the mass transfer of $\text{Ru}(\text{bpy})_3^{2+}$ and co-reactant tripropylamine (TPA). GNPs acted as conducting pathways to connect $\text{Ru}(\text{bpy})_3^{2+}$ sites to the electrode and accelerated the charge transport through the composite film.

Experimental

Apparatus

Cyclic voltammetric experiments were performed by using a CHI660C electrochemical working station (Chenhua Inc., China). All experiments were carried out with a conventional three-electrode system in a 10-mL electrochemical cell. The working electrode was a spectroscopic graphite electrode (Shanghai Carbon Works; diameter, 6 mm) coated with the composite films, and a platinum wire was the counter electrode. All the potentials quoted here were relative to a Ag/AgCl (Saturated KCl) reference electrode. Electrogenated chemiluminescence studies were performed using a MCDR-A ECL analytical system (Xi'an Remax Electronic Science Tech. Co. Ltd., Xi'an, China). The applied potential for electrolysis was achieved by the combination of a DJS-292 potentiostat with a XFD-8B ultra-low frequency signal generator. The ECL intensity was transformed into an electrical signal by an R456 photomultiplier (PMT) (Hamamatsu), which was operated at -700 V. The ECL cell was placed directly in front of the PMT window and was enclosed in a lighttight box. The sizes of GNPs were measured using a JEM-2100 transmission electron microscope (TEM) operated at an accelerating voltage of 200 kV.

Chemical and solutions

Nafion (perfluorinated ion-exchange resin, Fluka, purum 5% (w/v)). A 0.5% solution was prepared by dissolving Nafion in anhydrous ethanol, and chitosan (CHIT, medium molecular weight) was obtained from Sigma. CHIT solution 0.5% (w/v) was prepared by dissolving 0.5 g CHIT in 1% HAc with ultrasonication. $\text{HAuCl}_4 \cdot 4\text{H}_2\text{O}$ was obtained from Shanghai Reagent (Shanghai, China). A 0.1% HAuCl_4 stock solution was prepared by dissolving 0.1 g of HAuCl_4 in 100 mL of redistilled

Fig. 1 TEM of Nafion-CHIT/GNPs film on carbon-copper grid.

water and was stored at 4°C . A 1.0×10^{-3} mol/L stock solution of $\text{Tris}(2,2'\text{-bipyridyl})$ ruthenium(II) ($\text{Ru}(\text{bpy})_3^{2+}$) was prepared by dissolving a suitable amount of $\text{Ru}(\text{bpy})_3^{2+}$ (Sigma) in redistilled water without further purification; 1.0×10^{-2} mol/L tripropylamine (TPA 99%) stock solution was prepared by dissolving TPA in sodium phosphatic buffer. All other reagents were of analytical grade and the aqueous solutions were prepared with redistilled water.

Preparation of the composite film modified electrode

Firstly, a suitable amount of Nafion and CHIT was mixed homogeneously with an ultrasonic mixer. Then, 20 μL of mixing solution of Nafion-CHIT was hand-coated on the graphite electrode and the solvents were allowed to evaporate at room temperature in the air. Secondly, the composite film-modified electrode was immersed in 1% HAuCl_4 solution overnight. Thereafter, the composite film-modified electrode was further immersed in NaBH_4 for 30 min to form the gold nanoparticles within the composite film. Finally, the Nafion-CHIT/GNPs electrode was placed in 0.5 mM $\text{Ru}(\text{bpy})_3^{2+}$ solution for 3 h. After that, the film was carefully washed with water and was kept in dry state at room temperature when not in use.

Results and Discussion

Preparation of the Nafion-CHIT/GNPs composite films

In our study, we found that Nafion and CHIT could form a homogeneous solution based on ultrasonic assistance. Then, by using a coating film technique, one could form a stable and homogeneous composite film on the graphite electrode surface. The main reason of forming the composite film may be the electrostatic or hydrophilic interaction between CHIT and sulfonic groups of Nafion.³³ Furthermore, the previous research works demonstrated that the CHIT had stronger adsorbing ability for AuCl_4^- and could form more GNPs inside the CHIT film.³⁴ Based on these considerations, we investigated the possibility of preparing the Nafion-CHIT/GNPs composite film by the TEM and photography technique. As shown in Figs. 1 and 2, the results of both TEM and photo of the composite film showed that, after the Nafion-CHIT composite film coating carbon-copper grid or slide was immersed in 0.1% HAuCl_4 solution ($\text{pH} 4$) for about 10 h and further immersed in 0.02 mol/L NaBH_4 solution for 0.5 h, about 10 nm of GNPs was *in-situ* produced and dispersed well within the Nafion-CHIT composite film. These results indicated that CHIT still

Fig. 2 The photograph of GNPs generated in the Nafion-CHIT composite film on the glass (the process was the same as the process used for generation of the composite film-modified electrode).

Fig. 3 Cyclic voltammograms of $\text{Ru}(\text{bpy})_3^{2+}$ immobilized at Nafion-CHIT/GNPs composite film coating graphite electrode in phosphate buffer solution at the scan rate of 50 mV/s.

retained its chemical properties, in acidic condition ($\text{pH} < 6.3$) it charged to positive charge based on $-\text{NH}_3^+$ groups. So AuCl_4^- can be absorbed on the proposed composites film based on the electrostatic interaction between the CHIT and AuCl_4^- . Thus, CHIT could integrate GNPs into the composite film. In addition, due to the strong interaction of GNPs with CHIT, the GNPs could stably exist inside the Nafion-CHIT composite film. They presented better stability, and the aggregation effect between GNPs was greatly decreased. In this case, this effect offered the chance to make full use of the GNPs electrocatalytic function for $\text{Ru}(\text{bpy})_3^{2+}$ to design a better ECL sensor.

On the other hand, due to the strong adsorbing ability of Nafion for $\text{Ru}(\text{bpy})_3^{2+}$, our investigating results also showed that, after the Nafion-CHIT/GNPs composite film-modified graphite electrode was immersed into the 0.5 mM $\text{Ru}(\text{bpy})_3^{2+}$ solution for about 3 h and washed with water, this new composite film-modified electrode presented the typical electrochemical response of $\text{Ru}(\text{bpy})_3^{2+}$ in phosphate buffer medium ($\text{pH} 7.4$; the results are shown in Fig. 3). Such results indicated that $\text{Ru}(\text{bpy})_3^{2+}$ had been successfully immobilized into the composite film and presented stable electrochemical responses.

Electrochemistry and ECL behavior of immobilized $\text{Ru}(\text{bpy})_3^{2+}$
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Fig. 4 Cyclic voltammograms of $\text{Ru}(\text{bpy})_3^{2+}$ immobilized at the pure Nafion-modified electrode (green line) Nafion-CHIT film-modified electrode (red line) and the Nafion-CHIT/GNPs composite film-modified electrode (blue line) in 0.2 mol/L phosphate buffer solution ($\text{pH} 7.4$) at the scan rate of 50 mV/s.

$\text{Ru}(\text{bpy})_3^{2+}$ within the Nafion-CHIT/GNPs composite film-modified electrode was studied using cyclic voltammetry. As shown in Fig. 4, the charging current obtained at the Nafion-CHIT composite modified electrode was nearly the same as the current of the pure Nafion film-modified electrode, which indicated that CHIT did not affect the absorbing ability of Nafion for $\text{Ru}(\text{bpy})_3^{2+}$. But the charging current at the Nafion-CHIT/GNPs composite modified electrode was obviously enhanced. The main reason was the large electrode surface area due to the GNPs presented in the Nafion-CHIT composite film. It is remarkably that the redox peak current obtained at Nafion- $\text{Ru}(\text{bpy})_3^{2+}$ -CHIT/GNPs composite modified electrode was almost ~ 3 fold greater than that obtained at both pure Nafion-modified electrode and Nafion-CHIT composite films-modified electrode. These results indicated that the GNPs within the composite film offered the conducting pathways between the electrode and $\text{Ru}(\text{bpy})_3^{2+}$ within the composite film to accelerate the electron exchanges between $\text{Ru}(\text{bpy})_3^{2+}$ and electrode.

For further investigation of the electrochemical performances of the $\text{Ru}(\text{bpy})_3^{2+}$ within the proposed composite film, the diffusion coefficient, D_{app} , of $\text{Ru}(\text{bpy})_3^{2+}$ within the proposed composite film was investigated by the electrochemical method.³⁵ As shown in Fig. 5, it was obvious that, while the potential scanning rate was changed in the range of 10 – 200 mV/s, the anodic peak current was directly proportional to the square root of scan rate. This means that the current in the composite film is controlled by diffusion of $\text{Ru}(\text{bpy})_3^{2+}$, a similar situation was reported at the pure Nafion film-modified electrode.¹⁷ By using the previously reported method for measuring D_{app} , we calculated the apparent diffusion coefficient, D_{app} , for $\text{Ru}(\text{bpy})_3^{2+}$ in the Nafion-CHIT/GNPs composite films and pure Nafion film from the slope (from linear regression analysis) of the anodic peak current *versus* the square root of the scan rate plot by the Randles-Sevcik equation. The diffusion coefficient of $\text{Ru}(\text{bpy})_3^{2+}$ immobilized in the composite film-modified electrode was 65 times greater than that of the pure Nafion modified electrode, which further proved that the charge transport of the proposed composite films was faster due to the conducting pathway effect of the GNPs inside the composite film.

Fig. 5 Cyclic voltammograms of $\text{Ru}(\text{bpy})_3^{2+}$ immobilized at Nafion-CHIT/GNPs composite film-modified electrode in 0.2 mol/L phosphate buffer solution (pH 7.4) with different scan rates. Follow the arrowheads the scan rates respectively were: 10, 30, 50, 70, 90, 100, 120, 140, 160, 180, 200 mV/s. The inset shows the curve of anodic peak current with the square root of scan rate.

Fig. 6 ECL kinetic curves of $\text{Ru}(\text{bpy})_3^{2+}$ immobilized at pure Nafion-modified electrode (blue line) and Nafion-CHIT composite-modified electrode (red line), Nafion-GNPs composite film-modified electrode (gray line) and Nafion-CHIT/GNPs composite film-modified electrode (green line) in 5×10^{-6} mol/L TPA.

ECL behavior. The ECL behavior of $\text{Ru}(\text{bpy})_3^{2+}$ within the proposed composite film modified electrode has been studied with tripropylamine (TPA) as a representative analyte.^{36,37} Figure 6 shows the ECL kinetic processes of $\text{Ru}(\text{bpy})_3^{2+}$ -TPA system at a pure Nafion-modified electrode (blue line), a Nafion-GNPs composite film-modified electrode (gray line), a Nafion-CHIT composite film-modified electrode (red line) and Nafion-CHIT/GNPs composite film-modified electrode (green line) in 5.0×10^{-6} mol/L TPA. The ECL kinetic process of TPA in pure Nafion-modified electrode was the slowest due to the slow mass transfer speed of TPA into the pure Nafion film, whereas at the Nafion-CHIT composite film, the speed was obviously increased. Due to the interfusion of CHIT in Nafion, this composite film could form more hydrophilic

Fig. 7 Cyclic voltammograms of $\text{Ru}(\text{bpy})_3^{2+}$ immobilized in Nafion-CHIT/GNPs composite film-modified electrode in the absence (---) and presence (—) of 1 mM TPA in 0.2 mol/L phosphate buffer solution (pH 7.4) with a scan rate of 50 mV/s. Inset: Corresponding ECL-potential recorded during the CV scan.

channels based on the chitosan's hydrophilic property and its hydrogel ability in the pH 7.4 PBS buffer medium;³¹ these channels then increase the mass transfer speed of TPA in the composite film. The ECL kinetic process of TPA at Nafion-GNPs composite film-modified electrode was similar to that of Nafion-CHIT-modified electrode. These results indicated that the CHIT provided the hydrophilic channel to accelerate TPA diffusing the composite film to take part in the relating ECL reaction, because the CHIT could form the hydrogel within the proposed composite film.³¹

The electrocatalytic oxidation function of $\text{Ru}(\text{bpy})_3^{2+}$ for TPA at the composite film-modified electrode surface was also investigated. As shown in Fig. 7, the presence of TPA caused the anodic peak current to increase clearly while the cathodic peak current decreased, which was consistent with the electrocatalytic reaction mechanism. The result indicated that $\text{Ru}(\text{bpy})_3^{2+}$ incorporated in the Nafion-CHIT/GNPs composite film-modified electrode still had electrocatalytic oxidation for TPA. The corresponding ECL-potential profile was also recorded (as shown in Fig. 7), the ECL signal occurred at about 0.85 V, and then increased steeply until it reaches a maximum at 1.14 V, which was coincident with the oxidation potential of $\text{Ru}(\text{bpy})_3^{2+}$. The results showed that the immobilized $\text{Ru}(\text{bpy})_3^{2+}$ still presented the better ECL performances inside the composite film.

Choice of the amount of CHIT

In our initial test, it was found that the amount of CHIT in composite films greatly affected the electrochemical and ECL behaviors of the ECL sensor. Figure 8 shows the cyclic voltammograms (CVs) results of $\text{Ru}(\text{bpy})_3^{2+}$ immobilized on Nafion-CHIT/GNPs composite films-modified electrode with different amounts of CHIT used. While the amount of CHIT was above 50% ($V_{\text{chitosan}}/V_{\text{Nafion}}$), the cyclic voltammetry curve become more tail-shaped and the redox peak current was decreased. However while the amount of CHIT in the composite film was changed in the range of 30 – 10%, the shapes of the cyclic voltammetric curves were symmetrical and showed the better electrochemical performances than those of the pure Nafion modified electrode. All of these results indicated that,

Fig. 8 Cyclic voltammograms of $\text{Ru}(\text{bpy})_3^{2+}$ immobilized at Nafion-CHIT/GNPs composite film-modified electrode (with different amount of CHIT). The amounts of CHIT respectively are: 0 μL (green line), 10 μL (red line), 30 μL (blue line), 50 μL (yellow line), 70 μL (olive line), 90 μL (wine line).

although CHIT could modify the electrochemical properties of the developed composite film, its concentration was so high that the adherence of the composite film on the electrode surface was weakened due to both the better hydrophilic properties and the swelling ability of CHIT in the water solution. So, while the concentration of CHIT in the composite film was above 30%, the electrochemical performances become poor. In consideration of the electrochemical performance as well as the sensor's stability, 10% CHIT concentration was used throughout the experiments.

Analytical performances of the composite film

Calibration curves for TPA have been constructed using the present ECL sensor based on the Nafion-CHIT/GNPs composite films. The ECL intensity has good linearity, with the TPA concentration ranging from 3.0×10^{-9} to 1.0×10^{-5} mol/L with a detection limit ($S/N = 3$) of 4×10^{-10} mol/L. Compared with pure Nafion film-modified electrodes, the detection limit of Nafion-CHIT/GNPs composite films were four orders of magnitude lower.

We also investigated both the operational and the storage stability of the ECL sensor. When the potential was scanned continuously for 11 cycles in PBS (pH 7.4) containing 5.0×10^{-6} mol/L TPA with the scan rate of 50 mV/s (Fig. 9), there was almost no change concerning the ECL intensity. The long-term storage stability of the present sensor was studied over a month by monitoring its ECL response to 5.0×10^{-6} mol/L TPA in PBS (pH 7.4) with intermittent detection (every 2–3 days); the sensor was stored in the air at room temperature when not in use. The coating of the Nafion-CHIT/GNPs composite film does not come off during this period, implying that the Nafion-CHIT/GNPs composite film was well adhered to the graphite electrode. It was observed that the response of this ECL sensor decreased less than 5% of its initial value after a month, which was much better than the values of pure Nafion-based electrodes (stability less than a day). Reproducible ECL signals were observed under consecutive potential scanning for 11 cycles, as shown in Fig. 8. The relative standard deviation was less than 5% ($n = 11$, $C = 5.0 \times 10^{-6}$ mol/L TPA), suggesting good reproducibility of the ECL determination of TPA at the CHIT-Nafion/GNPs composite film-modified electrode.

Fig. 9 ECL emission from Nafion-CHIT/GNPs/ $\text{Ru}(\text{bpy})_3^{2+}$ composite film in phosphate buffer solution (pH 7.4) containing 5×10^{-6} mol/L TPA under continuous CVs for 11 cycles. Scan rate: 100 mV/s.

Conclusion

In this work, a new method for preparing the Nafion-CHIT/GNPs composite films was developed. The electrochemical and ECL activity of the composite material had been examined. The relating results showed that this ECL sensor not only presented the rapid mass-transfer and faster charge transport ability, but also offered the higher sensitivity and long-term stability than those of pure Nafion film-modified electrodes. In addition to these, due to the infusion of CHIT into this composite film, the biocompatibility of the proposed composite film and its chemical/biochemical modifying ability were greatly improved. Such better properties can offer the composite film a potential application in designing the $\text{Ru}(\text{bpy})_3^{2+}$ -based ECL or electrochemical biosensors.

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