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Nafion-stabilized magnetic nanoparticles (Fe_3O_4) for $[Ru(bpy)_3]^{2+}$ (bpy = bipyridine) electrogenerated chemiluminescence sensor†

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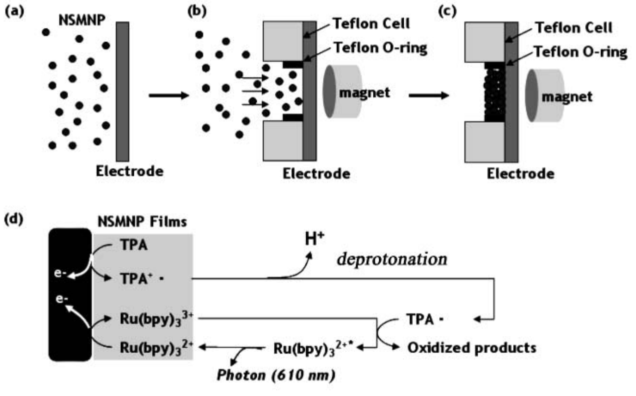
A highly sensitive and stable $[Ru(bpy)_3]^{2+}$ ECL sensor has been fabricated based on the multilayer films of Nafion-stabilized magnetic nanoparticles (Nafion/Fe_3O_4) formed on a platinum electrode surface by means of an external magnet.

$Ru(bpy)_3{}^{2+}$ 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 regenerable ECL-based chemical sensors, biosensors, and detection devices in flowing streams, much effort has been focused on the immobilization of $Ru(bpy)_3{}^{2+}$ on an electrode surface, thus recycling $Ru(bpy)_3{}^{2+}$ as seen in scheme 1 and making the detection system simpler. For example, relatively simple immobilization method based on ion-exchange of $Ru(bpy)_3{}^{2+}$ in cation-exchange polymer such as Nafion, perfluorosulfonated ionomer, has been reported.[2] The $Ru(bpy)_3{}^{2+}$ ECL sensor based on the Nafion matrix can be easily fabricated but its sensitivity and stability are problematic. Thus, the sol-gel ceramic/Nafion composite films such as silica/Nafion[3] and titania/Nafion[4] have been prepared in order to increase the sensitivity and stability of the $Ru(bpy)_3{}^{2+}$ ECL sensor. Some polymeric systems where Ru complexes are chemically attached in organic polymer[5] and inorganic silica[6] have also been reported to enhance the stabilities of the ECL sensors. However, the preparations of the ECL sensors are complex and their ECL signals are relatively low due to poor permeations of analyte molecules through the matrices. Recently, an ECL sensor based on the mulilayer films of nanoparticles (SiO_2 or clay)/$Ru(bpy)_3{}^{2+}$ fabricated by the layer-by-layer method on an ITO electrode was presented.[7] The high surface area of the nanoparticles in the multilayer films led to an increased ECL response compared to that of the pure Nafion-based ECL sensor.



Scheme 1 Formation of NSMNP (Nafion/Fe_3O_4)-modified electrode (a–c) and the ECL from $Ru(bpy)_3{}^{2+}$ immobilized in the multilayer films on a Pt electrode surface in the presence of coreactant tripropylamine (TPA) (d).

As an alternative new immobilization method, we present here the preparation of Nafion-stabilized magnetic nanoparticles (NSMNP, Nafion/Fe_3O_4, *ca.* ~20 nm in size) and the magnetic field-driven formation of the multilayer films of Nafion/Fe_3O_4 magnetic nanoparticles on a Pt electrode for the fabrication of a highly sensitive and stable $Ru(bpy)_3{}^{2+}$ ECL sensor. Magnetic particles of iron oxides (Fe_3O_4, *ca.* ~μm in size) functionalized with redox or biochemical components have been extensively used for the concentration and localization of chemical or biochemical components such as enzymes,[8] DNA,[9] and cells.[10] In addition, the magnetic attraction of the functionalized particles to the electrode surface by an external magnet can be used to initiate the electrocatalytic and biocatalytic processes. Thus, magnetic-field switching of electrocatalytic and biocatalytic processes have been easily performed for selective dual biosensing, immunosensing, DNA sensing, and luminol ECL.[11]

Scheme 1 outlines the present method to construct a multilayer film of Nafion/Fe_3O_4 magnetic nanoparticles on a Pt electrode surface by an external magnet and also shows the ECL from $Ru(bpy)_3{}^{2+}$ immobilized in the Nafion/Fe_3O_4 multilayer-modified electrode in the presence of coreactant tripropylamine (TPA). Nafion-stabilized magnetic nanoparticles were synthesized according to the published procedure[12] using Nafion as the surfactant in the reaction medium.‡ A similar system such as the Nafion-stabilized iridium oxide (Nafion/IrO_2) colloid has been reported.[13] The deep brown colloidal solution was stable over a period of one month. A TEM image of Nafion/Fe_3O_4 nanoparticles is shown in Fig. 1(A). Primary particles less than 20 nm in diameter form small clusters that have a diameter of 200–300 nm.

A certain amount of Nafion/Fe_3O_4 nanoparticles was introduced into an electrochemical cell containing 1 mL deionized water. Positioning of an external magnet behind the Pt electrode (surface area = 0.28 cm^2; thickness = 300 nm) on silicon wafer attracts the magnetic particles and the multilayer films of the magnetic nanoparticles are formed in less than 20 min as shown in scheme 1 (a–c). The thickness of the multilayer films can be easily controlled by the variation of the amount of the magnetic nanoparticles introduced into the aqueous solution. It is possible to control the mutillayer thickness from sub μm to several hundred

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† Electronic supplementary information (ESI) available: UV-visible absorption and XRD spectra of Nafion/Fe_3O_4 magnetic nanoparticles. See http://www.rsc.org/suppdata/cc/b5/b503358f/
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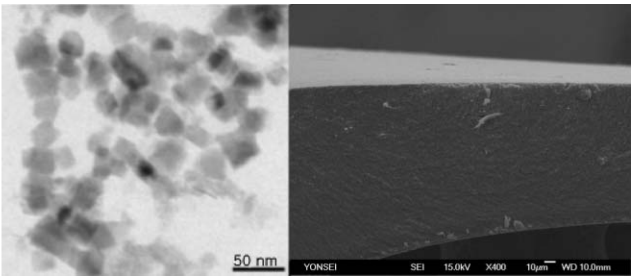

Fig. 1 TEM image of Nafion-stabilized magnetic nanoparticles (Nafion/Fe_3O_4) (left) and FE-SEM image of a cross-sectional view of the Nafion/Fe_3O_4 mutillayer films on a Pt electrode (right).

μm with high reproducibility. For example, the attraction of 3.0 mg Nafion/Fe_3O_4 nanoparticles on the electrode resulted in the Nafion/Fe_3O_4 multilayer films with about 140 μm thickness. A FE-SEM image of the cross-sectional view of the Nafion/Fe_3O_4 multilayer films is shown in Fig. 1 (right).

The Nafion/Fe_3O_4 multilayer-modified Pt electrode (0.03 mg Nafion/Fe_3O_4 used; film thickness = 1.0 μm) was then placed in 0.1 mM $Ru(bpy)_3^{2+}$ solution prepared in 0.05 M phosphate buffer at pH 7. As seen in Fig. 2 (inset), the incorporation of $Ru(bpy)_3^{2+}$ into the Nafion/Fe_3O_4 multilayer films was observed voltammetrically by continuously cycling the potential between +0.8 V and +1.3 V *vs.* Ag/AgCl (3 M NaCl) with a Pt wire auxiliary electrode. An EG&G 263A potentiostat was used for the electrochemical experiments. The anodic peak currents of $Ru(bpy)_3^{2+}$ were measured with time and were plotted as seen in Fig. 2 (●). For comparison, pure Nafion film was prepared by casting 10 μL of 0.5% Nafion solution onto the same Pt electrode (○). As can be seen, the uptake rate of $Ru(bpy)_3^{2+}$ into the Nafion/Fe_3O_4 multilayer films is much faster than that into the pure Nafion films. For the Nafion/Fe_3O_4 multilayer-modified electrode, the time taken to reach a steady-state peak current was only about 50 s, which is sevenfold shorter than that obtained with a corresponding pure Nafion-modified electrode (350 s) under identical experimental conditions.

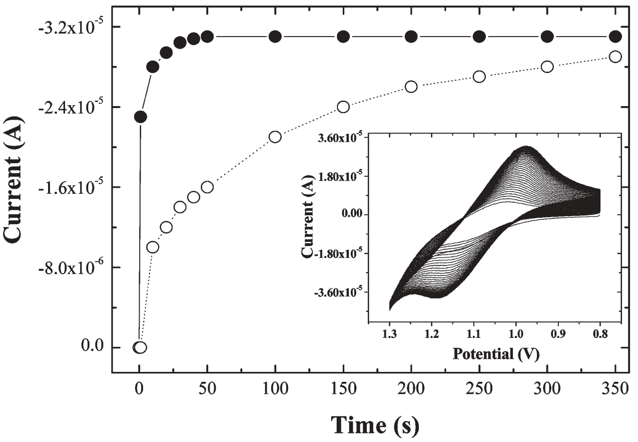

Fig. 2 Anodic peak currents as a function of loading time in the 0.1 mM $Ru(bpy)_3^{2+}$ solution. Nafion/Fe_3O_4 mulilayer-modified electrode (●), pure Nafion-modified electrode (○). Inset: cyclic voltammograms to monitor the incorporation of $Ru(bpy)_3^{2+}$ into Nafion/Fe_3O_4-modified electrode at a scan rate of 10 mV s^{-1}.

The increased uptake rate of $Ru(bpy)_3^{2+}$ into the multilayer films of Nafion/Fe_3O_4 nanoparticles relative to the pure Nafion films in the present study is possibly due to the greater accessibility of the ion-exchange site, SO_3^-, in the multilayer films, therefore, leading to a faster diffusion of $Ru(bpy)_3^{2+}$ into the multilayer films. The apparent diffusion coefficient, D_{app}, for $Ru(bpy)_3^{2+}$ into the multilayer films was calculated from the slope (from linear regression analysis) of the anodic peak current *vs.* square root of the scan rate plot by the Randles–Sevick equation. The calculated D_{app} is $\sim 3.7 \times 10^{-8}$ cm^2 s^{-1}, which is almost 2 orders of magnitude larger than the diffusion coefficient for the $Ru(bpy)_3^{2+}$ in the pure Nafion films (3.0×10^{-10} cm^2 s^{-1}). This result indicates that the high surface area-to-volume ratio in the multilayer films of Nafion/Fe_3O_4 nanoparticles leads to higher mass transport and faster ion-exchange kinetics in comparison to the pure-Nafion films.

The ECL behavior of $Ru(bpy)_3^{2+}$ incorporated into the Nafion/Fe_3O_4-modified electrode has been studied with tripropylamine (TPA) as a representative analyte. The ECL intensity *versus* potential profiles was monitored using a photomultiplier tube (Hamamatsu HC 135) in conjunction with an EG&G 263A potentiostat. Fig. 3 shows the cyclic voltammograms of $Ru(bpy)_3^{2+}$ incorporated into the Nafion/Fe_3O_4-modified electrode in the absence (—) and presence (- - -) of 0.5 mM TPA in 0.05 M phosphate buffer at pH 7. The presence of TPA causes the oxidation current to increase considerably while the reduction current decreases due to the catalytic oxidation of TPA. The corresponding ECL potential profile recorded during the CV scan is shown in the inset. The onset of ECL emission seen at ~1.0–1.1 V was coincident with the oxidation of $Ru(bpy)_3^{2+}$ complex.

The amount of Nafion/Fe_3O_4 nanoparticles coated in the Pt electrode surface strongly affects the amount of $Ru(bpy)_3^{2+}$ immobilized in the Nafion/Fe_3O_4 multilayer-modified electrode as well as its resulting ECL intensity, as shown in Fig. 4.

The anodic peak current of $Ru(bpy)_3^{2+}$ immobilized in the Nafion/Fe_3O_4-modified electrode increased consistently as the amount of Nafion/Fe_3O_4 nanoparticles coated in the modified electrode was increased (inset) due to the increased amount of the

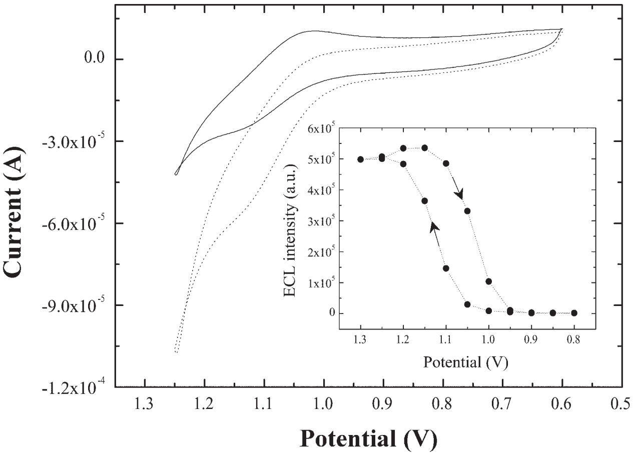

Fig. 3 Cyclic voltammograms of $Ru(bpy)_3^{2+}$ immobilized at Nafion/Fe_3O_4 -modified electrode in the absence (—) and the presence (- - -) of 0.5 mM TPA in 0.05 M phosphate buffer (pH 7) at a scan rate of 100 mV s^{-1}. Inset: ECL-potential profile recorded during the CV scan.

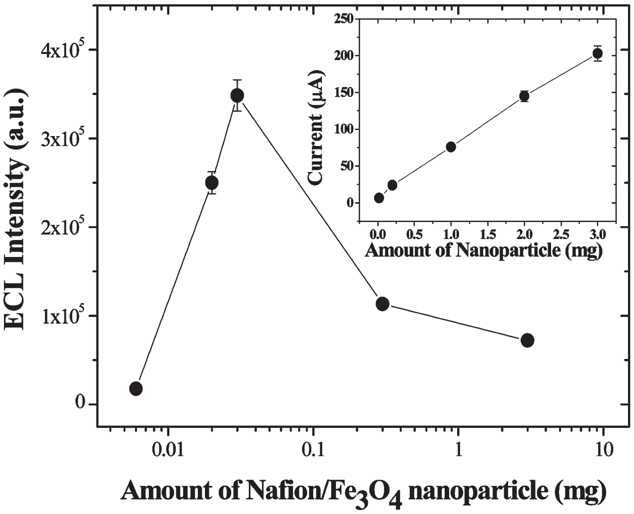

Fig. 4 Dependence of ECL intensity on the amount of Nafion/Fe_3O_4 coated on a Pt electrode. The ECL intensity was measured for 0.5 mM TPA solution prepared in 0.05 M phosphate buffer at pH 7. The points shown are the mean of at least five determinations ($\pm$SD). Inset: anodic peak current as a function of the amount of the Nafion/Fe_3O_4 coated on Pt electrode.

ion-exchange sites. However, the ECL intensity obtained with the present Nafion/Fe_3O_4-modified electrode increased sharply as the amount of Nafion/Fe_3O_4 nanoparticles in the modified electrode increased up to 0.03 mg, after which it then decreased at higher amounts of the Nafion/Fe_3O_4 nanoparticles although the films contain a greater amount of $Ru(bpy)_3^{2+}$. The reason why films with higher amounts of Nafion/Fe_3O_4 nanoparticles led to a decreased ECL intensity is not clear at the present time. However, the increased amount of nanoparticles might absorb and scatter the ECL emission within the films. (see UV-VIS absorption spectra of nanoparticles: ESI†).

The $Ru(bpy)_3^{2+}$ ECL sensor based on the mutilayer films (0.03 mg Nafion/Fe_3O_4 nanoparticles) gave a linear response ($R^2 = 0.999$) for TPA concentration from 0.1 μM to 1.0 mM with a remarkable detection limit (S/N = 3) of 50 nM while the pure Nafion-based ECL sensor gave a detection limit of 1 μM.

The long-term storage stability of the present $Ru(bpy)_3^{2+}$ ECL sensor was studied over a month by monitoring its ECL response to 1.0 mM TPA in 0.05 M phosphate buffer solution (pH 7) with intermittent usage (every 2–3 days) and storage in the buffer solution at room temperature when not in use. The coating of the Nafion/Fe_3O_4 multilayer films did not come off during the test period, indicating that the Nafion/Fe_3O_4 multilayer films are well stuck to the Pt electrode. It was found that the response of the ECL sensor gradually decreased to almost 80% of its initial value in a month. The stability of the present Nafion/Fe_3O_4-modified electrodes is much better than that of the pure Nafion-modified electrode[2] and even better than that of the titania–Nafion composite-modified electrode.[4] Reproducible ECL signals were observed for 10 consecutive injections of 0.5 mM TPA with a relative standard deviation of 3.9%.

In conclusion, we have presented a new method for the preparation of the Nafion/Fe_3O_4 magnetic nanoparticles and have demonstrated their use as an immobilization matrix for $Ru(bpy)_3^{2+}$. Due to the fast mass transport in the Nafion/Fe_3O_4 multilayer films, the ECL sensor based on the Nafion/Fe_3O_4 multilayer films is more sensitive than that based on pure Nafion films. While the use of Nafion/Fe_3O_4-modified electrode has been presented in connection to the ECL sensor, it could also be readily extended to the preparation of functional coating materials as well as preconcentration media for the trace monitoring of hydrophobic cations such as the heart imaging agent, $Re(DMPE)_3^+$ (DMPE = 1,2-bis(dimethylphosphino)ethane).[14]

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Notes and references

‡ Nafion-stabilized magnetic nanoparticle was prepared by the precipitation–oxidation method as follows: 1.0 g $FeSO_4 \cdot 7H_2O$ (Aldrich, 99%) and 500 mg of Nafion powder (Aldrich) were dissolved in the 40 mL of deionized water by mechanical stirring. The mixture was stirred under N_2 gas for 30 min, and followed by the addition of 0.5 mL of 40% H_2O_2 and 10 mL ethanol. The added ethanol enhanced the solubility of Nafion. The reaction was allowed to proceed for 6 h at 80 °C at pH 13 with a reflux condenser. The pH of the reaction mixture was regulated with 3 M NaOH solution. The precipitate was isolated and purified repeatedly by magnetic field separation, decantation, and redispersion in water. (see XRD spectrum of nanoparticles: ESI†).

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