



Short communication

3D nanostructured Ni(OH)₂ microspheres as an efficient immobilization matrix of Ru(bpy)₃²⁺ for high-performance electrochemiluminescence sensor

Kun Wang*, He-Nan Li, Chang Ju, Zhi-Jun Luo, Li-Na Dai, Hua-Ming Li

Key Laboratory of Modern Agriculture Equipment and Technology, School of Chemistry and Chemical Engineering, Jiangsu University, 301 Xuefu Road, Zhenjiang 212013, China

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ABSTRACT

The electrochemistry and electrochemiluminescence (ECL) of novel three-dimensional nanostructured Ru(bpy)₃²⁺/Ni(OH)₂ microspheres were investigated for the first time. The negatively charged porous Ni(OH)₂ microspheres composed of Ni(OH)₂ nanowires were specifically designed to interact with Ru(bpy)₃²⁺. The large surface area and porous structure of Ni(OH)₂ microspheres enhance loading of Ru(bpy)₃²⁺ and mass transport of the model analyte, tripropylamine (TPA). Excellent ECL performance of the presented sensor was achieved including good stability and wide linear range from 7.7×10^{-10} to 3.8×10^{-3} M with the detection limit of 2.6×10^{-10} M to TPA.

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1. Introduction

Nowadays, the immobilization of Ru(bpy)₃²⁺ for fabricating Ru(bpy)₃²⁺-based solid-state electrochemiluminescence (ECL) sensors has been one of the main focuses in the ECL field, which provides several advantages compared with those solution phase ECL procedures, such as reducing the consumption of expensive reagents, enhancing ECL signal, and simplifying experimental design [1]. And some Ru(bpy)₃²⁺-based solid-state ECL sensors have been successfully used as the detectors for flow-injection analysis [2], high-performance liquid chromatography [3], or capillary electrophoresis [4], and in biosensors [5].

The previous reports have demonstrated the migration of the Ru(bpy)₃²⁺ into the electrochemically inactive hydrophobic region of Nafion results in charge transport difficulty within the film and poor long-term stability of the ECL sensor [6]. As an alternative strategy, quite a lot of advanced nanostructured materials (such as silica, titania, zirconia, etc.) have been introduced to Ru(bpy)₃²⁺-Nafion composite film to improve the performance of ECL sensors [1,7], which allow fast diffusion of the analytes into the film and accelerated charge transportation. Meanwhile, many research studies have been also focused on the enhancement of quantity of the Ru(bpy)₃²⁺ immobilized to improve sensitivity of the ECL sensors including fabricating multilayer films [8], introducing negatively charged nanomaterial into the composite

films [9]. And also, porous materials due to their unique structure and property including large surface area and pore volume were adopted as the platform for constructing Ru(bpy)₃²⁺-based ECL sensors, such as Zeolite Y sieves [4], MCM-41 [10], porous gold film [11], porous titania [12], which enhance loading of Ru(bpy)₃²⁺ and mass transport of the analytes, and show a wide linear range to the model compound, tripropylamine (TPA) which is an important coreactant due to its ability to allow efficient ECL not only in aqueous media but also at physiological pH (~7.4), opening up a wide range of assays for molecules of diagnostic or biological relevance [13]. Though much progress has been made in this field, new immobilization methods and materials are still needed to make robust, novel, sensitive and regenerative solid-state ECL sensors [1].

Nickel hydroxide has been widely employed as the electrode material in the field of various electrochemical devices because of their good chemical and thermal stability, good chemical compatibility, low cost and toxicity [14]. The previous report [15] has proved the surface of nanostructured Ni(OH)₂ possesses strongly negative charges, which could be further used to fabricate the Ni(OH)₂-based electrodes by electrostatic layer-by-layer with some polycations or electrophoretic deposition methods. Herein, we first synthesized a novel kind of negatively charged three-dimensional (3D) nanostructured Ni(OH)₂ microspheres composed of ultrathin nanowires. Then it was employed as the immobilization matrix of Ru(bpy)₃²⁺ for preparing nanostructured Ru(bpy)₃²⁺/Ni(OH)₂ microspheres (RuNiM) by electrostatic assembly techniques, the as-prepared RuNiM was cast on a glassy carbon electrode (GCE) using Nafion as the dispersant, which showed fast

* Corresponding author. Tel.: +86 511 8791800; fax: +86 511 8791708.
E-mail address: wangkun@ujs.edu.cn (K. Wang).

charge transfer rate, good stability, and high sensitivity to the model compound TPA.

2. Experimental

2.1. Reagents

Tri(2,2-bipyridyl)dichlororuthenium(II) hexahydrate ($\text{Ru}(\text{bpy})_3\text{Cl}_2 \cdot 6\text{H}_2\text{O}$), TPA and 5% (w/v) Nafion was purchased from Sigma–Aldrich. $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ was purchased from Sinopharm Chemical Reagent Co., Ltd. Unless otherwise stated, reagents were of analytical grade and used as received. All aqueous solutions were prepared with doubly distilled water.

2.2. Apparatus and equipments

Cyclic voltammograms (CVs) and ECL curves were monitored by a CHI 660B workstation (Shanghai Chenhua, China) and a MCFL-A multifunctional chemiluminescent and bioluminescent analytical system (Remax Electronic Co., Ltd., China) with the voltage of the photomultiplier tube set was at 600 V. All experiments were carried out a conventional three-electrode system comprised of a platinum wire auxiliary, a saturated Ag/AgCl reference and the modified GCE working electrode. Zeta potential measurements were carried out with a ZetaPlus (Brookhaven Instruments Corp., USA). Characteristics were performed via Elemental analysis (Heraeus CHN-O-Rapid, Germany), Brunauer–Emmett–Teller (BET, Quntachrome NOVA2000, USA), field-emission scanning electron microscopy (FE-SEM, Hitachi S4800, Japan).

2.3. The preparation of $\text{Ni}(\text{OH})_2$ microspheres

In a representative procedure for $\text{Ni}(\text{OH})_2$ microspheres composed of nanowires, 2.4 mmol of $[\text{C}_{16}\text{mim}]\text{CF}_3\text{COO}$ and 0.6 mmol $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ were put into a 12 mL Teflon-sealed autoclave. After that, 9 mL of distilled water was also added into the 12 mL Teflon-sealed autoclave. The autoclave was sealed and maintained at 160 °C for 24 h. After cooling to room temperature, green precipitation was collected by centrifugation and washed several times with deionized water and absolute ethanol. The washed precipitation was dried in vacuum oven at 50 °C for 12 h.

2.4. The preparation of $\text{Ru}(\text{bpy})_3^{2+}/\text{Ni}(\text{OH})_2$ microspheres

$\text{Ru}(\text{bpy})_3^{2+}/\text{Ni}(\text{OH})_2$ microspheres were prepared by the following procedure: 0.5 mg/mL $\text{Ni}(\text{OH})_2$ microspheres were suspended in 0.1 M pH 8.2 phosphate buffer solution (PBS) by ultrasonication for the following experiments. 60 μL 0.1 M $\text{Ru}(\text{bpy})_3^{2+}$ solution was added into the mixture. The reaction was allowed to stir for 1 h under an ultrasonication condition. And then the RuNiM were isolated by acetone, following by centrifuging and washing with deionized water several times. Finally, the required RuNiM were obtained.

2.5. Preparation of the modified electrode

The GCE was first polished with 0.3 and 0.05 μm alumina slurry, and sonicated in ethanol and water successively. For the preparation of RuNiM/GCE, 0.3% Nafion/ethanol solution was used as a dispersant, and 1 mg RuNiM were dispersed 0.5 mL Nafion/ethanol solution under the condition of the ultrasonication, and then 5 μL of the resulting homogeneous suspension was cast on the pretreated GCE surface and dried in air at room temperature to form RuNiM modified GCE.

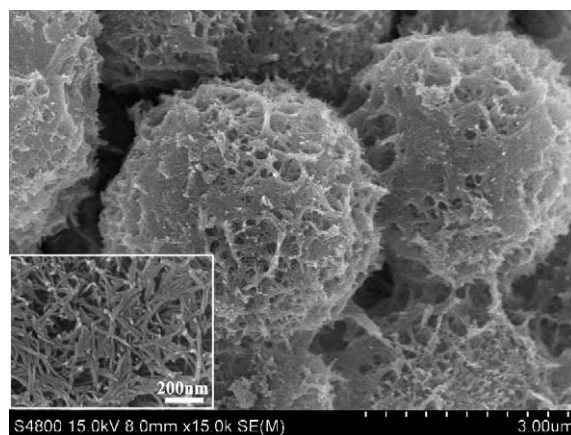


Fig. 1. SEM image of the nanostructured $\text{Ru}(\text{bpy})_3^{2+}/\text{Ni}(\text{OH})_2$ microspheres. Inset: SEM image of the surface of the $\text{Ru}(\text{bpy})_3^{2+}/\text{Ni}(\text{OH})_2$ microspheres.

3. Results and discussion

3.1. Characterization of the $\text{Ru}(\text{bpy})_3^{2+}/\text{Ni}(\text{OH})_2$ microspheres

The $\text{Ru}(\text{bpy})_3^{2+}/\text{Ni}(\text{OH})_2$ composite could be formed via electrostatic interactions between negatively charged $\text{Ni}(\text{OH})_2$ and positively charged $\text{Ru}(\text{bpy})_3^{2+}$. The zeta potential for the $\text{Ni}(\text{OH})_2$ microspheres is -14.8 mV at pH 8.2 solution. The $\text{Ru}(\text{bpy})_3^{2+}/\text{Ni}(\text{OH})_2$ composite was first characterized by FE-SEM. Fig. 1 showed that the as-obtained $\text{Ru}(\text{bpy})_3^{2+}/\text{Ni}(\text{OH})_2$ consisted of porous microspheres with the diameter of about 3 μm , and the microsphere was fabricated by a large quantity of nanowires randomly tangled up. The thickness of the nanowire building the microspheres is about 10 nm, the porous structure of the microsphere deriving from the intercrossing of the nanowires. BET surface area of the RuNiM is 207 m^2/g calculated from nitrogen adsorption isotherms, suggesting the RuNiM possess considerable large surface area. The pore diameter distribution of the RuNiM was measured by the Barret–Joyner–Halenda (BJH) method. The pore sizes are about 5 nm. Furthermore, the RuNiM constituent was ascertained by the EDS analysis, as shown in Fig. 2. The peaks of Ru, Ni, C, O, and N elements were successfully achieved (other peaks such as Al and Au elements originated from the substrate), demonstrating the constituent element of the as-prepared microspheres in accordance with $\text{Ru}(\text{bpy})_3^{2+}$ and $\text{Ni}(\text{OH})_2$. The as-prepared RuNiM was also measured by elemental analysis, verifying that 8 wt.% of the $\text{Ru}(\text{bpy})_3^{2+}$ presented in these $\text{Ni}(\text{OH})_2$ microspheres. It was worthwhile to mention that the formed struc-

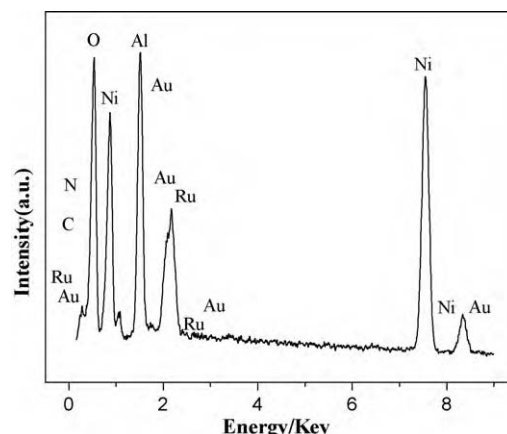


Fig. 2. EDS image of the $\text{Ru}(\text{bpy})_3^{2+}/\text{Ni}(\text{OH})_2$ microspheres.

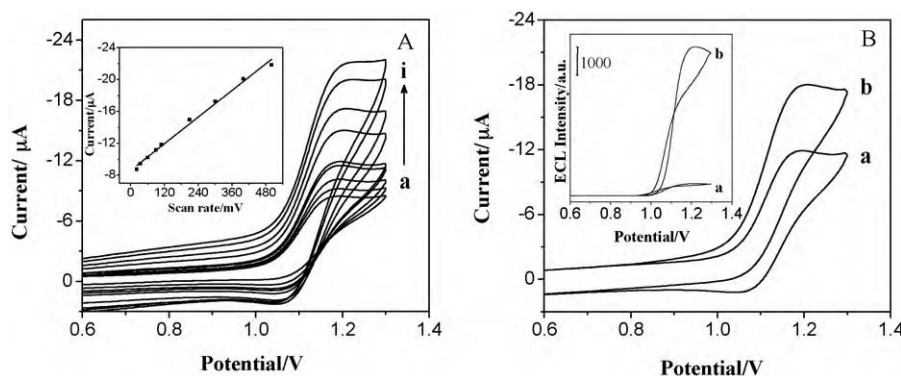


Fig. 3. (A) CVs of RuNiM/GCE at various scan rates (from inner curve to the outer curve: 20, 40, 60, 80, 100, 200, 300, 400, 500 mV/s) in pH 8.2 PBS. Inset: Relationship between the anodic peak current and the scan rate; and (B) CVs of the RuNiM/GCE in (a) pH 8.2 PBS and (b) containing 0.7 mM TPA. Inset: corresponding ECL-potential curves of them. Scan rate: 100 mV/s.

ture was robust enough to undergo an ultrasonication for about 1 h using a sonifier with 300 W. Such robust porous structures and large BET surface areas facilitated rapid diffusion of the substrate during ECL and enhance the rate of ECL reaction, as seen subsequently.

3.2. Electrochemistry and ECL behavior of the RuNiM

Fig. 3A showed CVs of various scan rates at the RuNiM/GCE in pH 8.2 PBS. There was a couple of redox peaks from 1.3 to 0.6 V with a change in potential (ΔE_p) of about 95 mV at 100 mV/s, which was ascribed to the oxidation and reduction of the Ru(bpy) $_3^{2+}$. ΔE_p was similar to that reported in the literature [8,9], and smaller than that for Nafion-TiO $_2$ composites containing Ru(bpy) $_3^{2+}$ modified electrodes [3], and Ru(bpy) $_3^{2+}$ doped SiO $_2$ @MWNTs coaxial nanocable [16]. From the integration of reduction peak of the RuNiM/GCE at 100 mV/s, the surface coverage of Ru(bpy) $_3^{2+}$ is calculated to be 1.24×10^{-9} mol/cm 2 [11]. The anodic peak currents exhibited a linear relationship with the scan rates from 20 to 500 mV/s (Fig. 3A inset), indicating a surface-confined process [8,17], and ΔE_p was independent of the scan rates, indicating that the electron transfer from Ru(bpy) $_3^{2+}$ immobilized inside Ni(OH) $_2$ microspheres to the electrode surface was fast [9].

ECL of the RuNiM/GCE was further investigated with TPA as a coreactant. The Ru(bpy) $_3^{2+}$ -TPA system has been well studied in solution due to its about 10-fold higher ECL compared to other commonly used reductants [18,19]. Fig. 3B showed CVs and corresponding ECL curves of the RuNiM/GCE without (curve a) and with (curve b) 0.7 mM TPA at a scan rate of 100 mV/s in 0.1 M pH 8.2 PBS, respectively. With the addition of 0.7 mM TPA, the oxidative peak current significantly increased with decreasing reductive current,

indicating that the Ru(bpy) $_3^{2+}$ /Ni(OH) $_2$ microspheres exhibited good electrocatalytic activity towards TPA, and as can be expected from the Ru(bpy) $_3^{2+}$ -TPA ECL mechanism, the onset of ECL seen at around 0.95 V and the ECL peak intensity at around 1.15 V, and the ECL intensity was about 6000 a.u., which showed that the Ru(bpy) $_3^{2+}$ immobilized in the Ni(OH) $_2$ microspheres could retain its ECL efficiency. In addition, the pH effect on the ECL intensity was examined in the range of 7.0–9.8. The ECL intensity of the RuNiM/GCE in the presence of 0.7 mM TPA was dependent on the solution pH, and the maximum ECL intensity appeared at pH 8.2.

Finally, the RuNiM/GCE was used to detect the content of TPA. Calibration curves for TPA have been constructed using the RuNiM/GCE in 0.1 M pH 8.2 PBS, as shown in Fig. 4A. Each point was an average of ECL signals obtained by consecutive cyclic potential scans (100 mV/s) at a given concentration. Calibration curves were plotted on logarithmic axes to show a very wide dynamic range (Fig. 4A inset). The linear range extended from 7.7×10^{-10} to 3.8×10^{-3} M. The detection limit for TPA at the RuNiM/GCE was 2.6×10^{-10} M ($S/N=3$), which was lower than most of the previously reported immobilization methods for the determination of TPA, and equivalent to that reported ultrasensitive ECL sensor for TPA based on Ru(bpy) $_3^{2+}$ immobilized in the CNT/Nafion composite film [20]. And the stability was further studied by continuous potential scanning for 11 cycles. Fig. 4B showed the ECL intensity from RuNiM/GCE in 0.1 M pH 8.2 PBS containing 0.7 mM TPA for 11 cycles, and the relative standard deviation was 0.45%. The RuNiM/GCE still kept over 91% activity towards 0.7 mM TPA after being stored in air for 20 days. The excellent ECL performance of the as-prepared RuNiM/GCE could be attributed to the following points [9,17,21]: the 3D structure of Ni(OH) $_2$ microsphere could

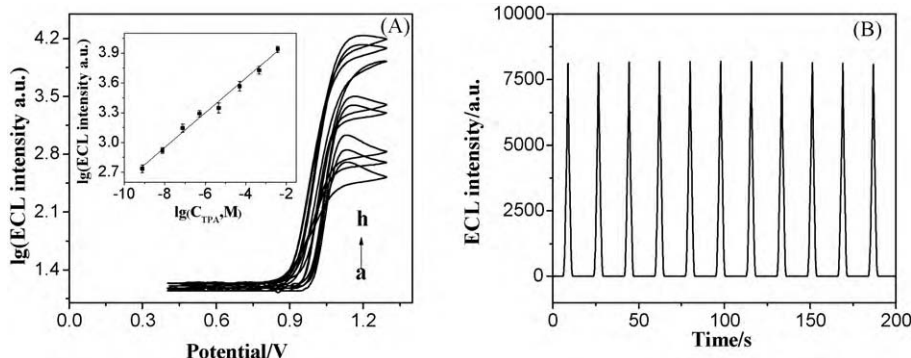


Fig. 4. (A) Effect of TPA concentration on ECL intensity of RuNiM/GCE in 0.1 M PBS containing (a) 7.7×10^{-10} , (b) 7.7×10^{-9} , (c) 7.7×10^{-8} , (d) 5.3×10^{-7} , (e) 4.6×10^{-6} , (f) 5.0×10^{-5} , (g) 4.6×10^{-4} and (h) 3.8×10^{-3} M TPA. Inset: corresponding calibration curves for TPA detection. (B) ECL emission of RuNiM/GCE in PBS containing 0.7 mmol TPA with continuous CVs.

immobilize a large amount of $\text{Ru}(\text{bpy})_3^{2+}$ molecules not only based on the strong electrostatic attraction between the negative $\text{Ni}(\text{OH})_2$ nanowires and the positive $\text{Ru}(\text{bpy})_3^{2+}$, but also on its considerable large surface area, and furthermore the 3D structure of $\text{Ni}(\text{OH})_2$ microspheres could effectively prevented $\text{Ru}(\text{bpy})_3^{2+}$ from coming off. In addition, the structured RuNiM with abundant pore canals could facilitate mass transfer of the analytes and enhance the rate of ECL reaction [8,11].

4. Conclusion

The 3D nanostructured $\text{Ru}(\text{bpy})_3^{2+}/\text{Ni}(\text{OH})_2$ microsphere was used in the field of ECL for the first time, and excellent performance of the presented sensor can be achieved including wide linear range, good stability and high sensitivity to TPA. According to our results, the 3D nanostructured $\text{Ni}(\text{OH})_2$ microsphere is the efficient immobilization matrix of $\text{Ru}(\text{bpy})_3^{2+}$ because it possesses larger surface area and strongly negative charges. And its porous structure with abundant canals also facilitates mass transfer of the analytes and enhances the rate of ECL reaction. It will provide us new kind of materials for solid-state ECL detection in high-performance liquid chromatography, flow-injection system, and capillary electrophoresis.

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