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Short communication

# Electrochemiluminescence detection of NADH and ethanol based on partial sulfonation of sol–gel network with gold nanoparticles

Liu Deng, Lihua Zhang, Li Shang, Shaojun Guo, Dan Wen, Fuan Wang, Shaojun Dong*

*State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Number 5625, Renmin Road, Changchun, Jilin 130022, PR China*

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ABSTRACT

We developed a stable, sensitive electrochemiluminescence (ECL) biosensor based on the synthesis of a new sol–gel material with the ion-exchange capacity sol–gel to coimmobilize the $Ru(bpy)_3^{2+}$ and enzyme. The partial sulfonated (3-mercaptopropyl)-trimethoxysilane sol–gel (PSSG) film acted as both an ion exchanger for the immobilization of $Ru(bpy)_3^{2+}$ and a matrix to immobilize gold nanoparticles (AuNPs). The AuNPs/PSSG/$Ru(bpy)_3^{2+}$ film modified electrode allowed sensitive the ECL detection of NADH as low as 1 nM. Such an ability of AuNPs/PSSG/$Ru(bpy)_3^{2+}$ film to promote the electron transfer between $Ru(bpy)_3^{2+}$ and the electrode suggested a new, promising biocompatible platform for the development of dehydrogenase-based ECL biosensors. With alcohol dehydrogenase (ADH) as a model, we then constructed an ethanol biosensor, which had a linear range of 5 μM to 5.2 mM with a detection limit of 12 nM.



## 1. Introduction

Reagentless electrochemiluminescence (ECL) biosensors which combines enzymatic selectivity with the sensitivity of $Ru(bpy)_3^{2+}$ detection have draw great attention [Jameison et al., 1996; Li et al., 2007; Lin et al., 2008a,b; Zhang et al., 2008]. But the coimmobilization of enzyme and $Ru(bpy)_3^{2+}$ in the same matrix on the electrode always lead to the loss of the enzyme activity [Xu et al., 2005; Zhang et al., 2007; Choi et al., 2007; Martin and Nieman, 1997]. The high concentration of $Ru(bpy)_3^{2+}$ affected the hydrophilicity of the film which might reduce the enzyme activity [Martin and Nieman, 1997]. In addition, the positive charges of $Ru(bpy)_3^{2+}$ could interact with the negative charges of the enzyme, which was quite unfavorable to enzyme orientation. Many efforts are made aiming at developing a sensitive, stable ECL-based biosensor. It has been reported both $Ru(bpy)_3^{2+}$ and enzyme were immobilized on one electrode by adjacent layers. Although such an approach could overcome the barrier to some extent, the two-layer design was quite complex. The sol–gel and Nafion hybrid film, $Ru(bpy)_3^{2+}$-gold nanoparticles (AuNPs) cluster and sol–gel titania–Nafion composite films doped with multiwalled carbon nanotube (MWNT) were applied as the matrix to coimmobilize enzyme and $Ru(bpy)_3^{2+}$ in previous reports [Xu et al., 2005; Zhang et al., 2007; Choi et al., 2007]. Although the reported ECL biosensors exhibited good performances for the detection of ethanol, developing the highly sensitive and long-term stable ECL biosensor is still drawing much attention. The thiolated silanes are extensively applied as the enzyme and nanoparticles matrix, and the enzyme biosensor exhibits high sensitivity and good stability [Jia et al., 2002; Raj and Jena, 2005]. We extended the thiolated silica into the ECL biosensing system once the latter was partially sulfonated. The partial sulfonation of (3-mercaptopropyl)-trimethoxysilane (MPS) sol–gel (PSSG) could provide not only a suitable environment to retain the enzyme activity, but also a matrix to immobilize the $Ru(bpy)_3^{2+}$ and gold nanoparticles (AuNPs). In this research, the integration of ion-exchange capacity sol–gel, nanoparticles and enzyme allowed the development of a highly sensitive and stable ECL biosensor.

## 2. Experimental

### 2.1. Chemicals

Alcohol dehydrogenase (ADH) (EC 1.1.1.1, from baker's yeast, 494 units/mg solid), nicotinamide adenine dinucleotide ($NAD^+$) (sodium salt, from yeast), (3-mercaptopropyl)-trimethoxysilane were purchased from Sigma. Tris(2,2-bipyridyl) dichlororuthenium(II) hexahydrate ($Ru(bpy)_3Cl_2{\cdot}6H_2O$) were purchased from Aldrich. Chloroauric acid trihydrate ($HAuCl_4{\cdot}3H_2O$) was purchased from EM Sciences. Ethanol was from Beijing Reagent Factory (Beijing, China). All other chemicals were of analytical

* Corresponding author. Tel.: +86 431 85262101; fax: +86 431 85689711.
*E-mail address:* dongsj@ciac.jl.cn (S. Dong).

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grade and aqueous solutions were prepared with doubly distilled water.

### 2.2. Apparatus and measurement

Cyclic voltammetric experiments were performed with a CHI 600B electrochemical analyzer. All experiments were carried out with a conventional three-electrode system. An Ag/AgCl (saturated KCl) reference electrode and a platinum wire counter electrode were used for all the measurements. The chemiluminescence signal produced was detected and recorded by a flow injection chemiluminescence analyzer (IFFD, Xi'an Remax Electronic Science Tech. Co. Ltd.), which was controlled by a personal computer. The photomultiplier tube (PMT) was biased at 600 V in the experiment. Scanning electron microscopy (SEM) images were determined with a Philips XL-30 ESEM. The accelerating voltage was 20 kV.

### 2.3. Synthesis of partial sulfonation of MPS sol–gel

The preparation procedure of partial sulfonation of MPS sol–gel was a modification of the literature [Zhang et al., 1999]. 1.5 mL MPS was mixed with 8 mL water. This mixture was sonicated for 0.5 h to ensure uniform mixing, followed by the addition of 0.5 mL concentrated nitric acid and sonication for 1 h. During the oxidation process of thiol groups to sulfonate ones, colloidal particles aggregated to some extent. After removal of the aggregated portion, the liquid was neutralized with concentrated NaOH to pH 7.0 and then transferred into a semipermeable packet. The packet was immersed in water to get rid of inorganic salt and the partial sulfonated sol was purified. For fabrication of the biosensor, 0.5 mL PSSG sol was mixed with 0.5 mL of ADH solution (1 mg/mL in 0.1 M PBS of pH 7.5). The resulting sol–gel biocomposite was stored at 4 °C.

### 2.4. Preparation and modification of Au electrodes

A gold disk electrode (1.0 mm diameter) was polished carefully with 1.0, 0.3, 0.5 μm alumina slurry and rinsed with water and absolute ethanol. Then the cleaned gold electrode was immersed in the PPSG for 30 min at room temperature. The resulting modified electrode was thoroughly rinsed with water to remove physically adsorbed sol–gel. Then it was immersed in the AuNPs solution for 10 h at 4 °C. Finally, the AuNPs/PSSG modified electrode was incubated in 0.1 mM $Ru(bpy)_3^{2+}$ for 0.5 h at 4 °C to immobilize the $Ru(bpy)_3^{2+}$ at the electrode surface. All resulting electrodes were washed with water and stored at 4 °C when not in use.

## 3. Results and discussion

### 3.1. Characterization of the PSSG

XPS measurements were performed to identify and characterize the prepared PSSG (Fig. 1A). The sulfur peaks are sensitive to the chemical environment. The lower binding energy peak at 162.4 eV was a characteristic binding energy for the sulfide components while the other peak at 167.7 eV had been assigned to the more oxidized form of sulfur in the film [Duchet et al., 1983; Hota et al., 2007]. Fig. 1B revealed the formation of a self-assembled silica network on the gold substrate and the distribution of the gold nanoparticles by the rest of thiol groups in partial sulfonated MPS. The homogenous distribution of the thiol groups in PSSG led to a uniform distribution of gold nanoparticles in silica network.

### 3.2. Electrochemistry and ECL of the sensor

The ion-exchange of $Ru(bpy)_3^{2+}$ into the AuNPs/PSSG was relatively fast, with the maximum peak current occurring within 15 min in 0.1 mM $Ru(bpy)_3^{2+}$ solution. The electrochemical stability of $Ru(bpy)_3^{2+}$ immobilized in the AuNPs/PSSG composite films was good. When the AuNPs/PSSG film modified electrode was repetitively cycled at 100 mV/s for 20 min, less than 5% loss in the current was observed (data not shown).

The ECL behavior of AuNPs/PSSG/$Ru(bpy)_3^{2+}$ film has been studied with NADH as a coreactant. This unique immobilization method led to a strong ECL signal (Fig. 2A). The onset of luminescence occurred near 1.0 V, and then the ECL intensity rose steeply until it reached a maximum near 1.15 V, which was consistent with the oxidation potential of $Ru(bpy)_3^{2+}$. The linear range of the NADH sensor was extended from 2.5 nM to 586 μM with a detection limit of 1 nM. This detection sensitivity was about two orders lower than sol–gel titania–Nafion-MWNT modified electrode [Choi et al., 2007], 800 times lower than at the CNT/Nafion composite film-modified electrode [Zhuang and Ju, 2005] and 50 times lower than that with single channel portable flow injection stacking on ECL test [Waseem et al., 2007]. This ultrasensitive detection to NADH could be ascribed to the introduction of AuNPs. For comparison, we prepared the PSSG/$Ru(bpy)_3^{2+}$ modified electrode under identical conditions except for the immobilization of AuNPs. It was obvious that the shape of curve in Fig. 3A was much sharper than that of Fig. 3B, which indicated that the ECL response at the AuNPs/PSSG/$Ru(bpy)_3^{2+}$ modified electrode was much faster than that of the PSSG/$Ru(bpy)_3^{2+}$ modified electrode. The reason was that the silica sol–gel acted as the inert electron and mass transfer blocking layer, and it hindered the diffusion of NADH toward the electrode surface. The same electrode displayed facile electron transfer kinetics with much sharper curve after the self-assemble of AuNPs. The accelerated ECL process was ascribed to the presence of AuNPs on the thiol groups of the PSSG network. As the thiol groups are distributed throughout the silicate network, AuNPs could be assembled both inside and on the surface of the network [Bharathi et al., 2001; Jena and Raj, 2008]. Those AuNPs on the electrode surface provided the necessary conduction pathways and allowed efficient electron tunneling, which facilitated electrical communication between $Ru(bpy)_3^{2+}$ and the electrode surface [Chen and Zu, 2007]. The impedance changes of the electrode surface confirmed the function of AuNPs. Compared to PSSG/$Ru(bpy)_3^{2+}$ film, the electron transfer resistance ($R_{ct}$) of AuNPs/PSSG/$Ru(bpy)_3^{2+}$ composite film electrode decreased obviously (data not shown). These results showed that the electronic conductivity and electrocatalytic properties of AuNPs provided conducting pathways to connect $Ru(bpy)_3^{2+}$ sites to the electrode and accelerated the speed of reaction. At the same time, the high surface area of AuNPs made the structure of the AuNPs/PSSG/$Ru(bpy)_3^{2+}$ composite film more open compared with that of the PSSG/$Ru(bpy)_3^{2+}$ film and allowed faster diffusion of analytes [Du et al., 2006]. The integration of AuNPs into the composite film greatly overcame the drawback of slow electron transfer through the film and enhanced the ECL intensity.

### 3.3. ECL biosensor for ethanol

The excellent performance of the present electrode toward the detection of NADH made it attractive for the development of dehydrogenase-based ECL biosensors. The amount of NADH generated during the enzymatic reaction is proportional to the substrate concentration, so that the substrates can be conveniently quantified by the enzymatically generated NADH. In the present investigation, the ADH–AuNPs/PSSG/$Ru(bpy)_3^{2+}$ composite film modified electrode was employed for the ethanol ECL biosensor. The ECL intensity had a good linearity with the ethanol concentration. The linear range extended from 5 μM to 5.2 mM with a detection limit of 12 nM. This detection limit was four orders lower than that at

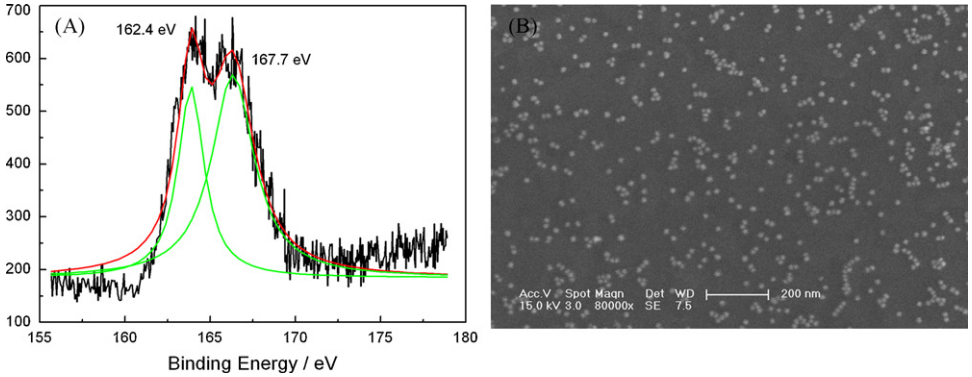


**Fig. 1.** (A) XPS spectrum of the S 2p region of the PSSG. (B) The SEM image for the assembled AuNPs on the PSSG network.

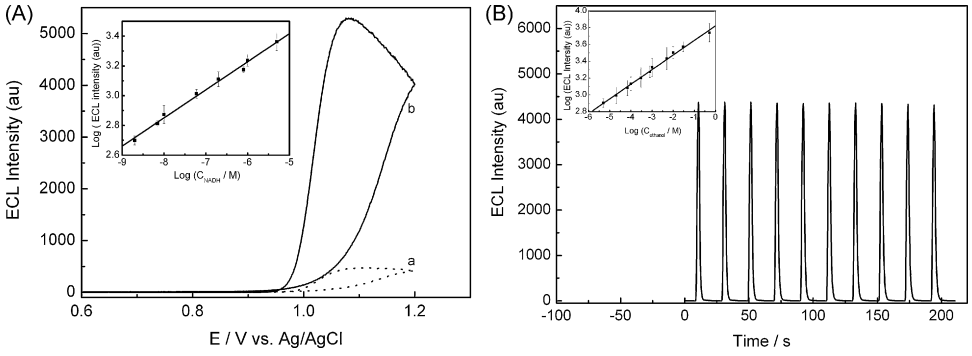


**Fig. 2.** (A) The ECL sensor without (a) and with (b) 0.2 mM NADH in PBS. Inset: calibration curve for NADH of the biosensors in 0.1 M PBS (pH 7.5). (B) ECL emission from the ECL biosensor in 0.1 M PBS (pH 7.5) containing 0.2 mM NADH and 4 mM ethanol under continuous CVs from 0.6 V to 1.2 V. Scan rate: 50 mV/s. Inset: calibration curve for ethanol of the biosensors with 0.2 mM NADH in 0.1 M PBS (pH 7.5).

the Nafion-sol–gel hybrid film-modified electrode [Xu et al., 2005], three orders lower than that at the $Ru(bpy)_3^{2+}$–AuNPs aggregates modified electrode [Zhang et al., 2007], two orders lower than that at sol–gel titania–Nafion-MWNT composite films [Choi et al., 2007]. The biosensor exhibited the advantages of ECL detection with higher sensitivity and wider linear range. Fig. 2B showed the ECL emission from ADH–AuNPs/PSSG/$Ru(bpy)_3^{2+}$ film modified electrode under continuous CVs for 10 cycles in 0.1 M PBS (pH 7.5) solution containing 0.2 mM NADH and 4 mM ethanol. The relative standard deviation was 0.6%. The results suggested that the charge transfer in the composite film was very fast and the film was quite stable. Moreover, the long-term stability of the biosensor was also investigated during a month. The biosensor was tested by repetitive measurements every 2 days. The sensor was stored in the dry state at 4 °C between measurements. After 1 week, the biosensor could retain 85% of the original response to 5 mM ethanol. After a month, the biosensor could retain 70% of the original response. In previous report using the Nafion-sol–gel to immobilize $Ru(bpy)_3^{2+}$ and ADH, the biosensor maintained only 50% of the original response after a 2-week storage [Xu et al., 2005]. Sol–gel and AuNPs provided a suitable environment to maintain the enzyme activity [Cui et al., 2007; Jia et al., 2002]. Such a method prevented the enzyme from direct spatial contact with $Ru(bpy)_3^{2+}$, which may greatly decrease the impact of positive charges of $Ru(bpy)_3^{2+}$ on the enzyme activity. Moreover, introduction of AuNPs to silica gel provided the necessary conduction pathways which facilitated the electron transfer between the substrate and electrode. The presence of sol–gel and AuNPs network increased not only the long-term stability of the biosensor but also the sensitivity of the ECL biosensor.

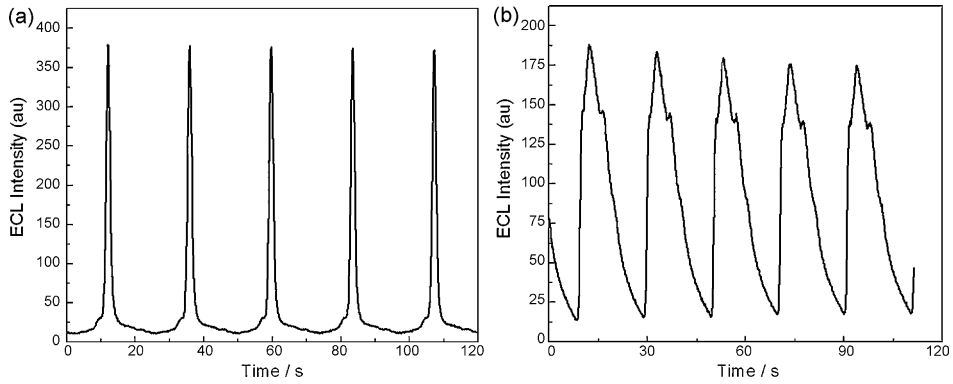


**Fig. 3.** ECL curves of AuNPs/PSSG/$Ru(bpy)_3^{2+}$ (A) and PSSG/$Ru(bpy)_3^{2+}$ (B) modified electrodes under continuous CVs from 0.6 V to 1.2 V in 0.1 M PBS (pH 7.5) containing 5 nM NADH. Scan rate: 50 mV/s.

## 4. Conclusion

The proposed approach in this paper was a universal fabrication method for ECL biosensor fabrication. Partial sulfonated thiol-containing silica gel could achieve a homogeneous distribution of sulfonic acid groups in the sol–gel and provide accessible ion-exchange sites for $Ru(bpy)_3^{2+}$. At the same time, the –SH groups in PSSG not only allowed sol–gel to assemble easily on a gold electrode but also provided attaching sites for AuNPs immobilization. The AuNPs and sol–gel network provided a suitable microenvironment to retain the activity of enzyme. What is more, these nanoparticles acted as tiny conduction centers and facilitated communication with the electrode. The PSSG was used as matrix to integrate three functional components (ECL agents, AuNPs and enzymes) and exhibited excellent ECL behavior, long-term stability and high sensitivity towards NADH and ethanol. The combination of AuNPs and ion-exchange capacity of sol–gel provided a wider scope for the design of nanoparticles modified electrodes with unique properties.

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