Synthesis of PtNPs/AQ/Ru(bpy)$_3^{2+}$ Colloid and Its Application as a Sensitive Solid-State Electrochemiluminescence Sensor Material

Yan Du, Bin Qi, Xiurong Yang,* and Erkang Wang*

State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Graduate School of the Chinese Academy of Sciences, Changchun, Jilin 130022, People's Republic of China

Received: August 2, 2006; In Final Form: August 29, 2006

The facile synthesis of the novel platinum nanoparticles/Eastman AQ55D/ruthenium(II) tris(bipyridine) (PtNPs/AQ/Ru(bpy)$_3^{2+}$) colloidal material for ultrasensitive ECL solid-state sensors was reported for the first time. The cation ion-exchanger AQ was used not only to immobilize ECL active species Ru(bpy)$_3^{2+}$ but also as the dispersant of PtNPs. Colloidal characterization was accomplished by transmission electron microscopy (TEM), X-ray photoelectron spectrum (XPS), and UV−vis spectroscopy. Directly coating the as-prepared colloid on the surface of a glassy carbon electrode produces an electrochemiluminescence (ECL) sensor. The electronic conductivity and electroactivity of PtNPs in composite film made the sensor exhibit faster electron transfer, higher ECL intensity of Ru(bpy)$_3^{2+}$, and a shorter equilibration time than Ru(bpy)$_3^{2+}$ immobilized in pure AQ film. Furthermore, it was demonstrated that the combination of PtNPs and permselective cation exchanger made the sensor exhibite excellent ECL behavior and stability and a very low limit of detection (1×10^{-15} M) of tripropylamine with application prospects in bioanalysis. This method was very simple, effective, and low cost.

1. Introduction

Electrochemiluminescence (ECL) has been paid considerable attention over the past several decades[1] due to the inherent essences such as low background signal, simple optical setup, versatility, high sensitivity, and selectivity.[2] Among the ECL systems, the most extensively studied and used ECL compound is ruthenium(II) tris(bipyridine) (Ru(bpy)$_3^{2+}$) for its excellent stability and high efficiency in the aqueous phase.[3] ECL based on Ru(bpy)$_3^{2+}$ has found many applications such as in immunoassays[4] and in detecting a number of biologically important species[5] without derivatization. Furthermore, it also has been used as a detector for high-performance liquid chromatography (HPLC),[6] for flow injection analysis,[7] for capillary electrophoresis (CE),[8] and as a CE microchip.[9]

Compared with the solution-phase ECL procedure, the immobilization of Ru(bpy)$_3^{2+}$ can provide several advantages such as reducing the consumption of expensive reagent, enhancing the ECL signal, and simplifying experimental design. Most importantly, as Ru(bpy)$_3^{2+}$ is regenerated during the ECL process, a reagentless ECL sensor can be constructed.[10] As a result, considerable effort has been made to immobilize Ru(bpy)$_3^{2+}$ on an electrode surface, including the Langmuir−Blodgett technique,[11] self-assembly technique,[12] and layer-by-layer assembly (LBL) technique.[13] Another popular method is the incorporation of Ru(bpy)$_3^{2+}$ in a perfluorosulfonated ionomer such as Nafion.[14] But the Nafion film has the drawback of slow mass transfer through the film and problematic stability. An improved approach is to incorporate Ru(bpy)$_3^{2+}$ into a composite film, such as carbon nanotube (CNT)/Nafion,[15] Nafion-silica,[16] Nafion-titania,[17] and Nafion-zirconia[18] composite films. Poly-(ester sulfonic acid) cation exchanger Eastman AQ55D (AQ) shows a greater hydrophilic character than Nafion[19] and appears to be an attractive material for sensor development due to the low cost, strong adherence to the surface, and simple coating procedure, as well as having permselective, ion-exchanging, and antifouling properties.[20]
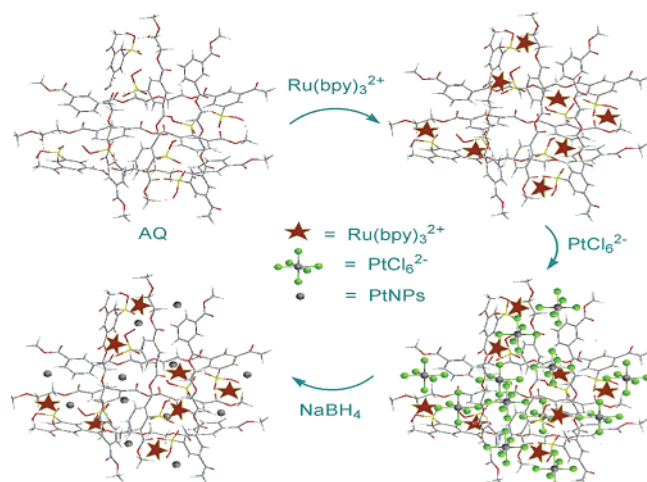
Metal nanoparticles have been attracting growing attention because of their unique physicaland chemical properties, as well as their application to biology, medicine, material science, and their different interdisciplinary fields.[21] The promising application of PtNPs as electrocatalysts or an electrochemical sensor has been investigated widely.[22] The combination of the electronic conductivity and electroactivity properties of PtNPs with high ion-exchange capacity of ion-exchange polymer provides a wider scope for the design of nanoparticle modified electrodes with novel properties and applications.[23] More recently, we have developed an immobilization method involving the formation of aggregates of citrate-capped gold nanoparticles and Ru(bpy)$_3^{2+}$ in aqueous solution and the following attachment of such aggregates on sulfhydryl-derivatized indium tin oxide (ITO) electrode surfaces.[24]

Here, the synthesis and characterization of novel PtNPs/AQ/Ru(bpy)$_3^{2+}$ colloidal material used for an ultrasensitive ECL sensor was reported for the first time, in which the cationic exchanger AQ was used not only to immobilize the ECL active reagent Ru(bpy)$_3^{2+}$ but also as a dispersant of nanoparticles. A directly coated as-formed colloidal material on an electrode surface used as an ECL sensor exhibited excellent ECL behavior, stability, and high sensitivity of tripropylamine (TPA).

2. Experimental Section

2.1. Materials. Tris(2,2′-bipyridyl)ruthenium(II) dichloride hexahydrate (Ru(bpy)$_3$Cl$_2$·6H$_2$O), tripropylamine (TPA), hexachloroplatinic acid (H$_2$PtCl$_6$), and sodium borohydride (NaBH$_4$)

* To whom correspondence should be addressed. E-mail: ekwang@ciac.jl.cn.

SCHEME 1: Preparation of Novel PtNPs/AQ/Ru(bpy)$_3^{2+}$ Colloid

Figure 1. TEM image of the PtNPs/AQ/Ru(bpy)$_3^{2+}$ colloid.

Figure 2. High-resolution XPS spectrum of the Pt (4f$_{7/2}$, 4f$_{5/2}$) region of the PtNPs/AQ/Ru(bpy)$_3^{2+}$ colloid.

were purchased from Aldrich (Milwaukee, WI). Eastman-AQ55D (28% dispersion, AQ) was purchased from Eastman Kodak Co. and was used as received. All other reagents were of analytical reagent grade. All solutions were prepared with water purified by a Milli-Q system (Millipore, Bedford, MA).

2.2. Synthesis of PtNPs Colloids. PtNPs/AQ/Ru(bpy)$_3^{2+}$ colloid was prepared as follows. In a typical experiment, 20 μL of AQ was diluted to 5 mL with water and 20 μL of 0.05 M Ru(bpy)$_3^{2+}$ aqueous solution was added under stirring at room temperature. Then 100 μL of 0.038 M H$_2$PtCl$_6$ was introduced into the resulting solution and the mixture was heated to 80 °C. After adding 1% NaBH$_4$ solution, the as-formed solution was maintained at 80 ± 0.5 °C for 20 min. The AQ-stabilized Pt nanoparticle (PtNPs/AQ) colloid was prepared the same as in the above procedure in the absence of Ru(bpy)$_3^{2+}$ before the reduction of H$_2$PtCl$_6$ with NaBH$_4$.

2.3. Preparation of the ECL Sensor. Before modification, the GC electrode (3 mm in diameter) was polished with a 0.3- and a 0.05-μm aluminum slurry, respectively, rinsed thoroughly with redistilled water between each polishing step, sonicated in ethanol and water successively, and then allowed to dry at room temperature. The resulting GC electrode was coated by hand with 10 μL of PtNPs/AQ/Ru(bpy)$_3^{2+}$ colloid or PtNPs/AQ colloid, and the solvents were allowed to evaporate at 30–35 °C leaving a cloudy orange or white film. The PtNPs/AQ film-modified electrode was then placed in a 1 mM Ru(bpy)$_3^{2+}$ aqueous solution for incorporation of Ru(bpy)$_3^{2+}$.

2.4. Characterization Techniques. Transmission electron microscopy (TEM) measurements were made on a JEOL 2010 transmission electron microscopy operated at an accelerating voltage of 200 kV. The X-ray photoelectron spectrum (XPS) was collected on an ESCLAB MKII (Thermo). UV−vis spectra were collected on a CARY 500 Scan UV−vis−near-infrared (UV−vis−NIR) spectrophotometer (Varian). A model 800 Electrochemical Analyzer (CH Instruments Co., Austin, TX) was used to perform cyclic voltammetric experiments. The ECL signals were recorded by a MCFL-A multifunctional chemi- and bioluminescent analytical system (Remax Electronic Co. Ltd., Xi'an, China) with the voltage of the photomultiplier tube (PMT) set at 800 V.

3. Results and Discussion

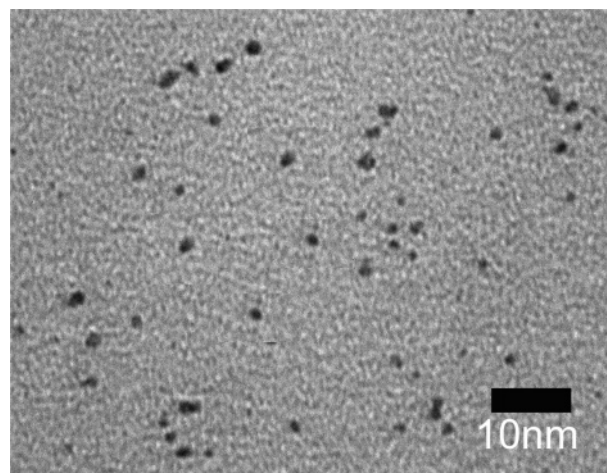**3.1. Characterization of the PtNPs/AQ/Ru(bpy)$_3^{2+}$ Colloid.** Scheme 1 illustrates the fabrication of the PtNPs/AQ/Ru(bpy)$_3^{2+}$
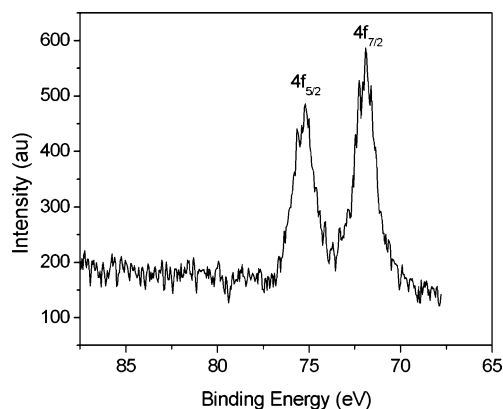
colloid. First, the Ru(bpy)$_3^{2+}$ solution was added into polymer AQ solution under stirring at room temperature for immobilization of Ru(bpy)$_3^{2+}$ based on the Wang report about the ion-exchange properties of AQ to cations of Ru(bpy)$_3^{2+}$.[20a] The solution was light orange and no precipitate developed. Second, the platinum salt added to the above solution was reduced with NaBH$_4$. The color of the solution slowly became dark orange, which indicates the formation of PtNPs. For control, on one hand, with the absence of AQ, precipitate occurred due to the electrostatic attractions between the positive charge of Ru-(bpy)$_3^{2+}$ and the negative charge of PtCl$_6^{2-}$. On the other hand, with the absence of Ru(bpy)$_3^{2+}$, black colloidal PtNPs was formed. So the above experimental results showed the polymer AQ can be used not only to immobilize the cations of Ru(bpy)$_3^{2+}$ but also as the dispersant of PtNPs.

Figure 1 shows a transmission electron micrograph (TEM) of the PtNPs/AQ/Ru(bpy)$_3^{2+}$. The dark spots and light features corresponded to PtNPs and the AQ matrix, respectively. One can see that PtNPs were highly dispersed in the composite film, and were small in size (~3 nm). Moreover such PtNPs were stable for several months without any observable aggregation, indicating that AQ serves as a very effective dispersant for the formation of PtNPs.

XPS measurements were also performed to identify and characterize the as-prepared PtNPs as shown in Figure 2. The XPS spectrum showed two binding energy peaks of the Pt (4f$_{7/2}$) and Pt (4f$_{5/2}$) electrons were at 71.8 and 75.2 eV, respectively, confirming the Pt particles in the film were in the Pt (0) state.[25]

The as-prepared PtNPs colloid was further characterized by means of a UV−vis spectrophotometer. Figure 3 shows UV−
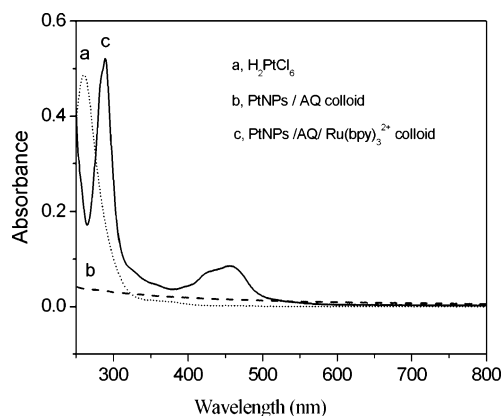
Figure 3. UV−visible spectra of 5 μM H_2PtCl_6 solution (a), dilute PtNPs/AQ colloid (b), and PtNPs/AQ/$Ru(bpy)_3^{2+}$ colloid (c).

vis spectra of 5 μM H_2PtCl_6 (a), dilute PtNPs/AQ colloid (b), and PtNPs/AQ/$Ru(bpy)_3^{2+}$ colloid (c) in deionized water. As shown in Figure 3a, there is a strong absorption band at 260 nm that corresponds to the absorption of $PtCl_6^{2-}$, while in the UV spectrum of PtNPs/AQ colloid (Figure 3b) the absorption peaks at 260 nm disappear completely, indicating the full reduction of H_2PtCl_6. The PtNPs/AQ/$Ru(bpy)_3^{2+}$ colloid (Figure 3c) exhibited a peak at 289 nm and a wide band at 450 nm as that of $Ru(bpy)_3^{2+}$ solution, which were due to electronic $\pi-\pi^*$ transition and MLCT adsorption of $Ru(bpy)_3^{2+}$,[13] respectively. The above experimental results showed the preparation procedure of PtNPs had no effect on the UV characterization of Ru-$(bpy)_3^{2+}$ ion-exchanged in the AQ polymer. The concentration of $Ru(bpy)_3^{2+}$ in PtNPs/AQ/$Ru(bpy)_3^{2+}$ colloid can be found by preparing a calibration curve of absorbance intensity at 289 nm vs $Ru(bpy)_3^{2+}$ concentration; the calculated concentration of $Ru(bpy)_3^{2+}$ is 5.22 μM.

3.2. Electrochemistry and ECL of the Sensor. The ECL sensor was prepared by directly coating 10 μL of PtNPs/AQ/$Ru(bpy)_3^{2+}$ colloid on the clean surface of a glassy carbon (GC) electrode. The high temperature (80 °C) at which the reduction of H_2PtCl_6 took place has no effect on the film-forming ability of AQ. While the $Ru(bpy)_3^{2+}$−TPA system has been well studied, it was chosen to examine the electrochemical and ECL behavior of the sensor. Martin[19] has reported that utilizing the AQ film for immobilization of $Ru(bpy)_3^{2+}$ has a longer equilibration time (about 2 h) before reproducible signals were recorded due to the initial loss of $Ru(bpy)_3^{2+}$. But in this work, the equilibration time of the PtNPs/AQ/$Ru(bpy)_3^{2+}$ modified electrode was very short (about 20 min). Figure 4 showed CVs and corresponding ECL curves of the sensor without (a) and with (b) 1 μM TPA in phosphate buffer solution (PBS). The CVs of the sensor exhibited a pair of characteristic redox waves of $Ru(bpy)_3^{2+}$ at around 1.1 V and the presence of TPA caused the anodic current to increase clearly while the cathodic current decreased due to the electrocatalytic reaction of $Ru(bpy)_3^{2+}$ with TPA. These results indicated that the $Ru(bpy)_3^{2+}$ had been incorporated into the composite film and retains good electroactivity. Moreover, as shown in Figure 5, the oxidation peak current was proportional to the square root of the scan rate, $v^{1/2}$, which indicates the immobilized $Ru(bpy)_3^{2+}$ underwent a diffusion process within the film. Similar results have been observed in previous work.[9b] The corresponding ECL intensity-potential curves for the sensor in 50 mM PBS during the cyclic voltammetric scan at a scan rate of 50 mV/s are shown in Figure 4B. The onset of luminescence occurred near 1.0 V and then the ECL intensity rose steeply. The ECL peak intensity occurred at 1.14 V, where $Ru(bpy)_3^{2+}$ was electrochemically oxidized.
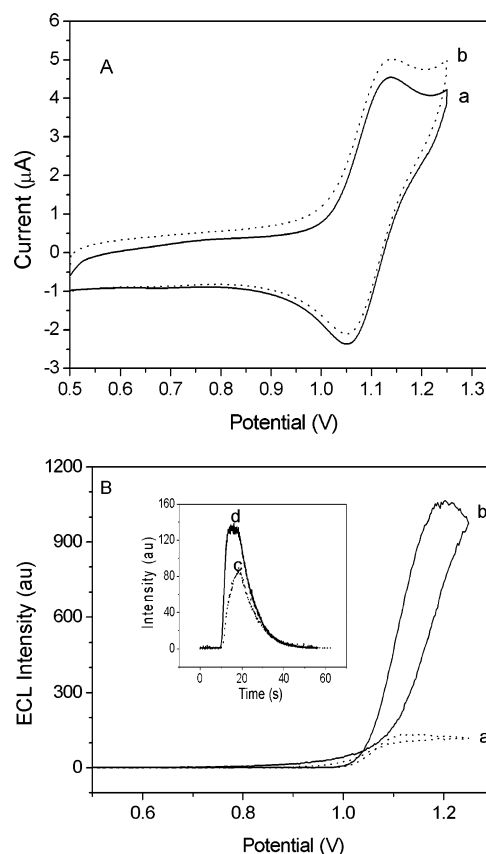

Figure 4. CVs (A) and corresponding ECL curves (B) of the ECL sensor without (a) and with (b) 1 μM TPA in PBS. Insert of B: ECL curves with (d) and without (c) 1×10^{-15} M TPA in 50 mM PBS (pH 7.5). Scan rate, 50 mV/s.
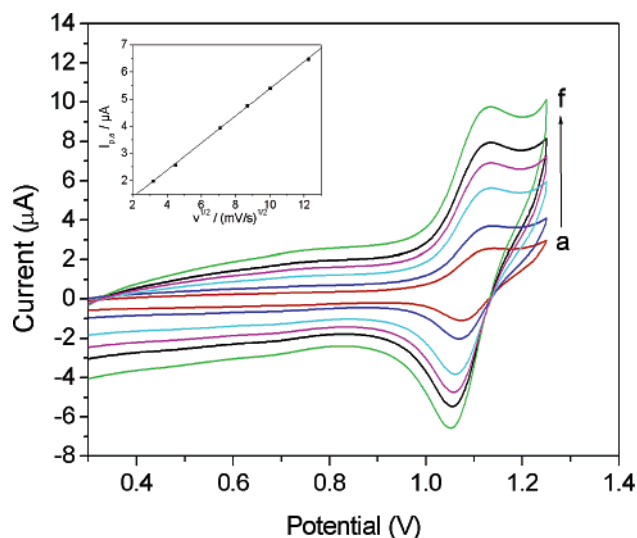

Figure 5. CVs of the sensor at various scan rates in 50 mM PBS (pH 7.5): (a) 10, (b) 20, (c) 50, (d) 75, (e) 100, and (f) 150 mV/s. Inset: The relationship between the anodic peak current and the square root of the scan rate.

The ECL signal increased greatly in the presence of TPA, which was consistent with the CVs. For sensitivity studies, the modified electrode was used to determine the TPA of low concentration. The insert in Figure 4B showed the ECL curves of the sensor without (a) and with (b) 1×10^{-15} M TPA in PBS. This detection result was six orders lower than that at the CNT/Nafion composite film-modified electrode,[15] four orders lower than that with analyte field-amplified sample stacking on capillary,[26] and
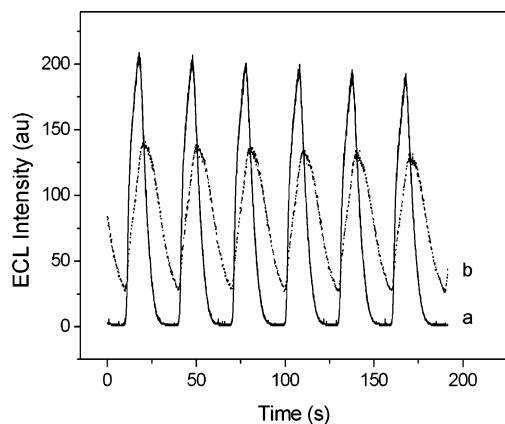
Synthesis of PtNPs/AQ/Ru(bpy)$_3^{2+}$ Colloid

J. Phys. Chem. B, Vol. 110, No. 43, 2006 **21665**

Figure 6. ECL curves of PtNPs/AQ/Ru(bpy)$_3^{2+}$ (a) and AQ/Ru(bpy)$_3^{2+}$ (b) modified electrodes under continuous CVs only in 50 mM PBS (pH 7.5). Scan rate, 50 mV/s.

Figure 7. ECL emission from the ECL sensor in 50 mM PBS (pH 7.5) containing 1 μM TPA under continuous CVs for 30 cycles. Scan rate, 50 mV/s.

about 500 times lower than that with TPA in a 100 nL ECL detector obtained by Manz's group.[27] The following reasons can explain the ultrasensitive detection. First, the high surface area PtNPs made the structure of the PtNPs/AQ/Ru(bpy)$_3^{2+}$ composite film more open compared with that of the AQ/Ru-(bpy)$_3^{2+}$ film and allowed faster diffusion of analyte. Second, the electronic conductivity and electrocatalytic properties of PtNPs provided conducting pathways to connect Ru(bpy)$_3^{2+}$ sites to the electrode and accelerated the speed of reaction. Third, the polymer AQ has been observed to exclude anionic species from the surface for substantial improvement in the selectivity.[20a] In our experimental condition, TPA (pK_α =10.7) is positively charged in pH 7.5 PBS.

For comparison, on one hand, we studied the ECL intensity of the PtNPs/AQ film modified electrode, which was then placed in a 1 mM Ru(bpy)$_3^{2+}$ aqueous solution for 20 min for the incorporation of Ru(bpy)$_3^{2+}$ (short by PtNPs/AQ+Ru(bpy)$_3^{2+}$) . Interestingly, with the same volume of colloid coating at the surface of the electrode, the relative ECL intensity on the PtNPs/AQ/Ru(bpy)$_3^{2+}$ modified electrode was almost 10-fold greater than that obtained on the PtNPs/AQ+Ru(bpy)$_3^{2+}$ modified electrode. This phenomenon may be explained as follows. AQ polymer was anionic ionomers with hydrophobic backbones and hydrophilic $-SO_3^-$ groups as Nafion. Ramaraj[5a] has reported that the formation of a nanostructured Pt electrode was found to be very high at the interior hydrophilic cluster regions of the Nafion film. If the same phenomenon described for Nation is also true for AQ, it is reasonable to consider that the formation of PtNPs is easier at hydrophilic regions of the AQ than at hydrophobic regions during the reduction of H$_2$PtCl$_6$. So in the preparation of PtNPs/AQ colloid without Ru(bpy)$_3^{2+}$ ion-exchanged into AQ at first, as-formed PtNPs took up preferentially the hydrophilic regions ($-SO_3^-$ groups), which decreased the number of ion-exchanged sites during the incorporation of Ru(bpy)$_3^{2+}$. In contrast, when the Ru(bpy)$_3^{2+}$ was added to AQ solution before the reduction of H$_2$PtCl$_6$, the AQ polymer preferentially binds Ru(bpy)$_3^{2+}$ cations and excludes negatively charged species (PtCl$_6^{2-}$) from entering the membrane. Moreover, the PtNPs/AQ/Ru(bpy)$_3^{2+}$ film formed on the surface of the electrode can be used directly as an ECL sensor, simplifying the experimental preparation.

On the other hand, we prepared the AQ/Ru(bpy)$_3^{2+}$ modified electrode under identical conditions except for the reduction of H$_2$PtCl$_6$ to PtNPs. Figure 6 showed ECL curves of PtNPs/AQ/Ru(bpy)$_3^{2+}$ and AQ/Ru(bpy)$_3^{2+}$ modified electrode under continuous CVs only in 50 mM PBS. It is obvious that the shape of Figure 6a with the addition of PtNPs is much sharper than
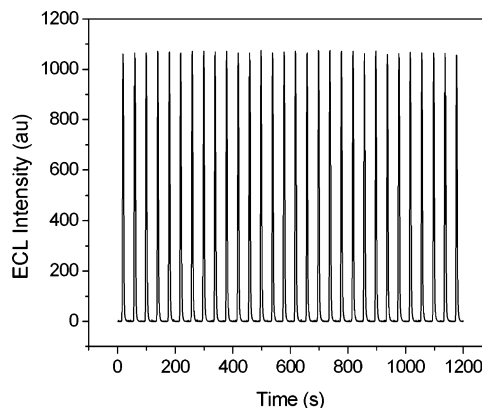
that of Figure 6b, which indicated that the ECL response at the PtNPs/AQ/Ru(bpy)$_3^{2+}$ modified electrode was much faster than that of the AQ/Ru(bpy)$_3^{2+}$ modified electrode due to the PtNPs in the film. The ECL reaction could take place from the reaction of oxidized Ru(bpy)$_3^{3+}$ and TPA at the outer surface of the film, from the reaction of oxidized Ru(bpy)$_3^{3+}$ and TPA throughout the film via permeation from the bulk solution, or from both.[15] Furthermore, direct amine oxidation at the electrode surface plays an important role in the ECL process.[4a] Therefore, the PtNPs/AQ/Ru(bpy)$_3^{2+}$ composite film exhibited a faster diffusion of TPA into the film and also increased charge transport.

To study the stability, the electrode was immersed in 50 mM PBS solution all the time. Figure 7 showed the ECL emission from a PtNPs/AQ/Ru(bpy)$_3^{2+}$ film modified electrode under continuous potential scanning for 30 cycles. The relative standard deviation was 0.6%. The results suggested that the charge transfer in composite film was very fast and the film was quite stable. The result was likely that the AQ showed a greater hydrophilic character than Nafion and restrained the migration of immobilized Ru(bpy)$_3^{2+}$ into the more hydrophobic regions with time.

4. Conclusions

In conclusion, we have developed a simple method to prepare the functional PtNPs colloid. Directly coating the as-formed colloid on the surface of the electrode was used as an ECL sensor, which showed fast charge transfer, excellent stability, and high sensitivity due to the high surface area, good electronic conductivity of PtNPs, or/and the selectivity of AQ. This method was very simple, effective, and low cost and demonstrated the application prospect of metal nanoparticle colloids.

References and Notes

(1) Faulkner, L. R.; Bard, A. J. In *Electroanalytical Chemistry*; Bard, A. J., Ed.; Marcel Dekker: New York, 1977; Vol. 10, p 1.

(2) O'Connell, C. D.; Juhasz, A.; Kuo, C.; Reeder, D. J.; Hoon, D. S. B. *Clin. Chem.* **1998**, *44*, 1161.

(3) Tokel, N. E.; Bard, A. J. *J. Am. Chem. Soc.* **1972**, *94*, 2862.

(4) (a) Richter, M. M. *Chem. Rev.* **2004**, *104*, 3003. (b) Wightman, R. M.; Curtis, C. L.; Flowers, P. A.; Maus, R. G.; McDonald, E. M. *J. Phys. Chem. B* **1998**, *102*, 9991. (c) Kozlov, V. G.; Bulovic, V.; Burrows, P. E.; Forrest, S. R. *Nature* **1997**, *389*, 362. (d) Fan, F. R. F.; Cliffel, D.; Bard, A. J. *Anal. Chem.* **1998**, *70*, 2941.

(5) Yin, X.; Dong, S.; Wang, E. *Trends Anal. Chem.* **2004**, *23*, 432.

(6) (a) Skotty, D. R.; Lee, W. Y.; Nieman, T. A. *Anal. Chem.* **1996**, *68*, 1530. (b) Ridlen, J. S.; Klopp, G. J.; Niemana, T. A. *Anal. Chim. Acta* **1997**, *341*, 195.

(7) Liu, J.; Yan, J.; Yang, X.; Wang, E. *Anal. Chem.* **2003**, *75*, 3637.

(8) Chiang, M. T.; Whang, C. W. *J. Chromatogr. A* **2001**, *934*, 59.

(9) (a) Qiu, H.; Yan, J.; Sun, X.; Liu, J.; Cao, W.; Yang, X.; Wang, E. *Anal. Chem.* **2003**, *75*, 5435. (b) Du, Y.; Wei, H.; Kang, J.; Yan, J.; Yin, X.; Yang, X.; Wang, E. *Anal. Chem.* **2005**, *77*, 7993.

(10) (a) Knight, A. W. *Trends Anal. Chem.* **1999**, *18*, 47. (b) Lee, W. Y. *Microchim. Acta* **1997**, *127*, 19.

(11) (a) Lyons, C. H.; Abbas, E. D.; Lee, J. K.; Rubner, M. F. *J. Am. Chem. Soc.* **1998**, *120*, 12100. (b) Wu, A.; Lee, T.; Rubner, M. F. *Thin Solid Films* **1998**, *663*, 327.

(12) (a) Zhang, Z.; Bard, A. J. *J. Phys. Chem.* **1988**, *92*, 5566. (b) Miller, C. J.; McCord, P.; Bard, A. J. *Langmuir* **1991**, *7*, 2781.

(13) Guo, Z.; Shen, Y.; Wang, M.; Zhao, F.; Dong, S. *Anal. Chem.* **2004**, *76*, 184.

(14) (a) Downey, T. M.; Nieman, T. A. *Anal. Chem.* **1992**, *64*, 261. (b) Rubinstein, I.; Bard, A. J. *J. Am. Chem. Soc.* **1980**, *102*, 6641. (c) Rubinstein, I.; Bard, A. J. *J. Am. Chem. Soc.* **1981**, *103*, 5007.

(15) Guo, Z.; Dong, S. *Anal. Chem.* **2004**, *76*, 2683.

(16) Khramov, A. N.; Collinson, M. M. *Anal. Chem.* **2000**, *72*, 2943.

(17) Choi, H. N.; Cho, S.-H.; Lee, W. Y. *Anal. Chem.* **2003**, *75*, 4250.

(18) Ding, S. N.; Xu, J. J.; Chen, H. Y. *Electrophoresis* **2005**, *26*, 1737.

(19) Martin, A. F.; Nieman, T. A. *Biosens. Bioelectron.* **1997**, *12*, 479.

(20) (a) Wang, J.; Golden, T. *Anal. Chem.* **1989**, *61*, 1397. (b) Wang, H.; Xu, G.; Dong, S. *Anal. Chim. Acta.* **2003**, *480*, 285.

(21) (a) Morris, C. A.; Anderson, M. L.; Stroud, R. M.; Merzbacher, C. I.; Rolison, D. R. *Science* **1999**, *284*, 622. (b) Tong, Y.; Rice, A.; Wieckowski, A.; Oldfield, E. *J. Am. Chem. Soc.* **2000**, *122*, 1123. (c) Wei, Y.; Cao, C.; Jin, R.; Mirkin, C. A. *Science* **2002**, *297*, 1536.

(22) (a) Zen, J. M.; Senthil Kumar, A.; Chung, C. R. *Anal. Chem.* **2003**, *75*, 2703. (b) Hrapovic, S.; Luong J. H. T. *Anal. Chem.* **2003**, *75*, 3308. (c) Antoine, O.; Bultel, Y.; Durand, R. *J. Electroanal. Chem.* **2001**, *499*, 85. (d) Solla-Gullon, J.; Montiel, V.; Aldaz, A.; Clavilier, J. *J. Electroanal. Chem.* **2000**, *491*, 69. (e) Jiang, J.; Kucernak, A. *J. Electroanal. Chem.* **2004**, *567*, 123.

(23) (a) Selvaraju, T.; Ramaraj, R. *J. Electroanal. Chem.* **2005**, *585*, 290. (b) Mao, L.; Zhang, D.; Sotomura, T.; Nakatsu, K.; Koshiba, N.; Ohsaka, T. *Electrochim. Acta* **2003**, *48*, 1015. (c) Yasuzawa, M.; Kunugi, A. *Electrochem. Commun.* **1999**, *1*, 459. (d) Michalska, A.; Walkiewicz, S.; Maksymiuk, K.; Hall, E. A. H. *Electroanalysis* **2002**, *14*, 1236.

(24) Sun, X.; Du, Y.; Dong, S.; Wang, E. *Anal. Chem.* **2005**, *77*, 8166.

(25) Drawdy, E.; Hoflund, G. B.; Gardner, S. D.; Yngvadottir, E.; Schryer, D. R. *Surf. Interface Anal.* **1990**, *16*, 369.

(26) Cao, W.; Liu, J.; Yang, X.; Wang, E. *Electrophoresis* **2002**, *23*, 3683.

(27) Arora, A.; deMello, A. J.; Manz, A. *Anal. Commun.* **1997**, *34*, 393.