Electrogenerated Chemiluminescence Sensors Using Ru(bpy)$_3^{2+}$ Doped in Silica Nanoparticles

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A novel electrogenerated chemiluminescence (ECL) sensor based on Ru(bpy)$_3^{2+}$-doped silica (RuDS) nanoparticles conjugated with a biopolymer chitosan membrane was developed. These uniform RuDS nanoparticles (∼40 nm) were prepared by a water-in-oil microemulsion method and were characterized by electrochemical and transmission electron microscopy technology. The Ru-(bpy)$_3^{2+}$-doped interior maintained its high ECL efficiency, while the exterior nanosilica prevented the luminophor from leaching out into the aqueous solution due to the electrostatic interaction. This is the first attempt to branch out the application of RuDS nanoparticles into the field of ECL, and since a large amout of Ru(bpy)$_3^{2+}$ was immobilized three-dimensionally on the electrode, the Ru-(bpy)$_3^{2+}$ ECL signal could be enhanced greatly, which finally resulted in the increased sensitivity. This sensor shows a detection limit of 2.8 nM for tripropylamine, which is 3 orders of magnitude lower than that observed at a Nafion-based ECL sensor. Furthermore, the present ECL sensor displays outstanding long-term stability.

Electrogenerated chemiluminescence (ECL) is the emission from an excited molecule generated by an electrochemical redox reaction.[1] It has attracted much attention during past several decades due to its versatility, simplified optical setup, and good temporal and spatial control. As a highly sensitive and selective detection method, ECL detection has been widely used in flow injection analysis, high-performance liquid chromatography, capillary electrophoresis (CE), and microchip CE to detect amino acids, oxalate, NADH, alkylamines, and nucleic acids.[2−10] Among various ECL systems, Ru(bpy)$_3^{2+}$-based ECL gained more importance due to its superior properties including high sensitivity and good

stability under moderate conditions in aqueous solution.[11] Moreover, the oxidation−reduction mechanism for Ru(bpy)$_3^{2+}$ ECL postulated by Rubinstein and Bard indicates that Ru(bpy)$_3^{2+}$ could be regenerated on the electrode surface.[12] Therefore, a variety of methods to immobilize Ru(bpy)$_3^{2+}$ on the electrode surface have been developed including the following: (I) Ru(bpy)$_3^{2+}$ is cation-exchanged to a surface-bound polymer such as ion exchanger Nafion and its composite film.[13−16] Although Nafion has proven to be a promising matrix, the migration of the Ru(bpy)$_3^{2+}$ into the electrochemically inactive hydrophobic region of Nafion causes a serious problem with the long-term stability of the ECL sensor. (II) Ru(bpy)$_3^{2+}$ is localized at the electrode surface by adsorption with negatively charged nanoparticles by a layer-by-layer method.[17,18] (III) The derivatized Ru(bpy)$_3^{2+}$ complex is covalently attached to the electrode surface.[19,20] Such methods possess good stability due to the strong interaction between Ru-(bpy)$_3^{2+}$ derivatives and the electrode surface. But the synthesis process is quite complicated and arduous. Though some of above methods could attain good sensitivity and stability, one inherent limitation that lies in all of these methods is the limited amount of Ru(bpy)$_3^{2+}$ immobilized on the electrode. If more Ru(bpy)$_3^{2+}$ is needed to be immobilized on the electrode, then the film must become thicker, which may retard the transfer of the electron and analyte.

The rapidly evolving field of nanoscience and nanotechnology has opened up a new and promising era, in which nanoparticles of various shapes, sizes, and compositions have been successfully used in sensing and labeling due to their unique properties. With manipulated composition and surface modification, these nanoparticle probes have enhanced the response signal, increased the sensitivity, and shown better reproducibility. Recently, Tan's group developed a new strategy for the preparation of uniform size, spherical-Ru(bpy)$_3^{2+}$-doped silica (RuDS) nanoparticles with a

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reverse microemulsion method.[21] Incorporation of $Ru(bpy)_3^{2+}$ molecules inside the silica matrix protects them from the surrounding environment, increases photostability, and provides signal enhancement due to an increase in the number of dye molecules doped per nanoparticle.[22] Furthermore, the ease of introducing functional groups such as amines, thiols, and carboxyls on the surface of $Ru(bpy)_3^{2+}$-doped silica nanoparticles makes them ideal for bioanalysis applications.[23] On the other side, considerable efforts were made to synthesize electroactive reagent-doped silica nanoparticles using a water-in-oil (W/O) microemulsion method, and the electroactive reagent doped in the silica nanoparticles showed high electron-transfer efficiency in electrochemical detection.[24,25] It is well known that $Ru(bpy)_3^{2+}$ is an excellent ECL reagent with superior properties, such as good stability, high sensitivity, and regenerative capability. Unfortunately, all of above-reported applications of RuDS nanoparticles were limited to the spectrofluorometric measurements. To our knowledge, no further investigations on electrochemistry or ECL of RuDS nanoparticles have ever been reported.

In the present work, we prepared the RuDS nanoparticles with the same microemulsion method and bound these nanoparticles with the chitosan (CHIT) by H-bonding interaction.[26] CHIT is a polysaccharide biopolymer, which displays excellent film-forming ability, high water permeability, and good adhesion to the electrode surface.[27] Then the mixture of RuDS nanoparticles and CHIT was cast on the electrode to fabricate ECL sensors. Compared with the reported ECL sensors, this novel sensor has outstanding advantages concerning both sensitivity and stability. Obviously, $Ru(bpy)_3^{2+}$ is three-dimensionally entrapped in the nanoparticles; in other words, a great deal of $Ru(bpy)_3^{2+}$ is entrapped in the RuDS–CHIT composite film, which finally results in the enhanced sensitivity. Furthermore, the $Ru(bpy)_3^{2+}$ immobilized in the composite film could maintain extraordinary stability since the leakage of $Ru(bpy)_3^{2+}$ from the silica nanoparticles is negligible, which is probably due to the strong electrostatic interaction between positively charged $Ru(bpy)_3^{2+}$ and negatively charged silica.[28]

EXPERIMENTAL SECTION

Materials. Tris(2,2′-bipyridyl)dichlororuthenium(II) hexahydrate ($Ru(bpy)_3Cl_2 \cdot 6H_2O$) and tripropylamine (TPA) were purchased from Aldrich. CHIT (low molecular weight, minimum 85% deacetylated) and Triton X-100 (TX-100) were purchased from Sigma. Tetraethyl orthosilicate (TEOS) was obtained from Beijing Yili Chemical Reagent Factory (Beijing, China); 1-hexanol, cyclohexane, and ammonium hydroxide (25 wt %) were purchased from Beijing Chemical Reagent Factory (Beijing, China). All other chemicals were of analytical grade, and the aqueous solutions were prepared with doubly distilled water.

Apparatus. Cyclic voltammetric experiments were performed with a CH Instruments 832 voltammetric analyzer. All experiments were carried out with a conventional three-electrode system. The working electrode was glassy carbon electrode (GCE) coated with RuDS–CHIT composite film. A platinum wire was used as the counter electrode, and an Ag/AgCl (saturated KCl) as a reference electrode. All the potentials were measured and reported according to this reference electrode.

The ECL signal produced in the electrolytic cell was detected and recorded by a flow injection chemiluminescence analyzer (IFFD, Xi'an Remax Electronic Science Tech. Co. Ltd.), which was operated by a personal computer. ECL intensities were measured through the bottom of the cell with a photomultiplier tube (PMT) window, and all of them were enclosed in a light-tight box. The PMT was operated in current mode, unless noted, otherwise the PMT was biased at 800 V.

The size of RuDS nanoparticles were measured using the JEOL 2010 transmission electron microscope (TEM) operated at an accelerating voltage of 200 kV.

The Barrett–Jouner–Halenda (BJH) pore diameter was measured by a nitrogen adsorption–desorption isotherm on a Quantachrome NOVA 1000 Ver 6.11 system at 77.4 K. The sample was first degassed in a vacuum before analysis.

Preparation of the Modified Electrode. GCE ($d = 4$ mm) was polished before each experiment with 1- and 0.3-μm alumina powder, respectively, and sonicated in acetone and doubly distilled water thoroughly. Then it was allowed to dry at room temperature.

The RuDS nanoparticles were prepared as previously described with a little modification.[23] The W/O microemulsion was prepared first by mixing 1.77 mL of TX-100, 7.5 mL of cyclohexane, 1.8 mL of 1-hexanol, and 340 μL of water. Then 80 μL 0.1 M $Ru(bpy)_3^{2+}$ aqueous solution was added into the mixture. In the presence of 100 μL of TEOS, a polymerization reaction was initiated by adding 60 μL of NH_4OH (25%). The reaction was allowed to stir for 24 h. After the reaction was completed, the RuDS nanoparticles were isolated by acetone, followed by centrifuging and washing with ethanol and water several times to remove any surfactant molecules. At last, some completely dry orange RuDS nanoparticles were obtained.

These RuDS nanoparticles were then suspended in phosphate buffer solution (PBS; pH 7.5) by ultrasonication for the following experiments. One percent CHIT solution was prepared by dissolving CHIT in 1% acetic acid solution with magnetic stirring for ~2 h. By mixing 1% CHIT with RuDS nanoparticles PBS, the silica surfaces could be used to bind covalently with biopolymer CHIT and the final concentration of the RuDS is 1 mg/mL. A 5-μL aliquot of RuDS–CHIT mixture was placed on the GCE and allowed to dry at room temperature.[24] The procedure yielded a robust and strongly adherent composite film. When not in use, the films were kept in dry state at room temperature.

RESULTS AND DISCUSSION

RuDS Nanoparticle Characterization. In a typical W/O microemulsion system, water droplets are stabilized by surfactant molecules and retain dispersed in bulk oil. The nucleation and growth kinetics of the silica are highly regulated in the water droplets of the microemulsion system, and the $Ru(bpy)_3^{2+}$ is

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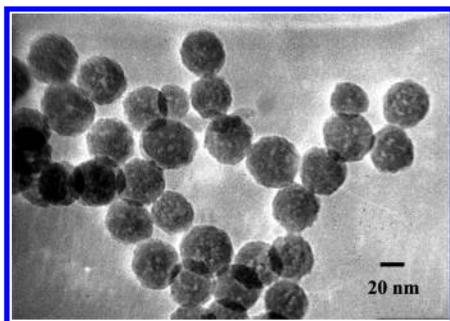
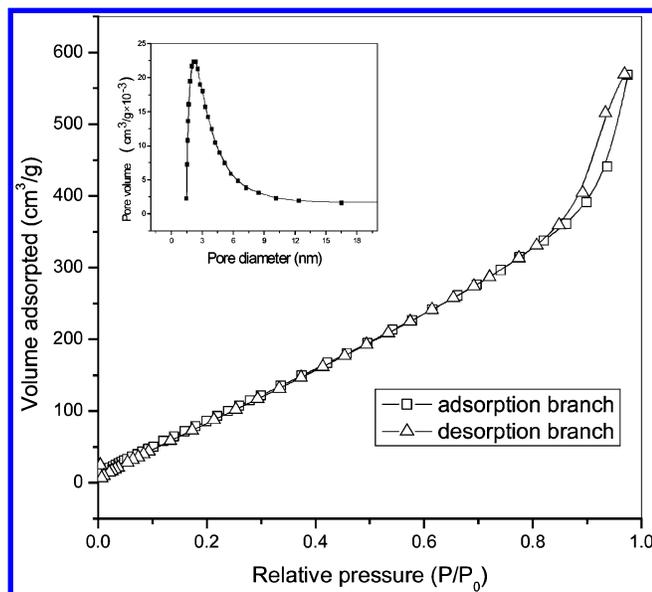**Figure 1.** TEM image of Ru(bpy)$_3^{2+}$-doped silica nanoparticles.

Figure 2. Nitrogen adsorption−desorption isotherm for the Ru-(bpy)$_3^{2+}$-doped silica nanoparticles. Inset: BJH pore diameter distribution of the Ru(bpy)$_3^{2+}$-doped silica nanoparticles.

physically encapsulated in the silica network, resulting in the production of highly monodisperse dye-doped silica nanoparticles.[29] The RuDS nanoparticles prepared by this method are uniform in size, ~40 nm as characterized by TEM in Figure 1. Moreover, we determined the pore diameter of the RuDS nanoparticles by nitrogen adsorption−desorption isotherm measurement at 77 K on a Quantachrome NOVA 1000 Ver 6.11 system. Figure 2 presents the nitrogen adsorption−desorption isotherm, and the inset of Figure 2 indicates BJH pore size distribution. The results showed that the pore diameter of RuDS nanoparticles is ~2.27 nm. Since TPA is a small molecule, the porous silica matrix could not retard TPA penetration severely but only to a certain extent. On the other hand, although the pore is bigger than the Ru(bpy)$_3^{2+}$ molecule, Ru(bpy)$_3^{2+}$ could not leach out from the silica matrix since there exists strong electrostatic interaction between Ru(bpy)$_3^{2+}$ and silica.

Electrochemistry and ECL of Immobilized Ru(bpy)$_3^{2+}$. The electrochemical and ECL behavior of the RuDS−CHIT composite film electrode has been investigated with TPA, since the Ru(bpy)$_3^{2+}$−TPA system has been well studied and gave much higher ECL compared with other commonly used reductants such
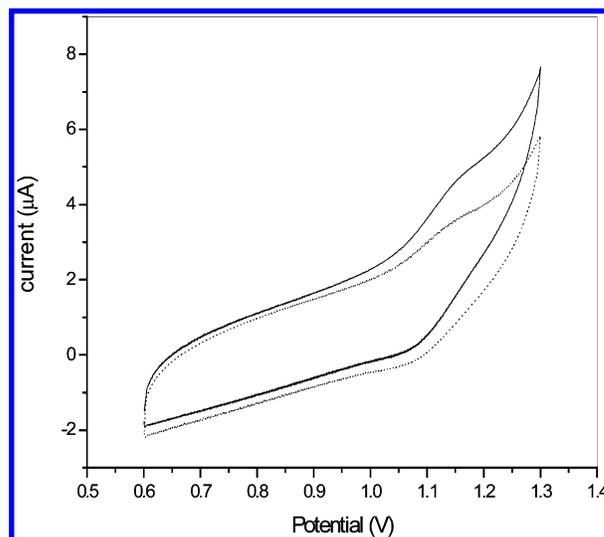
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Figure 3. Cyclic voltammograms of Ru(bpy)$_3^{2+}$ immobilized in RuDS−CHIT composite film electrode in the absence (dashed line) and presence (solid line) of 3 × 10^{-5} M TPA in PBS (pH 7.5) at the scan rate of 100 mV/s.

as oxalate.[30] Figure 3 shows cyclic voltammograms (CVs) of Ru-(bpy)$_3^{2+}$ in the absence (dotted line) and presence (solid line) of 3 × 10^{-5} M TPA at the scan rate of 100 mV/s in PBS (pH 7.5). Since the TPA is quite small, it could permeate into the silica nanoparticle through the pores of the nanoparticles to react with the oxidized Ru(bpy)$_3^{2+}$. The presence of TPA made the oxidation current of Ru(bpy)$_3^{2+}$ increase clearly while the reduction current decreased, which is consistent with the electrocatalytic reaction mechanism. Meanwhile, the ECL signal increased considerably in the presence of TPA. This result showed that the Ru(bpy)$_3^{2+}$ could retain its high ECL efficiency when doped in the silica nanoparticles. Moreover, with such a unique immobilization method, a great deal of Ru(bpy)$_3^{2+}$ could dope inside the silica nanoparticles, which led to the strong ECL signal as demonstrated in Figure 4. Such ECL signal enhancement could facilitate the ultrasensitive analyte determination. The onset of luminescence occurred near 1 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)$_3^{2+}$. This means that the oxidation of immobilized Ru(bpy)$_3^{2+}$ plays a key role in the process of ECL.

We investigated the effect of the amount of RuDS nanoparticle in the composite film and the amount of composite film on the ECL response, respectively. As can be seen from the Figure 1S (Supporting Information), the ECL increased with the increase of the RuDS nanoparticle amount in the composite film, leveled off after reaching 1 mg/mL; when it was higher than 1.5 mg/mL, ECL began to fall. On the other hand, ECL could attain the strongest response when the amount of composite film material cast on the electrode was 5 μL (Figure 2S, Supporting Information). This could be attributed to the fact that when the film is too thick or too many RuDS nanoparticles were immobilized, the ECL emission might be absorbed and scattered, which finally led to the ECL decrease. Therefore, we chose the RuDS nanoparticle amount as 1 mg/mL in the composite film and cast 5 μL of

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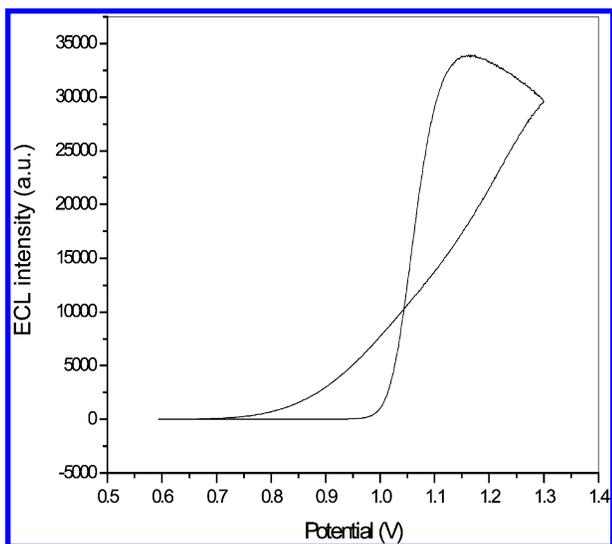
Figure 4. Corresponding ECL-potential curves for RuDS−CHIT composite film electrode in PBS (pH 7.5) containing 8×10^{-6} M TPA at the scan rate of 10 mV/s.
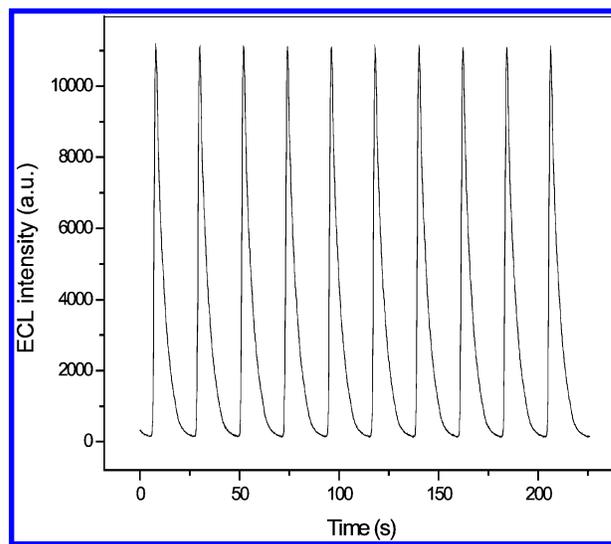

Figure 6. ECL intensity of RuDS−CHIT composite film electrode in PBS (pH 7.5) containing 7.5×10^{-6} M TPA under continuous CVs for 10 cycles with the scan rate of 100 mV/s.
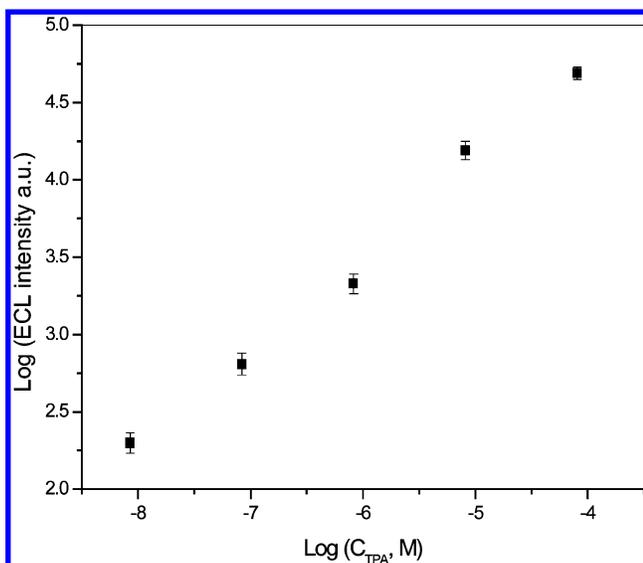

Figure 5. Calibration of TPA at the RuDS−CHIT composite film electrode in PBS (pH 7.5) at the scan rate of 100 mV/s.
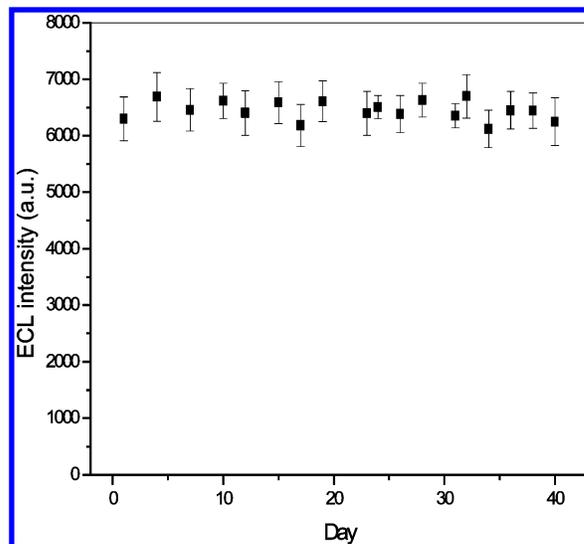

Figure 7. Stability of the RuDS−CHIT composite film electrode ECL sensor under storage.

composite film material on the electrode surface in our experiments.

A pH study has been carried out to investigate the pH effect on the $Ru(bpy)_3^{2+}$−TPA system (Figure 3S, Supporting Information). The ECL response for 2.5×10^{-6} M TPA obtained at the RuDS−CHIT composite film electrode increases from pH 6.5 to 7.5 and then decreases at higher pH. The ECL response increased from pH 5.5 to 7.5, implying that the deprotonation of TPA is required for the efficient ECL in RuDS−CHIT composite film, which was consistent with the mechanism proposed in the literature for solution-based ECL.[31] While with the pH increasing continuously, some decomposition of species might be expected, leading to a diminished ECL reagent available for ECL reaction. Therefore, the ECL intensity decreased.

The scan rate effect on the ECL intensity was also studied. The ECL intensity decreased with the increase of scan rate in

the range from 30 to 200 mV s^{-1}. Previous work has shown the system to be of intermediate reaction kinetics.[9] So formation of the ECL reaction intermediate and diffusion of TPA contributed to the variation of the ECL with the scan rate. Because the ECL signal increased as the scan rate decreased, the chemical kinetics of the system plays a major role in this process.

Calibration curves for TPA have been constructed using the present ECL sensor based on the RuDS−CHIT composite films. Each point as shown in Figure 5 is a mean of three ECL signals obtained by consecutively cyclic potential scanning (100 mV/s) at a given concentration in PBS (pH 7.5). It could be seen that ECL intensity had good linearity with the TPA concentration and the linear range was wide, extending from 8.5×10^{-9} to 8.1×10^{-5} M with a remarkable detection limit (S/N = 3) of 2.8 nM. Compared with the effective $Ru(bpy)_3^{2+}$ preconcentration medium Nafion, the detection limit of RuDS−CHIT composite films is 3 orders of magnitude lower. The improved sensitivity could be attributed to the large amount of $Ru(bpy)_3^{2+}$ doped in the composite film, which greatly increased ECL response.

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Another advantage of the present RuDS−CHIT-based Ru-(bpy)$_3^{2+}$ ECL sensor is the outstanding stability. Both operational and storage stability are important from the practical application point of view. Under continuously cyclic potential scanning for 10 cycles in PBS (pH 7.5) containing 7.5×10^{-6} M TPA with the scan rate of 100 mV/s, there was no detectable change for ECL intensity as demonstrated in Figure 6. The long-term storage stability of the present sensor was studied over a 40-day period by monitoring its ECL response to 5×10^{-6} M TPA in PBS (pH 7.5) with intermittent usage (every 2−3 days) and by storing in the air at room temperature when not in use. As can be seen from Figure 7, the ECL intensity changed slightly during this period. Eighty days later, the sensor could still maintain 80% of the original response. The good stability of the ECL sensor may be attributed to the two different kinds of interactions: the strong electrostatic interaction between positively charged Ru(bpy)$_3^{2+}$ and negatively charged silica nanoparticles; the H-bonding interaction between RuDS and CHIT.

CONCLUSION

Ru(bpy)$_3^{2+}$-doped silica nanoparticles were applied in the field of ECL for the first time, and based on it, we fabricated a novel ECL sensor. With such a special immobilization method, a great deal of Ru(bpy)$_3^{2+}$ is three-dimensionally immobilized onto the electrode, which finally leads to the increased ECL response and enhanced sensitivity. Moreover, with the unique characteristics of the Ru(bpy)$_3^{2+}$ and silica nanoparticle, such an ECL sensor displays distinguished stability. Since it is quite easy to modify the RuDS surface with functional groups, the combination of the sensitive ECL sensor with some biomolecules could be a perfect candidate in the application of bioanalysis.

SUPPORTING INFORMATION AVAILABLE

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.