

# Electrochemiluminescence Sensor Based on Partial Sulfonation of Polystyrene with Carbon Nanotubes

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Herein, homogeneously partial sulfonation of polystyrene (PSP) was performed. An effective electrochemiluminescence (ECL) sensor based on PSP with carbon nanotube (CNTs) composite film was developed. Cyclic voltammetry and electrochemical impedance spectroscopy were applied to characterize this composite film. The PSP was used as an immobilization matrix to entrap the ECL reagent  $\text{Ru}(\text{bpy})_3^{2+}$  due to the electrostatic interactions between sulfonic acid groups and  $\text{Ru}(\text{bpy})_3^{2+}$  cations. The introduction of CNTs into PSP acted not only as a conducting pathway to accelerate the electron transfer but also as a proper matrix to immobilize  $\text{Ru}(\text{bpy})_3^{2+}$  on the electrode by hydrophobic interaction. Furthermore, the results indicated the ECL intensity produced at this composite film was over 3-fold compared with that of the pure PSP film due to the electrocatalytic activity of the CNTs. Such a sensor was verified by the sensitive determinations of 2-(dibutylamino)ethanol and tripropylamine.

Electrochemiluminescence (ECL) has attracted much attention due to its acknowledged advantages such as versatility, simplified optical setup, very low background signal, and good temporal and spatial control.<sup>1–3</sup> As a sensitive detection method, ECL has been used in high-performance liquid chromatography (HPLC), flow injection analysis, capillary electrophoresis (CE), and CE microchip.<sup>4</sup> Among various ECL systems,  $\text{Ru}(\text{bpy})_3^{2+}$ -based ECL has been extensively investigated due to its superior properties including high sensitivity and good stability under moderate conditions in aqueous solution.<sup>5</sup>

The oxidation–reduction reaction mechanism for  $\text{Ru}(\text{bpy})_3^{2+}$ -based ECL postulated by Rubinstein and Bard elucidates that  $\text{Ru}$

$(\text{bpy})_3^{2+}$  can be regenerated in situ at the electrode surface during the ECL process.<sup>6</sup> In addition, the immobilization of  $\text{Ru}(\text{bpy})_3^{2+}$  can provide several advantages compared with the solution-phase ECL procedure, for example, reducing the consumption of expensive reagent, enhancing the ECL signal, and simplifying experimental design. Therefore, many efforts were made aiming at developing a cost-effective, regenerable chemical sensor as follows: (1)  $\text{Ru}(\text{bpy})_3^{2+}$  is incorporated to a cation exchanger Nafion and its composite film.<sup>7–11</sup> (2)  $\text{Ru}(\text{bpy})_3^{2+}$  is immobilized at the electrode surface by a layer-by-layer method.<sup>12,13</sup> (3)  $\text{Ru}(\text{bpy})_3^{2+}$  is entrapped in a sol–gel.<sup>14,15</sup> (4)  $\text{Ru}(\text{bpy})_3^{2+}$  is mingled with the complex incorporated by using a carbon electrode.<sup>16–18</sup> (5)  $\text{Ru}(\text{bpy})_3^{2+}$  is mixed with metal nanoparticle films.<sup>19–20</sup> Although all the above ECL sensors showed improved ECL characteristics, new materials and immobilization methods are still needed to develop highly sensitive and regenerable ECL-based sensors. To our knowledge, poly(sodium 4-styrenesulfonate) (PSS) has been applied in the immobilization of  $\text{Ru}(\text{bpy})_3^{2+}$ ,<sup>21,22</sup> however, due to its water solubility, it only acted as an enhancer for ion exchange of the composite film<sup>22</sup> and could not be used as an immobilization matrix for long-term stability ECL sensors. Whereas, partial sulfonation of polystyrene (PSP), which could achieve a homogeneous distribution of sulfonic acid groups in the polymer, could overcome the disadvantage of PSS. On one hand, it could be easily used as a membrane material by evaporation casting

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from appropriate solvents due to the residual unsulfonated polystyrene; on the other hand, it could also be used as an ion exchanger due to the electrostatic interactions between existing sulfonic acid groups and Ru(bpy)<sub>3</sub><sup>2+</sup> cations.<sup>23</sup> Nevertheless, these materials are nonelectroactive; therefore, improving the conductivity of the membrane material should be done.

Carbon nanotubes (CNTs) have inspired enormous interest due to their special properties such as high electrical conductivity, high chemical stability, and extremely high mechanical strength and modulus. One promising application of CNTs is their use in chemical sensors and nanoscale electronic devices. Such potential applications would greatly benefit from their ability to promote electron-transfer reactions of important molecules when used as an electrode material in electrochemical reactions.<sup>24–26</sup> Therefore, the interfusion of negatively charged CNTs into the PSP not only acted as a conducting pathway to accelerate the electron transfer but also acted as a proper matrix to immobilize Ru(bpy)<sub>3</sub><sup>2+</sup> on the electrode by hydrophobic interaction.

In the present work, what we reported is an alternative matrix to immobilize Ru(bpy)<sub>3</sub><sup>2+</sup> on a glassy carbon (GC) electrode surface by combination of PSP with CNTs. The composite film modified electrode also showed sensitivity for the ECL determination of tripropylamine (TPA) and 2-(dibutylamino)ethanol (DBAE). In this method, PSP dissolved in *N,N*-dimethylformamide (DMF) acted as a membrane material, ion exchanger, and dispersant for CNTs. The introduction of CNTs into the PSP can overcome the drawback of slow mass transfer through the film. Moreover, this kind of immobilization approach was simple and effective.

## EXPERIMENTAL SECTION

**Chemicals.** Tris(2,2'-bipyridyl)ruthenium(II) chloride hexahydrate (Ru(bpy)<sub>3</sub>Cl<sub>2</sub>·6H<sub>2</sub>O, 98%), DBAE, TPA, and polystyrene were purchased from Aldrich Chemical Co. (Milwaukee, WI). 1,2-Dichloroethane (DCE), acetic anhydride (AA), sulfuric acid, 2-propanol, and DMF were obtained from Beijing Chemicals Inc (Beijing, China). The single-wall carbon nanotubes were obtained from Shenzhen Nanotech. Port. Co. Ltd. with a typical diameter of 1.4 nm and length of 1–10 μm. The purity was ~80%. It was purified and functionalized by a well-established procedure.<sup>27</sup> The resultant CNTs were negatively charged under our experimental conditions.<sup>28</sup>

**Synthesis of PSP.** PSP is prepared as given in the previous literature.<sup>29</sup> First, the sulfonating reagent acetyl sulfate is freshly prepared. Briefly, 10 mL of AA is added to 20 mL of DCE in a test tube, and the mixture is cooled to ~0 °C. Then 4 mL of sulfuric acid in stoichiometric amount with respect to the desired virtual degree of sulfonation in the polymer is added. Finally, the test tube is capped and the resulting acetyl sulfate in DCE solution is ready to be used. Then, 5 g of atactic PS is dissolved in 80 mL

of DCE. The flask is capped, and the solution is then heated to 80 °C with reflux condensation. The prepared acetyl sulfate solution is syringed through the cap into the flask, and the sulfonation reaction proceeds for 2 h under stirring and is then stopped by adding 10 mL of 2-propanol. The resulting solution is cooled and concentrated by evaporating ~50 mL of DCE under vacuum. The sulfonated polymer is then precipitated by adding the solution to 1.5 L of distilled water drop by drop by means of a syringe. The polymer is filtered, washed for 2 h in distilled water at moderate temperature under reflux condensation, and then filtered again. Finally, the polymer is dried under vacuum at 60 °C for 2 days.

**Apparatus and Measurements.** A Fourier transform infrared (FT-IR) spectrum used to ascertain the presence of sulfonated groups attached to the phenyl rings was measured using a Perkin-Elmer model 580B IR spectrophotometer with the KBr pellet technique. Electrochemical impedance spectroscopy (EIS) measurements were carried out with an Autolab/PG30 electrochemical analyzer system (ECO Chemie B.V.) in a grounded Faraday cage at ambient temperature. The dc potential was 0.24 V versus Ag/AgCl (saturated KCl), and the conductivity was determined by ac impedance in the frequency range between 0.01 Hz and 1 MHz with a perturbation signal of 5 mV. Cyclic voltammetric experiments were performed with a CH Instruments 600 voltammetric analyzer. All experiments were carried out in a conventional three-electrode system: a GC electrode (*d* = 3 mm) as the working electrode, a Pt disk as the counter electrode, and an Ag/AgCl (saturated KCl) as the reference electrode. The ECL emission was detected by a model MPI-A capillary electrophoresis ECL system (Xi'an Remax Electronics Inc., Xi'an, China). ECL intensities were measured through the bottom of the cell with a photomultiplier tube (PMT) window, and the voltage of PMT was set at 800 V.

**Preparation and Modification of GC Electrodes.** The GC electrode used in the experiments was polished with 0.3- and 0.05-μm alumina powder, respectively, rinsed thoroughly with redistilled water, and then sonicated in redistilled water for 1 min. A 34-mg sample of PSP was dissolved in 2.5 mL of DMF solution (13.6 mg/mL). Then 14 μL of Ru(bpy)<sub>3</sub><sup>2+</sup> (50 mM) aqueous solution was added into the above solution and the resultant mixture sonicated for 10 min (referred as PSP/Ru).

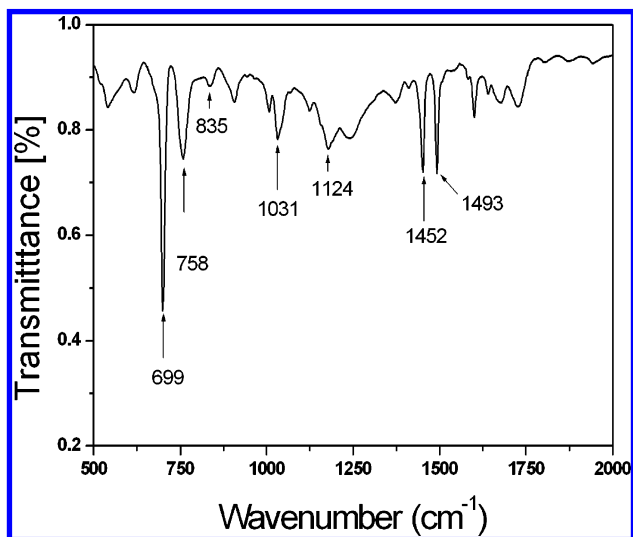
A 0.2-mg sample of CNTs was dispersed in 0.5 mL of PSP/Ru solution with ultrasonication for 20 min to get a homogeneous solution (PSP/CNT/Ru). The resulting GC electrode was coated by casting the same volume of PSP/Ru or PSP/CNT/Ru suspension (3 μL), and the solvents were allowed to evaporate at room temperature in the air.

## RESULTS AND DISCUSSION

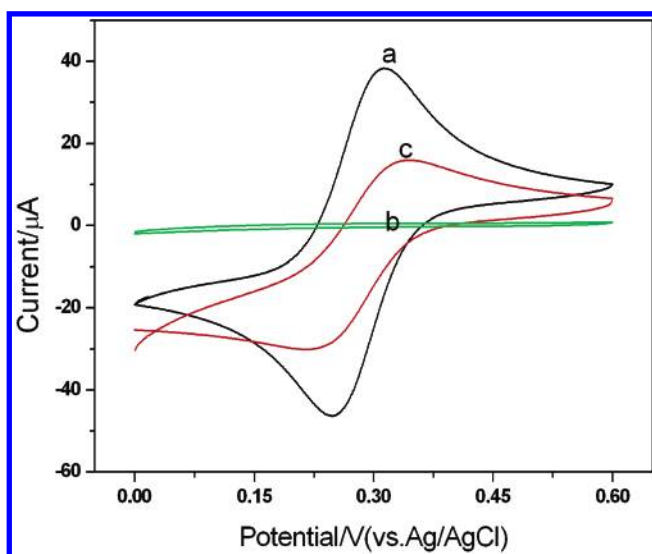
**Characterization of the Sample PSP.** Proof of partially sulfonation of polystyrene was provided by FT-IR spectrum shown in Figure 1. In the spectrum, the bands at 835, 1031 and 1124 cm<sup>-1</sup> are observed, which are ascribed to the typical absorption of the SO<sub>3</sub>H group and the typical PS absorption bands at 1493, 1452, 758, and 699 cm<sup>-1</sup> are also clearly observed.

**Characterizations of the Composite Film.** The electrochemistry experiments were applied to characterize the formation of the composite film at the surface of GC electrode. Different cyclic voltammetry (CV) responses at bare GC, PSP/Ru, and PSP/CNT/

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**Figure 1.** FT-IR spectrum of sample PSP.



**Figure 2.** CVs at bare GC (a), PSP/Ru GC (b), and PSP/CNT/Ru GC electrode (c) in 5 mM  $\text{Fe}(\text{CN})_6^{3-}$  in 0.1 M NaCl solution at the scan rate of 50 mV/s, respectively.

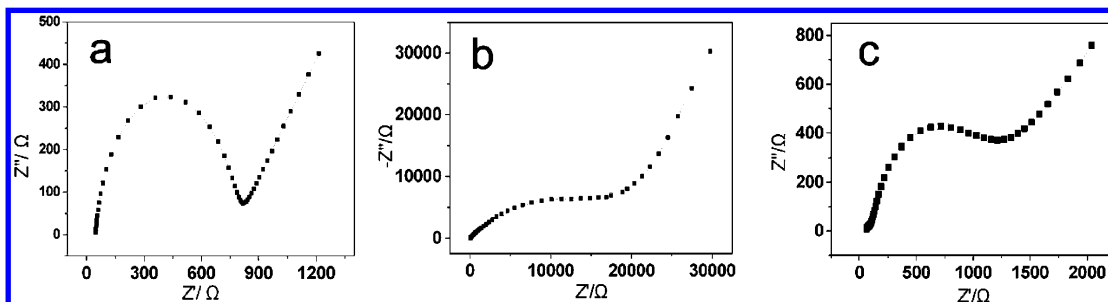
Ru are shown in Figure 2. A well-shaped cyclic voltammogram with a peak-to-peak separation of 65 mV was observed at the bare GC electrode (curve a). After being modified with PSP/Ru, the anodic and cathodic peaks almost disappeared (curve b), demonstrating that PSP acted as the blocking layer for the electron and mass transfer and hindered the diffusion of ferricyanide toward the electrode surface, although there existed electrostatic

interactions between  $\text{Ru}(\text{bpy})_3^{2+}$  cations and ferricyanide anions. However, at the PSP/CNT/Ru modified GC electrode (curve c), the peak current increases obviously compared with that at PSP/Ru (curve b), indicating that CNTs provided the conducting bridges for the accelerated electron transfer of ferricyanide due to the high conductivity of CNTs, although the current was smaller and the peak-to-peak separation was still larger than that at the bare GC electrode. The impedance changes of the electrode surface obtained by the EIS measurements further confirmed the function of CNTs. Figure 3 showed the ac impedance spectroscopy on bare GC (a), PSP/Ru (b), and PSP/CNT/Ru GC electrodes (c). By fitting suitable circuit and calculation, the electron-transfer resistance ( $R_{ct}$ ) obtained was about 0.737, 16.52, and 0.817 k $\Omega$ , respectively. The change of the resistance, which controlled the electron-transfer kinetics of the redox probe at the electrode, indicated that the CNTs added could reduce the  $R_{ct}$ . Considering PSP, on one hand, it favored the immobilization of  $\text{Ru}(\text{bpy})_3^{2+}$  as a result of the electrostatic interactions between sulfonic acid groups and  $\text{Ru}(\text{bpy})_3^{2+}$  cations; on the other hand, it could be used as a kind of membrane material.

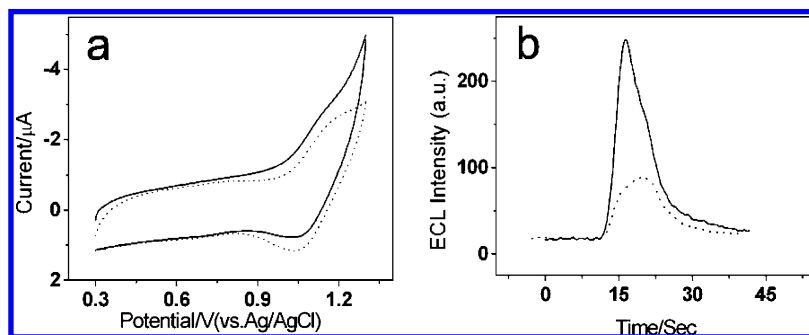
#### Electrochemistry and ECL of Immobilized $\text{Ru}(\text{bpy})_3^{2+}$ .

The different electrochemical behaviors of immobilized  $\text{Ru}(\text{bpy})_3^{2+}$  at different GC electrodes were studied using CVs. As depicted in the Figure 4, the ECL intensity and current response were both enhanced at PSP/CNT/Ru modified electrode. The enhancement of the current response of PSP/CNT/Ru (Figure 4a) and the enhanced ECL intensity (Figure 4b) might be attributed to the electrocatalytic oxidation of CNTs due to the interfusion of CNTs and the increased amount of the immobilized  $\text{Ru}(\text{bpy})_3^{2+}$  at PSP/CNT/Ru as a result of the electrostatic interactions between negative charges CNTs and positive charges  $\text{Ru}(\text{bpy})_3^{2+}$ .

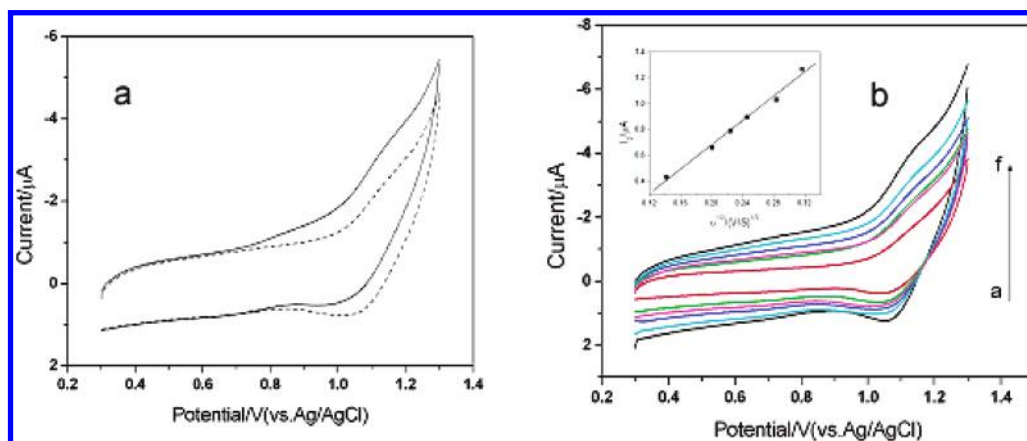
The ECL behavior of  $\text{Ru}(\text{bpy})_3^{2+}$  incorporated into the PSP/CNT/Ru film has been studied with DBAE as a coreactant. The  $\text{Ru}(\text{bpy})_3^{2+}$ -DBAE system was chosen because in solution DBAE is much less toxic, more soluble, and less volatile than TPA.<sup>30</sup> Figure 5a shows CVs of  $\text{Ru}(\text{bpy})_3^{2+}$  immobilized in the PSP/CNT/Ru modified electrode in the absence and presence of 0.2 mM DBAE at the scan rate of 50 mV/s in 0.1 M phosphate buffer solution (PBS; pH 7.5). The presence of DBAE causes the anodic peak current to increase clearly while the cathodic peak current decreased, which is consistent with an electrocatalytic reaction mechanism, and the oxidation peak current is proportional to the square root of the scan rate,  $\nu^{1/2}$ , which indicates the immobilized  $\text{Ru}(\text{bpy})_3^{2+}$  underwent a diffusion process within the film depicted in the Figure 5b. In addition, this unique immobilization method led to the strong ECL signal as demonstrated in Figure 6. Such



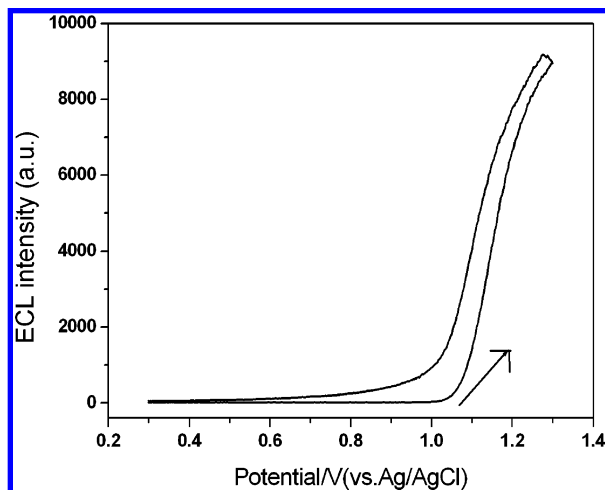
**Figure 3.** Nyquist plots of bare GC electrode (a), PSP/Ru (b), and PSP/CNT/Ru GC electrode (c) in 10 mM  $\text{Fe}(\text{CN})_6^{3-}$  in 0.1 M NaCl solution. The frequency range is from 1 MHz to 0.01 Hz, and the perturbation signal is 5 mV.



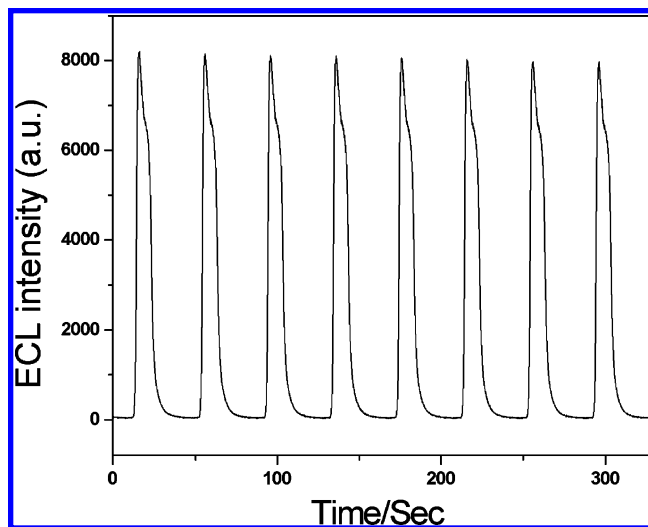
**Figure 4.** CVs (a) and corresponding ECL intensity curves of (b) PSP/Ru (dotted line) and PSP/CNT/Ru (solid line) in 100 mM PBS (pH 7.5) at the scan rate of 50 mV/s.



**Figure 5.** (a) CVs of  $\text{Ru}(\text{bpy})_3^{2+}$  immobilized in PSP/CNT/Ru modified electrode in the absence (dotted line) and presence of 0.2 mM DBAE (solid line) in 0.1 M PBS (pH 7.5) at the scan rate of 50 mV/s. (b) CVs of  $\text{Ru}(\text{bpy})_3^{2+}$  immobilized in PSP/CNT/Ru modified electrode at various scan rates in 100 mM PBS (pH 7.5): (a) 20, (b) 40, (c) 50, (d) 60, (e) 80, and (f) 100 mV/s. Inset: the relationship between the anodic peak current and the square root of the scan rate.



**Figure 6.** Corresponding ECL-potential curves for PSP/CNT/Ru composite film electrode in PBS (pH 7.5) containing 0.5 mM DBAE at the scan rate of 50 mV/s.



**Figure 7.** ECL intensity of  $\text{Ru}(\text{bpy})_3^{2+}$  immobilized in PSP/CNT/Ru composite film electrode in the presence of 0.25 mM DBAE under continuous CVs for 8 cycles at the scan rate of 50 mV/s.

ECL signal enhancement could facilitate the sensitive analyte detection. 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  $\text{Ru}(\text{bpy})_3^{2+}$ .

Figure 7 shows the ECL emission by immersing the PSP/CNT/Ru modified GC electrode in 0.1 M PBS (pH 7.5) solution, under continuously cyclic potential scanning for eight cycles in PBS (pH 7.5) containing 0.25 mM DBAE at the scan rate of 50

mV/s. There was no detectable change for ECL intensity, suggesting good stability of the ECL determination of DBAE. The strong electrostatic interactions between positively charged  $\text{Ru}(\text{bpy})_3^{2+}$  and negatively sulfonic acid groups and the fast charge-transfer due to the good conductivity and electrocatalytic activity of the CNTs may attribute to the good stability. Besides, due to the hydrophobic interaction with the surface of electrode, CNTs were also a proper matrix to immobilize  $\text{Ru}(\text{bpy})_3^{2+}$ .<sup>31</sup> The



calibration curve for DBAE is linear in the concentration range from 0.5  $\mu\text{M}$  to 1.0 mM with a detection limit ( $S/N = 3$ ) of  $2.5 \times 10^{-7}$  M for DBAE. Furthermore, this ECL sensor was also used to detect TPA, and it is showed high sensitivity with a detection limit of  $6.0 \times 10^{-9}$  M for TPA.

## CONCLUSION

The combination of PSP with CNTs provides an effective approach to fabricate a ECL sensor. Herein, PSP as a low-cost matrix played dual significant roles; on one hand, it could be used as a membrane material compared with the PSS due to the residual unsulfonated polystyrene; on the other hand, it could act as an ion exchanger for immobilization  $\text{Ru}(\text{bpy})_3^{2+}$  due to the electrostatic interactions. The interfusion of CNTs into the composite film greatly overcame the drawback of slow mass

transfer through the film due to the high electrical conductivity and enhanced the ECL intensity as a result of the increased amount of the immobilized  $\text{Ru}(\text{bpy})_3^{2+}$  at PSP/CNT/Ru due to the electrostatic interactions between negative charges CNTs and positive charges  $\text{Ru}(\text{bpy})_3^{2+}$ . The present ECL sensor also showed good stability. Furthermore, the present sensor has great potentials in applications of microfluidics, CE, and HPLC.

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