Enhanced electrochemiluminescence efficiency of Ru(II) derivative covalently linked carbon nanotubes hybrid[†]

Jing Li,^a Li-Rong Guo,^b Wei Gao,^a Xing-Hua Xia^{*a} and Li-Min Zheng^{*b}

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The synthesized derivative $Ru(bpy)_3$ covalently linked CNTs hybrid shows good electrochemical activity and *ca*. 17 times higher luminescence quantum efficiency than the adsorbed derivative $Ru(bpy)_3$. The Ru-CNTs based ECL sensor exhibits high stability toward determination of TPA with a detection limit as low as 8.75 pM.

Nowdays, one-dimensional nanostructured materials have been widely studied due to their potential applications in nano-scaled optical and electrical devices. As one of the representative materials, carbon nanotubes (CNTs) with unique structural, superior mechanical and excellent electronical properties, have been extensively explored for numerous applications in field emission, molecular electronics, and biomedicine.¹ The applications can be further broadened by functionalizing the surface properties of CNTs.² On the other hand, electrochemiluminescence (ECL) has been proven to be a powerful detection technique. The ECL signal may be significantly improved by immobilizing luminophores on electrode surfaces, forming solid state ECL detectors. A number of approaches including physical incorporation of luminophores into various sol-gels³ and nanomaterials-based composite films, have been attempted.⁴ However, such ECL detectors usually lack long-term stability due to the leakage of the luminophores. Covalent bonding of ruthenium complex luminophore onto an electrode surface or in silica nanoparticles could solve these problems.⁵ This strategy could enhance the ECL signal, however, poor conductivity of silica materials may limit their further applications.⁶ Up to now, the Ru(bpy)₃²⁺ complex exhibits the highest ECL efficiency, and has been extensively used in construction of solid state ECL sensors.^{3–6} In this communication, the ruthenium complex was used as a model molecule for constructing high performance solid state ECL sensors. In order to covalently bind this luminophore to CNTs, a Ru(bpy)₃ derivative was synthesized. The covalently bounded Ru(bpy)₃ derivative at CNTs (Ru-CNTs) shows good electrochemical activity and ca. 17 times higher luminescence quantum efficiency than the adsorbed

Ru(bpy)₃ derivative on CNTs. The present Ru-CNTs hybrid could be used as both electrochemical and luminescent labels for ultrasensitive bioanalysis.

The ruthenium(II) bipyridyl derivative, [Ru(bpy)2(AMbpy)]Cl2 (AMbpy = 4,4'-aminomethyl-2,2'-bipyridine) (Ru(II)) was synthesized using a modified method referring to literature.⁷ Products from each synthesis process were characterized by ¹H NMR and electrospray ionization mass spectrometry (ESI-MS). The results were in good agreement with those reported in the literature (see the ESI[†] for detail, Fig. S1 and S2). The Ru(II) was then covalently linked onto functionalized CNTs, forming the Ru-CNTs hybrid (illustrated in Fig. 1A). The absorption spectrum of Ru-CNTs shows obvious redshifts from 245 to 270 nm and from 454 to 472 nm (Fig. 1B) compared with those of free Ru(bpy)₃Cl₂⁸ which is similar to the case of Ru(bpy)₃Cl₂ immobilized silica nanoparticles.⁹ This phenomenon can be ascribed to the strong covalent force and electrostatic interaction between Ru(II) and the negatively charged carbon nanostructures. The maximum emission peak of the Ru-CNTs appears at about 640 nm (Fig. 1B), resulting from the triplet metal-to-ligand charge-transfer (MLCT) excited to the ground state.^{8a} This band exhibits an obvious red-shift by about 40 nm compared with the free Ru(II). The Ru-CNTs also shows distinct fluorescence (inset in Fig. 1B).

The FTIR spectrum of the oxidized CNTs shows two peaks at 1718 and 1567 cm⁻¹ due to the carbonyl stretching modes of COOH and COO⁻, respectively (Fig. S3,† curve a). After the Ru(II) was covalently bonded, the Ru-CNTs hybrid displays two new bands at 1650 and 1568 cm⁻¹ (Fig. S3,† curve b) corresponding to the C–O stretching modes (amide I) and a combination of the N–H bending and C–N stretching (amide II), respectively. In addition, another two peaks resulting from the stretching vibrations of C–H and N–H bonds in Ru(II) appear at 2920 and 3415 cm⁻¹, respectively. The decrease in the band intensity corresponding to the carbonyl stretching mode also indicates the effective reaction between –COOH and –NH₂.



Fig. 1 (A) Schematic illustration of the synthesis procedure of Ru-CNTs. (B) UV-Vis absorbance spectra of Ru(II) complex (curve a) and Ru-CNTs (curve b), and the fluorescence spectra of Ru(II) complex (curve c) and Ru-CNTs (curve d) (excitation at 450 nm). Inset shows the fluorescence photo of the Ru-CNTs.

^a Key Laboratory of Analytical Chemistry for Life Science, Chemistry School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210093, China. E-mail: xhxia@nju.edu.cn; Fax: +86-25-83597436; Tel: +86-25-83597436

^b State Key Laboratory of Coordination Chemistry, Chemistry School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210093, China. E-mail: lmzheng@nju.edu.cn

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These results demonstrate that the ruthenium complex has been successfully covalently linked to the carbon nanotubes.

According to the decrease of the UV-Vis absorption band intensity and element analysis, 0.19 mmol (0.133 g) Ru(II) per gram of oxidized CNTs with a loading density of 13.3% was calculated. This hybrid was modified on a glassy carbon electrode (GCE) for electrochemical and ECL characterizations as follows: the hybrid modified GCE (Ru-CNTs/GCE) was prepared by casting a 5 μ L of 1 mg mL⁻¹ Ru-CNTs aqueous suspension on a cleaned GCE followed by a 5 µL 1.0% Nafion solution coating. The amount of Ru(II) on the electrode was calculated as 9.5×10^{-9} mol (6.7 $\times 10^{-8}$ g). A pure CNTs modified electrode (CNTs/GCE) was prepared by the same procedures. The electrochemical results show that the GCE and the CNTs/GCE do not exhibit any Faradic signal in the potential region from 0.6 to 1.35 V (Fig. 2A, curves a, b). The increased current for the CNTs/GCE compared with the GCE could be due to the significant charging current of the CNTs matrix. While the Ru-CNTs/GCE shows a quasi-reversible redox wave with an anodic peak at ca. 1.22 V and a cathodic peak at ca. 1.13 V due to the electrochemical transition between Ru(III) and Ru(II) (Fig. 2A, curve d). This redox wave is similar to that of $Ru(bpy)_3^{2+}$. The result demonstrates that the Ru-CNTs has been successfully modified on the GCE and shows excellent electrochemical activity ascribed to the good conductivity of CNTs. Such electrochemical profiles could not be observed if the ruthenium complex was loaded in silica particles.^{5a,8c,9} For comparison, a modified electrode (Ru&CNTs/GCE) was prepared by immersing the CNTs/GCE in 4 mM Ru(II) solution for 17 h (saturation adsorption, Fig. 2A, curve c). In this case, the redox peak currents for Ru(II) are much smaller than those of the Ru-CNTs/GCE (compare Fig. 2A, curves c and d), demonstrating that covalent linking can increase the loading density of Ru(II) on CNTs.

Fig. 2B shows the ECL of the Ru-CNTs/GCE (curve a) and the Ru&CNTs/GCE (curve b) in the presence of a coreactant of tri-*n*-propylamine (TPA). As expected, both modified electrodes show significant ECL signals starting at potentials positive to 1.0 V which is consistent with the oxidation of Ru(π). The ECL peak intensity rises steeply until reaching a maximum at *ca.* 1.22 V. Interestingly, the ECL peak intensity of the Ru-CNTs/GCE is *ca.* 18 times larger than that of the adsorbed Ru(π). This increased ratio of ECL is much larger than that of the electrochemical currents (see next paragraph),



Fig. 2 (A) Cyclic voltammograms of a bare GCE (a), CNTs/GCE (b), Ru&CNTs/GCE (c) and Ru-CNTs/GCE (d) in 100 mM PBS (pH 7.4) containing 50 mM NaCl at a scan rate of 100 mV s⁻¹. (B) ECL-potential profiles of the Ru-CNTs/GCE (a) and Ru&CNTs/GCE (b) in 100 mM PBS (pH 7.4) containing 50 mM NaCl in the presence of 35 μ M TPA at a scan rate of 100 mV s⁻¹.

which could be due to the enhanced quantum emission efficiency of the Ru(II) covalently linked on CNTs.

The ECL quantum efficiency ($\varphi_{\rm ECL}$) for an annihilation system is commonly expressed as the number of photons emitted per redox event. However, the direct measurement of the ECL efficiency for the present coreactant system is difficult because of the complexity of the system, the irreversible nature of coreactant electrochemistry, and the high concentration of coreactant in solution.¹⁰ In the present case, we used the coulometric efficiency (φ_{coul} , photons generated per electron injected) for comparing the ECL efficiency of the Ru-CNTs ($\varphi_{Ru-CNTs}$) with that of Ru&CNTs ($\varphi_{Ru\&CNTs}$). The integrated charges were 1.85×10^{-5} and 1.11×10^{-5} C for the Ru-CNTs and Ru&CNTs modified electrodes (from Fig. 2A, curves c and d), respectively, and the integrated emission intensities were 1526.6 and 54.6 au (Fig. 2B). The ECL efficiency ratio ($\varphi_{Ru-CNTs}/\varphi_{Ru\&CNTs}$) was estimated by comparing the ratio of integrated emission intensity to the total coulombs passed for both systems at the GC electrodes. Under the same conditions, the integrated ECL intensity of the (Ru-CNTs)-TPA system was found to be about 28 times larger than that observed from the (Ru&CNTs)-TPA system. However, the oxidation charge passed on the modified electrode was 1.67 times for the former system larger than the latter one. Thus, it can be estimated that the coulometric ECL efficiency for the (Ru-CNTs)-TPA system is ca. 17 times higher than that of the (Ru&CNTs)-TPA. This demonstrates that the covalently conjugated Ru(II) on CNTs exhibits much higher ECL emission efficiency than the physically adsorbed Ru(II) in the CNTs matrix, which may indicate that possible electron transfer between the Ru(II) and CNTs occurs in the covalently conjugated hybrid.

The ECL of the (Ru-CNTs)-TPA system also shows good stability during continuous potential scans. Fig. 3A shows that the ECL signal decreases only by 1.6% in 22 potential cycles. The calibration curve displayed in Fig. 3B shows that the ECL signal varies linearly with the concentration of TPA from 0.05 nM to 50 μ M and 50 μ M to 57 mM. The detection limit of the present sensor is as low as 8.75 pM for TPA at a S/N of 3, which is 3 orders of magnitude lower than that of Nafion–Ru&CNTs film.^{2b}

In summary, a novel ruthenium(II) bipyridyl derivative, $[Ru(bpy)_2(AMbpy)]Cl_2$ (Ru(II)), was synthesized and then covalently conjugated onto CNTs, which can significantly increase the loading density of functional units on a single carbon nanotube. Due to the high conductivity, the Ru-CNTs modified electrode shows excellent electrochemistry. Meanwhile,



Fig. 3 (A) ECL emission of Ru-CNTs/GCE in a 100 mM PBS (pH 7.4) containing 50 mM NaCl with 35 μ M TPA at a scan rate of 100 mV s⁻¹. (B) Calibration curve of TPA at the Ru-CNTs/GCE in 100 mM PBS (pH 7.4) containing 50 mM NaCl.

the Ru-CNTs hybrid possesses unique electrochemiluminescence efficiency which is *ca.* 17 times larger than that of the physically adsorbed Ru(II) in the CNTs matrix, which is possibly due to efficient electron transfer between the ruthenium complex and the carbon nanotubes in the covalently conjugated system. Therefore, the Ru-CNTs hybrid based solid state ECL sensor exhibits excellent sensitivity and high stability toward TPA determination and the detection limit can be as low as 8.75 pM. The present Ru-CNTs hybrid could be used as both electrochemical and luminescent labels for ultrasensitive bioanalysis.

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