Electrogenerated Chemiluminescence Sensor Based on Tris(2,2'-bipyridyl) ruthenium(II) Immobilized in the Composite Film of Multi-walled Carbon Nanotube/Sol-gel Zinc oxide/Nafion

Eun Jun Choi, Chang Hoon Kang, Han Nam Choi, and Won-Yong Lee

Department of Chemistry and Center for Bioactive Molecular Hybrids, Yonsei University, Seoul 120-749, Korea. E-mail: forhamjun@gmail.com and wylee@yonsei.ac.kr

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A composite film of multi-walled carbon nanotube (MWCNT)/sol-gel-derived zinc oxide (ZnO)/Nafion has been utilized as an efficient immobilization matrix for the construction of a highly sensitive and stable tris(2,2'-bipyridyl) ruthenium(II) (Ru(bpy)$_3^{2+}$) electrogenerated chemiluminescence (ECL) sensor. The electrochemical and ECL behaviors of Ru(bpy)$_3^{2+}$ ion-exchanged into the composite film were strongly dependent upon the sol-gel preparation condition, the amount of MWCNT incorporated into the ZnO/Nafion composite film, and the buffer solution pH. The synergistic effect of MWCNTs and ZnO in the composite films increased not only the sensitivity but also the long-term stability of the ECL sensor. The present ECL sensor based on the MWCNT/ZnO/Nafion gave a linear response ($R^2 = 0.999$) for tripropylamine concentration from 500 nM to 1.0 mM with a remarkable detection limit (S/N = 3) of 15 nM. The present ECL sensor showed outstanding long-term stability (94% initial signal retained for 5 weeks). Since the present ECL sensor exhibits large response towards NADH, it could be applied as a transduction platform for the ECL biosensor in which the NADH is produced from the dehydrogenase-based enzymatic reaction in the presence of NAD$^+$ cofactor.

Key Words: Electrogenerated chemiluminescence, Ru(bpy)$_3^{2+}$, Sol-gel, Zinc oxide-Nafion composite film, Carbon nanotube

Introduction

Electrogenerated chemiluminescence (ECL) is the light emission from the generation of light emitting excited states via an electron transfer of an electrogenerated reactant. The oxidation-reduction ECL mechanism for tris(2,2'-bipyridyl)ruthenium(II) (Ru(bpy)$_3^{2+}$) has been well studied in which oxidized products of analyte (e.g., tripropylamine or NADH) can work as a reductant. Over the last two decades, Ru(bpy)$_3^{2+}$ ECL has attracted much attention as a powerful and analytical method due to its inherent sensitivity, selectivity, and wide dynamic range for a variety of analytes such as oxalate, alkylamines, amino acids, NADH, DNA, and a number of pharmaceutical compounds. In the Ru(bpy)$_3^{2+}$ ECL process, the starting material Ru(bpy)$_3^{2+}$ is regenerated and thus can be permanently reused in theory when it is immobilized on an electrode surface. Therefore, extensive efforts have been directed toward the immobilization of Ru(bpy)$_3^{2+}$ on electrode surfaces to fabricate a regenerable ECL sensor.

To date, a number of different approaches have been tried to immobilize Ru(bpy)$_3^{2+}$ on an electrode surfaces. The simplest method is relied on the use of cation-exchange polymer, Nafion. Another powerful approach is the immobilization of Ru(bpy)$_3^{2+}$ in sol-gel silica SiO$_2$/organic polymer composite modified electrode. The ECL sensor based on the silica/organic composite films exhibited greatly enhanced sensitivity and stability compared to pure Nafion-based ECL sensor. However, the brittle character of the sol-gel silica limited their practical applications. Therefore, less brittle and thus more stable non-silica-based sol-gel material is needed for the construction of robust Ru(bpy)$_3^{2+}$ ECL sensor. We previously reported the ECL sensors based on Ru(bpy)$_3^{2+}$ immobilized in sol-gel-derived titania (TiO$_2$/Nafion$^+$) and vanadium pentoxide (V$_2$O$_5$/Nafion) composite films. The TiO$_2$/Nafion and V$_2$O$_5$/Nafion composites are more stable and porous than the SiO$_2$/Nafion composite films, thus the ECL sensors based on TiO$_2$/Nafion and V$_2$O$_5$/Nafion composites showed much higher ECL response and improved stability relative to the SiO$_2$/Nafion-based ECL sensor.

Zinc oxide (ZnO) is special non-silica sol-gel material. Since ZnO film has a wide band gap and high isoelectric point for electrostatic interaction with protein, sol-gel ZnO is one of the attractive candidates as the immobilization matrix for biosensors. For example, tyrosinase$^{23-25}$ and glucose$^{26}$ biosensors were reported based on the pure ZnO film. In the present work, we utilized the composite films of CNT-doped sol-gel-derived ZnO/Nafion as an effective immobilization matrix for the construction of a highly sensitive and stable Ru(bpy)$_3^{2+}$ ECL sensor. In particular, the sol-gel-derived ZnO is an effective immobilization matrix for Ru(bpy)$_3^{2+}$ because of its large surface area, moderate pore size, and good electrical conductivity. MWCNTs were easily dispersed in the ZnO/Nafion composite, exhibiting the same behavior observed in the sol-gel titania/Nafion composite.$^{21}$ The synergistic effect of ZnO and MWCNT in the MWCNT/ZnO/Nafion composite films has increased both sensitivity and stability of the ECL sensor. The electrochemical and ECL behaviors of Ru(bpy)$_3^{2+}$ ion-exchanged into the composite film were strongly dependent upon the sol-gel preparation condition, the amount of MWCNT incorporated into the ZnO/Nafion composite film, and the buffer solution pH. The ECL characteristics obtained with the present Ru(bpy)$_3^{2+}$ ECL sensor based on MWCNT/ZnO/Nafion composite films will be compared to...
those obtained with pure Nafion-based as well as ZnO/Nafion-based ECL sensors for the determination of tripropylamine.

**Experimental**

**Reagents.** Zinc acetate (Zn(Ac))·2H2O, 99.99%, lithium hydroxide (LiOH·H2O, 99.99%), ethanol, Nafion (perfluorinated ion-exchange resin, 5% (v/v) solution in 90% aliphatic alcohol/10% water mixture), tripropylamine (99%), sodium oxalate (99.5%), and Ru(bpy)32+ (98%) were purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). Multiwalled CNT (95% purity, diameter: 10 ~ 20 nm, and length: 10 ~ 50 nm) were obtained from L-gin Nanotech (Seoul, Korea). Water for all solutions was purified using a Milli-Q water purification system (Millipore, Bedford, MA, USA).

**Instrumentation.** Cyclic voltammetric experiments were performed with an EG&G 273A potentiostat (Oak Ridge, TN, USA). A conventional three-electrode system was employed with a platinum wire as counter electrode, glassy carbon (0.07 cm2) electrode coated with the prepared composite films as a working electrode, and an Ag/AgCl (3 M NaCl) reference electrode. The photon counting system used was a Hamamatsu Photonics HC 155-02 photon counting module (Hamamatsu city, Japan) in conjunction with a computer for recording the output. The electrochemical cell was also used in the ECL experiments. The ECL cell was placed directly in front of the photomultiplier tube (PMT) window. The entire ECL cell was enclosed in a light-tight box. All experiments were conducted in ambient conditions at approximately 25 °C.

**Preparation of the ECL Sensor.** The sol-gel stock solution was prepared by mixing 1.5 mL of deionized water with 0.25 mL of 0.1 M Zn(Ac)2 dissolved in ethanol and 0.25 mL of 0.1 M LiOH dissolved in ethanol in a glass vial. The solution was stirred at 65 °C for complete dissolution until 40 min. After complete dissolution, it was stored in an ice bath at temp below 0 °C for 10 min. The LiOH ethanol solution was dissolved in an ultrasonic bath and then cooled to 0 °C. Once a homogeneous solution was obtained by a vigorous stirring at 0 °C for 4 h, the stock solution was stored at 0 °C.23,27-28 The ZnO/Nafion composite solution was prepared by mixing 2 mL of ZnO sol-gel stock solution with 3 mL of Nafion solution. A given amount of MWCNT was solubilized in the ZnO/Nafion composite solution. MWCNT was well dispersed in the ZnO/Nafion composite solution. An aliquot of 2 mL MWCNT-doped composite solution was hand-cast on the surface of a pre-cleaned glassy carbon (GC) electrode (A = 0.07 cm2). The thin composite films were dried for 20 min at room temperature and then swelled in 0.05 M phosphate buffer solution (pH 7) for 20 min. The MWCNT-doped electrode was then placed in an electrochemical cell (sample volume: 10 mL) containing 0.5 mM Ru(bpy)32+ solution in 0.05 M phosphate buffer solution (pH 7). The immobilization of Ru(bpy)32+ was monitored by running consecutive cyclic potential scans between +0.80 V and +1.25 V at a scan rate of 100 mV/s vs Ag/AgCl (3 M NaCl) reference electrode. The modified electrode was then placed in the phosphate buffer solution until use.

**Results and Discussion**

**Effect of Sol-Gel Preparation Condition.** Nafion can be easily mixed with the positively charged ZnO sol-gel matrix and thus the resulting ZnO/Nafion composite was stable.25-30 The sol-gel preparation condition strongly affects the properties of sol-gel metal oxide gel and finally ECL reaction of the immobilized Ru(bpy)32+ with analytes. In particular, the level of water employed during the LiOH-catalyzed sol-gel process strongly influences the porous nature of the sol-gel-derived electrodes for immobilization of Ru(bpy)32+. Therefore, the effect of sol-gel precursor Zn(Ac)2 to water ratio (v/v) in the stock sol-gel solutions on the performance of the ECL sensor based on the ZnO/Nafion composite (ZnO : Nafion = 2 : 3) film was examined. Table 1 shows anodic peak current and ECL intensity for tripropylamine (TPA) obtained with the ZnO/Nafion composite-based Ru(bpy)32+ ECL sensors fabricated from different Zn(Ac)2 to water ratios. When the content of water in the sol-gel Zn(Ac)2 stock solution was 1 : 3 (v/v), the amount of Ru(bpy)32+ immobilized in the composite films was the maximum and thus led to the highest ECL intensity for TPA. When the content of water in the sol-gel Zn(Ac)2 stock solution ranged between 1 : 1 and 1 : 2 (v/v), irregular sol-gel matrices were formed and thus the fabrication of the composite films on the GC electrode surface was not reproducible. In contrast, when the water content in the sol-gel Zn(Ac)2 stock solution ranged from 1 : 4 to 1 : 5, the sol-gel matrix were less dense, thus rendering the thickness of the resulting composite films on the electrode surface to be thin. Thin films resulted in low Ru(bpy)32+ loading and low ECL intensity for TPA although the uptake time became faster because of the increased mass transport rate in the thinner sol-gel matrix. In view of the optimum condition for Ru(bpy)32+ immobilization and ECL response, the optimum Zn(Ac)2 to water ratio was fixed at 1 : 3 for all subsequent studies.

**MWCNT-sol-gel ZnO/Nafion Composite.** MWCNT can be dispersed in composite solution of sol-gel ZnO/Nafion (ZnO : Nafion = 2 : 3), which is quite similar to the TiO2/Nafion composite solution. This fact indicates that the presence of the ZnO in Nafion solution does not prevent the ability of Nafion from dispersing MWCNT. The Nafion content in the composite ranging from 1.3 to 0.5 wt% was able to disperse the MWCNT. MWCNT-modified electrodes were prepared by simply hand-casting a small aliquot of composite solution containing the MWCNT (2 µL) onto the surface of a pre-cleaned glassy carbon (GC) electrode. Scanning electron microscopy (SEM) was employed to observe the nature of the new MWCNT-doped ZnO/Nafion composite (2 : 3, v/v). As shown in SEM image of Fig. 1,
the surface of the MWCNT/ZnO/Nafion composite is characterized with bundles of MWCNTs covering the ZnO/Nafion composite. The MWCNT fibers have the diameters ranging from 30 to 50 nm. The MWCNT/ZnO/Nafion composite films are highly porous.

Electrochemical Behavior. Ru(bpy)$_{3}^{2+}$ can be easily immobilized into the ZnO/Nafion and MWCNT/ZnO/Nafion composite films on a glassy carbon (GC) electrode via ion-exchange process of Ru(bpy)$_{3}^{2+}$ in cation-exchangeable sulfonate (SO$_3^-$) groups of Nafion in the ZnO/Nafion composite films. Tang, Jiang, and coworkers have reported the similar silica/Nafion composite structure. The Ru(bpy)$_{3}^{2+}$ was immobilized into the ZnO/Nafion and MWCNT/ZnO/Nafion composite films in 0.5 mM Ru(bpy)$_{3}^{2+}$ solution prepared in 0.05 M phosphate buffer at pH 7 by running consecutive cyclic potential scans from an initial potential of +800 mV to a high of +1350 mV and back to +800 mV at a scan rate of 100 mV/s. Figure 2 shows a series of cyclic voltammograms (CVs) obtained at a Nafion modified electrode (dot line), the ZnO/Nafion composite-modified electrode without MWCNT (dashed line), and the MWCNT/ZnO/Nafion composite-modified electrode (MWCNT content 0.75 mg/mL) (solid line) in 0.5 mM Ru(bpy)$_{3}^{2+}$ solution. The CVs for the composite-modified electrodes were recorded after those electrodes had reached a steady-state current in the Ru(bpy)$_{3}^{2+}$ solution by running consecutive cyclic potential scans. The CVs obtained at all composite-modified electrodes is similar in shape to those obtained at Nafion-modified electrode as observed with the TiO$_2$/Nafion, V$_2$O$_5$/Nafion$^5$ and MWCNT/TiO$_2$/Nafion$^5$ composite-modified electrodes. The oxidation current obtained at the MWCNT/ZnO/Nafion composite electrode was 2-fold greater than that obtained at the composite modified electrode without MWCNT and 5-fold greater than that obtained at the pure Nafion-modified electrode. In order to figure out the amount of Ru(bpy)$_{3}^{2+}$ immobilized in the prepared ECL sensors, the surface coverage, $\Gamma$, were determined for composite-modified electrodes by graphical integration of background-corrected cyclic voltammogram (1 mV/s). Since the ion-exchange sites are the same in the two types of composite films, the hydrophobic MWCNT in the ZnO/Nafion composite certainly increased the amount of Ru(bpy)$_{3}^{2+}$ immobilized in the ECL sensor possibly by adsorption of Ru(bpy)$_{3}^{2+}$ onto MWCNT surface (surface coverage $\Gamma = 2.3 \times 10^{-8}$ mol/cm$^2$) compared to that for the corresponding composite electrode without MWCNT $\Gamma = 6.4 \times 10^{-8}$ mol/cm$^2$), which is quite similar to those observed with the MWCNT/TiO$_2$/Nafion composite-modified electrode. The amount of Nafion immobilized into sol-gel derived ZnO sol significantly affected the electrochemical behavior of the composite films. The anodic peak current obtained from CVs was plotted versus the amount of Nafion incorporated into sol-gel-derived ZnO sol. As shown in Figure 3, the anodic peak current steadily increased as the amount of Nafion in the ZnO sol increased up to 60%. However, as the amount of Nafion in the ZnO sol further increased, the current suddenly decreased. This results are somewhat similar to the previous results of Ru(bpy)$_{3}^{2+}$ incorporated into TiO$_2$/Nafion and V$_2$O$_5$/Nafion composite films. The voltammetric current directly reflects both the diffusion coefficient of Ru(bpy)$_{3}^{2+}$ and the concentration of Ru(bpy)$_{3}^{2+}$ in the film on the electrode surface.
The amount of MWCNT immobilized into ZnO/Nafion composite films also affected the electrochemical behavior of the composite films. The anodic peak currents obtained from CVs were plotted as a function of the amount of MWCNT incorporated into the ZnO/Nafion composite. As shown in Figure 4, the anodic peak current steadily increased as the amount of MWCNT in the ZnO/Nafion composite increased up to 0.75 mg/mL. Since the bipyridyl ligands of the Ru(bpy)$_3^{2+}$ are hydrophobic, the hydrophobic MWCNT in the ZnO/Nafion composite might result in the increased adsorption of Ru(bpy)$_3^{2+}$ onto MWCNT surface via hydrophobic-hydrophobic interactions between the Ru(bpy)$_3^{2+}$ and the MWCNT, thus leading to the increased anodic peak current. However, as the amount of MWCNT in the ZnO/Nafion composite further increased up to 1.3 mg/mL, the anodic current remained almost constant. It is reasonable to assume that the amount of Ru(bpy)$_3^{2+}$ incorporated into the MWCNT/ZnO/Nafion composite films increases as the amount of MWCNT in the ZnO/Nafion increases. However, it is noted that the observed anodic peak current directly reflected both the diffusion coefficient of Ru(bpy)$_3^{2+}$ and the concentration of Ru(bpy)$_3^{2+}$ in the film on the electrode surface. Although the increased amount of MWCNT in the ZnO/Nafion composite contains the increased amount of Ru(bpy)$_3^{2+}$ in the film, the incorporation of MWCNT in the ZnO/Nafion composite could lead to the decreased mass transport within the film. The apparent diffusion coefficient, $D_{app}$ for Ru(bpy)$_3^{2+}$ in the MWCNT/ZnO/Nafion composite (MWCNT: 0.75 mg/mL) film was calculated from the slope (from linear regression analysis) of the anodic peak current vs square root of the scan rate plot by the Randles-Sevcik equation. The measured diffusion coefficient was around $1.37 \times 10^{-7}$ cm$^2$/s, which was slightly smaller than the diffusion coefficient for Ru(bpy)$_3^{2+}$ in the ZnO/Nafion composite without MWCNT (1.25 $\times 10^{-6}$ cm$^2$/s). However, it was much larger than the previously reported diffusion coefficient for the Ru(bpy)$_3^{2+}$ in the pure Nafion films ($5 \times 10^{-10}$ cm$^2$/s). This fact indicates that the presence of MWCNT in the ZnO/Nafion composite affects not only the amount of Ru(bpy)$_3^{2+}$ immobilized in the composite films but also the mass transport rate within the composite films. When the amount of MWCNT in the casting solution of the ZnO/Nafion composite further increased more than 1.3 mg/mL, the MWCNT dispersion gradually decreased in the ZnO/Nafion composite solution, which could be easily recognized by observing the precipitation of un-dispersed MWCNT in the solution. Therefore, the amount of MWCNT from 0.75 mg/mL up to 1.3 mg/mL can be used in all the subsequent experiments.

**ECL Behavior.** Similar to the voltammetric behavior of the composite-modified electrode, the amount of MWCNT incorporated into the ZnO/Nafion composite also strongly affected the ECL intensity as shown in Figure 4. The ECL intensity increased as the MWCNT content increased up to 0.75 mg/mL, after which it sharply decreased at higher MWCNT content. This trend observed in the ECL intensity as a function of MWCNT amount in the ZnO/Nafion composite is different to that observed in the anodic current of Ru(bpy)$_3^{2+}$ in the same composite films. The ECL could take place from the reaction of oxidized Ru(bpy)$_3^{3+}$ and TPA at the outer surface of the film and/or from the reaction of oxidized Ru(bpy)$_3^{3+}$ and TPA throughout the film via permeation from the bulk solution. Therefore, the composite film exhibiting greater open structure leads to the faster diffusion of Ru(bpy)$_3^{3+}$ and TPA, thus resulting in greater ECL response. Although the increased amount of hydrophobic MWCNT in the ZnO/Nafion composite (more than 0.75 mg/mL) results in the increased amount of Ru(bpy)$_3^{2+}$ immobilized in the composite films, the increased MWCNT in the composite films also hinder the ECL process between the oxidized TPA and Ru(bpy)$_3^{3+}$ in the composite films due to the decreased mass transport rate in the films as described in the previous section. So the ECL response for TPA was the maximum at the ECL sensor based on the composite film with 0.75 mg/mL CNT. Therefore, an optimum amount of MWCNT at 0.75 mg/mL was used in all the subsequent experiments.

A pH study has been carried out to determine the pH effect on the Ru(bpy)$_3^{2+}$-TPA system. The ECL intensity for TPA is greatly affected by the pH of the buffer solution. The background corrected ECL signals for TPA obtained at the ZnO/Nafion (60%) composite-modified electrode increased significantly from pH 5.0 up to pH 9.5 and slightly decreased at higher pH, as shown in Figure 5. The ECL intensity obtained at pH 9.5 was 3.5-fold larger than that at pH 5.0. Since the ZnO has the isoelectric point of 9.5, the ZnO/Nafion composite is positively charged at the pH below 9.5. Therefore, TPA cation radicals cannot be easily partitioned into the positively charged ZnO/Nafion composite, thus leading to decreased ECL response. Therefore, we used the buffer solution of pH 9.5 throughout the experiments to obtain the maximum response. Calibration curves for TPA have been constructed using the ZnO/Nafion (60%) composite-modified electrode and MWCNT/ZnO/Nafion-modified electrode immobilized with Ru(bpy)$_3^{2+}$. Calibration curves are plotted on logarithmic axes to show the wide dynamic ranges as shown in Figure 6. Each point is a mean
of three or more ECL signals obtained by consecutive cyclic potential scans (100 mV/s) at a given concentration. The linear range extended from 100 nM to 1.0 mM (R² = 0.999) at the MWCNT/ZnO/Nafion modified ECL sensor compared to that from 500 nM to 1.0 mM at the ZnO/Nafion-modified electrode and from 1.0 µM to 1.0 mM at the pure Nafion-modified electrode. The good detection limit (S/N = 3) of the ECL sensors based on MWCNT/ZnO/Nafion composite and ZnO/Nafion composite films gave a detection limit of 15 nM and 55 nM, respectively. Overall, the detection limit for TPA at the ECL sensor based on the MWCNT/ZnO/Nafion composite was two orders of magnitude lower than that at the ECL sensor based on pure Nafion films, which is similar magnitude than that obtained with other metal oxide/Nafion, TiO₂/Nafion, and V₂O₅/Nafion composite electrodes.

Selectivity, Stability and Reproducibility. ECL intensities were measured for a variety of amine compounds, and the results are listed in Table 2 in order to find out if the MWCNT/ZnO/Nafion and ZnO/Nafion composite-modified ECL sensor have response selectivity. As shown in Table 2, relative ECL intensities obtained with the present MWCNT/ZnO/Nafion composite based ECL sensor for analytes containing amine groups were larger than those obtained at TiO₂/Nafion composite-modified and V₂O₅/Nafion composite-modified electrode. The present MWCNT/ZnO/Nafion composite based ECL sensor exhibited greater ECL intensities for hydrophobic analytes such as NADH and promazine than those obtained at the ZnO/Nafion composite-based ECL sensor without MWCNT. This result can be quite similar to those obtained with MWCNT/TiO₂/Nafion composite-modified ECL sensor. In addition, the present MWCNT/ZnO/Nafion composite-based ECL sensor exhibited greater ECL intensities for amino acids and β-blockers such as atenolol. Therefore, the present ECL sensor could be used for monitoring the concentrations of antibiotics and β-blockers in biological fluids (urine and blood), and for detecting NADH and species coupled to NADH production.

The long-term storage stabilities of the MWCNT/ZnO/Nafion composite-modified and ZnO/Nafion composite-modified ECL sensors were studied by monitoring its ECL response to 0.5 mM TPA in 0.05 M phosphate buffer solution (pH 9.5) with intermittent usage (every 2 – 3 days) and storage in the buffer solution at room temperature when not in use as shown Figure 7. The ECL response of the present MWCNT/ZnO/Nafion composite-modified ECL sensor exhibited 86% signal for 1 month, which is much better than that obtained with the ZnO/Nafion composite-based ECL sensor without MWCNT (39% of initial activity retained in 3 weeks). This result indicates that the hydrophobic MWCNT present in electroactive region within the ZnO/Nafion composite films could prohibit the Ru(bpy)₃²⁺ from migrating into electroinactive hydrophobic region of Nafion because strong hydrophobic interactions between Ru(bpy)₃²⁺ and MWCNT favor Ru(bpy)₃²⁺ to stay in electroactive region. The present ECL sensor was also quite stable in flowing streams. Reproducible ECL signals were observed for 10 consecutive injections of 0.5 mM TPA (R.S.D. less than 4%).

Conclusions

A highly sensitive and stable Ru(bpy)₃²⁺ ECL sensor has been developed for the detection of alkylamine compounds using the

![Figure 5. Effect of the pH in the ZnO/Nafion composite films on ECL intensity obtained at a scan rate of 100 mV/s. The ECL intensity was measured for 0.5 mM TPA solution prepared in 0.05 M buffer. The points shown are the mean of at least five determinations (± S. D.).](image)

![Figure 6. Calibration curve for TPA obtained with the ECL sensor of Ru(bpy)₃²⁺ ion-exchanged in ZnO/Nafion (60%) (●) and MWCNT/ZnO/Nafion (□) modified electrode. The peak ECL intensity was obtained from the ECL-potential curves acquired at 100 mVs.](image)

Table 2. Selectivity study for amine compounds with the present MWCNT/ZnO/Nafion, ZnO/Nafion, and other metal oxide/Nafion compound-modified ECL sensor.

<table>
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<tr>
<th>Amine compounds</th>
<th>MWCNT/ZnO/Nafion</th>
<th>ZnO/Nafion</th>
<th>TiO₂/Nafion</th>
<th>V₂O₅/Nafion</th>
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<tr>
<td>TPA</td>
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<td>9.05 x 10⁶</td>
<td>6.64 x 10⁶</td>
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<td>Atenolol</td>
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<td>4.49 x 10⁵</td>
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<td>Proline</td>
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<td>3.52 x 10⁵</td>
<td>1.11 x 10⁵</td>
<td>7.77 x 10⁵</td>
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<tr>
<td>NADH</td>
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<td>3.35 x 10⁶</td>
<td>2.68 x 10⁶</td>
<td>1.20 x 10⁷</td>
</tr>
<tr>
<td>L-tryptophan</td>
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<td>1.18 x 10⁴</td>
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<td>1.08 x 10⁴</td>
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</tr>
<tr>
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<td>6.14 x 10⁴</td>
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<td>2.30 x 10⁴</td>
<td>1.01 x 10⁴</td>
<td>8.04 x 10⁴</td>
<td>4.67 x 10⁷</td>
</tr>
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</table>

*All concentrations 0.5 mM, 1 Absolute ECL intensity (counts).*
MWCNT/ZnO/Nafion composite film. The synergistic effect of MWCNTs and ZnO in the composite films increased not only the sensitivity but also the long-term stability of the ECL sensor. The present ECL sensor based on the MWCNT/ZnO/Nafion gave a linear response ($R^2 = 0.999$) for tripropylamine concentration from 500 nM to 1.0 mM with a remarkable detection limit ($S/N = 3$) of 15 nM. The present ECL sensor showed outstanding long-term stability (94% initial signal retained for 5 weeks). Furthermore, the present ECL sensor also showed improved response for NADH. Given the aforementioned benefits, the use of the MWCNT/ZnO/Nafion composite provides a useful means for the preparation of not only the present Ru(bpy)$_{3}^{2+}$ ECL sensor but also ECL biosensors based on dehydrogenase enzymes because of the large response towards NADH.

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