Tris(2,2′-bipyridyl)ruthenium(II) electrogenerated chemiluminescence sensor based on carbon nanotube dispersed in sol–gel-derived titania–Nafion composite films

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Abstract

A highly sensitive and stable tris(2,2′-bipyridyl)ruthenium(II) (Ru(bpy)32+) electrogenerated chemiluminescence (ECL) sensor was developed based on carbon nanotube (CNT) dispersed in mesoporous composite films of sol–gel titania and perfluorosulfonated ionomer (Nafion). Single-wall (SWCNT) and multi-wall carbon nanotubes (MWCNT) can be easily dispersed in the titania–Nafion composite solution. The hydrophobic CNT in the titania–Nafion composite films coated on a glassy carbon electrode certainly increased the amount of Ru(bpy)32+ immobilized in the ECL sensor by adsorption of Ru(bpy)32+ onto CNT surface, the electrocatalytic activity towards the oxidation of hydrophobic analytes, and the electronic conductivity of the composite films. Therefore, the present ECL sensor based on the CNT–titania–Nafion showed improved ECL sensitivity for tripropylamine (TPA) compared to the ECL sensors based on both titania–Nafion composite films without CNT and pure Nafion films. The present Ru(bpy)32+ ECL sensor based on the MWCNT–titania–Nafion composite gave a linear response (R2 = 0.999) for TPA concentration from 50 nM to 1.0 mM with a remarkable detection limit (S/N = 3) of 10 nM while the ECL sensors based on titania–Nafion composite without MWCNT, pure Nafion films, and MWCNT–Nafion composite gave a detection limit of 0.1 M, 1 M, and 50 nM, respectively. The present ECL sensor showed outstanding long-term stability (no signal loss for 4 months).

Keywords: Electrogenerated chemiluminescence; Ru(bpy)32+; Sol–gel; Titania–Nafion composite film; Carbon nanotube

1. Introduction

Electrogenerated chemiluminescence (ECL) is the light emission from the generation of emitting excited states via the electron transfer reaction of electrogenerated species at electrode surfaces [1]. In particular, tris(2,2′-bipyridyl)ruthenium(II) (Ru(bpy)32+) ECL has attracted much attention as a powerful analytical tool due to its inherent sensitivity, selectivity, and wide linear dynamic range for a variety of analytes [2–4] such as oxalate [5], alkylamines [6], amino acids [7–9], NADH [10–12], DNA [13], and a number of pharmaceutical compounds [14–19]. The ECL emission of the Ru(bpy)32+–TPA system arises when the deprotonated TPA radical (TPA•−) formed from reduction of Ru(bpy)33+ or via direct electrode oxidation reacts with another Ru(bpy)32+ or additional Ru(bpy)33+ to form [Ru(bpy)32+]∗, which then decays to produce orange emission. A simplified ECL reaction scheme is shown below [20–21]

\[
\begin{align*}
\text{Ru(bpy)3}^{2+} & \rightarrow \text{Ru(bpy)3}^{3+} + e^- \quad (1) \\
\text{Ru(bpy)3}^{3+} + \text{TPA} & \rightarrow \text{Ru(bpy)3}^{2+} + \text{TPA}^* \quad (2a) \\
\text{TPA} & \rightarrow \text{TPA}^* + e^- \quad (2b) \\
\text{Ru(bpy)3}^{3+} + \text{TPA}^* & \rightarrow [\text{Ru(bpy)3}^{2+}]^+ \quad (3) \\
[\text{Ru(bpy)3}^{2+}]^+ & \rightarrow \text{Ru(bpy)3}^{2+} + \text{light (610 nm)} \quad (4)
\end{align*}
\]

The reaction scheme for Ru(bpy)32+ ECL indicates that the starting material Ru(bpy)32+ is regenerated in situ when it is immobilized on an electrode surface. Therefore, much efforts have been focused on the immobilization of Ru(bpy)32+ on an electrode surface, thus recycling Ru(bpy)32+ permanently in theory and making the detection system simpler in ECL-based chemical sensors, biosensors, and detectors in flowing streams such as high-performance liquid chromatography and...
capillary electrophoresis. For example, some polymeric systems where Ru complexes are chemically attached in organic polymer [22, 23] and inorganic silicate [24] have been reported to enhance the stabilities of the ECL sensors. However, the preparations of the ECL sensors are complex and their ECL signals are relatively low due to poor permeations of analyte molecules through the matrices. The ECL sensors based on inorganic particle/Ru(bpy)$_3^{2+}$ multilayer films have been fabricated [25–27]. The ECL sensors exhibited high sensitivity due to the increased surface area of the inorganic particles. Relatively simple immobilization methods based on ion-exchange of Ru(bpy)$_3^{2+}$ in cation-exchange polymer such as perfluorosulfonated ionomer Nafton [10, 11, 28] and composite films of both sol–gel silica–Nafton [29] and sol–gel titania–Nafton [30] have been reported. The ECL sensor based on the Ru(bpy)$_3^{2+}$ immobilized in Nafton matrix has proven to be promising but the migration of Ru(bpy)$_3^{2+}$ into electrochemically inactive hydrophobic region of Nafton diminishes the stability of the ECL sensor [10, 11].

Carbon nanotube (CNT) has received considerable attention in various fields because of its unique properties such as high electrical conductivity, physical rigidity, and chemical stability [31]. In particular, single-wall (SWCNT) and multi-wall CNT (MWCNT) have been widely used in electrochemical sensors [32–37] and biosensors [38–42] because both CNTs can provide excellent electrocatalytic activity and minimization of surface fouling onto electrodes. Recently, Wang’s group reported on a simple method for the preparation of CNT-based electrochemical biosensors, in which CNTs were first solubilized by Nafton and the resulting CNT–Nafton composite was coated onto a glassy carbon surface [38]. Based on the composite films of SWCNT and Nafton, Dong’s group reported on the fabrication of CNT–Nafton composite films exhibiting improved ECL characteristics compared to those based on pure Nafton films, new matrix for the fabrication of the highly sensitive and stable CNT-based Ru(bpy)$_3^{2+}$ ECL sensors as well as the deeper understanding of their electrochemical and ECL behaviors are still needed.

Therefore, we report here on a new and simple method for the fabrication of CNT-based Ru(bpy)$_3^{2+}$ ECL sensor using sol–gel derived titania–Nafton mesoporous composite films. In this work, CNTs were first dispersed in the titania–Nafton composite films before coating them onto a glassy carbon electrode. In our previous report, the use of sol–gel titania–Nafton composite for the fabrication of the Ru(bpy)$_3^{2+}$ ECL sensor improved both sensitivity and long-term stability compared to those obtained at the pure Nafton-based ECL sensor because of the increased pore size and enhanced physical stability of the titania–Nafton composite films compared to that of Nafton films [30]. Therefore, the present Ru(bpy)$_3^{2+}$ ECL sensor based on the titania–Nafton composite films containing CNT has coupled the advantages of highly porous titania–Nafton composites with the advantages of CNT such as enhanced electrocatalytic activities towards the oxidation of analyte and the increased electronic conductivity of the composite films. The electrochemical and ECL behaviors of the present Ru(bpy)$_3^{2+}$ ECL sensor have been studied as a function of the amount of CNT incorporated into the sol–gel titania–Nafton composite. The ECL characteristics obtained with the present Ru(bpy)$_3^{2+}$ ECL sensor based on CNT–titania–Nafton composite films will be compared to those obtained with pure Nafton-based, titania–Nafton-based, as well as CNT–Nafton-based ECL sensors for the determination of tripropylamine and oxalate.

2. Experimental

2.1. Reagents

Titanium(IV) isopropoxide (Ti(OR)$_4$ (R = CH(CH$_3$)$_2$, 99.99%), Nafton (perfluorinated ion-exchange resin, 5% (w/v) solution in a solution of 90% aliphatic alcohol/10% water mixture), tripropylamine (TPA, 99%), sodium oxalate (99.5%), tris(2,2'-bipyridyl)dichlororuthenium(II) (Ru(bpy)$_3^{2+}$, 98%) were purchased from Aldrich. MWCNT (95% purity, diameter: 10–20 nm, and length: 10–50 μm) and SWCNT (purified grade, diameter: 1.2 nm, and length: 2.0 μm) were obtained from Il-gin Nanotech (Seoul, Korea). Water for all solutions was purified using a Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.2. Instrumentation

Cyclic voltammetric experiments were performed with a cDAQ-1604 multi-channel potentiostat (ELBIO Corporation, Seoul, Korea). All experiments were carried out with a conventional three-electrode system in a 15 mL electrochemical cell. The working electrode was glassy carbon (0.07 cm$^2$) coated with the composite films, and a platinum wire as the counter electrode. All the potentials quoted here were relative to an Ag/AgCl (3 M NaCl) reference electrode. The photon counting system used was a Hamamatsu Photonics HC 135-02 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.

BET surface area, pore diameter, and BJH cumulative pore volume were obtained using a particle size analyzer (UPA-150, Microtrac, USA) in Korea Basic Science Institute (KBSI). The thickness of the composite films was measured with a Tencor alpha step 500 surface profilometer.

2.3. Preparation of ECL sensor

The titania (TiO$_2$) sol was prepared by the hydrolysis and condensation by mixing 1 mL of 0.15 M Ti(OC$_4$H$_9$)$_4$ dissolved in propan-2-ol and 5 mL of deionized water and followed by 7 μL of 0.1 M HCl solution under vigorous stirring for at least 1 h similar to the previous report [30]. The titania sol was mixed with
Nafion (Aldrich, 5 wt.%) to form the titania–Nafion composite solution (1:1, v/v). A given amount of CNT was solubilized in the titania–Nafion composite solution. Both MWCNT and SWCNT were well dispersed in the composite solution. An aliquot of 2 µL CNT-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 1 h at room temperature (thickness: ~1.5 µm). The CNT-modified 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 incorporation of Ru(bpy)₃²⁺ was monitored by running consecutive cyclic potential scans between +0.6 and +1.35 V at a scan rate of 100 mV/s versus Ag/AgCl (3 M NaCl) reference electrode. The modified electrode was then placed in the phosphate buffer solution until use. The surface coverage, Γ, was determined for a CNT–titania–Nafion composite-modified electrode by graphical integration of background-corrected cyclic voltammogram (1 mV/s).

3. Results and discussion

3.1. Preparation of CNT–TiO₂–Nafion-modified electrode

CNT can be solubilized in solutions of Nafion in phosphate buffer or alcohol as reported by Wang group [38], because the Nafion contains both hydrophobic domain composed of fluorocarbon skeleton and hydrophilic domain composed of sulfonates [44]. Similar to Nafion, the composite solution composed of sol–gel-derived titania (TiO₂) and Nafion (1:1, v/v) can also solubilize both multi- and single-wall carbon nanotubes. The incorporation of the titania sol in Nafion solution does not impair the ability of Nafion to solubilize CNT. The Nafion content in the composite ranging from 5 to 0.7 wt.% can solubilize the SWCNT and MWCNT. In contrast, the composite containing 0.5 wt.% Nafion can solubilize SWCNT while it cannot solubilize MWCNT. This behavior is exactly the same as observed in pure Nafion solution [38]. CNT-modified electrodes were prepared by simply hand-casting a small aliquot of composite solution containing the CNT (2 µL) onto the surface of a pre-cleaned glassy carbon (GC) electrode.

3.2. Voltammetric behavior

Electrochemical behavior of Ru(bpy)₃²⁺ immobilized in the CNT–titania–Nafion composite films on a glassy carbon (GC) electrode were initially studied using cyclic voltammetry. Hydrophobic cation Ru(bpy)₃²⁺ can be easily incorporated into the present titania–Nafion composite films containing hydrophobic CNT via both hydrophobic interaction and ion-exchange process of Ru(bpy)₃²⁺ in cation-exchangeable sulfonate (SO₃⁻) groups of Nafion. The Ru(bpy)₃²⁺ was incorporated into the CNT–titania–Nafion composite films in 0.5 mM Ru(bpy)₃²⁺ 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. Fig. 1 shows a series of cyclic voltammograms which were used to monitor the incorporation of Ru(bpy)₃²⁺ into the MWCNT–titania–Nafion. With each scan taking 11 s, the rapid incorporation of Ru(bpy)₃²⁺ into the film is easily seen by the increase in peak current with each scan.

Fig. 2 shows typical cyclic voltammograms obtained at a bare GC electrode (a), the titania–Nafion composite-modified electrode without MWCNT (b), and the MWCNT–titania–Nafion composite-modified electrode (MWCNT content: 1.0 mg/mL) (c) in 0.5 mM Ru(bpy)₃²⁺ solution. The voltammograms for the composite-modified electrodes were recorded after those electrodes had reached a steady-state current in the Ru(bpy)₃²⁺ solution as seen in Fig. 1 by running consecutive cyclic potential scans. The voltammogram obtained at the MWCNT–titania–Nafion composite electrode is similar in shape to those obtained at bare GC and at the titania–Nafion composite-modified electrode without MWCNT. The oxidation...
current obtained at the MWCNT–titania–Nafion composite electrode is three-fold greater than that obtained at the composites-modified electrode without MWCNT and 2-fold greater than that obtained at the pure Nafion-modified electrode. Since the ion-exchange sites are the same in the two types of composite films, the hydrophilic MWCNT in the titania–Nafion composite certainly increases the amount of Ru(bpy)$_3^{2+}$ immobilized in the films, the hydrophobic MWCNT in the titania–Nafion composite might prevail. Since the amount of CNT incorporated into titania–Nafion composite films significantly affects the electrochemical behavior of the composite films, the effect of MWCNT amount in the composite films on the oxidation current of Ru(bpy)$_3^{2+}$ because of its greater hydrophobicity.

Since the amount of CNT incorporated into titania–Nafion composite films significantly affects the electrochemical behavior of the composite films, the effect of MWCNT amount in the composite films on the oxidation current of Ru(bpy)$_3^{2+}$ was studied. The anodic peak current obtained from cyclic voltamograms similar to those shown in Fig. 2 was plotted as a function of the amount of MWCNT incorporated into titania–Nafion composite. As shown in Fig. 4, the anodic peak current steadily increased as the amount of MWCNT in the titania–Nafion composite increased up to 1.0 mg/mL. The increased amount of hydrophobic MWCNT in the titania–Nafion composite might result in the increased adsorption of Ru(bpy)$_3^{2+}$ onto MWCNT surface, thus leading to the increased anodic peak current. However, as the amount of MWCNT in the titania–Nafion composite further increased, the current gradually decreased. In that case, the MWCNT were not evenly dispersed in the composite films containing more than 1.0 mg/mL, thus leading to poor film preparation. Therefore, an optimum amount of MWCNT at 1.0 mg/mL was used in all the subsequent experiments. The uptake rate of Ru(bpy)$_3^{2+}$ into the MWCNT–titania–Nafion composite films was relatively fast. However, the time to reach the steady-state current (loading time) at

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**Table 1**

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<td>$I'_{50}$ (mol/cm$^2$)</td>
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<td>$D_{app}$ (cm$^2$/s)</td>
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$^a$ CNT–titania–Nafion (1 mg/mL).

$^b$ Titania–Nafion (50%) composite film.

drastic increase in Ru(bpy)$_3^{2+}$ immobilized in the SWCNT–titania–Nafion composite. As shown in Table 1, the oxidation current obtained at the SWCNT–titania–Nafion composite electrode was 50% smaller than that obtained at the MWCNT–titania–Nafion composite electrode. The amount of Ru(bpy)$_3^{2+}$ immobilized in the SWCNT–titania–Nafion composite electrode ($I'_{50} = 2.1 \times 10^{-7}$ mol/cm$^2$) was lower compared to that at the corresponding MWCNT–titania–Nafion composite electrode as summarized in Table 1. This indicates that MWCNT is more effective than SWCNT for the immobilization of Ru(bpy)$_3^{2+}$ because of its greater hydrophobicity.

**Fig. 3.** Cyclic voltammograms of 0.5 mM Ru(bpy)$_3^{2+}$ in 0.05 M phosphate buffer solution (pH 7) at a MWCNT–titania–Nafion composite-modified electrode (solid line), at a SWCNT–titania–Nafion composite-modified electrode (dotted line) with a scan rate of 100 mV/s. Inset: plot of anodic peak current vs. immersion time in solution; (○) MWCNT–titania–Nafion composite-modified electrode; (□) SWCNT–titania–Nafion composite-modified electrode; and (●) MWCNT/Nafion composite-modified electrode.
the MWCNT–titania–Nafion composite (16 min) was slightly larger compared to that at the titania–Nafion composite without MWCNT (11 min), but still much shorter than that at pure Nafion films (33 min) as summarized in Table 1. The increased loading of Ru(bpy)32+ into the MWCNT–titania–Nafion composite films relative to the titania–Nafion composite films without MWCNT in the present study is probably due to the decreased accessibility of the ion-exchange sites, SO3−, in the MWCNT-dispersed composites, therefore leading to a slower diffusion of Ru(bpy)32+ in the composite films. The apparent diffusion coefficient, Dapp, for Ru(bpy)32+ in the MWCNT–titania–Nafion composite films was calculated from the slope (from linear regression analysis) of the anodic peak current versus square root of the scan rate plot by the Randles–Sevick equation. As summarized in Table 1, the calculated Dapp for the Ru(bpy)32+ in the MWCNT–titania–Nafion composite films is around 7.9 × 10−10 cm2/s, which is smaller than the diffusion coefficient for the Ru(bpy)32+ in the titania–Nafion composite films without MWCNT (4.1 × 10−10 cm2/s), but still larger than previously reported diffusion coefficient for the Ru(bpy)32+ in pure Nafion films (5.2 × 10−10 cm2/s) [45]. This fact might also explain why the presence of MWCNT in the titania–Nafion composite electrode causes the peak separation of Ru(bpy)32+ to increase to about 160 mV compared to 110 mV at the titania–Nafion composite electrode as shown in Fig. 2. The charge transport within the MWCNT-doped titania–Nafion composite is slower than in the titania–Nafion composite films without MWCNT. The pore size in MWCNT–titania–Nafion composite films has been examined and compared to those in both titania–Nafion composite films without MWCNT as summarized in Table 2. Therefore, the ion-exchange sites appear to be less easily accessible to Ru(bpy)32+ through the interconnected porous channels in the MWCNT–Nafion composite films. However, the MWCNT–titania–Nafion composite films are still more porous compared to the pure Nafion films. Therefore, the uptake rate of Ru(bpy)32+ in the MWCNT–titania–Nafion composite films is still much faster than in the pure Nafion films. In addition, the time to reach a steady-state cyclic voltammogram in the MWCNT–titania–Nafion composite films was much shorter than in the MWCNT–Nafion composite films (16 min versus 25 min) as shown in the inset of Fig. 3. This indicates that the titania–Nafion composite film is more porous than the pure Nafion film as reported previously [30].

The type of the CNT incorporated in the composite films also affects the uptake rate of Ru(bpy)32+. The uptake rate in the SWCNT–titania–Nafion composite films was slightly faster than in the MWCNT–titania–Nafion composite films. As shown in the inset of Fig. 3, the time to reach a steady-state cyclic voltammogram with the SWCNT–titania–Nafion composite films was 12 min, which was shorter than that with the MWCNT–titania–Nafion composite films (16 min). As expected, the calculated Dapp for the Ru(bpy)32+ in the SWCNT–titania–Nafion composite films was around 1.2 × 10−9 cm2/s, which was slightly greater than the diffusion coefficient for the Ru(bpy)32+ in the MWCNT–titania–Nafion composite films (7.9 × 10−10 cm2/s) as summarized in Table 1.

### 3.3. ECL behavior

The ECL behavior of Ru(bpy)32+ incorporated into the MWCNT–titania–Nafion composite-modified electrode has been studied with tripropylamine (TPA) as a representative analyte since Ru(bpy)32+–TPA system has been well characterized [46,47]. Fig. 5 shows cyclic voltammograms of Ru(bpy)32+ immobilized in MWCNT–titania–Nafion composite-modified electrode in the absence (solid line) and presence (dashed line) of 1 mM TPA in 0.05 M phosphate buffer at pH 7. In the presence of TPA, the anodic current increases significantly and the cathodic current decreases due to the catalytic oxidation of TPA. The corresponding ECL-potential profile recorded during the CV scan at a scan rate of 100 mV/s is shown in the inset of Fig. 5. As can be expected from the Ru(bpy)32+–TPA ECL mechanism, the onset of ECL seen at about 1.0–1.1 V, which is coincident with the oxidation of Ru(bpy)32+ immobilized in the MWCNT–titania–Nafion composite films. The peak ECL intensity remained relatively constant over the scan rate range of 10–100 mV/s.
Similar to the voltammetric behavior of the CNT–titania–Nafion composite-modified electrode, the amount of MWCNT incorporated into the titania–Nafion composite also strongly affects the ECL intensity as shown in Fig. 4. The ECL intensity obtained at a scan rate of 100 mV/s increased sharply as the MWCNT amount increased up to 1.0 mg/mL. However, as the MWCNT amount further increased, the ECL intensity gradually decreased. This trend observed in the ECL intensity as a function of MWCNT amount in the titania–Nafion composite is relatively similar to that observed in the anodic current of Ru(bpy)$_2^{3+}$ in the same composite films. The ECL intensity for TPA obtained with the present MWCNT–titania–Nafion composite-modified electrode was around 11-fold larger than that obtained with the titania–Nafion composite-modified electrode without MWCNT although the diffusion of Ru(bpy)$_2^{3+}$ in the MWCNT–titania–Nafion composite was slower than that in the titania–Nafion composite without MWCNT as described previously in the uptake rate study. In general, the response of the Ru(bpy)$_2^{3+}$ ECL sensor for TPA is proportional to the concentration of Ru(bpy)$_2^{3+}$ immobilized in the ECL sensor and TPA oxidation rate in the ECL sensor. The increased ECL intensity in the present ECL sensor based on the MWCNT–titania–Nafion composite is probably attributed to the combined effects such as an improved electroactivity of the hydrophobic MWCNT for the oxidation of hydrophobic analyte in the composite film and the increased Ru(bpy)$_2^{3+}$ amount immobilized in the films. In our experiment, the oxidation current of TPA at the MWCNT–titania–Nafion composite-modified electrode was around three-fold greater than that obtained at the titania–Nafion composite-modified electrode without MWCNT.

The ECL intensity for TPA obtained with the present MWCNT–titania–Nafion composite-modified electrode was even almost three orders of magnitude larger than that obtained with pure Nafion-based ECL sensor. The pore size of the MWCNT–titania–Nafion composite is greater than that of pure Nafion films, thus increased diffusion of Ru(bpy)$_2^{3+}$ and TPA in the MWCNT–titania–Nafion composite also contributed to the increase in the ECL intensity along with the above-mentioned positive effects induced by the MWCNT. In addition, the ECL intensity for TPA with the MWCNT–titania–Nafion-based ECL sensor was around two-fold greater than that obtained with SWCNT–titania–Nafion-based ECL sensor similar to the voltammetric behavior.

Calibration curves for TPA and oxalate have been constructed using the present ECL sensor based on the MWCNT–titania–Nafion composite films, and compared to those obtained with the ECL sensors based on both pure Nafion, titania–Nafion, and MWCNT–Nafion composite films. Each point as shown in Fig. 6 is a mean of three or more ECL signals obtained by consecutive cyclic potential scans (100 mV/s) at a given concentration. The Ru(bpy)$_2^{3+}$ ECL sensor based on the MWCNT–titania–Nafion composite gave a linear response ($R^2 = 0.999$) for TPA concentration from 50 nM to 1.0 mM with a remarkable detection limit (S/N = 3) of 10 nM while the ECL sensors based on titania–Nafion composite without MWCNT, pure Nafion films, and MWCNT–Nafion composite gave a detection limit of 0.1 M, 1 M, and 50 nM, respectively as shown in the inset of Fig. 6. Overall, the detection limit for TPA at the ECL sensor based on the MWCNT–titania–Nafion composite was two orders of magnitude lower than that at the ECL sensor based on pure Nafion films.

For oxalate, the linear range obtained at the present ECL sensor extended from 1 M to 1 mM ($R^2 = 0.999$) with a detection limit (S/N = 3) of 0.1 μM. The ECL intensity for oxalate is around four orders of magnitude lower than that for TPA. The decreased sensitivity for oxalate relative to that for TPA observed with the MWCNT–titania–Nafion composite films is consistent with that observed in the Ru(bpy)$_2^{3+}$ ECL sensor based on Nafion films [10,11] and titania–Nafion composite films [30].
3.4. Selectivity and stability

The response selectivity of the present MWCNT–titania–Nafion composite-based ECL sensor for various analytes were examined and compared to those obtained with the titania–Nafion composite-based ECL sensor without MWCNT. Of interest, the present MWCNT–titania–Nafion composite-based ECL sensor exhibited around 20-fold greater ECL intensities for hydrophobic analytes such as NADH, erythromycin, and promazine than those obtained at the titania–Nafion composite-based ECL sensor without MWCNT. This result can be attributed to enhanced Ru(bpy)$_3^{2+}$ amount in the present ECL sensor and also to the enhanced electroactivity of the hydrophobic MWCNT for the oxidation of hydrophobic analytes in the composite film. For example, the oxidation potential for NADH obtained at the MWCNT–titania–Nafion composite-based ECL sensor has been decreased by 200 mV and the oxidation current was seven-fold larger than the titania–Nafion composite-based ECL sensor without MWCNT. In contrast, hydrophilic analytes such as oxalate and ascorbic acid showed around three-fold increase in the ECL intensity in the present ECL sensor, which is mainly due to the increased Ru(bpy)$_3^{2+}$ amount in the present ECL sensor compared to that in the corresponding composite without MWCNT. The difference in the oxidation current in the two ECL sensors was almost negligible. The present ECL sensor did not respond to urea and neurotransmitters such as dopamine and serotonin. Therefore, the present ECL sensor could be preferably used for monitoring the concentrations of antibiotics such as erythromycin [48] and hydrophobic analytes, such as promazine derivatives [49,50], in biological fluids (urine and blood), and also for detecting NADH or species coupled to NADH production [51,52].

Another advantage of the present MWCNT–titania–Nafion composite-based (Ru(bpy)$_3^{2+}$)$_2$ ECL sensor is the outstanding long-term stability. The long-term storage stability of the present sensor was studied over a 4 month period by monitoring its ECL response to 0.5 mM TPA in 0.05 M phosphate buffer solution (pH 7) with intermittent usage (every 2–3 days) and storage in the buffer solution at room temperature when not in use. The coating of the MWCNT–titania–Nafion composite films did not come off during the test period, indicating that the MWCNT–titania–Nafion composites are well stick to the glassy carbon electrode. The ECL response of the present ECL sensor exhibited no signal loss for 4 months, which is much better than those of pure-Nafion-based electrode (stability less than a day) and the titania–Nafion composite-based ECL sensor without MWCNT (65% of initial activity retained in 3 weeks). This result indicates that the hydrophilic MWCNT present in electroactive regions within the titania–Nafion composite films could prohibit the Ru(bpy)$_3^{2+}$ to migrate into electroinactive hydrophobic regions of Nafion because strong hydrophobic interactions between Ru(bpy)$_3^{2+}$ and MWCNT favor Ru(bpy)$_3^{2+}$ to stay in electroactive regions. Similar result was also reported by Dong group [43]. 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%).

4. Conclusions

The ability of the titania–Nafion composite to solubilize the CNT permits an easy modification of electrode surfaces. MWCNT was more effective for the immobilization of larger amount of Ru(bpy)$_3^{2+}$ in the composite films on an electrode surface compared to the SWCNT because of the greater hydrophobicity of MWCNT than that of SWCNT. The present Ru(bpy)$_3^{2+}$ ECL sensor based on the MWCNT–titania–Nafion composite films has coupled the advantages of highly porous titania–Nafion composites with the advantages of MWCNT such as enhanced electrocatalytic activities towards the oxidation of analyte and the increased Ru(bpy)$_3^{2+}$ amount immobilized in the composite films. Therefore, the present ECL sensor based on the MWCNT–titania–Nafion showed improved ECL response compared to the ECL sensors based on the MWCNT–Nafion composite films as well as the titania–Nafion composite films without MWCNT. Furthermore, the present ECL sensor also showed improved long-term stability. Given the aforementioned benefits, the use of the titania–Nafion composite as a solubilizing matrix for CNT provides a useful means for the preparation of not only the present Ru(bpy)$_3^{2+}$ ECL sensor but also CNT-based electrochemical biosensors because of the superior biocompatibility of the titania–Nafion composite films and excellent electrocatalytic effects of CNT towards NADH. We are at present developing Ru(bpy)$_3^{2+}$ ECL-based biosensors using the present MWCNT–titania–Nafion composite films.

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