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Assembly of ruthenium(II) complex/layered double hydroxide ultrathin film and its application as an ultrasensitive electrochemiluminescence sensor

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ABSTRACT

An ruthenium(II) complex-containing ultrathin film, layered double hydroxide/poly(sodium 4-styrene sulfonate)/ruthenium(II) tris(bipyridine)/poly(sodium 4-styrene sulfonate) (denoted as LDH/PSS/Ru(bpy)₃²⁺/PSS), has been fabricated via the layer-by-layer (LBL) method, which was demonstrated as an solid-state electrochemiluminescence (ECL) sensor for dihydronicotinamide adenine dinucleotide (NADH). The growth of the LBL film was monitored by the UV–vis absorption spectroscopy. XRD measurements indicate the LDH/PSS/Ru(bpy)₃²⁺/PSS ultrathin film possesses a long range stacking order in the normal direction of the substrate, with an average repeating distance of 10.7 nm. SEM images show that the film surface displays a continuous and uniform morphology, with the root-mean square (rms) roughness of ~9.0 nm revealed by AFM. The ECL detection for NADH in phosphate buffer solution (pH = 7.4) was performed by using the LDH/PSS/Ru(bpy)₃²⁺/PSS film sensor, and a linear response was obtained in the range 3.16×10^{-6} to 3.23×10^{-4} M with a detection limit of 0.023 μ M. Therefore, it is expected that the LBL film based on ruthenium(II) complex in this work can be potentially used as an ultrasensitive ECL sensor in environmental and biological analysis.

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1. Introduction

Electrochemiluminescence (ECL) has received considerable attention over recent decades as a result of its great scientific and technological importance to, for example, clinical tests and biomolecule detection [1–3]. Among the ECL systems, the most extensively studied compound is ruthenium(II) tris(bipyridine) (Ru(bpy)₃²⁺) owing to its excellent stability and high efficiency in the aqueous phase [4,5]. Continuous delivery of the chemiluminescence reagent or catalyst into the electrochemical cell is required because the reagent is consumed, which limits the widespread applications of Ru(bpy)₃²⁺ ECL. It is believed that the immobilization of Ru(bpy)₃²⁺ on a solid support can overcome this limitation and provide several advantages compared with the solution-phase Ru(bpy)₃²⁺ ECL system: reducing the consumption of expensive reagent and simplifying experimental design. Therefore, it is highly essential to immobilize and stabilize the ECL active species without influencing the direct electron transfer between Ru(bpy)₃²⁺ and underlying electrodes.

Recently, many reports were focused on the immobilization of Ru(bpy)₃²⁺; several approaches based on incorporation of Ru(bpy)₃²⁺ with polymer matrices have been evaluated, including the method of Langmuir–Blodgett technique [6–8], self-assembly

techniques [9,10] and sol–gels [11–14]. Quite a few new materials, such as cation exchange polymers [15–17], silica nanoparticles [18–21], carbon nanotubes [22,23] and metal nanoparticles [24], have also been developed for solid-state ECL sensors. However, the analytical applications of these solid-state ECL sensors are limited because they easily desorb in organic solvents and lack stability in positively biased potential [25]. As a result, developing new materials and immobilization approaches still remain a significant challenge in order to improve sensitivity and long-term stability of solid-state ECL sensors.

Layered double hydroxides (LDHs), well-known as an important class of inorganic layered materials, can be represented by the general formula $[M^{2+}_{1-x}M^{3+}_x(OH)_2]^{x+}(A^{n-})_{x/n} \cdot mH_2O$, where M^{2+} and M^{3+} are divalent and trivalent metal ions, respectively, and A^{n-} is an exchangeable anion compensating for the positive charge of the hydroxide layers [26–29]. LDHs materials have been widely used in immobilization of photoactive and electroactive species as biosensors for metal ions and medicine [30–32]. LDH materials show the advantages of nontoxicity, high stability and compatibility with biomolecules [33–37], which inspires us to challenge the goal of fabricating ECL biosensors through alternate assembly of positively charged LDH nanosheets, Ru(bpy)₃²⁺ and negatively charged counterpart via the layer-by-layer (LBL) technique. The incorporation of Ru(bpy)₃²⁺ within LDH host matrix in such a triple-component system may possess the following advantages: (1) the LDH nanosheets offer a confined 2D environment for the immobilization and homogeneous distribution of Ru(bpy)₃²⁺ based on

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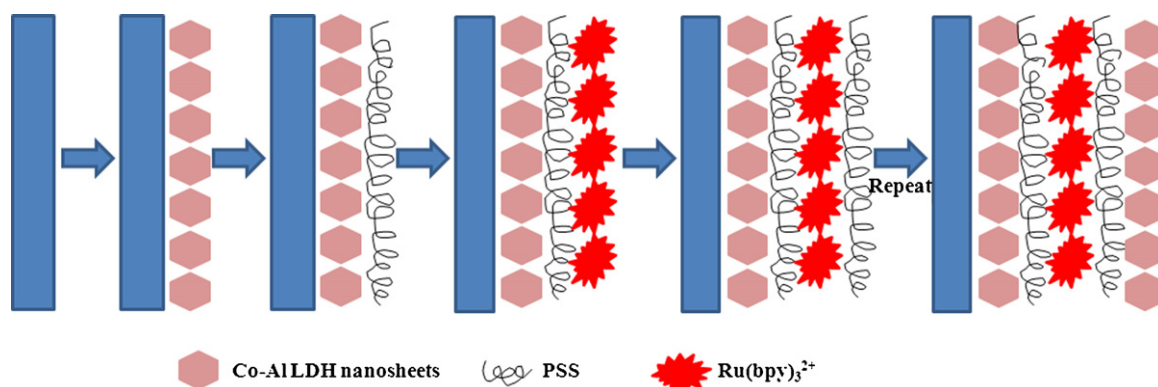


Fig. 1. Schematic representation for the LBL assembly of $(\text{LDH}/\text{PSS}/\text{Ru}(\text{bpy})_3^{2+}/\text{PSS})_n$ multilayer films.

host–guest interactions, which can reduce its aggregation; (2) the solid matrix would enhance the stability of $\text{Ru}(\text{bpy})_3^{2+}$ and suppress its leaching, which meets the prerequisite for the fabrication of solid-state ECL sensors.

In this work, the ECL active species, $\text{Ru}(\text{bpy})_3^{2+}$, was immobilized into the $(\text{CoAl-LDH}/\text{PSS}/\text{Ru}(\text{bpy})_3^{2+}/\text{PSS})_n$ multilayer films by the LBL assembly, which was applied as an ultrasensitive ECL sensor for NADH. The films show uniform surface morphology, low root-mean square (rms) roughness and a long-range stacking order in the normal direction of the substrate. The results demonstrate that CoAl-LDH nanosheets can serve as one promising building block for the fabrication of nanostructured ECL sensors, as a result of the uniform immobilization and homogeneous distribution of $\text{Ru}(\text{bpy})_3^{2+}$ in the 2D confined matrix. Moreover, the introduction of a positively charged ECL active species into an LDH-based multicomponent film system is another distinct feature in this work. It is expected that the novel strategy herein can be expanded to the fabrication of other functional cations, for the purpose of potential applications in electrochemical analysis and biosensors.

2. Experimental

2.1. Materials

Poly(styrene sulfonic acid) (PSS, $M_w = 70,000$) and tris(2,2-bipyridyl) ruthenium(II) chloride hexahydrate ($\text{Ru}(\text{bpy})_3\text{Cl}_2 \cdot 6\text{H}_2\text{O}$) were purchased from Alfa Aesar Chemical Co. Ltd. Dihydronicotinamide adenine dinucleotide (NADH) was purchased from Sangon Biotech (shanghai) Co., Ltd. Analytical grade chemicals including $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, $\text{Al}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$, NaOH, Na_2HPO_4 and NaH_2PO_4 were used without further purification. Deionized and decarbonated water was used in the preparation processes.

2.2. Synthesis and exfoliation of CoAl-LDH

CoAl- CO_3 LDH was synthesized according to a previous report [38]. CoAl- NO_3 LDH was prepared by the so-called salt-acid method. Typically, 1.0 g of CoAl- CO_3 LDH was treated with 1000 cm^3 of an aqueous salt-acid solution containing NaNO_3 (1.5 mol) and HNO_3 (0.005 mol) in a three-neck flask under nitrogen flow and continuous stirring at ambient temperature for 24 h. The resulting product was filtered, washed and vacuum dried at ambient temperature. Subsequently, formamide was used as solvent to swell and exfoliate the CoAl- NO_3 LDH particles. A colloidal suspension of LDH nanosheets was obtained by vigorously agitating 0.1 g of CoAl- NO_3 LDH in 100 cm^3 of formamide at room temperature under a N_2 gas flow for 2 days.

2.3. Fabrication of the $(\text{LDH}/\text{PSS}/\text{Ru}(\text{bpy})_3^{2+}/\text{PSS})_n$ and $(\text{PSS}/\text{Ru}(\text{bpy})_3^{2+})_n$ multilayer films modified electrode

ITO glass substrates (1 cm $\times$ 3 cm, 10 Ω per square) were cleaned in an ultrasonic bath using the following reagents in sequence: acetone, ethanol and water for 10 min each. After the cleaning procedure, the surface of substrates was hydrophilic and negatively charged. The substrates were stored in deionized water before use. Quartz glass (1 cm $\times$ 3 cm) substrates were cleaned by immersing in a fresh piranha solution ($\text{H}_2\text{SO}_4:\text{H}_2\text{O}_2$ (30%) = 3:1, v/v) (warning: piranha solution is very corrosive and must be treated with extreme care) for 40 min, followed by rinsing in deionized water and drying at 60 $^\circ\text{C}$.

The $(\text{LDH}/\text{PSS}/\text{Ru}(\text{bpy})_3^{2+}/\text{PSS})_n$ multilayer films were fabricated by applying the LBL assembly technique: the substrate was alternately dipped into an colloidal LDH nanosheets suspension (1.0 mg mL^{-1}), PSS solution (1.0 mg mL^{-1}), $\text{Ru}(\text{bpy})_3^{2+}$ solution (7.5×10^{-2} mg mL^{-1}) and PSS solution (1.0 mg mL^{-1}), for 10 min each time. Subsequently, a series of these four deposition operations were repeated n times to obtain a multilayer film of $(\text{LDH}/\text{PSS}/\text{Ru}(\text{bpy})_3^{2+}/\text{PSS})_n$ (Fig. 1). For comparison study, the $(\text{PSS}/\text{Ru}(\text{bpy})_3^{2+})_n$ multilayer films were prepared by alternate dipping into a PSS solution (1.0 mg mL^{-1}) and $\text{Ru}(\text{bpy})_3^{2+}$ solution (7.5×10^{-2} mg mL^{-1}) with a similar method described above.

2.4. Characterization techniques

X-ray diffraction (XRD) patterns were recorded by a Rigaku XRD-6000 diffractometer, using Cu K α radiation ($\lambda = 0.15418$ nm) at 40 kV, 30 mA. The UV–vis spectra were collected in a Shimadzu U-3000 spectrophotometer. The morphology of thin films was investigated using a scanning electron microscope (SEM Hitachi S-4700) with the accelerating voltage of 20 kV. The surface roughness was studied using the atomic force microscopy (AFM) software (Digital Instruments, Version 6.12). Fluorescence emission spectra were recorded on a RF-5301PC fluorophotometer (1.5 nm resolution) in the range 470–900 nm with the excitation wavelength of 450 nm and slit widths of 3 nm. Metallic elemental analysis was performed by Inductively Coupled Plasma-Atomic Emission Spectroscopy with a Shimadzu ICPS-7500 instrument (ICP-AES). A conventional three-electrode system was used, including a modified ITO glass as the working electrode, a platinum foil as the auxiliary electrode and a saturated Ag/AgCl electrode (0.799 V) as the reference electrode. The ECL signals were recorded by a MPI-B multifunctional chemiluminescent analytical system (Remax Electronic Co. Ltd., Xi'an, China) with the voltage of the photomultiplier tube (PMT) set at 800 V. The solutions were prepared with Milli-Q water (>18 M Ω cm) and purged with highly purified nitrogen. All measurements were performed at room temperature.

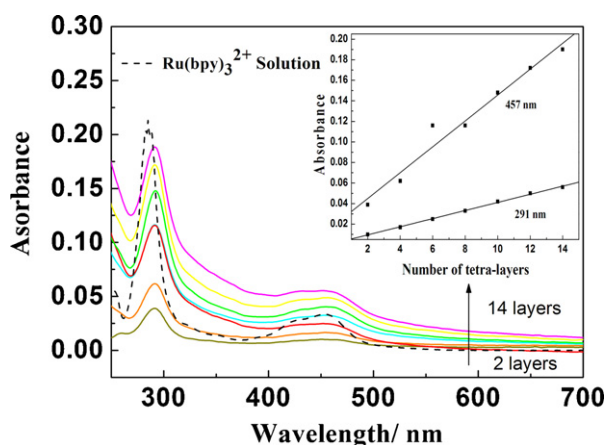


Fig. 2. UV-vis absorption spectra of the (LDH/PSS/Ru(bpy)₃²⁺/PSS)_n films along with different tetra-layer number (*n*) on quartz glass substrates. The inset shows plots of the absorbance at 291 and 457 nm vs. *n*, respectively.

3. Results and discussion

3.1. UV-vis absorption spectra of the (LDH/PSS/Ru(bpy)₃²⁺/PSS)_n multilayer films

The characterizations of LDH precursor (Co:Al = 2:1, molar ratio) and the resulting LDH nanosheets are described in ESI (Figs. S1–S3). A clear Tyndall light scattering effect was observed for the LDH nanosheets sol by a side-incident light beam. The LDH nanosheets display a size of 130–230 nm and a thickness of ~4 nm revealed by AFM. The fabrication process of the (LDH/PSS/Ru(bpy)₃²⁺/PSS)_n multilayer films was monitored by their UV-vis absorption spectra

(Fig. 2: *n* varies from 2 to 14). For the Ru(bpy)₃²⁺ solution sample, two bands at 286.0 and 452.0 nm were observed, which can be attributed to the ligand-centered transition and metal-to-ligand charge transfer (MLCT) of Ru(bpy)₃²⁺, respectively [39]. In the case of (CoAl-LDH/PSS/Ru(bpy)₃²⁺/PSS)_n films, a red-shift occurred for the two bands (from 286.0 nm to 291.0 nm and from 452.0 nm to 457.0 nm), which suggests the electrostatic interaction between Ru(bpy)₃²⁺ and PSS anion. The absorption intensity of the two bands increases gradually along with the film deposition process, and the inset in Fig. 2 shows a linear increase of the absorbance at 291.0 and 457.0 nm as a function of the tetra-layer number *n*, indicating a uniform growth procedure for the LBL film.

3.2. The surface morphology and structure of the (LDH/PSS/Ru(bpy)₃²⁺/PSS)_n films

A top-view of SEM image for the (LDH/PSS/Ru(bpy)₃²⁺/PSS)₁₀ (Fig. 3A) shows that the film surface is continuous and uniform with a thickness of ~110 nm revealed by its side-view image (Fig. 3B), corresponding to an average increment of ~11 nm per tetra-layer repeat unit. The AFM topographical image (Fig. 3C) displays a smooth surface of the (LDH/PSS/Ru(bpy)₃²⁺/PSS)₁₀ film, with a root-mean-square roughness of 9.0 nm (the roughness of ITO is 0.8 nm shown in Fig. S4). XRD patterns (Fig. 3D) exhibit a Bragg peak at $2\theta = 0.82^\circ$ and its intensity increases successively upon increasing the number of deposition cycles, demonstrating a superlattice structure of the (LDH/PSS/Ru(bpy)₃²⁺/PSS)_n films perpendicular to the substrate. The average repeating distance is ~10.7 nm based on this reflection, which is consistent with the result obtained from the cross-sectional SEM measurement.

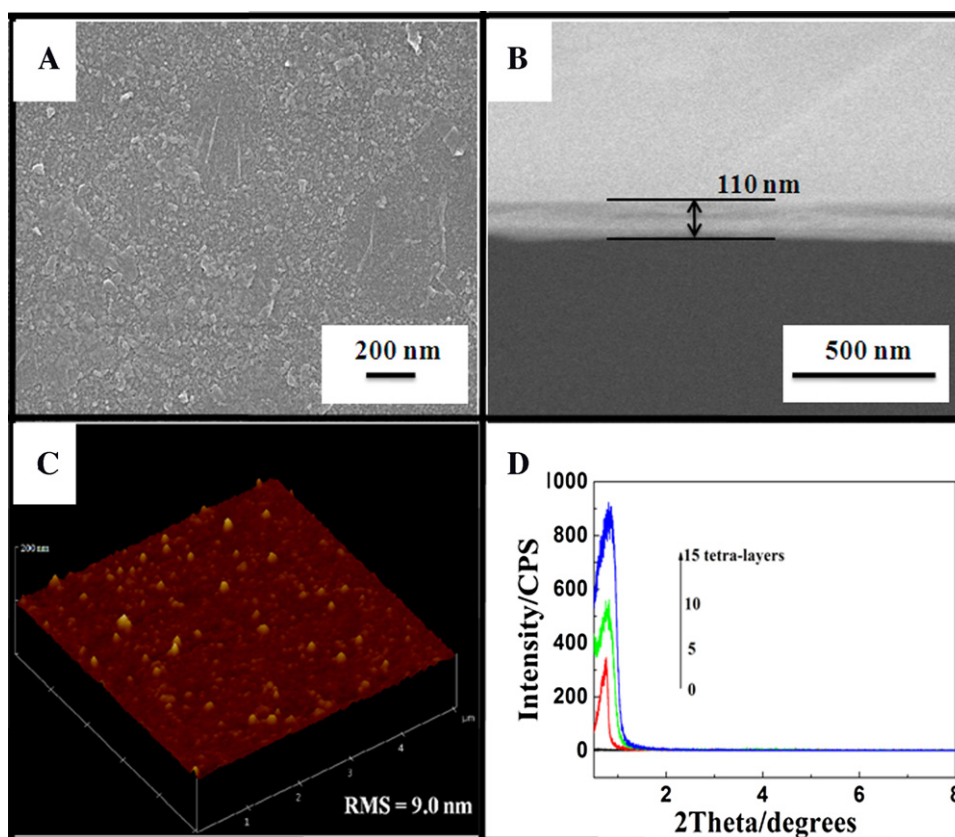


Fig. 3. (A) Top-view of SEM image, (B) cross-sectional view of SEM image for the (LDH/PSS/Ru(bpy)₃²⁺/PSS)₁₀ film, (C) tapping-mode AFM image of the (LDH/PSS/Ru(bpy)₃²⁺/PSS)₁₀ film and (D) XRD patterns for the (LDH/PSS/Ru(bpy)₃²⁺/PSS)_n films with *n* = 5, 10 and 15, respectively.

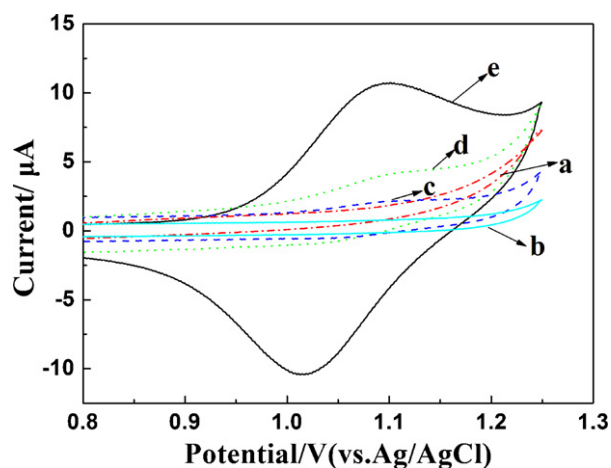


Fig. 4. Cyclic voltammograms of (a) the LDH modified ITO electrode, (b) the PSS modified ITO electrode, (c) the $\text{Ru}(\text{bpy})_3^{2+}$ modified ITO electrode, (d) the $(\text{PSS}/\text{Ru}(\text{bpy})_3^{2+})_{10}$ film modified ITO electrode, and (e) the $(\text{LDH}/\text{PSS}/\text{Ru}(\text{bpy})_3^{2+}/\text{PSS})_{10}$ film modified ITO electrode in 0.1 M phosphate buffer solution (pH = 7.4) with a scan rate of 100 mV s^{-1} .

3.3. Electrochemistry and ECL behavior of the sensor

Electrochemical performances of the $(\text{LDH}/\text{PSS}/\text{Ru}(\text{bpy})_3^{2+}/\text{PSS})_{10}$ film modified ITO electrode and several comparison samples were studied, and Fig. 4 shows their cyclic voltammograms (CVs) in phosphate buffer solution (PBS) (pH = 7.4, 0.1 M). No electrochemical response was observed at both the LDH modified electrode (curve a) and the PSS modified electrode (curve b); a rather weak anodic peak was found for the $\text{Ru}(\text{bpy})_3^{2+}$ modified (curve c) and the $(\text{PSS}/\text{Ru}(\text{bpy})_3^{2+})_{10}$ film modified ITO electrode (curve d). In contrast, the $(\text{LDH}/\text{PSS}/\text{Ru}(\text{bpy})_3^{2+}/\text{PSS})_{10}$ film modified ITO electrode (curve e) gives a couple of well-defined quasi-reversible redox peaks at 1.09 and 1.01 V. A rather low ΔE_p (80 mV) was obtained, and the ratio between the anodic and cathodic peak current is 1.03. Furthermore, according to the Laviron theory [40,41] and the linear relationship between E_p and $\log v$ (Fig. S5), the electron transfer coefficient (α) and the electron transfer rate constant (k_s) were calculated to be 0.71 and 0.78 s^{-1} , respectively, for the $(\text{LDH}/\text{PSS}/\text{Ru}(\text{bpy})_3^{2+}/\text{PSS})_{10}$ UTF modified electrode. The electronic conductivity in this work is close to that of the reported layered systems [42,43]. Electrochemical impedance spectroscopy (EIS) is an effective method to probe the features of surface-modified electrodes and has been successfully used to characterize the formation of multilayer films on different substrates [44,45]. The EIS response consists of the linear portion at lower frequencies corresponding to the diffusion-limited process and the semicircle part at higher frequencies representing the electron-transfer-limited process [46]. The Nyquist plots of the bare and modified ITO electrodes are presented in Fig. 5. The R_{ct} (the charge transfer resistance) tested in $\text{Fe}(\text{CN})_6^{4-/3-}$ solution can be obtained by measuring the diameter of the semicircle, which shows a sequence of $(\text{LDH}/\text{PSS}/\text{Ru}(\text{bpy})_3^{2+}/\text{PSS})_{10}/\text{ITO} < (\text{PSS}/\text{Ru}(\text{bpy})_3^{2+})_{10}/\text{ITO} < \text{bare ITO}$. This is consistent with their CV behavior shown in Fig. 4, further confirming that the $(\text{LDH}/\text{PSS}/\text{Ru}(\text{bpy})_3^{2+}/\text{PSS})_{10}$ modified electrode dramatically facilitates the electron transfer process. It has been reported that a high dispersion of electroactive species with uniform orientation provides electron transfer channel [31,47]. The results in this work can be attributed to the ordered arrangement of $\text{Ru}(\text{bpy})_3^{2+}$ in the 2D LDH matrix, which facilitates the charge transfer within the UTF.

Furthermore, self-quenching of the luminescence was also studied in this work. Fig. S6 displays the fluorescence emission spectra

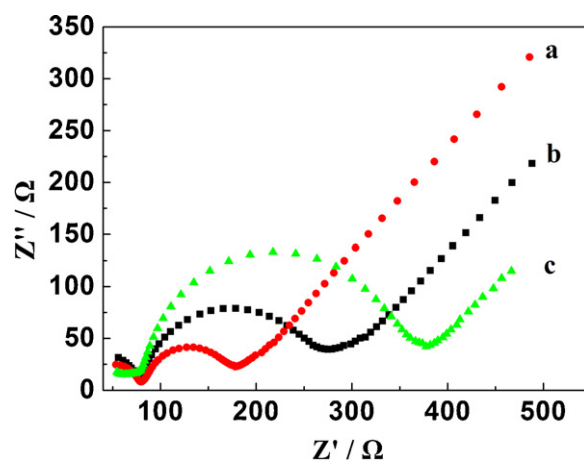


Fig. 5. Nyquist plots of EIS for the (a) $(\text{LDH}/\text{PSS}/\text{Ru}(\text{bpy})_3^{2+}/\text{PSS})_{10}/\text{ITO}$, (b) $(\text{PSS}/\text{Ru}(\text{bpy})_3^{2+})_{10}/\text{ITO}$ and (c) bare ITO in 5 mM $\text{Fe}(\text{CN})_6^{4-/3-}$ solution.

of the $(\text{LDH}/\text{PSS}/\text{Ru}(\text{bpy})_3^{2+}/\text{PSS})_{10}$ film and $(\text{PSS}/\text{Ru}(\text{bpy})_3^{2+})_{10}$ film, respectively, and the corresponding photographs under UV irradiation are illustrated in the inset. The fluorescence efficiency was employed to reveal the self-quenching of luminescence, which was calculated by the ratio of fluorescence intensity (determined from the integral of the peak) to absorbance at the excitation wavelength ($I_{\text{flu}}/A_{\text{exc}}$) [48]. It was found that the $(\text{LDH}/\text{PSS}/\text{Ru}(\text{bpy})_3^{2+}/\text{PSS})_{10}$ film (curve b, $I_{\text{flu}}/A_{\text{exc}} = 40,229$) exhibits a larger fluorescence efficiency than that of the $(\text{PSS}/\text{Ru}(\text{bpy})_3^{2+})_{10}$ film (curve a, $I_{\text{flu}}/A_{\text{exc}} = 32,946$), indicating a less degree of self-quenching in the former film. This is attributed to the existence of LDH nanosheets which provide a confined and stable microenvironment to suppress the aggregation of $\text{Ru}(\text{bpy})_3^{2+}$. Additionally, the content of $\text{Ru}(\text{bpy})_3^{2+}$ in these films was determined by ICP-AES. The $(\text{LDH}/\text{PSS}/\text{Ru}(\text{bpy})_3^{2+}/\text{PSS})_{10}$ film show the ruthenium concentration of 0.032 ppm, four times larger than that of the $(\text{PSS}/\text{Ru}(\text{bpy})_3^{2+})_{10}$ film (0.0072 ppm), indicating that $\text{Ru}(\text{bpy})_3^{2+}$ can be immobilized in the $(\text{LDH}/\text{PSS}/\text{Ru}(\text{bpy})_3^{2+}/\text{PSS})_n$ film more easily. LDH nanosheets provide a rigid and confined microenvironment for $\text{Ru}(\text{bpy})_3^{2+}$, which suppresses its aggregation and improves the fluorescence efficiency. Moreover, the electrostatic repulsion between positively charged LDH nanosheets and $\text{Ru}(\text{bpy})_3^{2+}$ would be beneficial to the ordered stacking of the active species. This accounts for the advantages of the $(\text{LDH}/\text{PSS}/\text{Ru}(\text{bpy})_3^{2+}/\text{PSS})_n$ system over the $(\text{PSS}/\text{Ru}(\text{bpy})_3^{2+})_n$ one.

The influences of the tetra-layer number (n) on the cyclic voltammogram and ECL response were investigated. It was found that the redox currents increased upon increasing the deposition cycle (n varies from 2 to 10); while a decrease in the currents was observed with further increase of tetra-layer number (Fig. S7-A). The ECL intensity of the $(\text{LDH}/\text{PSS}/\text{Ru}(\text{bpy})_3^{2+}/\text{PSS})_n$ film also shows a similar change along with n (Fig. S7-B). Consequently, the film with $n = 10$ is chosen as the optimum electrode for ECL determination in the following section. The solution pH plays a key role in the CV and ECL behavior of multilayer film, which was displayed in Fig. S8. An increase in both the redox currents and ECL intensity was observed from pH = 6.0 to 7.4; while they decreased from pH = 7.4 to 8.0. The results indicate that the $(\text{LDH}/\text{PSS}/\text{Ru}(\text{bpy})_3^{2+}/\text{PSS})_{10}$ film maintains good electrochemical and ECL performance at pH = 7.4, which is feasible for biological usage. In order to verify the effect of Co in the LDH nanosheets, the $(\text{MgAl-LDH}/\text{PSS}/\text{Ru}(\text{bpy})_3^{2+}/\text{PSS})_{10}$ film modified electrode was prepared as a comparison sample. The ECL-potential spectra (Fig. S9-A) demonstrate that the $(\text{CoAl-LDH}/\text{PSS}/\text{Ru}(\text{bpy})_3^{2+}/\text{PSS})_{10}$ film exhibits higher luminous intensity than the $(\text{MgAl-LDH}/\text{PSS}/\text{Ru}(\text{bpy})_3^{2+}/\text{PSS})_{10}$ film. In addition,

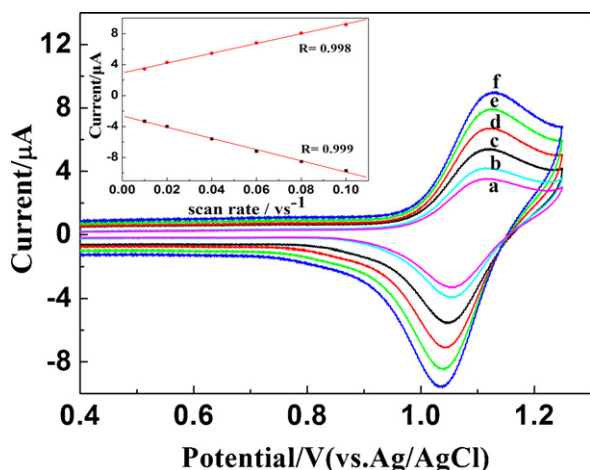


Fig. 6. CVs of the (LDH/PSS/Ru(bpy)₃²⁺/PSS)₁₀ film in 0.1 M PBS (pH = 7.4) at various scan rates (a) 10, (b) 20, (c) 40, (d) 60, (e) 80, (f) 100 mV s⁻¹. Inset: plots of peak current vs. scan rate.

electrochemical impedance spectroscopy (Fig. S9-B) reveals that the charge transfer resistance of the (CoAl-LDH/PSS/Ru(bpy)₃²⁺/PSS)₁₀ film is lower than the (MgAl-LDH/PSS/Ru(bpy)₃²⁺/PSS)₁₀ one, which can be attributed to the better conductivity of CoAl-LDH than MgAl-LDH. Therefore, the CoAl-LDH nanosheets were chosen in the following study. Fig. 6 shows CVs of the as-prepared (LDH/PSS/Ru(bpy)₃²⁺/PSS)₁₀ multilayer film in phosphate buffer solution (pH = 7.4) at various scan rates. Both the anodic and the cathodic peak currents were proportional to scan rate from 10 to 100 mV s⁻¹ (inset of Fig. 6), indicating a surface-controlled electrochemical process.

3.4. The ECL determination of NADH with the (LDH/PSS/Ru(bpy)₃²⁺/PSS)₁₀ film modified electrode

ITO electrode coated with (LDH/PSS/Ru(bpy)₃²⁺/PSS)₁₀ film used for the determination of NADH is shown in Fig. 7. The ECL peak intensity was obtained from the ECL-potential curves acquired at 100 mV s⁻¹ in phosphate buffer (pH = 7.4) while the potential scan was performed on the (LDH/PSS/Ru(bpy)₃²⁺/PSS)₁₀ multilayer film modified electrode. The results show that the ECL

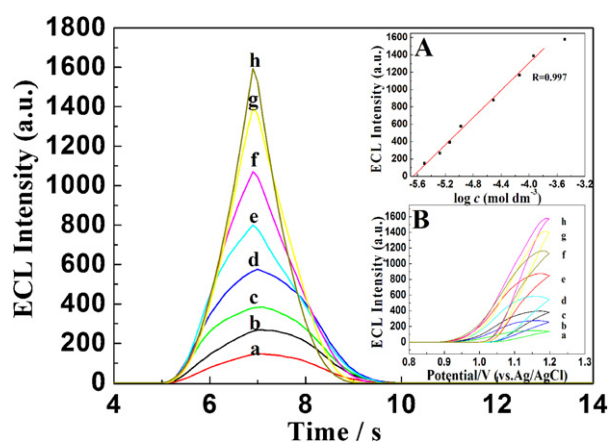


Fig. 7. ECL of the (LDH/PSS/Ru(bpy)₃²⁺/PSS)₁₀ modified electrode in 0.1 M PBS (pH = 7.4) in the presence of NADH with various concentrations (from a to h: 3.16×10^{-6} to 3.23×10^{-4} M). Scan rate: 100 mV s⁻¹. The inset A shows plot of ECL intensity vs. NADH concentration; the inset B displays ECL-potential curves of the (LDH/PSS/Ru(bpy)₃²⁺/PSS)₁₀ modified electrode in the presence of NADH with various concentrations.

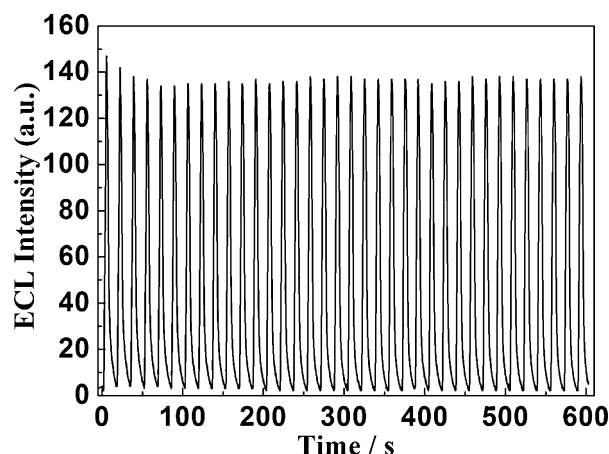
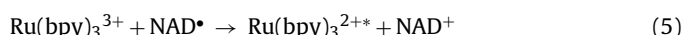
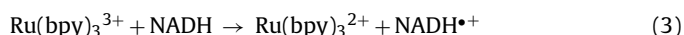


Fig. 8. The ECL emission of the (LDH/PSS/Ru(bpy)₃²⁺/PSS)₁₀ film modified ITO electrode in 0.1 M PBS (pH = 7.4) under continuous CV for 36 cycles. Scan rate: 100 mV s⁻¹.

intensity increased linearly with NADH concentration in the range 3.16×10^{-6} to 3.23×10^{-4} M, with the following linear regression equation: $I = 4451.7 + 786 \log c$ (M), $r^2 = 0.997$. The detection limit was 0.023 μM based on the signal to noise ratio of 3. In addition, CV curves reveal that remarkable increases both in anodic and in cathodic peak current were observed in the presence of NADH (from 7.2×10^{-6} to 1.1×10^{-4} M, shown in Fig. S10). A comparison study between this work and previously reported NADH chemosensors was performed (Table 1). It can be seen that the (LDH/PSS/Ru(bpy)₃²⁺/PSS)₁₀ ECL sensor in this work displays a wide linear range response and low detection limit based on the comparison [49–54]. The (LDH/PSS/Ru(bpy)₃²⁺/PSS)₁₀ modified electrode exhibits good measurement stability from 5 times of test using one electrode with a relative standard deviation (RSD) less than 2.5%. Additionally, the (LDH/PSS/Ru(bpy)₃²⁺/PSS)₁₀ modified electrode exhibits a good reproducibility from the data of five different electrodes with RSD less than 5.0%. Therefore, the (LDH/PSS/Ru(bpy)₃²⁺/PSS)₁₀ modified electrode possesses a wide linear response range, low detection limit as well as high stability for the determination of NADH.

Based on the results in this work and previous reports [49], the ECL behavior of the (LDH/PSS/Ru(bpy)₃²⁺/PSS)₁₀ modified electrode for the oxidation of NADH can be expressed as follows:



The mechanism of this ECL reaction involves reaction of Ru(bpy)₃³⁺ and an intermediate species formed upon oxidation of NADH. Eqs. (1)–(6) indicate that an homogeneous electron transfer from a neutral radical of a NADH analogue (NAD[•]) to Ru(bpy)₃³⁺ occurs to produce the excited state Ru(bpy)₃^{2+*} [55]. Eventually, Ru(bpy)₃^{2+*} returns to the ground state by emitting a photon ($k_{\text{max}} = 610$ nm).

The (LDH/PSS/Ru(bpy)₃²⁺/PSS)₁₀ modified electrode exhibits good stability of this solid-state ECL sensor under continuous potential scanning for 36 cycles (shown in Fig. 8, RSD = 5%). In order to give a further insight into the role of LDH nanosheets in the multilayer film, the (PSS/Ru(bpy)₃²⁺)₁₀ modified electrode was prepared under identical conditions for comparison

Table 1
Comparison study between the (LDH/PSS/Ru(bpy)₃²⁺/PSS)₁₀ sensor in this work and previously reported chemosensors.

Sensor	Working range (mol dm ⁻³)	Detection limit (mol dm ⁻³)	Method	Reference
LDH/PSS/Ru(bpy) ₃ ²⁺ /PSS	3.16 × 10 ⁻⁶ to 3.23 × 10 ⁻⁴	2.3 × 10 ⁻⁸	ECL	This work
PVP-Ru(bpy) ₃ ²⁺ hydrogel film	1.0 × 10 ⁻⁷ to 5.0 × 10 ⁻⁶	1.0 × 10 ⁻⁷	ECL	[49]
AuNPs/PSSG/Ru(bpy) ₃ ²⁺ film	2.5 × 10 ⁻⁹ to 5.8 × 10 ⁻⁸	1.0 × 10 ⁻⁹	ECL	[50]
PEDOT _{SDS} -nanoAg-MDB electrode	1.0 × 10 ⁻⁵ to 5.6 × 10 ⁻⁴	1.0 × 10 ⁻⁵	Electrochemistry	[51]
Graphite nanosheet/GC electrode	2.10 × 10 ⁻⁶ to 4.69 × 10 ⁻³	2.3 × 10 ⁻⁷	Electrochemistry	[52]
Chitosan-DA/MWCNTs film	1.0 × 10 ⁻⁵ to 6.0 × 10 ⁻⁴	1.2 × 10 ⁻⁷	Electrochemistry	[53]
GNSs/GC electrode	2.0 × 10 ⁻⁶ to 1.9 × 10 ⁻⁴	2.3 × 10 ⁻⁷	Electrochemistry	[54]

with (LDH/PSS/Ru(bpy)₃²⁺/PSS)₁₀. Fig. S11 shows ECL curves of the (LDH/PSS/Ru(bpy)₃²⁺/PSS)₁₀ and (PSS/Ru(bpy)₃²⁺)₁₀ modified electrode under continuous CVs in 0.1 M PBS, respectively. It is observed that the ECL intensity corresponding to the (LDH/PSS/Ru(bpy)₃²⁺/PSS)₁₀ film is much higher than that of the (PSS/Ru(bpy)₃²⁺)₁₀ film, resulting from the larger content of active species Ru(bpy)₃²⁺ in the former sample, which was revealed by elemental analysis mentioned above. Furthermore, the (LDH/PSS/Ru(bpy)₃²⁺/PSS)₁₀ film possesses a higher content of Ru(bpy)₃²⁺ than the (PSS/Ru(bpy)₃²⁺)₁₀ film, which can be demonstrated by its higher intensity of UV-vis absorption (Fig. S12) as well as stronger red fluorescence in brightness (Fig. S13). In addition, a comparison study between the (LDH/PSS/Ru(bpy)₃²⁺/PSS)₁₀ film and (PDDA/PSS/Ru(bpy)₃²⁺/PSS)₁₀ film was carried out to illustrate the stability of the former sample. After dipping the two modified electrodes into PBS (pH = 7.4) for 5 days, the ECL intensity of the (PDDA/PSS/Ru(bpy)₃²⁺/PSS)₁₀ film decreased by 24% (Fig. S14-A); while only 3% decrease was observed for the (LDH/PSS/Ru(bpy)₃²⁺/PSS)₁₀ film (Fig. S14-B). These merits can be attributed to the superlattice structure of the multilayer film in which LDH nanosheets provide a 2D confined microenvironment for the uniform dispersion and immobilization of Ru(bpy)₃²⁺ so as to suppress its aggregation. In the case of polymer/Ru(bpy)₃²⁺ system however, intertwining is inevitable between adjacent organic polymer stacking. Therefore, the (LDH/PSS/Ru(bpy)₃²⁺/PSS)₁₀ ECL sensor can be feasibly used for the determination of NADH.

4. Conclusion

In summary, multilayer films of (LDH/PSS/Ru(bpy)₃²⁺/PSS)_n were successfully fabricated by the LBL technique. The structural and morphological studies show that the film surface is continuous and uniform with long range stacking order in the normal direction of the substrate. The electrochemical and electrogenerated chemiluminescence behavior of the (LDH/PSS/Ru(bpy)₃²⁺/PSS)_n film modified electrode was studied in detail. Comparison studies demonstrate that the (LDH/PSS/Ru(bpy)₃²⁺/PSS)_n film displays improved electron transfer and fluorescence efficiency. The modified electrode exhibits remarkable electrocatalytic activity toward the oxidation of NADH with a wide linear response range, low detection limit and high stability, resulting from the superlattice structure of the multilayer films. This work provides a facile approach to immobilize electrochemiluminescence species within a 2D inorganic layered matrix for the purpose of potential application in biological field.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.electacta.2012.02.039.

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