

A Novel Electrochemiluminescence Modified Glass Carbon Electrode Based on Carboxylated Acetylene Black / Ru(bpy)₃²⁺ / Nafion for Detection of Isoniazid

Jing-yi Jiang¹, Qingyi Li², Wen-chang Wang³, Xiao-hui Chen⁴, Zhi-dong Chen^{5,*}

¹ School of Petrochemical Engineering, Changzhou University, China.

² Changzhou SIMM DRUG R&D Co., Ltd, Changzhou 213164, China

³ School of Petrochemical Engineering, Changzhou University, China

⁴ School of Chemistry and Material Engineering, Changzhou Vocational Institute of Engineering, China.

⁵ School of Petrochemical Engineering, Changzhou University, China.

*E-mail: zdchen@cczu.edu.cn

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A novel, rapid, and facile electrochemiluminescence sensor which based on carboxylated acetylene black / Ru(bpy)₃²⁺ / nafion modified glass carbon electrode (CAB/Ru(bpy)₃²⁺/NAF/GCE) was developed for detecting isoniazid (INH). The CAB/Ru(bpy)₃²⁺/NAF/GCE expresses an efficient electrochemiluminescence response for INH, because of the brilliant luminous efficiency of Ru(bpy)₃²⁺ and admirable electronic transmission of acetylene black. The electrochemiluminescence intensity was quenched significantly with addition of INH. Under the optimal conditions, the sensor for the detection of isoniazid exhibits a wide linear range of $1.0 \times 10^{-6} \sim 1.0 \times 10^{-3} \text{ mol}\cdot\text{L}^{-1}$ with the detection limit of $5.0 \times 10^{-7} \text{ mol}\cdot\text{L}^{-1}$. The sensor is expected to be a simple and convenient method for the determination of INH in pharmaceutical samples .

Keywords: electrochemiluminescence, acetylene black, Ru(bpy)₃²⁺, isoniazid

1. INTRODUCTION

Isoniazid (INH), which can get through the cells and inhibit and kill tuberculosis has been widely used for the treatment of various types of tuberculosis [1]. INH plays an extremely significant role in curing tuberculosis. However, poisoning incidents, even death, have occurred occasionally due to the overdose of INH. It is reported in 2016, a patient took excessive INH, leading to acute toxic white matter lesions [2]. It is widely agreed that one should lay great emphasis on the detection of INH with a high efficiency, high sensitivity and cost-effectiveness. Therefore, developing an efficient,

convenient and cost-effective method for the determination of INH is a hot topic that attracts many researchers attention.

At present, some traditional methods for Isoniazid determination have intensively investigated in several excellent studies, such as fluorescence [3], high performance liquid chromatography [4-7], capillary electrophoresis [8-10], spectrophotometric [11-15], chemiluminescence [16,17], polarography [18], and electroanalytical methods [19-23]. Unfortunately, it should be noted that very little optimisation work has been carried out on these methods. The traditional determination methods for INH are mostly expensive, more tedious and the process is much more cumbersome.

Electrochemiluminescence (ECL) is an effective analytical technique which has the characteristics of electrochemical and the chemical luminescence. Comparing to the traditional methods, this technique possesses the superiors of high sensitivity, good reproducibility, selectivity, wide linear range, low detection limit and high detection rate. In addition, the device for this method is simple and convenient [24]. It has been widely recognized that $\text{Ru}(\text{bpy})_3^{2+}$ plays a fundamental role in study, $\text{Ru}(\text{bpy})_3^{2+}$ is an active electrochemiluminescent substance with high luminous efficiency. In recent years, it has been widely used in the determination of organic acids, amino acids and drugs [25,26]. However, its application is limited because of pyridine ruthenium in the solution phase electrochemical luminescence system, the expensive reagents ruthenium continue to bring high cost, environmental pollution and experimental device complexity and other issues. Based on the characteristics of $\text{Ru}(\text{bpy})_3^{2+}$ in the electrochemiluminescence, the $\text{Ru}(\text{bpy})_3^{2+}$ immobilization on the surface of the electrode can not only overcome the above problems, but also improve the electrochemiluminescence intensity. With the extensive use of ruthenium in electrochemiluminescence sensors, the immobilization of $\text{Ru}(\text{bpy})_3^{2+}$ into solid carriers becomes more and more popular.

In this work, the modified electrode is prepared by immobilization $\text{Ru}(\text{bpy})_3^{2+}$ via the electrostatic interaction between $\text{Ru}(\text{bpy})_3^{2+}$ and carboxylated acetylene black. The modified electrode exhibits wide linear range, low detection limit, good reproducibility and considerable stability for detecting isoniazid.

2. EXPERIMENTAL

2.1 Materials

Acetylene black (AB) with diameter about 20 ~ 40nm were obtained from Shenzhen Nanotech Port Co, Ltd. Isoniazid (INH, 98%), Tris(2,2'-bipyridyl) ruthenium(II) chloride hexahydrate ($\text{Ru}(\text{bpy})_3\text{Cl}_2 \cdot 6\text{H}_2\text{O}$, 98%), potassium hexacyanoferrate(II) ($\text{K}_4[\text{Fe}(\text{CN})_6] \cdot 3\text{H}_2\text{O}$, 99.5%), potassium hexacyanoferrate(III) ($\text{K}_3[\text{Fe}(\text{CN})_6]$, 99.5%), potassium chloride (KCl, 99.8%), disodium hydrogen phosphate dodecahydrate ($\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 99.0%), sodium dihydrogen phosphate ($\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, 99.0%), sodium hydroxide (NaOH, 96.0%), phosphoric acid (H_3PO_4 , 85.0%) nitric acid (HNO_3 , 65%-68%) were purchased from Aladdin (Shanghai, China). The tablets of isoniazid purchased from Shenyang Hongqi Pharmaceutical Co.Ltd. Anhydrous ethanol ($\text{C}_2\text{H}_6\text{O}$, 99.7%) was purchased from Sinopharm Group Chemical Reagent Co., Ltd. Nafion (NAF, 5 wt%, $0.87 \text{ g} \cdot \text{mL}^{-1}$) was

obtained from Fluka Corporation. All others reagents were of analytical reagent grade and used without further purification. Double distilled water was used in all experimental process

2.2. Apparatus

Electrochemiluminescence (ECL) measurement was performed on MPI-B multifunctional ECL system (Xi'an Remex Analyse Instrument Co., Ltd., China). Electrochemical polymerization and electrochemical impedance spectroscopy (EIS) were conducted with a CHI 660D Electrochemical Workstation (Shanghai CH instrument Co., Ltd., China). A three-electrode system was used with carboxylated acetylene black / $\text{Ru}(\text{bpy})_3^{2+}$ / nafion modified glass carbon electrode (CAB/ $\text{Ru}(\text{bpy})_3^{2+}$ /NAF/GCE) as the working electrode, a Ag/AgCl electrode as the reference electrode, and a platinum coil ($\phi = 3$ mm) as the auxiliary electrode. Scanning electron micrographs of the electrode surfaces were obtained using a JEOL (JSM-6360LA, Japan) Scanning Electron Microscope (SEM).

2.3. Synthesis of CAB/ $\text{Ru}(\text{bpy})_3^{2+}$ /NAF.

20.0 mg AB was mixed with 50.0 mL concentrated nitric acid in a round bottom flask and was heated to 100 °C in an oil bath., while the mixture was continuously stirred by a magnetic bar stirrer. Then, condensed reflux was maintained 25 °C for 6 h. After filtering out the filtrate to leave the residue, washing to neutral and dried, carboxylated AB can be preserved.

Carboxylated AB (2.0 mg) and $\text{Ru}(\text{bpy})_3^{2+}$ aqueous solution (5.0 mL, 5.0×10^{-4} mol·L⁻¹) were mixed and stirred by a magnetic bar stirrer for 120 min, followed by removing excess $\text{Ru}(\text{bpy})_3^{2+}$ using centrifugal separation (8000 rpm, 15 min). After drying the precipitation at room temperature, the CAB/ $\text{Ru}(\text{bpy})_3^{2+}$ was uniformly dispersed in 500 μL , 0.2 wt % nafion solution to prepare 4.0 mg mL⁻¹ CAB/ $\text{Ru}(\text{bpy})_3^{2+}$ /NAF solution.

Prior to modification, the glassy carbon electrode (GCE, $\phi = 3$ mm) was polished with 0.3 μm and 0.05 μm alumina (Al_2O_3) polishing suspension respectively on the Suede. After polishing, the electrode was rinsed with double distilled water, and sonicated in ethanol, respectively, and then was dried at room temperature. The bare GCE was Pasted by 2.0 μL 4.0 mg·mL⁻¹ the solution of CAB/ $\text{Ru}(\text{bpy})_3^{2+}$ /NAF to obtain the CAB/ $\text{Ru}(\text{bpy})_3^{2+}$ /NAF modified electrode. In order to obtain the excellent electrochemical properties, the above conditions were already optimized.

2.4. Methods.

Cyclic voltammetry with potential range from 0 V to 1.3 V and scan rate of 100 mV·s⁻¹, was performed on the CAB/ $\text{Ru}(\text{bpy})_3^{2+}$ /NAF modified electrode in 0.1 mol·L⁻¹ phosphate buffer saline (PBS) (pH 8.0). to record the ECL intensity. The schematic diagram of the process to fabricate the modified electrode and ECL measurement for the determination of INH are presented in Figure 1.

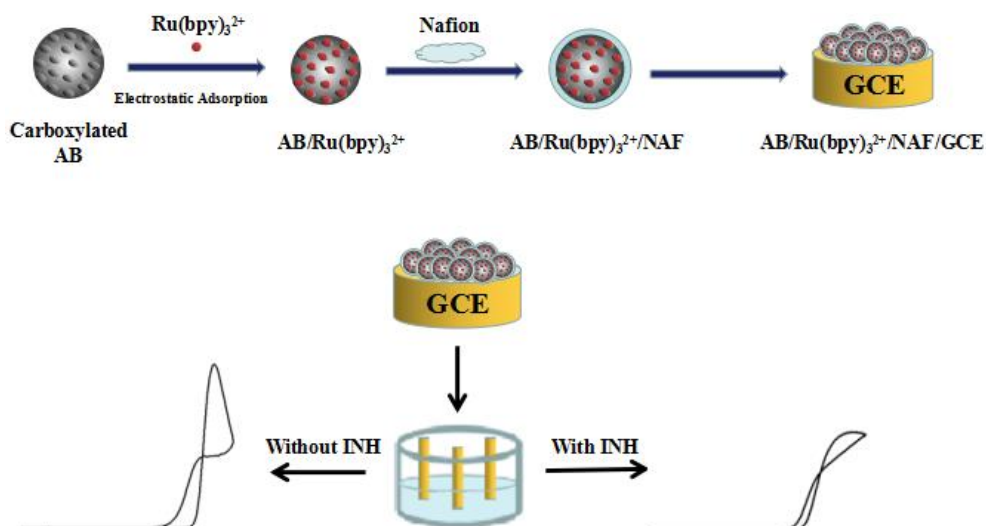


Figure 1. Schematic diagram of the fabrication process of preparing modified glassy carbon electrode (top) and ECL measurement (bottom)

3. RESULTS AND DISCUSSION

3.1. Characterization of Electrode

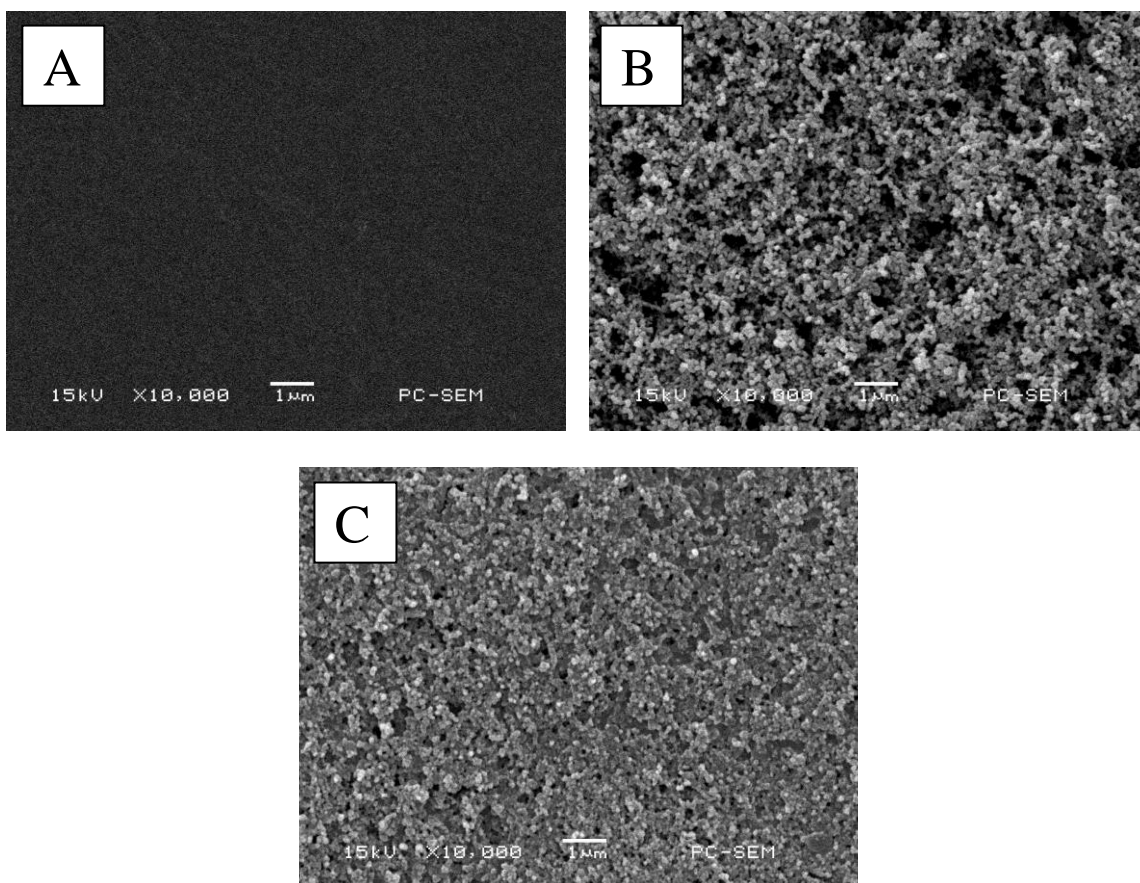


Figure 2. SEM images of bare glass carbon electrode (GCE) (A), and carboxylated acetylene black / nafion modified glass carbon electrode (CAB/NAF/GCE) (B) and carboxylated acetylene black / Ru(bpy)₃²⁺ / nafion modified glass carbon electrode (CAB/Ru(bpy)₃²⁺/NAF/GCE) (C).

The surface morphology of different modified electrodes was characterized by scanning electron microscope (SEM), as shown in Figure 2. The surface of bare glassy carbon electrode (GCE) is relatively smooth (Fig. 2A). The carboxylated acetylene black/nafion (CAB/ NAF) modified GCE possesses a typical wrinkled and sponge-like structure, indicating its large surface-to-volume ratio and flexibility (Fig. 2B). After the adsorption of $\text{Ru}(\text{bpy})_3^{2+}$, injected into the hole of carboxylated acetylene black, the morphology exhibited in Figure Fig. 2C, which indicates the CAB/ $\text{Ru}(\text{bpy})_3^{2+}$ /NAF/GCE has been successfully immobilized [27,28].

3.2. Electrochemical Characterization

The electrochemical behavior of the electrode before and after modification was studied. Figure 3A shows the cyclic voltammograms (CVs) of bare GCE(a), CAB/NAF/GCE(b) and CAB/ $\text{Ru}(\text{bpy})_3^{2+}$ /NAF/GCE(c) in $5.0 \times 10^{-3} \text{ mol L}^{-1} \text{ K}_3[\text{Fe}(\text{CN})_6]$ and $\text{K}_4[\text{Fe}(\text{CN})_6]$ solution containing $0.1 \text{ mol L}^{-1} \text{ KCl}$ as supporting electrolyte. A pair of well-reversible redox peaks appears at the bare GCE (curve a), which is attributed to the redox between $\text{Fe}(\text{CN})_6^{3-}$ and $\text{Fe}(\text{CN})_6^{4-}$. Compared with bare GCE, increased redox peak currents are observed on both CAB/NAF/GCE and CAB/ $\text{Ru}(\text{bpy})_3^{2+}$ /NAF/GCE. Besides, CAB/ $\text{Ru}(\text{bpy})_3^{2+}$ /NAF/GCE (curve c) shows larger redox peak current than CAB/NAF/GCE (curve b), suggesting that CAB/ $\text{Ru}(\text{bpy})_3^{2+}$ /NAF/GCE can facilitate the conductivity and the electron transfer process [29]. For the further investigation, impedance of the electrodes was studied by electrochemical impedance spectroscopy (EIS). The impedance spectra of the bare GCE (curve a), CAB/NAF/GCE (curve b) and CAB/ $\text{Ru}(\text{bpy})_3^{2+}$ /NAF/GCE (curve c) are shown in Fig. 3B. electron-transfer resistance of GCE is 121.9Ω (curve a), indicating the electroactive ion $\text{Fe}(\text{CN})_6^{3-}$ and $\text{Fe}(\text{CN})_6^{4-}$ are transported fast on the GCE interface.

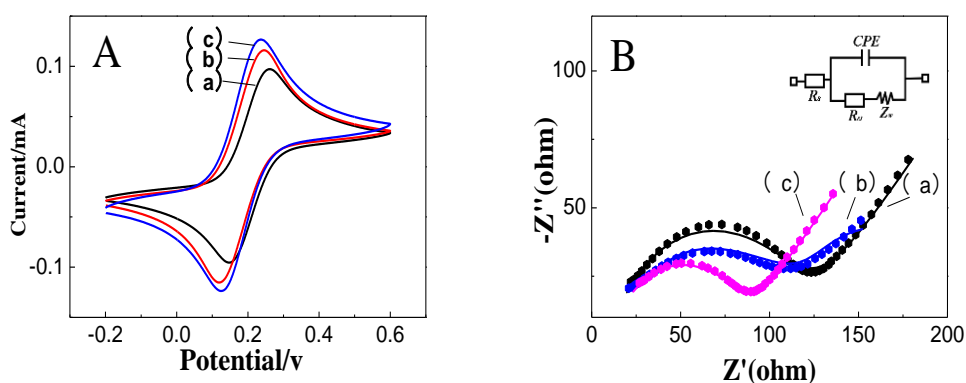


Figure 3. (A) Cyclic voltammograms of bare GCE (a), CAB/NAF/GCE (b), and CAB/ $\text{Ru}(\text{bpy})_3^{2+}$ /NAF/GCE (c) in $5.0 \text{ mmol/L Fe}(\text{CN})_6^{3-/4-}$ solution containing 0.1 mol/L KCl . (B) EIS represented by the Nyquist diagram of bare GCE (a), CAB/NAF/GCE (b) and CAB/ $\text{Ru}(\text{bpy})_3^{2+}$ /NAF/GCE (c) in the presence of $5.0 \text{ mM } [\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 M KCl . Inset: equivalent circuit (top).

The electron-transfer resistance decreased to 114.1Ω (curve b) with the introduction of CAB due to its porous structure and possesses many fascinating properties such as excellent electric

conductivity and large surface area [30]. The CAB/Ru(bpy)₃²⁺/NAF/GCE (curve c) exhibits a moderate electron-transfer resistance of 88.96 Ω (curve c) which could be because of the electrostatic interaction of positively charged ruthenium complex immobilized on the electrode and the negatively charged ferricyanide allowing more electrochemical probes access the surface of electrode for electron transfer reaction.

3.3. The ECL Behavior of INH on Different Modified Electrode

The electrochemiluminescence (ECL) behavior of the modified electrode was characterized in the solutions containing different amounts of INH. As shown in Figure 4, compared to the bare GCE(a), the ECL response of INH examined on the CAB/Ru(bpy)₃²⁺/NAF/GCE is significant. The modified electrode in the absence (curve b) and presence (curve c) of INH in PBS buffer (pH 8.0), when the potential region is from 0.1 to 1.3 V at a scan rate of 0.1 V·s⁻¹. We can discover that the quench of electrochemiluminescence intensity (ΔECL). The luminescence response peak is higher than the one after addition of INH, and this method can be successfully applied to detect isoniazid.

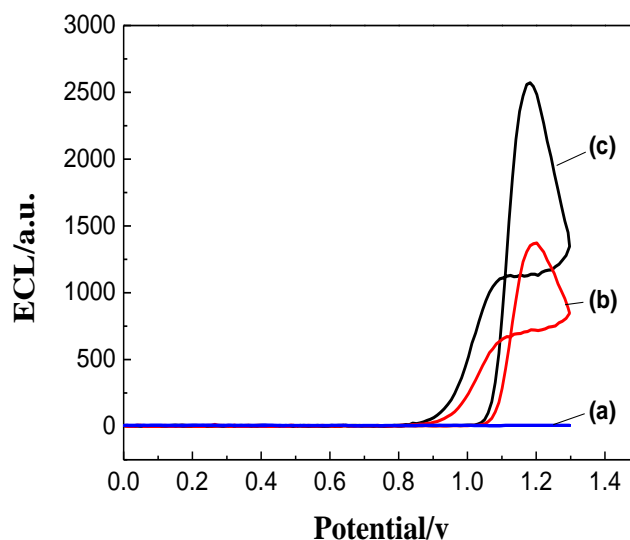


Figure 4. ECL of bare glass carbon electrode(a) and AB/Ru(bpy)₃²⁺/NAF modified electrode in 0.1 mol·L⁻¹ PBS buffer (pH = 8.0) with 0 mol·L⁻¹(b), 1.0×10⁻³ mol·L⁻¹ (c) isoniazid. at the scan rate of 100 mV·s⁻¹

3.4. Condition Optimization

In this paper, the concentration of carboxylated acetylene black (CAB) affects the amount of Ru(bpy)₃²⁺, adsorbs on the electrode and the ΔECL intensity depends on the amount of Ru(bpy)₃²⁺. It can be seen from Figure 5 that the ΔECL intensity gradually increased with the increase of CAB and reached maximum at the concentration of 4.0 mg mL⁻¹. With the increase of CAB concentration, more Ru(bpy)₃²⁺ ions were immobilized leading to an increase of the ECL intensity. However, when the

concentration of CAB was higher than 4.0 mg mL^{-1} , the CAB/Ru(bpy) $_3^{2+}$ /NAF film became too thick that slows down the rate of electron transfer between CAB/Ru(bpy) $_3^{2+}$ and GCE. Therefore, the concentration of 4.0 mg mL^{-1} of CAB was used for further study.

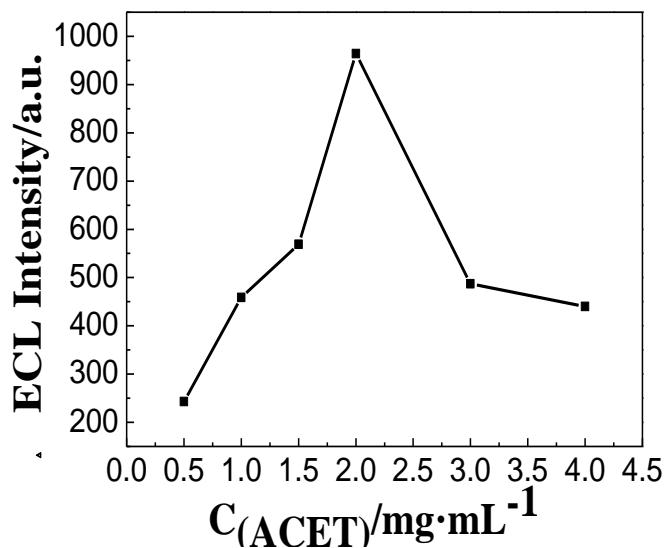


Figure 5. Influence of Different concentration of CAB ($1.0 \text{ mg}\cdot\text{mL}^{-1}$, $2.0 \text{ mg}\cdot\text{mL}^{-1}$, $3.0 \text{ mg}\cdot\text{mL}^{-1}$, $4.0 \text{ mg}\cdot\text{mL}^{-1}$, $6.0 \text{ mg}\cdot\text{mL}^{-1}$, $8.0 \text{ mg}\cdot\text{mL}^{-1}$) on ΔECL .

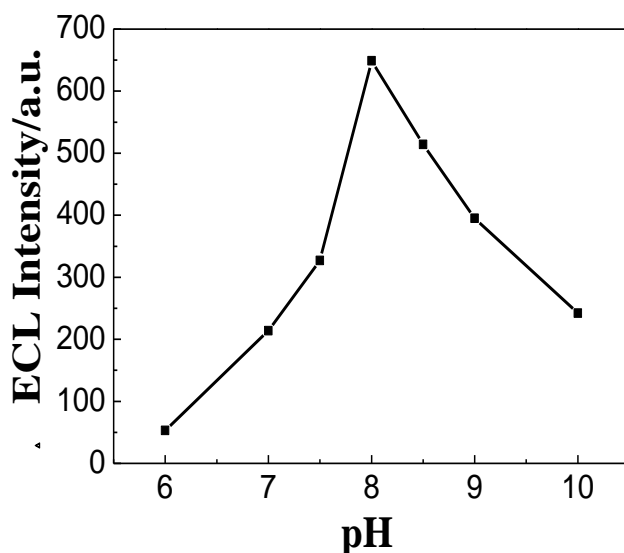


Figure 6. Influence of CAB/Ru(bpy) $_3^{2+}$ /NAF/GCE in $0.1 \text{ mol}\cdot\text{L}^{-1}$ PBS buffer with different pH(6.0~10.0) on ΔECL

Owing to the pH is a significance decisive for the presence of INH, which will be hydrolysis to nicotinic acid and hydrazine in a strong alkali solution [31]. The ECL process also depends on the pH

of the testing solution [32]. Hence, the influence of pH on ECL were investigated. As illustrated in Figure 6, when the pH of PBS increased from 6.0 to 10.0, the Δ ECL increased due to $\text{Ru}(\text{bpy})_3^{2+}$ have good luminescence properties under the alkaline condition [33]. The highest Δ ECL intensity appears at pH of 8.0. The experiment selected pH=8.0 phosphate buffer solution as optimum pH value of solution

The scan rate of cyclic voltammetry also affects the intensity and stability of the signal. Figure. 7 shows the Δ ECL at the scan rates from 25 mV s^{-1} to 150 mV s^{-1} . Because the ECL efficiency depends on the rate of generation/quench of the excited state $\text{Ru}(\text{bpy})_3^{2+*}$ [34] Δ ECL increases with the scanning rate. When the scan rate exceeds $100 \text{ mV}\cdot\text{s}^{-1}$, the Δ ECL starts to have a significant reduction, with the poor stability and reproducibility. Therefore, in this experiment, $100 \text{ mV}\cdot\text{s}^{-1}$ is chosen as the optimal condition.

Under the optimal conditions, the effect of different concentrations of INH on Δ ECL intensity was studied. The voltage of the photomultiplier was set at 800 V and the scanning voltage was 0.1 ~ 0.13 V. When the concentration of INH was in the range of $1.0 \times 10^{-6} \sim 1.0 \times 10^{-3} \text{ mol L}^{-1}$, the intensity of Δ ECL increase linearly with the concentration of INH (Fig. 8), When the concentration of INH in the range of $1.0 \times 10^{-6} \text{ mol L}^{-1}$ to $1.0 \times 10^{-5} \text{ mol L}^{-1}$ The linear regression equation is Δ ECL = $47.089 C + 553.21$ ($R = 0.9573$). Another one is Δ ECL = $0.27552 C + 1022.7268$ ($R = 0.2254$), while the concentration of INH is from 1.0×10^{-5} to $1.0 \times 10^{-3} \text{ mol/L}$. The detection limit was $5.0 \times 10^{-7} \text{ mol/L}$. Comparing with other reported methods to detect INH, the results are reported in Table 1. The proposed sensor exhibits a wide linear range and the sensor is much simpler and convenient.

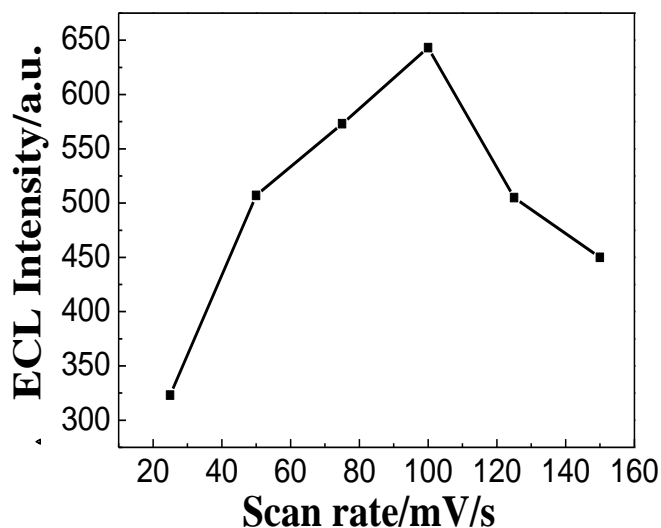


Figure 7. Influence of CAB/ $\text{Ru}(\text{bpy})_3^{2+}$ /NAF/GCE at different scan rate on Δ ECL

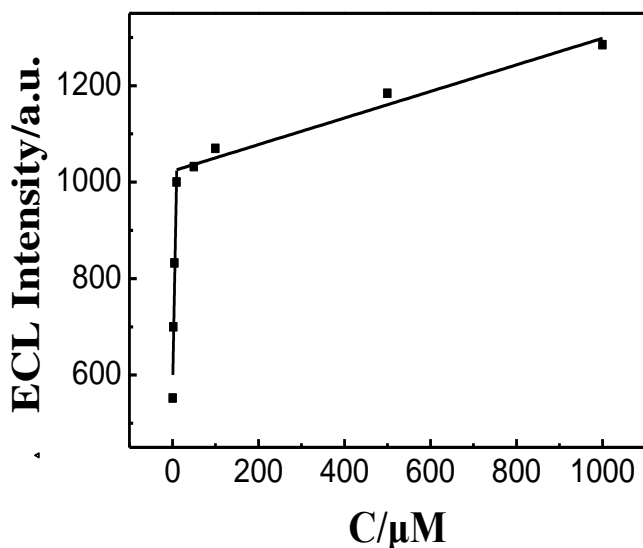


Figure 8. Δ ECL intensity with different concentrations of INH in 0.1 mol/L PBS buffer (pH = 8.0).

Table 1. Comparison between this ECL assay and other reported methods for the detection of INH.

Methods	Linear range mol/L ⁻¹	LOD mol/L ⁻¹	Ref.
HPLC	$1.3 \times 10^{-6} \sim 6.5 \times 10^{-5}$	4.0×10^{-8}	[7]
spectrophotometry	$3.6 \times 10^{-6} \sim 2.2 \times 10^{-4}$	2.9×10^{-6}	[14]
chemiluminescence	$4.4 \times 10^{-7} \sim 1.5 \times 10^{-6}$	2.0×10^{-8}	[16]
polarography	$6.0 \times 10^{-8} \sim 1.0 \times 10^{-4}$	-	[18]
electroanalytical methods	$2.0 \times 10^{-6} \sim 7.0 \times 10^{-5}$	1.7×10^{-7}	[22]
solid-state ECL	$1.0 \times 10^{-6} \sim 1.0 \times 10^{-3}$	5.0×10^{-7}	This work

3.5. Detection of INH with the Proposed Sensor

Table 2. Determination result of INH in tablets

Samples	INH Added (mmol/L)	Found (mmol/L)	Average (mmol/L)	Recovery (%)	RSD (%)
Tablets	0	0.103	0.1	97.4	2.65
		0.098			
		0.099			
	0.5	0.487	0.504	104.4	3.49
		0.522			
		0.502			

In order to examine the feasibility of the ECL sensor, modified electrode in the application of detecting INH in the medicine, The tablet samples of isoniazid (100 mg per tablet), appropriately diluted and determined by our sensor using the standard addition method. The recovery of INH for the samples is from 97.4 % to 104.4 %. The analytical results are shown in Table 2. It is obviously that the recovery was satisfactory at a CAB/Ru(bpy)₃²⁺/NAF/GCE. Each sample was tested 3 times in parallel and the RSD below 3.5 %.

4. CONCLUSIONS

In this paper, a novel type of electrochemiluminescence sensor for INH was successfully fabricated by using CAB/Ru(bpy)₃²⁺/NAF/GCE. Under the optimum experimental conditions, the method has the superiors of convenient, simple operation, cost-effectiveness and can be used for the detection of INH in the tablet samples

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