

Facile nano-free electrochemiluminescence biosensor for detection of sulphamethoxazole via tris(2,2'-bipyridyl)ruthenium(II) and *N*-methyl pyrrolidone recognition

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Xiyuan Tong¹, Shiyao Jin¹, Yingdai Zhao¹, Yue Gai¹, Yifeng E¹ ✉, Dan Li¹

¹Department of Physical Chemistry, School of Pharmaceutical Sciences, Jinzhou Medical University, Jinzhou, Liaoning, People's Republic of China

✉ E-mail: eyf@jzmu.edu.cn

Abstract: The electrochemiluminescence (ECL) system based on the ruthenium complex has become a powerful tool in the field of analytical chemistry. However, the non-aqueous ECL luminescence system, which does not involve complex nano-modification, has not been widely used for the determination of analytes. In this study, *N*-methyl pyrrolidone was selected as the solvent, and it could also act as a co-reactant of Ru(bpy)₃²⁺. Based on this, a simple ECL system without nanomaterials was established. Strong ECL was generated. Furthermore, a quenching effect between the excited state of Ru(bpy)₃²⁺ and sulphamethoxazole (SMZ) was observed. Based on this, a sensitive ECL sensor for detecting SMZ is constructed. A linear relationship between ECL signal quenching intensity (ΔI) and the logarithm of SMZ concentration (log C) in the concentration range of 1×10^{-7} – 1×10^{-5} mol/l is obtained. The limit of detection is as low as 3.33×10^{-9} mol/l. The method has been applied to the detection of SMZ in tap water samples with different concentration levels with satisfactory results, and the recovery was 95.3–102.6%.

1 Introduction

Electrochemiluminescence (ECL) has become an effective analytical technique in recent years [1, 2]. Tris(2,2'-bipyridyl)ruthenium(II) (Ru(bpy)₃²⁺) is one of the most important compounds to generate ECL [3]. It shows a reversible electrochemical reaction, good solubility in aqueous and non-aqueous solutions, low cytotoxicity and stable chemical properties [4]. However, Ru(bpy)₃²⁺ without a co-reactant only shows weak ECL. A highly efficient ECL is very important for sensitive analytical applications. Thus, multiple co-reactants have been investigated for enhancing ECL of Ru(bpy)₃²⁺ including inorganic molecules [5], organic amines [6] such as 2-(dibutylamino)ethanol [7], triethanolamine [8], tri-*n*-butylamine [9], L-cysteine [10], and nanomaterials [11]. Among these, we found that nanomaterials show excellent ECL enhancement effect compared to other systems and most of the studies were constructed in phosphate-buffered saline (PBS) solution [12]. However, in order to make nanomaterials interact with Ru(bpy)₃²⁺ to generate ECL signals, researchers often need complex procedures and overcome various interference factors [13]. For instance, sandwich structures were constructed by nanomaterials and Ru(bpy)₃²⁺ to enhance the ECL [14, 15]. First of all, this will make the experimental steps more complicated and requires professional technology to achieve, which limits its wide application. Secondly, this will hinder the transfer of electrons and increase the instability of the ECL transmitting signal [16] and even affect the accuracy of the analytical results. Finally, and most importantly, ECL sensors currently prepared in PBS systems will limit the application in water insoluble substances.

Sulphamethoxazole (SMZ) is an efficient antibiotic to treat bacterial infections in human and animal husbandry [17]. Owing to the wide applications, this antibiotic has been found in sewage treatment plants, hospital wastewater, and rivers [18, 19]. Considering the safety of drug use, it is very important to find a simple and feasible analytical method to detect SMZ. Traditional methods such as high-performance liquid chromatography (HPLC)–ultraviolet (UV) spectroscopy [20] and surface-enhanced

Raman spectroscopy (SERS) [21] are expensive, time consuming, and require complex instrumentation.

In this work, *N*-methyl pyrrolidone (NMP), as a solvent of the system, is also considered to be a simple replacement for the nanomaterials to significantly enhance the ECL of Ru(bpy)₃²⁺. Without introducing complicated elements, this enables the efficient transfer of electrons and reduces energy loss, and significant ECL enhancement can be observed. On this basis, the Ru(bpy)₃²⁺/NMP ECL sensor for SMZ detection has been successfully established by utilising this simple ECL system. Compared to traditional methods, the sensor shows high accuracy and sensitivity. It is expected that the Ru(bpy)₃²⁺/NMP ECL sensor can also be used for the facile analysis of a wide range of water-insoluble materials with high sensitivity.

2 Experiments

2.1 Materials

Tris(2,2'-bipyridyl)dichlororuthenium(II) hexahydrate (Ru(bpy)₃Cl₂·6H₂O, 98.0%) was obtained from Aladdin Chemistry Co., Ltd, tetrabutylammonium perchlorate (TBAP, 98.5%) and NMP (>99.0%) was purchased from Mcln Biochemical Technology Co., Ltd, SMZ (99%) was purchased from Shanghai Civic Chemical Technology Co., Ltd.

2.2 Instrumentation

ECL signals were recorded by using an MPI-E II ECL analyser (Xi'an Remax). Cyclic voltammetry (CV) scanning was performed using the electrochemistry work-station (CHI 650D, Shanghai Chenhua). Fluorescence emission spectra and excitation spectra were recorded by using the fluorescence spectrophotometer (F97Pro, Shanghai Zhongyong). UV–visible absorption spectra were recorded by using the UV-1600 UV-visible spectrophotometer (Shanghai Jingmi). A QPN-300 II Nitrogen generator (Shanghai Quanpu) was used to supply N₂. All experiments were carried out with a conventional three-electrode system. The platinum (Pt)

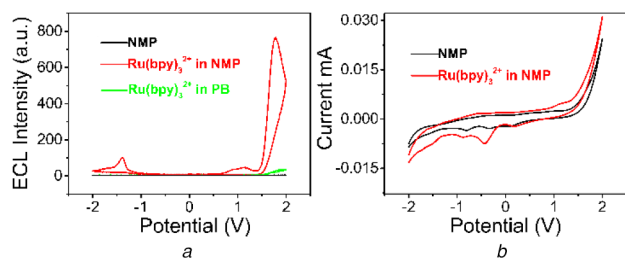


Fig. 1 Electroluminescence and electrochemical behaviors of $\text{Ru}(\text{bpy})_3^{2+}/\text{NMP}$ system

(a) ECL behaviours of 5 ml NMP, 0.1 mmol/l $\text{Ru}(\text{bpy})_3^{2+}$ dispersed in 5 ml NMP and 0.1 mmol/l $\text{Ru}(\text{bpy})_3^{2+}$ dispersed in 5 ml PB, (b) CV of 5 ml NMP (black line) and 0.1 mmol/l $\text{Ru}(\text{bpy})_3^{2+}$ dispersed in 5 ml NMP (red line). Scan rate, 0.1 V/s

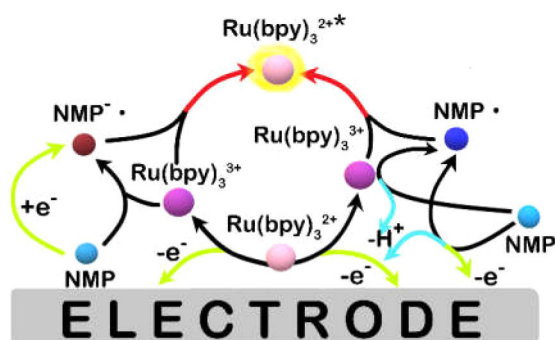


Fig. 2 Schematic illustration of NMP as novel co-reactant for enhancing the ECL of $\text{Ru}(\text{bpy})_3^{2+}$

electrode (2 mm in diameter) and the Pt wire are used as the working electrode and counter electrode, respectively, for all measurements. Since the system in this work used organic solvent, the reference electrode with an organic electrolyte (Ag/AgCl (0.01 mmol/l TBAP in NMP) was selected. In the instruction manual of this electrode, TBAP is recommended as the electrolyte. Acetonitrile (ACN) or other organic solvents are recommended as the solvents. Since the most commonly used 'ACN' solvents penetrated slowly into the electrolysis cell which affected our experimental results, we chose NMP as the solvent to decrease the interference.

2.3 Preparation of ECL luminescence system

0.0075 mg $\text{Ru}(\text{bpy})_3\text{Cl}_2 \cdot 6\text{H}_2\text{O}$ was dispersed in 10 ml NMP with ultrasonication for 20 min to obtain a well-distributed solution. The electrolyte solution was 0.01 mol/l TBAP solution prepared by NMP. Different volumes of the above $\text{Ru}(\text{bpy})_3^{2+}$ solution were added to the electrolyte solution to change the concentration of $\text{Ru}(\text{bpy})_3^{2+}$ in the system. The volume of the electrolyte cell should always be 5 ml for each measurement. The Pt electrode was carefully polished with 0.3 and 0.05 μm alumina slurries on a cloth pad. After ultrasonication of water to remove the alumina residues, the electrode was dried under a nitrogen stream. The Pt electrode was then placed in the NMP solution of $\text{Ru}(\text{bpy})_3^{2+}$ with different concentrations. ECL signals were recorded by cyclic scanning at different scan rates in the range of -2 V to $+2$ V. The voltage of the photomultiplier tube was 900 V.

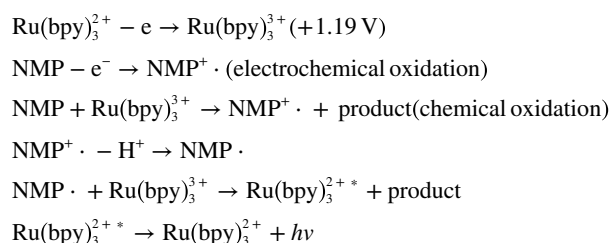
2.4 Detection of SMZ

20 μl of different concentrations of SMZ solution was added to the NMP electrolyte containing 0.1 mM $\text{Ru}(\text{bpy})_3^{2+}$. The ECL signals were cyclically scanned and recorded over a potential range of -2 V to $+2$ V. The photomultiplier tube voltage is 900 V. Oxygen was removed by nitrogen for 20 min before each measurement. Quantitative detection of SMZ was achieved based on the response of the $\text{Ru}(\text{bpy})_3^{2+}/\text{NMP}$ luminescence system ECL intensity to the concentration of SMZ.

3 Results and discussions

The Pt electrode was placed in NMP, phosphate buffer (PB) solution and the mixture of $\text{Ru}(\text{bpy})_3^{2+}$ and NMP solution, respectively. The ECL signal was recorded by cyclic scanning in the range of -2 V to $+2$ V with a 0.1 V/s scan rate. The ECL spectra of $\text{Ru}(\text{bpy})_3^{2+}/\text{PB}$ and $\text{Ru}(\text{bpy})_3^{2+}/\text{NMP}$ mixed solutions are shown in Fig. 1a. Their ECL spectra are similar in shape and location. In PB buffer solution, $\text{Ru}(\text{bpy})_3^{2+}$ only shows weak ECL intensity, when NMP is used as the non-aqueous solvent of $\text{Ru}(\text{bpy})_3^{2+}$, the ECL intensity of $\text{Ru}(\text{bpy})_3^{2+}$ can be remarkably enhanced than that of $\text{Ru}(\text{bpy})_3^{2+}/\text{PB}$ without adding any other materials. Also, there is no ECL phenomenon in the separate NMP, indicating that the illuminate in the system is $\text{Ru}(\text{bpy})_3^{2+}$, NMP sensitises the ECL of $\text{Ru}(\text{bpy})_3^{2+}$. The ECL curve shows that $\text{Ru}(\text{bpy})_3^{2+}$ begins to produce a strong ECL peak at $+1.5$ V in NMP, which is the characteristic electrochemical oxidation potential of $\text{Ru}(\text{bpy})_3^{2+}$. Then, it reaches a peak at $+1.75$ V with an intensity of 764 Au. Similar oxidations are observed in the co-reactant ECL systems such as $\text{Ru}(\text{bpy})_3^{2+}/\text{C}_2\text{O}_4^{2-}$, and $\text{Ru}(\text{bpy})_3^{2+}/\text{tripropylamine}$ [22, 23] in which $\text{Ru}(\text{bpy})_3^{2+}$ is electrochemically oxidised.

The cyclic voltammetric curves of the NMP and $\text{Ru}(\text{bpy})_3^{2+}/\text{NMP}$ mixed system are shown in Fig. 1b. In the absence of $\text{Ru}(\text{bpy})_3^{2+}$, the oxidation of NMP occurs at -0.5 V to generate $\text{NMP}^{\cdot+}$. When $\text{Ru}(\text{bpy})_3^{2+}$ was added, a significant oxidation peak appeared at $+1.19$ V. At this time, $\text{Ru}(\text{bpy})_3^{2+}$ is oxidised to $\text{Ru}(\text{bpy})_3^{3+}$. In addition, the oxidation peak of NMP was enhanced at -0.5 V, indicating that there were not only $\text{NMP}^{\cdot+}$ generated by directly electrochemical oxidation but also $\text{NMP}^{\cdot+}$ formed by chemical oxidation of $\text{Ru}(\text{bpy})_3^{3+}$. It is speculated that the possible luminescence mechanism is, firstly, NMP is oxidised by directly electrochemical oxidation or chemically oxidised by electrogenerated $\text{Ru}(\text{bpy})_3^{3+}$ to generate $\text{NMP}^{\cdot+}$ at -0.5 V, and deprotonated to form a reductive intermediate $\text{NMP}^{\cdot-}$, then $\text{Ru}(\text{bpy})_3^{3+}$ produced by electrochemical oxidation was reduced by $\text{NMP}^{\cdot-}$ at $+1.19$ V to form an excited state of $\text{Ru}(\text{bpy})_3^{2+*}$. When $\text{Ru}(\text{bpy})_3^{2+*}$ returned to the ground state, it emits light. To verify this hypothesis, the potential scanning window is reduced. The results show that when the $\text{Ru}(\text{bpy})_3^{2+}/\text{NMP}$ system was scanned in the range of 0 – 2 V and -0.4 V to 2 V, the ECL signal did not increase because NMP did not oxidise to produce $\text{NMP}^{\cdot+}$. The above results further prove the rationality of the hypothesis. This is the most common oxidation–reduction mechanism, as shown in scheme 1 (Fig. 2). The specific reaction process is as follows:



At the same time, in Fig. 1b, we found that the two reduction peaks of the mixed system were also significantly stronger than that of NMP, and the increase of reduction peaks of the $\text{Ru}(\text{bpy})_3^{2+}/\text{NMP}$ luminescent system at -0.45 V was larger than that at -0.87 V, which indicated that there was not only the process of electrochemical reduction of $\text{Ru}(\text{bpy})_3^{3+}$ by NMP but also other reduction reactions, which significantly improved the reduction peak of the mixed system. Therefore, we believe that the ECL process of $\text{Ru}(\text{bpy})_3^{2+}$ involves not only the redox pathway but also the reduction–reduction pathway [4]. The NMP electrochemically reduces to form the $\text{NMP}^{\cdot-}$. Then, $\text{Ru}(\text{bpy})_3^{3+}$ interacts with $\text{NMP}^{\cdot-}$.

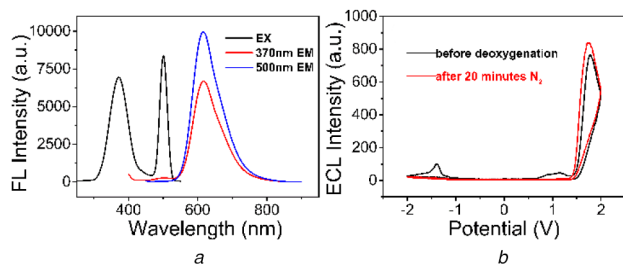


Fig. 3 Fluorescence spectra and influence of oxygen of $\text{Ru}(\text{bpy})_3^{2+}/\text{NMP}$ system

(a) Excitation (black line) and emission (red and blue lines) fluorescence spectra of $\text{Ru}(\text{bpy})_3^{2+}$, (b) ECL behaviours of 0.1 mmol/l $\text{Ru}(\text{bpy})_3^{2+}$ in NMP electrolyte before deoxygenation (black line) and after 20 min purged with nitrogen (red line)

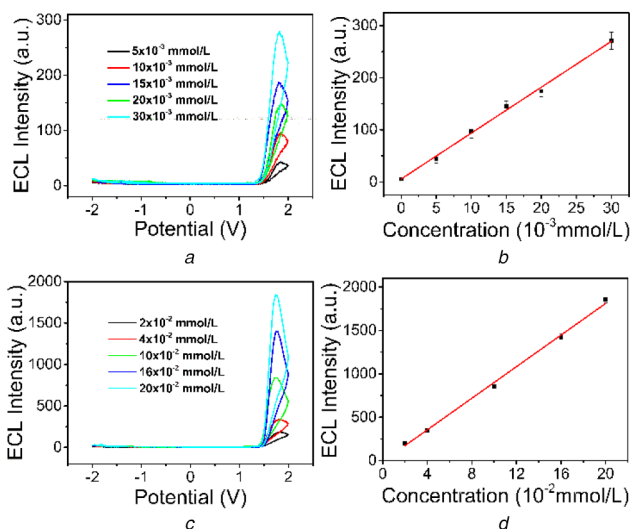


Fig. 4 Influence of different concentration ranges of $\text{Ru}(\text{bpy})_3^{2+}$ on the ECL intensity of $\text{Ru}(\text{bpy})_3^{2+}/\text{NMP}$

(a) ECL emission of the lower concentration of $\text{Ru}(\text{bpy})_3^{2+}$, (b) Calibration plot obtained with the ECL intensity and the lower concentration of $\text{Ru}(\text{bpy})_3^{2+}$, (c) ECL emission of the higher concentration of $\text{Ru}(\text{bpy})_3^{2+}$, (d) Calibration plot obtained with the ECL intensity and the higher concentration of $\text{Ru}(\text{bpy})_3^{2+}$

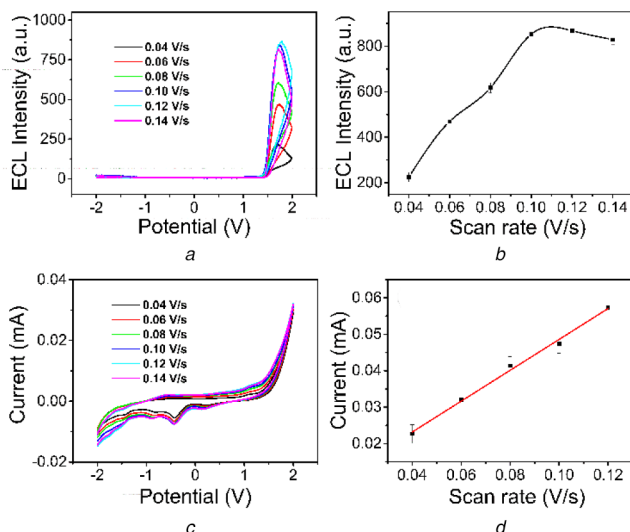
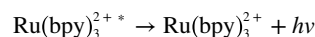
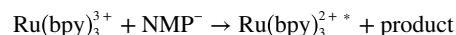


Fig. 5 $\text{Ru}(\text{bpy})_3^{2+}/\text{NMP}$ system behaviors at different scan rates

(a) Influence of different scan rates on the ECL intensity of $\text{Ru}(\text{bpy})_3^{2+}/\text{NMP}$ system, (b) The calibration plot obtained with ECL intensity and scan rate, (c) CV at different scan rate, (d) The calibration plot obtained with current and scan rate

and excited $\text{Ru}(\text{bpy})_3^{2+*}$ forms. The reduction–reduction mechanism of the $\text{Ru}(\text{bpy})_3^{2+}/\text{NMP}$ system is shown as follows:



The fluorescence spectra of the $\text{Ru}(\text{bpy})_3^{2+}/\text{NMP}$ system are shown in Fig. 3a. The excitation spectrum of the $\text{Ru}(\text{bpy})_3^{2+}/\text{NMP}$ system shows two sharp peaks at 370 and 500 nm. The mixed $\text{Ru}(\text{bpy})_3^{2+}/\text{NMP}$ solution has a characteristic absorption peak at 620 nm, which is the characteristic absorption peak of $\text{Ru}(\text{bpy})_3^{2+}$. No other absorption peak appears to indicate that there are no chemical reactions and no new substances formed in the mixing process between $\text{Ru}(\text{bpy})_3^{2+}$ and NMP. This also indicates that the ECL signal of the mixed system originates from $\text{Ru}(\text{bpy})_3^{2+}$, and the NMP plays an enhanced role in it. ECL spectra in the absence and presence of O_2 are shown in Fig. 3b. In an oxygen-rich environment, a weak ECL signal at -1.4 V is observed (Fig. 3b). It attributes to the emission of excited state NMP^* . Before the N_2 purging, there is dissolved oxygen in the system. The intermediate $\text{NMP}^\bullet$ which is formed by deprotonation can interact with O_2^- to generate excited state of NMP^* and illuminate when it returns to the ground state. The ECL signal near $+1.15 \text{ V}$ is derived from the excited state of $\text{Ru}(\text{bpy})_3^{2+*}$, formed by the interaction of $\text{Ru}(\text{bpy})_3^{3+}$ with dissolved O_2^- . It reveals that both the reductive intermediate $\text{NMP}^\bullet$ and $\text{Ru}(\text{bpy})_3^{3+}$ interact with O_2^- will result in insufficient amine groups and then affect the intensity and stability of $\text{Ru}(\text{bpy})_3^{2+}$ ECL. All experiments are performed after 20 min of continuous N_2 purging. After that, the two weak ECL signals disappeared and the strong ECL signal further increases. This again confirms that NMP plays a vital role in enhancing the ECL of $\text{Ru}(\text{bpy})_3^{2+}$.

The relationship between the concentration of $\text{Ru}(\text{bpy})_3^{2+}$ and its ECL intensity is investigated in Fig. 4. ECL intensity increases with the increase of concentration of $\text{Ru}(\text{bpy})_3^{2+}$ (Fig. 4a). It shows a linear relationship in the concentration range between 5×10^{-3} and $30 \times 10^{-3} \text{ mmol/l}$ and the correlation coefficient is 0.997 (Fig. 4b). Continuing to increase the concentration of $\text{Ru}(\text{bpy})_3^{2+}$, we can still observe a linear correlation at a higher concentration range (2×10^{-2} – $20 \times 10^{-2} \text{ mmol/l}$) (Fig. 4c). The correlation coefficient is 0.997.

The ECL intensity and peak current change are investigated as the function of the scan rate (Fig. 5). It can be seen that the ECL intensity of $\text{Ru}(\text{bpy})_3^{2+}/\text{NMP}$ increases steadily with the increase of the scan rate (0.04–0.1 V/s). Then the ECL intensity of $\text{Ru}(\text{bpy})_3^{2+}/\text{NMP}$ slightly decreases over the range of 0.1–0.14 V/s. This indicates that the ECL intensity depends not only on the chemical kinetics but also on the diffusion of free radicals [24]. The larger the scan rate, the more reductive intermediates (NMP^{+*}) are generated, and the more $\text{Ru}(\text{bpy})_3^{2+*}$ is formed. However, when the scan rate exceeds 0.1 V/s, the ECL intensity of $\text{Ru}(\text{bpy})_3^{2+}$ saturates.

It may be related to the diffusion rate of the co-reactant (NMP). An excessive scan rate leads to the consumption rate of co-reactant (NMP) on the surface of the electrode far exceeding its diffusion rate. This results in a lower concentration of NMP^{+*} on the surface of the electrode. Owing to the insufficient NMP^{+*} , ECL intensity does not increase or even decrease, leading to poor reproducibility. The CV shows the scan rate is proportional to the current (Figs. 5c and d). This indicates that an adsorption control process occurs on the surface of the electrode. In consideration of the reproducibility and intensity, 0.1 V/s is selected as the optimal scan rate.

The stability of the proposed $\text{Ru}(\text{bpy})_3^{2+}/\text{NMP}$ system was tested (Fig. 6). The concentration of $\text{Ru}(\text{bpy})_3^{2+}$ is 0.1 mmol/l with 0.1 V/s as the scan rate. During the repetitive CV cycle, the ECL intensity stabilises after the fifth scan. After that, no significant

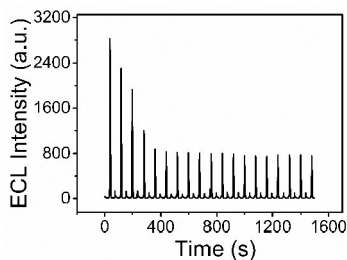


Fig. 6 ECL response of 0.1 mmol/l $\text{Ru}(\text{bpy})_3^{2+}$ dispersed in NMP electrolyte after 19 repetitive CV scans

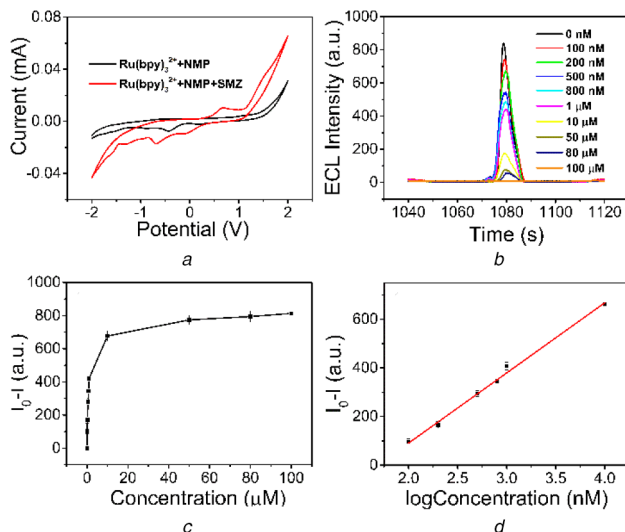


Fig. 7 Detection of SMZ by $\text{Ru}(\text{bpy})_3^{2+}/\text{NMP}$ ECL sensor

(a) Electrochemical behaviours of 0.1 mM SMZ in $\text{Ru}(\text{bpy})_3^{2+}/\text{NMP}$ system, (b) Effect of different concentrations of SMZ on ECL intensity of $\text{Ru}(\text{bpy})_3^{2+}/\text{NMP}$ system and, (c) The calibration plot obtained with between the difference (I_0-I) of the ECL signal and the concentration of the SMZ, (d) The calibration plot obtained with the difference (I_0-I) of the ECL signal and the logarithmic concentration of the SMZ in the range of 100 to 100,000 nM

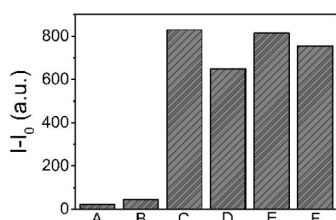


Fig. 8 Interference of coexisting substances on ECL determination of SMZ. 0.1 mM, A (glucose), B (β -cyclodextrin), C (SMZ), D (acetaminophen), E (bisphenol A) and F (catechol), respectively

change is found for the ECL intensity. This indicates the high stability of ECL. For accuracy, the data was scanned 1000 s each time.

A simple non-aqueous ECL system is constructed by $\text{Ru}(\text{bpy})_3^{2+}/\text{NMP}$. It is expected to be used in the analytical field conveniently and efficiently. In order to confirm this assumption, an ECL sensor of SMZ was constructed. The SMZ is found to inhibit ECL of $\text{Ru}(\text{bpy})_3^{2+}/\text{NMP}$ which attributes the energy transfer between $\text{Ru}(\text{bpy})_3^{2+}$ and SMZ [25]. After adding 0.1 mM SMZ to the $\text{Ru}(\text{bpy})_3^{2+}/\text{NMP}$ luminescence system, the CV curve of Fig. 7a shows that, compared with the CV curve of the $\text{Ru}(\text{bpy})_3^{2+}/\text{NMP}$ ECL system, when adding 0.1 mM SMZ, a distinct oxidation peak appeared at +0.7 V and the ECL of the mixed system was quenched. An oxidative quenching reaction occurs here, and the mechanism is that the aniline groups of SMZ can be electrochemically oxidised at +0.7 V to further form free

radicals, and then react with the intermediate $\text{Ru}(\text{bpy})_3^{2+}$ to transfer energy from $\text{Ru}(\text{bpy})_3^{2+}$ to free radicals, resulting in ECL quenching. We further studied the relationship between SMZ concentration and ECL intensity. As shown in Fig. 7b, increasing the SMZ concentration from 100 nM to 0.1 mM, we can find that the higher the concentration of SMZ in the system, the lower the intensity of ECL. Therefore, we can detect the content of SMZ in the system based on the relationship between the change of ECL intensity ΔI ($I-I_0$) and the concentration of SMZ (Fig. 7c). Here, I represents the ECL intensity in the absence of SMZ; I_0 represents the ECL intensity in the presence of SMZ. In the range of 1.0×10^{-7} – 1.0×10^{-5} mol/l, ΔI and the logarithmic value of SMZ concentration are in a linear relationship (Fig. 7d). The regression equation is $Y = 288.87 \log C - 487.15$ ($R^2 = 0.993$). The limit of detection (LOD) was calculated by the IUPAC method [26] and the formula is as follows: $\text{LOD} = 3\sigma/m$. Here, σ is the standard deviation of the blank, and m is the slope of the calibration curve. $S/N = 3$. The detection limit is as low as 3.33×10^{-9} mol/l.

Several commonly used methods for detecting SMZ were compared to the current method (Table 1). It can be found that the SMZ $\text{Ru}(\text{bpy})_3^{2+}/\text{NMP}$ ECL sensor is comparable to the highly sensitive SERS and HPLC. However, it is simple to operate. Compared to capillary electrophoresis with amperometry detection (CE-AD) and electrochemical detection (CV/differential pulse voltammetry (DPV) in Table 1) methods, the current ECL sensor is applied much more simply, but higher sensitivity is achieved. It can be concluded that the ECL sensor is promising for the facile analysis of the SMZ.

In order to evaluate the selectivity of electrochemical sensors [27], we used this $\text{Ru}(\text{bpy})_3^{2+}/\text{NMP}$ ECL sensor to test a variety of compounds but unexpectedly found that a variety of compounds can inhibit ECL luminescence. We selected several typical compounds for testing as shown in Fig. 8, including acetaminophen, bisphenol A, and catechol. These compounds all inhibit ECL luminescence and it can be seen that the changes of the ECL value caused by these interfering substances cannot be ignored. The quenching effect of these compounds on ECL is also found in other ECL luminescence systems [28, 29, 30]. After further research, we found that these compounds contain typical phenolic hydroxyl or arylamino structures. They can quench ECL because the above structures can be oxidised to form free radicals, and then energy is transferred from $\text{Ru}(\text{bpy})_3^{2+}$ to free radicals. Finally, the ECL is annihilated. Common natural compounds such as glucose and β -cyclodextrin have no significant effect on the $\text{Ru}(\text{bpy})_3^{2+}/\text{NMP}$ luminescence system. Therefore, when the above structure exists in the sample to be detected, the ECL luminescence intensity of the system can be quenched, and if the ECL intensity is not decreased, there is no relevant compound in the sample.

We used the $\text{Ru}(\text{bpy})_3^{2+}/\text{NMP}$ luminescence system to measure the SMZ solution at a concentration of 1×10^{-5} mol/l five times under the same conditions to verify the repeatability of the sensor. The measured ECL intensity is 175, 181, 173, 188, and 169, respectively, and the relative standard deviation (RSD) is 4.2%, indicating that the sensor has good reproducibility.

To evaluate the feasibility of this sensor in actual samples, we used the ECL sensor based on the $\text{Ru}(\text{bpy})_3^{2+}/\text{NMP}$ system to detect SMZ in tap water. We pretreated the sample by evaporating the tap water of the laboratory and redistributed it with NMP. However, since the concentration of SMZ in the tap water is too low, it has no effect on the ECL intensity of the system. Therefore, we added three different concentrations of SMZ to the tap water. The results are shown in Table 2, the recovery rate is in the range of 96.7–102.6%, with an RSD of 2.59–3.58%, which indicates that the SMZ sensor we developed is expected to be used for SMZ detection.

4 Conclusion

NMP significantly enhanced the ECL of $\text{Ru}(\text{bpy})_3^{2+}$. The mechanism involves free radicals transfer and redox reaction. This

Table 1 Comparison of different detection methods of SMZ

Methods	System	Detection limit	Ref
SERS	Raman microscope alpha300 RS/ aqueous environment (water)	2.2×10^{-9} mol/l	[21]
HPLC	Amethyst-C18 column/methanol– water	3.95×10^{-9} mol/l (1 μg/l)	[31]
Liquid chromatography– mass spectrometry	Synergi HydroRP column/ACN– ammonium acetate– formic acid	0.02×10^{-9} mol/l (5.1 ng/l)	[32]
CV/DPV	multi-walled carbon nanotube/PBnc/ Britton–Robinson buffer solution	38×10^{-9} mol/l	[33]
CE-AD	Fused-silica capillary/ Na ₂ B ₄ O ₇ – H ₃ BO ₃ /Na ₂ B ₄ O ₇ – KH ₂ PO ₄	201.37×10^{-9} mol/l (5.1×10^{-5} g/l)	[34]
ECL	Ru(bpy) ₃ ²⁺ /NMP	3.33×10^{-9} mol/l	this work

Table 2 Recoveries of SMZ spiked from tap water samples

Sample	Standard value of SMZ, M	Found, mM	Recovery, %	RSD, %, n = 5
Lake water	1×10^{-6}	9.53×10^{-7}	95.3%	3.37
	1×10^{-5}	9.67×10^{-6}	96.7%	3.58
	5×10^{-5}	5.13×10^{-5}	102.6%	2.59

system shows good stability. In addition, a sensitive SMZ sensor is developed based on the Ru(bpy)₃²⁺/NMP system. This facile strategy opens up new prospects for the wide application of ECL detection of non-water-soluble materials. It also provides excellent strategies for replacing nano-based enhancement methods.

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6 References

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