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The electrochemiluminescence (ECL) of Ru(bpy)$_3^{2+}$ (bpy = 2,2'-bipyridyl) and Ru(phen)$_3^{2+}$ (phen = 1,10-phenanthroline) at bare and graphene oxide (GO) modified glassy carbon (GC) electrodes has been employed for the determination of chlorpromazine hydrochloride (CPZ). The ECL intensity was gradually increased with the increasing concentration of CPZ under optimal condition. For Ru(bpy)$_3^{2+}$, a linear response was obtained over CPZ concentration range of $1.0 \times 10^{-5}$–$1.0 \times 10^{-2}$ mol•L$^{-1}$, with a detection limit of $1.0 \times 10^{-8}$ mol•L$^{-1}$ at 10 a bare GC electrode in phosphate buffer (pH = 7). The detection limit can be further down to $1.0 \times 10^{-10}$ mol•L$^{-1}$ after modification of the GC electrode with GO. In the case of Ru(phen)$_3^{2+}$, the linear response was acquired over a CPZ concentration range of $1.0 \times 10^{-11}$–$1.0 \times 10^{-7}$ mol•L$^{-1}$, and the detection limit can be reduced further to $1.0 \times 10^{-11}$ mol•L$^{-1}$ on the GO modified GC electrode. All these are much lower compared to other detection methods. The proposed method was applied to the determination of spiked CPZ in human serum; the recovery was quite satisfactory with good reproducibility and stability, providing the possibility of developing an ECL 15 detection method for CPZ.

Introduction

Antipsychotic drugs are widely used as therapeutic agents for treating various mental and personality disorders.$^1$ 20 Chlorpromazine hydrochloride (CPZ, Scheme 1) is such a compound and belongs to the phenothiazine neuroleptic group. The discovery of antipsychotic agent CPZ in the early 1950s and the advent of even more powerful phenothiazinic psychopharmacological agent represented a landmark in the history of the medical and psychiatric science.$^2$ CPZ is used for the control of psychoses including schizophrenia, mania and several disturbed or agitated behavior, it is also used for the relief of nausea, vomiting, preoperative anxiety and intractable hiccup.$^3,4$ However, excess CPZ has inhibiting effect on neural 30 system, respirometric system and circulative system. Generally, oral lethal dose is 15–150 mg CPZ kg$^{-1}$, and lethal blood concentration is 5–10 mg CPZ L$^{-1}$. Therefore, the determination of residual CPZ in human serum is of great significance in the clinical detection.

35 Many methods have been reported for the individual determination of CPZ, such as spectrophotometry,$^6,7$ colorimetry,$^8$ HPLC$^9$, titrimetry$^{10}$ and spectrofluorimetry.$^{11}$ However, it is still a great challenge to develop a simple but reliable detection method for the rapid and sensitive determination of CPZ. A potential approach is to utilize an electrochemiluminescence (ECL) method,$^{12–15}$ which can produce light at an electrode without involving any light source. Considering that CPZ has two N atoms in the structure, it can be detected by using of a ruthenium complex/CPZ ECL system. In our previous works,$^{16–18}$ melamine, malachite green, leucomalachite green and hydrazine hydrate were detected using ECL, where the determinand was employed to be an amine additive candidate of ruthenium complexes, providing strong evidence for the proposed ECL method.

50 To improve detecting efficiency, graphene oxide (GO) was immobilized on the glassy carbon (GC) electrodes, because GO has high Young’s modulus and hardness, excellent flexibility, and low cost compared with carbon nanotubes, which make it an effective reinforcement for electrode composites.$^{19}$ In particular, GO is negatively charged and has many π-conjugated aromatic domain in its basal plane,$^{20}$ thus it is a remarkable material for strongly immobilizing substances that are positively charged and have aromatic structure through both electrostatic interaction and π–π stacking interaction.$^{21}$ PVA (polyvinyl alcohol) has been chosen as a membrane material and solvent for GO, due to the reason that there are a large number of hydrophilic groups on GO surface, such as hydroxyl, carboxyl, and epoxy,$^{22}$ which can form hydrogen bonds with the PVA chains that contain even more hydrophilic groups, enhancing the interfacial adhesion ability and 65 mechanical performance of the resulting PVA/GO composite.$^{23}$ To make a comparison, Ru(bpy)$_3^{2+}$ (bpy = 2,2'-bipyridyl, Scheme 1) and its derivative Ru(phen)$_3^{2+}$ (phen = 1,10-phenanthroline, Scheme 1) were chosen here. The later has better adsorptive ability on the GO modified GC electrode because phen is a tricyclic aromatic ligand while bpy is a bidentate aromatic ligand.$^{24–26}$ Attributed to both the π–π stacking interaction and the electrostatic interaction, immobilization of the ruthenium complexes can be achieved easily by immersing GO 75 modified GC electrodes into the corresponding solution of the
ruthenium complex, and the interaction may accelerate the electron transfer and amplify ECL signals even at a lower concentration of CPZ. The results demonstrated that the logarithmic concentration of CPZ was linear over a CPZ concentration ranges of $1.0 \times 10^{-2}$ to $1.0 \times 10^{-6}$ mol·L$^{-1}$ for \( \text{Ru(bpy)}_{3}^{2+} \) and $1.0 \times 10^{-1}$ to $1.0 \times 10^{-7}$ mol·L$^{-1}$ for \( \text{Ru(phen)}_{2}^{2+} \) at GO modified GC electrode surface. All of these can provide the possibility of developing an ECL method for accurate determination of CPZ.

![Scheme 1 Structures of chlorpromazine hydrochloride (CPZ), \( \text{Ru(bpy)}_{3}^{2+} \) and \( \text{Ru(phen)}_{2}^{2+} \).](image)

### Experimental

#### Materials and reagents

15 The Cl$^-$ salt of \( \text{Ru(bpy)}_{3}^{2+} \) and \( \text{Ru(phen)}_{2}^{2+} \) were samples left in our previous work.\textsuperscript{29,30} GO was bought from Nanjing XFNANO Materials Tech Co., Ltd, China. CPZ was bought from Beijing century audiocodes biological technology Co., Ltd, China. PVA (average \( M_w \) 17 000, 99% hydrolyzed) was bought from Sinopharm Chemical Reagent Co., Ltd. Shanghai, China. Other chemicals and solvents were all of reagent grade and used as received. All experiments were performed in compliance with the relevant laws and institutional guidelines, and were approved by Dalian University of Technology. Informed consent was obtained for all human subjects. Blood samples were collected from healthy volunteers of appropriate age and sex.

#### Modified electrode preparation

GC working electrodes (3.0 mm in diameter) were first polished with a slurry of 0.05 mm alumina, then sonicated, and rinsed with deionized water. Then the electrode was successively sonicated in 1:1 nitric acid and doubly distilled water, and allowed to dry at room temperature. An amount of 0.75 mg of the treated GO\textsuperscript{29} was dispersed with the aid of ultrasonic agitation in 5 mL deionized water, then 1 mL was taken to mix with 2 mL 5% PVA aqueous solution, to obtain a homogeneous, well-distributed suspension, then 10 \( \mu \)L of this suspension was dropped onto the surface of the pretreated GC electrode, and the solvent was allowed to evaporate at room temperature in the air. Fig. 1 shows the SEM images of the fracture surface of PVA and PVA/GO films.

![Fig. 1 SEM images of the fracture surface of PVA/GO films, (a) PVA alone, (b) PVA with GO.](image)

### Results and discussion

#### Effect of pH on ECL

As is known that pH of the buffer solution has an important effect on aqueous ECL reactions.\textsuperscript{25} 0.1 mol·L$^{-1}$ phosphate buffer was employed and the pH of the solution was adjusted with phosphate acid and NaOH solution to the required pH value at first, then ECL performance for $2.0 \times 10^{-4}$ mol·L$^{-1}$ \( \text{Ru(bpy)}_{3}^{2+} \) and $1.0 \times 10^{-5}$ mol·L$^{-1}$ CPZ under different pH from 5 to 9 was studied at a GC electrode. As shown in Fig. S1, both the highest ECL signal and the highest signal-to-noise ratio can be reached at pH = 7. The measurements were repeated several times with a relative standard deviation (RSD) of less than 4.5%, suggesting good stability under this condition, so 0.1 M phosphate buffer (pH = 7) was chosen for all the ECL measurements in this study.

#### Effect of scan rate on ECL

According to the literature,\textsuperscript{25,36} the scan rate could affect the ECL over a wide range, because the ECL efficiency significantly depends on the rate of generation/annihilation of the excited state \( \text{Ru(bpy)}_{3}^{2+} \). To investigate the effect of different scan rate on ECL intensity, ECL performance for $2.0 \times 10^{-4}$ mol·L$^{-1}$ \( \text{Ru(bpy)}_{3}^{2+} \) and $1.0 \times 10^{-5}$ mol·L$^{-1}$ CPZ under different scan rate were also performed. The results are shown in Fig. S2. It is noted that the scan rate affected the ECL dramatically at GC electrode in 0.1 mol·L$^{-1}$ phosphate buffer. The best reproducibility and stability was reached when scan rate was 100 mV·s$^{-1}$. Therefore, 100 mV·s$^{-1}$ was employed for all the detections below.

#### ECL performance after addition of CPZ

Two obvious oxidation peaks can be observed in 0.84 V and 1.09 V from Fig. S3, because there are two amine groups in CPZ, so the two peak current can be determined to be the first step oxidation (0.84 V) and the second step oxidation (1.09V) of CPZ. After the addition of a certain amount of CPZ into $2.0 \times 10^{-4}$ mol·L$^{-1}$ \( \text{Ru(bpy)}_{3}^{2+} \) phosphate buffer (pH = 7) solution, cyclic voltammograms and the corresponding ECL performance for the system at the GC electrode under different potentials is shown in Fig. 2. When the electrode potential was scanned positively close to 1.25 V, upon the oxidation of Ru$^{2+}$, an ECL signal was observed.\textsuperscript{37,38} It is noted that the anodic current increased along with the increasing of the oxidation potential, and significant
enhancement can be observed when the electrode potential was close to 1.25 V, demonstrating that CPZ can be a candidate as an amine additive reductant, to build a Ru(bpy)$_3$/$\text{CPZ}$ ECL system. In the presence of CPZ, the original anode current increased around 0.84 V and 1.09 V. This indicates that CPZ has sensibilization effect on Ru(bpy)$_3$ oxidation current. However, the CPZ oxidation peak at 1.09 V couldn’t be observed, due to the overlap of the Ru(bpy)$_3$ oxidation peak at 1.25 V.

15 Similar to the mechanism of the ECL of the Ru(bpy)$_3$/TPrA system, CPZ undergoes oxidation at proper positive potential at the electrode surface, which leads to the formation of a radical cation intermediate. Then it reacts with the oxidized Ru(bpy)$_3$ to form the excited state Ru(bpy)$_3^{2+*}$ and light will be obtained when the excited Ru(bpy)$_3^{2+*}$ releases energy to return to Ru(bpy)$_3^{2+}$. The ECL sensing mechanism for CPZ can be proposed as follows:

25 Scheme 2 Proposed ruthenium/CPZ ECL mechanism.

Both the pH value and the scan rate on the ECL result were studied in detail following the above mentioned procedure, and the same trend can be observed compared with those of before the addition of CPZ, so pH = 7 and 100 mV/s were employed for the detections. For 2.0×10$^{-4}$ mol·L$^{-1}$ Ru(bpy)$_3^{2+}$ in 0.1 mol·L$^{-1}$ phosphate buffer (pH = 7) at the GO modified GC electrode, the ECL intensity of Ru(bpy)$_3^{2+}$ was observed to increase along with the increasing concentration of CPZ, and the logarithmic ECL increase [lgECL = lg(ECL$_{\text{after addition of CPZ}}$ - ECL$_{\text{before addition of CPZ}}$)] versus the logarithmic concentration of CPZ is linear over a CPZ concentration range of 1.0×10$^{-9}$-1.0×10$^{-6}$ mol·L$^{-1}$ (Fig. S5). Ru(phen)$_2^{2+}$ was used as an alternative luminophore at GO modified GC electrode, by contrast, demonstrated better quantitative examination for CPZ. The ECL increase (AECL = ECL$_{\text{after addition of CPZ}}$ - ECL$_{\text{before addition of CPZ}}$) versus the logarithmic concentration of CPZ is lineal over a certain range of the concentration of CPZ. A linear regression equation (∆I$_{\text{ECL}}$ = 134.698lg[CPZ] + 1502.99, R = 0.99656, n = 5) was obtained in the range of 1.0×10$^{-4}$-1.0×10$^{-6}$ mol·L$^{-1}$ and CPZ detection limit was 1.0×10$^{-8}$ mol·L$^{-1}$ at a signal to noise ratio of three (Fig. S3).

50 Fig. 3 Dependence of logarithmic ECL increase versus logarithmic concentration of CPZ in 0.1 mol·L$^{-1}$ phosphate buffer (pH = 7) at GC electrode.

Detection of CPZ on GO modified electrode

In order to make a comparison with that of the bare GC electrode, the same conditions as above mentioned were employed for the control experiment on the GO modified GC electrode, and good reproducibility and stability can be achieved when utilizing 0.15 mg·mL$^{-1}$ GO and 3.4% PVA to modify the GC Electrode.

Stability of the ECL measurement on the GO modified GC electrode
Fig. 4 shows the ECL performance by immersing the GO modified GC electrode in 0.1 mol\cdot L^{-1} phosphate buffered solution (pH = 7) containing 2.0×10^{-4} mol\cdot L^{-1} Ru(bpy)_{3}^{2+} and 3.0×10^{-6} mol\cdot L^{-1} CPZ, then continuously cyclic potential scanning for ten times at the scan rate of 100 mV\cdot s^{-1}. No significant change for the ECL intensity can be observed in the detection process, suggesting good reproducibility and stability of the ECL measurement on the GO modified GC electrode. Under the same condition, the GO modified GC electrode immersed into 2.0×10^{-4} 10 mol\cdot L^{-1} Ru(phen)_{3}^{2+} and 3.0×10^{-6} mol\cdot L^{-1} CPZ in 0.1 mol\cdot L^{-1} phosphate buffered solution (pH = 7), the experimental result that almost no change for the ECL intensity can be obtained also shows excellent reproducibility and stability of the GO modified GC electrode.

![Continuous cyclic scan of GO modified GC electrode for 10 cycles in pH = 7 phosphate buffer containing 2.0×10^{-4} mol\cdot L^{-1} Ru(bpy)_{3}^{2+} and 3.0×10^{-6} mol\cdot L^{-1} CPZ; the scan rate was 100 mV\cdot s^{-1}.](Image)

**Table 1** Recovery of CPZ determined by proposed method.

<table>
<thead>
<tr>
<th>Spiked (mol\cdot L^{-1})</th>
<th>Detected (mol\cdot L^{-1})</th>
<th>Average (mol\cdot L^{-1})</th>
<th>Recovery</th>
<th>RSDb</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.00×10^{-4}</td>
<td>0.98×10^{-4}</td>
<td>1.00×10^{-4}</td>
<td>100%</td>
<td>2.1%</td>
</tr>
<tr>
<td>1.05×10^{-4}</td>
<td>1.02×10^{-4}</td>
<td>1.01×10^{-4}</td>
<td>102%</td>
<td>3.5%</td>
</tr>
<tr>
<td>0.99×10^{-4}</td>
<td>0.98×10^{-4}</td>
<td>1.00×10^{-4}</td>
<td>99%</td>
<td>1.6%</td>
</tr>
</tbody>
</table>

Average of three measurements and the averaged readings were used. Applied potential: 0-1.8 V (vs. Ag/AgCl); Scan rate: 100 mV\cdot s^{-1}.

**Conclusions**

In conclusion, the ECL of Ru(bpy)_{3}^{2+}/Ru(phen)_{3}^{2+} at GO modified GC electrodes has been successfully employed for the determination of CPZ. The proposed method was applied to the determination of CPZ and the recovery is quite satisfactory with good reproducibility and stability. All of these results provide the possibility of developing a novel ECL detection method for CPZ.

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**Notes and references**

8  V. N. Gandhi, H. Shah, Indian Drugs, 1984, 21, 354.