

## Supporting Information

# A cathode electrochemiluminescence sensor based on graphite carbon nitride nanosheets for determination of buflomedil hydrochloride in mice plasma

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## Experimental

### *Reagent and materials*

Melamine was obtained from Nation Pharmaceutical Group Chemical Reagent Co., Ltd. (Beijing, China). Buflomedil hydrochloride was obtained from China Standard Material Net (Beijing, China).  $K_2S_2O_8$  was obtained from Guangzhou Chemical Reagent Factory (Guangzhou, China).  $Na_2HPO_4$ ,  $NaH_2PO_4$ , glucose, lactose, ascorbic acid, uric acid were obtained from Xilong Chemical Co., Ltd. (Shantou, China). All reagents were of analytical grade. All aqueous solutions are prepared with double-distilled water (DDW).

### *Apparatus*

The ECL signals were detected using an MPI-B multifunctional electrochemical and chemiluminescent analytical system (Xi'an Remex Electronic Science-Tech Co., Ltd., China). A modified glass carbon electrode (GCE) was employed as the working electrode. A platinum wire was used as the counter electrode and an Ag/AgCl (saturated KCl) electrode worked as reference electrode, respectively. Scanning electron microscopy (SEM) image was acquired on a model Vario Micro Cube scanning electron microscope (Elementar Company, Germany). X-ray diffraction (XRD) measurements were measured with a D/max-2500V/PC power X-ray diffractometer (Rigaku, Tokyo, Japan). Ultraviolet-visible (UV-vis) absorption spectra were measured on a Cary 60 UV-vis spectrophotometer (Agilent Technologies, USA). Fourier transform infrared spectra (FT-IR) were received on a PerkinElmer FT-IR spectrophotometer (Perkin-Elmer, UAS). Fluorescence spectra were recorded on a RF-5301 fluorescence spectrometer (Shimadzu, Japan). Electron paramagnetic resonance (EPR) experiments were performed on a Bruker EMX 10/12 spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany) with 5,5-dimethyl-pyrroline N-oxide (DMPO) as a spin-trapping agent. Electrochemical experiments were recorded on a CHI 660 electrochemical workstation (Chenhua Instrument Co. Ltd., Shanghai, China).

### *Synthesis of g-C<sub>3</sub>N<sub>4</sub> nanosheets*

The g-C<sub>3</sub>N<sub>4</sub> nanosheets were prepared according to the previous literature with some changes.<sup>1</sup> Briefly, 10 g melamine was put in an alumina crucible and heated for 2 h at 600 °C with a ramp rate about 5 °C/min in a muffle furnace. Thereafter cooled to ambient temperature and obtained yellow bulk g-C<sub>3</sub>N<sub>4</sub> powder. Then 50 mg of yellow bulk g-C<sub>3</sub>N<sub>4</sub> powder was dispersed into 50 mL DDW with ultrasonication for 10 h to obtain a white suspension. Thereafter, the white suspension was centrifuged at 10000 rpm to remove the residual unexfoliated g-C<sub>3</sub>N<sub>4</sub>. Finally, the supernatant was dried in air overnight to obtain g-C<sub>3</sub>N<sub>4</sub> nanosheets.

### *Construction of ECL sensor*

Prior to the fabrication, the GCE was successively polished in turn using 0.3 and 0.05  $\mu\text{m}$  alumina power to mirror. Then washing thoroughly with ethanol, the electrode was washed in depth with DDW and dried under nitrogen flow. 1 mg as-prepared g- $\text{C}_3\text{N}_4$  NS was dissolved in 10 mL double distilled water and was treated under ultrasonic conditions for 2h to obtain homogeneous white suspension. 8  $\mu\text{L}$  0.1 mg/mL g- $\text{C}_3\text{N}_4$  suspension was dropped on the cleaned GCE electrode, then dried in room temperature to obtain the ECL sensor of g- $\text{C}_3\text{N}_4$ /GCE (as shown in Scheme 1).

### *Measurement procedure*

The modified electrodes were in contact with 0.1 mol/L phosphate buffer solution (PBS) (pH 7.5) containing 10 mmol/L  $\text{K}_2\text{S}_2\text{O}_8$  and scanned from -0.2 V to -1.3 V with a scan rate of 0.1 V/s. The potential of the photomultiplier tube was operated at 800 V. With increasing Buf concentration, the ECL intensity gradually attenuated. Hence, the variations of ECL response ( $\Delta I = I_0 - I_t$ ) directly related to the concentration variations of Buf, which  $I_t$  and  $I_0$  are the ECL respond in the presence and absence Buf.

### *Plasma sample pretreatment*

Female BALB/c mice (6–8 weeks old) were provided by Hunan SJA Laboratory Animal Co., Ltd. (Changsha, China). All animal handling procedures were approved by the Animal Ethics Committee of Guangxi Normal University (Approval No. 202409-004). The mice were divided into experimental group and control group. The mice were fasting but freely drinking water the night before. The experimental mice were accepted an oral administration of Buf by stomach at 1mg/kg each and the control group were received the same amount of double distilled water. After 2 h of being kept, the blood samples of 0.5 mL were taken from eye socket of mice and added into centrifugal tubes, which were treated by heparin sodium. In order to separate the plasma, the plasma samples were centrifuged at 3500 rpm for 10 min. A particular amount of 200  $\mu\text{L}$  of plasma sample were precisely shifted into a test tube, thereafter 1 mL of ethanol and 50  $\mu\text{L}$  of 0.1 mol/L NaOH were put in the centrifugal tube. Then vortex oscillated for 5 minutes, the mixtures were centrifuged at 10,000 rpm for 10 min. The top layer was removed into another test tube and evaporated to desiccation under nitrogen flow in the water bath at 80  $^{\circ}\text{C}$ . Finally, the dry residual was redispersed in 200  $\mu\text{L}$  DDW.

### *Optimization of ECL conditions*

We next sought to investigate the optimum experiment conditions of the fabricated ECL sensor for determination of Buf. Several vital factor including the pH of phosphate buffer solution in ECL cell, the concentration of coreactant  $\text{K}_2\text{S}_2\text{O}_8$  and the scan rate were carried out to reach the best ECL performance. The ECL emission intensity of the proposed ECL sensing platform for Buf detection can be greatly affected by the pH of phosphate buffer solution. As shown in Fig. S1A, when the pH of PBS was 7.5, the ECL response reached the maximum. Because the pH value is low, the proton may be reduced, which inhibits the electrochemical reduction of g- $\text{C}_3\text{N}_4$ . Therefore, the ECL intensity is weak. At extremely high pH, the strong oxidant of  $\text{SO}_4^{\cdot-}$  (negatively charged radical of  $\text{K}_2\text{S}_2\text{O}_8$ ) could react with  $\text{OH}^-$ , leading to that the formation of excited state of  $\text{C}_3\text{N}_4$  (g- $\text{C}_3\text{N}_4^*$ ) was inhibited. As a result, the ECL emission decreased. Hence, pH 7.5 was chosen as the optimal pH of PBS. As illustrated in Fig. S1B, the ECL response was greatly enhanced with the increase of  $\text{K}_2\text{S}_2\text{O}_8$  concentration toward the peak when  $\text{K}_2\text{S}_2\text{O}_8$  was 10 mmol/L. The reason is that the negative charge of cathode may react with

excessive  $\text{K}_2\text{S}_2\text{O}_8$  leading to fewer electrons was gained by the  $\text{g-C}_3\text{N}_4$ . Thus the generation of the excited state  $\text{g-C}_3\text{N}_4$  was inhibited, and the ECL signal was decreased. Therefore, the optimal concentration of  $\text{K}_2\text{S}_2\text{O}_8$  was 10 mmol/L. The effect of scan rate on the ECL signal was evaluated in Fig. S1C. According to the results, with the increasing of scan rate the ECL signal enhanced toward the peak, which the scan rate was 0.1V/s. Because the coreactant  $\text{S}_2\text{O}_8^{2-}$  diffusing rate coming from the electrolyte solution to the electrode surface is less than the consuming rate of co-reactant  $\text{S}_2\text{O}_8^{2-}$  on the electrode surface with the scan rate was greater than 0.1 V/s.<sup>2</sup> In consequence, the ECL signal receded. Therefore, 0.1 V/s was chosen as the optimum scan to acquire maximum ECL response.

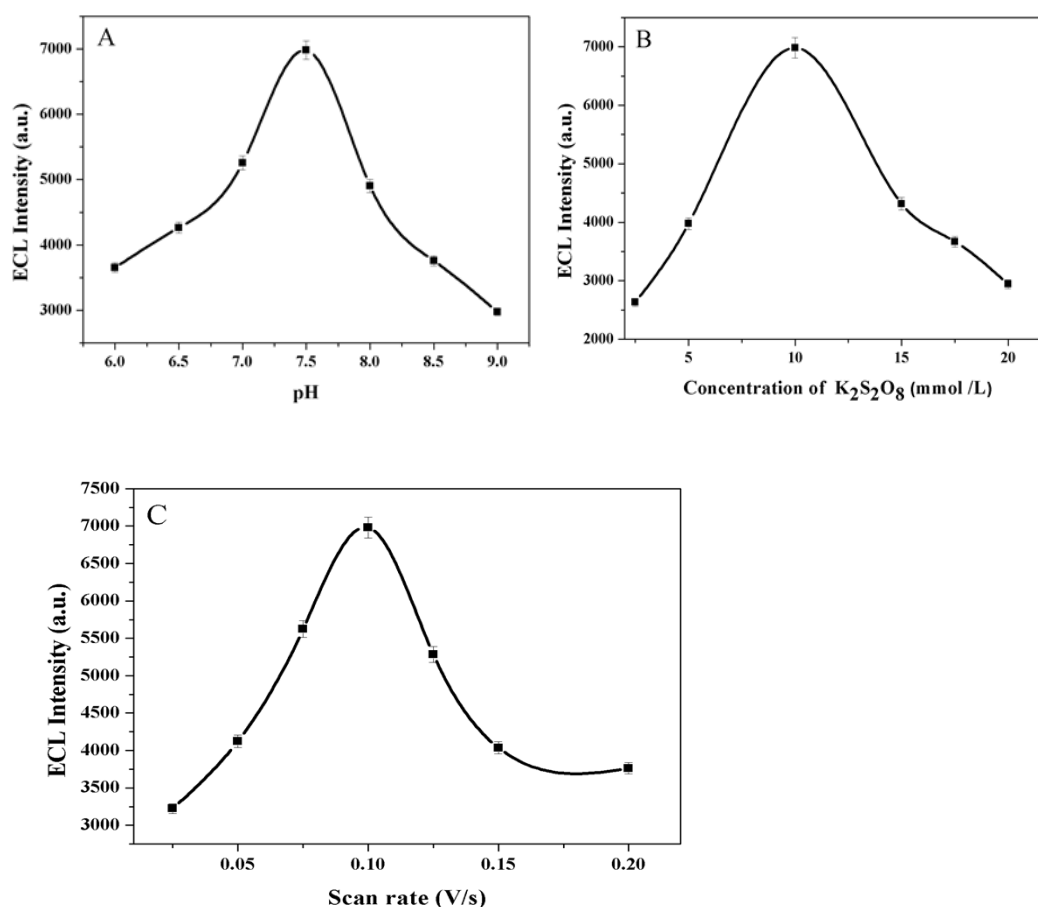


Fig. S1. Effect of experimental conditions on the ECL of  $\text{g-C}_3\text{N}_4$  modified electrode: (A) The pH of PBS in ECL cell, (B) The concentration of co-creactant  $\text{K}_2\text{S}_2\text{O}_8$ , (C) The potential scan rate.

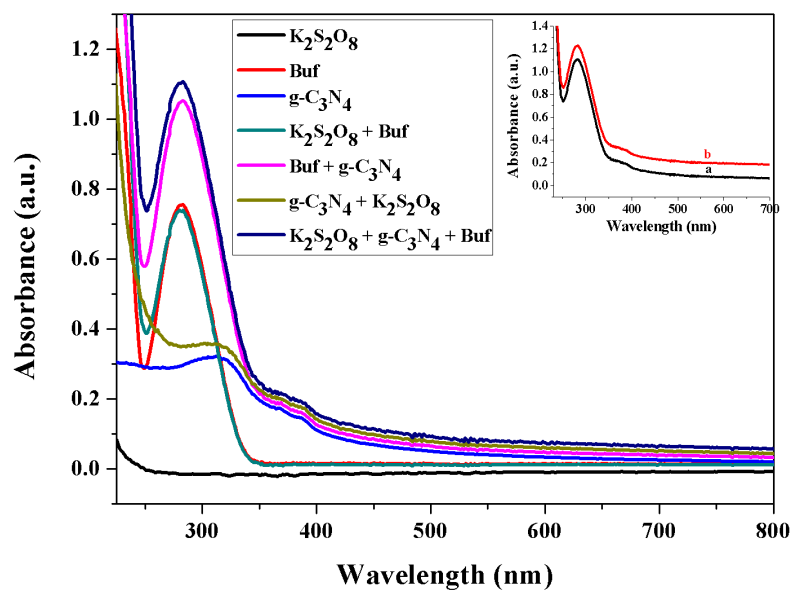


Fig. S2. UV-vis absorption spectra of g-C<sub>3</sub>N<sub>4</sub>/S<sub>2</sub>O<sub>8</sub><sup>2-</sup>/bufloxedil system.

Table S1 Comparison of different methods for the detection of bufloxedil

Detection method	Linear range (mol/L)	Detection limit (mol/L)	Ref.
Rapid HPLC	$5.8 \times 10^{-6}$ - $1.2 \times 10^{-4}$	$1.5 \times 10^{-6}$	3
Stripping voltammetry	$4 \times 10^{-9}$ - $4 \times 10^{-7}$	$1.2 \times 10^{-9}$	4
HPLC–tandem mass spectrometry	$6.9 \times 10^{-8}$ - $3.4 \times 10^{-6}$	$6.9 \times 10^{-8}$	5
Spectrophotometry	$3 \times 10^{-6}$ - $6 \times 10^{-5}$	$8 \times 10^{-7}$	6
Electrochemiluminescence	$3 \times 10^{-9}$ - $3 \times 10^{-6}$	$9.7 \times 10^{-10}$	This work

Table S2 The repeatability the g-C<sub>3</sub>N<sub>4</sub> ECL sensor to assess the interday, during five days in the presence of 0.3  $\mu\text{mol/L}$  Buf.

day	Known ( $\mu\text{mol/L}$ )	Obtained ( $\mu\text{mol/L}$ )	Average ( $\mu\text{mol/L}$ )	SD	RSD (%)	$t_{\text{calculated}}$	$t_{\text{critical}}$
day1	0.3	0.323	0.305	0.0147	4.82%	0.761	2.78
day2	0.3	0.318					
day3	0.3	0.290					
day4	0.3	0.295					
day5	0.3	0.298					

Table S3 The repeatability the g-C<sub>3</sub>N<sub>4</sub> ECL sensor to assess the intraday five times in the presence of 1.5  $\mu\text{mol/L}$  Buf.

day	Known ( $\mu\text{mol/L}$ )	Obtained ( $\mu\text{mol/L}$ )	Average ( $\mu\text{mol/L}$ )	SD	RSD (%)	$t_{\text{calculated}}$	$t_{\text{critical}}$
day1	1.5	1.507, 1.506, 1.516, 1.541, 1.475	1.509	0.024	1.59	0.839	2.78

Table S4 Determination results and recoveries of Buf in mice plasma (n=6)

Standard ( $\mu\text{mol/L}$ )	Average detected	Added	Average Founded	Relative error(%)	SD	RSD (%)	Recovery (%)	$t_{\text{calculated}}$	$t_{\text{critical}}$
0.043	0.0425 (0.0460, 0.0417, 0.0399, 0.0461, 0.0442, 0.0373)	0.020	0.0623 (0.0601, 0.0624, 0.0622, 0.0628, 0.0605, 0.0659)	1.1	0.0019	3.1	96.5	0.902	2.57
		0.040	0.0838 (0.0807, 0.0873, 0.0849, 0.0832, 0.0829, 0.0838)	0.96	0.0020	2.4	102.1	0.979	
		0.080	0.1215 (0.1162, 0.1205, 0.1278, 0.1183, 0.1251, 0.1211)	1.6	0.0043	3.5	98.13	0.854	

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