Supporting Information

A covalent organic framework/reduced graphene oxide composites-based electrochemical sensing platform for the detection of enrofloxacin

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1. Experimental section

1.1. Reagents and apparatus

Enrofloxacin Standard solution, 1,3,5-tris(4-aminophenyl) benzene (APB, 98%), p-phthalaldehyde (PDA, 98%), tetrahydrofuran (MSDS, 97%), triethylamine ($C_6H_{15}N$, ≥99.5%), reduced graphene oxide (RGO), and acetonitrile (C_2H_3N, ≥99.9%) were purchased from Aladdin industrial corporation (Shanghai, China). Ethanol (C_2H_5OH), acetic acid (CH_3COOH), trisodium citrate ($Na_3C_8H_5O_7·2H_2O$), disodium hydrogen phosphate (Na_2HPO_4), sodium chloride (NaCl), potassium chloride (KCl), potassium dihydrogen phosphate (KH_2PO_4), potassium nitrate (KNO_3), and potassium ferricyanide ($K_3Fe(CN)_6$) were obtained from China Pharmaceutical Group Chemical Reagent Co., Ltd. (Shanghai, China). Chloroauric acid ($HAuCl_4·4H_2O$) was supplied by Nanjing Chemical Reagent Co., Ltd. (Jiangsu, China). Phosphate-buffered solution (PBS, 0.1 mol/L, pH 5-9) was prepared by KH_2PO_4, Na_2HPO_4, NaCl and KCl. All solutions were prepared using ultrapure water (resistance > 18.2 MX·cm).

Electrochemical measurements were performed using a CHI 660E electrochemical workstation (Chenhua Instrument Co., Ltd., Shanghai, China). All electrodes and polishing materials were also procured from the same supplier. A conventional three-electrode system was employed, consisting of a TAPB-PDA-COFs/RGO/GCE as the working electrode, an Ag/AgCl reference electrode (saturated KCl, 3.0 mol/L), and a platinum wire as the counter electrode.

1.2. Synthesis of TAPB-PDA-COFs

TAPB-PDA covalent organic frameworks (COFs) were synthesized according to a previously reported method[1]. Briefly, PDA (0.05 g) and TAPB (0.095 g) were dissolved in 50 mL of DMSO, followed by ultrasonication for 5 min to obtain a homogeneously dispersed solution. Subsequently, 1.8 mL of acetic acid was slowly added under continuous ultrasonication, and the mixture was allowed to stand for 10 min. The reaction solution was then sealed and incubated at ambient temperature for 30 min. The resulting TAPB-PDA-COFs were collected by centrifugation at 10,000 rpm for 10 min and washed three times each with tetrahydrofuran and methanol. The

purified COFs were dried at 55 °C for 6 h, ground into a fine powder, and stored under dry conditions at room temperature until further use.

1.3. Characterization

The morphological characteristics of TAPB-PDA-COFs, RGO, and TAPB-PDA-COFs/RGO were observed and analyzed using a US FEI Titan G260-300 transmission electron microscope (TEM). Crystallographic structures of TAPB-PDA-COFs, GO, RGO, and TAPB-PDA-COFs/RGO were examined by powder X-ray diffraction (XRD) using a Bruker D8 Advance diffractometer (Germany). Scanning was performed over a 2θ range of 5-90° at a rate of 10°/min[1] to determine the crystalline nature of the materials and to assess whether the composite retained the structural features of its individual components. Fourier transform infrared (FTIR) spectroscopy was conducted using a Bruker Vertex 70 spectrometer (Germany) to identify functional groups in TAPB, PDA, TAPB-PDA-COFs, GO, RGO, and TAPB-PDA-COFs/RGO, thereby providing insight into chemical bonding and framework formation. X-ray photoelectron spectroscopy (XPS) was carried out using a Thermo Fisher ESCALAB Xi+ system (USA) to analyze the elemental composition and chemical states of TAPB-PDA-COFs, RGO, and TAPB-PDA-COFs/RGO. Full-spectrum and high-resolution scans were used to detect the presence of new elements and investigate changes in binding energies and valence states, elucidating the chemical interactions involved in composite formation. Raman spectroscopy was performed with a HORIBA LabRAM HR Evolution spectrometer (France) to analyze GO, RGO, and TAPB-PDA-COFs/RGO. The D and G bands were evaluated to assess structural defects, graphitization degree, and disorder within the carbon lattice, thereby offering insights into structural modifications induced by composite synthesis. Nitrogen adsorption-desorption measurements were conducted using a Quantachrome Quadrasorb SI-3MP surface area and porosity analyzer (BET method) to evaluate the specific surface area and pore structure of TAPB-PDA-COFs, RGO, and TAPB-PDA-COFs/RGO. These measurements were used to determine whether the composite exhibited enhanced porosity and surface area relative to its monomeric constituents,

contributing to improved electrochemical performance. Thermal stability was assessed using a TA Instruments TGA55 thermogravimetric analyzer (TG). Samples of TAPB-PDA-COFs, RGO, and TAPB-PDA-COFs/RGO were heated at a rate of 10 °C/min under a nitrogen atmosphere, and the resulting thermal decomposition profiles were analyzed to evaluate the composite's thermal resistance compared to the individual materials.

2. Figure S1-S4

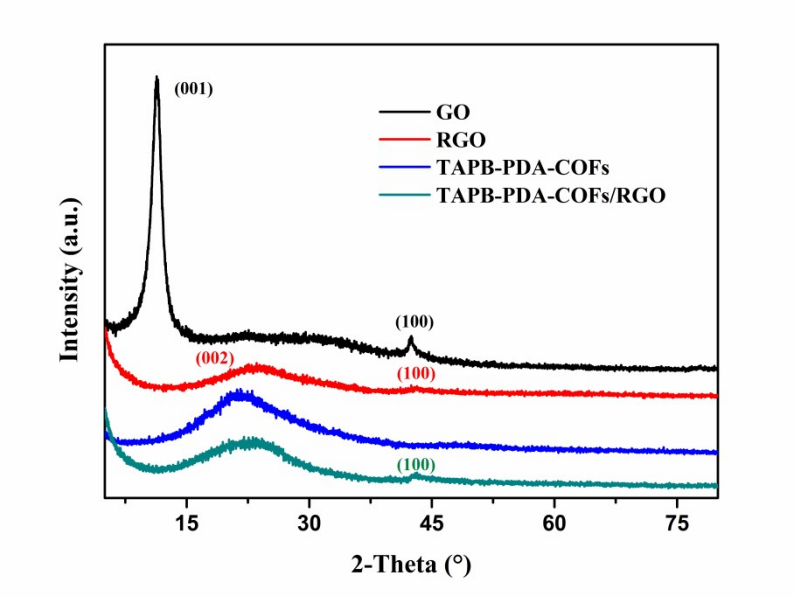

Fig. S1 XRD of GO, RGO, TAPB-PDA-COFs and TAPB-PDA-COFs/RGO composite.

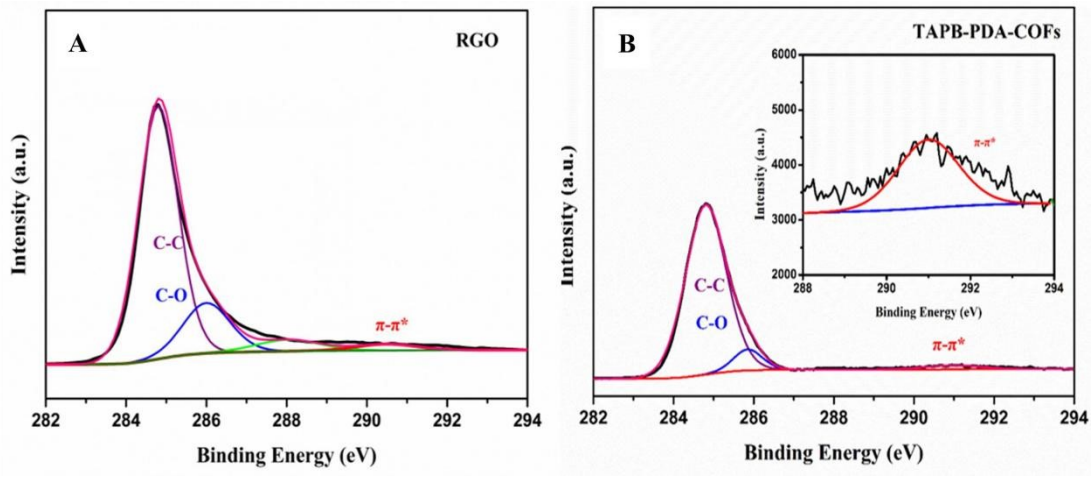

Fig. S2 C 1s XPS analysis of (A) RGO, and (B) TAPB-PDA-COFs.

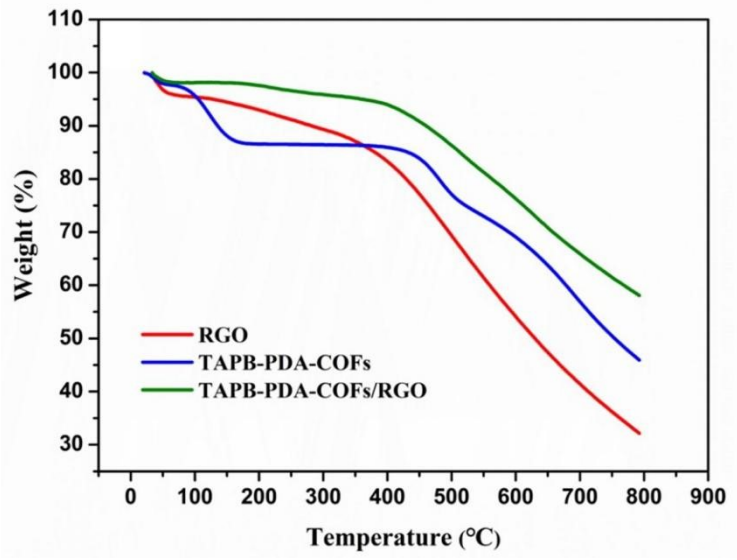

Fig. S3 TGA plots of TAPB-PDA-COFs, RGO and TAPB-PDA-COFs/RGO.

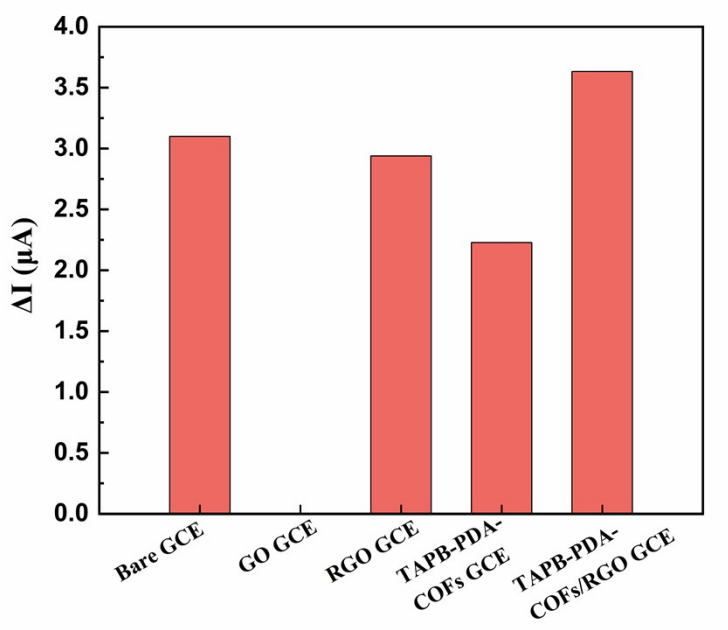

Fig. S4 Current response of different modified electrode with ENR.

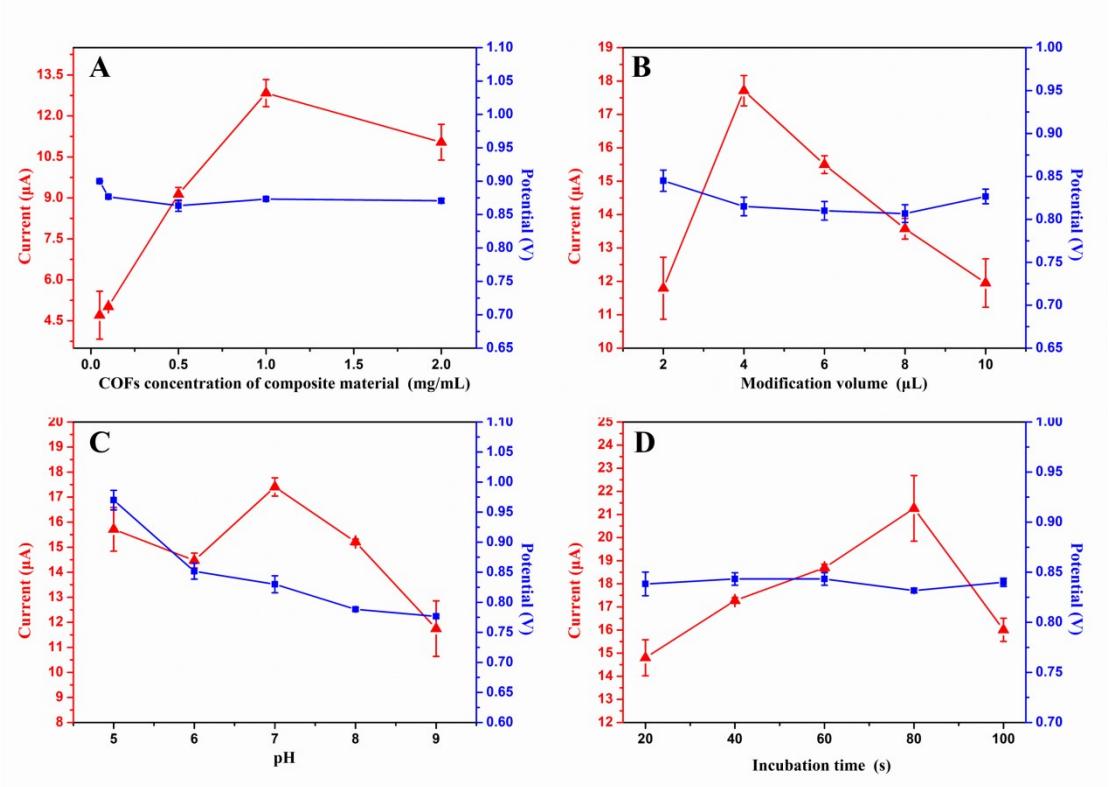

Fig. S5 Optimization of reaction conditions for the TAPB-PDA-COFs/RGO-based electrochemical sensing platform. (A) Concentration of TAPB-PDA-COFs solution, (B) Volume of TAPB-PDA-COFs/RGO composite modification, (C) pH of buffer solution, (D) Incubation time of TAPB-PDA-COFs/RGO/GCE.

3. Table S1-S3

Table S1 Box Behnken design for variables and levels.

Independent variables	Coded values	Range and level		
		-1	0	1
The modified volume of materials (μL)	X_1	2.0	4.0	6.0
pH	X_2	6.0	7.0	8.0
Incubation time (s)	X_3	60	80	100

Table S2 The experimental design and results for response surface analysis.

Run	A: Composite modified volume (μL)	B: pH	C: Incubation time (s)	i_p (μA)
1	0	1	1	14.85
2	-1	1	0	14.13
3	0	0	0	19.66
4	0	0	0	21.38
5	1	0	1	16.99
6	1	0	-1	18.87
7	-1	0	-1	15.31
8	0	1	-1	15.29
9	-1	-1	0	14.28
10	1	1	0	14.59
11	0	-1	-1	14.23
12	1	-1	0	13.39
13	-1	0	1	17.94
14	0	-1	1	12.70
15	0	0	0	19.88
16	0	0	0	20.74
17	0	0	0	20.57

Table S3 ANOVA of regression model.

Source	Sum of squares	Degree of freedom	Mean square	*F* Value	*P* Value	
Model	131.10	9	12.57	22.28	0.0002	significant
A	0.59	1	0.59	0.91	0.3722	
B	2.27	1	2.27	3.47	0.1048	
C	0.19	1	0.19	0.28	0.6102	
AB	0.46	1	0.46	0.70	0.4313	
AV	5.09	1	5.09	7.78	0.0269	
BC	0.30	1	0.30	0.45	0.5219	
A^2	11.73	1	11.73	17.95	0.0039	
B^2	92.19	1	92.19	141.04	＜0.0001	
C^2	9.46	1	9.46	14.48	0.0067	
Residual	4.58	7	0.65			
Lack of Fit	2.66	3	0.89	1.86	0.2775	not significant
Pure Error	1.91	4	0.48			
Cor Total	135.67	16				

References

1. Lu S, Wang S, Wu P, Wang D, Yi J, Li L, Ding P, Pan H. A composite prepared from covalent organic framework and gold nanoparticles for the electrochemical determination of enrofloxacin. Adv. Powder Technol. 2021; 32 (6): 2106-2115.