Supporting Information

Ferrocene-functionalized COF-derived Fe single-atom dispersed N-doped porous carbon for enhanced electrochemical sensing of chloramphenicol

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1. Experimental section

1.1 Materials and reagents

Terephthalaldehyde (TPAL, 98%), tetrakis(4-aminophenyl)ethene (ETTA, 97%), 1,1'ferrocenedicarboxylic Acid (Fc(COOH)₂, 98%), chloramphenicol (CAP), gentamicin (GM), penicillin (PG), and kanamycin (Kana) were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. 1,4-dioxane (C₄H₈O₂, > 99%), N,N-dimethylformamide (C₃H₇NO, 99.5%), tetrahydrofuran (C₄H₈O, > 99%), and acetonitrile (C₂H₃N, > 99%) were purchased from Tianjin Damao Chemical Reagent Co., Ltd.

1.2 Apparatus

Transmission electron microscopy (TEM, JEM-2100, JEOL), scanning electron microscopy (SEM, S-4800, Hitachi), high-angle annular dark-field scanning transmission electron microscopy (HAADF-STEM, ARM200F, JEOL), powder X-ray diffraction (XRD, XRDynamic500, Anton Paar), Raman spectroscopy (LabRAM HR Evolution, Horiba), X-ray photoelectron spectroscopy (XPS, K-Alpha, Thermo Fisher), Fourier transform infrared spectrum (FT-IR, Nicolet iS20, Thermo Fisher), and automatic surface area and porosity analyzer (BET, ASAP 2460, Micromeritics), were employed to characterize the prepared nanomaterials. Electrochemical experiments were conducted using a CHI660D electrochemical workstation (Shanghai CH Instrument Co., Ltd., China) with a conventional three-electrode configuration, consisting of a 3-mm diameter glassy carbon working electrode (GCE), a platinum wire counter electrode, and an Ag/AgCl reference electrode.

1.3 Preparation of Fc-COF

30.0 mg of ETTA, 22.0 mg of Fc(COOH)₂ and 20.5 mg of TPAL were weighed into 5 mL

of acetonitrile and sonicated for 30 min to obtain homogeneous solutions. Then 0.4 mL of 6 M acetic acid was added and dispersed by sonication. After uniform dispersion, the mixed solution was transferred to a reaction kettle and heated for 96 h at 120 °C. After heating, the solid was collected by centrifugation, washed several times with N,N-dimethylformamide and tetrahydrofuran, soaked in 1:1 tetrahydrofuran and dichloroethane for 12 h, and dried under vacuum overnight to give a brown Fc-COF.

The synthesis of COF closely resembled that of Fc-COF. Nevertheless, a key distinction was that $Fc(COOH)_2$ was not employed as a reactant during the COF synthesis process.

1.4 Preparation of FeSA-NC

To prepare FeSA-NC, the Fc-COF was placed in a tube furnace, sealed and evacuated, after which the Ar gas was passed through to keep the Fc-COF in an Ar atmosphere. Then, the tube furnace was heated to 900 °C with a heating rate of 10 °C min⁻¹. After continuously calcining at 900 °C for 2 h and cooling to room temperature, the FeNP-NC powder was obtained. Afterwards, FeSA-NC was obtained by placing FeNP-NC in 1 M sulfuric acid, stirring at 300 rpm for 10 h at 80 °C, and washing with water and ethanol three times, respectively. As a control, NC was produced by pyrolysis of COF.

1.5 Construction of modified electrodes

Before modification, the bare GCE working electrode was successively polished with 1.0, 0.3 and 0.05 μ m α -Al₂O₃ abrasive powders, and then ultrasonically treated with ethanol and ultrapure water respectively. For the catalytic material dispersion, 1 mg of FeSA-NC was weighed and sonicated with 1 mL of deionized water for 30 min to form a sufficiently dispersed suspension to prepare the catalyst ink. Subsequently, 6 μ L of FeSA-NC material dispersion

was uniformly drip-coated on the electrode surface by the drop coating method and allowed to dry naturally to obtain FeSA-NC/GCE. As a control, Fc-COF/GCE, NC/GCE and FeNP-NC/GCE were produced by the same method.

1.6 Detection of CAP in real samples

The milk sample was centrifuged at 10,000 rpm for 10 min to isolate the precipitate, and the resulting supernatant was filtered using a sterile Millipore membrane for subsequent use. For other samples, such as honey and tap water, a 10-fold dilution was performed using 0.1 M PBS (pH 7.0), followed by centrifugation at 10,000 rpm for 10 min to collect the supernatant. The final sample preparation involved diluting the samples appropriately with 0.1 M PBS and spiking them with known concentrations of CAP.

2. Results and discussion



2.1 Fourier transform infrared (FT-IR) spectroscopy of Fc-COF

Fig. S1 FT-IR spectra of Fc-COF, ETTA, TPAL, and Fc(COOH)₂.

As presented in the FT-IR spectrum of Fc-COF (Fig. S1), a characteristic peak of C=N appeared at 1606 cm⁻¹, while the N-H peaks from ETTA at 3356 and 3423 cm⁻¹ and the C=O peak from TPAL at 1687 cm⁻¹ disappeared, indicating the successful amine-aldehyde

condensation reaction between ETTA and TPAL. Moreover, the characteristic vibration peaks of ferrocene (1030 and 828 cm⁻¹ attributed to the cyclopentadiene ring)¹ can be observed in the Fc-COF spectrum, demonstrating the successful preparation of Fc-COF.



2.2 SEM image of Fc-COF and FeSA-NC

Fig. S2 SEM images of (A) Fc-COF and (B) FeSA-NC.

2.3 HAADF-STEM image of FeSA-NC



Fig. S3 HAADF-STEM image of FeSA-NC.



2.4 N₂ adsorption-desorption isotherm and pore size distribution of FeSA-NC

Fig. S4. (A) N₂ adsorption-desorption isotherm and (B) pore size distribution of FeSA-NC.



2.5 X-ray photoelectron spectroscopy analysis of FeNP-NC and FeSA-NC

Fig. S5 (A) Full XPS spectrum, (B) C 1s spectrum, (C) N 1s spectrum, and (D) Fe 2p spectrum of FeNP-NC and FeSA-NC.

2.6 Randles-Sevcik equation²

$$I_{\rm P} = 2.69 \times 10^5 n^{3/2} A D^{1/2} c v^{1/2}$$

In this equation, $I_{\rm P}$ represents the peak current, *n* is the number of electrons involved in the reaction, *A* is the effective surface area of the modified electrode (cm²), *D* is the diffusion coefficient of the [Fe(CN)₆]³⁻ solute (6.70 × 10⁻⁶ cm² s⁻¹), *c* is the concentration of the [Fe(CN)₆]³⁻ solution (5 × 10⁻⁶ mol cm⁻³), and *v* is the scan rate of the CV curve (V s⁻¹).

2.7 EIS diagrams of different catalysts

The EIS data were simulated with an equivalent circuit model by using ZSimpWin software. As shown in Fig. S6, the electron-transfer resistance (Rct) value of the bare GCE was simulated to be approximately 97.4 Ω . After modifying the GCE with Fc-COF, the Rct

increased to 119.9 Ω , mainly because of the intrinsic low conductivity of Fc-COF. While after carbonization of Fc-COF, a distinctly decreased Rct value was observed for FeNP-NC/GCE (47.88 Ω), indicating that the FeNP-NC possessed excellent electrical conductivity. Compared with the FeNP-NC/GCE, the Rct of FeSA-NC/GCE further decreased to 10.94 Ω , suggesting that the FeSA-NC exhibited even more excellent electrical conductivity, which may be attributed to the synergistic effects of the NC framework, Fe single-atom active sites, and the large surface area.



Fig. S6 EIS diagrams of different catalysts in 5.0 mM [Fe(CN)₆]^{3-/4-} solution containing 0.1 M KCl.

2.8 DFT calculation

Spin-polarized DFT calculations were carried out using Vienna ab initio simulation package with a cutoff energy of 400 eV.^{3,4} All calculations used the Perdew-Burke-Ernzerhof functional⁵ and projector augmented wave method^{6,7} to describe the ions-electrons interaction. The DFT-D3 method was chose to correct the systems with long-range van der Waals interactions.⁸ Monkhorst-Pack scheme k-point grids of 3×3×1 was used to sample the Brillouin zones. For geometry optimization, the tolerances of total energy and force were set to 10⁻⁵ eV and 0.02 eV/Å, respectively. The GGA+U method considering coulomb repulsion and exchange interactions was used to describe the strongly 3d-electrons correlation effect in Fe atoms, with U= 4.0 eV and J=1.0 eV employed.^{9,10}

The species were adsorbed on FeSA-NC and FeNP-NC surfaces, respectively. To avoid the falsification by periodic boundaries, a vacuum region of 20 Å was selected to separate surface slab. The FeNP-NC model was constructed in a 3×3 super cell with 54 atoms.

In this study, the Gibbs free energy is defined as:¹¹

$$G = E_{DFT} + E_{ZPE} - TS$$

where E_{DFT} represents DFT total energy, E_{ZPE} denotes the zero-point energy, and S is the entropies of a adsorbate. The temperature (T) was set to 298.15 K for Gibbs free energy and entropies calculations, which was the typical experiment condition.



Fig. S7 FeSA-NC and FeNP-NC respectively undergo reduction procedures with CAP to CAP-NHOH.

2.9 Optimization of conditions

As shown in Fig. S8 and Fig. 2F, the FeSA-NC/GCE exhibited the best current response for CAP when the FeSA-NC drop-coating amount was 6 μ L, PBS was used as the electrolyte buffer solution, and the pH was 7.0.



Fig. S8 The influence of (A) the drop coating amount of FeSA-NC (1.0 mg mL⁻¹) dispersion solution and (B) the different supporting electrolytes on the peak current of CAP R1 reduction.

2.10 Comparison of different CAP electrochemical sensors

Electrodo motorials	Method	Linear range	LOD	Sensitivity	Ref.
		(µM)	(µM)	$(\mu A \ \mu M^{-1} \ cm^{-2})$	
AuNPs/GO	Amperometry	1.5-2.95	0.25	3.81	12
MoS ₂ /PANI	DPV	0.1-100	0.069	_	13
β-CD/CMK-3@PDA	SWV	0.5-500	0.2	_	14
3D CNTs@Cu NPs	CV	10-500	10	_	15
MoS ₂ -MWCNTs	DPV	1-35	0.4	2.588	16
NiCo ₂ O ₄ /C	DPV	0.5-320	0.035	0.46	17
DUT-4@rGO	SWV	1-1000	0.0769	0.7295	18
MnO ₂ /ErGO	LSV	1-20	0.58	4.750	19
SrTiO ₃ /GO	LSV	10-550	6.08	2.77	20
FeSA-NC	SWV	0.5-800	0.015	13.51	This work

Table S1 Comparison of the performance of different CAP electrochemical sensors.



2.11 Selectivity, reproducibility and stability

Fig. S9 (A) Selectivity, (B) Reproducibility, and (C) Stability of the CAP electrochemical sensor.

2.12 Analysis of CAP in real samples

Sample	Added	Found	Recovery (%)	RSD (%)
Milk	40.0 μΜ	38.62 μM	96.9	1.9
	50.0 µM	47.41 μΜ	96.6	0.9
	80.0 μΜ	81.28 μΜ	101.5	1.1
Honey	40.0 μΜ	41.10 μΜ	102.5	2.3
	50.0 µM	48.59 μM	98.8	2.4
	80.0 μΜ	80.22 μM	100.3	0.8
Tap water	40.0 μΜ	40.30 µM	100.7	3.6
	50.0 µM	48.67 μM	99.0	1.5
	80.0 µM	79.65 μM	99.6	0.4

Table S2 Determination of CAP in milk, honey and tap water samples.

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