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An ultrasensitive high-performance baicalin sensor based on C₃N₄-SWCNTs/reduced graphene oxide/cyclodextrin metal-organic framework nanocomposite

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ABSTRACT
Baicalin (Bn) obtained from natural plants has been found to exhibit significant antiviral activity against Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Herein, a novel ultrasensitive Bn electrochemical sensor was proposed based on graphitized carbon-nitride - single-walled carbon nanotube nanocomposites (C₃N₄-SWCNTs), reduced graphene oxide (rGO) and electrodeposited cyclodextrin-metal organic framework (CD-MOF). The sensing nanomaterials were characterized by X-ray diffraction spectroscopy, X-ray photoelectron spectroscopy, Raman spectroscopy, scanning electron microscopy, and transmission electron microscopy. Under optimal conditions, the sensor exhibited sensitive detection of Bn in a wide linear range of 1 × 10⁻⁹–5 × 10⁻⁷ M with an LOD of 4.6 × 10⁻¹⁰ M and a sensitivity of 220 A/M, and it showed satisfactory stability and accuracy for detecting Bn in real samples (human serum and bear bile scutellaria eye drops). In addition, the electrochemical reaction sites and redox mechanism of Bn were revealed through electrochemical behavior and density functional theory. This work provided an insightful solution for detecting Bn, and extensive potential applications could be further expected.

1. Introduction

Flavonoids, a class of polyphenolic compounds that can reduce the risk of cardiovascular disease and cancer, have aroused great interest of researchers. Among these, baicalin (Bn) is an important biologically active flavonoid extracted from Scutellaria baicalensis Georgi, which exhibits versatile physiological activities such as anti-inflammatory, antioxidant, anti-cancer, anti-microbial and anti-fungal activities. Therefore, as a medicine, Bn has been widely used to treat diphtheria, viral hepatitis, nephritis, upper respiratory tract infections, dysentery, scarlet fever, and prevent cerebrovascular dysfunction. Notably, recent researches have shown that Bn exhibits excellent antiviral activity against Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), which provides a great opportunity for the development of much-needed drugs for treating Coronavirus Disease 2019 (COVID-19). However, the amount of Bn must be strictly controlled, otherwise excessive Bn will cause some adverse physiological reactions such as muscle soreness, low-grade fever, diarrhea, and leukopenia. Therefore, it is necessary and urgent to develop accurate, sensitive and innovative methods for detecting Bn. Currently, the quantitative detection of Bn mainly relies on traditional methods such as HPIC and LC-MS, but these methods have many disadvantages including high cost, complex operation and cumbersome pre-treatment processes. Electrochemical techniques have
become an attractive method due to their low cost and high sensitivity [12]. Moreover, the sensitivity of the electrochemical sensor can be greatly improved by synthesizing and using different new materials to modify the electrode surface. Therefore, a constructed ultrasensitive Bn electrochemical sensor has good application prospects.

Single-walled carbon nanotubes (SWCNTs), an excellent charge conductor and charge semiconductor, have the advantages of good mechanical strength, good chemical stability, and large specific surface area [13,14]. Graphene has high electronic conductivity, excellent electrocatalytic activity, and a wide range of applications [15,16]. Similarly, graphite carbon nitride (C$_3$N$_4$) is a soft material with a two-dimensional graphene-like structure, and it is considered as a promising material due to its simple synthesis, low cost, abundant nitrogen content, and many active sites [17]. Due to their great performance, these carbon nanomaterials have been attracted the attention of researchers in the field of electrochemical sensors [18,19]. For example, Duoc et al. [20] have used double-walled carbon nanotubes and graphene hybrid films (DWCNTs-Gr), and cholesterol oxidase (ChOx) to construct sensitive arsenic (V) electrochemical sensor. Alizadeh et al. [21] constructed a citric acid sensor based on g-C$_3$N$_4$, Fe$_3$O$_4$ and BiOI. Thus, it is possible to use these carbon nanocomposites to enhance the electrochemical and electrocatalytic properties of sensors.

Cyclodextrin-metal-organic framework (CD-MOF) based on alkali metal ions and cyclodextrin (CD) has been reported as a new type of green MOF [22]. Because of the unique adsorption and encapsulation abilities of CD-MOF, it has usually been used as a carrier and adsorbent [23]. For instance, Liu et al. [24] used β-cyclodextrin MOF as precursor to synthesize porous carbon for the adsorption of herbicides. Kritkii et al. [25] used γ-cyclodextrin-metal organic framework for encapsulation of leflunomide. However, the application of CD-MOF in the field of electrochemical sensors has been rarely reported. Therefore, it is novel to improve the sensitivity and detection capability of sensors by the powerful enrichment performance of CD-MOF.

Herein, a C$_3$N$_4$-SWCNTs/rGO/CD-MOF composite has been developed on bare glass carbon electrode (GCE) as novel high-sensitivity sensor for Bn (Fig. 1). First, the cavity of CD-MOF can adsorb a large amount of Bn, thus increasing the concentration of Bn on the sensor surface to amplify the signal. Next, the active site on C$_3$N$_4$ can accelerate the rapid redox of Bn on the electrode surface. However, the strong bonding between SWCNTs can greatly affect the high charge mobility and stability of C$_3$N$_4$-SWCNTs. After the addition of rGO, the strong π-π interaction between rGO and SWCNTs provides good dispersion and stability of the nanocomposites. In addition, rGO can be loaded with various nanomaterials at high capacity to improve the efficiency of the sensing materials and thus the sensitivity of the sensors [26]. Thus, the sensor shows excellent detection performance for Bn with the help of synergistic effects of these materials and can be applied to the analysis in actual samples.

2. Material and methods

2.1. Reagents and apparatus

Melamine (C$_6$H$_6$N$_6$) and short single-walled carbon nanotubes (SWCNTs) were purchased from Tianjin Guangfu Fine Chemical Research Institute and Chengdu Organic Chemistry Co., Ltd., Chinese Academy of Sciences. β-cyclodextrin, baicalin, and Na$_2$CO$_3$ were obtained from Aladdin. The phosphate buffered saline (PBS) was prepared with NaCl, H$_3$PO$_4$, NaH$_2$PO$_4$, and Na$_2$HPO$_4$.

CHI 660D was used to study the electrochemical behavior of the sensors. JEM-2100 transmission electron microscope, Bruker D8 Advance X-ray diffractometer, JEOL JSM-6610LV scanning electron microscope, Thermo ESCALAB 250Xi X-ray photoelectron spectrometer, Thermo Nicolet 6700 FT-IR spectrometer, and Renishaw inVia Raman microscopes were used to characterize the materials.

2.2. Preparation of C$_3$N$_4$-SWCNTs

First, a 50 mL crucible containing 5 g of melamine was placed in a tube furnace. The tube furnace was heated to 550 °C at a rate of 10 °C min$^{-1}$ and held for 2 h. After cooling, pale yellow solid was collected and ground into a powder to obtain g-C$_3$N$_4$.

Next, 100 mg of SWCNTs and 50 mg of g-C$_3$N$_4$ were added to 80 mL of mixed acid (V(H$_2$SO$_4$):V(HNO$_3$) = 3: 1) and ultrasonically dispersed for 1 h. The dispersion was refluxed at 60 °C for 2 h and cooled to room temperature. Next, the dispersion was diluted with deionized water and filtered using a polytetrafluoroethylene (PTFE) microfiltration membrane (220 nm), and this process was repeated three times. C$_3$N$_4$-SWCNTs was collected after vacuum drying at 60 °C.

2.3. Preparation of reduced graphene oxide

Graphite oxide (GO) was synthesized by a modified Hummers method [27]. GO was dispersed in H$_2$O by ultrasound for 1 h. Then NaBH$_4$ was added into the suspension and kept stirring for 24 h at 80 °C. A black solid was obtained after filtration and washing. The reduced
graphite oxide (rGO) was collected after vacuum drying at 60 °C.

2.4. Preparation of CD-MOF

CD-MOF was prepared by referring to the previous literature [24, 28], 1.0 equiv of β-CD and 8.0 equiv of Na2O2 were dissolved in H2O and filtered. Methanol was slowly diffused into the filtrate within 14 days. The colorless crystals were collected and washed three times with methanol. CD-MOF was obtained after vacuum drying at 35 °C. In addition, γ-CD-MOF was synthesized under the same conditions as β-CD-MOF, only replacing β-CD with γ-CD.

2.5. Preparation of modified electrodes

Dispersions of CβN4-SWCNTs (N, N-dimethylformamide as dispersant, 1 mg mL⁻¹), rGO (H₂O as dispersant, 1 mg mL⁻¹), and CD-MOF (PBS as dispersant, 2 mg mL⁻¹) were prepared and sonicated for 1 h. Then, 6 μL CβN4-SWCNTs suspension and 4 μL rGO suspension were mixed and sonicated for 30 min. Next, the mixed suspension was dropped on a clean glassy carbon electrode (GCE, Diameter = 3 mm) and dried under an infrared lamp to obtain CβN4-SWCNTs/rGO/GCE. CD-MOF was electrodeposited on CβN4-SWCNTs/GCE, CβN4-SWCNTs/rGO/GCE, CβN4-SWCNTs/GCE, and CβN4-SWCNTs/rGO/γ-CD-MOF/GCE were prepared in similar method.

3. Results and discussion

3.1. Materials characterization

The X-ray diffraction (XRD) patterns of CβN4 and CβN4-SWCNTs are shown in Fig. S2. CβN4 exhibits two distinct diffraction peaks at 13.1° and 27.5°, corresponding to the stacking of in-planner repeating layers and the stacking of conjugated aromatic systems (JCPDS 87-1526) [29]. Two characteristic peaks can be observed at 43.2° and 26.0° in the XRD pattern of CβN4-SWCNTs, corresponding to the (100) and (002) diffraction planes of SWCNTs, which indicate that SWCNTs have been loaded on the CβN4 [30].

The X-ray photoelectron spectroscopy (XPS) spectra of CβN4, CβN4-SWCNTs and CβN4-SWCNTs/rGO have been obtained (Fig. 2 and S3). The contents of C and O in CβN4-SWCNTs are significantly higher than those in CβN4, and Na in CβN4-SWCNTs/rGO is attributed to NaBH₄ in rGO, which proves that CβN4 has been bound to SWCNTs and rGO (Fig. 2A and S3A). The peaks of CβN4 at 284.8 eV and 288.4 eV correspond to the C–C/C–O bond and N–C–N bond [31], and the intensity of the C–C/C–C bond is greatly enhanced after binding to SWCNTs and rGO (Fig. 2B and S3B). In N 1s spectra, the intensity of the N–(C₃) bond and H–N–C bond of CβN4-SWCNTs/rGO is higher than that of CβN4 due to the covalent bonding of CβN4 with SWCNTs and rGO. (Fig. 2C and S3C) [32]. In the O 1s spectrum (Fig. 2D and S3D) of CβN4, the binding energy peak at 532.4 eV mainly comes from the adsorbed water molecules in the CβN4 sample, and the intensity of the peak is low [33]. The O 1s binding energy peaks of CβN4-SWCNTs and CβN4-SWCNTs/rGO at

![Fig. 2. (A) XPS survey spectra of CβN4 and CβN4-SWCNTs. High-resolution XPS spectra of (B) C 1s, (C) N 1s, (D) O 1s of CβN4 and CβN4-SWCNTs.](image-url)
532.1 eV and 533.5 eV are mainly attributed to the C=O bond and C–O bond [34,35]. Compared with C$_3$N$_4$, the intensity of the C=O/C–O peaks of C$_3$N$_4$-SWCNTs is so high that the peaks corresponding to the adsorbed water molecules could hardly be found (Fig. 2A). The XPS results indicate C$_3$N$_4$ has been synthesized and successfully covalently bonded to SWCNTs and rGO.

The Raman spectra of C$_3$N$_4$, C$_3$N$_4$-SWCNTs, rGO and C$_3$N$_4$-SWCNTs/rGO are shown in Fig. S4A. It can be seen that C$_3$N$_4$ has a wide band at ~1687 cm$^{-1}$, which is attributed to conjugate vibration of carbon and nitrogen in C$_3$N$_4$ [36]. The Raman spectrum of pure SWCNTs shows two typical bands, D band and G band at 1349 cm$^{-1}$ and 1539 cm$^{-1}$. Fig. S4B shows the radial breathing mode (RBM) Raman scattering of SWCNTs, which can be used to estimate diameter of nanotube. The diameter of SWCNTs can be estimated according to the following analytical expression [37]:

$$\omega_{\text{RBM}} = \frac{A}{d_t} + B$$

where $\omega_{\text{RBM}}$ and $d_t$ are the Raman frequency of RBM and the diameter of SWCNTs, respectively. A and B are coefficients related to the substrate (in this experiment, A and B are 234 nm cm$^{-1}$ and 10 cm$^{-1}$) [38]. By calculation, the diameters of SWCNTs are mainly 1.56 nm and 0.91 nm. The intensity ratio $I_D/I_G$ of SWCNTs and C$_3$N$_4$-SWCNTs are almost identical, indicating that the bound C$_3$N$_4$ has no effect on the structure of SWCNTs [39]. Moreover, the $I_D/I_G$ of C$_3$N$_4$-SWCNTs/rGO is significantly lower than that of rGO, which proves that C$_3$N$_4$-SWCNTs/rGO has a high graphitization degree and fewer defects.

The FT-IR spectrum of C$_3$N$_4$ shows several strong bands at 1200–1700 cm$^{-1}$, which is a typical stretching mode of C–N heterocycles (Fig. S5A) [40]. Peaks of 810 and 896 cm$^{-1}$ are attributed to characteristics of the typical breathing pattern of the s-triazine ring and the deformation mode of N–H, respectively [41]. The peak at 3000–3350 cm$^{-1}$ belongs to amino groups and physically adsorbed water molecules [42]. For the FT-IR spectrum of C$_3$N$_4$-SWCNTs, there is a broader peak at 3000–3600 cm$^{-1}$ than C$_3$N$_4$, which is due to stretching vibration of more -OH [43]. In addition, the tensile vibration of C=C /
C=O and C–O (at 1500–1700 cm⁻¹ and 1005 cm⁻¹), CH stretching vibration (at 2953 / 2893 cm⁻¹), bending vibration of C–H (at 854 cm⁻¹) can also be observed [44]. On the other hand, a typical vibrational band of β-cyclodextrin can be found in the FT-IR spectrum (Fig. S5B). The vibration bands at 1024 (C–O / C–C stretching / C–H bending vibrations), 1646 (C=C stretching vibrations), 2924 (CH₂ stretching vibrations), and 3302 cm⁻¹ (OH stretching vibrations) can be observed [45]. In addition, the -OH peak intensity of CD-MOF is significantly lower than that of pure CD, which is due to the coordination effect of -OH. This result is consistent with previous reports, indicating that CD-MOF has been successfully synthesized [22,24].

3.2. Micromorphology of materials

The surface micromorphology of materials was obtained by scanning electron microscope (SEM) and transmission electron microscope (TEM) (Fig. 3). C₃N₄ and SWCNTs show blocky and nanofibrous tubular structures, respectively (Fig. 3a, b). The image of C₃N₄-SWCNTs displays that there are carbon nanotubes around C₃N₄, which proves that C₃N₄ and SWCNTs are well combined (Fig. 3c, d). In addition, rGO has a layered and wrinkled structure, which is its typical feature (Fig. 3e). The CD-MOF is arranged in an orderly manner and has a long block shape (Fig. 3f). Fig. 3g shows that rGO has successfully encapsulated C₃N₄-SWCNTs, and lamellar, massive, and fibrous morphologies can be observed. After electrodeposition of CD-MOF on C₃N₄-SWCNTs/rGO, many blocky particles are found on the surface, indicating successful and effective deposition (Fig. 3h).

3.3. Electrochemical behavior of electrodes

Electrochemical impedance spectroscopy (EIS) and the classical electrochemical model (Rs(Rct-Zw)-CPE) have been used to study the electron transfer resistance (Rct) of electrodes. As can be seen from Fig. 4, bare GCE (Rct = 550 Ω) and C₃N₄/GCE (Rct = 1072 Ω) have poor electrochemical performance. Conversely, the Nyquist plots of SWCNTs/GCE and C₃N₄-SWCNTs/GCE show almost no semicircle on the high-frequency region, which indicates that the electron transfer of the probe is extremely fast on these electrodes. In addition, compared with pure β-CD (Rct = 649.9 Ω), the Rct of CD-MOF (Rct = 1338 Ω) is significantly enhanced, which indicates that CD-MOF successfully formed rigid skeleton structure and reduced electron transfer performance. Due to the poor conductivity of CD-MOF, the electron transfer performance of the C₃N₄-SWCNTs/rGO/CD-MOF/GCE (Rct = 70.94 Ω) is slightly reduced compared to C₃N₄-SWCNTs/GCE. This result can also confirm that CD-MOF has been deposited on C₃N₄-SWCNTs/rGO/GCE.

Fig. S6 shows CV curves of various electrodes in K₃[Fe(CN)₆]. Peak current observed on the C₃N₄-SWCNTs/GCE is higher than currents of CD-MOF/GCE and C₃N₄/GCE, indicating that C₃N₄-SWCNTs demonstrates a better electrocatalytic and sensing activity. In addition, the peak current observed on the C₃N₄-SWCNTs/rGO/CD-MOF/GCE is slightly smaller than that observed on the C₃N₄-SWCNTs/GCE and C₃N₄-SWCNTs/rGO/GCE, which means that CD-MOF modified by electrodeposition dose not significantly reduce the electrochemical performance.

Further, the electroactive surface area (Aeff) of the different modified electrodes has been calculated (the calculation procedure has been recorded in Supplementary Material) and the results show that C₃N₄-SWCNTs/rGO/CD-MOF/GCE have the largest electroactive surface area (Fig. S7).

3.4. Electrochemical behavior of Bn on different electrodes

To study electrocatalytic of different modified electrodes on Bn, CV curves and differential pulse voltammetry (DPV) curves in PBS have been recorded (Fig. 5). The curves of GCE and C₃N₄/GCE have shown very weak peak currents (curves a, b), indicating that poor electrochemical processes have occurred on these electrode surfaces. SWCNTs/GCE has large background currents, excellent conductivity, but weak catalytic performance for Bn (curve f). Compared with curves b and f, C₃N₄-SWCNTs/GCE shows suitable background currents and good electrocatalytic performance for Bn with the help of good catalytic properties of C₃N₄ and great electrochemical properties of SWCNTs (curve e). Because of large specific surface area, good biocompatibility and stability of rGO, electrocatalytic ability of C₃N₄-SWCNTs/rGO/GCE for Bn has been further enhanced (curve d). In addition, CD-MOF can adsorb Bn on the electrode surface because of its excellent enrichment ability, which can greatly enhance the response current of Bn. Therefore, under the synergistic effect of various materials, C₃N₄-SWCNTs/rGO/CD-MOF/GCE has the largest redox current and the best catalytic performance for Bn (curve e).

To investigate the enrichment performance of different CD and CD-MOF for Bn, CV and DPV curves of Bn have been recorded on C₃N₄-SWCNTs/rGO/β-CD/GCE, C₃N₄-SWCNTs/rGO/γ-CD/GCE, C₃N₄-SWCNTs/rGO/β-CD-MOF/GCE, and C₃N₄-SWCNTs/rGO/γ-CD-MOF/GCE (Fig. S8). Compared with pure β-CD, γ-CD shows better enrichment and detection performance for Bn, which is consistent with the previous literature (curve a, b) [46]. When CD is replaced with CD-MOF (both γ-CD and β-CD), the enrichment capacity of these electrodes is further enhanced, which is attributed to the high specific surface area, large pore size, strong biodegradability and strong encapsulation capacity of CD-MOF (curve c, d) [47]. In addition, the redox currents on C₃N₄-SWCNTs/rGO/β-CD-MOF/GCE and C₃N₄-SWCNTs/rGO/γ-CD-MOF/GCE are similar, indicating that both γ-CD-MOF and β-CD-MOF have analogous adsorption properties. Due to the simple preparation and low cost of β-CD, β-CD-MOF has been chosen to construct this sensor.

3.5. Effect of scan rate on C₃N₄-SWCNTs/rGO/CD-MOF/GCE

Fig. 6a shows the CV curves of Bn on C₃N₄-SWCNTs/rGO/CD-MOF/GCE at different scan rates (v). Redox currents increase linearly with the increasing v (MEP L_fig6Fig. 6B), and the calibration equations for oxidative peak (iₚₒ) and cathodic peak (iₚₗ) can be found as follows.

\[ iₚₒ (μA) = 0.06 v (mV·s⁻¹) + 1.11 \quad (R² = 0.9985) \]  
\[ iₚₗ (μA) = -0.06 v (mV·s⁻¹) - 3.30 \quad (R² = 0.9994) \]  

These characteristics indicate that the redox reaction of Bn is adsorption-controlled process on C₃N₄-SWCNTs/rGO/CD-MOF/GCE.
Oxidation peak potential ($E_{pa}$) shifts negatively with the increasing $v$, which indicates redox process of Bn tends to be irreversible on C$_3$N$_4$-SWCNTs/rGO/CD-MOF/GCE (Fig. 6C). The relationship between scan rate and $E_{pa}$ can be depicted as:

$$E_{pa}(V) = 0.021 \ln v \ (\text{mV} \cdot \text{s}^{-1}) + 0.37 \ (R^2 = 0.9943)$$  \hspace{1cm} (4)

According to the Laviron equation [48,49]:

$$E_{pa}(V) = E^0 + \left(\frac{RT}{\alpha n F}\right) \ln \left(\frac{RTk_0}{\alpha n F}\right) + \left(\frac{RT}{\alpha n F}\right) \ln v$$  \hspace{1cm} (5)

where, $E^0$ is the formal standard potential, R is the universal gas constant (8.314 J mol$^{-1}$ K$^{-1}$), T is the absolute temperature (298 K), $\alpha$ is the transfer coefficient, n is the electron number, F is the Faraday constant (96485 C mol$^{-1}$), and $k_0$ is the rate constant. According to Eqs. (4) and (5), we have calculated that the value of $\alpha n$ is 1.22. The normal range of $\alpha$ is 0.3–0.7 [50], so the $\alpha$ is 0.61 and n is 2. Therefore, the redox electron transfer number of Bn is 2 on the surface of C$_3$N$_4$-SWCNTs/rGO/CD-MOF/GCE, which is consistent with the previous report [51].

Fig. 5. (A) CVs and (C, D, E) DPVs of (a) GCE, (b) C$_3$N$_4$/GCE, (c) C$_3$N$_4$-SWCNTs/GCE, (d) C$_3$N$_4$-SWCNTs/rGO/GCE, (e) C$_3$N$_4$-SWCNTs/rGO/CD-MOF/GCE, and (f) SWCNTs/GCE in 0.1 M PBS (pH=2.5) containing 2.5 $\times$ 10$^{-7}$ M Bn. (B, F) Redox peak current of different electrodes. Scan rate of CV: 100 mV s$^{-1}$, pulse period of DPV: 0.2 s.
3.6. Effect of pH on $C_3N_4$-SWCNTs/rGO/CD-MOF/GCE

As shown in Fig. 7, the redox potential shifts to the negative potential with increasing electrolyte pH. Calibration equations of $E_{pa}$, formal potential ($E^\theta$), and reduction peak potential ($E_{pc}$) can be expressed as follows.

\[
E_{pa}(V) = -0.049 \text{pH} + 0.60 \quad (R^2 = 0.9902)
\]

\[
E^\theta(V) = -0.052 \text{pH} + 0.59 \quad (R^2 = 0.9967)
\]

\[
E_{pc}(V) = -0.055 \text{pH} + 0.58 \quad (R^2 = 0.9981)
\]

According to Nernst Equation [52]:

\[
\frac{dE_{pc}}{d\text{pH}} = \frac{2.303 R T}{n F}
\]

Where, $m$ and $n$ are the proton and electron number, respectively. The value of $m/n$ is 1.1 through Eqs. (7) and (9), indicating that number of $m$ and $n$ are equal in the redox process of Bn on $C_3N_4$-SWCNTs/rGO/CD-MOF/GCE.

The above electrochemical studies of scan rate and pH indicate that the redox process of Bn on the sensor involves the transfer of two protons and two electrons. Gaussian 09 and Multiwfn [53] have been used to study the redox process and the reaction sites of Bn (Density functional theory study has been recorded in Supplementary Material), and the results show that the two hydroxyl groups on the benzene ring of Bn are possible sites for oxidation reactions (Fig. S9 and S10).

3.7. Optimization of conditions

3.7.1. Optimal electrolyte pH

It can be observed from Fig. 7C that the redox peak currents increase as the pH increases from 1.5 to 2.5, and the maximum value is obtained at pH 2.5. Then the redox current decreases with increasing electrolyte pH. Furthermore, analyzing the DPV curves of Bn at different pH on $C_3N_4$-SWCNTs/rGO/CD-MOF/GCE, the relationship between currents and pH is the same as the result of CV curves (Fig. 8). Since the maximum redox currents can be obtained in PBS with pH = 2.5, it has been selected as the supporting electrolyte for experiments.

3.7.2. Optimal electrodeposition cycle for CD-MOF

The number of electrodeposition cycles is an important factor affecting the amount of CD-MOF modification and the enrichment performance of $C_3N_4$-SWCNTs/rGO/CD-MOF/GCE. Therefore, the influence of different electrodeposition cycles on the detection ability of Bn has been studied. It can be seen from Fig. S11 that the oxidation current of Bn gradually increases as the number of electrodeposition cycles increases from 0 to 25. This shows that CD-MOF has been successfully modified and significantly improved the enrichment capacity of the
modified electrode. The peak current decreases significantly when the number of electrodeposition cycles is increased from 25 to 35, which is due to the weakened electrochemical and electrocatalytic properties of the modified electrodes caused by excessive CD-MOF. Therefore, 25 cycles have been chosen as the optimal modification cycles.

Fig. 7. (A) CVs of C₃N₄-SWCNTs/rGO/CD-MOF/GCE at different pH (from a to j: 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0) in 0.1 M PBS containing 2.5 × 10⁻⁷ M Bn. (B) The dependence of oxidation peak potential (Epa), formal potential (Eθ), reduction peak potential (Epc) on pH. (C) The dependence of redox peak currents on pH. Scan rate of CV: 100 mV s⁻¹.

Fig. 8. (A) DPVs of C₃N₄-SWCNTs/rGO/CD-MOF/GCE at different pH (from a to j: 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0) in 0.1 M PBS containing 2.5 × 10⁻⁷ M Bn. (B) The dependence of oxidation peak currents on pH. Pulse period of DPV: 0.2s.
3.7.3. Optimal accumulation time and accumulation potential

Since CD-MOF has excellent enrichment ability, the accumulation time and accumulation potential also affect the electrocatalytic ability of C$_3$N$_4$-SWCNTs/rGO/CD-MOF/GCE for Bn. Fig. S12A shows that the oxidation current gradually increases with the accumulation time from 1 min to 26 min. However, when the accumulation time is increased from 26 min to 34 min, the current is almost unchanged, which indicates that the enrichment has reached saturation. Therefore, 26 min is selected as the optimal accumulation time. Similarly, Fig. S12B shows that the peak current increases significantly as the accumulation potential changes from 0.2 V to 0 V and reaches a maximum at 0 V, and gradually decreases from 0 V to 0.2 V. Hence, 0 V is used as the optimal accumulation potential for the entire experiment.

\[ i_{pa} (\mu A) = 0.22 C_{Bn}(nM) + 0.24 \ (1 \times 10^{-9} M \leq C_{Bn} \leq 3 \times 10^{-7} M, \ R^2 = 0.9976) \]  
\[ i_{pa} (\mu A) = 72.11 \log(C_{Bn})(nM) - 112.91 \ (3 \times 10^{-7} M \leq C_{Bn} \leq 5 \times 10^{-7} M, \ R^2 = 0.9943) \]

3.8. The detection performance for Bn on C$_3$N$_4$-SWCNTs/rGO/CD-MOF/GCE

Under the optimal experimental conditions, the linearity and limit of detection (LOD) of Bn on C$_3$N$_4$-SWCNTs/rGO/CD-MOF/GCE has been obtained (Fig. 9A). The current and concentration of Bn show good linear relationship between $1 \times 10^{-9} M$ to $5 \times 10^{-7} M$, and the LOD (S/N = 3) is $4.6 \times 10^{-10} M$ (Fig. 9B, C). The linear regression equation can be described as:

As shown in Table 1, C$_3$N$_4$-SWCNTs/rGO/CD-MOF/GCE has high sensitivity, good linear range and ultra-low LOD comparing with reported electrodes. So, the C$_3$N$_4$-SWCNTs/rGO/CD-MOF/GCE has great detection performance for Bn.

![Fig. 9. (A) DPVs of Bn quantitative analysis on C$_3$N$_4$-SWCNTs/rGO/CD-MOF/GCE, concentrations of Bn (a to o- 1, 2, 5, 10, 30, 60, 100, 150, 200, 250, 300, 350, 400, 450, 500 nM). (B, C) DPVs of linear calibration between the current responses and the concentration of Bn. Pulse period of DPV: 0.2 s.](image-url)
3.9. Repeatability, reproducibility, anti-interference, and stability

C₃N₄-SWCNTs/rGO/CD-MOF/GCE has been employed to detect 2.5 \times 10^{-7} M Bn by DPV for twelve times to evaluate its repeatability. The relative standard deviation (RSD) of currents is 1.0%, indicating C₃N₄-SWCNTs/rGO/CD-MOF/GCE has good repeatability (Fig. 10A). To evaluate reproducibility, ten independently modified electrodes have been prepared under the same conditions to detect 2.5 \times 10^{-7} M Bn by DPV. Fig. 10B shows that the prepared sensors have satisfactory reproducibility with the RSD of 2.3%.

In addition, influences of some possible interferences on the oxidation current of Bn have been investigated by recording the DPV curves without or with some substances (Fig. 10C, D). It can be found that one hundred equivalents of galactose (Gal), fructose (Fru), sucrose (Suc), glucose (Glu), citric acid (CA), ascorbic acid (AA), potassium chloride (KCl), ammonium chloride (NH₄Cl), and twice equivalents of chrysin (Chr) has almost no effect on the DPV response and oxidation current of Bn, which indicates that C₃N₄-SWCNTs/rGO/CD-MOF/GCE has good anti-interference performance for Bn.

To study storage performance of C₃N₄-SWCNTs/rGO/CD-MOF/GCE, seven modified electrodes under the same conditions have been prepared. Then they are stored at room temperature (25 °C), and an electrode is taken out every five days to detect Bn and record the oxidation current (Fig. 11A). The results show that the modified electrode still has excellent electrocatalytic performance for Bn after being stored at room temperature for one month. Similarly, in order to study the stability of

### Table 1
Comparison of various modified electrodes for detection of Bn.

| Electrode                     | Technique | Linear range (mol L⁻¹) | LOD (mol L⁻¹) | Sensitivity (A/M) | Refs. |
|-------------------------------|-----------|------------------------|---------------|-------------------|-------|
| Ta₂O₅-Nb₂O₅@CTS/CPE           | DPV       | 8 \times 10^8, 8 \times 10^6 | 3 \times 10^8  | 8.49              | [51]  |
| DM-β-CD-GNs/GCE               | DPV       | 4 \times 10^8, 1.25 \times 10^6 | 1 \times 10^8  | 55.3              | [54]  |
| Co-amino-Gc/GCE               | SWV       | 1 \times 10^8, 8 \times 10^7 | 5 \times 10^8  | 53.2              | [55]  |
| MoSe₂/GCE                     | DPV       | 1.25 \times 10^7, 5.2 \times 10^6 | 5 \times 10^8  | 1.88              | [56]  |
| SS-b-CD-P4@RGO/GCE            | DPV       | 2 \times 10^8, 2 \times 10^5 | 2.6 \times 10^7 | 1.05              | [57]  |
| boron doped diamond           | SWV       | 1 \times 10^6, 9.5 \times 10^7 | 6 \times 10^9  | 7.36              | [58]  |
| TRGO/GCE                      | DPV       | 1 \times 10^8, 1 \times 10^5 | 4.6 \times 10^{10} | 220              | [59]  |
| C₃N₄-SWCNTs/rGO/CD-MOF/GCE    | DPV       | 1 \times 10^9, 5 \times 10^7 | 5 \times 10^7   | 4.6 \times 10^{10} | This work |

Fig. 10. (A) DPV responses of the same modified electrode toward 2.5 \times 10^{-7} M Bn collected from twelve repeat measurements. (B) DPV responses of ten independent electrodes prepared under the same condition toward 2.5 \times 10^{-7} M Bn. (C) The DPVs of C₃N₄-SWCNTs/rGO/CD-MOF/GCE in 0.1 M PBS (pH 2.5) containing different substances. (D) Oxidation peak current of DPV on C₃N₄-SWCNTs/rGO/CD-MOF/GCE in PBS containing different substances. Pulse period of DPV: 0.2 s.
C₃N₄-SWCNTs/rGO/CD-MOF/GCE, the same electrode has been used to obtain the oxidation current of Bn every two days and stored it at 4 °C (Fig. 11B). It shows oxidation current decreased by 11.9% after two weeks, indicating that the sensor has good stability.

3.10. Detection of Bn in actual samples on C₃N₄-SWCNTs/rGO/CD-MOF/GCE

3.10.1. Detection in human serum

Since Bn often has been used as medicament, to evaluate applicability of manufactured electrochemical sensor in actual samples, C₃N₄-SWCNTs/rGO/CD-MOF/GCE has been detected the Bn in human serum through a standard addition method. Human serum samples have been obtained from Xiangtan University Hospital and diluted 100 times with PBS (pH 2.5). C₃N₄-SWCNTs/rGO/CD-MOF/GCE has been obtained the oxidation current in the blank sample and the spiked sample, and the Bn concentration is calculated through the calibration equation. Table 2 shows that the recovery rate of Bn is 97.7–106.2%, which indicates that C₃N₄-SWCNTs/rGO/CD-MOF/GCE can be used to the detection of Bn in human serum.

3.10.2. Detection in sold medicine

Bear bile scutellaria eye drops (a kind of medicine) have been purchased from a local pharmacy and diluted 20 times with methanol. Then, 20 µL of diluent has been added to 20 mL PBS (pH 2.5). Next, DPV curves for unspiked and spiked samples have been obtained on C₃N₄-SWCNTs/rGO/CD-MOF/GCE, and the concentration of Bn has been calculated by the calibration equation. Table 3 shows that the recovery of Bn is 96.7–104.9% in the presence of actual medicine, indicating that the sensor can be used to detect actual medicines.

4. Conclusion

In this work, C₃N₄-SWCNTs have been successfully synthesized by a one-pot method, and a novel ultrasensitive Bn electrochemical sensor based on C₃N₄-SWCNTs/rGO/CD-MOF/GCE, and the concentration of Bn has been calculated by the calibration equation. This work provides a novel method for the detection of flavonoids and proposes an interesting scheme for the application of cyclodextrin metal organic frameworks in electrochemical sensors.
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Appendix A. Supporting information
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