Construction of molecularly imprinted electrochemical sensor and penicillin detection based on FeS$_2$@C

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Abstract: This paper proposes for the first time a molecularly imprinted electrochemical sensor (MIECS) based on graphene-loaded FeS$_2$@C modified glass electrode (GCE) for the efficient, rapid and sensitive detection of penicillin (PNC). Iron disulfide coated with biomass carbon has a very high specific surface area and volume effect, excellent electron transfer efficiency and conductivity, and is used to modify electrodes.In the experiment, electropolymerization was employed, with penicillin as the template molecule and o-phenylenediamine as the functional monomer, and the imprinted polymer membrane was successfully anchored on the surface of the FeS$_2$@C composite material to specifically adsorb and detect penicillin. The materials were characterized by XRD, CV, DPV and other characterization methods. At the same time, CV and DPV were used to optimize the experimental conditions such as the molar ratio of functional monomer and template, elution time, resorption time, the number of polymerization cycles, and pH. In the end, it is concluded that under the experimental conditions of pH=3, molar ratio of 1:5, and polymerization circle number of 20, MIECS has good sensitivity, selectivity, reproducibility and stability to PNC.

1. Introduction

Due to their unique antibacterial activity, antibiotics have been widely used in the treatment and control of biological infections and diseases caused by bacteria [1]. Penicillin is an important antibiotic with high efficiency, low toxicity and extensive clinical application. However, penicillin is unstable and can be decomposed into penicillin thiazolic acid and penicillonic acid. The former can be polymerized into penicillin thiazolinic acid polymer, combined with polypeptides or proteins to form penicillium thiazolinic acid protein, which is an immediate allergen and the main cause of allergic reactions; The latter can also be combined with the cysteine in the body, forming a delayed allergen-penicillin acid protein, which is related to serum sickness-like reactions [2]. Therefore, it is essential to develop a highly selective and sensitive and economical detection method for the determination of the residual amount of penicillin in animal-derived foods.

A plethora of methods are available for PNC determination, including liquid chromatography-mass spectrometry [3], high performance liquid chromatography [4], capillary electrophoresis [5], enzyme-linked immunoassay [6], fluorescence analysis [7], etc.Although these methods can accurately and repeatedly detect PNC, they also have drawbacks such as troublesome pretreatment, complex analysis methods, and high costs [8]. Therefore, the development of a low-cost detection

method with high selectivity and sensitivity to PNC has become a research hotspot. Electrochemical sensors have been widely used in the detection of antibiotics and other environmental pollutants.In addition, electrode modification materials for electrochemical sensors can improve the stability and anti-interference ability of electrochemical signals. These advantages make it have huge development potentials and broad application prospects in biomedical equipment, wearable devices, etc. [9]

In this paper, an electrochemical method for detecting PNC based on the molecularly imprinted polymer modified by the $FeS_2@C$ nanocomposite is constructed.Firstly, graphite oxide (GO) was prepared by the modified Hummers method, and then the prepared GO and an appropriate amount of $FeCl_3 \cdot 6H_2O$ were dispersed in deionized water and stirred for 24 h.The obtained GO@FeOOH was subjected to ultrasonic treatment inwater, and $NH_3 \cdot H_2O$ and cetyltrimethylammonium bromide (CTAB) aqueous solution were added and stirred at room temperature. The product was filtered and washed with water, dried overnight, and carbonized in an Ar atmosphere at a high temperature to obtain $G@Fe_3O_4@C$ composite material. Then, the prepared composite material sample was dispersed in the mixed dense agent, stirred, rinsed with ethanol and deionized water, filtered, and dried to finally obtain the graphene-supported hollow carbon material $G@-YS\ Fe_3O_4@C$.Finally, an appropriate amount of $G@YS\ Fe_3O_4@C$ and sulfur powder were evenly mixed, sealed in a quartz tube, calcined and dried at high temperature for 24 hours, and ground into powder to obtain $GO@FeS_2@C$ nanocomposite material. Dissolve the $G@FeS_2@C$ nanocomposite material in 1 mL of chitosan solution, and then apply it evenly on the surface of the pretreated glassy carbon electrode after ultrasonic treatment. Place it in the air to dry naturally at room temperature, and scan 20 cycles at a scanning rate of 100 mV/s within the potential range of -0.4-1.2V to obtain the modified electrode with molecularly imprinted polymer on the surface of $G@FeS_2@C$.

2. Experimental section

Reagents and apparatus

Ultrasonic cleaner, magnetic stirrer, constant temperature water bath, analytical balance, suction filter device, high-speed centrifuge, multi-function oscillator, vacuum drying oven, electrochemical workstation, horse boiling furnace, X-ray powder diffractometer (XRD), etc.

Natural graphite powder (AR), concentrated sulfuric acid (AR), sodium nitrate (AR), $Ba(NO_3)_2$(AR), $FeCl_3 \cdot 6H_2O$(AR), $NH_3 \cdot H_2O$(AR), cetyltrimethyl bromide Ammonium chloride (CTAB) (AR), sublimed sulfur (AR), concentrated nitric acid (AR), concentrated hydrochloric acid (AR), potassium permanganate (AR), hydrogen peroxide (30%, AR), sodium hydroxide (AR), Methanol (AR), absolute ethanol (AR), acetic acid (AR), o-phenylenediamine (AR), deionized water (AR), penicillin (AR).

Synthesis of GO

Use the modified Hummers method to prepare graphite oxide, specific experimental steps: add a mixture of graphite powder and $NaNO_3$ to concentrated H_2SO_4.Under ice bath conditions, slowly add $KMnO_4$ to the reaction solution while stirring. After stirring, slowly add water to the mixed solution within 30 minutes while controlling the temperature. After the reaction, add deionized water and H_2O_2 to the mixture, and then collect the precipitate in the mixture by centrifugation. Dry the resultant product under vacuum conditions for 24 hours and grind it into powder.

Synthesis of $G@Fe_3O_4@C$ composite materials

Disperse 1 g GO and 1.254 g $FeCl_3 \cdot 6H_2O$ in deionized water and stir for 24 h.Treat the obtained GO@FeOOH ultrasonically in water. Add $NH_3 \cdot H_2O$ and cetyltrimethylammonium bromide (CTAB) aqueous solution, stir at room temperature. The product was filtered and washed with water, dried overnight, and carbonized in an Ar atmosphere at a high temperature to obtain $G@Fe_3O_4@C$ composite material.

Synthesis of G@-YS Fe₃O₄@C composite materials

Disperse the obtained G@Fe$_3$O$_4$@C sample in the mixed solvent and stir it. After a period of time, use ethanol and deionized water to wash, filter, and dry. The graphene-supported hollow carbon material was obtained by etching FeS$_2$.

Synthesis of GO@FeS₂@C composite materials

Mix an appropriate amount of G@YS Fe$_3$O$_4$@C and sulfur powder evenly, seal it in a quartz tube, and calcinate at a certain temperature of 400°C for 2 hours. After taking it out, dry it in a vacuum drying oven for 24 hours and grind it into powder.

Preparation of the modified electrode with molecularly imprinted polymer on the surface of G@FeS₂@C.

Take 3 mg G@FeS$_2$@C nanocomposite material and dissolve it in 1mL chitosan solution, and treat it ultrasonically until the material was evenly mixed. Take 10 μL of the above solution and evenly apply it on the surface of the pretreated glassy carbon electrode, and let it dry naturally at room temperature. During this period, add a certain molar ratio of penicillin and o-phenylenediamine to the PBS buffer solution to dissolve, and treat it ultrasonically. Place the modified electrode in it, and scan 20 cycles at a scanning rate of 100 mV/s within the potential range of -0.4-1.2V to obtain the modified electrode with molecularly imprinted polymer on the surface of G@FeS$_2$@C.

3. Results and discussion

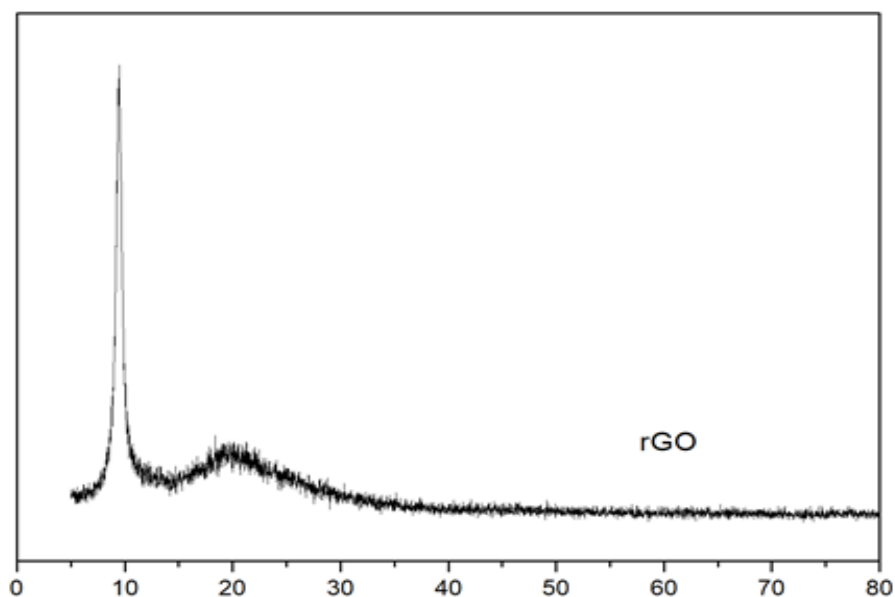**Morphology and element characterization of nanocomposites**

Figure 1: Graphene (rGO) X-ray diffraction pattern (XRD)

The XRD pattern of graphene is basically the same as the standard XRD pattern, indicating that rGO has been successfully synthesized, see Figure 1.
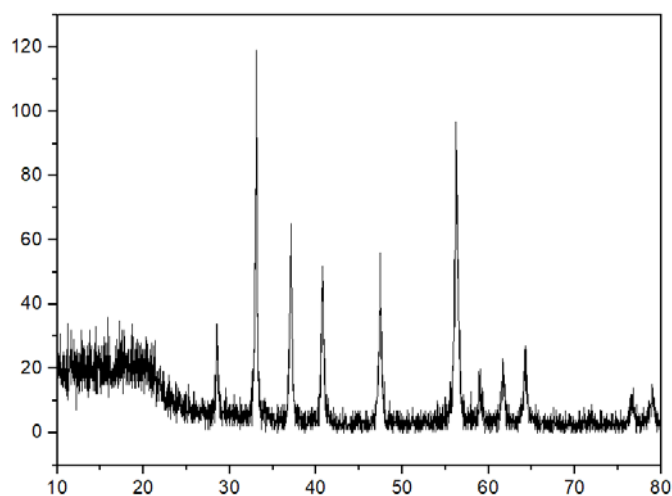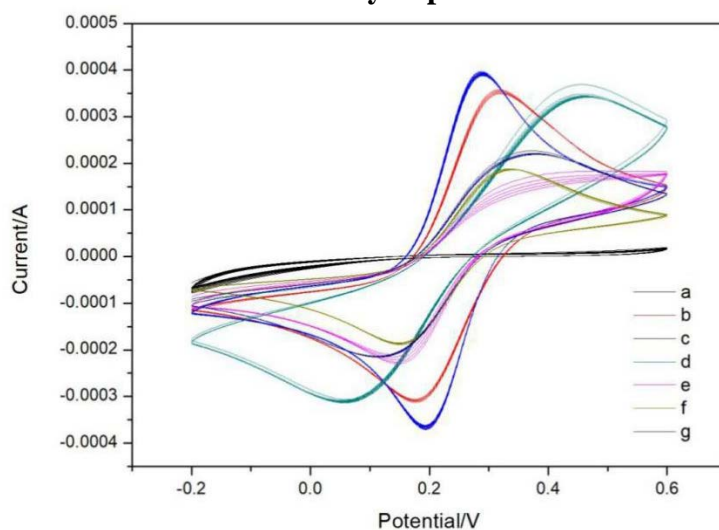
Figure 2: FeS$_2$@C X-ray diffraction pattern

It can be seen from the Figure 2 that the FeS$_2$@C XRD diffraction image is consistent with the actual one, and the peak shape is consistent, indicating that the FeS$_2$@C nanocomposite has been successfully synthesized.

Construction characterization of molecularly imprinted electrochemical sensors

(a) GO/FeS$_2$@C@ GCE/MIPs, (b) FeS$_2$@C@GCE, (c) GO/FeS$_2$@C@GCE,
(d) GO/FeS$_2$@C@GCE/ MIPs eluted, (e) GO/FeS$_2$@C@GCE/MIPs resorbed,
(f) bare GCE, (g) rGO/GCE.

Figure 3: The CV graph of 0.5 M KCl solution containing 5.0 mM [Fe(CN)$_6$]$^{3-/4-}$

It can be seen from Figure 3 that using a mixture of 5.0 mM [Fe(CN)$_6$]$^{3-/4-}$ and 1.0 M KCl to characterize the conductivity of the electrode, the conductivity of the electrode changes with the different supporting materials. When the electrode is loaded with GO, it is the best, followed by FeS$_2$@C, and the smallest when loaded with GO, but both are greater than the conductivity of the bare electrode. This shows that the experimental material has good electrical conductivity and a certain degree of excellence, and the conductive effect can be superimposed. When molecularly imprinted polymerization is performed, the surface of the electrode (GCE) is covered by the molecularly imprinted membrane, and electrons cannot pass through the imprinted membrane,

resulting in reduced conductivity. When the template molecule PNC is eluted, an imprinted cavity appears on the polymeric membrane, and electrons can pass through the void and reach the surface of the electrode, resulting in the increased conductivity .From the comparison of curves d and e, it can be seen that when PNC is re-adsorbed, the conductivity decreases again, which shows that the molecularly imprinted membrane was successfully prepared and the specific detection of penicillin was achieved.

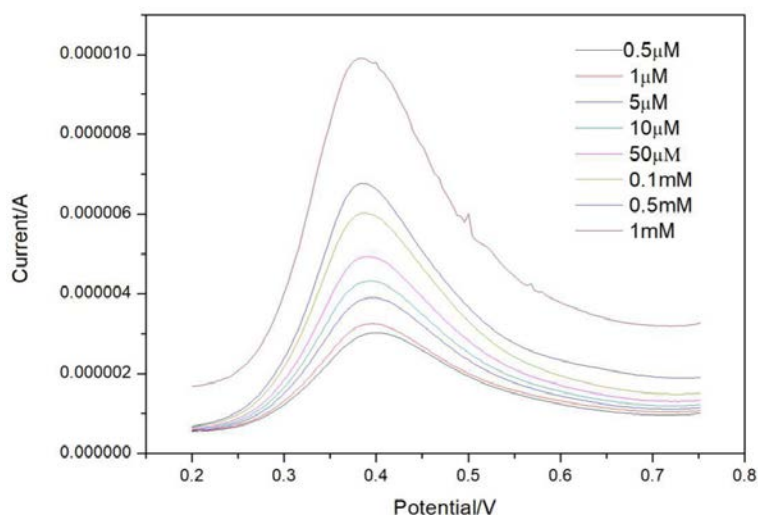**The response of DPV under different concentrations of PNC**

Figure 4: The effect of different concentrations of PNC on DPV in 0.1 M PBS (pH 3.0)

It can be seen from Figure 4 that the peak response of the detected substance is between 0.3 and 0.5, and with the increase of the PNC concentration, the peak value also increases gradually, which indicates that the experimentally constructed molecularly imprinted electrochemical sensor (MIECS) has a good detection effect on PNC.

Optimization of pH value of buffer solution

Provide a series of different pH environments for the molecular imprinted electrochemical sensor detection system, from 2.0 to 9.0 per 1.0 pH as a unit, adjust the pH value of the PBS buffer solution, and detect the response of the molecular imprinted electrochemical sensor to penicillin. The experimental results are as follows.
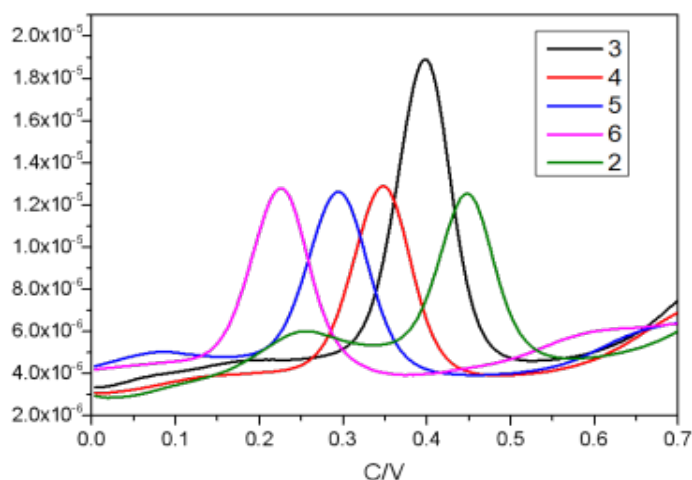

Figure 5: pH optimization experiment result graph

It can be seen from Figure 5 that when pH=3, the DPV response is the strongest, so the pH is adjusted to 3 in this experiment.

Optimization of the number of electropolymerization cycles

Using different polymerization cycles, the response of the molecularly imprinted electrochemical sensor to penicillin was detected. The experimental results are as follows.
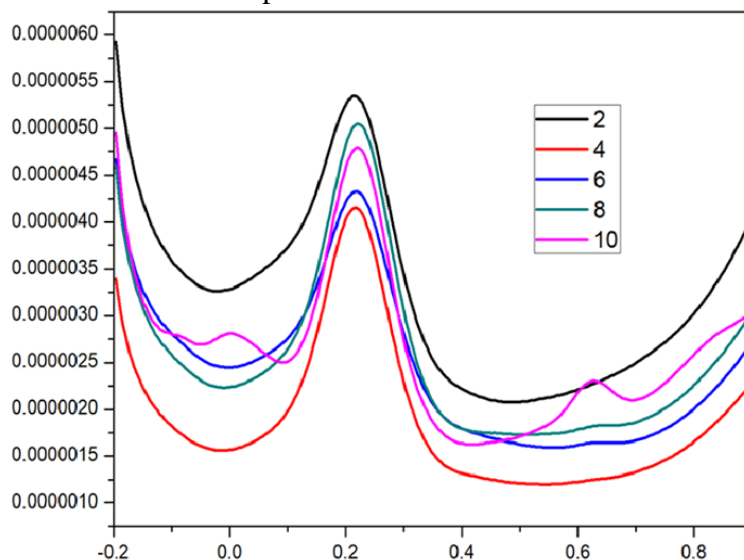

Figure 6: The influence of the number of electropolymerization cycles on the detection system

It can be seen from Figure 6 that when the number of polymerization turns is 2, the DPV response is the strongest.
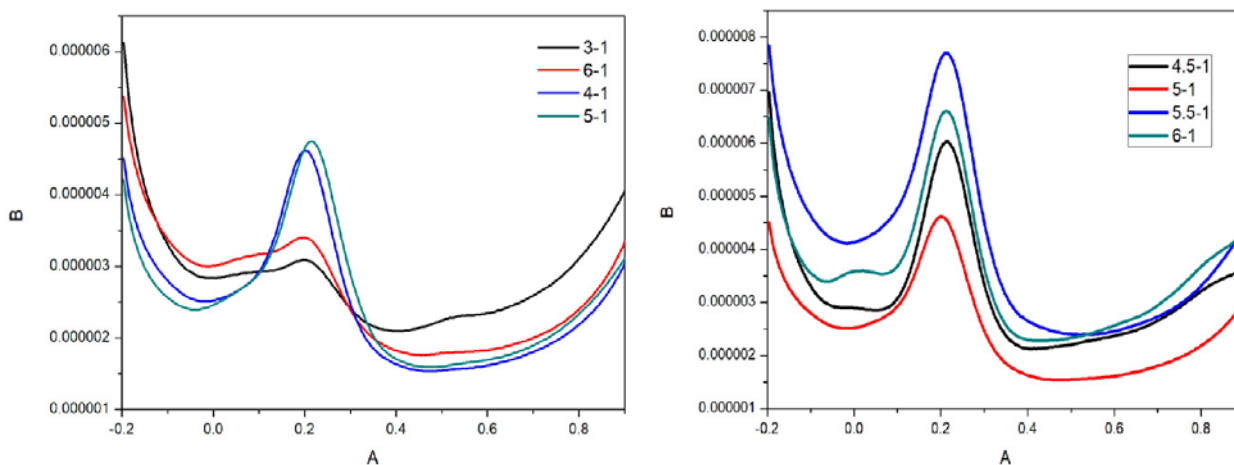

Figure 7: Optimization of the molar ratio of PNC to o-phenylenediamine

It can be seen from Figure 7 that when the molar ratio of PNC to o-phenylenediamine is 5.5:1, the DPV is the highest.

Optimization of resorption time

Using the DPV detection method, molecular imprinting is done once for each group, and the resorption time interval is 2 minutes.
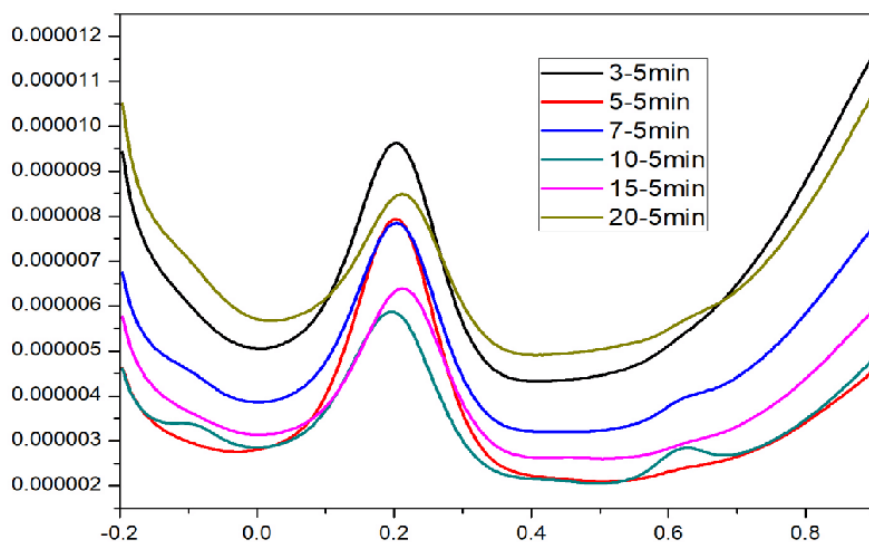
Figure 8: The effect of resorption time on the detection system

It can be seen from Figure 8 that when the resorption time is 3-5 minutes, the DPV response is the strongest.
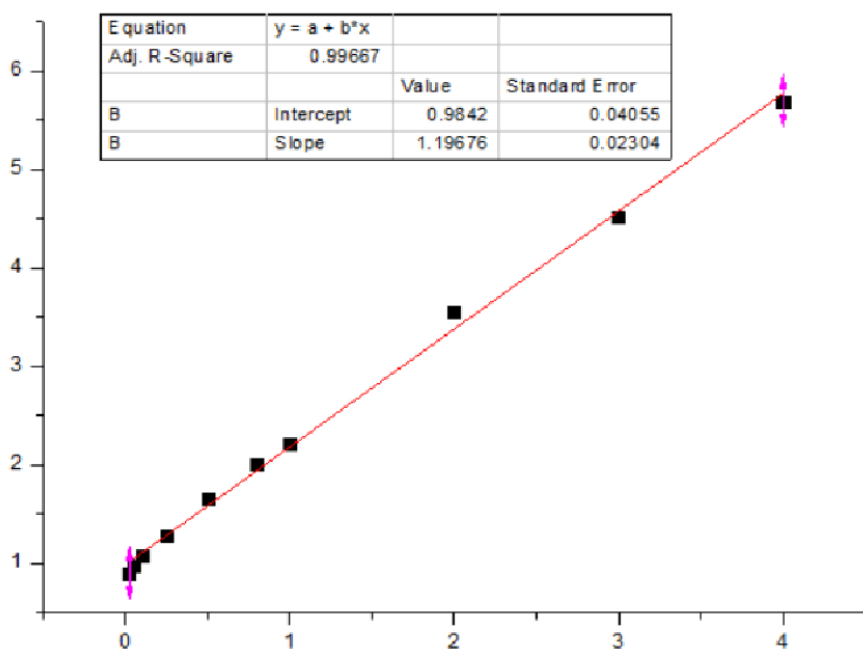

Figure 9: The Linear fitting

It can be seen from the figure 9 that within a certain range, the detection response of the electrochemical sensor increases with the increase of the measured concentration, which conforms to the linearity condition.

4. Conclusion

This paper proposes an electrochemical sensor based on graphene-loaded $FeS_2@C$ nanocomposite imprinted film for rapid, sensitive and selective detection of PNC. The surface of the polished glassy

carbon electrode (GCE) is coated with nanomaterials to increase its conductivity and electron transfer rate, thereby improving the sensitivity of the sensor. Afterwards, the template molecule PNC and the functional monomer o-phenylenediamine are polymerized by cyclic voltammetry (CV) electrochemically to form a molecularly imprinted polymer (MIP) film. The construction of GO@FeS$_2$@C/MIPs/GCE complex significantly enhanced the electrochemical response of the sensor to PNC, and the electrochemical performance of the sensor was evaluated by cyclic voltammetry (CV) and differential pulse voltammetry (DPV). The detection conditions such as the molar ratio of the template molecule to the functional monomer, the pH of the buffer solution, the number of electropolymerization cycles, the elution time, etc were optimized so as to obtain the relationship between the DPV current response and the PNC concentration under the optimal conditions and the best linear fitting image.

References

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