Construction of dibutyl phthalate molecularly imprinted electrochemical sensor based on multi-walled carbon nanotubes-modified boron-doped diamond electrode

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Abstract—In order to construct a dibutyl phthalate (DBP) molecularly imprinted electrochemical sensor, the DBP was used as the template molecule, due to the characteristics of multi-walled carbon nanotubes (MWCNTs) loaded with nano-gold to enhance the electrical conductivity of the composite material, a molecularly imprinted film was prepared on the surface of boron-doped diamond electrode (BDD) by potential deposition. The morphology of the composite material MIP/AuNPs/MWCNTs/BDD was analyzed and characterized by scanning electron microscopy (SEM), and the performance of the electrochemical sensor was characterized by cyclic voltammetry (CV), and differential pulse voltammetry (DPV) was used to detect DBP. The electrochemical sensor had a linear range of $1 \times 10^{-8}$ ~ $1 \times 10^{-5}$ mol/L and a detection limit of $3.3 \times 10^{-9}$ mol/L. The sensor was applied to the detection of DBP in water samples.

1. Introduction
In the past ten years, phthalates have been widely used as plasticizers in synthetic materials. The environmental estrogens of plasticizers were released into the environment and stored in the human body in various ways, threaten to human health and other organisms[1-2]. At present, the main methods for the detection of phthalates include liquid chromatography and gas chromatography-mass spectrometry[3-5]. Compared with the above technologies, the electrochemical method was characterized by simple operation, portable equipment, low cost, fast analysis speed and high sensitivity[6]. Electrochemical sensing methods have been widely used in organic pesticides, biological macromolecules, etc[7]. However, the low selectivity of electrochemical methods has greatly affected their practical application in complex samples. Nowadays, researchers were interested in modified electrode technology, due to various characteristics, convenient design and easy miniaturization, modified electrode have become a hot research issue in the field of sensor analysis[8].

Molecular imprinted technology (MIT) had selective specificity for specific target molecules, which could be applied in molecular recognition, solid phase extraction, chromatographic separation and so on. Sensors prepared based on molecularly imprinted polymers (MIPs) had the advantages of simplicity, strong specificity, high sensitivity, fast response, low cost, portability and easy operation[9-10]. It has been widely used in clinical, food and environmental monitoring[11]. However, there are few reports on the application of molecularly imprinted nanofiber or metal nanoparticles modified electrodes in the detection of plasticizer residues.
Metal nanoparticles, such as gold, platinum, silver, copper, and nickel, have become the most popular electrocatalysts to increase electrochemical activity\cite{12}. Gold nanoparticles have a large specific surface area, and using it as a sensitizing material can effectively improve the sensitivity, biocompatibility\cite{13} and stability of electrochemical sensors. In this study, multi-walled carbon nanotubes and nano-gold particles were used to modify the surface of boron-doped diamond electrode (BDD), and dibutyl phthalate (DBP) was used as the template molecule to form electrochemical in-situ electropolymerization technology on the surface of the BDD electrode. A layer of molecularly imprinted film was used to fabricate multi-walled carbon nanotubes loaded with gold nano-modified boron-doped diamond electrode molecularly imprinted film sensor with DBP as the template molecule. So, a new type of sensor was constructed and which was used to determinate DBP in the water sample.

2. Experiment

2.1 Reagents and Instruments
Chloroauric acid (HAuCl₄), p-mercaptoaniline (p-ATP), dibutyl phthalate (DBP), multi-walled carbon nanotubes (MWCNTs), and phosphate buffer (PBS) were purchased from (Aladdin), other reagents were analytical grade, and all experimental water was deionized water.
Electrochemical analysis was performed on CHI600 electrochemical workstation (Shanghai Chenhua). Scanning electron microscope (SEM) images were taken with a Hitachis-4800 Scanning Electronics Microscope, JEOL Co.LTD.

2.2 Preparation of dibutyl phthalate imprinted electrode

2.2.1 Preparation of MWCNTs dispersion
Accurately weighed 1g of MWCNTs, which was immersed in concentrated nitric acid solution, and ultrasonically dispersed at 65°C for 4h continuously. The solution was centrifuged and washed with deionized water to neutrality, filtered and dried in a 45°C drying oven for 24h to prepare acidified MWCNTs. Weighed 2 mg of acidified MWCNTs and dispersed them uniformly into 2 mL of N,N-dimethylformamide (DMF) to prepare a dispersion solution of MWCNTs, placed it at 4°C for used.

2.2.2 BDD electrode activation
The BDD electrode was ultrasonically cleaned in deionized water, 0.5mol/L nitric acid solution, acetone, and deionized water for 5 minutes successively, and then immersed in a 0.5mol/L sulfuric acid solution for CV scanning to obtain stable curve. and the electrode was rinsed with deionized water repeatedly and dried dry for later use.

2.2.3 Preparation of AuNPs/MWCNTs/BDD electrode
Took 10μL of MWCNTs dispersion and coated it on the surface of the BDD evenly, dried it to obtain the MWCNTs/BDD electrode. A three-electrode system was established with platinum wire electrode as auxiliary electrode, saturated KCI electrode as reference electrode, MWCNTs/BDD electrode as working electrode, which immersed in a 0.1g/L chloroauric acid aqueous solution at a potential of -0.2V for 2 minutes to prepare AuNPs/MWCNTs/BDD electrode in accordance with constant potential deposition method .

2.2.4 Preparation of molecularly imprinted membrane working electrode (MIP/AuNPs/MWCNTs/BDD)
The AuNPs/MWCNTs/BDD electrode (prepared in 2.2.3) was used as the working electrode, used a three-electrode system, immersed in a phosphate buffer solution containing 1 mmol/L DBP and 10 mmol/L p-ATP, at a potential range of -0.2~0.6V. The scanning speed was 50mV/s for 10 cycles of cyclic voltammetry. An electrode with a polymer film with DBP imprinted function was prepared. The
A molecularly imprinted membrane working electrode was placed in a refrigerator at 4°C for later use.

As a control, the non-imprinted electrode was prepared without adding DBP under the same conditions.

2.3 Electrochemical method to detect different concentrations of dibutyl phthalate

The MIP/AuNPs/MWCNTs/BDD electrode was immersed in the dibutyl phthalate solution of different concentrations. After 10 minutes, the electrode was removed and gently washed with deionized water several times to remove possible impurities on the surface of the electrode. At room temperature, MIP/AuNPs/MWCNTs/BDD electrode was transferred to buffer solution containing PBS (pH 6.86). The peaks were recorded when the MIP/AuNPs/MWCNTs/BDD electrode adsorbed DBP of different concentrations by differential pulse method.

3. Results and discussion

3.1 Scanning electron microscopy characterization

The surface morphology of different modified electrodes was studied by SEM. Figure (A) showed that the MWCNTs were evenly distributed on the surface of the BDD electrode with almost no agglomeration. In Figure (B), electrodeposited AuNPs were evenly dispersed on the surface of MWCNTs. The structure of lamellar aggregate was beneficial to increase the transfer rate of electrons, thereby increasing the sensitivity of the sensor, as shown in figure (C).
3.2 Electrochemical performance

Figure 2 showed the CV characterization of different electrodes in 1 mmol/L K₃[Fe(CN)₆] solution and 0.1 mol/L KCl buffer solution. The electric current of AuNPs/MWCNTs/BDD modified electrode was higher than that of MWCNTs/BDD electrode and bare BDD electrode significantly. It showed that AuNPs/MWCNTs/BDD had higher conductivity and sensitivity than MWCNTs/BDD and bare electrodes. The MIP film was electropolymerized on the surface of AuNPs/MWCNTs/BDD modified electrode. The peak current of MIP during elution was higher than that of bare electrode and MWCNTs/BDD modified electrode. That was due to the imprinted holes on the surface of MIP promote the probe molecule Fe(CN)₆³⁻ electron transfer between the modified electrode. The peak current of MIP-resorption was lower than that of MIP. The reason was the template molecule refilled the imprinted pores. Due to the non-conductivity of MIP, the electron transfer between MIP and the substrate was hindered, and the peak current decreased.

Figure 2 Cyclic voltammograms of different electrodes

a: bare BDD electrode, b: MWCNTs/BDD electrode, c: AuNPs/MWCNTs/BDD electrode, d: MIP/AuNPs/MWCNTs/BDD electrode before elution, e: MIP/AuNPs/MWCNTs/BDD electrode after elution, f: MIP/AuNPs/MWCNTs/BDD electrode recombined with DBP molecules g: nMIP/AuNPs/MWCNTs/BDD electrode
3.3 Optimization of preparation conditions

3.3.1 Optimization of scan rate and number of scan cycles
In the electropolymerization process, the polymerization rate and the number of polymerization turns had a direct effect on the formation of the polymer film. When the peak current difference was large, the imprinted electrode had a better effect. The results showed that when the scanning speed was 50 mV/s and the number of scanning circles was 10, the peak current difference was the largest and the polymerization effect was the best. Therefore, the optimal polymerization rate was selected as 50 mV/s, and the optimal number of polymerization cycles was 10 cycles.

3.3.2 Optimization of enrichment and elution time
The electrode was immersed in a DBP solution containing $5 \times 10^{-6}$ mol/L, and the DPV was measured in PBS solution after taking it out every 2 minutes, the system did not stable until 10 minutes later, which indicated that the imprinted hole on the electrode had been basically filled. The electrode was immersed in the 0.5mol/L hydrochloric acid solution, stirred, and the DPV response value was used as an indicator in a 0.2mol/L PBS solution every 5 minutes, the DBP molecule had been completely removed after 20 minutes. Therefore, an elution time of 20 minutes and an enrichment time of 10 minutes were used.

3.3.3 pH optimization
Different electrodes were prepared by changing the pH (4-9) of the polymerization solution, and the DPV response was used to compare the differences in the results of different pH buffer solutions. The results were shown in figure 3, and the maximum value appeared when the pH was 7. As the pH value increased, the anodic peak (Ep) potential shifted to the negative direction, and the oxidation potential (Epa) had a good linear relationship with the change of pH. The linear equation was $Epa(V) = 0.9329 - 0.031pH$, and the correlation coefficient was 0.9996.

![Figure 3 The electrochemical response diagram of DBP under different pH](image)

3.4 Electrochemical detection of different concentrations of DBP
The DPV method was used to analyze different concentrations of DBP, and the response performance to different concentrations of DBP was shown in Figure 4. In the DBP concentration range of $1 \times 10^{-8}$~$1 \times 10^{-5}$ mol/L, the sensor had a good linear relationship between the response current and the logarithm of the concentration. The linear equation was $I (\mu A) = 47.82 + 4.178 \log C$ (mol/L), the correlation coefficient was 0.9983, and the detection limit was $3.3 \times 10^{-9}$ mol/L (S/N=3). Table 1 showed the performance comparison of different electrodes for detecting DBP. The research indicated MIP/AuNPs/WMCNTs/BDD electrode had a relatively wide linear range and lower detection limit.
Figure 4 DPV method to detect the concentration of DBP aqueous solution

Table 1 performance of different electrodes

| Electrode                          | Detection method | Linear range   | Detection limit |
|------------------------------------|------------------|----------------|-----------------|
| Co(bpyp)/ERGO/GCE                  | DPV              | 0.05-1700 μmol/L | 0.0029 μmol/L   |
| ZrO2-CNFCNF/GCE                    | DPV              | 2×10^{-8}-2×10^{-7} g/L | 3.4×10^{-10} g/L |
| Pd-TPP/RGO/GCE                     | DPV              | 0.1-125 μmol/L   | 7.4 nmol/L      |
| AChE/ERGO/GCE                      | DPV              | 2×10^{-7}-7×10^{-7} mol/L | 1×10^{-9} mol/L |
| MIP/AuNPs/MWCNTs/BDD              | DPV              | 1×10^{-8}-1×10^{-7} mol/L | 3.3×10^{-9} mol/L |

3.5 Stability, anti-interference and reproducibility of the sensor

After the molecularly imprinted electrode was eluted, it was stored in a closed centrifuge tube at room temperature for 7 days. Under the same conditions, the DPV method was used to test the imprinted electrode every 24 hours. The results showed that the peak current was only reduced by 8.6% after being placed for 7 days.

Bisphenol A (BPA), dimethyl phthalate (DMP), diethyl phthalate (DEP), dioctyl phthalate (DOP) and dibutyl phthalate (DBP) were similar in structure, these substances were added to a DBP solution respectively with a concentration of ten times that of DBP, the current responses were observed by the DPV method (Figure 5). It could be seen from the figure that the addition of other substances had little effect on the current response, and the relative standard deviation (RSD) was <3.5%, it indicated that the electrode had good anti-interference.

Figure 5 The peak current of the mixed solution of DBP and different interference substances

The same method was used to prepare 10 MIP/AuNPs/MWCNTs/BDD electrodes, the DPV method was used to detect DBP, and the relative standard deviation of the results of 10 current values was used as an indicator to investigate the reproducibility of MIP/AuNPs/MWCNTs/BDD. The results showed that the relative standard deviation of 10 tests was only 1.91%, indicated that the sensor had good reproducibility.
3.6 Sample analysis
Taking lake water and tap water as actual samples, tested DBP among them, before tested, filtered the water samples to remove particulate impurities in the water. The standard addition recovery method was used to detect the content of DBP in the above water samples. The results were shown in Table 2. The recovery rate of standard addition was between 96.4% and 100.3%, and the RSD was less than 6.7% (n=3), indicated that the method had high accuracy and precision and could be applied to actual water samples.

| Sample    | Blank value (umol/L) | Added (umol/L) | Found (umol/L) | Recovery (%) | RSD%(n=3) |
|-----------|----------------------|---------------|---------------|--------------|-----------|
| Lake-water| 102.5                | 100           | 195.3         | 96.4         | 5.1       |
|           | 300                  | 400.1         | 99.4          | 3.4          |           |
|           | 500                  | 604.2         | 100.3         | 2.8          |           |
|           | 100                  | 98.89         | 98.89         | 6.7          |           |
| Tap-water | 0.0                  | 300           | 290.8         | 96.93        | 4.5       |
|           | 500                  | 500.2         | 100.04        | 3.2          |           |

4.Conclusion
Multi-walled carbon nanotubes and nano-gold particles were used to modify boron-doped diamond electrode (BDD), and dibutyl phthalate (DBP) was used as the template molecule. When the scanning speed was set to 50mV/s and the scanning circle was 10 laps, the MIP film was prepared by electropolymerization, and then MIP/AuNPs/MWCNTs/BDD was successfully constructed. The results showed that the sensor had good reproducibility, stability and selectivity. The linear range was from 1×10⁻⁸ to 1×10⁻⁵ mol/L, the corresponding current and the logarithm of the DBP concentration also showed a good linear relationship. The detection limit was as low as 3.3×10⁻⁹ mol/L(S/N=3), which could be used for trace analysis of DBP in water environment. This method removed the complicated sample pretreatment process, and the equipment was simple to use, which was expected to meet the needs of the scene detection requirements, and provided a new reference for the trace detection of other substances.

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