**Electrochemical sensor based on graphene-modified GCE for rapid quantification of benzo(a)pyrene in dark rubber materials**

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**Abstract.** Electrochemical sensor was constructed by depositing graphene on glassy carbon electrode (GCE), and the electrochemical properties of benzo(a)pyrene (BaP) were characterized. An oxidation peak was observed at 1.13 V according to the cyclic voltammogram with no reduction peak appeared. The differential pulse voltammetry revealed that sorption time and acid concentration play a prominent role in the oxidation of BaP-acetonitrile-NaClO₄. A good linear relationship between oxidation peak current and concentration within the range from 0.5 to 30 ng mL⁻¹ was developed. The limit of detection was calculated as 0.08 ng mL⁻¹. Further, this reliable method was applied for BaP determination of the dark rubber materials on graphene-modified GCE. The advantages of its sensitivity and portability were noteworthy for the sample pretreatment and rapid quantification of BaP compared with gas chromatography-mass spectrometry method.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are persistent organic pollutants owing to their high mutagenicity, teratogenicity and carcinogenicity [1-3]. They constitute a hazard to the human health through food chains being diffused in different ecosystems [4,5]. Among PAHs, benzo(a)pyrene (BaP) exhibits the greatest carcinogenic activity, and it always exists in PAHs contaminated environmental specimens [6]. BaP is usually utilized as an index for evaluating the standard of PAH contamination [7]. Also, oxidation route offers one of the appropriate ways to the removal of hydrocarbons [8-10].

BaP is widely found in rubber products, especially in the dark rubbers which include preformed rubber rolls, antiskid rubber particles and filling rubber particles. Present identification methods for BaP included high-performance liquid chromatography [11,12], gas chromatography-mass spectrometry (GC-MS) [13,14] and liquid chromatography-mass spectrometry [15]. However, most of them involved the employment of expensive and huge instruments. The complicated pretreatment steps make these techniques unsuitable for routine quality control [16-18]. However, the electrochemical method has many advantages such as high sensitivity, low cost, high efficiency and convenience, which has become the research hotspot of scholars [19-21]. For glassy carbon electrode (GCE), the design and synthesis of functional materials specific to BaP remain challenges.

In this paper, the electrooxidation of BaP-acetonitrile-NaClO₄ was adopted. BaP was adsorbed on the graphene-modified GCE in open circuit. Then mass concentrations of BaP (C_{BaP}) were prepared for differential pulse voltammetry (DPV) measurements using electrochemical sensor. The results were
compared with those obtained by GC-MS method. The achievement of selective and portable methods for the quantification of BaP was essential, which attempted to establish simplified and miniaturized assays. Thus, an electrochemical analysis of BaP in rubber particles was demonstrated, and meaningful progress had been made.

2. Experimental section

2.1. Instruments and reagents

The instruments used included a CorrTest CS350H electrochemical workstation (Wuhan, China), and the GC-MS system consisted of a Thermo Scientific Trace 1300 gas chromatograph coupled to an ISQ 7000 mass spectrometer (Waltham, MA, USA). A FEI Quanta FEG 650 scanning electron microscope (Hillsboro, USA), an Eppendorf 5430 desktop high-speed centrifuge (Hamburg, Germany), and an Ida Instrument 6-mm diameter GCE (Tianjin, China) were also utilized.

Water (resistivity of 18.2 MΩ cm) was prepared by the Dura pro 24 water purifier (Shanghai, China). Flaky graphene was purchased from Feynman Nano (Zhejiang, China). 99% ethylene glycol and chromatographic grade acetonitrile were purchased from Macklin Biochemical (Shanghai, China). 100 μg mL⁻¹ BaP in methanol was purchased from Best Reagent (Sichuan, China). The other reagents such as hydrochloric acid and NaClO₄ were of at least analytical grade.

2.2. Preparation of graphene-modified GCE

GCE surface was thrown into a mirror style by polishing with 0.05 μm Al₂O₃/water suspension on filter paper [22]. Ultrasonic cleaning for the electrode was successively performed with anhydrous ethanol and water. A three-electrode system was composed of GCE, Ag/AgCl electrode and platinum electrode. The GCE was immersed in 0.25 mol L⁻¹ hydrochloric acid for 2 min.

Next, 0.02 g graphene was added to 15 mL ethylene glycol (via ultrasonic treatment for 10 min), and a graphene suspension i.e., modified liquid was obtained. 10 μL modified liquid was applied to the surface of the preactivated GCE, and dried under an infrared lamp.

The surface state of the electrode will affect the electrochemical reaction. Therefore, the electrodes used were freshly made and well preserved to prevent contamination. In addition, the prepared electrodes were disposable, which needed to be polished after use during each measurement.

2.3. DPV measurement

To investigate the electrooxidation of BaP, a three-electrode system is employed and the DPV parameters are set in Table 1. The electrochemistry tests were carried out in normal atmosphere.

| Variables                     | Conditions                  |
|-------------------------------|----------------------------|
| Working electrode             | Graphene-modified GCE      |
| Reference electrode           | Ag/AgCl electrode          |
| Counter electrode             | Platinum electrode         |
| Potential (E) range           | 0.7–1.5 V                  |
| Pulse amplitude               | 50 mV                      |
| Pulse width                   | 0.05 s                     |
| Voltage step time             | 0.1 s                      |
| Potential scan rate           | 40 mV s⁻¹                  |

The C_{BaP} at 0.5, 2, 5, 10, 20 and 30 ng mL⁻¹ were prepared with 0.2 mol L⁻¹ electrolyte (NaClO₄ was dissolved in acetonitrile as supporting electrolyte; hydrochloric acid was used to adjust the pH value of
electrolyte at 2.5). The graphene-modified GCE was immersed in above-mentioned BaP standards for 15 min in an open circuit state, and then performed for DPV measurement. The average value of corresponding peak current was recorded as the oxidation peak current \( I \). The electrochemical tests were collected in triplicate, and the error bars represent standard deviations (SD).

2.4. Sample pretreatment and GC-MS analysis

0.4 g rubber particles with 20 mL acetonitrile was incubated in an ultrasonic water bath device, and extracted at 60 °C for 40 min. After cooling to room temperature, the mixture was centrifuged at 3000 rpm for 10 min, and then filtered by 0.2 μm membrane. 1 mL extract was transferred into NaClO₄-acetonitrile for electrochemical test.

1 mL extract in injection bottle was prepared for GC-MS analysis. The peak areas were determined based on both retention times and mass-to-charge ratios, and data were acquired in the selected ion monitoring mode. The average integral areas of chromatographic peaks \( (n = 3) \) were used to calculate \( C_{\text{BaP}} \) of the real samples, comparing with the results of the electrochemical sensor method.

3. Results and discussion

3.1. Morphology of modified electrode surface and redox behavior of BaP

The GCE was modified with 10 μL graphene suspension, and then dried under an infrared lamp. Scanning electron microscopy (SEM) was used to observe the surface morphology of graphene-modified GCE, and the accelerating voltage was 10.0 kV. The original GCE surface was smooth before treatment, but changes occurred on the surface after modification (Figure 1A), and the graphene sheets were packed in ethylene glycol dispersion (Figure 1B).

The bare GCE and graphene-modified GCE were immersed in 10 ng mL⁻¹ BaP-acetonitrile-NaClO₄ for 15 min in open-circuit condition, and then performed for cyclic voltammetry (CV). No oxidation peaks were observed in NaClO₄-acetonitrile electrolyte (Figure 2A) and 10 ng mL⁻¹ BaP-acetonitrile-NaClO₄(Figure 2B) on bare GCE, while a distinct peak at 1.13 V (vs. Ag/AgCl) was observed (Figure 2C) that was attributed to the oxidation of adsorbed BaP on modified GCE. The well-defined oxidation peak was observed with no reduction peak, indicating that the reaction was an irreversible redox process. After modification, graphene provided a microchannel for the electron transfer due to its large specific surface area. Hence, graphene-modified GCE has better electrochemical properties. The redox potential of BaP was a basis for distinguishing BaP from other substances to ensure its high selectivity.
Figure 2. CV curves of 0.2 mol L\(^{-1}\) NaClO\(_4\)-acetonitrile (A) and 10 ng mL\(^{-1}\) BaP-acetonitrile-NaClO\(_4\) (B) on bare GCE; CV curve of 10 ng mL\(^{-1}\) BaP-acetonitrile-NaClO\(_4\) on graphene-modified GCE (C) at potential from 0 to 1.5 V.

3.2. Effect of acid concentration and sorption time

The concentration of hydrochloric acid was directly related to the stability of BaP in solution. When the acid concentration was from 0.1 to 0.35 mol L\(^{-1}\) in bottom solution, the \(I\) of 10 ng mL\(^{-1}\) BaP-acetonitrile-NaClO\(_4\) increased quickly during the inception phase, revealing that the hydrochloric acid provided protons to promote the oxidation reaction of BaP. But hydrochloric acid continued to be added, it had little effect on the peak current (Figure 3A). Thus, protons needed for the oxidation reaction was saturated, and the hydrochloric acid concentration was selected as 0.25 mol L\(^{-1}\).

Subsequently, the sorption time at 3–24 min was discussed (Figure 3B), and the result demonstrated that BaP sorption was an essential step for achieving the oxidation of BaP at low concentrations. When the time was \(\geq 15\) min, BaP on electrochemical sensor has reached a saturation state. The sorption time affects amount of BaP adsorbed on the electrode surface.

During the immersion stage, BaP was adsorbed and enriched on the electrode surface. The redox reaction of BaP occurred at a specific potential, and the peak current was generated in the energized phase. The magnitude of the peak current was proportional to the concentration of BaP.
3.3. Electrochemical detection of BaP in dark rubber particles
The electrochemical sensor was immersed in BaP-acetonitrile-NaClO₄ at 0.5–30 ng mL⁻¹, and then the BaP standards were determined using DPV (Figure 4A). The relationship between \( I \) and \( C_{\text{BaP}} \) was acquired: \( I = 1.439C_{\text{BaP}} + 15.72 \), squared correlation coefficient \( R^2 = 0.991 \) (Figure 4B). The limit of detection (LOD) was calculated as 0.08 ng mL⁻¹ based on the following equations: LOD = \( 3s/k \); where \( s \) is the SD of the peak currents for blank sample \( (n = 11) \), and \( k \) is the slope of the calibration curve. Thus, this electrochemical sensor constructed has good sensitivity and can be used as a rapid analytical method for BaP.

\[ I = 1.439C + 15.72 \]
\[ R^2 = 0.991 \]

C_{\text{BaP}} of the black and grey rubber particles were detected by electrochemical sensor and GC-MS method. The spike-in sample had a specific concentration of BaP (2, 6, 10 ng mL⁻¹) sequentially added to the mixture. The mean \( C_{\text{BaP}} \) of the black and grey rubber particles were 10.1 and 5.9 ng mL⁻¹ by electrochemical method, while they were 10.3 and 6.2 ng mL⁻¹ by GC-MS, whose deviations were \( \leq 5.1\% \). The mean recoveries with electrochemical method were 95.8%–107.6%, while the mean recoveries for GC-MS method were 96.9%–107.3% (Table 2). Their difference was small, and electrochemical method is convenient. The relative standard deviations (RSD) of graphene-modified GCE and GC-MS method were \( \leq 5.3\% \) and \( \leq 4.7\% \), respectively. The accuracy and reproducibility of graphene-modified GCE were relatively good in terms of electrochemical detection, demonstrating that this electrochemical sensor system can be applied for determination of BaP.
Table 2. Recoveries of modified GCE and GC-MS method for BaP determination of real samples \((n = 3)\), and \(n\) stands for repetitions.

| Sample               | Added (ng mL\(^{-1}\)) | Found (ng mL\(^{-1}\)) | Recovery (%) | RSD (%) |
|----------------------|--------------------------|-------------------------|--------------|---------|
|                      |                          | Electrochem | GC-MS | Electrochem | GC-MS | Electrochem | GC-MS |
| Black rubber particles | 10                       | 21.5        | 21.1  | 107.0       | 103.9 | 4.3         | 3.7   |
|                      | 6                        | 16.8        | 17.3  | 104.3       | 106.1 | 5.3         | 4.6   |
|                      | 2                        | 11.7        | 12.0  | 96.7        | 97.5  | 4.1         | 4.0   |
| Grey rubber particles | 10                       | 16.3        | 15.7  | 102.5       | 96.9  | 4.5         | 3.6   |
|                      | 6                        | 11.4        | 12.5  | 95.8        | 102.4 | 3.9         | 4.3   |
|                      | 2                        | 8.5         | 8.8   | 107.6       | 107.3 | 5.0         | 4.7   |

4. Conclusion

A graphene-modified GCE was successfully applied for the adsorption and subsequent oxidation of BaP-acetonitrile-NaClO\(_4\). Surface functionalities investigated by electrochemical behaviors of BaP on bare GCE and modified GCE indicated that the reaction was an irreversible redox process. Analytical procedures for the DPV measurement of BaP using the modified GCE were developed. The acid concentration and sorption time affected the current of oxidation peak. The mean recoveries of BaP in dark rubber materials via electrochemical method were 95.8\%–107.6\%, and the RSD were \(\leq 5.3\%\). The results verify this quantitative approach for BaP as well as other PAHs, serving as a facile detection of trace risk chemicals of environmental interest. Due to its good sensitivity, the designed assay is promising to open a novel perspective in the field of PAHs analysis.

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