Quick determination of phenanthrene in coloured latexes using graphene-modified glassy carbon

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Abstract. A facile detection system was devised by attaching graphene to glassy carbon, and the electrooxidation of phenanthrene (Phe) was investigated. The linear sweep voltammetry revealed that the modified liquid quantity and electrolyte concentration affected the oxidation of Phe-acetonitrile-NaClO₄ whose applicability conditions were optimised. The peak oxidation current in the range of 1-120 nmol L⁻¹ has a good linear relationship with Phe concentration (I= 0.1066cₚhe +19.04, R²=0.9975). Furthermore, we reported an electrochemical assay for Phe in coloured latexes on a graphene-modified glassy carbon. It was meaningful for quick determination of Phe, especially in simpleness and detection time compared with GC–MS.

1. Introduction
Polycyclic aromatic hydrocarbons are environmental pollutants with carcinogenic and mutagenic effects [1,2]. They are present in water, soil and latexes, constituting a danger to human beings [3-5]. Phenanthrene (Phe) is a chemical carcinogen with strong carcinogenicity [6,7]. Phe is not intentionally added to the latexes but is produced in the manufacturing process. They are widely found in synthetic latexes, especially in coloured latexes. Electrooxidation supplies available ways to reduce the toxicity of organic compounds [8,9]. Given the issues of the high social popularity rate of latex products, it is important to investigate the Phe in dark coloured latexes.

Existing techniques for determination of Phe include GC–MS [10,11], fluorescence spectrometry [12], HPLC [13] and LC–MS [14]. Although the above methods involve large scale valuable instruments, the complex pretreatment steps make these techniques less attractive [15,16]. The preparation of proper materials on glassy carbon electrodes (GCE) is the main development direction for the quick determination of polycyclic aromatic hydrocarbons.

Here, the sorption of Phe-acetonitrile-NaClO₄ on modified GCE and the electrooxidation of Phe were evaluated. Different molar concentrations of Phe (Cₚhe) were analysed using linear sweep voltammetry (LSV). The electrochemistry (EC) data were compared with those detected by GC–MS. The selective EC method for Phe determination was necessary, establishing an economical and mini-type approach. The EC analysis of Phe in latexes was expected to provide a theoretical basis for the quality control of latexes.
2. Experimental section

2.1. Instruments and materials
The main instruments used were CH Instrument s CHI660E electrochemical workstation (Shanghai, China) and Agilent 7890B/5977B gas chromatograph/mass spectrometer (Palo Alto, USA). Additionally, a Hitachi SU3500 scanning electron microscope (Tokyo, Japan), a Grant LMC-3000 centrifuge (Cambridge, UK) and an ID 4-mm diameter GCE (Tianjin, China) were also used.

Graphene (97%), glycol (99.5%) and chromatographic grade acetonitrile were purchased from Macklin Biochemical (Shanghai, China). Phenylalanine (200 μg mL\(^{-1}\)) was purchased from Biow (Beijing, China). The other reagents (e.g. hydrochloric acid, sodium perchlorate, ethanol) have at least an analytical grade. Water (resistivity of 18.2 M\(\Omega\)·cm) was prepared by the Millipore Milli-Q system (Boston, MA, USA).

2.2. Modified GCE fabrication
The surface of GCE was polished with 0.3 μm alumina powder/water suspension on metallographic sandpaper. The three-electrode system consists of GCE, platinum electrode and Ag/AgCl electrode. The GCE was put in 1.0 mol L\(^{-1}\) HCl and the oxidation-reduction cycles were studied from −0.4 to 0.7 V. The potential scan rate was repeated as 0.5 V s\(^{-1}\) until the cyclic voltammetry (CV) characteristics remained stable and the activation was completed.

Under normal stirring conditions, the modified liquid was obtained by adding 0.03 g graphene to 20 mL glycol under normal stirring conditions. Then, 8 μL of the modified liquid was applied to the surface of the preactivated GCE and dried under an infrared lamp.

2.3. Electrooxidation measurement
For deoxygenation, high purity nitrogen was bubbled above the solution for more than 10 min to separate the reaction from the air. Here, the three-electrode system was employed: graphene-modified GCE, Ag/AgCl electrode and platinum electrode were the working, reference and counter electrodes, respectively. To investigate the electrooxidation of Phenylalanine in this system, LSV measurements were performed within the range from 0.7 to 1.3 V at a potential scan rate of 50 mV s\(^{-1}\).

The \(C_{\text{Ph}}\) of 0, 20, 40, 60, 80, 100, 120, 150, 210 and 250 nmol L\(^{-1}\) was prepared with 0.25 mol L\(^{-1}\) acetonitrile-NaClO\(_4\) (adjusting the pH of the electrolyte to strong acid condition). The modified GCE was immersed in the Phenylalanine standards with open circuit for 20 minutes. The average value of the LSV peak current was expressed as the oxidative current (\(I\)). All EC tests were conducted at 25 °C.

2.4. Sample pre-treatment and chromatographic analysis
For sample pre-treatment, 0.5 g latex in 10 mL acetonitrile was sealed in a thermostatic water tank and extracted at 65 °C for 30 minutes. The process was repeated twice with 20 mL acetonitrile, centrifuged at 2000 rpm for 15 min. Then, it was filtered by organic filtration and brought to volume. The extraction solution was added to acetonitrile for LSV.

Thus, 0.8 mL extract was ready for chromatographic analysis and the data were acquired in SIM mode to reach high selectivity (Table 1). The average integral areas of the GC peaks were used to calculate \(C_{\text{Ph}}\) of the latex samples.
Table 1. Analysis conditions of GC–MS.

| Apparatus            | Conditions                                                                 |
|----------------------|-----------------------------------------------------------------------------|
| Gas chromatograph    | Column: HP-5MS (30 m × 0.25 mm × 0.25 µm)                                    |
|                     | Carrier gas: He (99.999%) at 1.0 mL min⁻¹                                   |
|                     | Temperature programme: 150 °C (2 min) at a rate of 10 °C min⁻¹ to 280 °C (10 min) |
|                     | Injector mode and volume: splitless, 1 µL                                  |
|                     | Ionisation mode: EI (70 eV)                                                |
| Mass spectrometer    | Ion source temperature: 250 °C                                             |
|                     | MS interface temperature: 280°C                                            |
|                     | Solvent delay: 7 min                                                        |

3. Results and discussion

3.1. Glassy carbon morphology and Phe redox behaviour

GCE was treated with 8 µL of the modified liquid under infrared drying. Scanning electron microscopy was used to observe the microstructures of the modified GCE. After modification, GCE changes and graphene surface deposits to a certain extent (Figure 1).

The modified GCE was placed in 100 nmol L⁻¹ Phe-acetonitrile-NaClO₄ under open circuit and characterised by CV and LSV analysis. No oxidative peak was observed in 0.25 mol L⁻¹ electrolyte (Figure 2A); an oxidative peak appeared, which was caused by the electrooxidation of Phe on the electrodes (Figure 2B). The oxidative peak at 1.07 V (vs. Ag/AgCl) was due to graphene combined with GCE possessing a large specific surface area and outstanding sorption capacity (Figure 2C).

Figure 1. Surface microstructures of the bare GCE (A) and graphene-modified GCE (B).
Figure 2. CV of 0.25 mol L\(^{-1}\) electrolyte (A) and 100 nmol L\(^{-1}\) Phe (B) on modified GCE within 0–1.3 V; LSV of 100 nmol L\(^{-1}\) Phe on modified GCE (C) within 0.7–1.3 V.

3.2. Effect of the modified liquid quantity and electrolyte concentration
The modified liquid quantity has a significant effect on the electronic transfer [17]. For this experiment, 3–11 μL graphene suspension was applied to the surface of the preactivated GCE. The \(I\) of Phe was measured by LSV. Within the volume ranging from 3 to 8 μL, the \(I\) continued to increase (Figure 3A). When the volume was 8 μL, the \(I\) reached a maximum. By increasing the volume from 8 to 11 μL, the \(I\) decreased gradually. This was because the modified film was too thick, which hindered the electron transfer.

The \(I\) under different electrolyte concentrations (0.1–0.4 mol L\(^{-1}\)) is shown in Figure 3B. In the beginning, the \(I\) increased significantly. When the electrolyte concentration was 0.25 mol L\(^{-1}\), the \(I\) reached a maximum. The peak current tended to be stable, exhibiting a final equilibrium. Therefore, the concentration of NaClO\(_4\)-acetonitrile played an important role in the solution conductivity.
3.3. EC assay of Phe in dark coloured latex

The modified electrodes were submerged in 1–250 nmol L\(^{-1}\) for 20 min and the standard solutions were detected. As shown in Figure 4, the relationship between \(I\) and \(C_{\text{Phe}}\) was determined by the equation: 
\[
I = 0.1066C_{\text{Phe}} + 19.04, \quad R^2 = 0.9975.
\]
For the LSV analysis of Phe, the linear range was 1–120 nmol L\(^{-1}\) and the limit of detection was calculated to be 0.7 nmol L\(^{-1}\) for S/N = 3. Thus, this EC system has good sensitivity, which can be applied to Phe determination as a fast method.

**Figure 4.** Current response at different concentrations; embedded graph: linear relationship between \(I\) and \(C_{\text{Phe}}\) when the concentration ranges from 1 to 120 nmol L\(^{-1}\).

Coloured latexes were pretreated according to Section 2.4 to get the extract. \(C_{\text{Phe}}\) of the specimens were characterised using EC and GC–MS. The spike-in specimens had the concentrations 0.2, 0.8, 1.4 \(\mu\)mol L\(^{-1}\) in the preparation. The \(C_{\text{Phe}}\) of the latex was 1.40 \(\mu\)mol L\(^{-1}\) by EC and 1.45 \(\mu\)mol L\(^{-1}\) by GC–MS, of which the deviation was <3.6%. The mean recoveries with EC ranged from 94.3% to 106.4%, while the mean recoveries for the chromatographic method ranged from 98.2% to 105.8% (Table 2).

The relative standard deviations of EC and GC–MS were \(\leq 5.1\%\) and \(\leq 4.5\%). The reproducibility and accuracy of the glassy carbon were comparatively good in the field of EC, indicating that this system can be used for quantitative study of Phe in real samples.
### Table 2. Mean recoveries of EC and GC–MS for Phe \((n = 3)\).

| Specimen Added \((\text{μmol L}^{-1})\) | Found \((\text{μmol L}^{-1})\) | Recovery (%) | Relative standard deviation (%) |
|--------------------------------------|--------------------------------|--------------|---------------------------------|
|                                       | EC                             | GC–MS        | EC                             | GC–MS             |
| Dark coloured latex                   | 1.4                            | 2.98         | 2.91                           | 106.4             | 102.1             | 4.9             | 3.8             |
|                                      | 0.8                            | 2.33         | 2.38                           | 105.9             | 105.8             | 5.1             | 4.1             |
|                                      | 0.2                            | 1.51         | 1.62                           | 94.3              | 98.2              | 4.3             | 4.5             |

### 4. Conclusion

Electrooxidations of Phe-acetonitrile on the graphene-modified glassy carbon were investigated in this study and a low oxidative peak potential \((1.07 \text{ V vs. Ag/AgCl})\) was achieved. The modified liquid quantity and electrolyte concentration affected the oxidative peak current. The mean recoveries of Phe in coloured latex by EC ranged from 94.3\% to 106.4\%, whose relative standard deviations were \(<5.1\%\). The recovery experiment indicated that the EC method possessed practical applicability to Phe analysis and the oxidising characteristics of Phe showed an excellent selectivity. Hence, the Phe determination in latexes was well developed, and these results can assist future investigations of polycyclic aromatic hydrocarbons.

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