Ferrocene-Graphene nanocomposite modified glassy carbon electrode for amaranth detection

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Abstract. A non-covalent nanohybrid of Ferrocene (Fc) with grapheme (Gr) via π-π interaction was proposed for amaranth detection. The synthesized Fc-Gr composite was modified on glassy carbon electrode (GCE) and characterized by cyclic voltammetry (CV) as well as differential pulse voltammetry (DPV). Cyclic voltammogram and differential pulse voltammogram demonstrated successful fabrication of Fc-Gr composite-modified GCE for amaranth detection. DPV was used to obtain a linear range of amaranth detection. The linear range of amaranth detection with a modified sensor was expressed from 10 to 250 µM of amaranth. The limit of detection was obtained at 155 nM of amaranth. Based on these results, the developed sensor provides a good alternative for the determination of amaranth. Moreover, the developed sensor can hopefully be utilized and adapted for detection of other substances.

Keywords: Amaranth; Ferrocene; Graphene; Cyclic voltammetry; Differential pulse voltammetry

1. Introduction

In recent years, several food additives have been permitted in the food industry. Amaranth is one of them. Amaranth is a synthetic food colouring substance that provides red colorant. It has been widely used to make more fascinating food. However, excessive consumption of amaranth has become a concern because it has the potential to cause many adverse health effects such as nausea, vomiting, dizziness. Further, it is hazardous to internal organs such as the liver and kidneys. Moreover, amaranth has been reported to be hazardous to fetuses [1]. Therefore, the European Food Safety Authority (EFSA) defined an acceptable daily intake of amaranth at 0.15 mg/kg of body weight per day [2]. As amaranth’s structure contains azo group -(N=N)- which can be reduced at the electrode surface, many researches have proposed electrochemical methods with different modification electrodes to determine amaranth levels in food [3-7].

Graphene is comprised of a single layer of carbon atoms arranged in a honeycomb structure. Due to its properties including excellent conductivity, electron transfer promotion and large surface area [8, 9],

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it has been widely used to modify electrodes in the electrochemistry field to enhance sensor effectiveness. However, it is prone to restack due to strong π-π interaction between graphene sheets [10, 11]. Therefore, it is imperative to decorate graphene to enhance its dispersibility and stability.

Ferroocene (Fc) consists of two cyclopentadienyl rings bound on opposite sides of a central iron atom [8]. A π-electron system also exists in the molecule [12]. Thus, it is possible to intercalate Fc between layers of graphene sheet via π-π interaction. There have been numerous reports concerning the non-covalent hybridization of Fc on graphene oxide [10, 13, 14], reduced graphene oxide [8, 9, 15] and carbon nanotubes [16] to enhance electrochemical catalytic properties. However, few reports exist concerning research on the hybridization of Fc on graphene [11].

In this work, facile synthesis of Fc functionalized graphene is proposed. Fc-Gr nanocomposite was modified on glassy carbon electrode for amaranth detection. The modified electrode was characterized by cyclic voltammetry (CV) and differential pulse voltammetry (DPV).

2. Experiment details

2.1. Reagents and apparatus

Amaranth and Ferrocene (Fc) were purchased from Sigma Aldrich. Pristine graphene with diameter between 20-100 nm was obtained from cheaptubes.com. Electrochemical measurement was performed on an AutoLab PGSTAT128N system (EcoChemie, Netherlands). The three electrodes system consisted of modified glassy carbon electrode; GCE (3 mm in diameter) as working electrode, Ag/AgCl as reference electrode and Platinum wire as counter electrode. 0.1M phosphate buffer solution (PBS) pH 7.0 containing 0.1M KCl was used as electrolyte for electrochemical measurement. All solutions were prepared with deionized (DI) water and purged with nitrogen gas for 30 minutes to eliminate dissolved oxygen.

2.2. Synthesis of ferrocene-graphene (Fc-Gr) nanocomposites

20 mg of graphene was dispersed in 10 mL of ethanol by sonication for 30 min. 80 mg of Fc was dissolved in 10 mL of ethanol. Then, the dissolved Fc solution was added into a dispersed graphene solution and sonicated for 4 h. After that, the Fc-Gr nanocomposites were collected by centrifugation at 13,000 rpm for 10 min. The pellet was washed several times with ethanol to eliminate non-hybridized Fc. Finally, the precipitated Fc-Gr nanocomposites were dried at 37°C and stored under dry conditions.

2.3. Preparation of ferrocene-graphene (Fc-Gr) modified glassy carbon electrode

The bare GCE was polished on 0.05 alumina powder until a mirror-like surface was obtained. Then, it was rinsed with DI water followed by sonication in 70% ethanol and DI water for 5 min. The synthesized Fc-Gr nanocomposites were dispersed in DI water to a final concentration of 1 mg/mL. Fc-Gr modified GCE was prepared by dropping 5 µL of Fc-Gr solution on the surface of cleaned GCE followed by drying at room temperature for at least 1 h.

3. Results and discussion

3.1. Characterization of electrode modification

To characterize the assembly process, CV was studied on bare GCE, Gr modified electrode and Fc-Gr modified GCE. Fig. 1 represents cyclic voltammograms of three types of electrode in 10 mM Fe(CN)₃⁻⁴ containing 0.1 M KCl at a scan rate of 50 mV/s. The presented current was the signal of Fe(CN)₆⁻⁴⁺ in electrolyte on different electrodes. The current increased from bare GCE, Gr modified electrode and Fc-Gr modified GCE. Increasing current was the effect of Gr on electrode. The large surface area can help to increase the electron transfer rate and conductivity performance of electrode, which affects the current increment. At the same time, intercalation of Fc molecule can prevent restacking of graphene and help to form a larger conjugated system. Therefore, it can accelerate the electron transfer.
rate on Fc-Gr modified GCE. The highest current on Fc-Gr modified GCE demonstrates that Fc is hybridized on Gr sheet by π-π interaction and the composite is stacked on GCE surface by physical absorption.

Figure 1. Comparison of cyclic voltammograms for bare GCE (solid line), Gr-modified GCE (dash line) and Fc-Gr modified GCE (dot line) in 10 mM Fe(CN)$_6^{3-4+}$ containing 0.1 M KCl at a scan rate of 50 mV/s.

Figure 2. Comparison of differential pulse voltammograms for 10 µM amaranth on bare GCE (solid line), Gr modified GCE (dash line) and Fc-Gr modified GCE (dot line) in 0.1 M PBS containing 0.1M KCl.
3.2. Electrochemical behaviour of amaranth on modified electrode

The electrochemical behaviour of amaranth was tested on three different electrodes to study the performance of the modified electrodes. Differential pulse voltammograms of 10 µM amaranth in 0.1 M PBS containing 0.1 M KCl were recorded on bare GCE, Gr modified GCE and Fc-Gr modified GCE at a scan rate of 50 mV/s. Fig. 2 demonstrates that the anodic peak current of amaranth was observed on all three types of electrode with slightly different potential. Anodic peak current of amaranth appeared at potential 0.64, 0.67 and 0.66 on bare GCE, Gr modified GCE and Fc-Gr modified GCE, respectively. The results were consistent with the characterization of electrode modification by CV. The highest current was obtained from Fc-Gr modified GCE, while the current of Gr modified GCE was higher than bare GCE. The higher current was the result of Gr and Fc-Gr on the electrode. The increment of the anodic peak current can prove that Fc-Gr composite helps to accelerate electron transfer between amaranth and electrode.

3.3. Determination of linear range and limit of detection

Standard solutions of amaranth with various concentrations at 0.41, 0.82, 1.23, 1.64, 2.05, 2.46, 2.87, 3.28, 3.69, 4.10, 6.15, 8.20, 10.25, 12.30 and 14.35 mM were prepared in 0.1 M PBS containing 0.1 M KCl. 250 µL of each solution was injected into an electrochemical cell to obtain the desired concentration in bulk for analysis. DPV of each concentration was studied at potential 0.4 to 0.9 V and at step potential 0.01 V. The current signal increased when amaranth concentration was increased. However, current signal tended to be stable when amaranth concentration reached 300 µM as demonstrated in Fig.3. Thus, the linear range was obtained at amaranth concentration ranging from 10 to 250 µM. The linear equation can be expressed as $y = 0.818x - 4.327$ ($R^2 = 0.971$). The detection limit was calculated from the ratio between 3 times the standard deviation of the blank ($n = 10$) and slope from the linear range. A detection limit of 155 nM was obtained from this developed sensor.

![Figure 3. Linear relationship between the obtained current and concentration of amaranth in a range from 10 to 250 µM (n=3).](image)

However, when comparing the modified sensor with a previous work that applied Fc-Gr modified GCE for chloromycetin detection [11], our modified sensor still has an advantage of facile synthesis.
Although, both our modified and the chloromycetin sensors utilized the \( \pi-\pi \) interaction to intercalate between graphene layer but their synthesized composite was not immobilized onto GCE via physical absorption. It was mixed with graphite and polyvinylidene fluoride (PVDF) for the formation of conductive polymer to coat on GCE. Although, PVDF has good binding property but it has poor conductivity. Thus, excessive PVDF can inhibit electron transfer on the electrode. Moreover, the modified sensor can be optimized by other factors, for examples, pH of electrolyte, scan rate or accumulation time. These factors influence the catalytic reaction of amaranth, therefore the developed sensor can be optimized to improve the performance of the sensor.

4. Conclusion
In summary, Fc-Gr composite modified GCE was successfully used as a sensor for amaranth detection. The synthesis of the composite was easily successful by \( \pi-\pi \) interaction between graphene and Fc without hazardous reagent. The preparation and fabrication of the sensor can be achieved with an easy method that is cost-effective and environmental friendly. The developed sensor worked well and demonstrated a wide linear range with low detection limit. However, this work did not test using a real sample. Thus, there are many other factors that should be optimized and adjusted by future research in order to obtain greater performance from the sensor.

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