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Sensitive Artemisinin Electrochemical Sensor Based on Polymerized Molecularly Imprinted Membranes

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An electrochemical molecular imprinted sensor was successfully developed for ultra-sensitive detection of artemisinin (AN). The electrochemical sensor was fabricated on a poly(diallyldimethylammonium) chloride-reduced graphene oxide (PDDA-RGO) functionalized glassy carbon electrode (GCE) using acrylamide (AM) and ethylene glycol dimethacrylate (EGD) as monomer and cross-linking agent, respectively. The fabricated AN sensor was characterized by different techniques. After optimization of experimental parameters, the proposed AN sensor showed a wide detection linear range, low detection limit, excellent sensitivity and selectivity. The practical performance of the AN sensor showed an accurate determination of AN in *Artemisia annua* L extract.

Keywords: Molecularly imprint; Artemisinin; Graphene; Acrylamide; Electrochemical sensor

1. INTRODUCTION

Artemisinin (AN) is an important substance extracted from *Artemisia annua* L, which has been widely used for treating malaria [1-3]. In fact, AN is the most effective drug to fight with malaria. The discovery of the AN saves millions peoples life in Asian and Africa. The detection and determination of AN are considerably important in the field of clinical and pharmaceuticals [4]. Therefore, it is a great need for developing a simple, accurate, low-cost and selective technique for AN detection. The detection of AN cannot be achieved using optical analysis due to its lacks of characteristic absorption peak. Chromatography is the most common method for AN determination, such as HPLC-MS and HPLC-MS/MS [5-8]. However, chromatography based methods require a high-cost apparatus and skilled operators. Moreover, these methods usually require a complex pre-treatment of samples. Other

combined methods also developed for AN analysis such as TCL and FASA-HPCE-PDA [9]. But the TCL cannot be used for quantitive AN detection and the FASA-HPCE-PDA method lacks of sensitivity. Based on the existence problems, electrochemical method shows a quick, simple and low-cost performance because it only requires simple sample pretreatment, high sensitivity and wide detection range [10-15]. However, the AN is a poor electrocatalytic active species, which cannot be detected using a common commercially available electrode. Electrode surface modification was adopted for improving the performance of the common electrode. For example, Gong and co-workers fabricated a [FeT(o-glu)PPCl]/Au NPs modified electrode for successful determination of AN in plant sample [16]. Reys and co-workers demonstrated an AN electrochemical sensor based on the hemin immobilized on a titanium oxide modified silica [17]. Although these proposed electrochemical sensor can be used for AN detection, the detection sensitivity and linear range still unsatisfactory. Therefore, further improving the performance of the electrochemical AN sensor is an important issue in this field.

Molecular imprinting approach, which is a method uses polymeric substances for recognition of target molecules. Molecularly imprinted membrane is one of the molecular imprinting approach has surface cavities could recognition of structure similar molecules with template molecules. So far, the molecularly imprinted membrane has been successfully used for electrochemically determination of various substances, including glutathione [18], dapsone [19], propyl gallate [20], dicyandiamide [21], metolcarb [22], cholesterol [23], estradiol [24], furosemide [25], tulathromycin [26], norepinephrine [27] and glyphosate [28]. In this study, AN was successfully determined using a molecularly imprinted membrane method. Before the molecularly imprinted membrane assembly, the grassy carbon electrode was firstly modified using poly(diallyldimethylammonium) chloride (PDDA) functionalized reduced graphene oxide (RGO). The AN imprinted membrane was fabricated using acrylamide (AM) and AN as functional monomer and template, respectively. The analytical performance of fabricated AN sensor was studied and discussed. Moreover, the proposed AN sensor was then used for analyzing AN content in *Artemisia annua* L extract.

2. EXPERIMENTS

Synthetic graphite (average diameter <30 μm), poly (diallyldimethylammonium chloride) (20 wt. % in H_2O), artemisinin (AN; 3R,5aS,6R,8aS,9R,12S,12aR)-Octahydro-3,6,9-trimethyl-3,12-epoxy-12H-pyrano[4,3-j]-1,2-benzodioxepin-10(3H)-one; $C_{15}H_{22}O_5$), AM, EGD and azobisisobutyronitrile (AIBN) were purchased from Sigma. Other chemical reagents are analytical grade.

Graphene oxide (GO) was prepared with the modified Hummers method with little modification [29, 30]. Briefly, graphite (4 g) was added into 100 mL of concentrated H_2SO_4 and followed adding 2.5 g $NaNO_3$. Then, $KMnO_4$ (15 g) was added into the above solution under stirring. The reaction was maintained below 20 °C. 200 mL water was then slowly added into above solution until boiling and maintained at this state for half hour and then stopped by adding excess water and H_2O_2 solution. Low concentration of HCl was used for washing sample. $BaCl_2$ was used as an indicator for determining the sample was neutralized. Then, the solid sample was centrifuged and dried in an oven. The surface negatively charged oxygen containing groups on GO make it could simply

disperse into water. PDDA-RGO was synthesized using an one-pot hydrothermal approach. Briefly, 2 mL PDDA was added into 10 mL GO (1 mg/mL) and went 2 h bath sonication. 20 mg urea was then added into the mixture and further sonicate for 1 h. The whole mixture was then transferred into a 50 mL Teflon-lined stainless steel autoclave. The autoclave was then heated at 120 °C for 10 h. After naturally cool down, the result dispersion was centrifuged at washed by water and ethanol twice. RGO without PDDA functionalization was also prepared using a similar approach except adding PDDA. For electrochemical measurement, a glassy carbon electrode (GCE) was polished by different size of alumina powder dispersion. The GCE surface modification was according to following procedures: 5 μL of PDDA-RGO (1 mg/mL) was dropped on the GCE surface and the solvent was evaporated in a fume hood. The electrochemical determination experiments were carried out at an electrochemical workstation (CHI 660a) using three electrodes system, which a GCE as working electrode, a platinum electrode as the auxiliary electrode and an Ag/AgCl (3M KCl) as the reference electrode. The DPV measurements were carried out at scan range between —0.2-0.6 V. The modulation time was set as 0.05s with a time interval of 0.2s and a step potential of 0.8mV/s.

The AN imprint was prepared through the polymerization of AM. Specifically, 0.02 mM of AN and 0.08 mM AM were dissolved in to 1 mL acetonitrile. After a half hour sonication, 0.3 mM EGD and 0.02 mM azobisisobutyronitrile were added into the above solution. After further a half hour sonication, above solution was dropped on the PDDA-RGO modified GCE and covered with a glass. The molecular imprinted membrane was formed after drying the electrode in an oven. The AN was then removed by inserting the electrode into the 1:1 (v/v) acetic acid/methanol. The result electrode was denoted as MI-PDDA-RGO/GCE. Figure 1 shows the schematic diagram of the fabrication process.

The morphology of sample was observed using a ZEISS, SUPRA 55 field emission scanning electron microscopy (FESEM) and a high-resolution transmission electron microscopy (HRTEM) a JEOL (JEM-2100) at an acceleration voltage of 200kV.

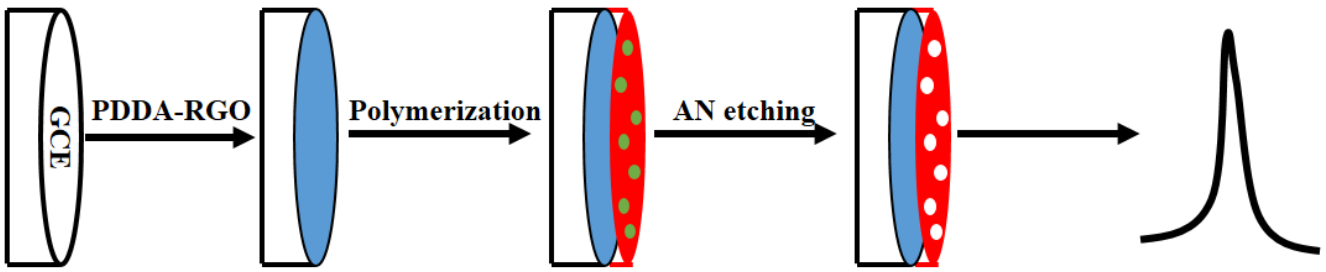

Figure 1. Schematic diagram of the fabrication of molecular imprinted membrane AN electrochemical sensor.

For real sample test, 2.0 g *Artemisia annua* L was added into 20 mL water and heated at 70 °C for 1 h. Then, the extract was filtered and dried in an oven. The solid sample was then dissolved into 10 mL ethanol and used as a stock solution. For comparison purpose, HPLC (Shimadzu Prominence) detection was also carried out. An HPLC instrument (Shimadzu Prominence). The protocol was based on a 50:30:20 (%, v/v) acetonitrile:water:methanol mobile phase with a column temperature of 45 ◦C.

Columns used were: (1) Shimadzu XR-ODS 50 mm × 2 mm with a 2.2 m deactivated type B silica, 12 nm pores at a column flow-rate 0.5 mL min^{-1} and (2) a Betasil C18 5 m 250 mm × 4.6 mm at column flow-rate 1.0 mL min^{-1}.

3. RESULTS AND DISCUSSION

Figure 2A and B shows the SEM and TEM images of PDDA-RGO. As shown in the figure, the PDDA-RGO remains excellent dispersibility. The nanosheets presented a crumpled and winkled structure, which is the natural morphology of the graphene, indicating the functionalization of PDDA could prevent the re-stacking effect commonly caused in RGO. Figure 2C and D shows the SEM images of the MI-PDDA-RGO before and after removal of AN. It can be seen that the morphology of the mixture are different. A thinker film was observed before the AN removal, indicating the successful polymerization. After removal AN using acetic acid and methanol, the film surface seems destruction due to the dissolve of AN molecules. The removal of AN results a very rough surface morphology of the electrode surface, which provides a larger surface area for target molecule detection.

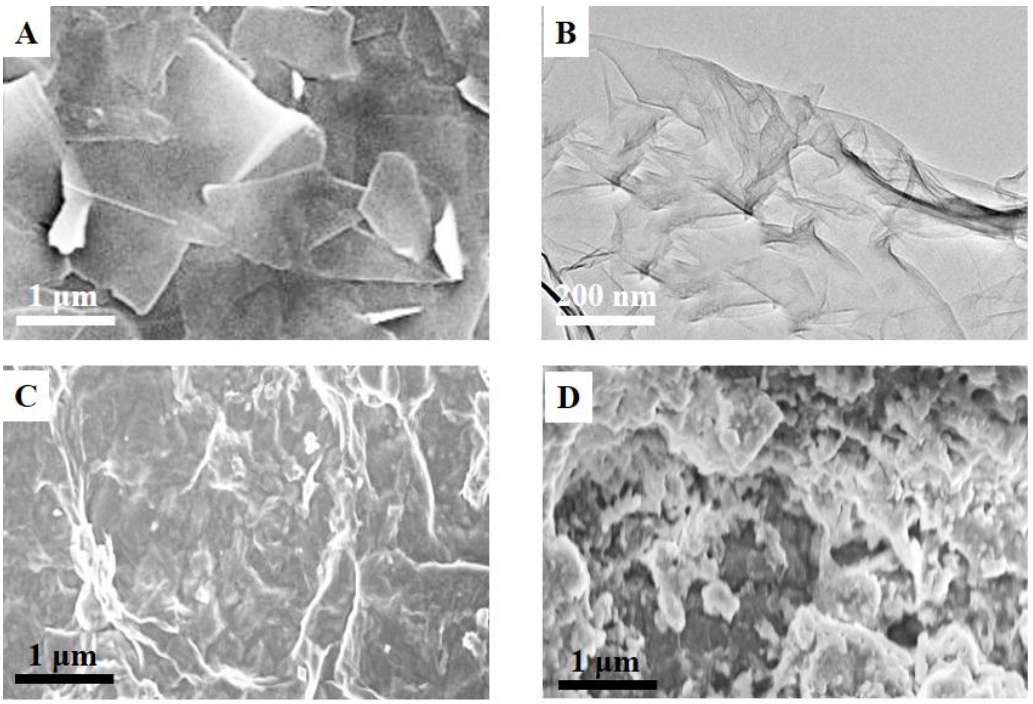

Figure 2. (A) SEM and (B) TEM images of PDDA-RGO. SEM images of MI-PDDA-RGO (C) before and (D) after AN etching.

The degree of cross-linking during the polymerization and the AM-AN interaction before the polymerization are important factors for determining the quantity of the host-guest sites finally present in the matrix. Therefore, the amount of the AN and EGD should be optimized in order to enhance the

final determination performance. We first studied the effect of the EGD by fixing the molar ratio of AN and AM at 1:5. We tested different amount of EGD introduced during the polymerization and found the most stable membrane could be formed at the AN to EGD ratio of 1:15. If this ratio is less than 1:15, the membrane cannot be stably assembled on the PDDA-RGO modified GCE surface. On the other hand, if this ratio is higher than 1:15, the removal of AN from the membrane need takes a long time. Therefore, the ratio of AN and EGD was fixed at 1:15. Similarly, the different ratio of AN and AM was also tested. The electrochemical performance of a series of MI-PDDA-RGO/GCE with different AN:AM ratio were tested using DPV method in a 1 mM $K_3[Fe(CN)_6]$ solution containing 10 μM AN due to the DPV is a sensitive technique and has been widely used for electrochemical analysis. Figure 3A displays the oxidation peak current collected using DPV scans at different AN:AM molar ratios, the highest current response was achieved in the AN:AM molar ratio at 1:4. As can be expected, the less AM addition could result the insufficient host-guest sites. In contrast, larger amount of AN will cause the high degree of the cross-linking, which will impede the molecule access. Therefore, the ratio between AN and AM was fixed at 1: 4.

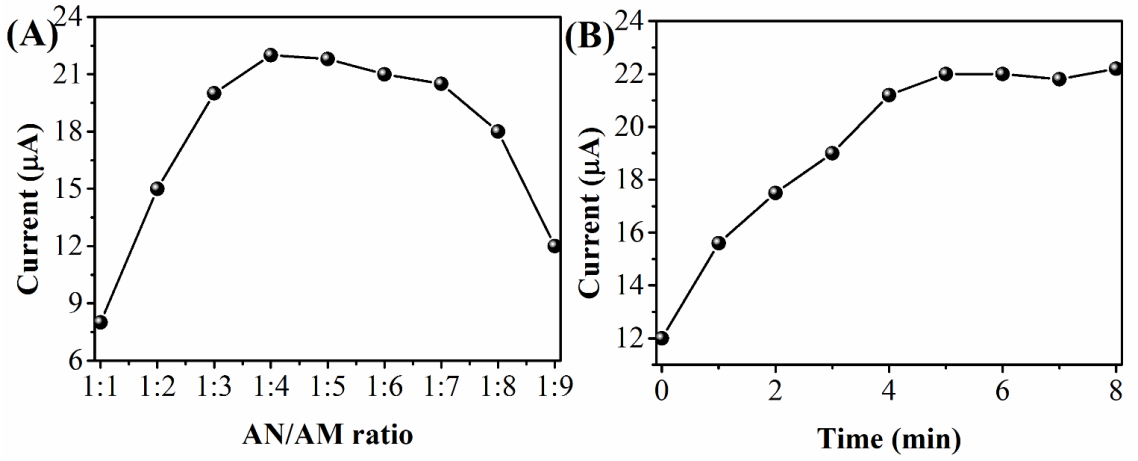

Figure 3. (A) Effect of the molar ratio of AN to AM in the 1 mM $K_3Fe(CN)_6$ containing 10 μM AN. (B) Effect of the incubation time in the 1 mM $K_3Fe(CN)_6$ containing 10 μM AN.

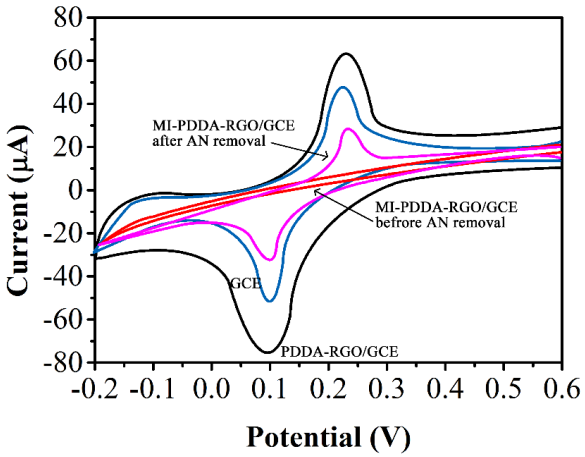

Figure 4. CVs of bare GCE PDDA-RGO/GCE and MI-PDDA-RGO/GCE before and after AM removal in 1 mM $K_3Fe(CN)_6$ solution.

Incubation time is another important parameter should consider. Figure 3B shows the MI-PDDA-RGO/GCE with different incubation time before detection of 10 μM AN. As can be seen from the figure, the current response increased when the incubation time increased from 0 to 5 min and then remained a constant value. Therefore, incubation time of 5 min was used in this study.

In order to investigate the electrochemical behaviors of the MI-PDDA-RGO, different electrodes were tested by cyclic voltammetric (CV). Figure 4 shows the CV profiles of bare GCE, PDDA-RGO/GCE and MI-PDDA-RGO/GCE before and after AM removal in 1 mM $K_3Fe(CN)_6$. It can be clearly seen that the bare GCE exhibited a pair of redox peaks. After surface medication with PDDA-RGO, the redox current significantly increased due to the excellent conductivity of the PDDA-RGO as well as a large specific surface area. On the other hand, almost no peaks were observed in the CV scan using the MI-PDDA-RGO/GCE before the AM removal, indicating the surface membrane is not electro-conductive. In contrast, a pair of redox peaks was observed when using MI-PDDA-RGO/GCE after AM removal, which indicating imprinted cavities can enhance the $K_3Fe(CN)_6$ diffusion after removal of AM.

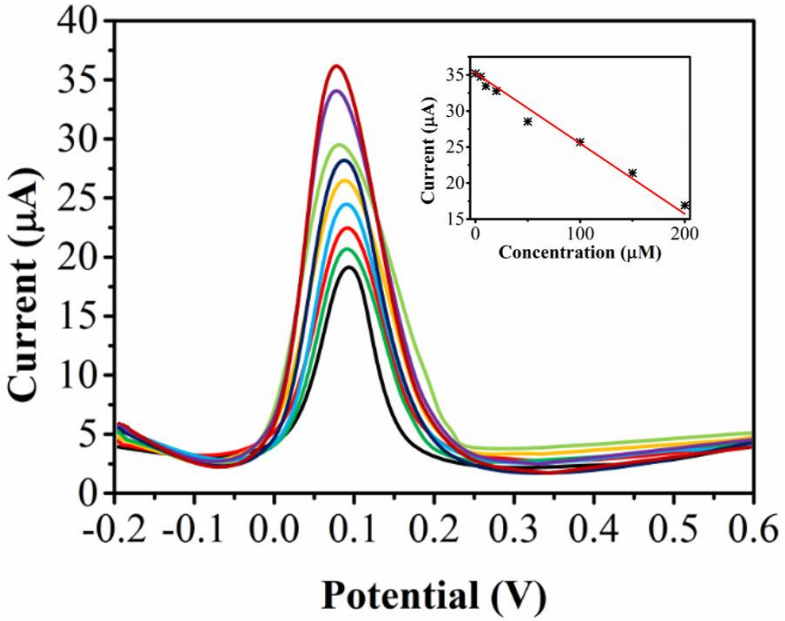

Figure 5. DPV responses of different concentrations (0.01, 0.1, 5, 10, 20, 50, 100, 150 and 200 μM) of AN using MI-PDDA-RGO/GCE. Inset: Fitting linear curve of AN concentrations and current response.

Under optimum condition, DPV was used for investigating the electrochemical performance of the AN on the MI-PDDA-RGO/GCE. As shown in Figure 5, the DPV responses showed a decreasing when the concentration of AN increasing. A linear relationship was observed between the concentrations of AN and current response from 0.01 to 200 μM (Inset of Figure 5). The regression equation could be expressed as: I (μA) = -0.0915C (μM) +35.212 (R^2 = 0.987). The detection limit of the MI-PDDA-RGO/GCE towards AN detection can be estimated as 3 nM. Table 1 shows the comparison of our proposed AN sensor with two existing reported electrochemical sensors. Results showed that the MI-PDDA-RGO/GCE is an effective tool for AN determination.

Table 1. Comparison of AN detection using our proposed method with other literatures.

Electrode	LDR[a] (μM)	LOD[b] (μM)	Reference
Polyhydroxyalkanoate–Au/ITO[c]	4-80	0.036	[31]
Hemin biosensor	—	0.011	[32]
MI-PDDA-RGO/GCE	0.01-200	0.003	This work

[a] Linear Dynamic Range
[b] Limit of Detection
[c] indium-tin oxide

The selectivity of the MI-PDDA-RGO/GCE was tested some common species in the biological system as well as the analogs. Results showed 100-fold of dihydroartemisinin, glucose, ascorbic acid, artemether, uric acid, sucrose, glycine, citric acid and artesunate had no influence of the detection performance. Therefore, the proposed MI-PDDA-RGO/GCE has an excellent selectivity due to the molecular imprinting recognition of AN molecules. The stability was tested by six success detection of 10 μM AN using one MI-PDDA-RGO/GCE. A decreasing of 5.23 % of current was observed. The reproducibility was tested by the ten individually MI-PDDA-RGO/GCE. A RSD of 3.27% was observed. Based on these results, our proposed MI-PDDA-RGO/GCE exhibited an excellent performance towards AN detection.

The real sample test using proposed MI-PDDA-RGO/GCE was conducted in *Artemisia annua* L extract and compared with HPLC. The concentration of AN determined by our proposed MI-PDDA-RGO/GCE was 68.5 μM, which was consistent with the result determined using HPLC (67.6 μM).

Table 2. Determination of AN using MI-PDDA-RGO/GCE in real sample followed by standard addition approach.

Sample	Added (μM)	Found (μM)	RSD (%)	Recovery (%)
1	5	72.4	3.12	98.50
2	10	78.1	5.08	99.49
3	20	87.4	1.98	98.76
4	50	119.2	0.96	100.59

Table 2 shows the results of the detection of AN in real samples by the standard addition method. It can be seen that the recoveries of our proposed MI-PDDA-RGO/GCE was in the range of 98.5-100.59% with RSD less than 5.08%, suggesting the proposed electrochemical AN sensor could be used for real sample test.

4. CONCLUSION

In this work, a novel electrochemical molecular imprinting sensor was fabricated for ultra-sensitive determination of AN. The commercial GCE was firstly modified with PDDA-RGO. After polymerization process, the MI-PDDA-RGO/GCE was formed. The degree of cross-linking during the polymerization, the AM-AN ratio and the incubation time were optimized. The proposed MI-PDDA-RGO/GCE showed an excellent detection performance towards AN with outstanding selectivity. Moreover, this molecular imprinting electrochemical sensor was successfully applied for detecting AN in the *Artemisia annua* L extract.

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