An electrochemical sensing interface based on aptamer conjugated gold nanoparticles/reduced graphene oxide for the determination of malathion

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Abstract

The widespread use of organophosphorous pesticides primarily in agriculture has raised human health and environmental concerns which has led us to the development of a sensing interface that is able to detect the organophosphorous (OP) compound. Herein, we report gold nanoparticles (AuNPs) decorated reduced graphene oxide (rGO) modified on screen printed carbon electrode (SPCE) as the support for aptamer immobilization to develop an electrochemical impedimetric aptasensor for the detection of malathion, one of the most common used OP compounds. Graphene oxide was electrochemically reduced on SPCE and further modified with AuNPs to produce AuNPs/rGO-modified SPCE which was then utilized for the immobilization of thiolated DNA aptamer by self-assembly method. The modified SPCE was characterized for structural changes as compared to bare SPCE by using Fourier-transform infra red (FTIR) and scanning electron microscopy (SEM) for surface morphology. The stepwise modification process was characterized by cyclic voltammetry (CV). The detection was carried out by using EIS where it is based on the variation of electron transfer resistance associated with the formation of malathion-aptamer complex at the modified electrode surface. The result shows that the value of charge transfer resistance increased upon binding of malathion to the aptamer-modified electrode indicating the successful formation of malathion-aptamer complex that blocks the electron transfer.

Keywords: *Electrochemical Impedance, Chemical Sensor, Gold Nanoparticles*

1. Introduction

The threat posed by chemical warfare agents (CWAs) for both civilian populations and military personnel is growing, thus the need for effective methods for detection and measurement of CWA is critical. CWAs are extremely toxic synthetic chemicals that can dispersed as a gas, liquid or aerosol or as agents adsorbed to particles to become a powder. These CWAs have either lethal or incapacitating effects on human [1]. Since CWAs are highly toxic and their use is restricted in non-surety laboratories, research on the CWAs is often conducted using simulant compounds [2, 3]. Among different types of CWAs, nerve agents have raised current interest in the detection of CWAs, especially one of the highly toxic V-series member, VX nerve agent. It is approximately ten times more deadly than the infamous Sarin (GB), while Tabun (GA) belongs to the G-series agents and has Sarin-like toxicity [4]. A single droplet of VX can cause human death in 15 minutes and its persistence makes it into a long-lasting environmental hazard.

Previous studies on the detection of VX nerve agent have employed malathion (Figure 1) as simulant, due to its physical and chemical similarities to VX, but at a much lower toxicity [2]. Analytical methods for the detection of malathion are mostly based on the conventional techniques such as gas chromatograph coupled mass spectrometry (GC-MS) or high performance liquid chromatography (HPLC) [2, 5]. These methods are capable of measuring the target analyte within satisfactory limit of detection, however, they often involve time- and labor- intensive sample preparation [5], as well as expensive instrumentations. Therefore, an alternative for rapid, sensitive, and simple detection method of malathion is needed. Biosensors are one of technology used in developing devices, use biological components as recognition elements to provide affinity to desired target. The recognition element is coupled to a transducer, which transforms the biological event into an electrical signal. In this proposed research work, the biological recognition element that will be used is DNA aptamer, which offers great properties including stability over a wider range of experimental and storage conditions, and because it can bind targets based on structure recognition with high affinity and specificity [6].

While most electrochemical techniques have been employed in developing biosensors, Electrochemical Impedance Spectroscopy (EIS) technique has recently gain an attention owing to its fast, label-free technique to measure the properties of electrode surfaces and bulk electrolytes [7]. Combination of impedimetric technique with aptasensor in this proposed research work, allowing impedance changes following the binding of DNA aptamer to malathion without using enzyme labels. In recent years, label-free electrochemical impedance aptamer sensors have attracted the interest of researchers as an alternative strategy for the rapid and sensitive quantification of target analytes due to the advantages in terms of simplicity, sensitivity as well as simple operation [8, 9]. To best of our knowledge, this research work is the first to investigate the impedimetric aptasensing technique for the determination of malathion for potential application as VX nerve agent detector.

Another challenge in developing biosensor for the determination of malathion is the sensitivity of the aptasensor. The accuracy and sensitivity of the aptasensors are influenced directly by the amount of the immobilized aptamer on the surface of electrode [10]. The use of nanomaterials can be applied to overcome this challenge, and among of nanomaterials, graphene and gold nanoparticles are widely used because of its superior properties. By having larger surface area, high conductivity, and good biocompatibility [11], they can be used as material to fabricate the electrode surface for the development of aptasensor. By combining the unique properties of these hybrid nanomaterials with a specific DNA aptamer, we can amplify redox electrical signal and show high selectivity and sensitivity towards malathion. In this study, we propose DNA aptamer conjugated AuNPs/rGO as a novel molecular recognition sensing for impedimetric determination of malathion. This proposed research will be focused on the investigation of the impedimetric behaviour of DNA aptamer conjugated AuNPs/rGO nanocomposites-modified electrode towards the binding with malathion.

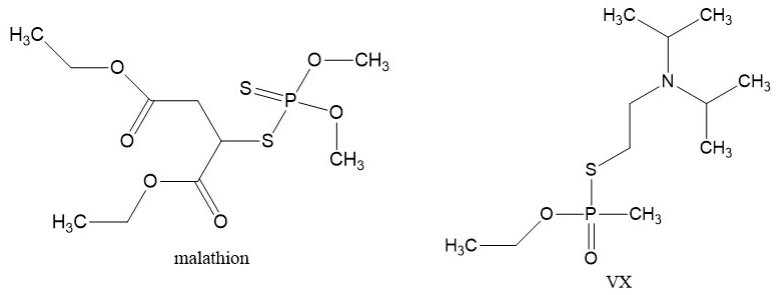

Fig. 1. Chemical structure of malathion (left) and VX nerve agent (right)

2. Materials and Method

2.1 Materials and reagents

Graphene oxide (GO) (code: 777676) in water suspension (4 mg/ml), gold chloroauric acid salt (HAuCl4.4H2O), potassium ferricyanide (III) ($K_3[Fe(CN]_6$), sodium citrate, phosphatebuffered saline (PBS) were purchased from Merck, USA. All other chemicals were of analytical reagent grades. Preparation of AuNPs suspension with an average diameter of 20 nm was prepared according to our previous work [12]. All aqueous reagents were prepared in sterilized ultrapure water (Milli Q ultrapure water system (18 M Ω cm^{-1}), Millipore Billerica, MA, USA).

2.2 Apparatus

The electrochemical experiment was carried out using a screen-printed carbon electrode (SPCE) from Rapid Genesis (Malaysia) featuring Carbon as the working and auxiliary electrode and Ag/AgCl as a reference electrode. The electrochemical analysis was performed using a potentiostat/impedance analyzer (STAT-I 400s, Metrohm, Netherlands) controlled by

electrochemistry software NOVA version 2.1 (Metrohm). The characterization of the modified electrode surface was performed using ZEISS GeminiSEM 500 Field Emission Scanning Electron Microscope (FESEM).

2.3 Preparation and charaterization of DNA aptamer conjugated AuNPs/rGO-modified electrode

The first part of the project was to synthesize materials that will be used for the preparation of sensing platform. In this stage, gold nanoparticles (AuNPs) was synthesized, and transmission electron microscopy (TEM) and UV-absorbance was used for characterisation.

The SPCEs were prepared with 15 µL graphene oxide (2 mg/mL GO) and left overnight to bind, and then electrochemically reduced to rGO-modified SPCE by using cyclic voltammetry (CV) technique in 0.1 M KCl solution, pH 7.4 for 11 cycles with a potential range of -2.0 V to 0.0 V vs. Ag/AgCl at a scan rate of 50 mV/s until a stable CV response was observed at both oxidation and reduction potential. The modified electrode was rinsed with distilled water and dried at room temperature. Following that, AuNPs is drop casted on rGO-modified SPCE for minimum of 2 hours at room temperature. The modified electrode after this step is denoted as AuNPs/rGO-modified SPCE. Several characterizations of the prepared rGO/AuNP-modified SPCE were carried out to determine the successful modification of gold electrode with the hybrid nanomaterials of rGO and AuNP including electrochemical technique, and surface morphology studies.

The thiolated DNA aptamer was then immobilized on the AuNP/rGO-modified electrode via the S-Au bond forming DNA aptamer/AuNP/rGO-modified AuE. Further surface characterization at this stage was carried out to confirm the attachment of DNA aptamer to the AuNP/rGO-modified electrode. CV and EIS were used for the electrochemical behaviour studies, while FTIR will be used for the elemental composition study. Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) was used to monitor the electrochemical behaviour of the stepwise modification process.

2.4 Electrochemical behaviour of the DNA aptamer/AuNPs/rGO-modified electrode towards the interaction with malathion

The aptamer selection for specific binding to target OP (malathion) has been reported previously where the aptamer was developed using the modified Systematic Evolution of Ligands by Exponential enrichment (SELEX) method of Bruno et al.[13]. Malathion is chosen as a simulant for VX due to its physical and chemical similarities to VX, but a much lower toxicity [14]. In this stage, the ability for the DNA aptamer/AuNPs/rGO-modified electrode to capture malathion was studied by using the electrochemical impedance spectroscopy (EIS) which it measures the charge-transfer resistance (R_{ct}) value before and after the aptamer-malathion binding in the presence of a redox species to provide the electrochemical response.

3. Results and Discussion

3.1 Surface morphological studies of AuNPs/rGO-modified SPCE

Surface morphology of AuNPs/rGO-modified SPCE was characterized by SEM. Figure 2 depicts the attachment of AuNPs on the rGO layer with an even distribution of the nanoparticles that has an average diameter of 20 nm.

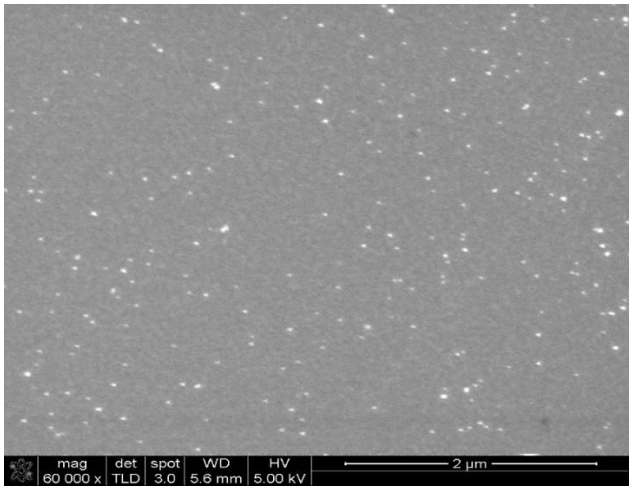

Fig. 2. SEM images of AuNPs/rGO-SPCE at 60k magnification.

3.2 Electrochemical characterization of different modified electrodes

Electrochemical characterization of the electrodes were carried out by using cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). Figure 3 shows the cyclic voltammogram of each modification step in 5 mM ferri/ferrocyanide solution prepared in 0.1 M KCl. In the CV, it was observed that AuNPs/rGO-modified SPCE (yellow curve) recorded the highest anodic and cathodic peak, 307.54 µA and -267.625 µA at 0.4 V and -0.07 V, respectively as compared to rGO- or GO- modified SPCE. This indicates that the couple of AuNPs to rGO could enhance the electron transfer between the redox species and electrode surface.

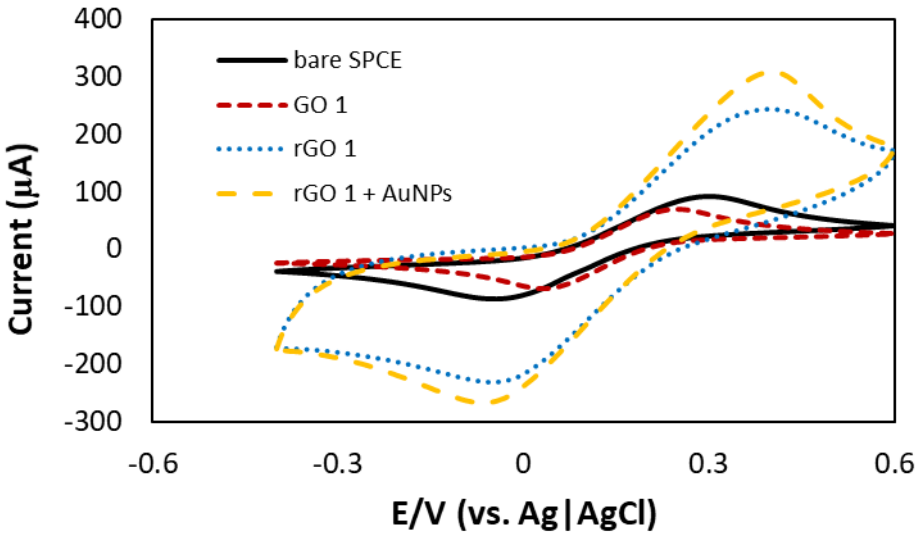

Fig. 3. CV of the different modified electrode in 5 mM $[Fe(CN)_6]^{3-/4-}$ in 100 mM KCl. Scan rate: 100 mV s^{-1}

Nyquist plot observed from the same modified surfaces was further confirmed the result obtained in CV as shown in Figure 4. The Nyquist plots including a semicircle portion at higher frequencies corresponding to the electron-transfer-limited process and a linear part at lower frequency range representing the diffusion-limited process [15]. Charge transfer resistance (Rct) of the electrode can be obtained from the diameter of the semicircle. The curves of rGO/SPCE and AuNPs/rGO-SPCE exhibits smaller radius of semicircles compared to GO/SPCE and bare SPCE, which showed that the result obtained is in a good agreement with CV in Figure 3.

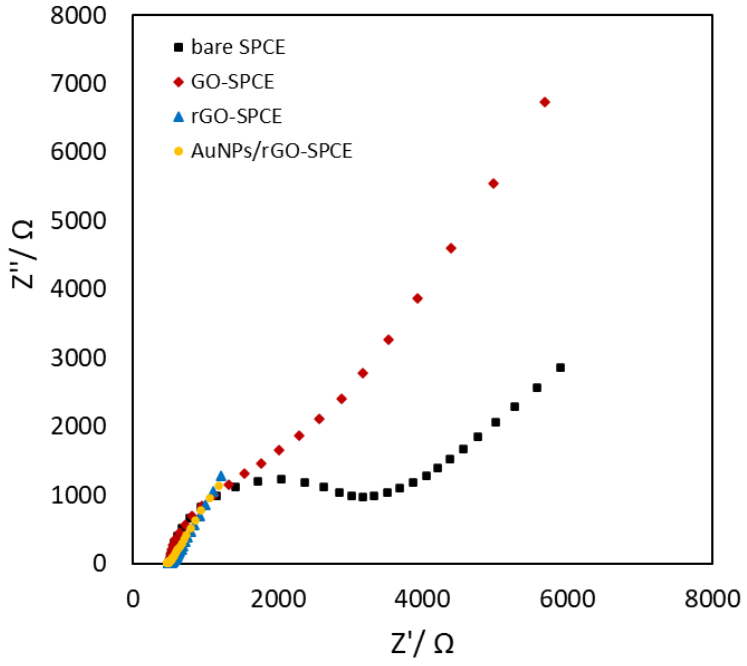

Fig. 4. Nyquist plot of different modified surfaces in 5 mM $[Fe(CN)_6]^{3-/4-}$ in 100 mM KCl.

3.3 Electrochemical behaviour of redox probe on DNA aptamer conjugated AuNPs/rGO-modified SPCE upon binding to malathion

Further electrochemical characterization was carried out to compare the radius of semicircle of DNA aptamer attached to AuNPs/rGO-modified SPCE before and after binding to malathion as shown in Figure 5. The large increase of semicircle after the attachment of aptamer indicates the successful formation of Apt/AuNPs/rGO-modified SPCE (curve a) which hindering the electron transfer. When the malathion has been captured by the aptamer, the semicircle of the EIS curve significantly increased (curve b), suggesting that the malathion on the modified electrode has block the electron transfer.

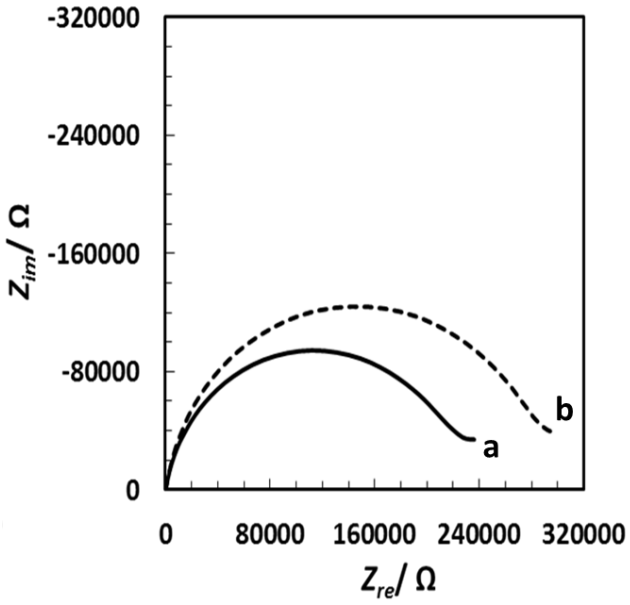

Fig 5. Nyquist plot of Apt/AuNPs/rGO-modified SPCE before (a), and after binding to malathion (b), in 5 mM $[Fe(CN)_6]^{3-/4-}$ in 100 mM KCl. Scan rate: 100 mV s^{-1}.

4. Conclusion and Future Direction

An aptamer-based functionalized AuNPs/rGO-SPCE was developed for the electrochemical determination of malathion. The selected aptamer exhibits high affinity to malathion, which was covalently bonded to AuNPs via thiolated end of the DNA aptamer. The stepwise modification of SPCE was characterized by CV and EIS, as well as SEM for the morphological studies. Based on the results obtained, AuNPs/rGO-modified SPCE provide rather a good sensing platform which was shown in the increase in electrochemical response, due to the couple of AuNPs and rGO that enhance the electron transfer. The results from EIS also demonstrate that the Apt/AuNPs/rGO-SPCE can bind malathion which is observed from the increase of semicircle in Nyquist plot. To generate a highly sensitive method with low detection limit for the determination of malathion, several parameter studies will be carried out to choose

the optimum condition for the construction of aptasensors. These include the aptamer concentration, the reaction time, and the binding time between DNA aptamer and malathion.

Acknowledgement

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