Electrochemical Sensor Based on Reduced Graphene Oxide, Gold Nanoparticles and DNA Aptamer for the Detection of Malathion

Safura Taufik[a], Muhammad Amir Irfan Azizan[a,b], Jahwarhar Izuan Abdul Rashid

[a]*Centre for Defence Foundation Studies, Universiti Pertahanan Nasional Malaysia, Kem Sungai Besi, 57000 Kuala Lumpur, Malaysia*
[b]*Faculty of Defence Science and Technology, Universiti Pertahanan Nasional Malaysia, Kem Sungai Besi, 57000 Kuala Lumpur, Malaysia*

Abstract

The widespread utilization of organophosphorous (OP) compounds in the environment has raised significant concerns regarding both human health and the well-being of our ecosystems. These OPs find common application as pesticides and insecticides, but their potential use as chemical warfare agents (CWAs) by terrorists has further escalated these concerns. Consequently, the development of chemical sensors with exceptional sensitivity and specificity towards OPs has become a matter of utmost importance. Among the various available techniques, electrochemical sensors have emerged as particularly valuable due to their stability, adaptability for on-site detection, and straightforward measurement protocols. In this study, we have engineered an electrochemical sensor designed for the detection of malathion. This sensor leverages the unique properties of gold nanoparticles (AuNPs) coupled with reduced graphene ooxide (rGO), which are integrated onto a screen-printed carbon electrode (SPCE) to serve as the sensing platform. The process involved the electrochemical reduction og graphene oxide on the SPCE, followed by modification with AuNPs to yield an AuNPs/rGO-modified SPCE. This modified electrode was then employed for the immobilization of thiolated DNA aptamer through a self-assembly technique. Characterization techniques such as cyclic voltammetry (CV),Fourier-Transform Infra-Red spectroscopy (FTIR) and field-emission scanning electron microscopy (FESEM) were employed to confirm the successful surface modification of the SPCE. Detection of malathion was achieved using a differential pulse voltammogram (DPV) revealing a noteworthy decrease in the Faradaic peak current upon the binding of malathion to the aptamer-modified electrode. This outcome underscores the successful formation of a malathion-aptamer complex, which impedes electron transfer, thus demonstrating the sensor's efficacy in detecting malathion.

Keywords: electrochemical sensor, organophosphorous compound, malathion,

1. Introduction

Malathion is an organophosphate (OP) insecticide commonly used in agricultural fields and domestic settings for eradication and control of pests. The toxicity level of malathion in birds and mammals are considered moderate, however, due to widespread usage, their persistence in environment has raised serious human health and environmental concerns (Liu et al., 2019; Moore et al., 2010). Malathion and other OPs nerve agents (sarin, soman, tabun and VX) inhibit the activity of acetylcholinesterase (AChE), the enzyme involved in the hydrolysis of neurotransmitter acetylcholIne (Ach) and impact to the function of the central nervous system, eventually leading to respiratory paralysis and death (Mulchandani & Rajesh, 2011). Therefore, a method for the easy, sensitive, and rapid detection of malathion is necessary to be developed for both human health and environmental safety. There are traditional analytical methods have been developed for the detection of OP compounds including gas or liquid chromatoghraphy (Cai et al., 2021; Khalifa et al., 2017; Sapahin et al., 2019), mass spectrometry (Banoub et al., 1995; Picó et al., 2004; Sun et al., 2018), and enzyme linked immune system analysis (Hongsibsong et al., 2020; Yue et al., 2022). Most of these methods are selective and accurate, but they require expensive instrumentation and time-consuming, as well as limitation due to poor stability of the enzyme or antibodies. (McConnell et al., 2020). Recently, there are great numbers of research on aptamer-based biosensors (aptasensor) have been developed for the determination of OPs (Liu et al., 2019). In addition to the advantage of aptasensor, coupling with the fast development of nanotechnology and analyst methods, such as optical and electrochemical aptasensors, have tremendously enhance the performance of biosensor specifically developed for the detection of OPs (Rapini & Marrazza, 2017). Previous works done on colorimetric assays based on gold nanoparticles (AuNPs) for detection of omethoate and malathion have been reported by Wang et al. (P. Wang et al., 2016) and Bala et al. (Bala et al., 2017), respectively. The detection

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* Corresponding author. Tel.:; fax:.
E-mail address: safura@upnm.edu.my

of omethoate was carried out by observing and analyzing the color changes in AuNPs resulted from the disconnection of aptamer molecules from AuNPs in the presence of analyte, and showed in their aggregation. By using the aptamer, this biosensor showed high selectivity towards omethoate, and good linearity between 0.1 uM and 10 uM, with limit of detection (LOD) of 0.1 uM (P. Wang et al., 2016). A similar approach was developed for colorimetric detection of malathion utilizing aptamer, cationic peptide and unmodified AuNPs. This aptasensor was found to be linear in the range of 0.01 – 0.75 nM with LOD of 1.94 pM (Bala et al., 2017).

Electrochemical techniques have been employed in developing biosensors, and in this work, differential pulse voltammogram (DPV) technique has been applied for the detection technique. Combination of voltammetric technique with aptasensor in this proposed research work, allowing current 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(Malecka et al., 2021; Y. Wang et al., 2021). 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 (Oberhaus et al., 2020). 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 (Wu et al., 2014), 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 shows the chemical structure of malathion.

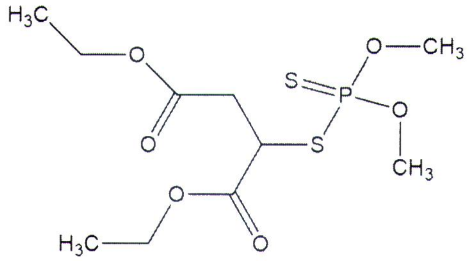

Fig. 1. The chemical structure of malathion

2. Materials and methods

2.1 Materials

Graphene oxide (GO) (code: 777676) in water suspension (4 mg/ml), hydrogen tetrachloroaurate (III) trihydrate. gold chloroauric acid salt ($HAuCl_4$.3H2O), potassium ferricyanide (III) ($K_3[Fe(CN]_6$), sodium citrate, phosphatebuffered saline (PBS) were purchased from Merck, USA. The aptamer was purchased from Apical Scientific Sdn. Bhd. (Selangor Malaysia); had the following sequence: *5'-ThioMC6-ATC CGT CAC ACC TGC TCT TAT ACA CAA TTG TTT TTC TCT TAA CTT CTT GAC TGC TGG TGT TGG CTC CCG TAT-3'* (Barahona et al., 2013). The aptamer contained a thiol C-6 modifier on the 5'-end for conjugation to gold nanoparticles attached to the surface of reduced graphene oxide. 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 (Taufik et al., 2016). All aqueous reagents were prepared in sterilized ultrapure water (Milli Q ultrapure water system ($18\ M\ \Omega\ cm^{-1}$), Millipore Billerica, MA, USA).

2.2 *Instrumentation*

The electrochemical experiment was carried out using a screen-printed carbon electrode (SPCE) from Metrohm (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 characterization of DNA aptamer conjugated AuNPs/rGO-modified electrode*

The screen printed electrodes (SPCEs) were prepared with 15 µL graphene oxide (0.5 mg/mL GO) and left overnight to bind. Then, the GO is electroreduced by CV in 100 µL 0.1 M KCl, with the parameter of 50 mV/s in potential range between -1.5 V and 0.0 V for 6 cycles. Upon reduction, the reduced graphene oxide (rGO) layer is modified with 25 µL of 2 nM gold nanoparticles (AuNPs) and left air dried for 24 hours. The modified electrode after this step is denoted as AuNPs/rGO-modified SPCE. Several characterizations of the prepared AuNPs/rGO-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 SPCE. 10 µL of 0.75 µM aptamer is dropped and left to dry for an hour before washed with distilled water. 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) was used to monitor the electrochemical behaviour of the stepwise modification process. Fig. 2 shows the schematic diagram of stepwise electrode surface modification to produce Apt/rGO/AuNP-SPCE.

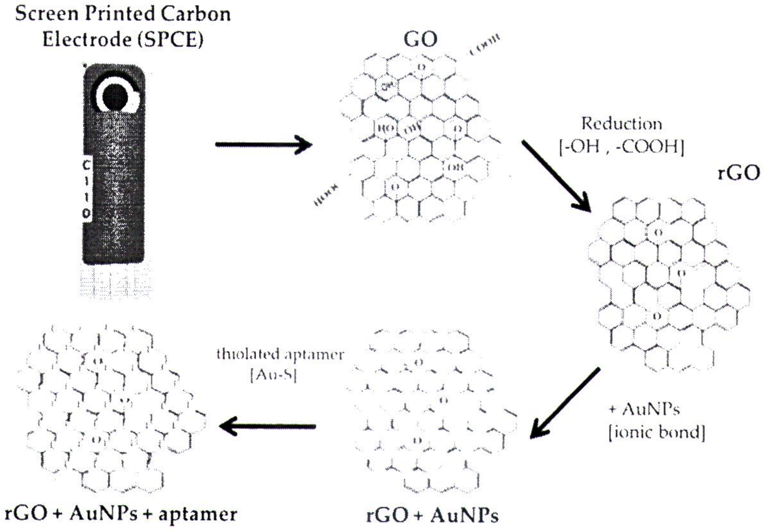

Fig. 2. Schematic diagram of the fabrication of sensing interface for detection of malathion

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 primarily developed in 1990 (Ellington & Szostak, 1990). In this stage, the ability for the DNA aptamer/AuNPs/rGO-modified electrode to capture malathion was studied by using the differential pulse voltammogram (DPV) which it measures the peak current changes of the sensing interface before and after the aptamer-malathion binding in the presence of redox species to observe the electrochemical response.

3. Results and discussions

3.1 FTIR analysis of different modified electrodes.

Fourier-transform infrared (FTIR) analysis was performed to study the structural changes in the rGO modified electrode as compared to its bare surface. Graphene oxide is a form of graphene that have oxygenated functional groups with sp^2 hybridization, resulting in insulation properties. From the GO sample (Fig. 3a), the existence of these oxygenated groups can be interpreted by the existence of strong absorbance peaks at 3332 and 1637 cm^{-1} for the stretching hydroxyl and carbonyl/carboxyl groups, respectively.

To obtain the hexagonal lattice structure and recreated graphene-like properties, the removal of oxygenated groups can be performed by reduction of graphene oxide. The sample of reduced graphene oxide (rGO) shows the absence of absorbance peaks for oxidized functional groups that existed from GO before, although the presence of carbonyl group residual still existed on the 1088 cm^{-1} absorbance peak. Even though rGO is lesser in quality than graphene, the structural defects and residual oxygen make the material suitable for modification while maintaining similar conducting properties as graphene. The FTIR spectrum for rGO is as shown in Fig. 3b.

Upon addition of gold nanoparticles (AuNPs), the presence of peaks at 3366, 1564, and 1388 cm^{-1} display the characteristic bands for citrate as can be seen in Fig. 3c. The stretching hydroxyl [-OH] group of 3366 cm^{-1} and stretching carboxyl [C=O] group from 1564 cm^{-1}, implied the interaction between hydrogen bonds of sodium citrate and residual oxygenated groups from rGO surface. Additionally, the emergence of peak at 1388 cm^{-1} indicated the [C-OH] deformation of surface-bound sodium citrate onto the rGO surface.

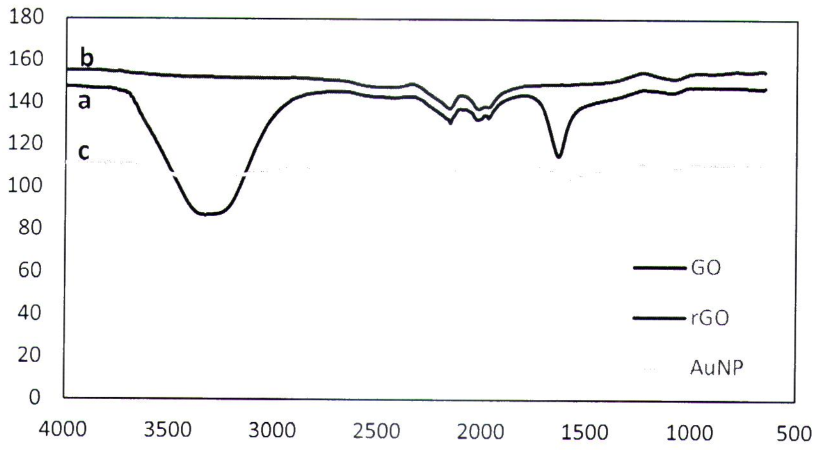

Fig. 3. FTIR spectra of (a) GO-SPCE, (b) rGO-SPCE and (c) AuNP/rGO-SPCE

Table 1. Summary of FTIR spectrum analysis.

Sample	Absorbance Peak (cm^{-1})	Functional group	Type of vibration	Peak remarks
GO	3332	Hydroxyl [O-H]	Stretch	Strong + broad
	2158	[C≡C]	Stretch	Present
	2030	[C≡C]	Stretch	Present
	1637	Carbonyl, Carboxyl [C=O, C-OH]	Stretch	Medium
	1088	Carbonyl [C-O, C-O-C]	Stretch	Strong

rGO	2025	[C≡C]	Stretch	Present
	1088	Carbonyl [C-O, C-O-C]	Stretch	Strong
rGO + AuNPs	3366	Hydroxyl [O-H]	Stretch	Strong + broad
	1564	Aromatic [C=C], [C-OH]	Stretch	Strong
	1388	[C-H, C-OH]	Bending, stretch	Strong medium
	1081	[C-O-C, C=C]	Stretch	Medium

3.2 FESEM

Characterization of the different modified SPCE was monitored through Field Emission Scanning Electron Microscopy (FESEM). The morphology of the modified two-dimensional thin layer observed was seen to be folded/crumples structure (Fig. 1a and 1b) and with an even distribution of AuNOs of 20 nm average diameter throughout the layer (Fig. 1c). The formation of creases on the background indicates that the rGO layer has a large surface area ration, which enhances the embedding of AuNPs onto the layer for an amplified current conductivity. Fig. 1d depicts the attachment of AuNPs onto the rGO bed.

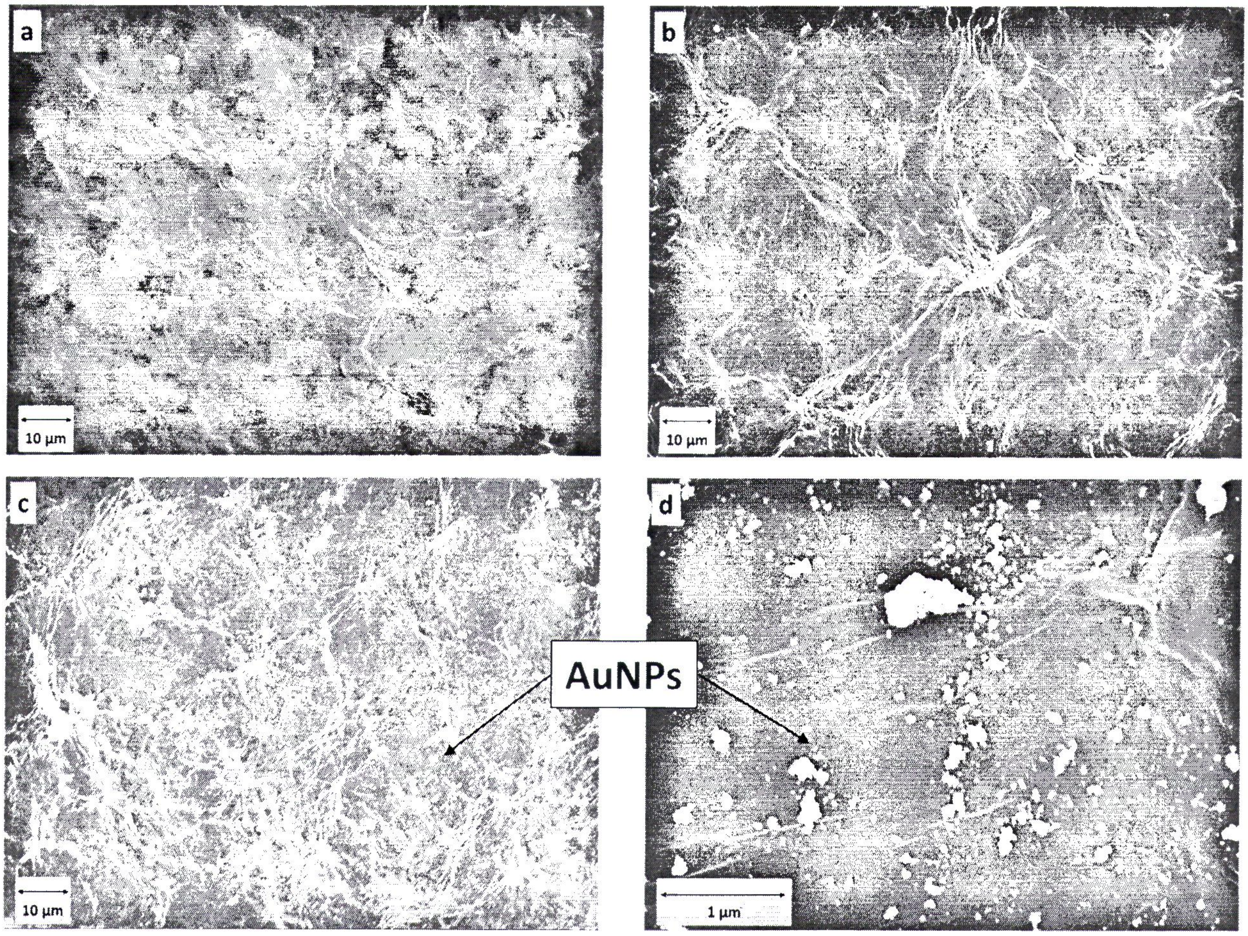

Fig. 4. FESEM images of: (a) GO, (b) rGO, (c) AuNPs/rGO layer at 1k magnification, and (d) AuNPs/rGO layer at 25k magnification, respectively.

3.3 Electrochemical characterization of different modified electrodes

Electrochemical characterization of the electrodes was carried out by using cyclic voltammetry (CV). Fig. 5 shows the cyclic voltammogram of each modification step in 5 mM ferri/ferrocyanide solution prepared in 0.1 M KCl. In the CV as shown in Fig. 5, it was observed that AuNPs/rGO-modified SPCE (yellow curve) recorded the highest anodic and cathodic peak, 248.0667 μA and -250.45 μA at 0.108 V and -0.02 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.

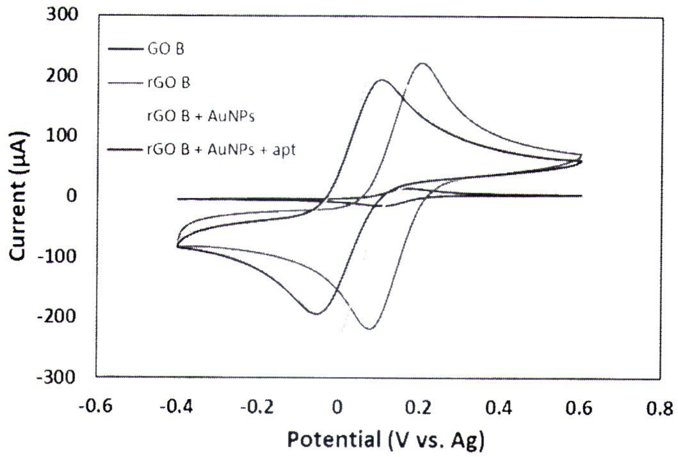

Fig. 5. CV of the different modified electrode in 5 mM $[Fe(CN)_6]^{3-/4-}$ in 100 mM KCl. Scan rate: 100 mV s^{-1}

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

Electrochemical characterization of Apt/AuNPs/rGO-modified SPCE in 25 nM malathion was assessed using DPV at the range of applied potential of –0.5 V to 0.5 V (Fig. 6). At 0.085 V, the peak current recorded for Apt/AuNPs/rGO-modified SPCE was 135.9167 μA, and at 0.04 V, the formation of malathion-aptamer complex due to the binding recorded a significant reduction in peak current with a value of 77.83334 μA. The large decrease of peak current after the binding of malathion to the aptamer-modified SPCE suggesting that the formation of aptamer-malathion complex hindered the electron transfer of redox species to the electrode surface.

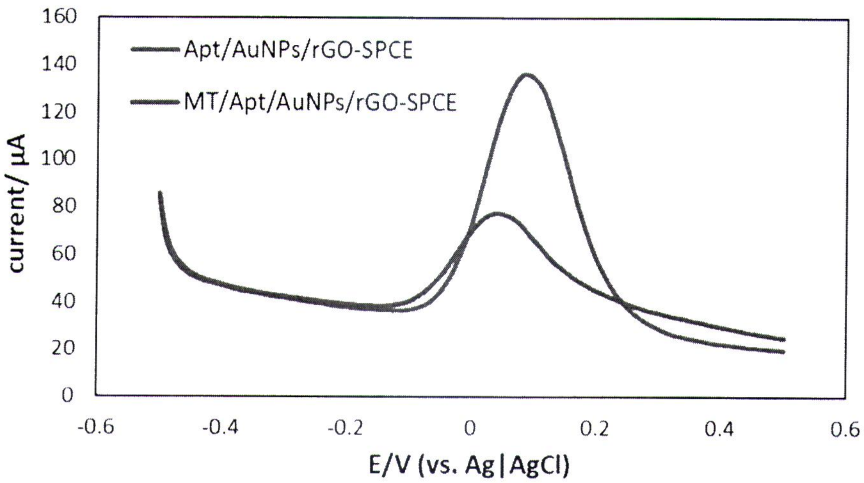

Fig. 6. DPV characterization of Apt/AuNP/rGO-modified SPCE in the presence of malathion (MT, 25 nM) in 0.5 M PBS at pH 7.4 measured at -0.5 V to 0.5 V.

4. Conclusion

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, as well as FTIR and FESEM 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 DPV also demonstrate that the Apt/AuNPs/rGO-SPCE can bind malathion which is observed from the significant decrease in the Faradaic current. To generate a highly sensitive method with low detection limit for the determination of malathion, several parameter studies will be carried out in future 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.

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