

MODIFICATION OF SCREEN-PRINTED CARBON ELECTRODE FOR DETECTION OF
PESTICIDES



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for the Degree of Master of Science in Chemistry

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การดัดแปรข้าวไฟฟ้าคาร์บอนพิมพ์สกรีนสำหรับการตรวจวัดสารฆ่าศัตรูพืชและสัตว์



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เมทธิลพาราไรเออนเป็นสารกำจัดศัตรูพืชในกลุ่มออร์กาโนฟอสเฟตที่ใช้กันอย่าง
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Methyl parathion (MP) is an organophosphate pesticide broadly used in agriculture to protect crops and fruits from the damages caused by insects and pests. However, when it is used in large quantities, it can be potentially toxic to humans and animals, causing many poisoning effects. Moreover, its residues can also penetrate the food chain (*via* agriculture food products) as well as contaminating the environment, leading to air, water and soil pollution. Therefore, rapid determination and reliable quantification of trace level of MP are crucially needed for food safety and environmental monitoring. In this work, a low-cost and portable electrochemical sensor was simply developed using a screen-printed carbon electrode (SPCE) for determination of MP residues with enhanced sensitivity. Typically, the SPCEs fabricated in-house was modified with polyaniline (PANI) using electro-polymerization technique, followed by the electrodeposition of gold nanoparticles (AuNPs), to obtain the AuNPs/PANI/SPCE sensor for MP detection. The modified AuNPs with electrocatalytic activity could increase the electrochemical performance of the detection, while the characteristics of PANI could make the sensor more conductive. The determination of MP was thoroughly investigated using the modified electrode by cyclic voltammetry (CV) and differential pulse voltammetry (DPV). Under optimal conditions, the AuNPs/PANI/SPCE showed high sensitivity, good stability, and reproducibility with acceptable limit of detection (LOD). The proposed AuNPs/PANI/SPCE sensor could then be applied for MP detection in real samples with satisfactory recoveries (107.30% - 110.83%).

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LIST OF ABBREVIATIONS

μg	Microgram
μL	Microliter
μm	Micrometer
$^{\circ}\text{C}$	Degree Celsius
A	Ampere
AC	Alternative current
Ag/AgCl	Silver-silver chloride electrode
C	Concentration
CV	Cyclic Voltammetry
DPV	Differential pulse voltammetry
LOD	Limit of detection
LOQ	Limit of quantitation
M	Molar
min	Minute
mL	Milliliter
mM	Millimolar
PB	Phosphate buffer
PBS	Phosphate buffer saline
PVC	Polyvinyl chloride substrate

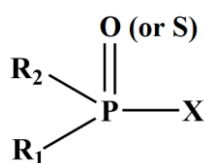
R^2	Correlation coefficient
RSD	Relative standard deviation
s	Second
SD	Standard deviation
SPCE	Screen-printed carbon electrode
T	Temperature (K)
ν	Scan rate (Vs^{-1})



CHAPTER I INTRODUCTION

1.1 Introduction

Organophosphate pesticides (OPs) are a group of synthetic chemical substances, typically containing a pentavalent phosphorus atom double bonded to either an oxygen or sulfur atom, as shown in Fig 1.1 [1].



organophosphates

Figure 1.1 The generic chemical structure of OPs.

Generally, OPs have been widely used in agriculture to control pests including insects, mites, rodents, weeds and other harmful organisms [2]. For example, methyl parathion (MP) is one of the organophosphate insecticides that has been extensively used for agriculture crops [3]. Basically, these OPs are highly neurotoxic as they can irreversibly inhibit the activity of acetylcholinesterase (AChE) in the central and peripheral nervous system, leading to cholinergic dysfunction which possibly causes some abnormal symptoms in humans such as diarrhea, headache, muscular paralysis, cognitive decline, sleep behavior disorder, depression and neuropsychiatric manifestations [4]. In addition, OP residues in agricultural products, as well as those contaminating into the environment (*via* soil and water), causing long-term accumulation in ecosystems and food chains, can lead to numerous physiological disorders in human health. Therefore, the control and monitoring of OP residues polluting the environment and food products has been widely recognized as an important issue for public health [5]. As a result, the development of a reliable and sensitive analytical method for trace analysis of OP residues is crucially needed.

There are many methods developed for OPs detection including gas chromatography (GC) [6]. and liquid chromatography (LC) [7], combined with either ultraviolet-visible (UV-vis) or mass spectrometric (MS) detection, and electrochemical

technique [8-9]. Among these various detection methods, electrochemistry seems to have many potential advantages in terms of simplicity, high sensitivity, fast analysis time, cost effectiveness, small-volume sample measurements, capability of miniaturization, and suitability for on-site applications. In addition, electrochemical detection of OPs could be directly performed at the electrode surface without any prior sample separation or sample purification and pre-treatments [10-11]. Moreover, the electrochemical responses and the detection sensitivity could be enhanced simply by modifying the surface of electrode (usually carbon-based substrates) with a wide range of materials including conducting polymers [13-15]. and metal nanoparticles [16-18].

Conducting polymers, which are a class of polymeric materials that are electrically conductive, have attracted increasing interest in electroanalysis where they have been prominently used as electrode modifiers for the fabrication of highly sensitive electrochemical sensors due to their unique electronic, chemical and biochemical properties with high environmental stability [19]. Among those conducting polymers, polyaniline (PANI) has attracted much attention not only because of its low cost, but also due to the fact that it could be simply synthesized and deposited onto the electrode surface by electrochemical polymerization of aniline (acted as an organic monomer) [20]. Typically, PANI possesses π electrons delocalized across its entire conjugated structure, resulting in a polyconjugated polymer that can be easily oxidized or reduced [21]. Additionally, PANI film tethered onto the electrode can be incorporated with other functional materials such as metal nanoparticles in order to further improve the electrochemical responses, as well as increasing the detection sensitivity.

In the past few decades, metal nanoparticles have been the subject of a large number of publications in almost every scientific field including electrochemistry owing to their unique electronic, optical, magnetic and catalytic properties [22]. Basically, gold nanoparticles (AuNPs), which are widely available in more homogeneous nanomaterials with high surface-to-volume ratio, have been extensively employed as electrode modifiers for the fabrication of electrochemical sensors as they can increase the electrode surface area, enhance the electron transfer rate, and subsequently

amplify the sensor signal [23]. Literally, it has been previously reported that AuNPs and PANI hybrid could offer remarkable and synergistic combination, leading to the excellent catalytic performance of the sensor [24]. Furthermore, the interface between AuNPs and the target analyte (MP) is mainly based on the affinity between either thiol functional groups of MP molecules and gold atoms to form S–Au bonds, and the affinity between amine functionalities of MP molecules and gold atoms to generate HN–Au bonds [25].

Recently, the fabrication of the electrochemical sensors by screen-printing technique has been gaining much attention since it offers several inherent advantages of manufacturing process, consisting of the inexpensiveness, high throughput, facileness, good reproducibility, and capability of miniaturization [26].

In this work, an electrochemical sensor based on screen-printed carbon electrode (SPCE), which is inexpensive, easy to fabricate, portable, and disposable, was developed using PANI and AuNPs as the electrode modifiers for the determination of MP with enhanced sensitivity. The proposed AuNPs/PANI/SPCE sensor was extensively characterized and optimized for the MP detection accordingly.

1.2 Objective

To modify screen-printed carbon electrodes (in-lab fabricated) for methyl parathion detection with increased sensitivity and selectivity.

CHAPTER II

THEORY AND LITERATURE REVIEW

This chapter is concerned with the definitions and theoretical electrochemistry which is used in this work. The important of the pesticide in the environment analysis are described. Additionally, the modifications of the working electrode with graphene are defined. Finally, the literature review of the current analytical methods for the determination of pesticides are presented.

2.1 Pesticides

A pesticide is any substance used to kill, repel, or control certain forms of plant or animal life that are considered to be pests. Pesticides include herbicides for destroying weeds and other unwanted vegetation, insecticides for controlling a wide variety of insects, fungicides used to prevent the growth of molds and mildew, disinfectants for preventing the spread of bacteria, and compounds used to control mice and rats. Because of the widespread use of agricultural chemicals in food production, people are exposed to low levels of pesticide residues through their diets.

Organophosphates are a group of human-made chemicals that poison insects and mammals. Organophosphates are the most widely used insecticides today. They are used in agriculture, the home, gardens, and veterinary practice. Organophosphate insecticides (such as diazinon) are one type of pesticide that works by damaging an enzyme in the body called acetylcholinesterase. This enzyme is critical for controlling nerve signals in the body. The damage to this enzyme kills pests and may cause unwanted side effects in exposed humans. All organophosphates have a common mechanism of toxicity and can cause similar symptoms in humans who have too much exposure.

2.1.1 Methyl parathion

Methyl parathion is an insecticide with broad applications. It is no longer used in the European Union, China, Japan, and other countries. Uses of methyl parathion in the United States are being canceled effective 31 December 2012. Methyl parathion does remain in use in some countries, however, as with other organophosphorus

insecticides, methyl parathion elicits toxicity in both target and nontarget species by inhibiting acetylcholinesterase. Methyl parathion has a history of misuse and illegal applications in homes in the United States, leading to substantial contamination issues and cases of human intoxication.

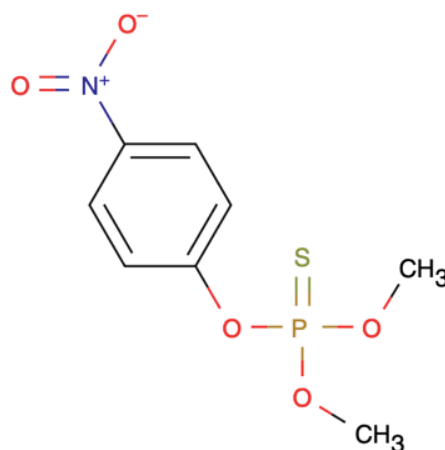


Figure 2.1 Structure of methyl parathion

The acute toxicity of methyl parathion is due to inhibition of acetylcholinesterase by the active metabolite (i.e., methyl paraoxon), resulting in stimulation of the central nervous system, the parasympathetic nervous system, and the somatic motor nerves. It is generally agreed to be somewhat less toxic than its diethyl analog, parathion [26]. Affect to human eith repeated exposures, acetylcholinesterase inhibition can persist without indications of toxicity. In most cases, cholinesterase inhibition is without overt effects. Methyl parathion does not cause delayed neurotoxicity. As noted, methyl parathion has been used illegally in the past for residential pest control. In the 1980s and 1990s, several misuses of methyl parathion in residential settings in the United States led to criminal prosecution of those involved. Substantial costs were associated with decontamination and clean-up. Deaths of a number of household pets were reported. Some follow-up findings suggest persistent neuropsychiatric sequelae in persons exposed with these residential exposures.

2.2 Electrochemical method

Electrochemical methods are the analytical methods which depend on the measurement of the current or potential during electrochemical reaction on the electrode surface. Normally employed electrochemical methods can be used in environment analysis. These methods have many advantages such as simplicity, excellent sensitivity with a very large useful linear concentration range for inorganic and organic species, short analysis time and the simultaneous determination of various analytes such as pesticides. The choice of the electrochemical methods depends on the character of the compound to be determined and the matrix components of the sample. The electrochemical methods described in this section involved the methods that were used in this work.

2.2.1 Cyclic voltammetry

CV [27] is a kind of voltammetric methods which is widely used for qualitative analysis, such as evaluated adsorption process, characterization of thermodynamic and kinetic reactions, and coupled chemical reaction, as well as redox reaction. In the detection terms, various potentials are applied between two values at a fixed scan rate, and the scan is reversible. Figure 2.2 (left) shows the potential waveform of CV including a forward scan which potential is changed from A to D, and a reverse scan where the potential is then changed in reversed from D to G. Subsequently, the cyclic voltammetric response can be obtained, and is shown in Figure 2.2 (right). The forward scan of this case is reduction (A to D) and the reverse scan is oxidation (D to G), resulting in a cathodic peak and an anodic peak, respectively. The scan rate is also important because the duration of the scan must provide an adequate time for the reaction to occur. Analytes can diffuse to the surface of working electrode, leading to the electrochemical signal once the electrode has either received or lost electrons. The potential is measured between working electrode and reference electrode, while the resulting current is measured between the working electrode and counter electrode.

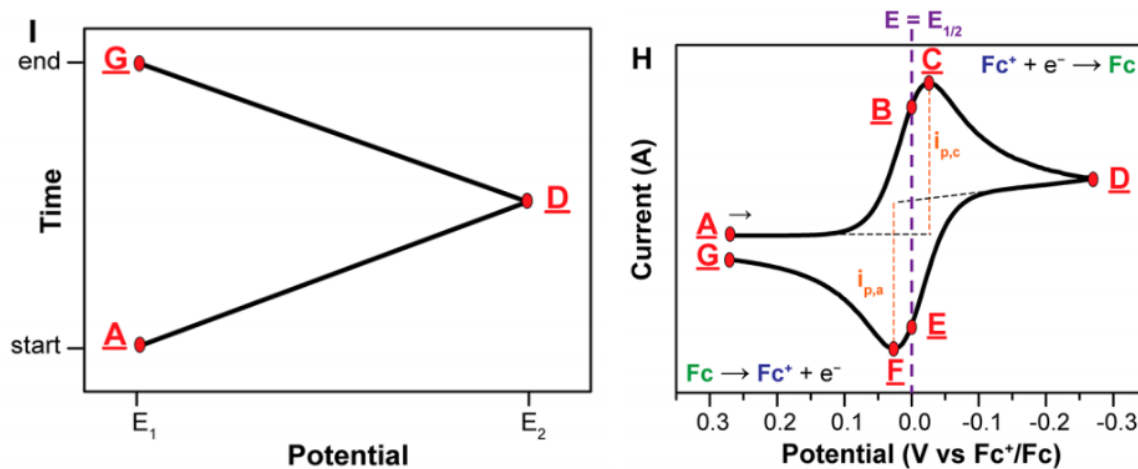


Figure 2.2 potential waveform applied (left) and voltammetric response (right) of cyclic voltammetry.

2.2.2 Differential pulse voltammetry

Differential pulse voltammetry (DPV) is used for both quantitative chemical analysis and to study the mechanism, kinetics, and thermodynamics of chemical reactions. DPV used as an analytical tool offers advantages when compared to other electrochemical techniques. DPV is very sensitive, often allowing direct analyses at the ppb (parts per billion) level. When used in a stripping mode, ppt (parts per trillion) measurements are possible. DPV owes its sensitivity to the relatively short pulse time and its differential nature. The short pulse time increases the measured currents, while the differential measurement discriminates against background processes. Figure 2.3 (left) shows the potential waveform of differential pulse voltammetry and Figure 2.3 (right) shows the differential pulse voltammetric response of the half-redox reaction, either oxidation or reduction [28].

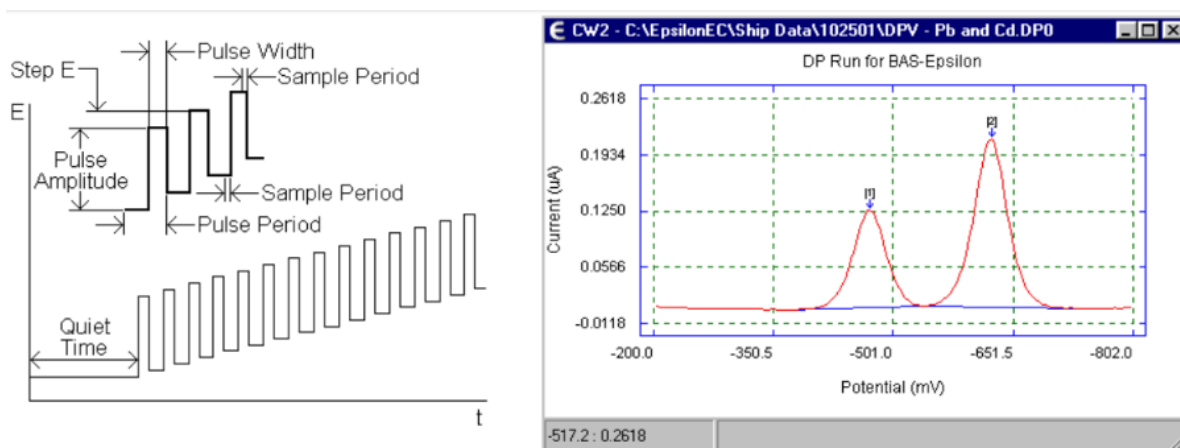


Figure 2.3 Potential waveform applied (left) and voltammetric response (right) of differential pulse voltammetry [28]

2.3 Electrode

The electrochemical cell, where the voltammetric experiment is carried out consist of a working electrode, a reference electrode, and a counter electrode. In general, the electrode provides the interface across which a charge can be transferred. At the appropriate applied potential, the reduction or oxidation of an analyte occurs at the surface of a working electrode, results in the mass transport of new material to the electrode surface and the generation of a current.

2.3.1 Screen-printed carbon electrodes (SPCEs)

Screen-printed electrode (SPE) is one of the electrodes that has been developed from the three-electrode system, used in a large scale of solution, to a smaller scale. It typically has three types of electrodes, which are RE, CE and WE, together in one piece of substrate. Thus, it has many advantages as follows: it is simple, disposable, inexpensive and minimal, which are the important factors for the development of the electrochemical sensors. Figure 2.4 shows an explanation of SPCE where RE is Ag/AgCl, and both CE and WE are carbon.

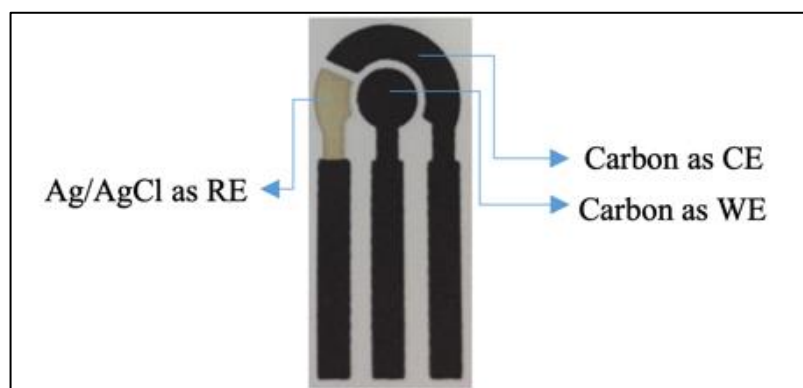


Figure 2.4 Screen-printed carbon electrodes (SPCEs)

Recently, materials are developed in several electrochemical analysis to increase sensitivity of detection methods. This section contains the theory of materials investigated in this work such as gold nanoparticles and polyaniline.

2.3.2 Gold nanoparticles

Colloidal gold nanoparticles have been utilized for centuries by artists due to the vibrant colors produced by their interaction with visible light. More recently, these unique optoelectronic properties have been researched and utilized in high technology applications such as organic photovoltaics, sensory probes, therapeutic agent, drug delivery in biological and medical applications, electronic conductor, and catalysis.

Gold nanoparticles are used as catalysts in several chemical reactions. The surface of gold nanoparticles can be used for selective oxidation. Gold nanoparticles are being developed for sensor. These technologies would be useful in the electrochemical applications.

2.3.3 Polyaniline

PANI is an organic conducting polymeric material that captured research interest after the discovery of conjugated polyacetylene. PANI has advantages such as ease of synthesis process, low-price, and excellent polymerization yield. It has been

researched due to its high electrical conductivity, and reversible (acid and base) doping and dedoping routes recognized with polymer chain containing nitrogen atom.

PANI polymer is a model of phenylene derivative based polymeric material that consists of a chemically flexible amino group in the polymeric chain, which is bound on any side of the phenylene ring. The transferring of protons (protonation), deprotonation and different physicochemical characteristics of PANI is due to presence of NH group in the polymer.

Among all the organic conjugated polymers, PANI has an exceptional representation, because of the following: (1) inexpensive aniline monomer; (2) easy synthesis; (3) environmental stability; (4) simple doping by protonic acids; (5) polymerization progress has excellent yield; (6) obtained salt is highly stable and shows good electrical conductivity; (7) when the salt reacts with a base like NaOH or KOH solution, PANI salt changes into PANI base; (8) PANI is the only conjugated polymeric material whose electrical conductivity and electronic structure can reversibly be optimized by both reactions of chemical oxidation and protonation; (9) PANI shows an excellent stability and the electrochemical characteristics; and (10) a conducting polymer with nitrogen atoms occupies the bridging to the benzenoid and quinoid rings in its backbone structure and plays an important role in π -bond formation and electrical intrachain conduction.[29]

2.4 Literature review

Wang et al. [30] fabricated ZrO_2/Au nano-composite film electrode and studied its sensing performance towards organophosphate pesticide, parathion, with square wave voltammetry. The nano- ZrO_2 showed a strong affinity toward the phosphate group on parathion molecules, which provides sensitivity and selectivity of the sensing film.

Kang et al. [31] developed methyl parathion sensor based on gold nanoparticles and Nafion film modified glassy carbon electrode. The results demonstrated that nafion improve ionic and electronic conductivity and gold nanoparticles possess extreme small size, high surface-to-volume ratio and high

electrocatalytic activity towards the reduction of methyl parathion. Square wave voltammetric method for determination methyl parathion was developed.

Tchemi et al. [32] investigated the use of a glassy carbon electrode coated with a thin film of a gemini surfactant intercalated smectite clay for the electroanalysis and detection of the methyl parathion pesticide by square-wave voltammetry.

Wu et al. [33] developed sorbent based electrochemical sensors using electrochemical reduced bata-cyclodextrin dispersed graphene as a sorbent for the preconcentration and electrochemical sensing of methyl parathion by differential pulse voltammetric analysis. The sensor shows ultra-high sensitivity, good selectivity and fast electron transfer rate and good electrochemical response to methyl parathion due to the ultra-large surface area and the superconductivity of graphene.

Gong et al. [34] fabricated enzymeless organophosphate pesticide sensor by using gold nanoparticles decorated graphene nanosheets modified glassy carbon electrode as solid phase extraction. The developed electrode was used in square wave stripping voltammetric analysis of methyl parathion. The combined the advantages of nanoassembly of Au nanoparticles and two-dimensional graphene provides a fast, simple, and sensitive electrochemical method for detecting nitroaromatic OPs.

Subramanian et al. [35] developed electrochemical biosensor for pesticides based on acetylcholinesterase immobilized on polyaniline deposited on vertically assembled carbon nanotubes wrapped with ssDNA. The biosensor has been high sensitivity, good selectivity and applied for the determination of methyl parathion and chlorpyrifos in spiked river water samples.

Shalini et al. [36] This research presents a novel strategy for the real-time detection of organophosphate pesticides by imprinting parathion molecules at the surface of conductive functional polyaniline nanoparticles (PANIs). It has been demonstrated that the introduction of vinyl group on the surface of PANIs is not only beneficial to the selectivity of imprinting polymerization, but also to the preconcentration of parathion template into the polymer by charge attraction between the template and the functionalized PANIs. Through the above two effects, parathion imprinted polymer was successfully prepared on the surface of PANIs. When the novel material was used to detect parathion in vegetable samples, its selectivity

coefficient was observed to be nearly ten times than that of its non-imprinted counterpart. It also possessed high selectivity toward parathion in comparison to structurally similar pesticide compounds.

Although some researchers had reported the highly sensitive methods for the determination of pesticides, these methods have several disadvantages such as long analysis time and complication. Consequently, the rapid and simple methods for the determination of pesticides are required.



CHAPTER III

EXPERIMENTAL

This chapter shows the list of chemicals, instruments, and apparatuses, as well as explaining the experimental details of this work, including fabrication of SPCEs, the modification of SPCEs, the optimization of experimental parameters and the detection methods for methyl parathion.

3.1 Instrument and apparatuses

Instrument and apparatuses used in this work are summarized in Table 3.1.

Table 3.1 List instrument and apparatuses used in this work.

Instruments / apparatuses	Suppliers
Potentiosta , PGSTAT 101	Potentiostat, PGSTAT 101
MS Semi-Micro Balances, MS205DU	Mettler toledo, Thailand
Screen-printed block	Chaiyaboon, Thailand
Hot air oven	Memmert, USA
Milli-Q ultrapure water purification system, R ≥ 18.2 MΩ·cm	Millipore, USA
Ultrasonic bath, GTSONIC VGT-1613QTD	GTSONIC, China
pH meter, FiveEasy Plus	Mettler toledo, Thailand
Micro pipette, Eppendorf research plus, 100- 1000 μL	Eppendorf, UK

3.2 chemical and reagent

The chemical and reagent involving in this work were analytical grade. They are listed in Table 3.2.

Table 3.2 List of chemical and reagents.

Chemicals	Suppliers
Chloroauric acid (HAuCl ₄)	Sigma Aldrich, Singapore
Methyl parathion	Sigma Aldrich, Singapore
Methanol (MeOH)	Sigma Aldrich, Singapore
Hydrochloric acid (HCl)	MERK, Germany
Sulfuric acid (H ₂ SO ₄)	MERK, Germany
Silver/silver chloride paste	Gwent group
Carbon paste	Gwent group
Aniline	Sigma Aldrich, Singapore
Sodium hydroxide (NaOH)	Sigma Aldrich, Singapore
Disodium hydrogen phosphate (Na ₂ HPO ₄)	MERK, Germany
Sodium dihydrogen phosphate (NaH ₂ PO ₄)	MERK, Germany
Graphene oxide	Sigma Aldrich, Singapore
Titanium oxide	Sigma Aldrich, Singapore

3.3 Chemicals and reagent preparation

3.3.1 Preparation of solution for the determination methyl parathion

3.3.1.1 Stock solutions of methyl parathion solution 1000 mg/L

A 1000 mg/L stock solution of methyl parathion was prepared by dissolving 2 mg of methyl parathion in 2 mL methanol (stored in the refrigerator).

3.3.1.2 Preparation 0.5 mM of methyl parathion in 0.1 mM phosphate buffer (PBS) pH 7.0

0.5 mM of methyl parathion was prepared by diluting stock solution of methyl parathion in 0.1 M phosphate buffer (PBS) pH 7 and 10% methanol.

3.3.1.3 Supporting electrolyte: 0.1 M phosphate buffer pH 7.0

Supporting electrolyte: 0.1 M phosphate buffer pH 7.0 prepared by 1.4196 g of NaH_2PO_4 was dissolved in 50 mL of Milli-Q water and 0.276 g of Na_2HPO_4 was dissolved in 10 mL of Milli-Q water. Then 40.5 mL of first solution and 9.5 mL of the second one was mixed together with Milli-Q to a final volume.

3.3.2 Preparation of 2 mM KAuCl_4 in 0.1 M H_2SO_4 solutions

A 2 mM KAuCl_4 solution was prepared by dissolving 7.55 mg of KAuCl_4 in 10 mL 0.1 M sulfuric acid.

3.3.3 Aniline solution, 5 mM

A 5 mM aniline was prepared by diluting 4.56 mL of 0.1 M aniline in 5 mL of 1 M sulfuric acid.

3.4 Fabrication of screen-printed carbon electrodes (SPCEs)

SPCEs were fabricated in-house by screening the conductive inks onto a polyvinylchloride (PVC) substrate. Reference electrode and both working and counter electrodes were screened using silver/silver chloride (Ag/AgCl) and carbon inks (conductive inks, Sun Chemical, UK), respectively. Each step in the SPCEs fabrication was performed as follows: the PVC substrate was cut in the size of 14x13 cm. Then, Ag/AgCl ink was screened onto the surface of PVC substrate to generate the RE, and the substrate was dried in the oven at 55 °C for 1 h. Next, the carbon ink was screened onto the substrate to form the WE and CE parts. The obtained PVC substrate was then dried in the oven at 55 °C for 1 h. Finally, the in-lab fabricated SPCEs were stored at room temperature in the desiccator. The fabricated SPCEs was shown in Fig 3.1.

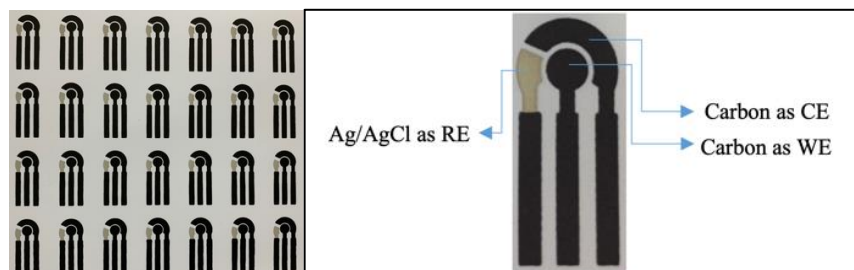


Figure 3.1 SPCEs fabricated in-lab.

3.5 Modification of screen-printed electrodes with nanomaterials.

3.5.1 Modification with titanium oxide nanoparticles (titanium (IV) oxide nanoparticle, TiO_2)

Method for preparing screen-printed carbon electrodes modified with titanium (IV) oxide, (TiO_2 /SPCE) modified screen-printed carbon electrode.

1. Wash the template block with acetone to clean the template block and let it dry before screening the electrodes.

2. Apply silver ink to the template block as a reference electrode and bake at 55 C for 1 hour.

3. Then mix 1.5 g of carbon ink per 0.15 g of TiO_2 , screen for working electrode and counter electrode, and bake at 55 C for 1 hour to obtain polarity. Electro-carbon screen printing modified with TiO_2 /SPCE.

3.5.2 Modification with gold nanoparticles (AuNPs).

Two types of screen-printing electrodes were modified: screen printed carbon electrode (SPCE) and screen-printed carbon electrode modified with TiO_2 /SPCE with gold nanoparticles. by using electrochemical deposition method. A solution of 2 mM KAuCl_4 in 100 μL of 0.1 M HCl was applied to the electrode surface covering all three electrodes. Then give a constant electric potential (applied potential) at -0.2 V (vs Ag/AgCl) for 300 s to allow gold nanoparticles to deposit on the electrode surface. Then rinse the electrodes with distilled water and dry. Screen-printed carbon electrodes modified with gold nanoparticles modified screen-printed carbon electrode (Au/SPCE) and screen-printed carbon electrodes modified with titanium oxide

nanoparticles and gold nanoparticles were obtained. (Au-TiO₂/SPCE), then bring the prepared electrodes to test their performance.

Performance testing of four prepared screen-printed carbon electrodes as follows:

1. Screen Printing Carbon Electrode (SPCE)
2. SPCEs modified with gold nanoparticles (Au/SPCE).
3. Screen-printed carbon electrodes modified with TiO₂/SPCE.
4. SPCEs modified with titanium oxide nanoparticles and gold nanoparticles. (Au-TiO₂/SPCE)

A 100 µL solution of 1 M NaCl (blank solution) was applied to the electrode surface. Cover all three electrodes. Then use the cyclic voltammetry technique to analyze by setting the following parameters: Then absorb the 1 M NaCl solution, rinse the electrode with water and dry it. Then, 100 µL of 1 mM K₃Fe(CN)₆ (analyte) was added using the parameters in Table 1 for analysis.

Table 3.3 CV staircase

1.Start potential	0.9 V
2.Upper vertex potential	0.9 V
3.Lower vertex potential	-1.0 V
4.Stop potential	0.89 V
5.Number of scans	3
6.Scan rate	0.1 V/s
7.Step	-0.01 V

3.5.3 Modification with Graphene oxide (graphene oxid, GO)

Two types of screen-printed electrodes were modified: screen-printed carbon electrodes (SPCE) and screen-printed carbon electrodes modified with gold nanoparticles (Au/SPCE) with graphene oxide. By drop-cast method, 0.1 mg of graphene

oxide was dissolved in 5 ml of distilled water and sonicated for 5 min. The graphene oxide solution was then dripped onto the screen-printed carbon electrode surface and electrode. A screen-printed carbon electrode modified with gold nanoparticles to cover the entire working electrode, 8 μL volume, was heated at 50 $^{\circ}\text{C}$ until dry, a screen-printed carbon electrode modified with graphene oxide was obtained. modified screen-printed carbon electrode, GO/SPCE) and graphene oxide modified gold nanoparticles screen-printed carbon electrode, GO/Au/SPCE).

3.5.4 Modification with Titanium oxide nanoparticles (titanium (IV) oxide nanoparticle, TiO_2)

Two types of screen-printed electrodes were modified: screen-printed carbon electrode (SPCE) and screen-printed carbon electrode modified with gold nanoparticles (Au/SPCE) with titanium oxide nanoparticles. By drop-cast method, 1 mg of titanium oxide per 0.1 mg of graphene oxide was dissolved in 5 ml of distilled water and sonicated for 5 min. The graphene oxide solution was then dripped onto the electrode surface. Screen-printed carbon and screen-printed carbon electrodes were modified with gold nanoparticles to cover the entire working electrode, 8 μL volume, and heated at 50 $^{\circ}\text{C}$ until dry. Screen-printed carbon electrodes modified with nanoparticles were obtained. Titanium oxide and graphene oxide (graphene oxide-titanium (IV) oxide nanoparticle modified screen-printed carbon electrode, GO- TiO_2 /SPCE) and gold nanoparticle modified screen-printed carbon electrodes. Titanium oxide and graphene oxide (graphene oxide-titanium (IV) oxide nanoparticle modified gold nanoparticles screen-printed carbon electrode, GO- TiO_2 /Au/SPCE). No phosphate using 6 types of screen-printed electrodes developed as follows:

1. Screen -printed Carbon Electrode
2. Screen-printed carbon electrodes modified with gold nanoparticles (Au/SPCE).
3. Screen-printed carbon electrodes modified with graphene oxide-modified gold nanoparticles (GO/Au/SPCE).
4. Screen-printed carbon electrode modified with gold nanoparticles modified with titanium oxide and graphene oxide nanoparticles. (GO- TiO_2 /Au/SPCE)
5. Graphene Oxide Modified Screen Printing Carbon Electrode (GO/SPCE)

6. Screen-printed carbon electrode modified with titanium oxide and graphene oxide nanoparticles. (GO-TiO₂/SPCE)

The study of electrochemical properties of organophosphates using 6 types of screen-printed electrodes developed as follows:

A 100 µl solution of 0.1 molar phosphate buffer solution (PBS) pH 7 (blank solution) was applied to the terminal surface. Cover all three electrodes. The cyclic voltammetry technique was used in the analysis by setting the following parameters:

Table 3.4 CV staircase for analysis

1.Start potential	1.0 V
2.Upper vertex potential	1.0 V
3.Lower vertex potential	-1.8 V
4.Stop potential	0.99 V
5.Number of scans	3
6.Scan rate	0.1 V/s
7.Step potential	-0.01 V

Then, a 100 µL solution of 1 mM methyl parathion in 0.1 molar pH 7 phosphate buffer (analyte) was applied to the terminal surface. Cover all three electrodes. using new electrodes and then use the parameters in Table 3.4 for analysis.

3.6 Electrodeposition of AuNPs onto the modified PANI/SPCE

The modification of AuNPs onto the PANI/SPCE was carried out in a 0.1 M H₂SO₄ containing 2 mM KAuCl₄ using an electrodeposition method. The fixed potential of -0.3 V vs. Ag/AgCl was applied to the PANI/SPCE for 180 s, resulting in the reduction of Au(III) to Au(0), and subsequently the deposition of AuNPs onto the modified

PANI/SPCE. The obtained AuNPs/PANI/SPCE was then rinsed with distilled water and dried in the oven at 55 °C for 3 min.

3.7 Electropolymerization of aniline onto SPCE

Electrochemical polymerization of aniline on the electrode surface was performed in a 1 M H₂SO₄ solution containing 5 mM aniline using cyclic voltammetry (CV) at the SPCE. The potential cycle was scanned between -0.3 and +0.9 V vs. Ag/AgCl at a scan rate of 50 mV/s, with 10 consecutive scans as the optimal condition. The polymeric PANI was simultaneously synthesized and deposited onto the SPCE. The resulting PANI/SPCE was then rinsed with distilled water and dried in the oven at 55 °C for 3 min.

3.8 Characterization of the modified SPCE

The modified electrodes including the PANI/SPCE and the AuNPs/PANI/SPCE were characterized by electrochemical technique. Typically, cyclic voltammetry (CV) was performed using those modified SPCEs in a MeOH : 0.1 M PBS pH 7.0 (10:90; v/v) solution containing 0.5 mM MP, recorded at a scan rate of 100 mV/s. The responses obtained from the modified SPCEs were also compared with the unmodified SPCE.

3.9 Electrochemical pretreatment of the AuNPs/PANI/SPCE prior to use

The AuNPs/PANI/SPCE was subsequently pretreated prior to use in the electroanalysis by cyclic voltametric technique in a MeOH : 0.1 M PBS pH 7.0 (10:90; v/v) solution (a blank solution). The CV potential was applied between +0.2 and -1.4 V vs. Ag/AgCl at a scan rate of 100 mV/s for 5 consecutive scans. After that, the modified electrode was washed with distilled water and dried in the oven at 55 °C for 3 min. Then, the pretreated AuNPs/PANI/SPCE was ready to use for further electrochemical studies.

3.10 Electrochemical measurement of MP

The quantitative determination of MP was performed at the AuNPs/PANI/SPCE using differential pulse voltammetry (DPV) under the optimal conditions as follows: an applied potential of -1.4 V vs. Ag/AgCl for 2 min; a step potential of 50 mV; a modulation amplitude of 0.2 V; a modulation time of 0.2 s, and an interval time of 0.5 s.

3.11 Real sample

3.11.1 water sample

Water sample was obtained from Lopburi province (agriculture water). Prior of the study, the water sample was filtered through a 0.45 μm filter membrane 3 times to remove the particulate matter. Next, each filtered water sample water 0.5 mL was mixed with 0.1 M PBS pH 7 of a 1.3 mL and 10% MeOH 0.2 mL (total volume 2 mL) mixed standard solution of methyl parathion. Then, the External standard method was applied to determine methyl parathion in the prepared sample solution. The obtained results were based on triplicate measurement ($n=3$). The percent recovery can be obtained from the following formula:

$$\text{Percent recovery} = \frac{\text{Found concentration of spiked sample}}{\text{Known concentration of spiked sample}} \times 100$$

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CHAPTER IV

RESULTS AND DISCUSSION

This chapter presents the results and discussion of the characterization of AuNPs and PANI, characterization of AuNPs/PANI/SPCE, optimization of the DPV determination of methyl parathion at AuNPs/PANI/SPCE, analytical performance and analytical applications.

4.1 Performance of screen-printed electrodes modified with nanomaterials.

The results of the performance of the four screen-printed electrodes are shown in fig 4.1. The screen-printed carbon electrode modified with titanium oxide nanoparticles and gold nanoparticles. (Au-TiO₂/SPCE) gave the highest cathodic current

(Fe³⁺ + e⁻ → Fe²⁺) and anodic current (Fe²⁺ → Fe³⁺ + e⁻) compared to other screen printed electrodes due to the nanoparticle properties.

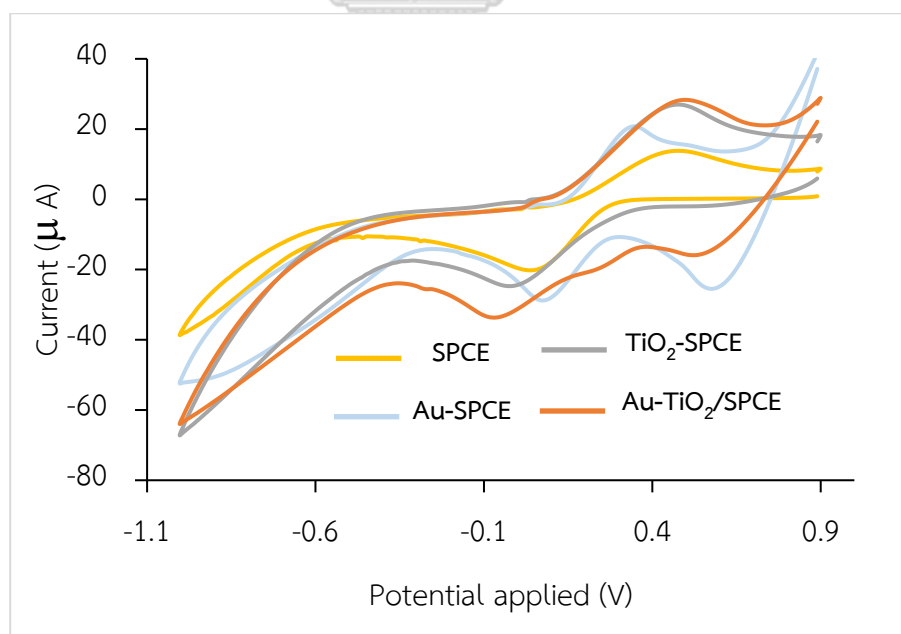


Figure 4.1 Cyclic voltammograms of 1 mM K₃Fe(CN)₆ in 1 M NaCl on electrode.

Condition; potential applied +0.9 to -1 V scan rate; 0.1 V/S

Highly conductive titanium oxide and gold nanoparticles. The authors expected that Au-TiO₂ NPs would improve specificity (selectivity) in the analysis of chemicals in the organophosphate group, and this expectation was supported by the numerous experiments described, including the increased surface area of the working electrode. However, after conducting the experiment again, it was discovered that mixing the titanium oxide and carbon ink resulted in a poor dispersion of the nanomaterial, suggesting a modification to the procedure.

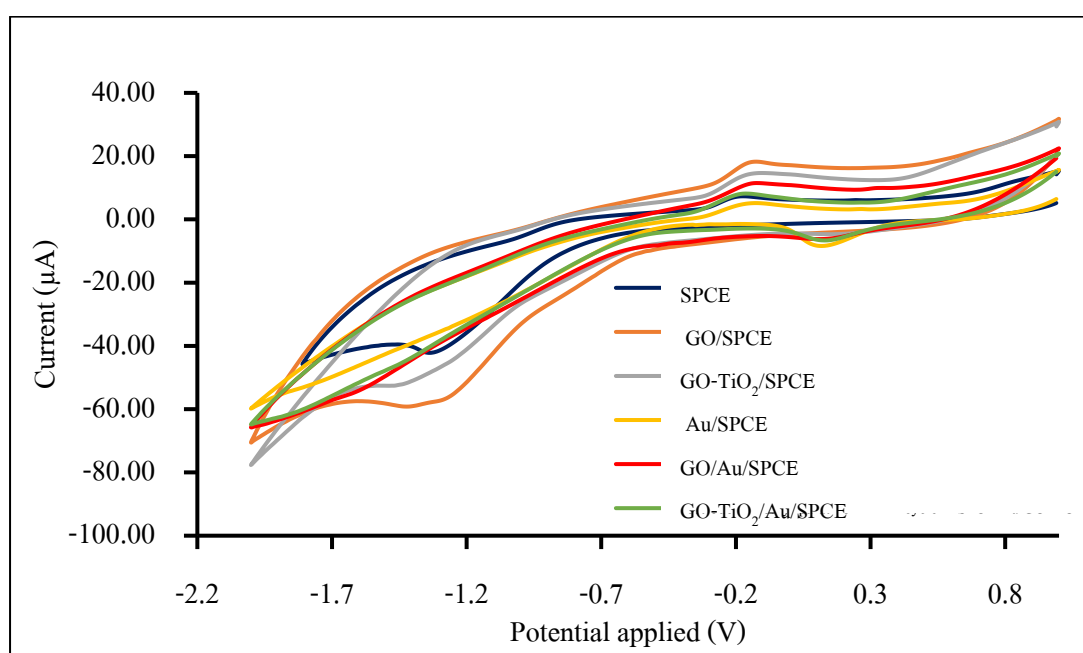


Figure 4.2 Cyclic voltammograms of 1 mM methyl parathion in 0.1 M PBS pH 7 cycle 1

Based on the electrochemical properties studies with the five modified screen-printed electrodes, it can be seen that (Shown in Fig 4.2) the graphene oxide-modified carbon screen-printed electrode (GO/SPCE) yielded the cathodic current E_{pc1} and the E_{pa1} anodic current is the highest in comparison with other screen-printed electrodes due to the good conductivity of graphene oxide. including the increased surface area of the working electrode make sensitivity increased, but the polarity with gold nanoparticles and titanium oxide nanoparticles gave lower than actual current values. This may be due to improper methods and conditions for modifying screen printing electrodes, resulting in poor electron transfer. Therefore, the researcher studied

various studies and found that gold binding PANI is used for modifications electrode for high sensitive and specific to further organophosphate measurements.

4.2 Electrochemical behavior of MP

The electrochemical behavior of MP, a commonly used nitroaromatic OP compound, was first investigated at the SPCE using CV. Fig. 4.3 displays the cyclic voltammograms of 1 mM MP in MeOH : 0.1 M PBS pH 7.0 (10:90; v/v), recorded with two consecutive scans in the two potential ranges of System I and System II.

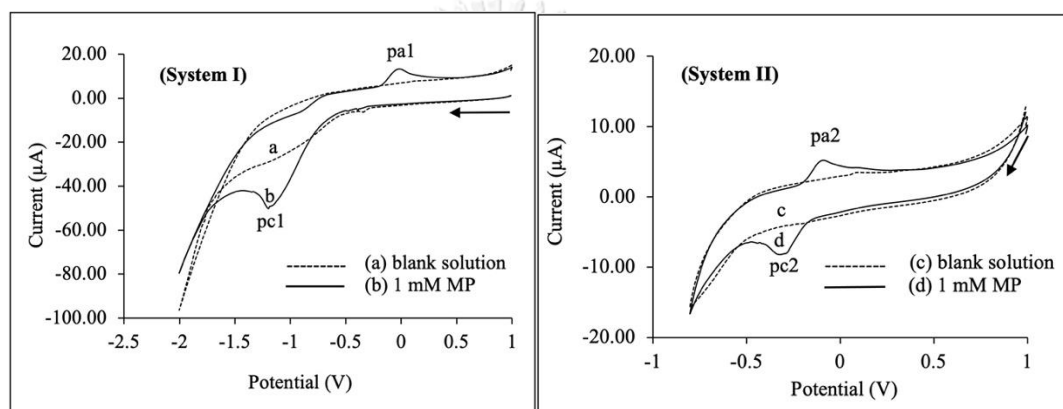


Figure 4.3 Cyclic voltammograms of 1 mM MP in MeOH : 0.1 M PBS pH 7.0 (10:90; v/v) at the SPCE, with a scan rate of 100 mV/s. Two consecutive CV cycles were recorded over two regions of System I (1st cycle) and System II (2nd cycle).

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In the first CV cycle (System I) when the potential was scanned between +1.0 V and -2.0 V vs. Ag/AgCl, a reduction peak (labelled pc1) was clearly observed at ca. -1.175 V, corresponding to the reduction of the nitro group to hydroxylamine (ArNHOH). When the potential was reversed, an oxidation peak (labelled pa1) could be seen at ca. -0.057 V, attributed to the oxidation of hydroxylamine (the product electrogenerated in the forward scan) to nitroso group (ArNO). In the second CV scan (System II) when the potential range of +1.0 V to -0.80 V vs. Ag/AgCl was recorded, a reversible redox couple could be observed at ca. -0.319 V (labelled pc2) and -0.067 V (labelled pa2), corresponding to the reversible ArNHOH/ArNO redox process. Note that no such electrochemical response could be observed in the blank solution.

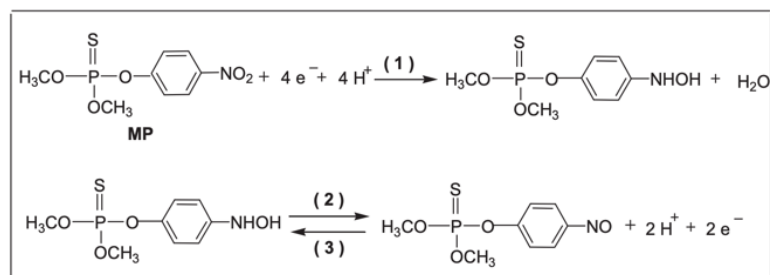


Figure 4.4 General mechanism of the electrochemical reaction of MP in aqueous solution, illustrating the reduction of the nitro group to the hydroxylamine group (System I), and the reversible redox couple of hydroxylamine and nitroso functionalities (System II) [37].

Typically, the electrochemical behavior of MP observed at the SPCE was comparable to those previously reported in the literature for the nitroaryl group [37]. Fig. 4.4 exhibits the general mechanism of the electrochemical reaction of MP in aqueous media, where the reduction process on the first scan (System I) which corresponds to the four-electron and four-proton reduction of nitro group to hydroxylamine (Scheme 1), and the corresponding redox couple of hydroxylamine and nitroso on the second scan (System II) based on two-electron and two-proton reversible process (Scheme 2 and 3) are depicted.

However, the voltametric response of MP at the SPCE was somewhat poor in terms of sensitivity and peak shape (not quite well-defined), likely due to the slow electron transfer at the unmodified electrode. Therefore, the modification of SPCE surface with PANI and AuNPs were subsequently carried out in order to improve the sensitive in the determination of MP.

4.3 Electropolymerization of aniline onto SPCE

PANI was simply synthesized and tethered onto the SPCE by electropolymerization method using CV, as previously described in the literature.²⁸ Basically, the aqueous solution of 5 mM aniline (monomer) in 1 M H₂SO₄ was

electropolymerized onto the SPCE for a number of successive cycles in the potential range of +0.9 V and -0.3 V vs. Ag/AgCl, at a scan rate of 50 mV/s. Fig. 4.5 shows the cyclic voltammograms obtained during the electropolymerization of aniline for 10 successive cycles. As seen in Fig. 4.5, a well-defined oxidation peak at ca. 0.417 V on the forward scan and a reduction peak at ca. 0.0540 V on the backward scan could be clearly observed, corresponding to the oxidation of aniline and the reduction of its reduced product formed during the forward scan, respectively. Upon successive scans, the peak currents, for both anodic and cathodic reactions, continuously increased, demonstrating that the growth of PANI film was occurring and the electropolymerized PANI was successfully deposited onto the SPCE.

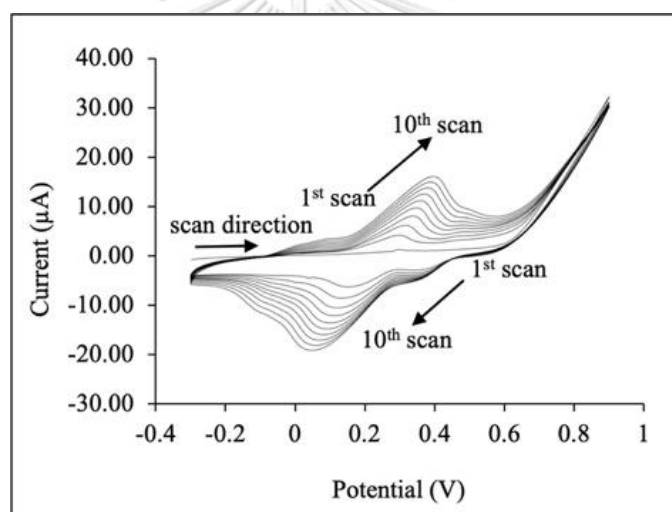


Figure 4.5 Cyclic voltammograms of PANI obtained from the electropolymerization of 5 mM aniline in 1 M H_2SO_4 solution at the SPCE, recorded in a potential range between +0.9 V and -0.3 V vs. Ag/AgCl, with 10 successive cycles at a scan rate 50 mV/s.

Next, the effect of film thickness of PANI modified onto the SCPE on the detection of MP was thoroughly investigated by varying the number of successive CV scans from 0 to 20 cycles. The resulting PANI/SPCEs were then rinsed with distilled water, dried at 55 °C for 3 min, and used to determine the electrochemical process of 5 mM MP in MeOH : 0.1 M PBS pH 7.0 (10:90; v/v), by CV at a scan rate of 50 mV/s. Fig.

6 demonstrates the plot of anodic peak current of MP (referring to the pa1 peak in System I) versus the number of successive CV cycles. It can be clearly seen from the plot that the anodic peak current of MP noticeably increased with successive scanning up to 10 cycles. When the electropolymerization of aniline was performed for 20 cycles, the anodic peak current of MP dropped dramatically, indicating that the PANI film formed onto the SPCE was too thick, and this potentially resulted in the slow electron transfer kinetics at the electrode surface with large overpotential. According to these results, CV scanning for 10 cycles in the electropolymerization process was therefore chosen as the optimum experimental condition of the formation of PANI film on the SPCE.

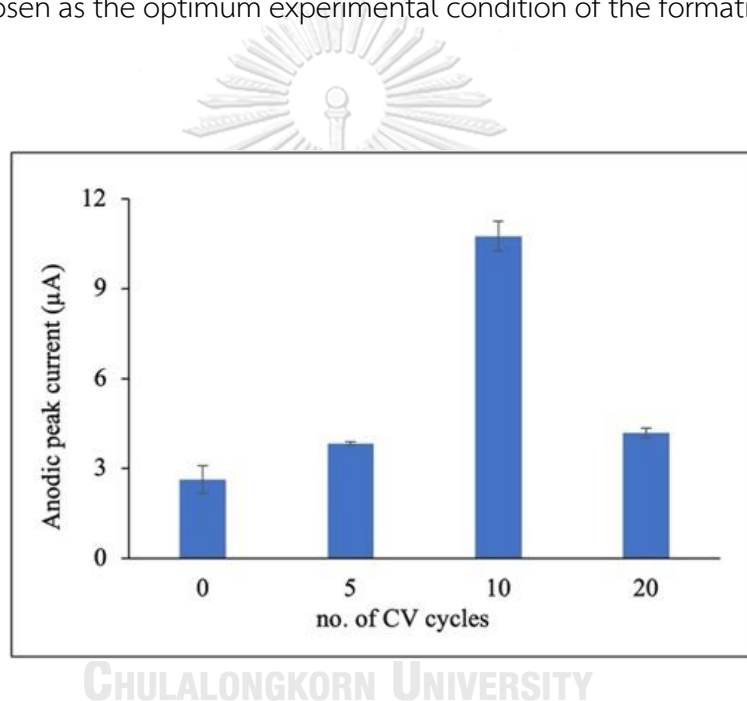


Figure 4.6 Plot displaying the effect of number of successive CV cycles (0, 5, 10 and 20 cycles) on the electropolymerization of 5 mM aniline in 1 M H_2SO_4 solution, at a scan rate of 50 mV/s, resulting in PANI formed onto the SPCE.

4.4 Electrodeposition of AuNPs onto the modified PANI/SPCE

The PANI/SPCE was further modified with AuNPs by the electrodeposition method in order to enhance the electrocatalytic performance of the sensor. Basically, the AuNPs were deposited onto the PANI/SPCE at a fixed potential of -0.3 V vs. Ag/AgCl for a range of deposition time including 0, 120, 180, and 300s. The resulting AuNPs/PANI/SPCEs were then rinsed with distilled water, dried at 55 °C for 3 min, and

used to determine the electrochemical response of 0.5 mM MP in MeOH : 0.1 M PBS pH 7.0 (10:90; v/v), by CV at a scan rate of 100 mV/s. Fig. 4.6 shows the plot of anodic peak current of MP (referring to the pa1 peak in System I) versus the deposition time. As clearly seen in Fig. 4.6, the anodic peak current of MP increased noticeably with increasing deposition time up to 180 s, confirming the presence of AuNPs on the surface of PANI/SPCE which provided an increase of the electroactive surface area and subsequently enhanced the electrocatalytic measurement of MP. However, when the deposition time was increased to 300 s, the anodic peak current of MP declined markedly, probably indicating a large amount of AuNPs attached onto the PANI/SPCE, making the electrode surface become thicker and passivated. Therefore, the deposition of 180 s was found to be optimum for the electrodeposition of AuNPs onto the PANI/SPCE.

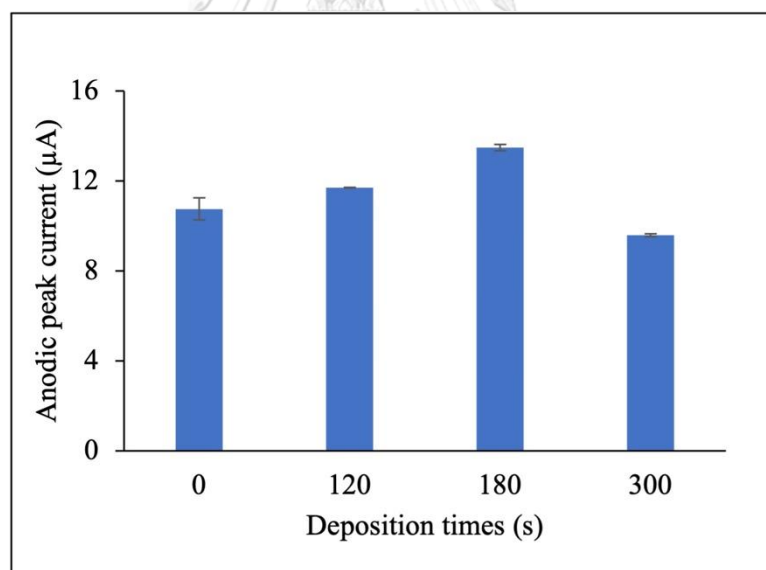


Figure 4.7 Plot showing the effect of deposition time (0, 120, 180 and 300 s) on the formation of AuNPs at the PANI/SPCE with the applied potential of -0.3 V vs. Ag/AgCl, in 0.1 M H_2SO_4 solution containing 2 mM $KAuCl_4$.

4.5 Electrochemical measurement of MP using DPV

In order to determine MP quantitatively, the differential pulse voltametric technique (DPV), possessing some advantages over the CV method such as low capacitive/background charging currents, high sensitivity and low detection limits [38] was employed in this study. The DPV parameters were extensively optimized using the AuNPs/PANI/SPCE, and the optimal conditions were found to be as follows: an applied potential of -1.4 V vs. Ag/AgCl for 2 min; a step potential of 50 mV; a modulation amplitude of 0.2 V; a modulation time of 0.2 s; and an interval time of 0.5s. (shown in fig 4.8) Fig. 4.9 displays the differential pulse voltammogram of 50 ppm MP in MeOH : 0.1 M PBS pH 7.0 (10:90; v/v), recorded at the AuNPs/PANI/SPCE under the optimal experimental conditions. Note that the background subtraction technique was applied to all DPV responses obtained in this study. As seen in Fig. 4.9, a well-defined oxidation peak could be clearly observed *ca.* -0.2496 V vs. Ag/AgCl, highlighting the oxidation of hydroxylamine to nitroso (as previously mentioned in System I). In addition, the anodic peak current of *ca.* 9.1596 μA corresponding to the voltametric response of 50 ppm MP could be measured, indicating high sensitivity of the detection.

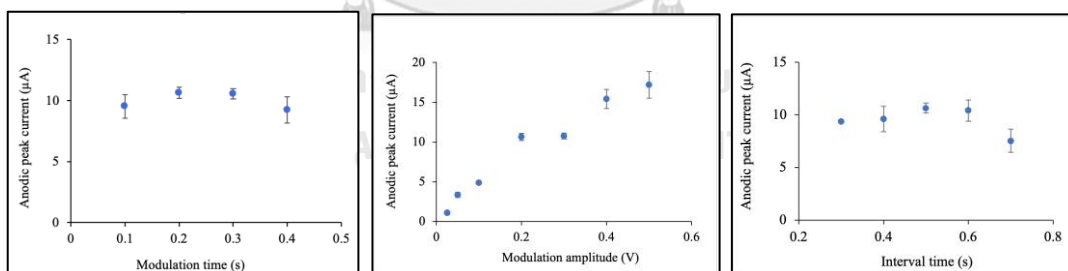


Fig 4.8 Optimization of DPV determination of MP

4.6 Linearity, LOD and LOQ

Under the optimal conditions, the analytical performance of the proposed AuNPs/PANI/SPCE was subsequently investigated for quantitative analysis of MP by DPV. Different concentrations of MP ranging from 1 to 50 ppm were measured for

constructing a calibration curve. Note that all studies were performed using the AuNPs/PANI/SPCE under optimal conditions with three repetitive measurements.

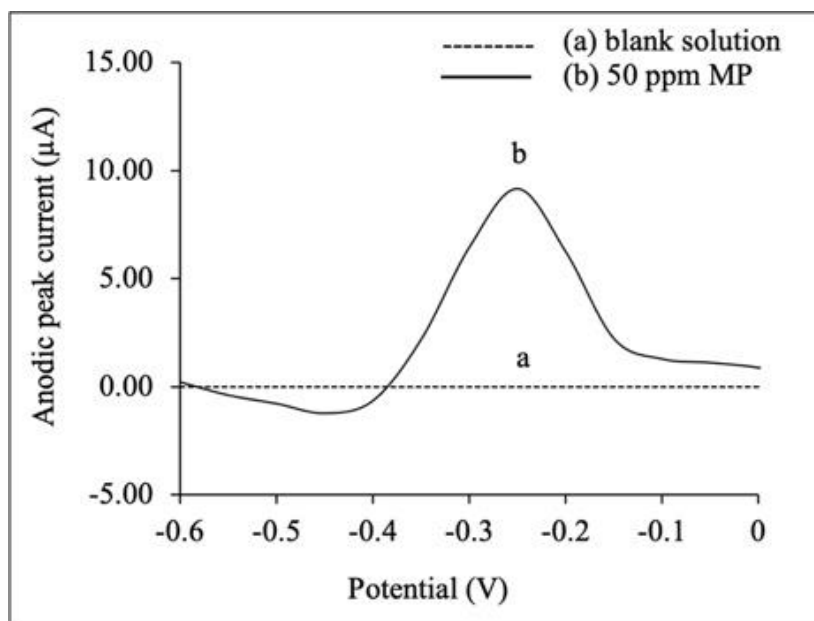


Figure 4.9 Differential pulse voltammograms of the AuNPs/PANI/SPCE in a MeOH : 0.1 M PBS pH 7.0 (10:90; v/v) solution in the absence (a), and the presence (b) of 50 ppm MP.

Fig. 4.10 presents the calibration plot of the anodic peak current of MP versus the concentration of MP. It could be clearly seen that the plot exhibited good linearity in the range of 1 to 50 ppm ($i_{pa} (\mu A) = 0.1126 C_{MP} (ppm) + 2.9517$) with linear regression (R^2) of 0.995. The limit of detection (LOD) was determined from the analyte concentration corresponding to the three-time signal-to-noise ($3S/N$) method, which was found to be 0.1719 ppm. Moreover, the limit of quantitation (LOQ) could be calculated using 3σ method (where σ is the standard deviation of the blank solution), and it was found to be 0.5131 ppm. Consequently, the developed AuNPs/PANI/SPCE sensor could be potentially applied for the determination of MP residues in the environmental monitoring and food safety control applications.

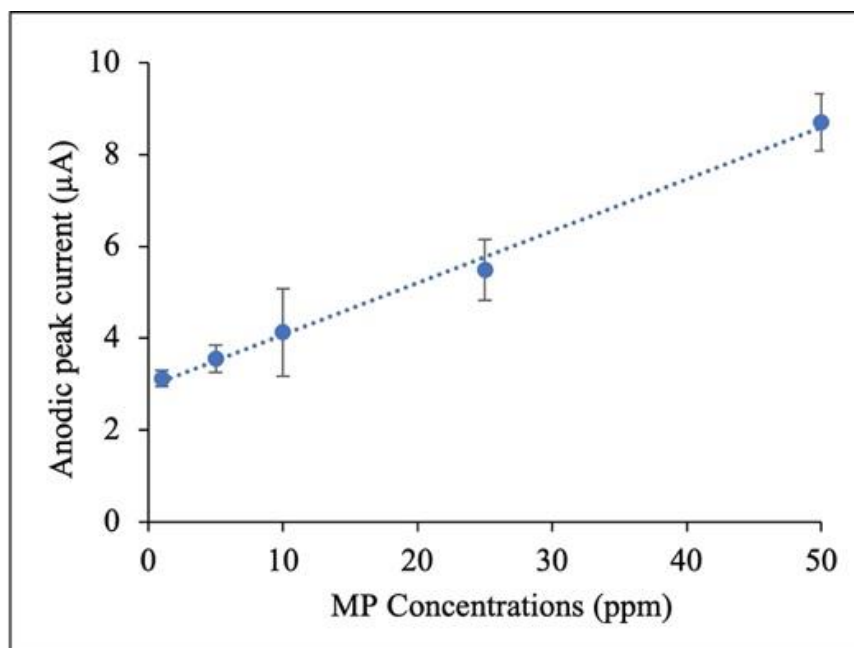


Figure 4.10 Calibration plot of anodic peak current versus MP concentrations ranging from 1 to 50 ppm (in a MeOH : 0.1 M PBS pH 7.0 (10:90; v/v) solution), recorded at the AuNPs/PANI/SPCE by DPV as follows: an applied potential of -1.4 V vs. Ag/AgCl for 2 min; a step potential of 50 mV; a modulation amplitude of 0.2 V; a modulation time of 0.2 s, and an interval time of 0.5 s.

4.7 Stability of the modified AuNPs/PANI/SPCE for the determination of MP

The stability and reproducibility of the proposed AuNPs/PANI/SPCE sensor was also studied over a storage period up to 7 days through the electrochemical detection of 50 ppm MP in MeOH : 0.1 M PBS pH 7.0 (10:90; v/v) using the modified electrodes. All measurements in this study were performed in triplicate by DPV under optimal conditions. Fig. 4.11 illustrates the plots of anodic peak current of MP (referring to the pa1 peak in System I) versus the storage time of the AuNPs/PANI/SPCE ranging from 0 to 7 days. As seen from the results, when the modified electrodes were prepared and kept up to 7 days in the desiccator, the voltametric responses of MP slightly increased by less than 8.27 % of its initial signal (Day 0), indicating high stability of the fabricated AuNPs/PANI/SPCE sensor up to 1 week after production. Furthermore, the relative standard deviations (RSDs) of the intra-day and inter-day experiments were found to

be in the ranges of 4.7% -19.1%, demonstrating the acceptable reproducibility of these modified electrodes.

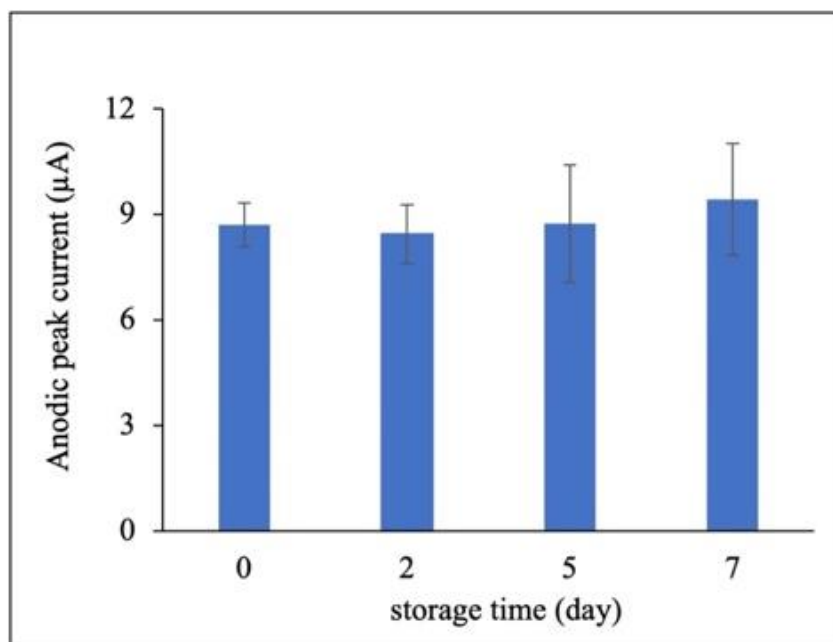


Figure 4.11 Stability study of the fabricated AuNPs/PANI/SPCE within the storage time up to 1 week on the quantitative determination of 50 ppm MP in MeOH : 0.1 M PBS pH 7.0 (10:90; v/v) by DPV under the optimal conditions.

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4.8 Determination of real samples

The real water samples collected from Chulalongkorn university were filtered with a 0.42 µm filter to eliminate particulate matters and spiked with a certain concentration of MP solution. Result show in table 4.1.

Table 4.1 The determination of methyl parathion in agricultural water by differential pulse voltammetry.

		DPV on AuNPs/PANI/SPCE
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Sample	Spiked (ppm)	Found (ppm)	Recovery (%)
agricultural water	-	Not detected	-
	10	11.08±1.22	110.83
	25	26.82±0.34	107.30

Data are shown as the mean \pm SD (N=3) and derived from three repeats.



CHAPTER V

CONCLUSIONS

The AuNPs/PANI/SPCE sensor was successfully developed for the determination of methyl parathion (MP) by differential pulse voltammetry (DPV) with enhanced sensitivity. This developed electrochemical sensor shows good electrocatalytic activity toward the quantification of MP, and the fabricated AuNPs/PANI/SPCE offers high stability and satisfactory reproducibility of the electrode surface for MP measurement. Under the optimal conditions, the proposed AuNPs/PANI/SPCE-based sensor possessed good linearity in the range of 1 to 50 ppm with limits of detection (LOD) and quantification (LOQ) of 0.17 and 0.51 ppm, respectively. Furthermore, the AuNPs/PANI/SPCE sensor applied to determine methyl parathion in agriculture water by external standard method with acceptable recoveries (107.3-110.8).

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