การพัฒนาวิธีตรวจวัดไอโซโพรทูรอนและคาร์เบนดาซิมโดยใช้ขั้วไฟฟ้าดัดแปรด้วยกราฟีน

นางสาวปิยนันท์ น้อยรอด

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METHOD DEVELOPMENT FOR DETERMINATION OF ISOPROTURON AND CARBENDAZIM USING GRAPHENE MODIFIED ELECTRODES

Miss Peeyanan Noirod

A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy Program in Chemistry
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Thesis Title	METHOD DEVELOPMENT FOR DETERMINATION OF ISOPROTURON AND CARBENDAZIM USING GRAPHENE MODIFIED ELECTRODES
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Field of Study	Chemistry
Thesis Advisor	Assistant Professor Suchada Chuanuwatanakul, Ph.D.
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ปิยนันท์ น้อยรอด : การพัฒนาวิธีตรวจวัดไอโซโพรทูรอนและคาร์เบนดาซิมโดยใช้ขั้วไฟฟ้าดัดแปรด้วยกราฟีน. (METHOD DEVELOPMENT FOR DETERMINATION OF ISOPROTURON AND CARBENDAZIM USING GRAPHENE MODIFIED ELECTRODES) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. ดร.สุชาดา จูอนุวัฒนกุล, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ศ. ดร.อรวรรณ ชัยลภากุล, 94 หน้า.

งานวิจัยนี้ได้พัฒนาวิธีตรวจวัดสารฆ่าศัตรูพืชและสัตว์ 2 ชนิดคือ ไอโซโพรทูรอนและคาร์เบนดาซิม ซึ่งเป็นสารประกอบที่เป็นอันตรายในคราวเดียวกันด้วยเทคนิคสแควร์เวฟสทริปปิงโวลแทมเมตรีโดยใช้ขั้วไฟฟ้าพิมพ์สกรีนดัดแปรด้วยกราฟีน ได้ศึกษาหาพารามิเตอร์ที่เหมาะสมในการตรวจวัดโดยใช้วิธีตัวแปรเดี่ยว ได้แก่ ปริมาณกราฟีนในขั้วไฟฟ้าใช้งาน ชนิดและความเข้มข้นของสารละลายอิเล็กโทรไลต์เกื้อหนุน ปริมาตรสารตัวอย่างที่ใช้ ศักย์ไฟฟ้าที่เริ่มต้นสแกน ศักย์ไฟฟ้าที่ใช้ในการตกสะสม เวลาที่ใช้ในการตกสะสม และสแควร์เวฟพารามิเตอร์ ที่ภาวะที่เหมาะสมนี้ ไอโซโพรทูรอนและคาร์เบนดาซิมให้พีกออกซิเดชันที่ชัดเจน โดยพบว่ากราฟีนมีผลต่อออกซิเดชันของไอโซโพรทูรอนและคาร์เบนดาซิมอย่างเด่นชัด เพราะนอกจากจะทำให้กระแสพีกออกซิเดชันเพิ่มขึ้นแล้ว ยังทำให้ค่าศักย์ไฟฟ้าออกซิเดชันลดลงอีกด้วย จากนั้นได้ประเมินลักษณะเฉพาะของวิธีวิเคราะห์ที่พัฒนาขึ้น พบว่าการตรวจวัดเชิงเส้นตรงอยู่ในช่วง 0.02 - 10.0 มิลลิกรัมต่อลิตร (R^2 = 0.9991) สำหรับไอโซโพรทูรอน และ 0.50 - 10.0 มิลลิกรัมต่อลิตร (R^2 = 0.9990) สำหรับคาร์เบนดาซิม โดยมีขีดจำกัดต่ำสุดของการตรวจวัดและขีดจำกัดต่ำสุดของการวิเคราะห์ปริมาณ 0.02 และ 0.07 มิลลิกรัมต่อลิตร สำหรับไอโซโพรทูรอน และ 0.11 และ 0.38 มิลลิกรัมต่อลิตร สำหรับคาร์เบนดาซิม ตามลำดับ นอกจากนี้ ค่าเบี่ยงเบนมาตรฐานสัมพัทธ์ระหว่างขั้วไฟฟ้า 10 ขั้ว เท่ากับ 9.2 และ 10 เปอร์เซ็นต์ สำหรับไอโซโพรทูรอนและคาร์เบนดาซิมตามลำดับ ในขณะที่ การวิเคราะห์ไอโซโพรทูรอนและคาร์เบนดาซิมในตัวอย่างน้ำ ดิน และผักที่เติมไอโซโพรทูรอนและคาร์เบนดาซิมได้ค่าการคืนกลับเป็นที่น่าพอใจ (81.4 - 107 เปอร์เซนต์) ดังนั้น วิธีที่พัฒนาขึ้นนี้เป็นวิธีที่ดี ใช้ขั้วไฟฟ้าที่ง่ายและราคาไม่แพง นอกจากนั้นยังใช้เวลาในการตรวจวิเคราะห์สั้น

ภาควิชา เคมี
สาขาวิชา เคมี
ปีการศึกษา 2556

ลายมือชื่อนิสิต ....
ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก ....
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KEYWORDS: ISOPROTURON / CARBENDAZIM / PESTICIDES / GRAPHENE-BASED ELECTROCHEMICAL SENSOR / STRIPPING VOLTAMMETRY

PEEYANAN NOIROD: METHOD DEVELOPMENT FOR DETERMINATION OF ISOPROTURON AND CARBENDAZIM USING GRAPHENE MODIFIED ELECTRODES. ADVISOR: ASST. PROF. SUCHADA CHUANUWATANAKUL, Ph.D., CO-ADVISOR: PROF. ORAWON CHAILAPAKUL, Ph.D., 94 pp.

Simultaneous determination of two pesticides, isoproturon and carbendazim, as the potentially hazardous compounds, by square wave anodic stripping voltammetry using graphene modified screen-printed electrodes was developed. The operational parameters, in terms of the graphene content of the working electrode, type and concentration of supporting electrolyte, applied sample volume, initial scan potential, accumulation potential, accumulation time, and square wave parameters, were optimized using a univariate approach. Under these optimal conditions, isoproturon and carbendazim yields well-defined oxidation peak. It is found that the graphene exhibits obvious activity toward the oxidation of isoproturon and carbendazim since it not only increases the oxidation peak current but also lowers the oxidation potential. The analytical characteristics of the proposed method were then evaluated. A linear detection was obtained in the range of 0.02 - 10.0 mg/L (R^2 = 0.9991) for isoproturon and 0.50 - 10.0 mg/L for carbendazim (R^2 = 0.9990), with the limits of detection and quantification being 0.02 and 0.07 mg/L for isoproturon and 0.11 and 0.38 mg/L for carbendazim, respectively. In addition, the relative standard deviations of detection between ten electrodes were 9.2 and 10 % for isoproturon and carbendazim, respectively, whilst satisfactory recoveries were obtained in the analysis of isoproturon and carbendazim in spiked water, soil and vegetable samples (81.4–107%). Consequently, the proposed method showed a great promise as an inexpensive and simple electrode, with additionally a shorter analysis time.

Department: Chemistry
Field of Study: Chemistry
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Student's Signature ....
Advisor's Signature ....
Co-Advisor's Signature ....

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LIST OF ABBREVIATIONS

A	ampere
CV	cyclic voltammetry
E	potential
E_{acc}	accumulation potential
GE	graphite electrode
L	liter
LOD	limit of detection
LOQ	limit of quantification
mg	milligram
MRL	maximum residue limit
mV	millivolt
PVC	polyvinylchloride
RSD	relative standard deviation
SEM	scanning electron microscope
SD	standard deviation
SPE	solid phase extraction
SWV	square wave voltammetry
t_{acc}	accumulation time
V	volt
µg	microgram
µL	microliter
°C	degree Celsius

CHAPTER I
INTRODUCTION

1.1 Introduction

Thailand is an agricultural country where pesticides are used for protection of a large variety of crops such as rice, fruits and vegetables. During the past decade, the Kingdom of Thailand has experienced an approximate four-fold increase in pesticide use [1]. Pesticides are chemicals that are specifically developed and produced for use in the control of agricultural pests. These compounds and the products derived from them by degradation or metabolism give rise to residues that may spread through the environment and are particularly frequent contaminants in superficial and ground water, soil as well as agricultural and food products, leads to an accumulation of pesticide residues that can impose a serious risk to human health and the environment worldwide. Thus, the determination of pesticide levels is a very important issue from an environmental point of view. For that reason, the development of simple, portable, sensitive, selective, and green analysis methods for the rapid and precise detection of pesticide residues in environmental samples has become increasingly important for homeland security and health protection.

There is a wide range of studies concerned with analytical methods for monitoring of pesticides in environmental samples. Traditional methods of analysis, such as ultraviolet spectrometry [2-4], capillary electrophoresis [5, 6], gas chromatography [7, 8], and liquid chromatography [9-13]. Most applications of chemical analysis to pesticide control involve methods with high sensitivity accompanied by sufficient selectivity, precision, and accuracy. Easy sample pretreatment and rapid analytical procedures are also desirable. When selecting the method, the cost of the instrumentation and the possibility of performing measurements in the field are also important factors to be considered. Since electrochemical methods satisfy all the above criteria, they were a good choice for the analysis and control of environmental pesticides [14, 15]. As many organic compounds used as pesticides contain electroactive groups, voltammetry can be used for their mechanistic and analytical studies [16-22].

A screen-printed electrode is a low-cost disposable electrode commonly used for electrochemical applications. Its ease for fabrication and modification with various materials, flexibility in design, and efficiency in large-scale production make it is increasingly being used in the determination of pesticides. Moreover, screen-printed electrodes chemically modified with various materials, such as clay [23, 24], multi-walled carbon nanotubes [25-27], and graphene [28], have been developed for the determination of pesticides.

Graphene, a carbon material, is a 2D crystal, composed of monolayers of carbon atoms arranged in a honeycombed network with six-membered rings. It has attracted increasing research interest. One of the promising applications of graphene is in electrochemical sensing, where it is an excellent material in terms of its electrochemical properties, especially in electrochemical sensors and biosensors, because of its high electrical conductivity and large surface area which can effectively promote the electron transfer between the target molecule and the electrode. Thus, graphene was selected as an electrode modifier. Encouragingly, the combination of the graphene modified screen-printed electrodes and square wave stripping voltammetry may open up new opportunities for a fast, simple, and low-cost electrochemical detection based method for the simultaneous determination of some pesticides in environmental water, vegetables or soil samples.

Isoproturon, one of the most commercially significant agricultural herbicides, mainly enters the environment during its application to soils before plants emerge and also to soils around growing crops. It is used for pre- and post-emergence control of grasses and weeds in cereals; slightly it can be used in other crops. The current use of isoproturon is explained by the fact that it is a relatively cheap and easy-to-use herbicide. This herbicide has been classified as very toxic to aquatic organisms and is mobile in soil [29]. Carbendazim is a sort of benzimidazole based fungicide. It is widely used in agriculture for protecting from and eradicating a variety of pathogens which affect cereals (rice), fruits and vegetables. This compound, when applied on soils, can persist for a long time, because the benzimidazolic ring is difficult to break and consequently its degradation is slow. In Thailand, isoproturon and carbendazim are hazardous substances controlled by Department of Agriculture, Ministry of Agricultural and Cooperatives. Therefore, the ability to rapidly and accurately determine trace amount of isoproturon and carbendazim has become increasingly important for the environment and health protection. Most of the previous electroanalytical studies of pesticides are focused on the determination of individual compounds rather than for their simultaneous analysis. Besides, the aforementioned voltammetric determination of individual isoproturon and carbendazim was performed in a conventional electrochemical cell that requires a large sample and reagent volume. For that reason, the development of the simultaneous determination method for isoproturon and carbendazim, that is sensitive, less time-consuming and less expensive are still needed.

This work intends to fabricate the low-cost disposable screen-printed sensor with three integrated electrodes, graphene-modified working electrode, silver/silver chloride reference electrode and carbon counter electrode, and develop a fast, simple, and low-cost method for the simultaneous quantification of isoproturon and carbendazim by square wave stripping voltammetry using the developed sensor. There is no requirement to use a conventional electrochemical cell because the screen-printed sensor could be used as an electrochemical cell [30]. Only a single drop of the sample solution is required to be dispensed onto the surface of the electrodes for each stripping voltammetric measurement. Various experimental parameters were optimized. Then the analytical performance of the developed method was studied. Finally, the developed method was applied to real sample such as water, soil and vegetable samples.

1.2 Objective of the research

This research consists of three main goals for development and improvement as follows:

1. To develop the screen-printed sensor with graphene-modified working electrodes for the determination of some pesticides, isoproturon and carbendazim.
2. To optimize experimental parameters for the simultaneous determination of isoproturon and carbendazim by square wave stripping voltammetry using the developed sensor.
3. To apply the developed method for the determination of isoproturon and carbendazim in real samples.

CHAPTER II

THEORY AND LITERATURE SURVEY

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

2.1 Pesticides

Thailand will continue to be a major agricultural exporter; Thai agricultural products are an important part of Thai economy. Pesticides, in common, are chemical or biological active substances, which are of anthropogenic origin, used for killing or controlling unwanted organisms. In this dissertation, two pesticides including isoproturon and carbendazim are interested.

2.1.1 Isoproturon

Isoproturon, belongs to the group of substituted phenylurea, is a selective systemic herbicide mainly used in the control of annual grasses and broad-leaved weed in cereals. This chemical is absorbed by the roots and the leaves of weeds and inhibits photosynthesis due to the inhibition of electron transport process. The current use of isoproturon is explained by the fact that it is a relatively cheap and easy-to-use herbicide.

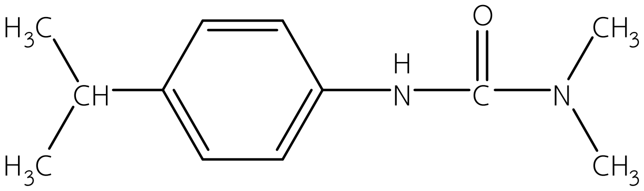

Figure 2.1 Structure of isoproturon

Table 2.1 Identity and property of isoproturon [31]

IUPAC name	3-(4-isopropylphenyl)-1,1-dimethylurea	
Molecular formula	$C_{12}H_{18}N_2O$	
Molecular mass	206.28 g/mol	
Activity	Herbicides (phenylurea herbicides)	
Appearance	Colorless crystals	
Melting point	158 °C	
Vapor pressure	3.15×10^{-3} mPa at 20 °C	
Specific density	1.2 at 20 °C	
Solubility at 20 °C	Water	65 mg/L
	Methanol	75 g/L
	Dichloromethane	63 g/L
	Acetone	38 g/L
	Benzene	5 g/L
	Xylol	4 g/L
	n-Hexane	0.2 g/L

Isoproturon mainly enters the environment during its application as an agricultural herbicide, but releases may also occur both diffuse and point sources. Emissions to water come mostly from surface runoff and farm point sources (runoff from farmyards, storage facilities), and at a lesser extent from field drain flow and spray drift during field application. Isoproturon has a low tendency to adsorb to soils and is therefore quite able to enter in water bodies despite its rather low water solubility. It has a half-life about 30 and 40 days for water and soils, respectively.

2.1.2 Carbendazim

Carbendazim is a systemic benzimidazole fungicide and a metabolite of benomyl, which plays a very important role in plant disease control in cereals and fruits, such as citrus, bananas, strawberries, pineapples, and pomes. It is absorbed through the roots and green tissues with translocation acropetally. It is also used in post-harvest food storage, and as a seed pre-planting treatment. Carbendazim works by inhibiting the development of fungi probably by interfering with spindle formation at mitosis.

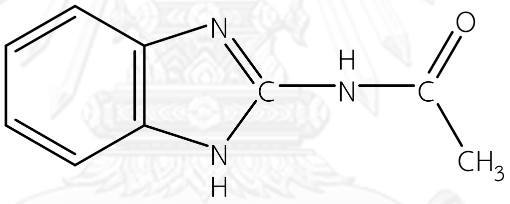

Figure 2.2 Structure of carbendazim

In Thailand, isoproturon and carbendazim are hazardous substances class III controlled by the Department of Agriculture, Ministry of Agricultural and Cooperatives. According to Commission Regulation (EU) No 212/2013, the maximum residue limits (MRLs) for isoproturon and carbendazim in almost fruits and vegetables are 0.05 and 0.1 mg/kg, respectively.

Table 2.2 Identity and property of carbendazim [32]

IUPAC name	Methyl benzimidazol-2-ylcarbamate	
Molecular formula	$C_9H_9N_3O_2$	
Molecular mass	191.20 g/mol	
Activity	Fungicides	
Appearance	Light gray powder	
Melting point	302-307 °C (decomposed)	
Vapor pressure	$< 1 \times 10^{-7}$ Pa at 20 °C	
Acidity (pKa)	4.48	
Solubility at 20 °C	Water pH 4	28 mg/L
	pH 7	8 mg/L
	pH 8	7 mg/L
	Hexane	0.5 mg/L
	Benzene	36 mg/L
	Dichloromethane	68 mg/L
	Ethanol	300 mg/L
	Dimethylformamide	5,000 mg/L
	Acetone	300 mg/L
	Chloroform	100 mg/L

2.2 Electrochemical methods

Electrochemical methods are the analytical methods which depend on the measurement of the current or potential during electrochemical reactions on the electrode surface. All of the normally employed electrochemical methods can be used in environmental analyses. 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 describe in this section involved the methods that were used in this work.

2.2.1 Voltammetric methods

Voltammetry is a part of electrochemical methods based on current measurement as a function of the potential applied to a small electrode under conditions of complete concentration polarization in which the rate of oxidation or reduction of the analyte is limited by rate of mass transfer of the analyte to the electrode surface. Therefore, voltammetric methods belong to the prior methods, it useful primarily for defining the electrochemical behavior of the target analyte. It is widely used by analytical, inorganic, physical and biological chemists for fundamental studies of three parts in redox reactions, first: oxidation and reduction processes in different media, second: adsorption processes on electrode surface and third: electron transfer mechanisms at chemically modified electrode surface. The characteristic of voltammetric methods is that concerns the application of a potential to an electrode dipped in a solution containing electroactive species and the measuring of the current in electrochemical cell, such as the applied potential is varied or the current is monitored over a period of time. Therefore, the voltammetric methods can be explained as function of potential, current and time; the recording result is called a voltammogram.

In this dissertation, voltammetric methods including cyclic voltammetry, square wave voltammetry and stripping voltammetry were used for the determination of two pesticides, isoproturon and carbendazim. These methods are described in the following section.

2.2.1.1 Cyclic voltammetry

Cyclic voltammetry (CV) has become an important in electrochemistry, it is widely used in electrochemical methods for the study of the redox reaction (oxidation and reduction behavior over a wide potential range) based on varying the applied potential of the working electrode in both forward and reverse directions (opposite direction) at fix scan rate while monitoring the signal current. It is a simple method that needs relatively little experimental effort and provides a good deal of useful information about the electrochemical behavior. However, it is rarely used for quantitative determination. CV is often the first experiment performed in electroanalytical methods in order to study the electrochemical properties of an analyte in solution. CV experiment consists of scanning linearly the potential of a stationary working electrode in an unstirred solution by a triangular potential waveform shown in Figure 2.3. The triangular waveform produces the forward and then the reverse scan. The experiment of one full cycle, a partial cycle, or several cycles can be carried out, depending on the analysis. The current at the working electrode is plotted versus the applied voltage in termed of cyclic voltammogram. CV is a complicated, time dependent function of a large number of physical and chemical parameters.

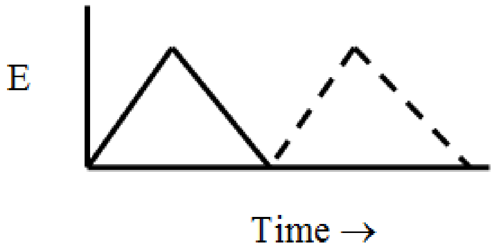

Figure 2.3 Cyclic voltammetric excitation signal [33].

2.2.1.2 Square wave voltammetry

Square wave voltammetry (SWV) is one of the major voltammetric methods, a great amplitude differential method, a further improvement of staircase voltammetry in which a waveform composed of symmetrical square wave, superimposed on the potential staircase sweep. This potential is applied to the working electrode. The current is measured twice during each square wave cycle, once at the end of the forward pulse and once at the end of the reverse pulse. Oxidation or reduction of species is registered as a peak or trough in the current signal at the potential at which the species begins to be oxidized or reduced. Excellent sensitivity accrues from the fact that the net current is larger than either the forward or the reverse components current. The sensitivity of this method can be increased by enhancing the amplitude or the frequency of the square wave. The limit of the enhancing is severely related to the kinetics aspects of the redox processes, it has not to be slower than the velocity of the scanning of potential. The speed of SWV coupled with computer control and current signal averaging, allows for experiments to be performed repetitively and increases the signal-to-noise ratio. Figure 2.4 shows the excitation signal in square wave voltammetry.

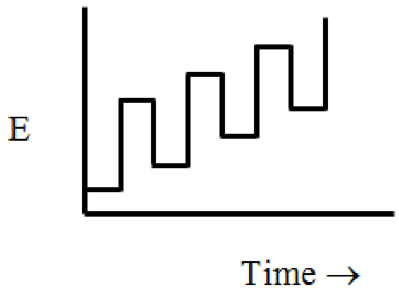

Figure 2.4 Square wave voltammetric excitation signal [33].

2.2.1.3 Stripping voltammetry

Stripping voltammetric methods have the lowest limits of detection of any of the commonly used electrochemical method. Stripping voltammetry consists of two steps.

1) Deposition step or accumulation step

The analyte species in the sample solution is accumulated onto or into a surface of the working electrode, either by the electrolysis or adsorption by controlled-potential electrolysis for a definite time under reproducible hydrodynamic conditions in the solution.

2) Stripping step

After the chosen period of time, the accumulated analyte is measured or stripped from the electrode by the application of a potential scan and the resulting current-voltage curve is recorded. Any number of potential waveforms can be used for the stripping step. The most common are differential pulse and square wave due to the discrimination against charging current. Though, square wave has the added advantages of faster scan rate and increased sensitivity relative to differential pulse.

As with any quantitative method, care must be taken in trace analysis so that reproducible results are obtainable. Important conditions, such as the electrode surface and accumulation time, should be held constant. Moreover, every effort should be made to minimize contamination.

Stripping voltammetry is an extremely sensitive electrochemical method for the determination of pesticides in a wide variety of environmental matrices [34, 35]. This has been rendered possible largely by the advent adsorption interfacial accumulation.

In this dissertation, square wave stripping voltammetry was selected for the simultaneous determination of isoproturon and carbendazim. Stripping voltammetry was carried out in 1 M $HClO_4$ solution as a supporting electrolyte solution. Therefore, the analytes were protonated. In an accumulation step, a negative potential was applied to the working electrode. The protonated analytes were induced to migrate to and accumulate onto the electrode surface by electrostatic interaction. In a stripping step, the electrode potential was scanned in a positive direction. The analytes were oxidized and released electrons which could be measured as a current. The proposed mechanism of electrochemical reactions of isoproturon and carbendazim are shown in Figure 2.5 and 2.6 and the applied potential waveform as well as the typical voltammogram of the simultaneous determination of isoproturon and carbendazim are shown in Figure 2.7.

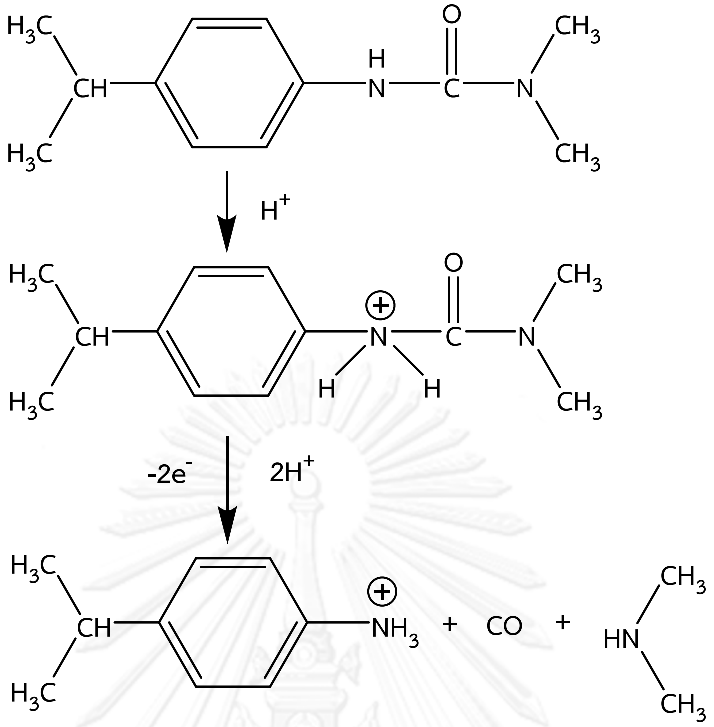

Figure 2.5 The proposed mechanism of electrochemical reaction of isoproturon [24].

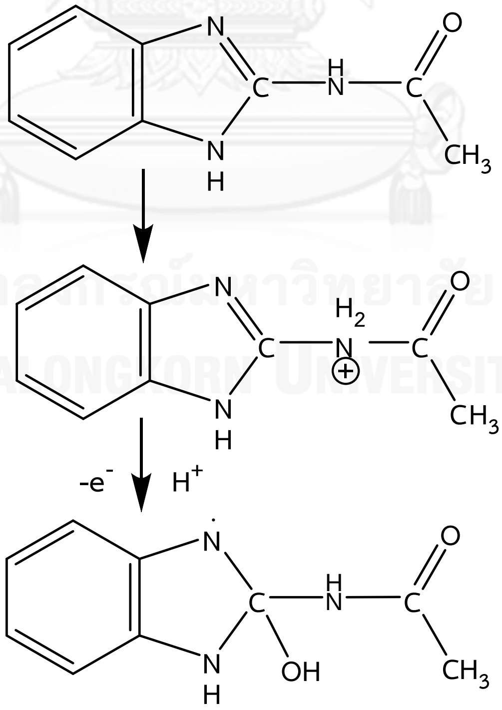

Figure 2.6 The proposed mechanism of electrochemical reaction of carbendazim [36].

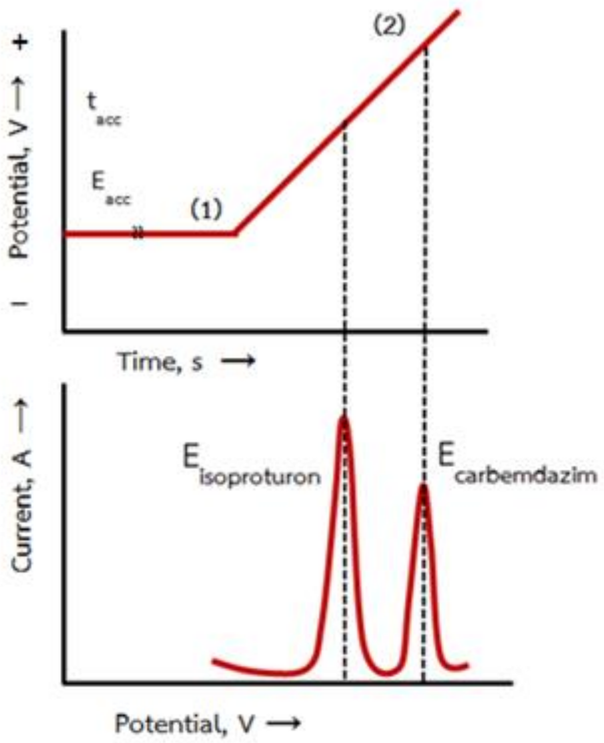

Figure 2.7 Stripping voltammetry: the potential-time waveform (top), voltammogram (bottom).

2.2.2 Electrodes

The electrochemical cell, where the voltammetric experiment is carried out consists 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.

2.2.2.1 Screen-printed electrode

Screen-printing technology is a method for used in the fabrication of electrodes for the development of disposable electrochemical sensor.

A screen-printed electrode is a planar device based on multiple layers of a graphite power based ink printed on polyvinyl chloride substrates.

The main advantages of the screen-printed electrode include simplicity, low-cost, portability, ease of operation, reliability, small size and mass production capabilities, which lead to its development in various applications in the electroanalytical field [37]. This thesis selected screen-printed method for used in the simultaneous determination of isoproturon and carbendazim.

2.2.2.2 Working electrode

The working electrode is a one of the components in an electrochemical method in which the interesting reaction of target analyte occurs. The occurred reaction at the working electrode can be referred to as either cathodic or anodic. A working electrode has a potential that varies in a known way with variations in the concentration of target analyte. In voltammetric method, the small surface area of the working electrode is used to enhance polarization and to minimize depletion of the analyte. The performance of this method is strongly influenced by the material of the working electrode. The ideal working electrode should provide a high signal-to-noise characteristic. Thus, its selection depends mainly on two influences including the redox behavior of the electroactive species and the background current over the potential region required for the measurement. A variety of materials has been applied as working electrodes.

2.2.2.3 Reference electrode

A reference electrode is an electrode having a known electrode potential that remains constant at constant temperature and is independent of the composition or concentration of the analyte solution. This electrode acts as reference point along the potential axis by which the oxidizing or reducing power of the working electrode is judged. In ideally, the reference electrode should be constant over time, provide a stable potential and temperature independent. In addition, the ideal reference electrode should be easy to make and to use. The most widely used is the silver/silver chloride reference electrode because it is simple, inexpensive, very stable and non-toxic. Then, this work used a silver/silver chloride (Ag/AgCl) electrode as a reference electrode for the simultaneous determination of isoproturon and carbendazim. The potential of this electrode was determined by the reaction [38]:

$$AgCl(s) + e^{-} \rightleftharpoons Ag(s) + Cl^{-}$$

2.2.2.4 Counter electrode

The counter electrode usually employed to minimize errors from cell resistance in controlling the potential of the working electrode. The counter electrode is made of a chemically inert conducting material with large surface area.

2.2.2.5 Screen-printed electrochemical sensor

Screen-printing is a process that has been widely used in artistic applications and more recently in the production of electronic circuits and sensors. It consists of squeezing an ink or paste through a patterned screen onto an inert substrate such as polyvinyl chloride (PVC), polycarbonate, polyester or ceramic. Successive layers can be deposited by this procedure through the corresponding mask providing a specific pattern [39].

The main advantages of the screen-printed sensor include simplicity, low-cost, small size, ease of operation, reliability, mass production capabilities and a wide range of designs, which lead to its development in various applications in the electroanalytical field. Screen-printed electrochemical sensors can be used as either single electrodes or whole electrode systems (working, reference and counter electrode). Furthermore, the disposable screen-printed electrochemical sensors can efficiently overcome the drawbacks of conventional electrodes such as carry-over and surface fouling because they are normally used only once. For those reasons, screen-printed sensors were used in the simultaneous determination of isoproturon and carbendazim [40].

2.2.2.6 Graphene

A screen-printed sensor is a planar device based on multiple layers of a graphite powder based ink printed on PVC substrates. As an attempt to promote the electron transfer reaction at the surface of working electrode and improve the sensibility and selectivity of a screen-printed sensor, numerous substances and materials either nanoparticles or nanostructured material can be used to modify electrode surface. In this work, graphene was used as a modifying material.

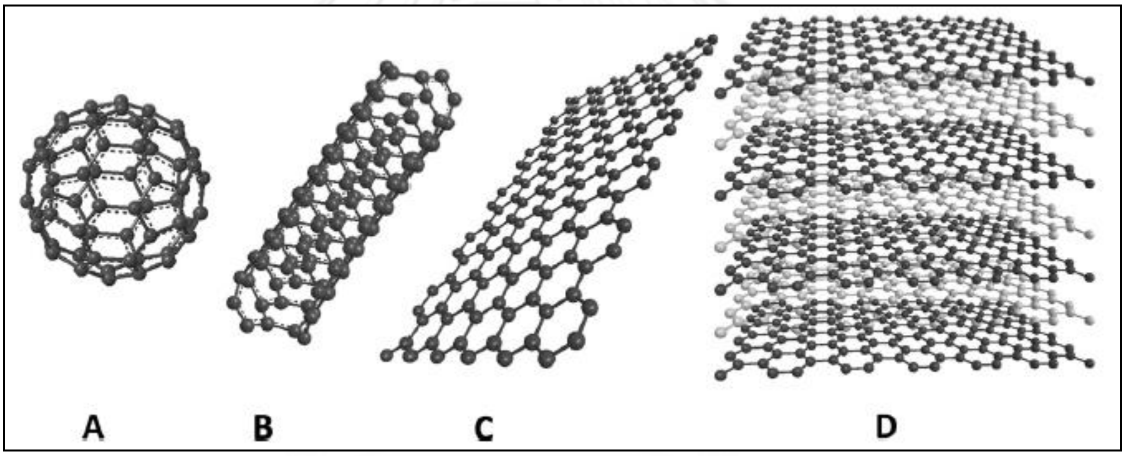

Figure 2.8 Various forms of sp^2-bonded carbon: (A) fullerene, (B) single-wall carbon nanotubes, (C) graphene, and (D) graphite [41].

Graphene is the most recent member of the multi-dimensional carbon-nanomaterial family, starting with fullerenes as zero-dimensional (0-D) material, single-wall carbon nanotubes as one-dimensional (1-D) nanomaterial, and ending with graphite as three-dimensional (3-D) material. Graphene fills the gap for two-dimensional (2-D) carbon nanomaterial (Figure 2.8).

Graphene has proved to be an excellent nanomaterial for applications in electrochemistry, emerging as a true two-dimensional (2-D) sheet of carbon atoms in a hexagonal pattern. In graphene, carbon atoms are densely packed in a regular sp^2-bonded. These bonds and this electron configuration are the reasons for the extraordinary properties of graphene as shown in Table 2.3. High-quality graphene is strong, light, nearly transparent and an excellent conductor of heat and electricity. Its interactions with other materials and with light and its inherently two-dimensional nature produce unique properties, such as the bipolar transistor effect, ballistic transport of charges and large quantum oscillations [42]. This has recently been at the scientific focus due to its remarkable mechanical and electronic properties.

Table 2.3 Graphene properties [43]

Appearance	Black powder
Thickness	6-8 nm
Average particle diameter	15 μm
Surface area	120-150 m^2/g
Carbon	99.5+%
Electrical conductivity	10^7 siemens/meter (parallel to surface)
	10^2 siemens/meter (perpendicular to surface)
Thermal conductivity	3000 watts/meter-K (parallel to surface)
	6 watts/meter-K (perpendicular to surface)

In comparison to carbon nanotube (CNTs), graphene has the advantages of high thermal and electrical conductivity due to small thickness and large surface area. As the electrochemistry of graphene sheets is driven by its edges (either in planar graphene or in rolled-up graphene – CNTs) where heterogeneous electron transfer is fast, when looking at the structure of CNTs and graphene, one might expect higher observed on graphene sheets simply because there is a larger number of edges per mass of the material.

Major additional premises of graphene compared to other carbon based nanomaterials are low cost and large production scale in biosensor development. Therefore, the excellent electrochemical behaviors of graphene indicate that graphene is a promising electrode material in electroanalysis. Several electrochemical sensors based on graphene and graphene composites for bioanalysis and environmental analysis have been developed.

2.3 Literature survey

In 2006, Manisankar *et al.* [24] developed the heteropolyacid montmorillonite clay-modified glassy carbon electrode for determination isoproturon, carbendazim and methyl parathion by square wave anodic stripping voltammetry. The presence of surfactant enhanced the peak currents of all three pesticides. The limits of detection were 1, 10 and 20 μg/L, respectively.

In 2008, Du *et al.* [44] used the bismuth-film electrode prepared by *ex situ* depositing bismuth onto glassy carbon electrode for the detection of methyl parathion. The sufficiently wide negative potential window available made the bismuth-film electrode a potentially suitable electrode for cathodic electrochemical detection of pesticides. The bismuth-film electrode showed similar or even favorable behavior compared to that of mercury and bare electrode. Under the optimum experimental conditions, the cathodic voltammetric response was proportional to the concentration of methyl parathion range from 3.0 to 100 ng mL^{-1}, with a correlation coefficient of 0.9981. The limit of detection was 1.2 μg/L. In this same year, Du *et al.* [45] reported the developed multiwalled carbon nanotube as a new sorbent for solid-phase extraction of organophosphate pesticides. A combination of SPE with square-wave voltammetric analysis resulted in a fast, sensitive, and selective electrochemical method for determination of methyl parathion. The linear range of 0.05-2 μg/L and the detection limit of 5 μg/L were obtained.

In 2008, Wang *et al.* [46] 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. The limit of detection was 3 µg/L.

In 2008, Manisankar *et al.* [25] reported the determination of isoproturon, voltage and dicofol by differential pulse voltammetric method using multiwalled carbon nanotubes modified glassy carbon electrode with polyaniline and polypyrrole. The limits of detection were 0.1, 0.01 and 0.05 µg/L for isoproturon, voltage and dicofol, respectively.

In 2009, Gong *et al.* [47] reported the use of Ni/Al layered double hydroxides modified glassy carbon electrode for the determination of organophosphate pesticide, methyl parathion, by square-wave stripping voltammetric method coupled with solid-phase extraction. The limit of detection was 0.6 µg/L.

In 2010, Parham and Rahbar [48] reported a carbon paste electrode modified with ZrO_2-nanoparticles. This electrode shows a strong affinity toward the phosphate group on methyl parathion molecules, which provides sensitive and selective method for the determination of methyl parathion by square wave voltammetry. The limit of detection and limit of quantification were 2.0 and 5.7 µg/L respectively.

In 2010, Kang *et al.* [49] developed methyl parathion sensor based on gold nanoparticles and Nafion film modified glassy carbon electrode. The results demonstrated that Nafion improve ionic and electronic conduction and gold nanoparticles possess extreme small size, a high surface-to-volume ratio and high electrocatalytic activity towards the reduction of methyl parathion. Square wave voltammetric method for determining methyl parathion was developed and the limit of detection of 0.03 µg/L was achieved.

In 2010, Tcheumi *et al.* [50] 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. The limit of detection was 0.02 µg/L.

In 2011, Gong *et al.* [51] 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 combining the advantages of nanoassembly of Au nanoparticles and two dimensional graphene provides a fast, simple, and sensitive electrochemical method for detecting nitroaromatic OPs. The detection limit for the stripping analysis of methyl parathion was 0.6 µg/L.

In 2011, Wu *et al.* [52] developed sorbent based electrochemical sensors using electrochemical reduced ß-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, large delocalized π-electron system and the superconductivity of graphene. The limit of detection was 0.05 µg/L.

In 2011, Guo *et al.* [28] used cyclodextrin-graphene hybrid nanosheets as an enhanced material for ultrasensitive detection of carbendazim by electrochemical method. The peak currents of carbendazim on the graphene modified glassy carbon electrode and the cyclodextrin-graphene modified glassy carbon electrode were increased by 11.7 and 82.0 folds compared to the bare modified glassy carbon electrode, respectively. This indicates the nanocomposite film shows the excellent electrical properties of cyclodextrin-graphene hybrid nanosheets and exhibits high supramolecular recognition capability of cyclodextrin. The limit of detection for the differential pulse voltammetric analysis of carbendazim was 0.38 µg/L. Moreover, the developed electrochemical sensor exhibited good stability and reproducibility for the detection of carbendazim.

In 2011, Ion *et al.* [53] fabricated a chitosan–grafted exfoliated graphite nanoplatelet hybrid chemically modified electrode for the determination of quintozen, an organochlorine pesticide by differential pulse voltammetry. This sensor exhibits good sensitivity with a detection of 0.003 µg/L.

In 2011, Sundari and Manisakar [26] used a modified electrode fabricated by coating multiwalled carbon nanotubes/poly(3-methyl thiophene) film on a glassy carbon electrode for the adsorptive stripping voltammetric determination of different classes of pesticides including herbicide (isoproturon), insecticides (voltage, cypermethrin, deltamethrin, fenvalerate) and an acaricide (dicofol). The limits of detection were 0.008, 0.0081, 0.0015, 0.0019, 0.0061 and 0.0054 µg/L, respectively.

In 2012, Liang *et al.* [54] developed Ni/Al layered double hydroxides decorated graphene nanosheets hybrid on a cathodic substrate for stripping voltammetric detection of methyl parathion. The detection limit was 0.6 ng/L.

In 2013, Dornellas *et al.* [55] presented the metal film (Bi-film) modified on a glassy carbon electrode for determination of picoxystrobin fungicide using differential pulse anodic stripping voltammetry. The detection limit was 8.4 µg/L.

In 2013, Chen *et al.* [56] reported that glassy carbon electrode modified with water-soluble β-cyclodextrin polymer functionalized reduced-graphene oxide showed excellent electrochemical performance for a pesticide-imidacloprid. The detection limit for differential pulse voltammetric measurement of imidacloprid at this electrode was 4.12 µg/L.

In 2013, Luo *et al.* [57] fabricated graphene oxide–multi-walled nanotubes hybrid nanomaterial modified glassy carbon electrode for determination of carbendazim by differential pulse voltammetry. The detection limit was 1.08 µg/L.

Although some aforementioned researchers had reported the highly sensitive methods for the determination of pesticides, these methods has a number of disadvantages such as long analysis time [25, 48, 53] and complication [52]. Consequently, the rapid and simple methods for the simultaneous determination of pesticides are required.

CHAPTER III

EXPERIMENT

The information of the chemicals, instruments, sample preparations and modification of the electrode in this work are explained in this chapter.

3.1 Instruments and apparatus

3.1.1 Fabrication of the graphene-based electrochemical sensors

The instruments and apparatus used in the fabrication of the graphene-based electrochemical sensors are listed in Table 3.1.

Table 3.1 List of instruments and apparatus involved in the fabrication of the graphene-based electrochemical sensors

Instruments and apparatus	Suppliers
Screen-printed blocks	Chaiyaboon, Thailand
Analytical balance, Mettler Toledo	Mettler, Switzerland
Ultrasonic bath, ULTRA Asonik 28H	ESP Chemical, USA
Hot air oven	Memmert, USA
Glassware	

3.1.2 Characterization of the graphene-based electrochemical sensors

The instruments and apparatus used in the characterization of the graphene-based electrochemical sensors are listed in Table 3.2

Table 3.2 List of instruments and apparatus involved in the characterization of the graphene-based electrochemical sensors

Instruments and apparatus	Suppliers
Scanning electron microscope (SEM)	JEOL, Japan

3.1.3 Sample preparations

The instruments and apparatus used in the sample preparations are listed in Table 3.3.

Table 3.3 List of instruments and apparatus involved in the sample preparations

Instruments and apparatus	Suppliers
Analytical balance, Mettler Toledo	Mettler, Switzerland
Blender, HR1701	Philips, Indonesia
Hot air oven	Memmert, USA
Nylon membrane filters 0.45 μm	Whatman, UK
Micropipette and tips	Eppendorf, Germany
Milli-Q ultrapure water purification system	Millipore, USA
Glasswares	

3.1.4 Electrochemical measurement of isoproturon and carbendazim

The instruments and apparatus used in the electrochemical measurement of isoproturon and carbendazim are listed in Table 3.4.

Table 3.4 List of instruments and apparatus involved in the electrochemical measurement of isoproturon and carbendazim

Instruments and apparatus	Suppliers
PalmSens potentiostat	Palm Instruments BV, The Netherlands
AutoLab PG 30 potentiostat/galvanostat	Metrohm, The Netherlands
Milli-Q ultrapure water purification system	Millipore, USA
Micropipette and tips	Eppendorf, Germany
Faraday cage	
Glasswares	

3.2 Chemicals

3.2.1 Fabrication of the graphene-based electrochemical sensors

The chemicals for the fabrication of the graphene-based electrochemical sensors are listed in Table 3.5

Table 3.5 List of the chemicals for the fabrication of the graphene-based electrochemical sensors

Chemicals	Suppliers
2-butoxyethyl acetate (AR grade)	Sigma-Aldrich, Germany
Carbon ink	Electrodag PF-407C, Acheson, USA
Diethylene glycol monobutyl ether (AR grade)	Fluka, UK
Ethanol (AR grade)	Emsure®, Merck, Germany
Graphene	SkySpring Nanomaterials, USA
Graphite	Sigma-Aldrich, Switzerland
Silver/silver chloride paste 70/30	Gwent, UK
Nail polish (as an insulator)	Skinfood, Thailand

3.2.2 Electrochemical measurement of isoproturon and carbendazim

The chemicals used in the electrochemical measurement of isoproturon and carbendazim are listed in Table 3.6

Table 3.6 List of the chemicals used in the electrochemical measurements of isoproturon and carbendazim

Chemicals	Suppliers
Carbendazim (AR grade)	Sigma-Aldrich, Germany
Isoproturon (AR grade)	Sigma-Aldrich, Germany
Perchloric acid (AR grade, 70-72% w/w)	Merck, Germany
Ethanol (AR grade)	Merck, Germany
Milli-Q ultrapure water ($R \geq 18.2$ MΩ·cm)	Milli-Q ultrapure water purification system, Millipore, USA

3.2.3 Sample preparations

The chemicals used in the sample preparations are listed in Table 3.7.

Table 3.7 List of the chemicals in the sample preparations

Chemicals	Suppliers
Carbendazim (AR grade)	Sigma-Aldrich, Germany
Isoproturon (AR grade)	Sigma-Aldrich, Germany
Perchloric acid (AR grade)	Merck, Germany
Dichloromethane (AR grade)	Merck, Germany
Ethanol (AR grade)	Merck, Germany
Milli-Q ultrapure water ($R \geq 18.2$ MΩ·cm)	Milli-Q ultrapure water purification system, Millipore, USA

3.3 Chemical preparations

3.3.1 Graphene dispersion

Dispersion of graphene in optimum solvent is an important process to improve the electrical conductivity of graphene-modified electrode. In this work, commercial graphene nanopowder was directly dispersed in a solvent by ultrasonication for 2 h to give a more homogeneously dispersed graphene suspension. Next, the well-dispersed graphene suspension was dried by evaporation of solvent in a vacuum oven at 100 °C for 1 h.

3.3.2 Preparation of the graphene-modified graphite ink

The graphene-modified graphite ink was prepared by weighing out of 10.7 mg graphene into a 25 mL ethanol to form a homogenous dispersion under ultrasonication for 2 h. Next, graphene was dried in an oven at 100 °C for about 1 h and ground in a mortar. After that, the graphene powder was mixed with 1.0 g carbon ink, 0.2 g graphite powder and 20 drops of the of 1:1 (v/v) diethylene glycol monobutyl ether: 2-butoxyethyl acetate for 20 min to produce the graphene-modified graphite ink.

Figure 3.1 The graphene-modified graphite ink

3.3.3 Preparation of solutions for the determination isoproturon and carbendazim

3.3.3.1 Stock solution of isoproturon solution, 100 mg/L

A 100 mg/L stock solution of isoproturon was prepared by dissolving 10.0 mg of isoproturon in 100 mL of ethanol.

3.3.3.2 Stock solution of carbendazim solution, 100 mg/L

A 100 mg/L stock solution of carbendazim was prepared by dissolving 10.0 mg of isoproturon in 100 mL of ethanol.

3.3.3.3 Perchloric acid solution, 1.0 M

A 1.0 M perchloric acid was prepared by diluting 8.38 mL of 70-72% w/w perchloric acid in 100 mL of milli-Q water.

3.4 Fabrication of the graphene-based electrochemical sensors

The in-house graphene-based electrochemical sensor with integrated three-electrode configuration consisted of a graphene-modified graphite working electrode, a carbon counter electrode and a silver/silver chloride reference electrode (Figure 3.2), was fabricated as follows. The silver ink was printed through the fine mesh or screen onto the polyvinyl chloride (PVC) substrate to form the reference electrode and connector (Figure 3.3), and then dried in an oven at 55 °C for 1 h. Next, the mixture of 1.0 g carbon ink, 0.2 g graphite powder and 20 drops of 1:1 (v/v) diethylene glycol monobutyl ether: 2-butoxyethyl acetate was and printed onto the counter electrode position (Figure 3.4) and dried at 55 °C for 1 h. Then, graphene-modified graphite ink described in Section 3.3.1 was printed onto the working electrode position (Figure 3.5) and dried at 55 °C for 1 h. Each printing step was repeated twice. Finally, the nail polish was printed as a protective insulating layer to cover the non-working and non-connector regions of the graphene-based electrochemical sensor (Figure 3.6), and dried at 55 °C for 1 h. This layer defined the shape and area of the electrodes exposed to the measurement solution.

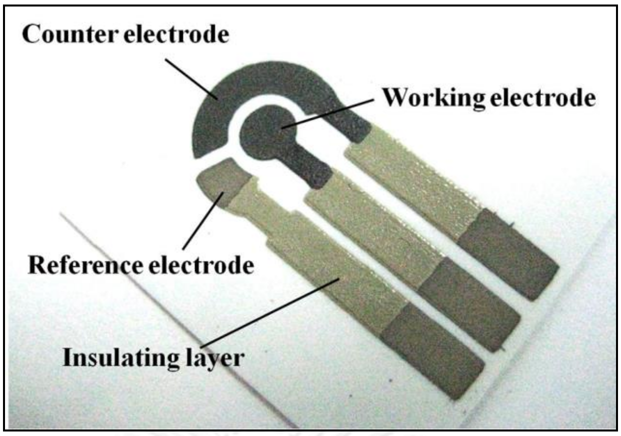

Figure 3.2 In-house graphene-based electrochemical sensor with three integrated electrodes

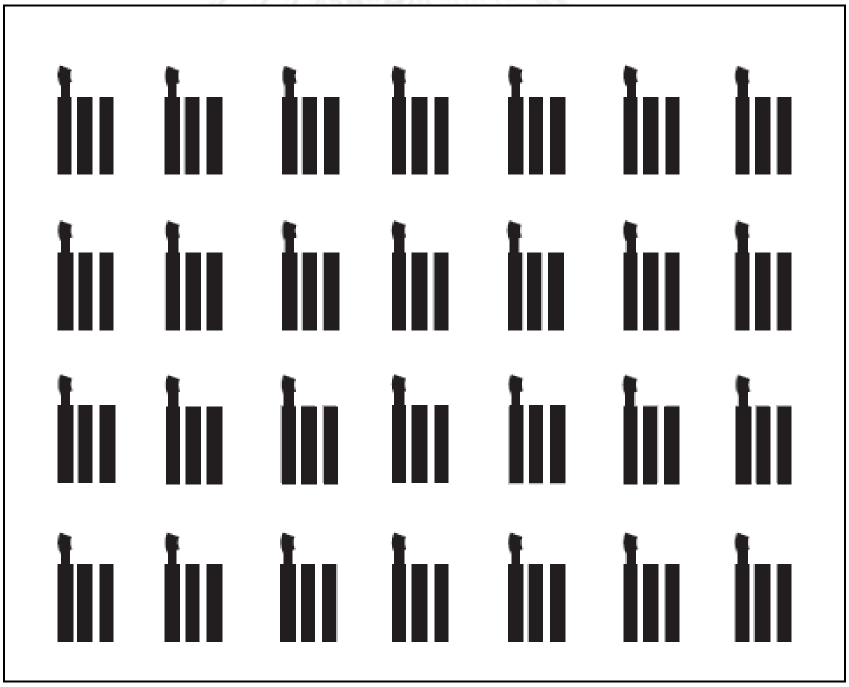

Figure 3.3 Template of the reference electrode and connector

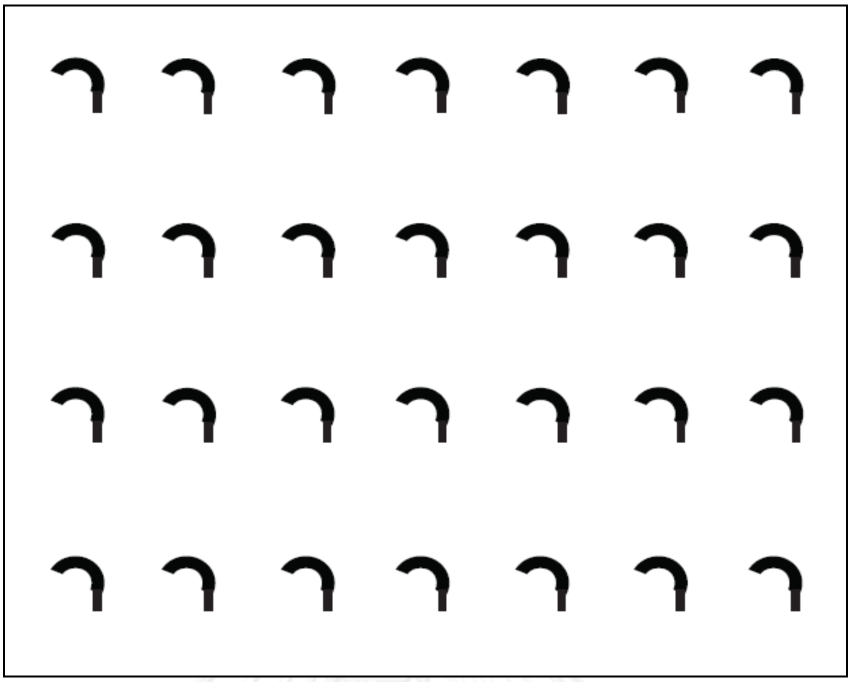

Figure 3.4 Template of the counter electrode

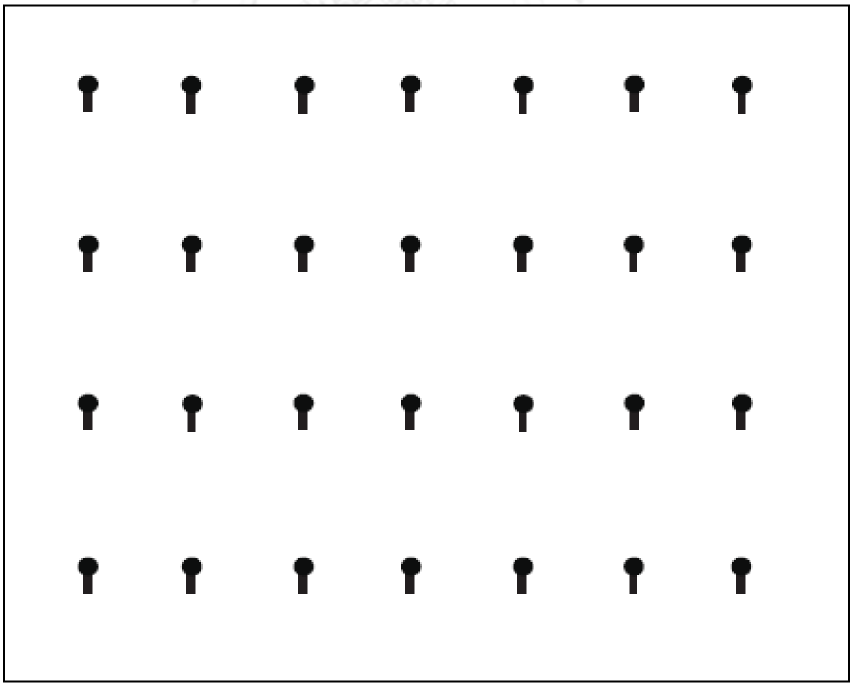

Figure 3.5 Template of the working electrode

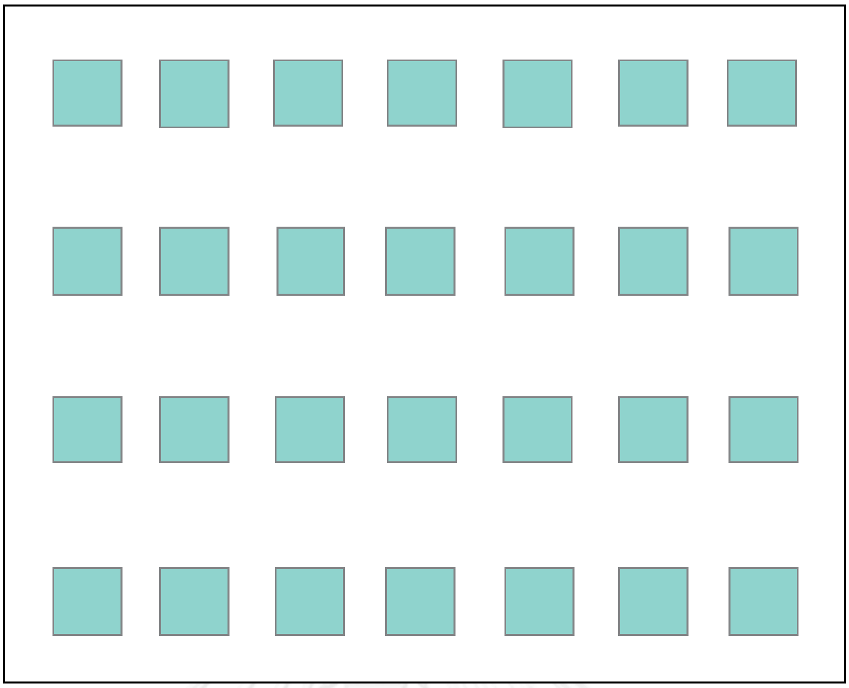

Figure 3.6 Template of the insulator

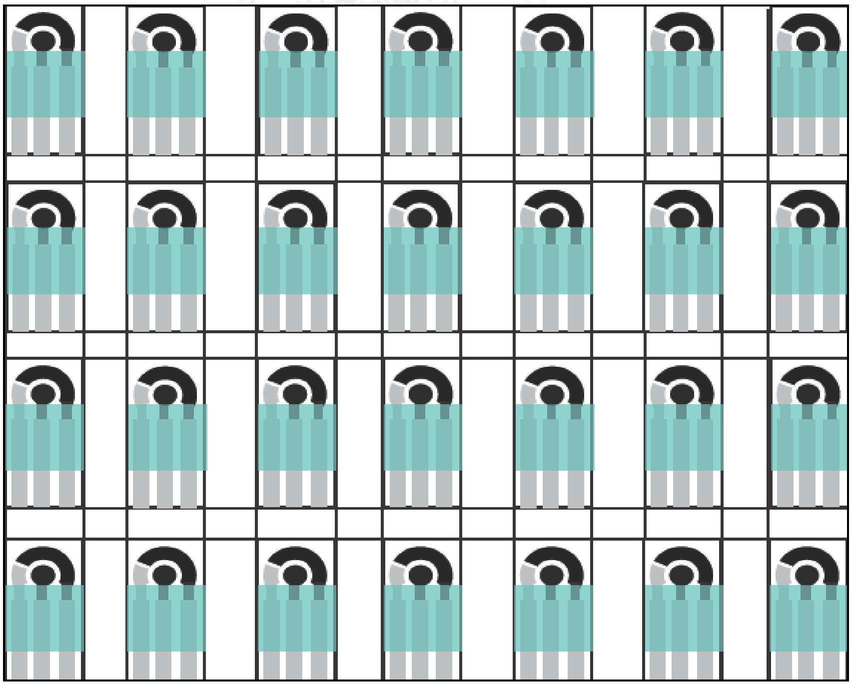

Figure 3.7 Scheme of the developed graphene-based electrochemical sensor

3.5 Electrochemical measurement procedure

Each electrochemical measurement was performed by dispensing 60 µL of the electrolyte solution onto the surface of the graphene-based electrochemical sensor via a micropipette and then the background voltammogram was recorded. After that, 60 µL of a solution containing isoproturon and carbendazim at the indicated concentrations was dispensed onto the graphene-based electrochemical sensor surface via a micropipette. Then, the cyclic voltammetry or square wave stripping voltammetry was carried out with an AutoLab PG 30 potentiostat /galvanostat and PalmSens potentiostat, respectively. The cyclic voltammetric parameters are shown in Table 3.8 and square wave stripping voltammetric parameters are shown in Table 3.9. All electrochemical experiments were studied at room temperature and were housed in a Faraday cage to prevent electrical noise.

Table 3.8 Experimental parameters of cyclic voltammetry

Pretreatment	
First conditioning potential (V)	0
Duration (s)	0
Equilibration time (s)	5
Measurement	
Number of scans	5
Standby potential (V)	0
Potentials	
start potential (V)	1.0
First vertex potential (V)	0.2
Seconds vertex potential (V)	1
Step potential (V)	0.00244
Scan rate (V/s)	0.100008

Table 3.9 Square wave stripping voltammetric parameters

Accumulation step	
Accumulation potential (V)	-0.3
Accumulation time (s)	120
Stripping step	
Initial scan potential (V)	0.5
End potential (V)	1.3
Initial potential (V)	0.5
Frequency (Hz)	25
Step potential (mV)	4
Amplitude (mV)	20

3.6 Optimization of the working electrodes

3.6.1 Type of working electrode

To enhance the performance of the working electrode, graphite powder was added into commercial carbon ink in order to increase the electrical conductivity and surface area of the working electrode. The cyclic voltammetry of a solution containing 10 mg/L isoproturon in 0.1 M phosphate buffer (pH 7.0) was performed on the developed screen-printed sensor with graphite working electrode. The screen-printed sensor with carbon working electrode was also used in the comparative study. Table 3.10 shows the composition of inks used for screen-printing of graphite and carbon working electrode.

Table 3.10 The composition of inks used for screen-printing of carbon and graphite working electrode

Type of working electrodes	Carbon ink (g)	Graphite powder (g)
Carbon working electrode	1.0	-
Graphite working electrode	1.0	0.2

3.6.2 The type of modified materials

The type of materials used in the modification of the working electrodes was investigated by mixing modifying materials, including graphene and crown ether, into the ink before screen-printing onto PVC substrate. The compositions of inks used for printing of the working electrodes are shown in Table 3.11. The cyclic voltammetry of a solution containing 10 mg/L isoproturon in 0.1 M phosphate buffer (pH 7.0) were carried out on these developed sensors.

Table 3.11 The composition of modified inks used for screen-printing of the working electrodes

Type of working electrodes	Carbon ink (g)	Graphite powder (g)	Graphene (mg)	Crown ether (mg)
Graphite	1.0	0.2	-	-
Graphene-graphite	1.0	0.2	6.7	-
Crown ether-graphite	1.0	0.2	-	10.0
Graphene-crown ether-graphite	1.0	0.2	6.7	10.0
	1.0	0.2	10.0	6.7
	1.0	0.2	10.0	10.0

3.6.3 Optimum solvent for graphene dispersion

Commercial graphene was dispersed in two different solvents, 2-propanol or ethanol. The resulted graphene from two dispersion solvent were used to prepare graphene-modified graphite ink and fabricate electrochemical sensor. Then, these sensors were used to analyze 1 mg/L isoproturon solution in 0.1 M phosphate buffer (pH 2) by cyclic voltammetry.

3.6.4 The amount of graphene

The effect of amount of graphene as the electrode modifying material on the electrochemical responses of isoproturon and carbendazim was studied by mixing various amounts of graphene, 0.7, 5.7, 10.7, 15.7, 20.7, 25.7 and 30.7 mg with 1.0 g carbon ink and 0.2 g graphite powder, then screen-printing onto the substrate. The square wave stripping voltammetry of a 1 mg/L mixed standard solution of isoproturon and carbendazim in 0.1 M $HClO_4$ were carried out on these developed sensors.

3.7 Optimization of the square wave stripping voltammetric conditions

3.7.1 The type of supporting electrolyte

The effect of type of supporting electrolyte on the square wave voltammetric response of isoproturon and carbendazim was investigated by variation of type of supporting electrolyte including phosphate buffer, perchloric acid ($HClO_4$), hydrochloric acid (HCl), nitric acid (HNO_3) and sulfuric acid (H_2SO_4), all supporting electrolytes were set at pH 2.

3.7.2 The concentration of supporting electrolyte

The influence of the stripping peak currents of isoproturon and carbendazim on the supporting electrolyte concentration was studied by variation of concentration of the supporting electrolyte at 0.001, 0.01, 0.10, 0.25, 0.50, 1.0, 1.5 and 2.0 M.

3.7.3 The effect of sample volume

The influence of the current responses of isoproturon and carbendazim on the sample volume dropped onto the surface of the graphene-based electrochemical sensors was studied at 40, 50, 60, 70, 80, 90 and 100 µL.

3.7.4 The initial scan potential

The effect of initial scan potential of square wave stripping voltammetry on the signals of isoproturon and carbendazim was studied by variation of the initial scan potential at 0.2, 0.3, 0.4, 0.5, and 0.6 V.

3.7.5 The effect of the accumulation potential

The effect of the accumulation potential on the square wave stripping voltammetric peak currents of isoproturon and carbendazim was studied by variation of the accumulation potential at -0.1, -0.2, -0.3, -0.4, -0.5, and -0.6 V.

3.7.6 The effect of the accumulation time

The effect of the accumulation time on the responses of isoproturon and carbendazim was studied by variation of the accumulation time at 0, 60, 120, 180 and 240 s.

3.7.7 The effect of the square-wave (SW) voltammetric parameters

3.7.7.1 The effect of the frequency

The effect of the square wave frequency on the signals of isoproturon and carbendazim was studied by variation of the frequency at 15, 20, 25, 30, 35, 50 and 75Hz.

3.7.7.2 The effect of the step potential

The effect of the step potential on the stripping peak currents of isoproturon and carbendazim was studied by variation of the step potential at 2.0, 3.0, 3.5, 4.0, 4.5 and 5.0 mV.

3.7.7.3 The effect of the amplitude

The effect of the square wave amplitude on the stripping peak currents of isoproturon and carbendazim was studied by variation of the amplitude at 5.0, 7.0, 9.0, 10, 20 and 30 mV.

3.8 The interference effect

The effect of other ions in the test sample solution as potential sources of interference for the determination of isoproturon and carbendazim were investigated with the concentration of 100-fold excess quantity of CN^-, CO_3^{2-}, NO_3^-, PO_4^{3-}, SO_4^{2-}, Ca^{2+}, Cd^{2+}, Co^{2+}, Cu^{2+}, K^+, Mg^{2+}, Na^+, Ni^{2+}, Pb^{2+}, Zr^{4+} and Zn^{2+}, with a mixed solution of isoproturon and carbendazim at 1 mg/L. Moreover, the influences of two dithiocarbamate fungicides, disulfiram (tetraethylthiuram disulfide) and thiram (tetramethylthiuram disulfide) were studied with the concentration of 10-fold excess quantity.

3.9 The analytical performance

3.9.1 Linearity

The mixed standard solutions of isoproturon and carbendazim in the concentration range of 0.02–10.0 and 0.50–10.0 mg/L, respectively were analyzed by square wave voltammetry using graphene-based electrochemical sensors under the optimized conditions. The average peak currents for triplicate measurements were used to plot the calibration curves for the simultaneous determination of isoproturon and carbendazim levels which linear range can be obtained.

3.9.2 Limit of detection (LOD) and limit of quantification (LOQ)

The limits of detection (LOD) and the limits of quantification (LOQ) were determined statistically approach from the calibration curves in the range of 0.02–10.0 and 0.50–10.0 mg/L for isoproturon and carbendazim, respectively. The

LOD and LOQ were calculated from $3S_b/S$ and $10S_b/S$, where S_b is the standard deviation from ten replicate measurements of standard solution with lowest concentration at which the peak currents can be measured (0.02 mg/L isoproturon and 0.5 mg/L carbendazim) and S is the slope of the calibration curve [58].

3.9.3 Repeatability

The fabrication repeatability of the screen-printed sensors was estimated by determination of isoproturon and carbendazim levels in a solution containing 0.02 mg/L isoproturon and 0.50 mg/L carbendazim by square wave stripping voltammetry at ten different sensors.

3.10 Analytical applications

The proposed procedure was applied for the simultaneous determination of isoproturon and carbendazim in two water samples (Chao Phraya River water and rice-field water), a soil sample from the same rice-field, and vegetable samples (tomatoes and lettuce) and compared with HPLC-UV method.

3.10.1 Sample preparations

3.10.1.1 Water samples

Water samples were obtained from two different parts of Nakhonsawan Province including the Chao Phraya River and a rice-field. Prior to study, the water samples were filtered through a 0.45 µm filter membrane to remove the particulate matter. Next, each filtered water sample was mixed with 2.00 mL of a 100 mg/L mixed standard solution of isoproturon and carbendazim and 8.34 mL of 6 M $HClO_4$ in 50 mL volumetric flask [23, 28].

3.10.1.2 Vegetables samples

Vegetables samples including tomatoes and lettuce were purchased from a local supermarket. Each sample of tomatoes and lettuce was chopped into small pieces using a blender. Then, 10 g of tomatoes and lettuce samples were spiked with 200 µL of a 100 mg/L mixed standard solution of isoproturon and carbendazim and kept overnight. After that, the vegetable samples were extracted with 60 mL of dichloromethane for 30 min. The extract was filtered through a 0.45 µm membrane and then evaporated to dryness. The residue was dissolved in 300 µL of ethanol and diluted to 10 mL with 1.0 M $HClO_4$ [14, 49].

3.10.1.3 Soil sample

Soil sample was obtained from the rice-field in Nakhonsawan Province. Prior to study, the soil sample was washed repeatedly with deionized water and exposed to the atmosphere. Next, the dried soil was ground into small size. Then, 10 g of soil sample was spiked with 200 µL of a 100 mg/L mixed standard solution of isoproturon and carbendazim and kept overnight. Thereafter, the soil sample was extracted with 60 mL of dichloromethane for 30 min. The extract was filtered through a 0.45 µm membrane and then evaporated to dryness. The residue was dissolved in 300 µL of ethanol and diluted to 10 mL with 1.0 M $HClO_4$ [23-25].

3.10.2 Accuracy and precision

The concentrations of isoproturon and carbendazim in aforementioned sample solutions were determined by the standard addition method using added concentrations of 0, 2.00, 4.00, 6.00 and 8.00 mg/L and then analyzed by the proposed method and the HPLC-UV method, as the standard method.

The accuracy is evaluated in terms of recovery, using the following formula:

$$\text{Recovery} = \left(\frac{C_{SS} - C_{US}}{C_S}\right) \times 100$$

Where C_{SS} is the concentration of pesticide found in spiked sample, C_{US} is the concentration of pesticide in unspiked sample and C_S is the concentration of pesticide that spiked into the sample.

For precision study, the repeatability of the developed graphene-based electrochemical sensors was examined by ten replicate measurements of solution containing 0.02 mg/L isoproturon and 0.50 mg/L carbendazim standard solution. The signal currents were measured. Then, the precision is evaluated in terms of the relative standard deviation (RSD), using the following formula:

$$\%\text{RSD} = \frac{\text{Standard deviation}}{\text{Mean}} \times 100$$

CHAPTER IV

RESULTS AND DISCUSSION

This chapter presents the results of the modification screen-printed electrodes, comparison of the electrochemical response between SPCE and SPGE. Optimization of the graphene-based electrochemical sensor, square wave stripping voltammetric conditions, analytical parameter and analytical applications.

4.1 Optimization of working electrodes

4.1.1 Type of working electrode

In this research, the performance of working electrodes were enhanced by adding graphite powder into the commercial carbon ink before fabricating three-electrode sensors with graphite working electrodes by screen-printing process. For comparison, the screen-printed sensors with carbon working electrodes were also used.

Figure 4.1 shows the cyclic voltamograms of a solution containing 10 mg/L isoproturon in 0.1 M phosphate buffer (pH 7.0) at the screen-printed sensor with graphite working electrode (curve a) and carbon working electrode (curve b) in the potential range of 0.2 to 1.0 V. The results showed that, the graphite working electrodes exhibited much higher peak currents than the carbon working electrode. These results indicated that, graphite could be used to increase the conductivity and surface area of the working electrodes. Therefore, the graphite working electrode was used for the next experiment.

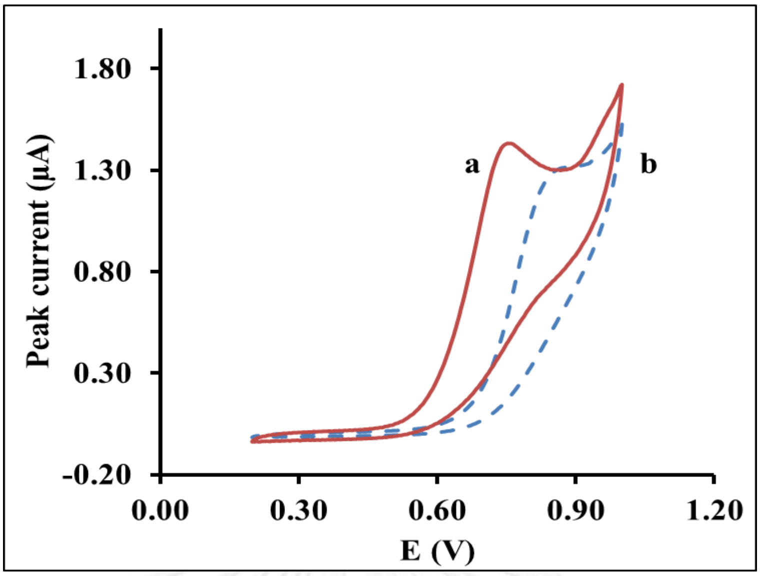

Figure 4.1 Cyclic voltammograms of 10 mg/L isoproturon solution in 0.1 M phosphate buffer (pH 7.0) at the screen-printed sensor with (a) graphite working electrode and (b) carbon working electrode.

4.1.2 The type of modified material

Two types of modifying materials, graphene and crown ether, were used to modify the graphite working electrode in order to improve the performance of the electrode. Graphene is a two dimensional material consisting of a single layer of carbon atoms arranged in a honeycomb structure. It has advantages of excellent electrical conductivity, high chemical stability and large surface. Hence, it is quite promising as modifying material. Crown ether, dibenzo-24-crown-8, is a macrocyclic polyether consisting of a number of oxygen atoms. The ring of crown ether creates an electron-rich cavity which can selectively form complex with protonated analytes based on the cavity and cation size. Crown ethers had been used for preparation of high extraction efficiency solid-phase microextraction fibers for monitoring of organophosphorus pesticide residues in food samples by solid-phase microextraction and gas chromatography-flame photometric detector [59]. Moreover, crown ether modified hybrid silica monolithic column was developed as a separation column and the bonded phase of hybrid silica monolith exhibited superior performance for analysis of imidacloprid and carbendazim in tomatoes by capillary electrochromatography [60]. Thus, these modifiers can preconcentrate the analytes on the electrode surface and improve sensitivity and selectivity for the determination of isoproturon and carbendazim.

The electrochemical performances of these graphene and crown ether modified electrodes were studied by cyclic voltammetry. Cyclic voltammograms of 10 mg/L isoproturon in 0.1 M phosphate buffer (pH 7.0) at the screen-printed sensor with various modified working electrode are shown in Figure 4.2. It was found that the graphite electrode modified with graphene (6.7 mg) and the graphite electrode modified with graphene (10 mg) and crown ether (6.7 mg) exhibited much higher peak currents than the other electrodes. However, graphene-modified graphite working electrode was selected because it has almost the same sensitivity to graphene-crown ether-modified electrode.

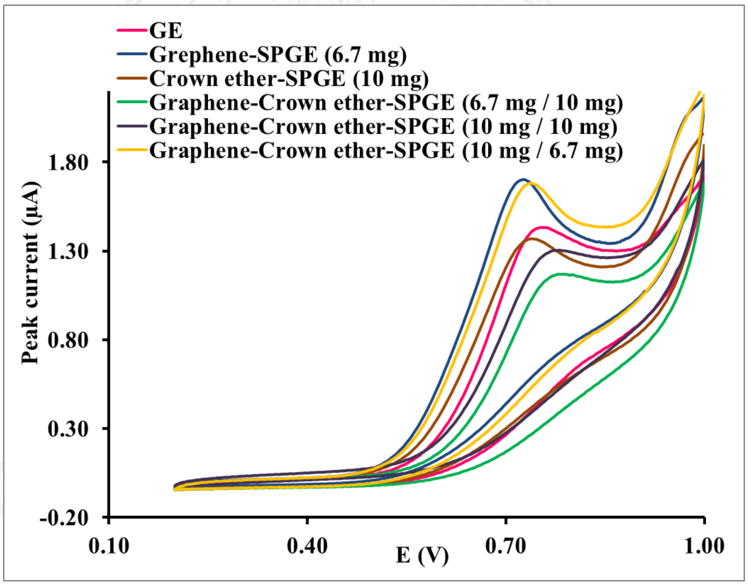

Figure 4.2 Cyclic voltammograms of 10 mg/L isoproturon solution in 0.1 M phosphate buffer (pH 7.0) on various types of modified graphite electrodes (GE).

4.1.3 Optimum solvent for graphene dispersion

Due to the small size and flat shape of graphene, graphene nanopowder is sensitive to van der Waal's interactions and conglomerates in the dry state, leading to poor electrical conductivity [61, 62]. Consequently, to obtain the unique and desired properties of graphene based sensors, the prevention of graphene aggregation is an important process [63]. Accordingly, dispersion of commercial graphene in an optimum solvent is a good way to improve it. In this research, the effect of dispersion in two different solvents, 2-propanol and ethanol, on the electrochemical response of isoproturon was studied because of their low toxicity. The results in Table 4.1 show that the higher peak currents with the lower standard deviation were obtained on the working electrode modified with graphene dispersed in ethanol. Therefore, ethanol was selected as optimum solvent for graphene dispersion in this work.

Table 4.1 The average peak currents for 1 mg/L isoproturon solution in 0.1 M phosphate buffer (pH 2) detected by cyclic voltammetry at graphite electrodes modified with graphene dispersed in different solvent

Type of electrode	Graphene-disperion solvent	Peak current ± SD (μA)
Graphite electrode	-	0.3497 ± 0.078
Graphene-graphite electrode	2-propanol	0.3859 ± 0.020
Graphene-graphite electrode	ethanol	0.3890 ± 0.002

4.1.4 The amount of graphene

The dependence of the square wave stripping voltammetric responses of isoproturon and carbendazim on the amount of graphene, as the electrode modifying material, was studied in the range of 0 to 30.7 mg, with a fixed amount of carbon ink and graphite powder at 1.0 g and 0.2 g, respectively. As shown in Table 4.2 and Figure 4.3, the anodic peak currents of carbendazim at various content of graphene were not significantly difference, nevertheless the highest peak current was obtained at 10.7 mg graphene. The anodic peak currents of isoproturon gradually increased with increasing graphene content from ~0.43 μA at 0.7 mg graphene to a maximum of ~0.46 μA at a graphene content of 10.7 mg. Further increasing the amount of graphene then led to a marked decrease, presumably due to the aggregation of the dispersed graphene which can be seen from the SEM images as shown in Figure 4.4. Such aggregated graphene clusters would result in a lower surface area and electron transfer of the modified electrode. Accordingly, 10.7 mg was selected as the optimum amount of graphene in the graphene-modified graphite ink for screen-printing. Representative square wave stripping voltammograms of a mixed standard solution of isoproturon and carbendazim (5 mg/L each) in 1 M $HClO_4$ at graphene-modified graphite electrode, graphite electrode, and a background are shown in Figure 4.5.

Table 4.2 Anodic peak currents of 1 mg/L isoproturon and carbendazim solution in 1.0 M $HClO_4$ analyzed by square wave stripping voltammetry at the modified graphite electrode with different amount of graphene. Amounts of carbon ink and graphite powder were fixed at 1.0 g and 0.2 g, respectively.

Amount of graphene (mg)	Peak current (μA) ± SD	
	Isoproturon	Carbendazim
0.7	0.435 ± 0.021	0.192 ± 0.002
5.7	0.439 ± 0.013	0.195 ± 0.004
10.7	0.458 ± 0.012	0.199 ± 0.013
15.7	0.429 ± 0.020	0.197 ± 0.025
20.7	0.400 ± 0.018	0.192 ± 0.004
25.7	0.377 ± 0.028	0.190 ± 0.006
30.7	0.348 ± 0.029	0.183 ± 0.010

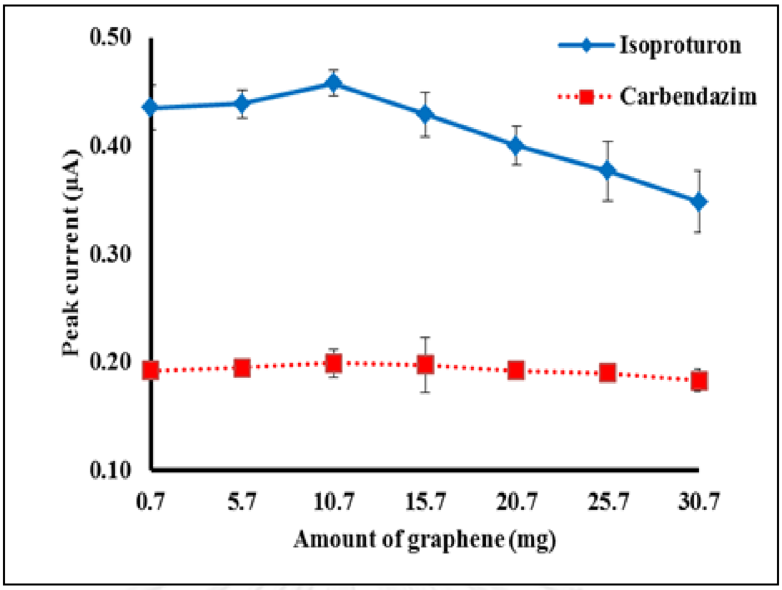

Figure 4.3 Effect of the graphene content in the modified graphite ink used to print the working electrode on the peak currents of 1 mg/L isoproturon and carbendazim solution in 1.0 M $HClO_4$ determined by square wave stripping voltammetry. Data are shown as the mean ± SD and are derived from three replicates. Measurement parameters: sample volume 60 µL, deposition potential -0.3 V, deposition time 120 s, initial scan potential 0.5 V, end potential 1.3 V, frequency 25 Hz, step potential 4 mV, amplitude 20 mV.

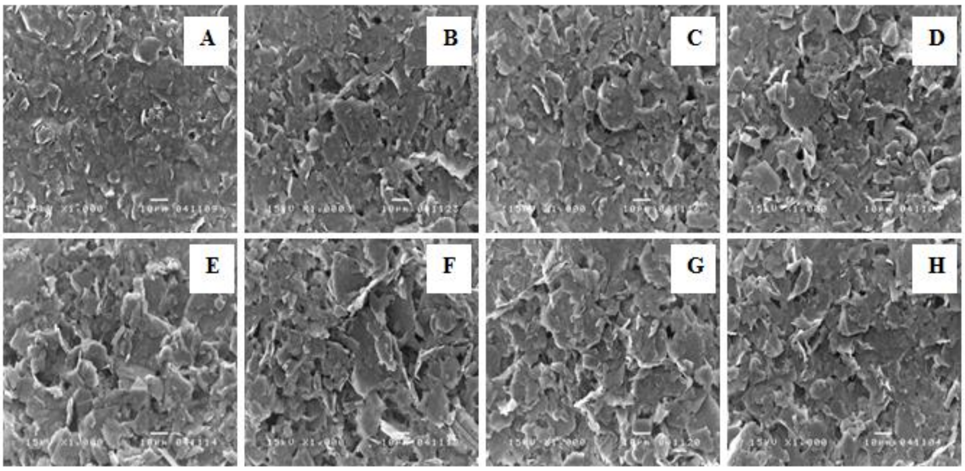

Figure 4.4 SEM images of the surface of electrodes prepared by screen printing with the modified graphite inks containing (A) 0, (B) 0.7, (C) 5.7, (D) 10.7, (E) 15.7, (F) 20.7, (G) 25.7 and (H) 30.7 mg of graphene.

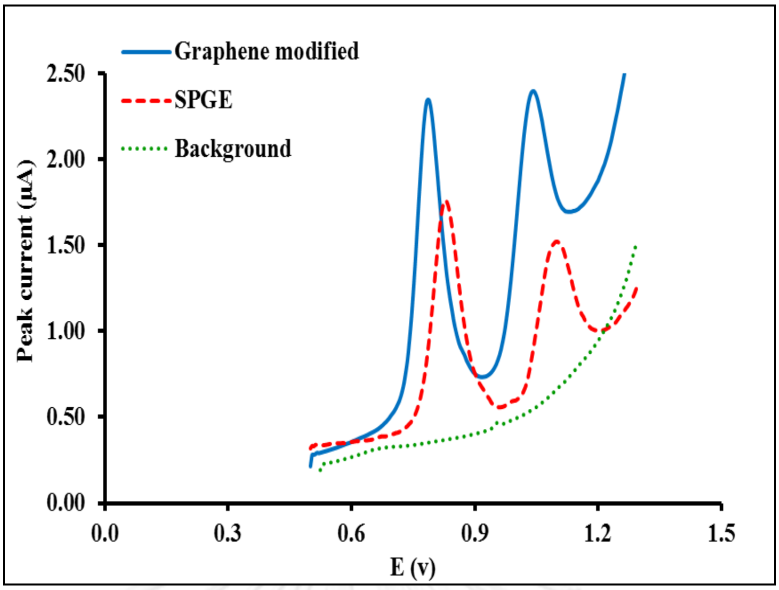

Figure 4.5 Square wave stripping voltammograms of 5 mg/L isoproturon and carbendazim in 1 M $HClO_4$ at the graphene-modified graphite electrode and graphite electrode as well as background.

Table 4.3 Signal currents for determination of 5 mg/L isoproturon and carbendazim by square wave stripping voltammetry at graphene-modified and non-modified graphite electrode

Type of electrode	Peak current (µA) ± SD	
	Isoproturon	Carbendazim
Graphene-modified graphite electrode	1.792 ± 0.081	1.230 ± 0.077
Graphite electrode	1.275 ± 0.009	0.774 ± 0.056

The results in Figure 4.5 and Table 4.3 show that, the developed electrochemical sensor with graphene-modified graphite working electrode exhibited well-defined peaks at lower potentials (0.78 V and 1.08 V for isoproturon and carbendazim, respectively), with a 1.4- and 1.6-fold higher sensitivity for isoproturon and carbendazim, respectively, than the sensor with non-modified graphite working electrode, indicating the higher efficiency of the developed graphene-based electrochemical sensor for the determination of isoproturon and carbendazim.

4.1.5 Modification method

Additionally, two methods of graphene-modification including drop-casting of dispersed graphene on the surface of screen-printed graphite electrode and mixing of dispersed graphene into an ink and then screen-printing were compared. As shown in Figure 4.6, the working electrode modified by drop-casting of graphene gave lower peak current than the other. Moreover, some of graphene could be removed when the analyte solution was dropped onto the surface of the electrode for the electrochemical measurement. Therefore, graphene-modified ink was used for screen-printing of the working electrodes in this research.

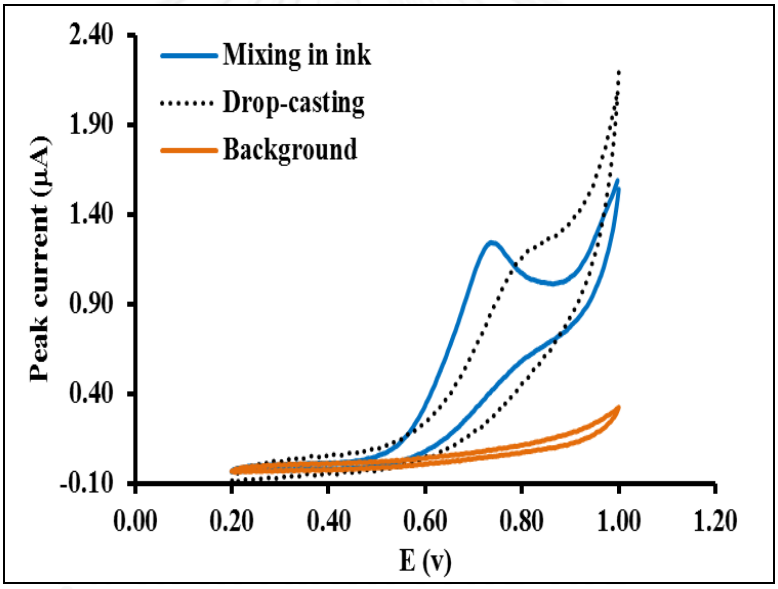

Figure 4.6 Cyclic voltammograms of 1.0 mg/L isoproturon in 1.0 M $HClO_4$ on the working electrode modified by drop casting of graphene and mixing of graphene into ink.

4.2 Optimization of the square wave stripping voltammetric conditions

4.2.1 The type of supporting electrolyte

The amino group in the isoproturon molecule is easily oxidized at a low pH [19], thus the pH of test solutions was adjusted to pH 2 with various supporting electrolytes including 0.1 M phosphate buffer pH 2 and 0.01 M strong acid such as $HClO_4$, HCl, HNO_3, and H_2SO_4. The results in Figure 4.7 and Table 4.4 indicated that the highest peak currents of isoproturon and carbendazim were obtained in 0.01 M $HClO_4$. Moreover, isoproturon and carbendazim in 0.01 M $HClO_4$ and 0.01 M HNO_3 showed the lower peak potentials than the ones obtained in the other supporting electrolyte solutions. It implies that the oxidation of isoproturon and carbendazim occurred easier in $HClO_4$ and HNO_3 which is probably due to the oxidizing properties of $HClO_4$ and HNO_3. Consequently, $HClO_4$ was selected for use as the supporting electrolyte.

Furthermore, it can be noticed that the stripping peaks of isoproturon and carbendazim in 0.01 M HCl were considerably shifted to more positive potential and the signal of carbendazim occurred at the upper limit of the potential window of the electrode. This indicates that the oxidation of both pesticides on the working electrode is slow. Therefore, Cl^- in real sample has an effect on the determination of isoproturon and carbendazim and need to be removed before analysis.

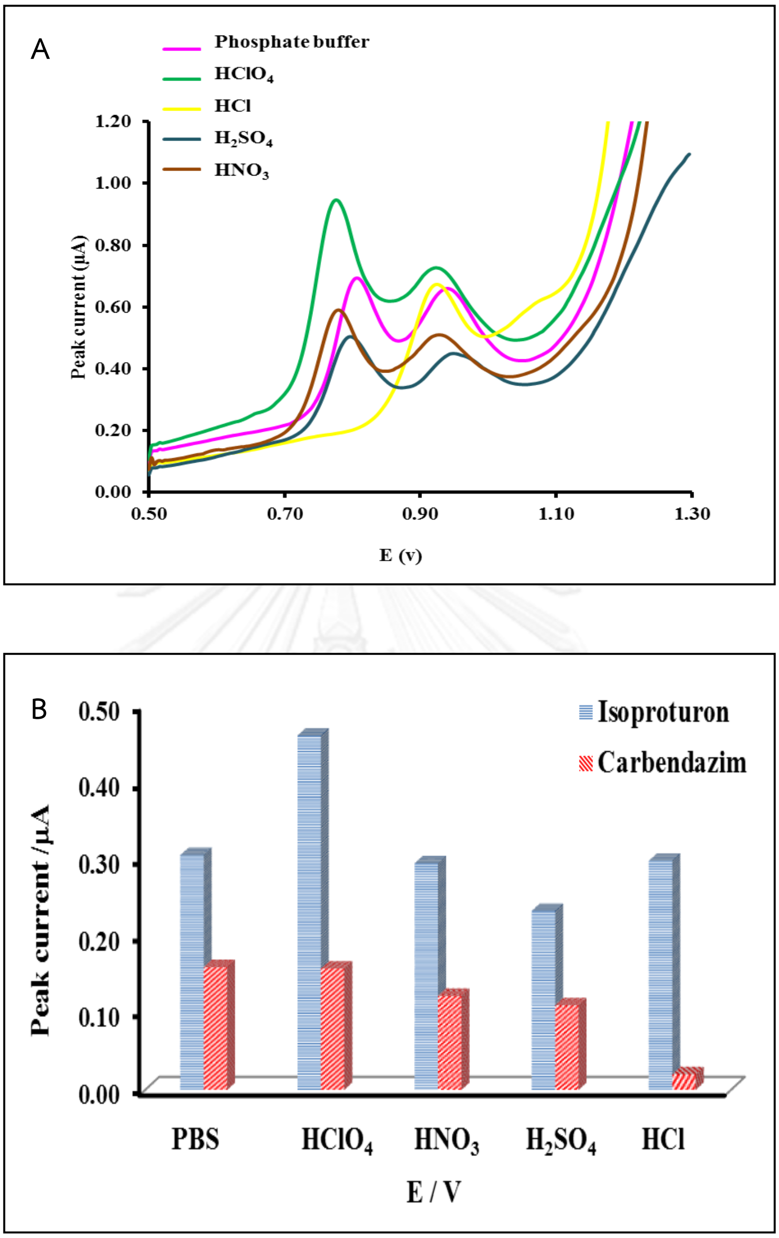

Figure 4.7 (A; Top) Square wave voltammograms of solutions containing 1.0 mg/L each of isoproturon and carbendazim in various supporting electrolyte, pH 2. (B; Bottom) Effect of the type of supporting electrolyte (pH 2) on peak currents of solutions containing 1.0 mg/L each of isoproturon and carbendazim.

Table 4.4 The square wave stripping voltammetric results of 1 mg/L isoproturon and carbendazim in different supporting electrolytes (pH 2).

Type of supporting electrolyte	Peak current (μA) ± SD	
	Isoproturon	Carbendazim
0.1 M Phosphate buffer	0.305 ± 0.003	0.160 ± 0.004
0.01 M $HClO_4$	0.461 ± 0.012	0.158 ± 0.007
0.01 M HNO_3	0.295 ± 0.009	0.122 ± 0.003
0.01 M H_2SO_4	0.233 ± 0.006	0.110 ± 0.006
0.01 M HCl	0.298 ± 0.015	0.020 ± 0.006

4.2.2 The concentration of supporting electrolyte

The effect of concentration of $HClO_4$, as a supporting electrolyte, on the electrode response in the range of 1 mM to 2.0 M was investigated. The relationship between the average current and $HClO_4$ concentrations are shown in Table 4.5 and Figure 4.8. The peak currents of both isoproturon and carbendazim increased markedly with increasing concentrations of $HClO_4$ and reached up to a maximum at 1.0 M $HClO_4$. The peak currents gradually decreased slightly and markedly thereafter with increasing concentrations of $HClO_4$ above 1.0 and 1.5 M, respectively, but there was no significant variation over the $HClO_4$ range of 0.5 to 1.5 M for both analytes. This decrease likely reflects the effect of the excess produced hydrogen gas that then forms a gas interface separating the electrolyte solution from the electrode and so limiting the electron transfer between the solvated isoproturon or carbendazim molecules and the electrode surface. Accordingly, 1.0 M $HClO_4$ was used as a supporting electrolyte.

Table 4.5 The square wave voltammetric peak currents of 1 mg/L isoproturon and carbendazim in $HClO_4$ supporting electrolyte at different concentrations

Concentration of $HClO_4$ (M)	Peak current (μA) ± SD	
	1 mg/L isoproturon	1 mg/L carbendazim
2.0	0.445 ± 0.036	0.239 ± 0.047
1.5	0.540 ± 0.006	0.296 ± 0.049
1.0	0.544 ± 0.036	0.328 ± 0.054
0.50	0.512 ± 0.039	0.313 ± 0.020
0.25	0.418 ± 0.034	0.290 ± 0.050
0.10	0.377 ± 0.012	0.271 ± 0.042
0.01	0.312 ± 0.016	0.147 ± 0.008
0.001	0.186 ±0.042	0.046 ±0.007

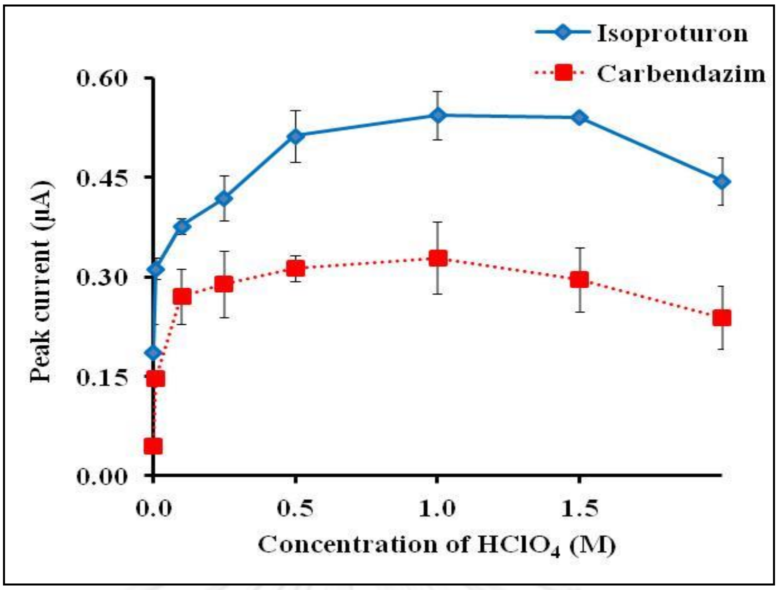

Figure 4.8 Effect of the concentration of $HClO_4$ as a supporting electrolyte on the square wave stripping voltammetric peak currents of solutions containing 1 mg/L each of isoproturon and carbendazim. Data are shown as the mean ± SD and are derived from three replicates. Measurement parameters: sample volume 60 μL, accumulation potential -0.3 V, accumulation time 180 s, frequency 25 Hz, step potential 4 mV, amplitude 20 mV.

4.2.3 The effect of sample volume

The effect of different sample volume dropped onto the surface of graphene-based electrochemical sensor was studied by applying different sample volume of 1 mg/L mixed isoproturon and carbendazim solution in the range of 40 to 100 μL. The applied sample volume clearly affected the obtained square wave stripping voltammetric response, with the maximum peak current for isoproturon being observed with an applied sample volume of 60 μL and declining thereafter as shown in Table 4.6 and Figure 4.9. The square wave stripping voltammetric peak currents for carbendazim also increased when the applied sample volume was increased from 40 to 50 μL, but then remained essentially unchanged with increasing applied sample volumes. Therefore, an applied sample volume of 60 μL was selected for the simultaneous determination of isoproturon and carbendazim, which will likely reflect the maximum electrode surface coverage under this condition.

Table 4.6 The square wave stripping voltammetric peak currents of a solution containing 1 mg/L isoproturon and carbendazim at different sample volume

Sample volume (μL)	Peak current (μA) ± SD	
	1 mg/L isoproturon	1 mg/L carbendazim
40	0.234 ± 0.009	0.132 ± 0.011
50	0.300 ± 0.012	0.187 ± 0.020
60	0.326 ± 0.015	0.191 ± 0.013
70	0.318 ± 0.008	0.191 ± 0.009
80	0.314 ± 0.012	0.190 ± 0.011
90	0.302 ± 0.007	0.182 ± 0.024
100	0.290 ± 0.009	0.206 ± 0.027

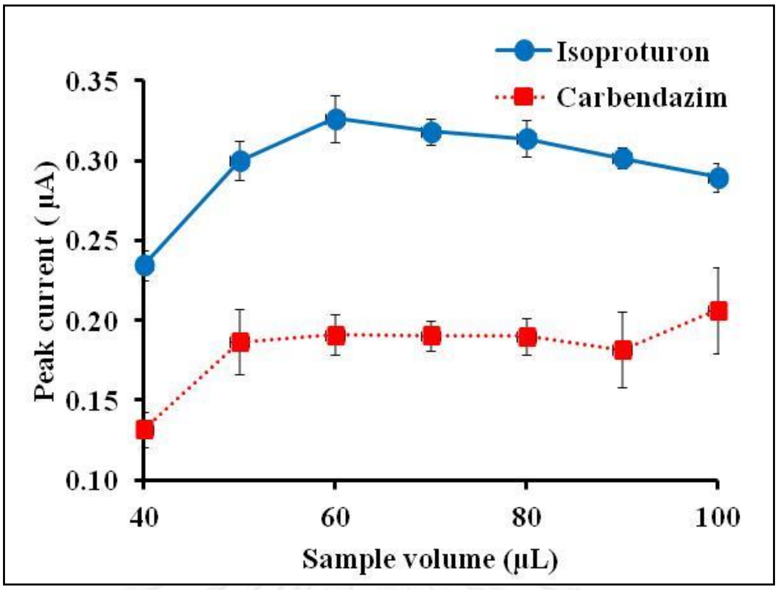

Figure 4.9 Effect of the applied sample volume on the square wave stripping voltammetric peak currents of a solution containing 1 mg/L each of isoproturon and carbendazim in 1.0 M $HClO_4$. Data are shown as the mean ± SD and are derived from three replicates. Measurement parameters are the same as in Fig. 4.8.

4.2.4 The initial scan potential

The initial scan potential is also important parameter in controlling the peak characteristics. In this work, the initial scan potential was varied in the range of 0.2-0.6 V. From the results in Table 4.7 and Figure 4.10, the initial scan potential of 0.5 V was chosen for stripping voltammetric studies of isoproturon and carbendazim because of maximum current signals.

Table 4.7 Square wave stripping voltammetric results at different initial scan potential of a solution containing 1 mg/L isoproturon and carbendazim in 1.0 M $HClO_4$

Scan potential range	Peak current (μA) ± SD	
	Isoproturon	arbendazim
0.2 - 1.3 V	0.291 ± 0.021	0.148 ± 0.023
0.3 - 1.3 V	0.318 ± 0.004	0.172 ± 0.029
0.4 - 1.3 V	0.335 ± 0.008	0.184 ± 0.008
0.5 - 1.3 V	0.354 ± 0.021	0.195 ± 0.021
0.6 - 1.3 V	0.321 ± 0.035	0.176 ± 0.032

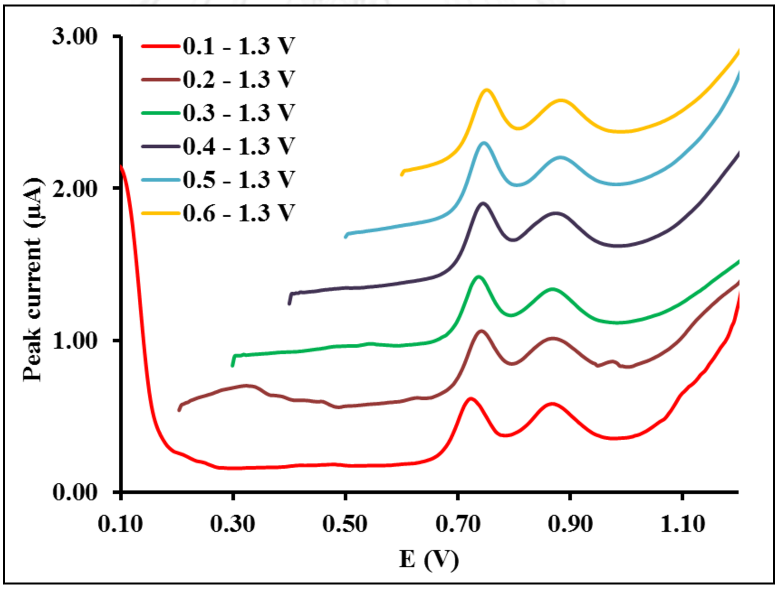

Figure 4.10 **(A)** Square wave stripping voltammetric peak currents at different initial scan potential of a solution containing 1 mg/L each of isoproturon and carbendazim in 1.0 M $HClO_4$

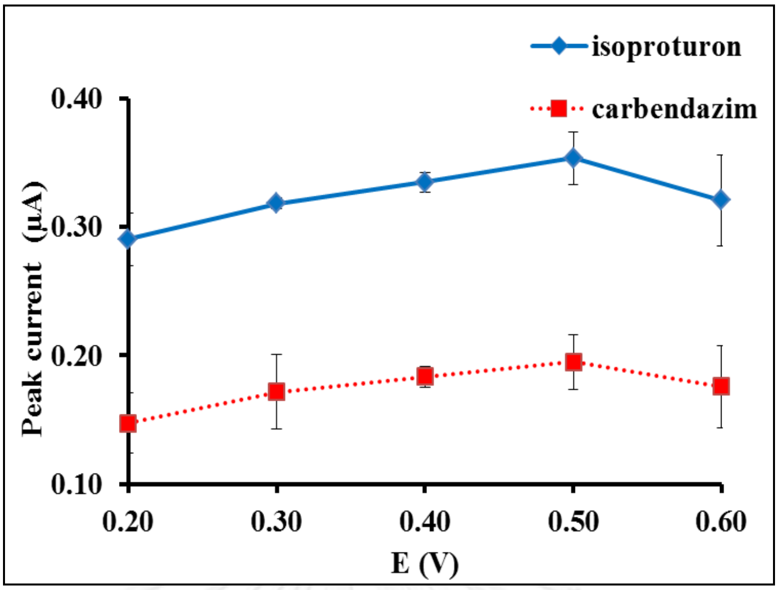

Figure 4.10 **(B)** Effect of the initial scan potential on the square wave stripping voltammetric peak currents of a solution containing 1 mg/L each of isoproturon and carbendazim in 1.0 M $HClO_4$. Data are shown as the mean ± SD and are derived from three replicates. Measurement parameters are the same as in Fig. 4.8.

4.2.5 The effect of the accumulation potential

The effect of the accumulation potential applied to the graphene-based electrochemical sensor on the square wave stripping voltammetric peak currents of a mixed solution of 0.25 mg/L isoproturon and 0.50 mg/L carbendazim in 1.0 M $HClO_4$ was investigated over the range of -0.6 to -0.1 V. Table 4.8 and Figure 4.11 show that the peak currents increased with increasing accumulation potential to a maximum at -0.3 V. This increase in the peak current with increasing accumulation potential might be due to the electrostatic interaction between the cationic isoproturon and carbendazim with the electron rich electrode at this potential. However, at higher accumulation potentials than -0.3 V the peak currents gradually decreased numerically, although this was not statistically significant. Accordingly, an accumulation potential of -0.3 V was selected.

Table 4.8 The square wave stripping voltammetric peak currents of 0.25 mg/L isoproturon and 0.50 mg/L carbendazim in 1.0 M $HClO_4$ at different accumulation potential

Accumulation potential (V)	Peak current (µA) ± SD	
	0.25 mg/L isoproturon	0.50 mg/L carbendazim
-0.6	0.141 ± 0.014	0.041 ± 0.018
-0.5	0.163 ± 0.011	0.092 ± 0.019
-0.4	0.184 ± 0.007	0.104 ± 0.017
-0.3	0.196 ± 0.004	0.110 ± 0.015
-0.2	0.195 ± 0.016	0.105 ± 0.019
-0.1	0.183 ± 0.014	0.095 ± 0.015

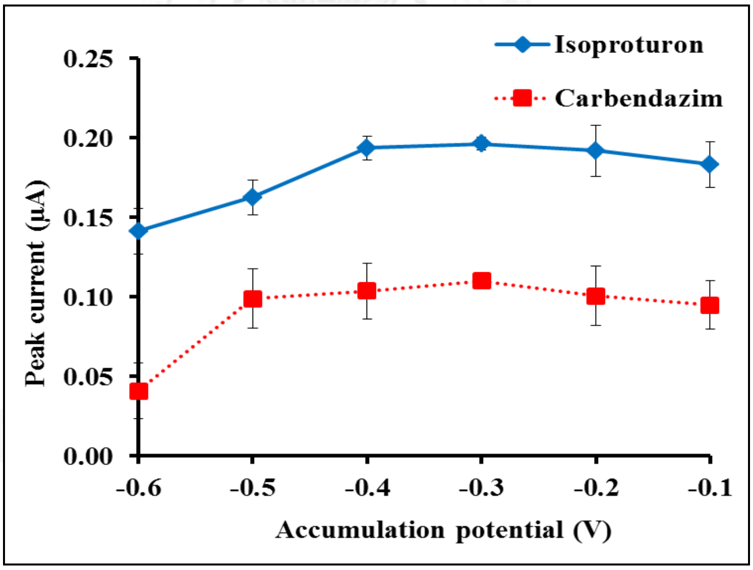

Figure 4.11 Effect of the accumulation potential on the square wave stripping voltammetric peak currents of a solution containing 0.25 mg/L isoproturon and 0.50 mg/L carbendazim in 1.0 M $HClO_4$. Data are shown as the mean ± SD and are derived from three replicates. Measurement parameters are the same as in Fig. 4.8, except the initial scan potential was 0.5 V.

4.2.6 The effect of the accumulation time

The effect of the accumulation time on the square wave stripping voltammetric peak currents of a mixed solution of isoproturon and carbendazim (0.25 mg/L and 0.5 mg/L, respectively, in 1.0 M $HClO_4$) was studied over the range of 0 to 240 s. The square wave stripping peak currents of isoproturon and carbendazim at various accumulation times are shown in Table 4.9 and Figure 4.12. The results indicated that the stripping peak currents increase with increasing the accumulation time up to 120 s for both pesticides and then decreased with further increases in the accumulation time indicating the saturation of the electrode surface and blocking the product forming. Therefore, an accumulation time of 120 s was chosen.

Table 4.9 The square wave stripping voltammetric peak currents of 0.25 mg/L isoproturon and 0.50 mg/L carbendazim in 1.0 M $HClO_4$ at different accumulation time

Accumulation time (s)	Peak current (μA) ± SD	
	Isoproturon	Carbendazim
0	0.195 ± 0.015	0.105 ± 0.025
60	0.209 ± 0.003	0.116 ± 0.021
120	0.248 ± 0.003	0.197 ± 0.013
180	0.210 ± 0.003	0.167 ± 0.008
240	0.183 ± 0.009	0.144 ± 0.020

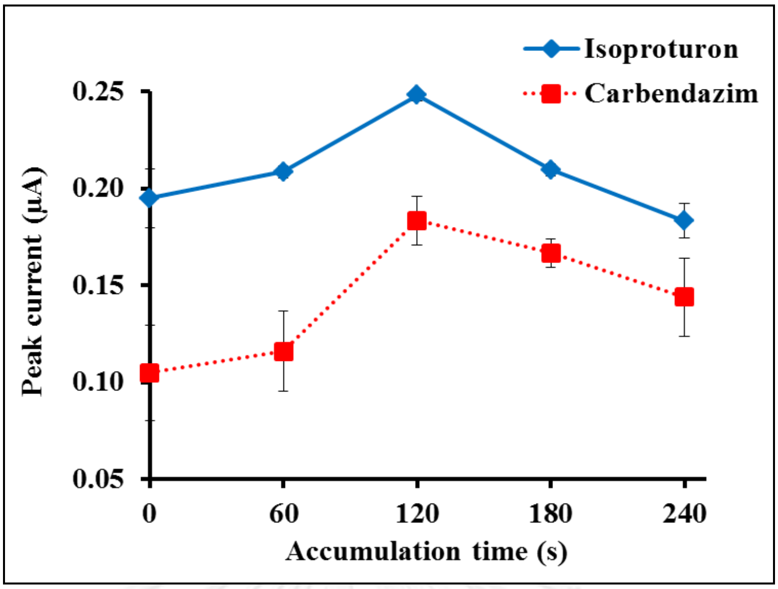

Figure 4.12 The effect of the accumulation time on the square wave stripping voltammetric peak currents of a solution containing 0.25 mg/L isoproturon and 0.50 mg/L carbendazim in 1.0 M $HClO_4$. Data are shown as the mean ± SD and are derived from three replicates. Measurement parameters are the same as in Fig. 4.8.

4.2.7 The effect of the square-wave (SW) voltammetric parameters

The effects of the SW parameters were evaluated sequentially using a mixed solution of 0.25 mg/L and 0.5 mg/L in 1.0 M $HClO_4$ for isoproturon and carbendazim, respectively.

4.2.7.1 The effect of the frequency

The dependence of the peak currents of isoproturon and carbendazim on the SW frequency was evaluated between 15 and 75 Hz. The results are shown in Table 4.10 and Figure 4.13. The peak currents of isoproturon increased almost linearly with increasing SW frequency over the tested 15–75 Hz range, whereas that for carbendazim reached a maximum at a SW frequency of 25 Hz and significantly decreased at higher frequencies. Therefore, a SW frequency of 25 Hz, which provided a sufficiently sensitive albeit non-optimal analytical signal for isoproturon, was selected for the codetermination of both pesticides.

Table 4.10 The square wave stripping voltammetric peak currents of 0.25 mg/L isoproturon and 0.50 mg/L carbendazim in 1.0 M $HClO_4$ at different SW frequency

SW frequency (Hz)	Peak current (µA) ± SD	
	Isoproturon	Carbendazim
15	0.166 ± 0.006	0.087 ± 0.044
20	0.189 ± 0.016	0.129 ± 0.013
25	0.209 ± 0.006	0.154 ± 0.021
30	0.216 ± 0.021	0.078 ± 0.001
35	0.255 ± 0.012	-
50	0.306 ± 0.020	-
75	0.419 ± 0.024	-

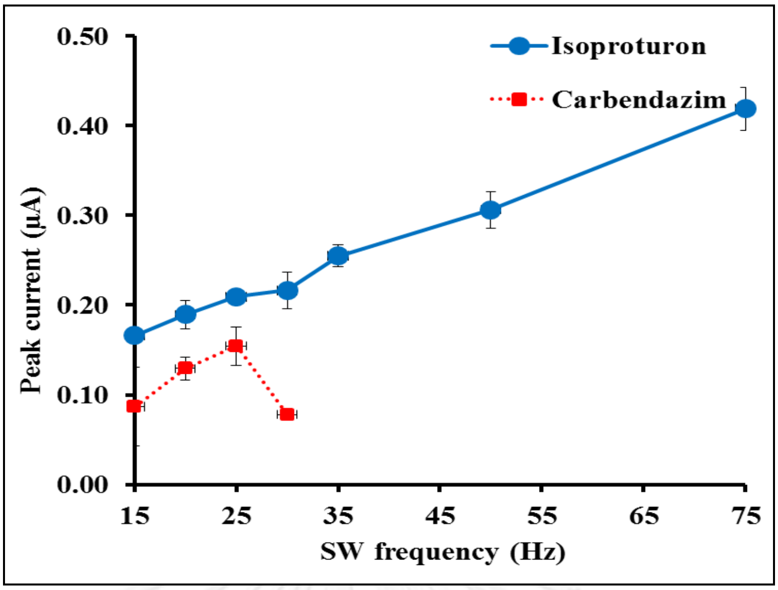

Figure 4.13 Effect of the SW frequency on the square wave stripping voltammetric peak currents of a solution containing 0.25 mg/L isoproturon and 0.50 mg/L carbendazim in 1.0 M $HClO_4$. Data are shown as the mean ± SD and are derived from three replicates. Measurement parameters: sample volume 60 μL, accumulation potential -0.3 V, accumulation time 120 s.

4.2.7.2 The effect of the step potential

The effect of the step potential variation on the square wave stripping voltammetric peak currents of isoproturon and carbendazim was studied over the range of 2.0 to 5.0 mV. Table 4.11 and Figure 4.14 show that increasing the step potential up to 4.0 mV caused an increase in the peak current, whereas increasing the step potential to more than 4.0 mV then decreased the peak current intensity of isoproturon and carbendazim. Accordingly, the optimum step potential value was selected to be 4.0 mV.

Table 4.11 The square wave stripping voltammetric peak currents of 0.25 mg/L isoproturon and 0.50 mg/L carbendazim in 1.0 M $HClO_4$ at different step potential

Step potential (mV)	Peak current (μA) ± SD	
	Isoproturon	Carbendazim
2.0	0.131 ± 0.022	0.036 ± 0.006
3.0	0.140 ± 0.001	0.083 ± 0.009
3.5	0.195 ± 0.007	0.089 ± 0.010
4.0	0.250 ± 0.008	0.141 ± 0.004
4.5	0.229 ±0.010	0.094 ± 0.014
5.0	0.222 ± 0.009	0.080 ± 0.036

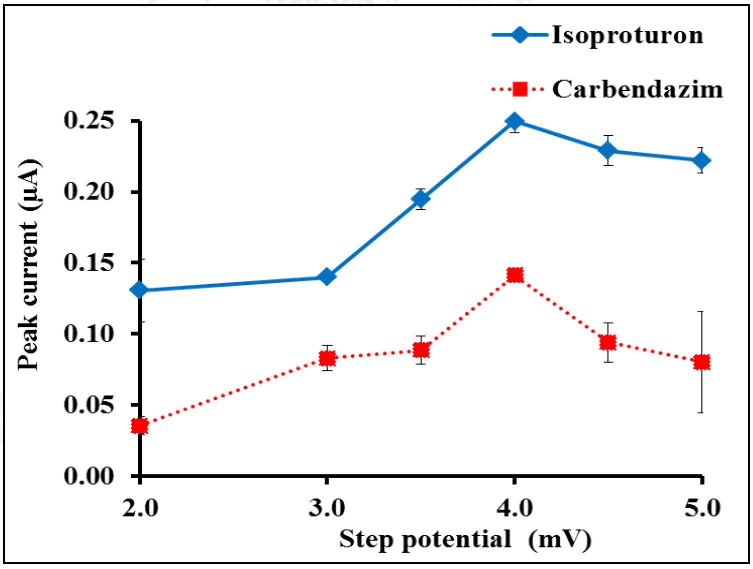

Figure 4.14 Effect of the step potential on the square wave stripping voltammetric peak currents of a solution containing 0.25 mg/L isoproturon and 0.50 mg/L carbendazim in 1.0 M $HClO_4$. Data are shown as the mean ± SD and are derived from three replicates. Measurement parameters: sample volume 60 μL, accumulation potential -0.3 V, accumulation time 120 s, SW frequency 25 Hz.

4.2.7.3 The effect of the amplitude

The effect of the SW amplitude variation on the peak currents of isoproturon and carbendazim was studied over the range of 5.0 to 30 mV, and was found to increase with increasing SW amplitude from 5.0 to 20 mV but then decreased dramatically and slightly at a SW amplitude of more than 20 mV for carbendazim and isoproturon, respectively (Table 4.12 and Figure 4.15). Accordingly, the optimum SW amplitude value was selected as 20 mV because of maximum current peak response.

The univariate optimization of these operational parameters for the simultaneous determination of isoproturon and carbendazim levels by square wave stripping voltammetry with this in-house fabricated graphene based sensor are summarized in Table 4.13.

Table 4.12 The square wave stripping voltammetric peak current of 0.25 mg/L isoproturon and 0.50 mg/L carbendazim in 1.0 M $HClO_4$ at different amplitude

Amplitude (mV)	Peak current (μA) ± SD	
	Isoproturon	Carbendazim
5.0	0.056 ± 0.035	0.037 ± 0.009
7.0	0.110 ± 0.012	0.026 ± 0.014
9.5	0.146 ± 0.013	0.095 ± 0.024
10	0.166 ± 0.011	0.113 ± 0.009
25	0.262 ± 0.012	0.197 ± 0.006
30	0.254 ± 0.005	0.080 ± 0.024

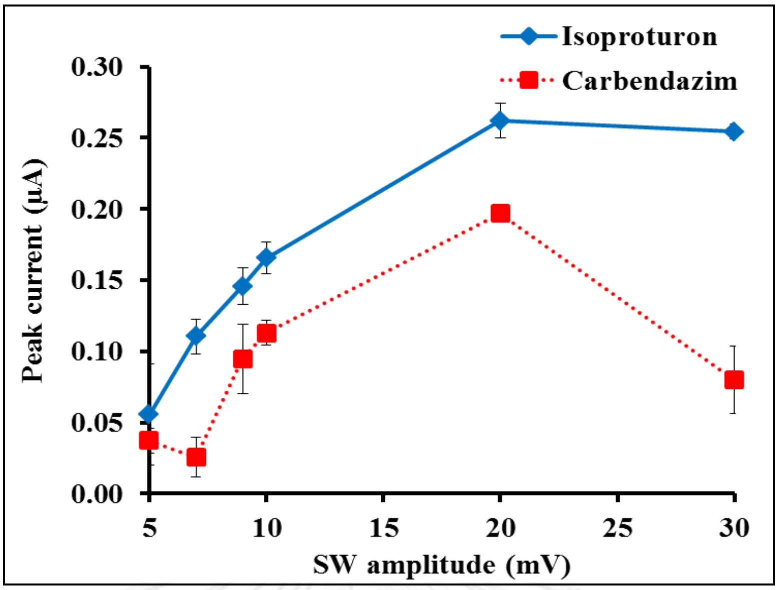

Figure 4.15 Effect of the SW amplitude on the square wave stripping voltammetric peak currents of a solution containing 0.25 mg/L isoproturon and 0.50 mg/L carbendazim in 1.0 M $HClO_4$. Data are shown as the mean ± SD and are derived from three replicates. Measurement parameters: sample volume 60 µL, accumulation potential -0.3 V, accumulation time 120 s, SW frequency 25 Hz, step potential 4 mV.

Table 4.13 Optimal operational parameters selected from the univariate analysis.

Parameter	Parameter examined	Optimized value
Graphene content in the working electrode[a]	0 to 30.7 mg	10.7 mg
Type of supporting electrolyte	6 types	$HClO_4$
Concentration of supporting electrolyte	0.001 to 2.0 M	1.0 M
Applied sample volume	40 to 100 µL	60 µL
Initial scan potential	0.2 to 0.6 V	0.5 V
Accumulation potential	-0.1 to -0.6 V	-0.3 V
Accumulation time	0 to 240 s	120 s
SW frequency	15 to 75 Hz	25 Hz
SW step potential	2 to 5 mV	4 mV
SW amplitude	5 to 30 mV	20 mV

[a] Graphene content applied to make the sensor is with 1.0 g carbon ink and 0.2 g graphite powder

4.3 The interference effect

The effect of other ions in the test sample solutions as potential sources of interference for the determination of isoproturon and carbendazim by square wave stripping voltammetry using the developed graphene-based electrochemical sensor were investigated [27, 29, 64]. No interference could be observed for a 100-fold excess quantity of CN^-, CO_3^{2-}, NO_3^-, PO_4^{3-}, SO_4^{2-}, Ca^{2+}, Cd^{2+}, Co^{2+}, Cu^{2+}, K^+, Mg^{2+}, Na^+, Ni^{2+}, Pb^{2+}, Zr^{4+} and Zn^{2+}, even with a mixed solution of isoproturon and carbendazim at only 1 mg/L (see Appendix A). However, Cl^- can interfere in the determination of isoproturon and carbendazim. Therefore, Cl^- in real samples must be removed before determination. Moreover, the influences of two dithiocarbamate fungicides which were frequently used in rice-field, disulfiram (tetraethylthiuram disulfide) and thiram (tetramethylthiuram disulfide) on the determination of isoproturon and carbendazim were investigated. A 10-fold of disulfiram and thiram had no significant effect on the square wave stripping voltammetric signal of isoproturon (Figure 4.16), and so do not interfere, while had an interfering influence on the signal of carbendazim analytes (Figure 4.17). Nevertheless, dithiocarbamates are decomposed easily and very fast in the presence of acid [65]. For this reason, disulfiram and thiram residues at the concentration of 1 mg/L do not interfere the determination of carbendazim in real samples.

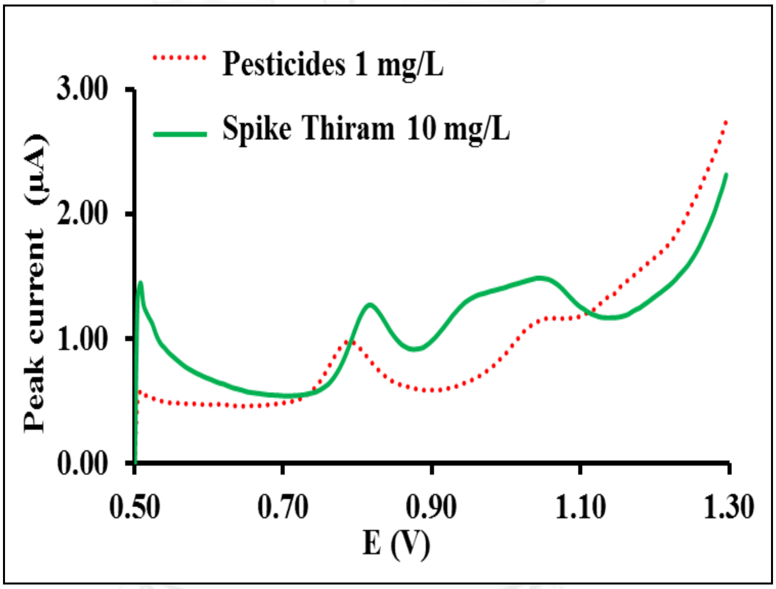

Figure 4.16 The square wave stripping voltammograms of a solution containing 1 mg/L isoproturon and carbendazim with and without 10 mg/L thiram.

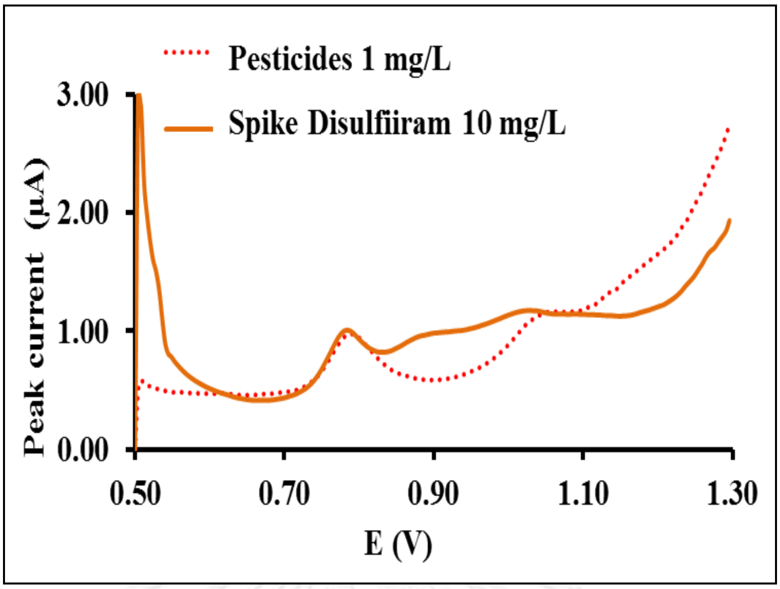

Figure 4.17 The square wave stripping voltammograms of a solution containing 1 mg/L isoproturon and carbendazim with and without 10 mg/L disulfiram.

4.4 The analytical performance

4.4.1 Linearity

The mixed standard solutions of isoproturon and carbendazim in the concentration range of 0.02–10.0 and 0.50–10.0 mg/L, respectively were analyzed by the proposed square wave voltammetric method using graphene-based electrochemical sensors under the optimized conditions. Well-defined peaks, with the peak current being linearly proportional to the concentration of isoproturon or carbendazim were observed as shown in Figure 4.18. Linear calibration curves for isoproturon and carbendazim were established over the range of 0.02–10.0 and 0.50–10.0 mg/L, respectively, with a correlation coefficient (r^2) of 0.9991 and 0.9990, respectively.

Table 4.14 Resulting peak currents of isoproturon and carbendazim at various concentrations

Concentrations (mg/L)	Peak current (µA) ± SD	
	Isoproturon	Carbendazim
0.02	0.021 ± 0.006	-
0.50	0.297 ± 0.019	0.104 ± 0.009
1.00	0.459 ± 0.015	0.203 ± 0.014
3.00	1.337 ± 0.042	0.709 ± 0.021
5.00	2.108 ± 0.041	1.172 ± 0.088
7.00	3.041 ± 0.060	1.706 ± 0.008
10.00	4.268 ± 0.092	2.435 ± 0.049

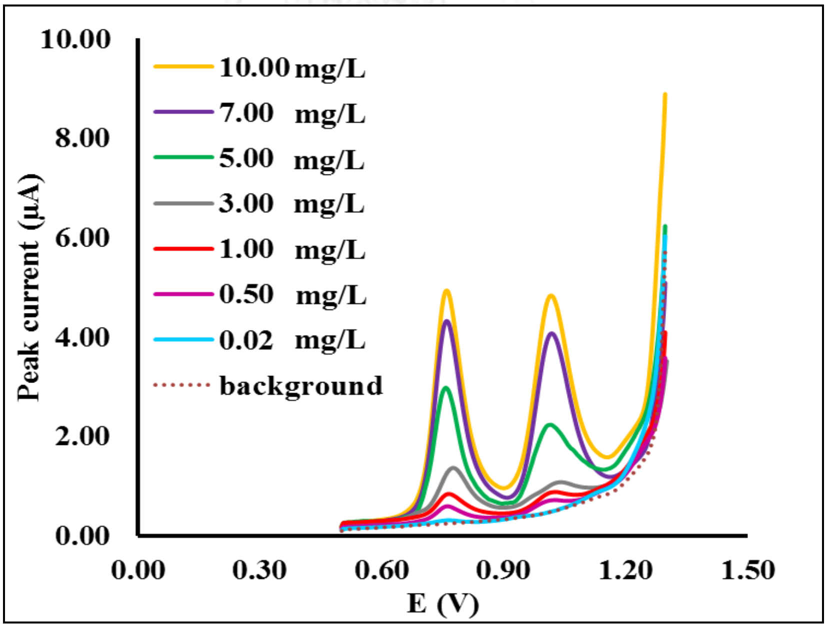

Figure 4.18 Square wave stripping voltammograms for different concentrations of isoproturon and carbendazim in 1.0 M $HClO_4$ measured under the optimal experimental conditions. Plots are representative of those seen from three independent repeats.

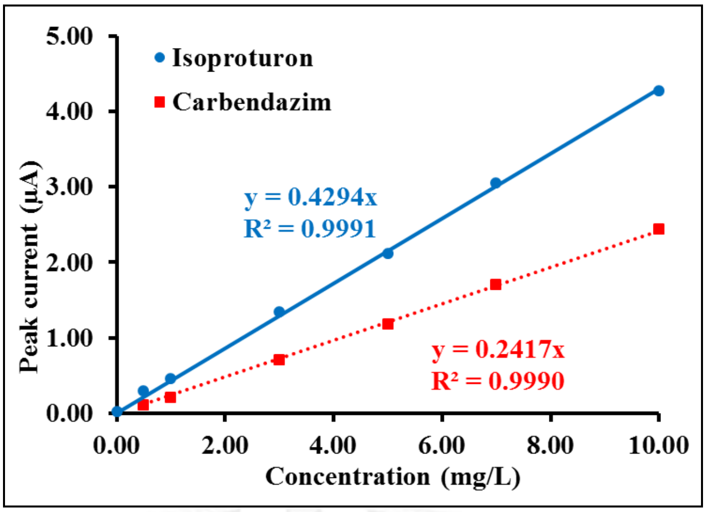

Figure 4.19 Calibration curves of isoproturon and carbendazim in 1.0 M $HClO_4$ measured under the optimal experimental conditions. Data are shown as the mean ± SD and are derived from three replicates.

4.4.2 Limit of detection (LOD) and limit of quantification (LOQ)

LOD were evaluated from $3S_b/S$, where S_b is the standard deviation of 0.02 mg/L isoproturon and 0.5 mg/L carbendazim responses (n=10) and S is the slope of the calibration curve whereas LOQ were evaluated from $10S_b/S$. LOD and LOQ were determined to be 0.02 and 0.07 mg/L, respectively, for isoproturon and 0.11 and 0.38 mg/L, respectively, for carbendazim.

4.4.3 Repeatability

The fabrication repeatability of the screen-printed sensors was estimated by determination of isoproturon and carbendazim in a solution containing 0.02 mg/L isoproturon and 0.50 mg/L carbendazim by square wave stripping voltammetry at ten different sensors, and was found to have a relative standard deviation of 9.2% and 10% for isoproturon and carbendazim respectively. Thus, the repeatability of the sensor was acceptable according to AOAC International guidelines.

The analytical performance data of the graphene-based electrochemical sensor for the determination of isoproturon and carbendazim are summarized in Table 4.15.

Table 4.15 Summary of the analytical performance data of the proposed method for the determination of isoproturon and carbendazim.

Pesticides	Linearity (mg/L)	r^2	LOD (mg/L)	LOQ (mg/L)	RSD (%)
Isoproturon	0.02–10	0.9991	0.02	0.07	9.2
Carbendazim	0.50–10	0.9990	0.11	0.38	10

4.5 Analytical applications

In order to test the validity of the method, the proposed procedure was applied for the simultaneous determination of isoproturon and carbendazim in two water samples (one each from Chao Phraya River and a rice-field water), a soil sample from the same rice-field, and two vegetable samples (tomato and lettuce) under the optimum conditions and compared to that determined by the HPLC-UV method. No anodic peak current of isoproturon or carbendazim was observed in any of the as-prepared samples, nor detected by the HPLC-UV method. Therefore, these samples were spiked with standard isoproturon and carbendazim. After extraction, sample solutions contained 2.00 mg/L each of isoproturon and carbendazim. The concentrations of isoproturon and carbendazim in these sample solutions were then determined by standard addition method, which is used when sample matrix could influence the analytical signal, using the concentrations of 0, 2.00, 4.00, 6.00 and 8.00 mg/L and then analyzed by both methods. The spiked sample was analyzed in triplicate runs. The analytical results are listed in Table 4.17 and the recovery efficiencies were obtained in the range of 88.9–107% for isoproturon and 84.4–85.5% for carbendazim, which are acceptable according to AOAC International guidelines. In addition, the results of the simultaneous determination of isoproturon and carbendazim in the Chao Phraya River water, rice-field water, soil, tomato, and lettuce samples by the method of this report were comparable to those obtained from the HPLC-UV method with no significant difference between the two methods (student's *t*-test at the 95% confidence level gave $t_{\text{calculated}}$ below t_{critical} at 2.776 with 4 degrees of freedom). Therefore, this proposed method is useful for the simultaneous determination of isoproturon and carbendazim in actual samples. According to Commission Regulation (EU) No 212/2013, the maximum residue limits (MRLs) for isoproturon and carbendazim in almost fruits and vegetables are 0.05 and 0.1 mg/kg, respectively. Hence, the sensitivity of the proposed method is high enough to detect them. In addition, larger amount of sample or less volume of supporting electrolyte (1 M $HClO_4$) may be used for the preparation of sample solution in order to increase the concentration of isoproturon and carbendazim.

Table 4.16 The determination of isoproturon and carbendazim levels in Chao Phraya River and rice-field water samples (mg/L), and in soil, tomato and lettuce samples (mg/kg) by the square wave stripping voltammetry and HPLC-UV.

Sample	Spiked[a]	Isoproturon		Carbendazim	
		Found	Recovery (%)	Found	Recovery (%)
<u>Square wave stripping voltammetry</u>					
Chao Phraya river water	2.00	1.84 ± 0.21	92.1	1.69 ± 0.16	84.4
Rice-field water	2.00	2.16 ± 0.18	107	1.71 ± 0.15	85.5
Soil	2.00	1.89 ± 0.20	94.6	1.64 ± 0.26	82.2
Tomato	2.00	1.78 ± 0.20	88.9	1.63 ± 0.23	81.4
Lettuce	2.00	2.02 ± 0.19	101	1.66 ± 0.21	82.8
<u>HPLC-UV</u>					
Chao Phraya river water	2.00	1.79 ± 0.15	89.4	1.59 ± 0.17	79.5
Rice-field water	2.00	1.85 ± 0.06	92.6	1.99 ± 0.14	99.8
Soil	2.00	1.85 ± 0.15	92.7	1.73 ± 0.26	86.6
Tomato	2.00	1.88 ± 0.32	93.8	1.65 ± 0.30	82.6
Lettuce	2.00	1.60 ± 0.31	80.0	1.56 ± 0.11	77.9

[a] After extraction, sample solutions contained 2.00 mg/L each of isoproturon and carbendazim.

[b] Data are shown as the mean ± SD (N = 3) and are derived from three repeats.

Table 4.17 The student's *t*-test values for the determination of isoproturon and carbendazim in various samples by the proposed square wave stripping voltammetric method and HPLC-UV method

Sample	***t*-calculated**		***t*-critical**
	Isoproturon	**Carbendazim**	
Chao Phraya river water	0.248	0.374	2.776 (df = 4)
Rice-field water	1.316	1.180	
Soil	0.414	0.064	
Tomato	1.761	0.230	
Lettuce	0.200	0.154	

CHAPTER V

CONCLUSIONS AND SUGGESTION FOR FUTURE WORK

5.1 Conclusions

In this work, low-cost disposable sensors with three integrated electrodes, graphene-modified graphite working electrode, silver/silver chloride reference electrode and carbon counter electrode were fabricated by screen-printing silver/silver chloride ink, graphite ink and graphene-modified graphite ink onto the PVC substrate. The optimum composition of graphene-modified graphite ink used for screen-printing of the working electrode was 10.7 mg graphene, 1.0 g carbon ink and 0.2 g graphite powder. The developed graphene-based electrochemical sensors were characterized by SEM. It is obvious that the graphene-modified electrode has more surface area than the non-modified electrode, thus it can more effectively promote the electron transfer between the target molecules and the electrode. It was found that the developed sensor exhibited well-defined peaks with a 1.4- and 1.6-fold higher sensitivity for isoproturon (1.79 *vs*. 1.28 µA) and carbendazim (1.23 *vs*. 0.77 µA), respectively.

Furthermore, a fast, simple, and low-cost method for the simultaneous quantification of isoproturon and carbendazim by square wave stripping voltammetry using the graphene-based electrochemical sensor was developed. There is no requirement to use a conventional electrochemical cell because the screen-printed sensor could be used as an electrochemical cell. Only a single 60 µL drop of the sample solution is required to be dispensed onto the surface of the sensor for each stripping voltammetric measurement. Various experimental parameters were optimized. Under the optimal conditions, the calibration curve for isoproturon and carbendazim were over the range of 0.02–10.0 and 0.50–10.0 mg/L, respectively, with a correlation coefficient (r^2) were 0.9991 and 0.9990, respectively. LOD were 0.02 mg/L for isoproturon and 0.11 mg/L for carbendazim and LOQ were 0.07 for isoproturon and 0.38 mg/L for carbendazim. The relative standard deviations were 9.2% and 10% for isoproturon and carbendazim, respectively.

In order to validate the method, the proposed method was applied for the simultaneous determination of isoproturon and carbendazim in the Chao Phraya River water, rice-field water, soil, tomato and lettuce samples by the standard addition method, the acceptable recoveries were obtained in the range of 88.9–107% for isoproturon and 84.4–85.5% for carbendazim. These samples were also analyzed by the standard HPLC-UV method. The recoveries were 80.0–93.8% for isoproturon and 77.9–99.8% for carbendazim. In addition, the student's *t*-test at the 95% confidence level gave $t_{\text{calculated}}$ below $t_{critical}$ at 2.776 with 4 degrees of freedom which show that no significant difference between the two methods. Therefore, this proposed method is useful for the simultaneous determination of isoproturon and carbendazim in actual samples. Moreover, the very simple measurement protocol and short analysis time of water (or post-extracted solid) samples are clear advantages of the proposed approach.

5.2 Suggestion for future work

In the future, graphene-based electrochemical sensor should be applied for the simultaneous determination of other pesticides. Improving the sensitivity of the graphene-based electrochemical sensor is another interesting aspect. Moreover, graphene-based electrochemical sensor can be applied for the other samples such as rice, fruits.

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APPENDIX

The interference study

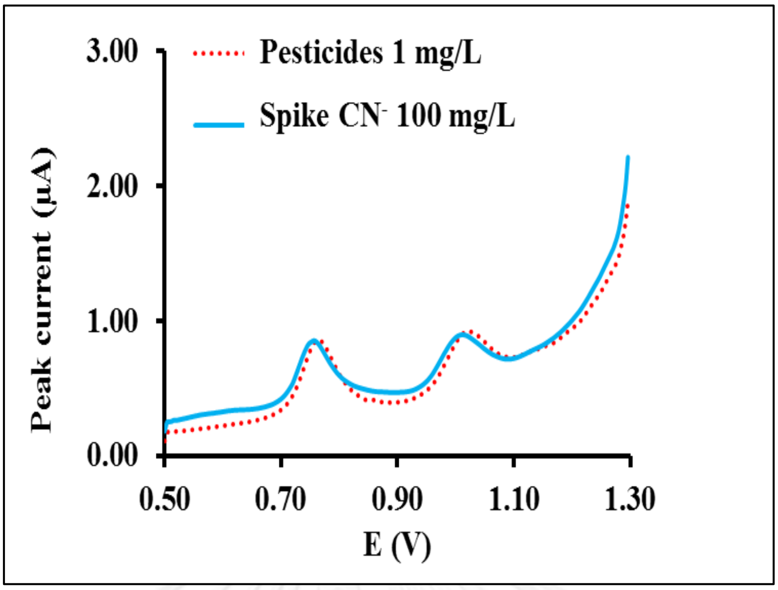

Figure A-1 Square wave stripping voltammograms of solutions containing 1 mg/L isoproturon and carbendazim in 1.0 M $HClO_4$ in the presence and absence of 100 mg/L CN^- ions

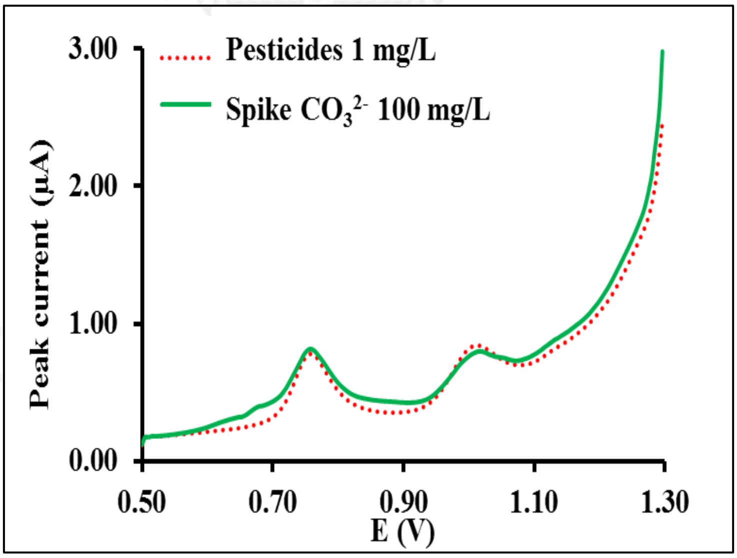

Figure A-2 Square wave stripping voltammograms of solutions containing 1 mg/L isoproturon and carbendazim in 1.0 M $HClO_4$ in the presence and absence of 100 mg/L CO_3^{2-} ions

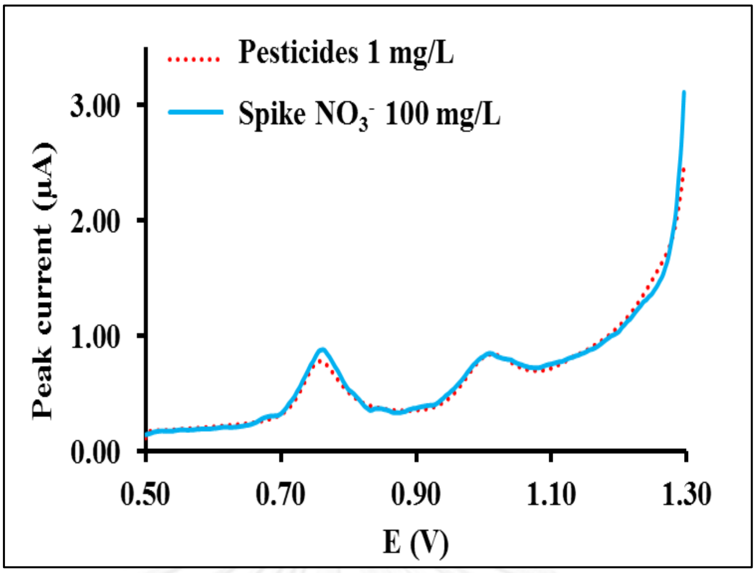

Figure A-3 Square wave stripping voltammograms of solutions containing 1 mg/L isoproturon and carbendazim in 1.0 M $HClO_4$ in the presence and absence of 100 mg/L NO_3^- ions

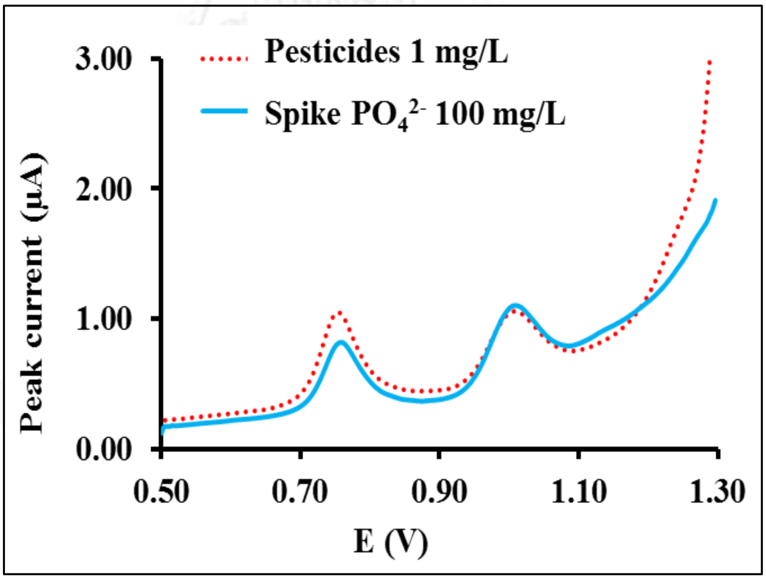

Figure A-4 Square wave stripping voltammograms of solutions containing 1 mg/L isoproturon and carbendazim in 1.0 M $HClO_4$ in the presence and absence of 100 mg/L PO_4^{3-} ions

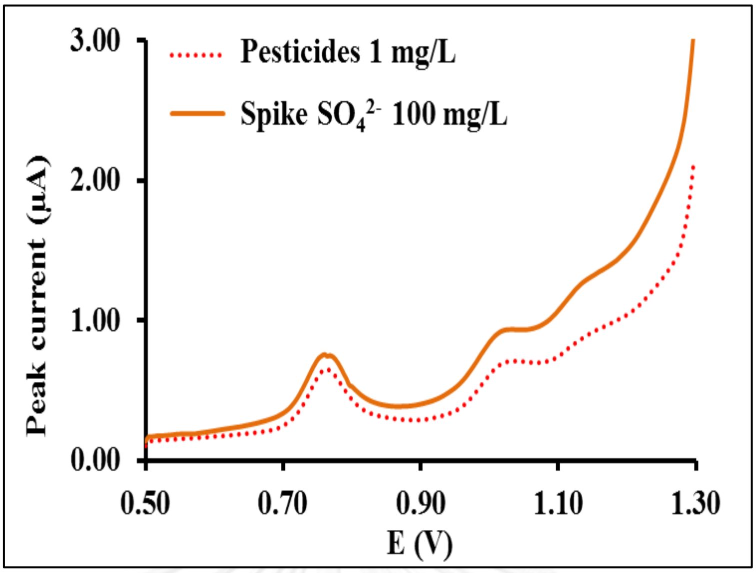

Figure A-5 Square wave stripping voltammograms of solutions containing 1 mg/L isoproturon and carbendazim in 1.0 M $HClO_4$ in the presence and absence of 100 mg/L SO_4^{2-} ions

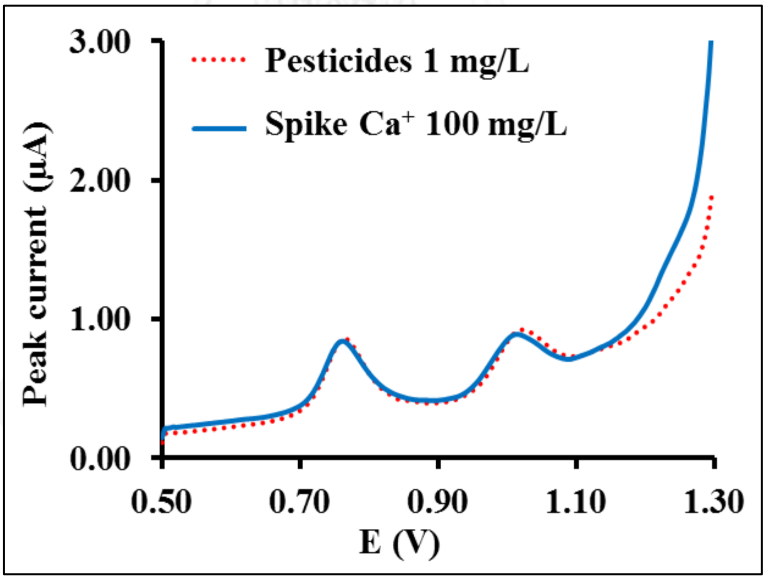

Figure A-6 Square wave stripping voltammograms of solutions containing 1 mg/L isoproturon and carbendazim in 1.0 M $HClO_4$ in the presence and absence of 100 mg/L Ca^{2+} ions

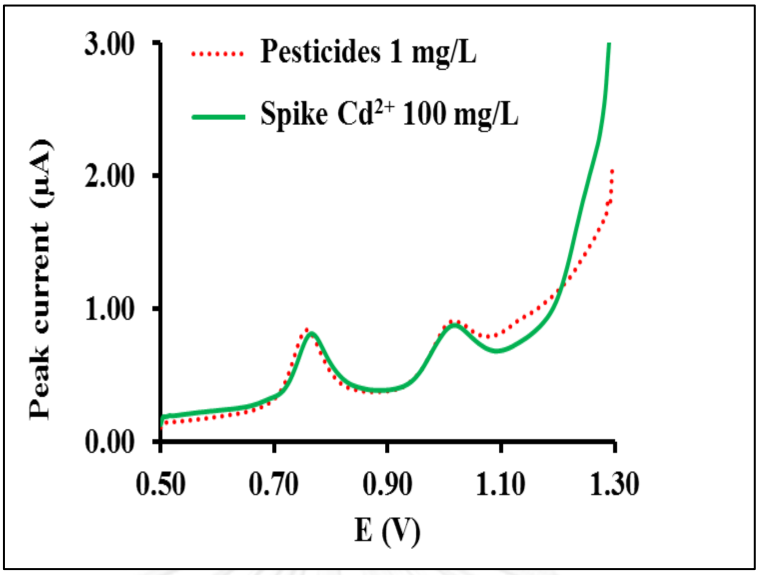

Figure A-7 Square wave stripping voltammograms of solutions containing 1 mg/L isoproturon and carbendazim in 1.0 M $HClO_4$ in the presence and absence of 100 mg/L Cd^{2+} ions

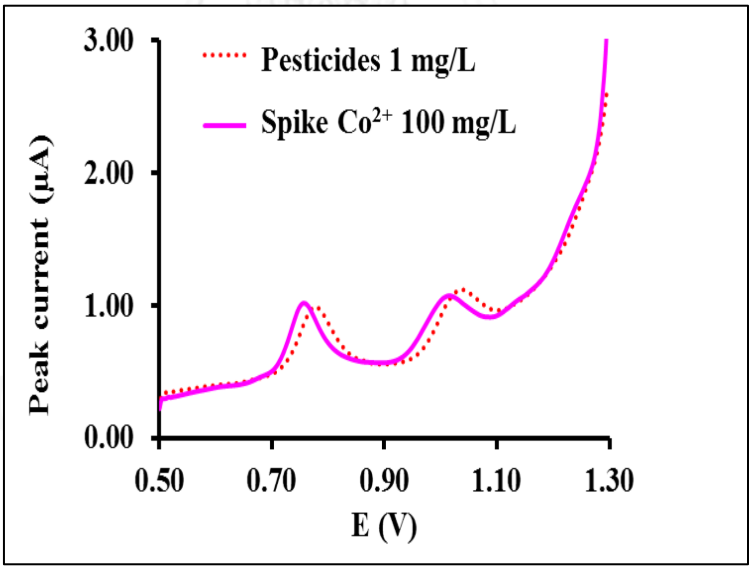

Figure A-8 Square wave stripping voltammograms of solutions containing 1 mg/L isoproturon and carbendazim in 1.0 M $HClO_4$ in the presence and absence of 100 mg/L Co^{2+} ions

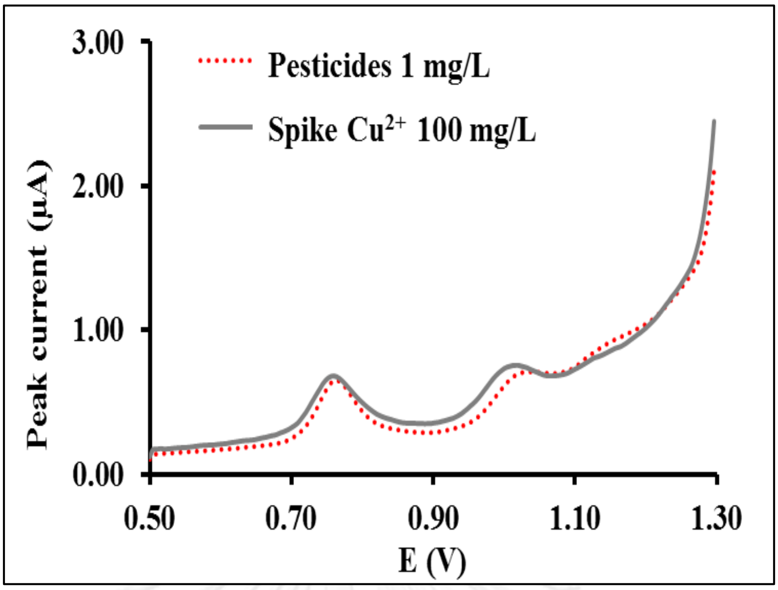

Figure A-9 Square wave stripping voltammograms of solutions containing 1 mg/L isoproturon and carbendazim in 1.0 M $HClO_4$ in the presence and absence of 100 mg/L Cu^{2+} ions

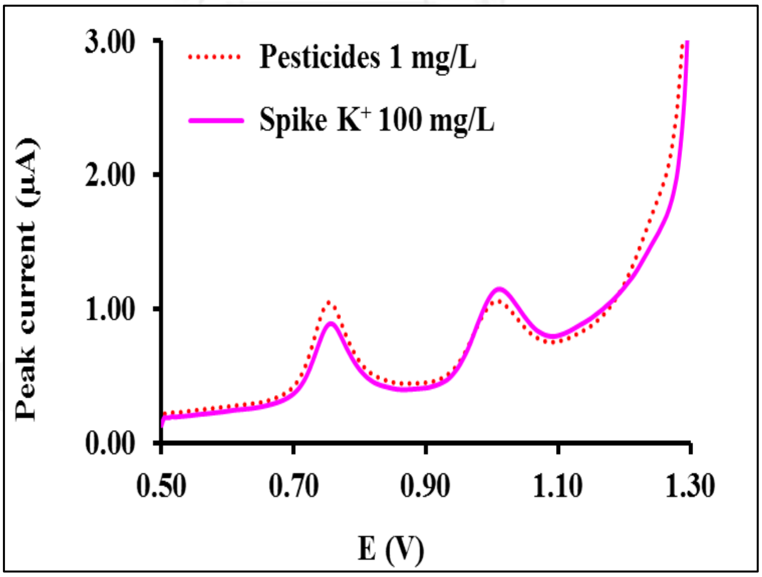

Figure A-10 Square wave stripping voltammograms of solutions containing 1 mg/L isoproturon and carbendazim in 1.0 M $HClO_4$ in the presence and absence of 100 mg/L K^+ ions

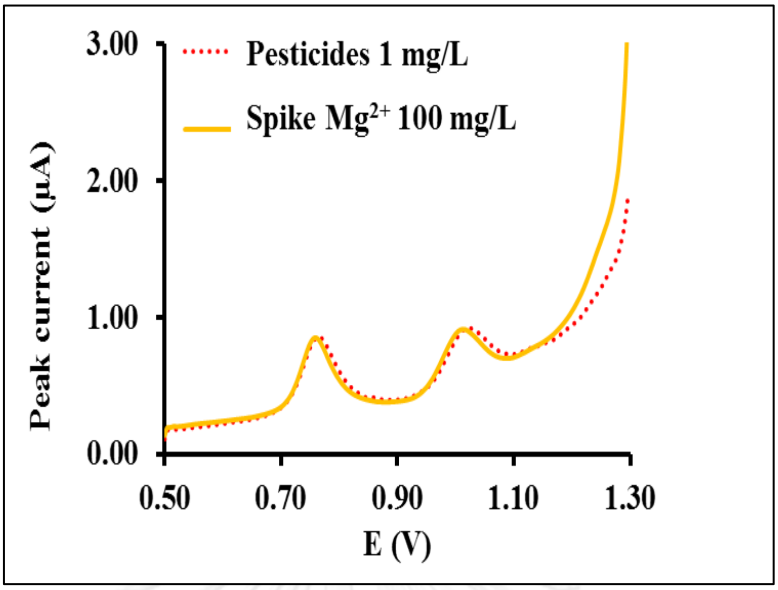

Figure A-11 Square wave stripping voltammograms of solutions containing 1 mg/L isoproturon and carbendazim in 1.0 M $HClO_4$ in the presence and absence of 100 mg/L Mg^{2+} ions

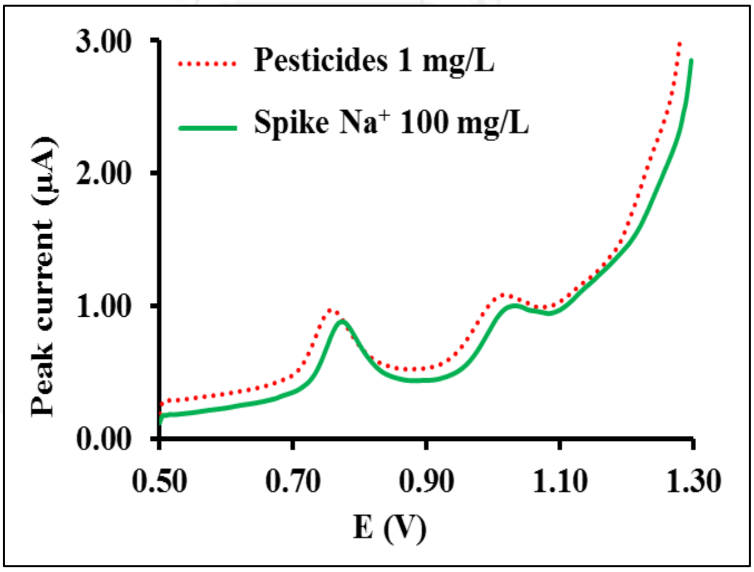

Figure A-12 Square wave stripping voltammograms of solutions containing 1 mg/L isoproturon and carbendazim in 1.0 M $HClO_4$ in the presence and absence of 100 mg/L Na^{+} ions

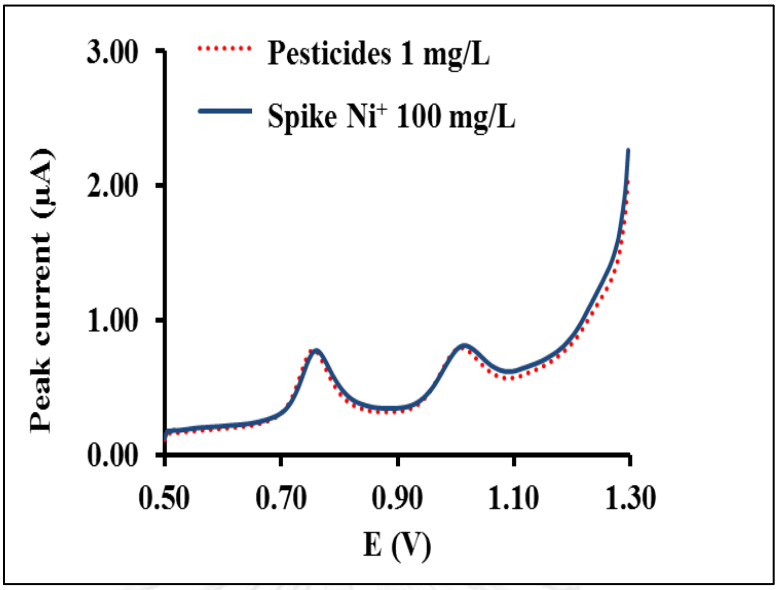

Figure A-13 Square wave stripping voltammograms of solutions containing 1 mg/L isoproturon and carbendazim in 1.0 M $HClO_4$ in the presence and absence of 100 mg/L Ni^{2+} ions

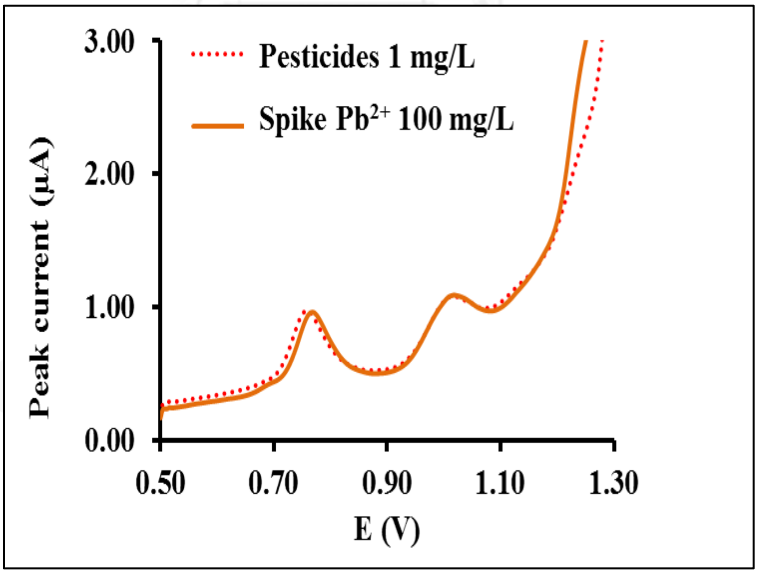

Figure A-14 Square wave stripping voltammograms of solutions containing 1 mg/L isoproturon and carbendazim in 1.0 M $HClO_4$ in the presence and absence of 100 mg/L Pb^{2+} ions

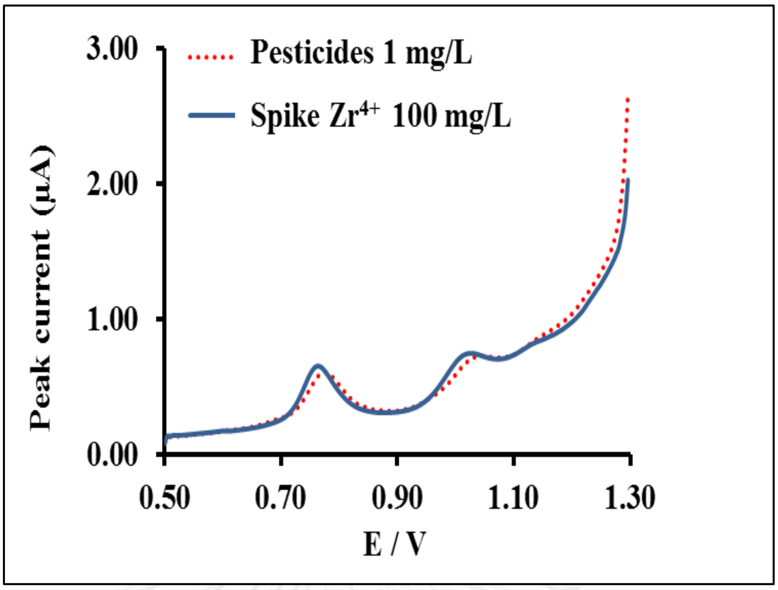

Figure A-15 Square wave stripping voltammograms of solutions containing 1 mg/L isoproturon and carbendazim in 1.0 M $HClO_4$ in the presence and absence of 100 mg/L Zr^{4+} ions

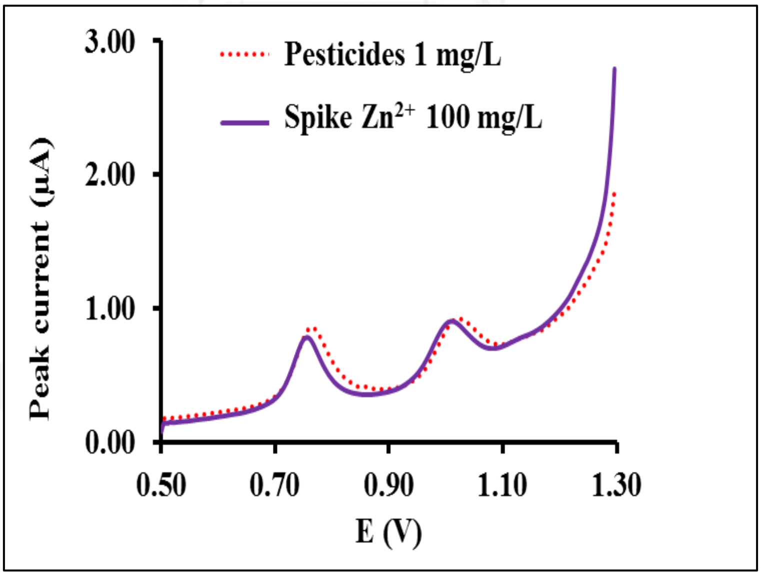

Figure A-16 Square wave stripping voltammograms of solutions containing 1 mg/L isoproturon and carbendazim in 1.0 M $HClO_4$ in the presence and absence of 100 mg/L Zn^{2+} ions

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Publications:

1.Uthaitip Injang, Peeyanun Noyrod, Weena Siangproh, Wijitar Dungchai, Shoji Motomizu, Orawon Chailapakul, Determination of trace heavy metals in herbs by sequential injection analysis-anodic stripping voltammetry using screen-printed carbon nanotubes electrodes, Analytica Chimica Acta 668 (2010) 54–60.

2.Peeyanun Noyrod , Orawon Chailapakul , Wanida Wonsawat , Suchada Chuanuwatanakul, The simultaneous determination of isoproturon and carbendazim pesticides by single drop analysis using a graphene-based electrochemical sensor, Journal of Electroanalytical Chemistry 719 (2014) 54–59.