

การดัดแปรชีวไฟฟ้าด้วยรีดิวซ์แกรฟีนออกไซด์นาโนคอมพอสิตสำหรับการตรวจวัด
ไดโคฟีแนค

นายกฤษณ กิมอ่วม



จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY

บทคัดย่อและแฟ้มข้อมูลฉบับเต็มของวิทยานิพนธ์ตั้งแต่ปีการศึกษา 2554 ที่ให้บริการในคลังปัญญาจุฬาฯ (CUIR)

เป็นแฟ้มข้อมูลของนิสิตเจ้าของวิทยานิพนธ์ ที่ส่งผ่านทางบัณฑิตวิทยาลัย

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต

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สาขาวิชาปิโตรเคมีและวิทยาศาสตร์พอลิเมอร์

คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

ปีการศึกษา 2559

ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

ELECTRODE MODIFICATION USING REDUCED GRAPHENE OXIDE
NANOCOMPOSITES FOR DICLOFENAC DETECTION

Mr. Kritthana Kimuam



A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Petrochemistry and Polymer

Science

Faculty of Science

Chulalongkorn University

Academic Year 2016

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Thesis Title ELECTRODE MODIFICATION USING REDUCED GRAPHENE OXIDE NANOCOMPOSITES FOR DICLOFENAC DETECTION

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กฤตธน กิมอ่วม : การดัดแปรขั้วไฟฟ้าด้วยรีดิวซ์แกรฟีนออกไซด์นาโนคอมพอสิตสำหรับการตรวจวัดไดโคลฟีแนค (ELECTRODE MODIFICATION USING REDUCED GRAPHENE OXIDE NANOCOMPOSITES FOR DICLOFENAC DETECTION) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ. ดร.นาตยา งามโรจนวิชัย, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ศ. ดร.อรรพรรณ ชัยลภากุล, ดร.นาฏนิตดา รอดทองคำ, 64 หน้า.

ตัวตรวจวัดทางเคมีไฟฟ้าได้ถูกพัฒนาขึ้นโดยแกรฟีนนาโนคอมพอสิตสำหรับการตรวจวัดทางเคมีไฟฟ้า (ซัลไฟต์และไดโคลฟีแนค) ในงานวิจัยนี้ได้แบ่งเป็น 2 ส่วน ส่วนแรกคือการพัฒนาตัวตรวจวัดทางเคมีไฟฟ้าสำหรับการตรวจวัดซัลไฟต์ ซึ่งขั้วไฟฟ้าที่ถูกดัดแปรด้วยแกรฟีนจะให้ค่าสัญญาณการตรวจวัดสูงกว่าขั้วไฟฟ้าที่ไม่ถูกดัดแปร ส่วนที่สองคือการใช้รีดิวซ์แกรฟีนออกไซด์/อนุภาคนาโนของแพลตินัมาดัดแปรขั้วไฟฟ้าสำหรับตรวจวัดไดโคลฟีแนค โดยลักษณะทางสัณฐานวิทยาของผิวหน้าขั้วไฟฟ้าที่ถูกดัดแปรสามารถศึกษาได้ด้วยกล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราด และใช้เทคนิคโวลแทมเมตรีแบบรอบในการศึกษาผลทางไฟฟ้าเคมี อีกทั้งยังศึกษาปัจจัยของระบบที่ส่งผลต่อสัญญาณการตรวจวัด โดยภายใต้สภาวะที่เหมาะสมพบว่า ขั้วไฟฟ้าที่ถูกดัดแปรจะให้ค่าสัญญาณการตรวจวัดสูงกว่าขั้วไฟฟ้าที่ไม่ถูกดัดแปร ซึ่งแสดงให้เห็นว่าขั้วไฟฟ้าที่ดัดแปรมีความไวในการตรวจวัดสูง นอกจากนี้ขั้วไฟฟ้าที่ดัดแปรยังให้ขีดจำกัดในการตรวจวัดที่ 0.04 ไมโครโมลาร์ และมีช่วงความเป็นเส้นตรงอยู่ที่ 0.1-100 ไมโครโมลาร์ สุดท้ายนี้ขั้วไฟฟ้าที่ดัดแปรยังสามารถนำไปประยุกต์ใช้ในการตรวจวัดปริมาณไดโคลฟีแนคในปัสสาวะได้

สาขาวิชา	ปิโตรเคมีและวิทยาศาสตร์พอลิ	ลายมือชื่อนิสิต
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5672098323 : MAJOR PETROCHEMISTRY AND POLYMER SCIENCE

KEYWORDS: REDUCED GRAPHENE OXIDE / NANOCOMPOSITES / DICLOFENAC

KRITTHANA KIMUAM: ELECTRODE MODIFICATION USING REDUCED GRAPHENE OXIDE NANOCOMPOSITES FOR DICLOFENAC DETECTION.

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RODTHONGKUM, Ph.D., 64 pp.

The electrochemical sensors based on graphene nanocomposite modified electrode for electrochemical detection (e.g. sulfite, diclofenac) were fabricated. This study is divided into two main parts. The first part is development of electrochemical sensor for sulfite detection. The modified electrode based on graphene showed the higher sensitivity than an unmodified electrode. However, the second part used reduced graphene oxide/platinum nanoparticles (RGO/PtNPs) modified electrode for the determination of diclofenac. The surface morphology of the modified electrode was characterized by using scanning electron microscopy (SEM). The cyclic voltammetry (CV) was employed to investigate the electrochemical performances of electrodes. All the factors affecting the sensitivity of this system were systematically evaluated. Under the optimized conditions, the modified electrode showed a higher current response signal compared to an unmodified electrode indicating the high sensitivity of this sensor. Moreover, the modified electrode provided a low limit of detection of 0.04 μM with a wide linearity of 0.1-100 μM . Eventually, this system was successfully applied for the determination of diclofenac in urine samples and the satisfactory results were obtained.

Field of Study: Petrochemistry and Student's Signature

Polymer Science Advisor's Signature

Academic Year: 2016 Co-Advisor's Signature

Co-Advisor's Signature

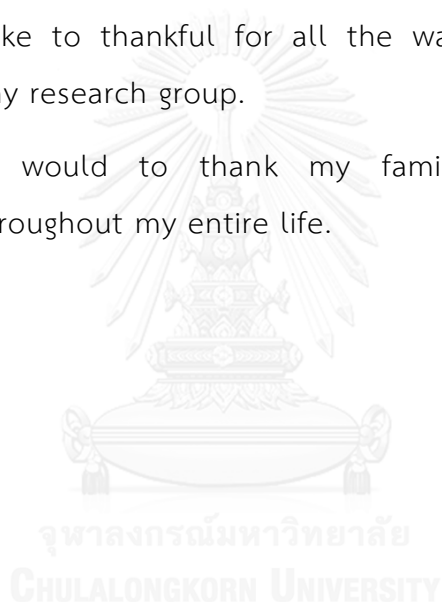
ACKNOWLEDGEMENTS

I would like to thank all my thesis advisor and co-advisors including Associate Professor Dr. Nattaya ngamrojanavanich, Professor Dr. Orawan Chailapakul, Dr. Nadnudda Rodthongkhum for their useful advices and encouragement throughout the thesis.

I am grateful to all the thesis committee members for their comments and interested advices.

I would like to thankful for all the warm friendship and great motivation from my research group.

Finally, I would to thank my family for their love and encouragement throughout my entire life.



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CHAPTER I

INTRODUCTION

1.1 Introduction

Electrochemical sensor is one of the interesting devices used for rapid determination of target analytes due to its high sensitivity, simple procedure, inexpensive equipment, short analysis time and portable size [1]. To prepared portable electrochemical sensor, a screen printing technique was used for fabricating of small sized of electrode. Due to small size of electrochemical sensor, this sensor has a limited electrode surface area leading to low sensor sensitivity. To solve this problem, electrode modification is a necessary step to improve the sensitivity of an electrochemical system. The simplest way to increase the electrode surface area is modification of working electrode surface by using nanomaterials.

Carbon based nanomaterials such as carbon nanotube (CNT) [2], carbon nanofiber (CNF) [3], carbon nanodot (CND) [4], fullerenes [5] and graphene (G) have been commonly used for electrode modification [6-8]. Among carbon based nanomaterials, G, a new member of nanocarbon family, has become a much attention material in several decades [9]. G is a two-dimensional material consisting of carbon atom in hexagonal configuration which bonded by sp^2 bond [10]. This configuration is a reason for remarkable properties of G such as high conductivity, fast electron transfer, high thermal stability and large specific surface area [11]. These outstanding properties have made G an attractive candidate for sensor application. Nevertheless, G has a strong Van der waals interaction with its neighbor sheets leading a self-agglomeration and re-stacking leading to lose a electrochemical sensitivity [12, 13]. To prevent the re-stacking of G, graphene oxide (GO), one of the most important G derivatives has become an alternative material. GO has similar structure to G but it was functionalized by several oxygen groups such as hydroxyl, carboxyl and epoxy groups resulting in possess of both

hydrophobic and hydrophilic properties [14, 15]. The hydrophilic groups make GO disperse well in water. However, GO is generally categorized as an insulating material resulting from disruption of oxygen groups [16]. Thus, GO has to be reduced into pristine G denoted as reduced graphene oxide (RGO) before it is used to modify electrode surface. The best methods to reduce GO is an electrochemical reduction technique because it provides a high quality yield with no contamination of reducing agents when compared with other techniques. Thus, RGO has attracted much attention to use for electrode modification for determination of various analytes.

Metallic nanoparticles such as gold [17], silver [18], palladium [19] and platinum [20] are widely used to modify electrode surface because they provide a high surface to volume ratio, good electrocatalytic activity [21]. Among these metallic nanoparticles, platinum nanoparticle (PtNPs) is one of attractive materials for electrochemical detection [22,23]. It is an excellent catalyst for electroactive molecules owing to its extraordinary electrocatalytic activity, high electrical conductivity and biocompatibility. In addition, PtNPs also has been successfully used to modify electrode for biological molecule and drug molecule [24-26]. In this study, nanomaterial modified electrodes were developed for the determination of target analytes, which is sulfite (contaminant in food and beverage) and diclofenac (common drug molecule). The parameters affecting the performance and sensitivity of the electrochemical sensor were optimized. Moreover, the proposed system was applied for the determination of analyte in real sample (human urine). This work was divided into 2 main parts;

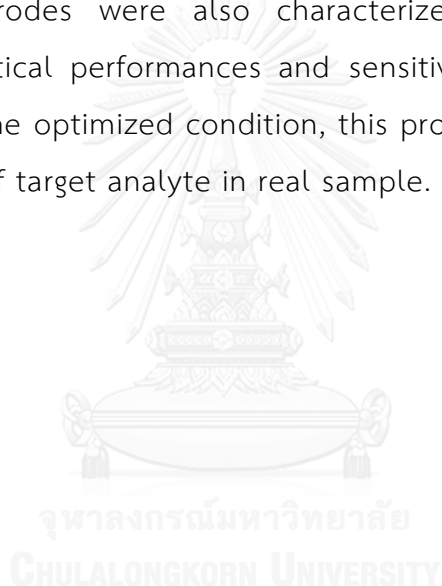
- Electrode modification using graphene/polyvinylpyrrolidone nanocomposites for sulfite detection
- Electrode modification using simultaneously reduced graphene oxide/platinum for diclofenac detection

1.2 Objectives

- To develop electrochemical sensor by using nanomaterial modified electrode surface
- To use the modified electrode for the determination of target analytes in real sample.

1.3 Scope of research

In this study, nanomaterial modified electrode surfaces for sensitive determination of target analytes were developed. The surface morphologies of modified electrodes were also characterized. Moreover, all factors affecting the analytical performances and sensitivities of this sensors were evaluated. Under the optimized condition, this proposed system was applied for determination of target analyte in real sample.



CHAPTER II

THEORY

In this chapter, the definitions and basic principles of electrochemical technique are described. In the first part, it focuses on electrochemical system, electrode and voltammetry. The second part of this chapter, it describes about modification of electrode surface by using nanomaterial especially carbon based nanomaterial (fullerene, carbon nanotube, graphene, graphene oxide) and metallic nanoparticles. Finally, the importance of target analytes (e.g. sulfite and diclofenac) is explained.

2.1 Electrochemistry

2.1.1 Basic principles of electrochemical technique

Electrochemistry is a brunch of chemistry that studies an interaction of electricity and chemical reaction. The electrochemists focus on studying a chemical changes caused by electron transportation and production of electricity by chemical reaction. Generally, electrochemistry has been wildy used in a variety of purposes including corrosion, environmental monitoring, batteries, fuel cells, industrial quality control chemical analysis and biomedical diagnosis.

Electrochemical methods are analytical techniques that concern with measurement of potential, charge or current signal to evaluate a target analyte's concentration. That is a qualitative and quantitative analysis. The electrochemical methods may be divided into two broad classes which are potentiometric methods (static technique) and voltammetric methods (dynamic technique) (Figure 1.1).

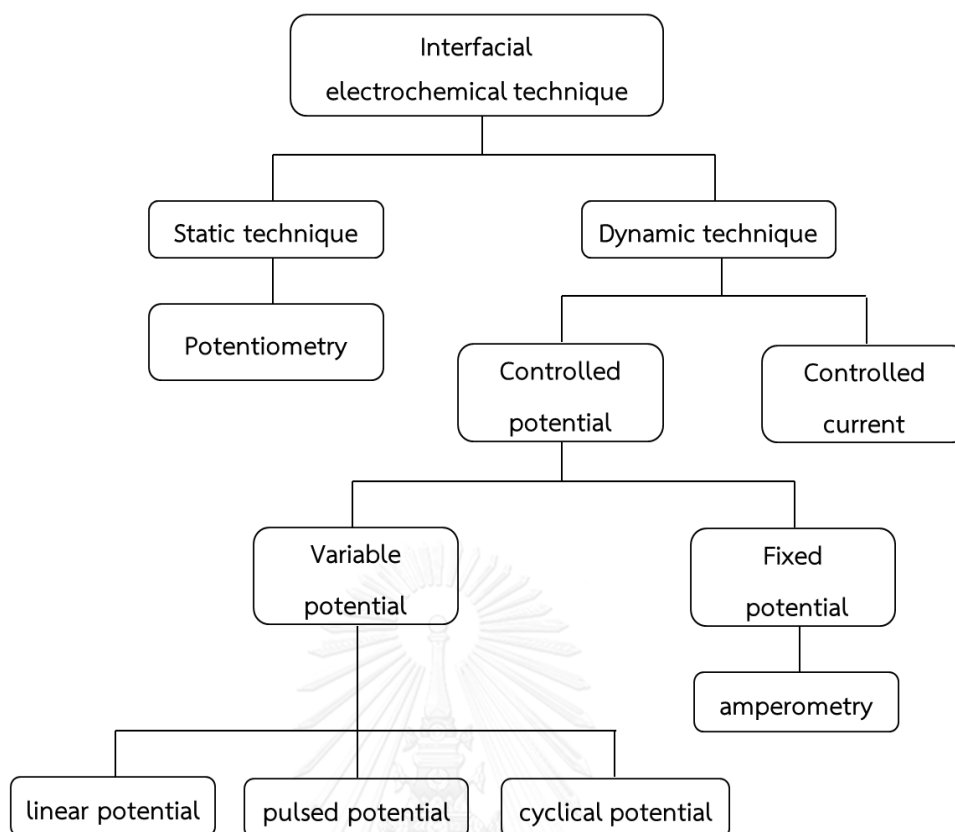


Figure 1.1 Family tree for interfacial electrochemical technique.

Potentiometric is a technique that performs under station condition (zero current). This technique bases on measurement of potential of a solution between working electrode and reference electrode. The potential of working electrode depends on concentration of analyte calculated from the equation below.

$$E_{\text{cell}} = E_{\text{ind}} - E_{\text{ref}} + E_j$$

Where

- E_{cell} = cell potential
- E_{ind} = potential of indicator electrode
- E_{ref} = potential of reference electrode
- E_j = junction potential

Conversely, the other technique; voltammetric methods are based on dynamic technique that measure current response as a function of applied potential and the results were plot between currents and applied potentials

namely, voltammogram. Basically, the principle of this technique is applying a various potential to electrochemical cell. While potential were applying to electrochemical cell, the currents are generated by the oxidation and reduction of redox analyte at interfacial of working electrode (Figure 1.2). This method can use to detect any target analytes that were oxidized and reduced because current signal obtained from this method is related to the concentration of analytes. The redox reaction occurring at electrode surface is shown below:



Where Ox is an oxidized form and Red is a reduced form of redox species. This reaction take place when the potentials were applied that make the electron transfer thermodynamically and kinetically favored. Following the law of thermodynamic, the concentration of the electroactive species can be obtained from potential of electrode by Nernst equation below:

$$E = E^0 + \frac{2.3RT}{nF} \log \frac{C_{\text{O}}(0,t)}{C_{\text{R}}(0,t)}$$

Where	E^0	=	standard potential of redox reaction
	R	=	gas constant ($8.314 \text{ JK}^{-1} \text{ mol}^{-1}$)
	T	=	temperature (K)
	n	=	number of electrons transferred
	F	=	Faraday constant (96,487 C)
	C_{O}	=	concentration of oxidized species
	C_{R}	=	concentration of reduced species

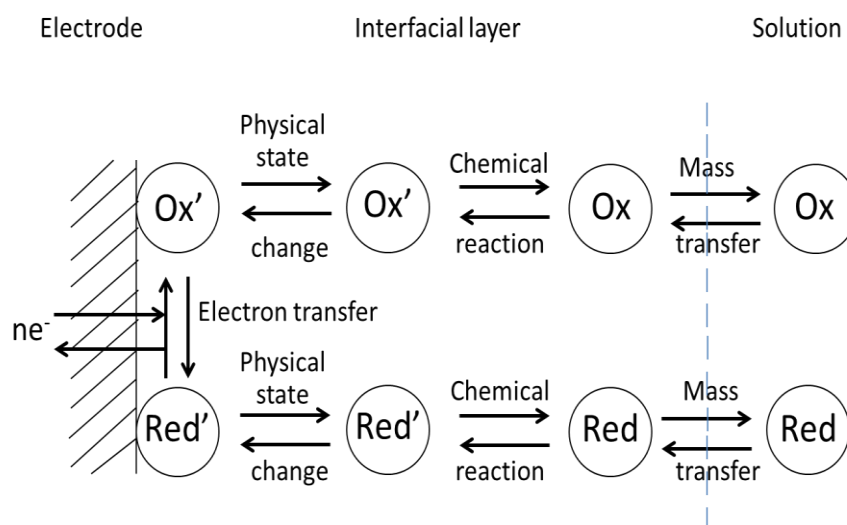


Figure 1.2 A schematic of the reaction at electrode.

For the development of electrochemical analysis, the methods based on potentiostatic technique such as cyclic voltammetry (describe in section 2.1.3) and differential pulse voltammetry (describe in section 2.1.4) were used for determination of target analytes.

2.1.2 Electrochemical cell

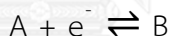
Generally, studying the electrochemical analysis requires three components which consist of electronic instrument (potentiostat), electrolyte and electrodes. The electrolyte and electrode is called electrochemical cell or electrolytic cell. The electrodes consist of three electrodes: reference electrode, working electrode and counter electrode. The reference is a stable electrode and known electrode potential. It uses to measure the potential of working electrode without passing current through. The working electrode is use for detection of target analytes. The counter electrode is used for supplying the current to working electrode. It serves to create a closed circuit. To complete the electrical circuit, the three electrodes connected with power supply have to immerse in electrolyte solution. In the past, the conventional three electrodes system was used but it has a drawback like large-scale large amount of sample volume. Recently the conventional electrode system was miniaturized to a small electrode called

screen printed electrode (SPE). Because of small size of electrode, it can be portable to detect anywhere and requires small amount of sample that make it suitable for biomedical detection.

2.1.3 Cyclic voltammetry (CV)

Cyclic voltammetry is a one of popular technique used for the study of electroactive species, redox event and determination of reaction mechanisms. Cyclic voltammetry is extensively employed because it rapidly provides the information of thermodynamic and kinetic of redox behavior over a wide range of potential. Cyclic voltammetry is often the first technique performed for developing and optimized electrochemical sensor.

For cyclic voltammetry experiments, the potential is swept linearly with time. The wave form of cyclic voltammetry is triangular or cyclic wave form that is scan forward then switched and swept backward. It can repeat over many cycles. This potential wave form is shown in Figure 1.3



In general experiment, when the potential was scanned linearly forward from starting potential which scans from positive to negative, the electron transfer occurs and makes a reduction of A to produce B. This result gives a cathodic current signal. Then the potential swept backward making electron transfer in opposite direction and the oxidation of B to A occurs. This result gives an anodic current signal. The output of cyclic voltammetry is known as cyclic voltammogram that plot between swept applied potential and obtained current. Cyclic voltammogram generally show the two peak of oxidation and reduction of electroactive species. According to Figure 1.4, the cyclic voltammogram show the response of redox couple. At first half cycle, it illustrate the cathodic peak shape caused by reduction process of O. besides, the height of that peak is called cathodic peak current (i_{pc}) and the potential at top of peak is called cathodic peak potential (E_{pc}). Afterwards, the other half cycle show reverse peak when re-oxidation of R to O occurs

which is anodic peak. The height of that peak is called anodic peak current (i_{pa}) and the potential at vertex is called anodic peak potential. The peak current is calculated by the equation below:

$$i_p = kn^{3/2} AD_0^{1/2} C_0 v^{1/2}$$

Where

i	=	peak current
n	=	number of electron transfer
A	=	electron transfer area
D_0	=	diffusion coefficient
C_0	=	bulk concentration
v	=	scan rate

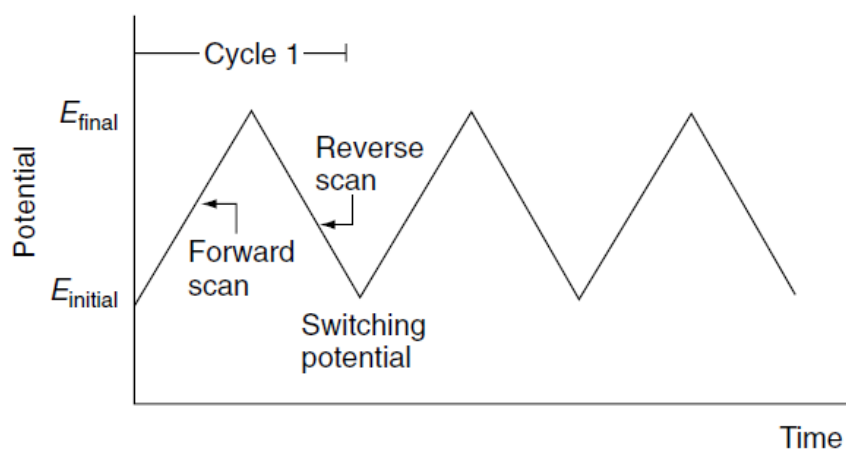


Figure 1.3 wave form of cyclic voltammogram.

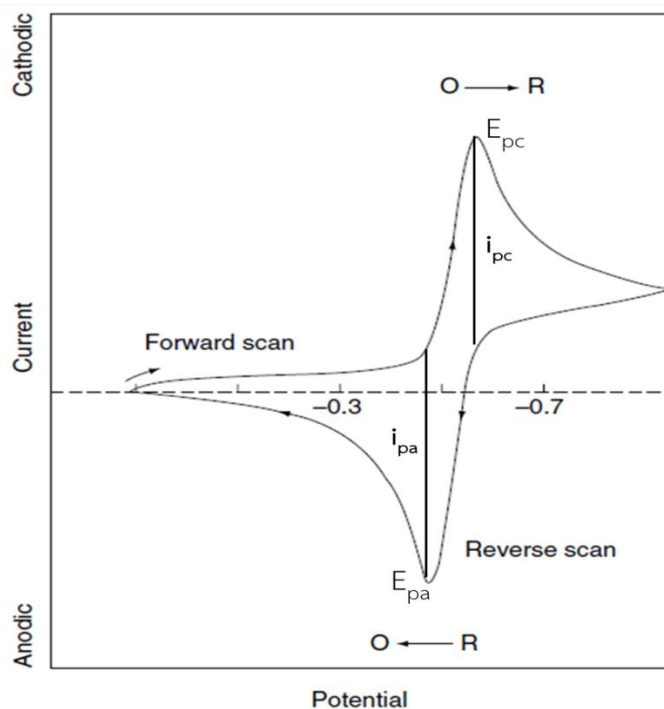


Figure 1.4 Cyclic voltammogram of reversible redox process.

2.1.3.1 Reversible system

For reversible system, the cathodic peak and anodic peak is well-defined with symmetry peak shape confirming that the electroactive species can oxidize and reduce completely. It is used for determination of number of electron transferred. The peak current for reversible was calculated by Randles-sevcik equation shown below:

$$i_p = (2.69 \times 10^5) n^{3/2} A D_0^{1/2} C v^{1/2}$$

Where

- i_p = peak current
- n = number of electron transferred
- A = surface area of electrode
- D_0 = diffusion coefficient
- C = concentration of electroactive species
- v = scan rate

Generally, the peak current is direct variation with concentration and scan rate implying that the electrode reaction controlled by mass transport. The

position of peak potential is located at center between E_{pa} and E_{pc} relating to the potential of redox process.

$$E_0 = (E_{pa} + E_{pc})/2$$

$$\Delta E_p = E_{pa} - E_{pc} = (0.059/n) \times V$$

2.1.3.2 Quasi-reversible system

For the irreversible system, the cathodic peak current and anodic peak current are not symmetry. The reaction at electrode is very slow. So that, the current signal is controlled by both mass transport and charge transfer. The individual peaks are reduced shown in Fig. 1.5 and the current peak is calculated by equation below:

$$i_p = (2.99 \times 10^5) n(\alpha n_a)^{1/2} A D_0^{1/2} C v^{1/2}$$

Where α is calculated from;

$$|E_p - E_{p/2}| = 47.7/\alpha(25^\circ \text{C})$$

Where $E_{p/2}$ is the potential of half peak current

2.1.4 Differential pulse voltammetry (DPV)

Differential pulse voltammetry is a technique used for quantitative analysis. This technique provides enhanced sensitivity. It is used for detecting a low concentration of analytes. Though, cyclic voltammetry is a first method for studying a redox process, it is difficult to use for measuring the faradaic currents in the presence of background current. But differential pulse voltammetry can discriminate between faradaic current and charging current. The principle of differential pulse technique is applying a potential in wave form shown in Figure 1.5A and the current is measured before and end the application of each pulse. The different between two measured current is plotted as a function of the potential (Figure 1.5B). The results peak is called differential pulse voltammogram and the height of peak is proportional to the concentration of electroactive species calculated by equation as shown below;

$$i_{\max} = \frac{nFACD^{\frac{1}{2}}}{\pi^{\frac{1}{2}}(\tau - \tau^1)^{\frac{1}{2}}} \left(\frac{1 - \sigma}{1 + \sigma} \right)$$

Where $\sigma = \exp[(nf/RT)(\Delta E/2)]$, (ΔE is the pulse amplitude) and the maximum value of the quotient $(1 - \sigma)/(1 + \sigma)$ is unity.

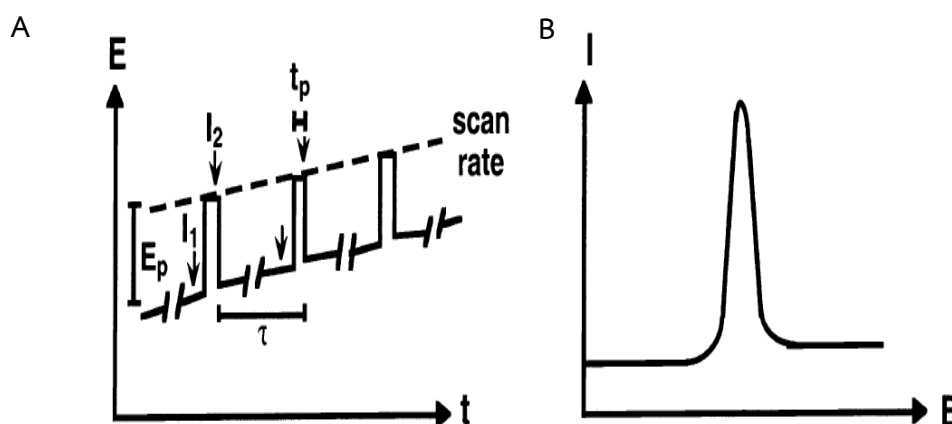


Figure 1.5 Applied potential-time wave form for differential pulse voltammetry (A) along with differential pulse voltammogram (B).

2.2 Nanomaterial

Nanomaterial is substance which having one dimension is less than proximately 100 nanometers. Due to its small size in nanoscale, the nanomaterials have a greater specific surface area to volume ratio than others form making a good chemical reactivity, greater electrical property and affecting their strength. The nanomaterials are divided into several types depending on its structures. The structures of nanomaterials are single form aggregated form or agglomerated form with spherical or tubular. The nanomaterials are become a material of interest in recent year and extensively used in diverse fields including anti-wrinkle, cosmetics, sunscreens, textile, electronics, paints, corrosion, drug delivery and electrochemical analysis etc.

2.2.1 Nanomaterial for modification of electrode surface

For electrochemical sensor, the nanomaterials are used to modify electrode surface because of the high specific surface area, electrocatalytic activity, good electrical conductivity, and good physical property. So, this study focuses on carbon-based nanomaterial and metallic nanomaterial.

2.2.2 Carbon based nanomaterial

Carbon nanomaterials are one of the most promising materials for various applications. It consists of wholly conjugated π electron system. The important carbon based nanomaterials are fullerene, carbon nanotube, carbon nanofiber and graphene. The chemical and physical properties depend on each structure.

Fullerene is the first carbon-based nanomaterial that serendipitously discovered in 1985. In addition, the discovery of fullerene is the beginning of an age to synthesis of other carbon allotropes. The structure of fullerene is a hollow ball made of sp^2 hybridized carbon atom. There are many types of fullerene such as C_{24} , C_{28} , C_{32} , C_{50} , C_{60} and C_{70} (Figure 2.1). Among fullerenes, C_{60} is the smallest stable and very prominent which composes of 60 hybridized carbon atoms form with 12 pentagons and 20 hexagons [5]. Fullerenes have several properties such as non-toxic and soluble in many organic solvent besides the functionalized fullerene increases the solubility of fullerene.

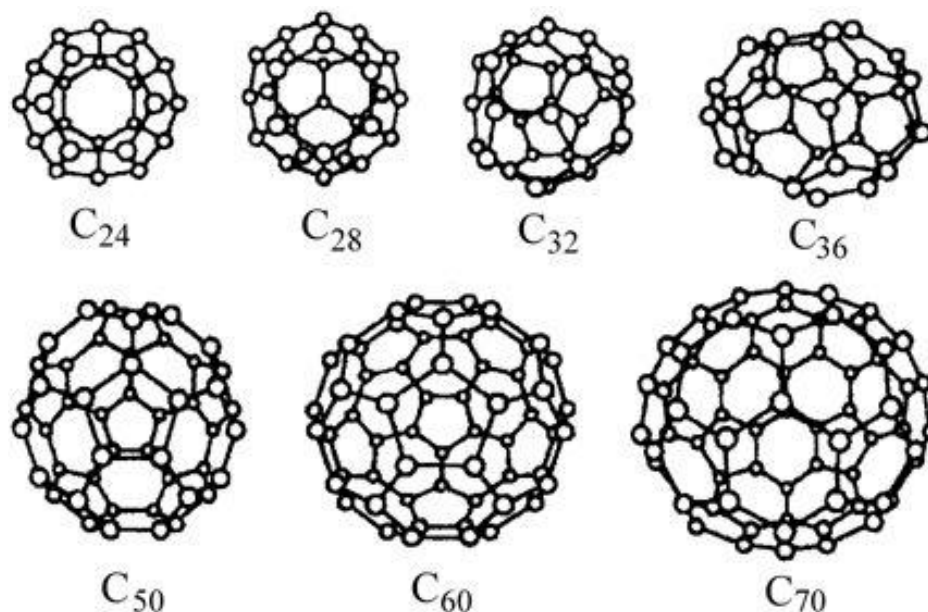


Figure 2.1 The structure of various formulas of fullerenes.

Carbon nanotubes (CNTs) are a rolled up graphene to tubular structure which made of hexagonal ring of carbon (Figure 2.2). The carbon nanotubes are classified as single wall carbon nanotube (SWCNT) and multiwall carbon nanotube (MWCNT). Due to the roll direction, CNT is a semiconductor or conductor. The CNTs provide extraordinary electrical, mechanical and physical properties. It was often used to facilitate the electron transfer between electrode and analytes that increasing the sensitivity and limit of detection. So the CNTs have become an attractive much attention in the scientific research in many application especially electrochemical sensor.

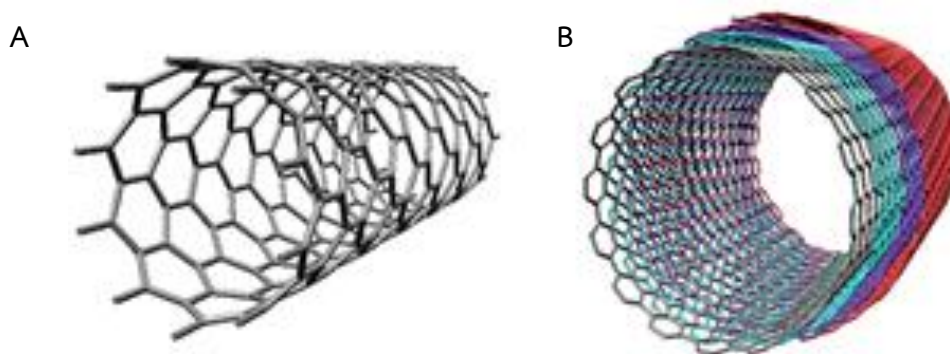


Figure 2.2 Structure of single walled carbon nanotube (A) and multi walled carbon nanotubes (B).

Tsierkezos, N et al. [27] developed the nitrogen doped carbon nanotube decorated with metallic nanoparticle including rhodium, palladium, iridium, platinum and gold. The modified electrodes were used for simultaneous determination of ascorbic (AA), dopamine (DA) and uric acid (UA). According to the limit of detection, the results showed that LOD of simultaneous AA, DA and UA tend to decrease with an order of N-MWCNTs, N-MWCNTs/RhNPs, N-MWCNTs/PdNPs, N-MWCNTs/IrNPs, N-MWCNTs/PtNPs and N-MWCNTs/AuNPs, respectively.

Buleandra, M et al. [28] used multi-walled carbon nanotube modified screen-printed electrode (MWCNTs-SPCE) for the determination of kojic acid (KA). On the MWCNTs-SPCE, the oxidation peak of kojic acid was observed at potential of 0.791 V. the linearity and LOD were found to be 0.02-5 mM and 0.016 mM, respectively. In addition, this system was successfully applied for determination of kojic acid in vinegar samples.

Graphene is a new allotrope of carbon based nanomaterial. Graphene is a two-dimensional material consisting of carbon atom in hexagonal configuration which bonded by sp^2 bond (Figure 2.3). This configuration is a reason for remarkable properties of graphene including:

- charge carrier mobility; graphene has a high charge carrier mobility ranging from 10,000 – 200,000 VS/cm^2 . So graphene is good candidate for electronic devices.

- Strength property; graphene is one of the strongest materials. It has a breaking strength 200 times greater than steel. The breaking strength of graphene is 42 N/m.

- Electrical conductivity; the conductivity of 2D graphene sheet is calculated by $\sigma = en\mu$. The mobility is theoretically limited to $\mu = 200,000 \text{ cm}^2/\text{Vs}$ by acoustic phonon at carrier density (n) = 10^{12} cm^2 . The resistance of 2D sheet is 31 Ω .

Thermal conductivity; the thermal conductivity of graphene is found to be 5,000 W/mK. The thermal conductivity of copper is 401 W/mK. So, the thermal conductivity of graphene is greater than copper for 10 times.

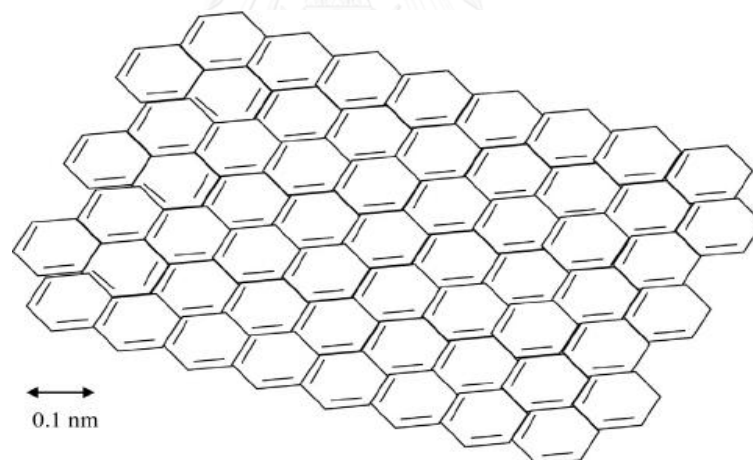


Figure 2.3 The chemical structure of graphene.

Due to outstanding properties, graphene has become an attractive candidate for sensor application. There are many publication reported the useful of graphene. However, using pure of graphene has a some problem because graphene has a strong Van der waals interaction with a neighbor sheet making a self-agglomeration and re-stacking causing to lose a electrochemical sensitivity. Thus, many researchers try to develop the

soluble graphene or using some materials to intercalate the graphene sheets. Graphene oxide, one of most important graphene derivatives has become an alternative material. Graphene oxide has similar structure to graphene but graphene oxide was functionalized by several oxygen groups such as hydroxyl, carboxyl and epoxy groups resulting in possess of both hydrophobic and hydrophilic properties (Figure 2.4). The hydrophilic groups make GO disperse well in water. However, Graphene oxide is generally categorized as an insulating material resulting from disruption of oxygen groups. So, the graphene oxide has to reduce into graphene form before used to modified electrode. There are many ways to reduce graphene oxide. Initially, graphene was reduced by chemical reduction using hydrazine or NaBH_4 . But this method provides a low yield product, contaminated with reducing agent and using hydrazine is harmful to environment. Another way to reduce graphene oxide is thermal reduction. This method requires high temperature up to 500°C to reduce graphene oxide. This way gives a graphene with high surface area but using high temperature may damage the structure of graphene sheets. The other method is electrochemical reduction. This method uses the electrochemical technique that provides a high quality and high purity. Besides, this method is done without harmful chemicals.

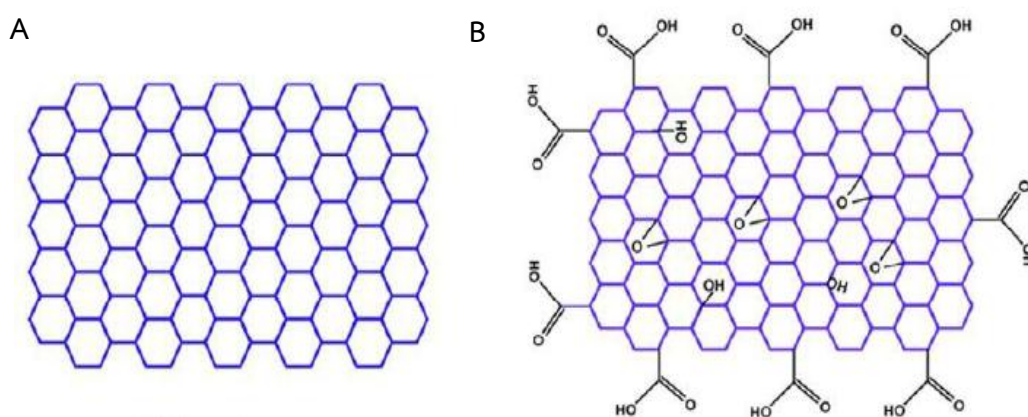


Figure 2.4 Structure of graphene (A) and graphene oxide (B).

Zhang, X et al. [29] developed a highly selective electrochemical sensor based on 3D reduced graphene oxide for chloramphenicol detection. This system showed high sensitivity, excellent anti-interferences ability and good reproducibility. In addition, this sensor exhibited a remarkable performance with a detection limit of 0.15 μM and linearity range from 1-113 μM .

2.2.3 Metallic nanoparticle

In the last few years, reported researches on metallic nanoparticles for electrochemical analysis have published numerous because of their electrocatalytic activity. The preparation of metallic nanoparticle is divided into 2 main processes, namely physical and chemical methods. There are many metallic nanoparticles used for electrode surface modification toward chemical sensors such as gold, platinum, palladium, silver and rhodium. The properties of each metallic nanoparticle depend on size, shape and stabilizing agents controlled by preparation process. Focusing on platinum nanoparticle, it facilitates electron transfer, catalyzes redox process and provides an excellent electrocatalytic activity for chemical sensor. It is used to modify electrode for both enzymatic sensors and non-enzymatic sensors.

Cui, Z and co-workers [30] developed electrochemical immunosensors for multiplexed determination based on mesoporous nanoparticles as a non-enzymatic label. They synthesized mesoporous platinum nanoparticle by ultrasonic method. The mesoporous platinum is a new kind of nanoparticle which has a high conductivity and electrocatalytic activity. This modified sensor showed a low limit of detection and processed excellent clinical value in cancer screening.

Heli, H et al. [31] fabricated the highly sensitive non-enzymatic sensor based on platinum nanoflowers for hydrogen peroxide detection. The platinum hierarchical nanoflowers were produced by sonoelectrodeposition

method. The catalytic activity and sensitivity of modified electrode for hydrogen peroxide were excellent without deterioration. The morphology of platinum nanoflowers was shown in Figure 2.5. This system is simple preparation and provided high sensitivity and excellent electrocatalytic activity.

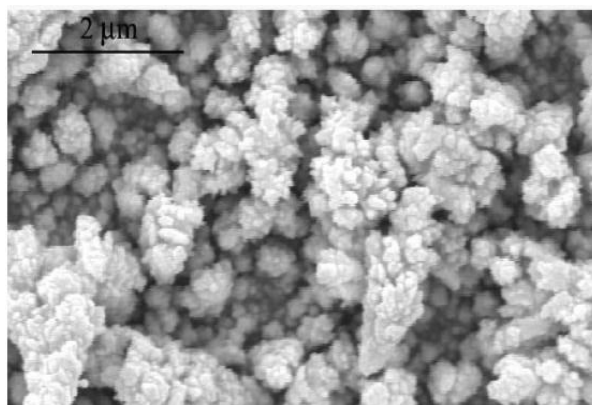


Figure 2.5 SEM images of the sonoelectrodeposited platinum surface.

Guo, M.Q. and co-workers [32] fabricated platinum nanoflowers on Au electrode by electrodeposition method. The morphology of platinum nanoflowers is uniform (Figure 2.6). The platinum modified electrode exhibited a high electrocatalytic activity for glucose determination with LOD of 48 μM .

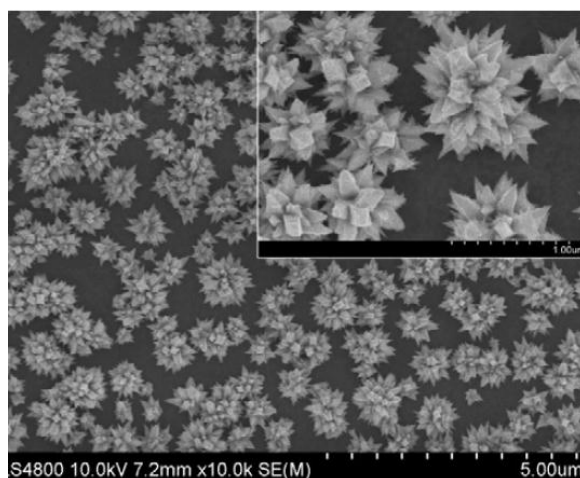


Figure 2.6 SEM image of platinum nanoflowers deposited on electrode.

Moreover, many researchers are interested in nanocomposites of carbon based nanomaterials and platinum nanoparticles because of the outstanding electrochemical property of carbon based nanomaterial and excellent electrocatalytic activity of platinum toward electroactive species. There are previous reports on the use of carbon and platinum nanocomposites;

Tajabadi, M. T. and co-workers [33] developed a sensitive non-enzymatic biosensor for hydrogen peroxide by electrodeposited platinum on nitrogen doped graphene. The electroanalytical results showed a high sensitivity, stability of the electrode for hydrogen peroxide because of the synergistic effect of platinum and nitrogen doped graphene.

Kalambate, P.K. and co-workers [34] fabricated the nanocomposites of multi-walled carbon nanotube/platinum nanoparticles modified carbon electrode for simultaneous detection of paracetamol, cetirizine and phenylephrine. The results found that using MWCNT/PtNPs can increase in sensitivity and improves the separation of overlap shape peak of those analytes. This system has been successfully applied for detection of paracetamol, cetirizine and phenylephrine in commercial pharmaceutical formulations, human blood serum and urine samples.

Chang, H.W. and co-workers [35] fabricated the non-enzymatic glucose biosensor by multi-walled carbon nanotube supported platinum. The platinum/multi-walled carbon nanotube modified electrode exhibited an excellent electrocatalytic activity for direct electrochemical oxidation of glucose in alkaline solution. This proposed system displays a good ability of anti-interferences (acetaminophen, uric acid and ascorbic acid).

2.3 Screen printed technique

Nowadays, miniaturization is an essential method for the development of analytical devices. Focusing on the electrochemical sensors, the conventional electrode system is large and requires large amount of volume

of analyte. So, the conventional electrode is miniaturized into small size via screen-printed technique. The screen-printed technique was employed for fabricating the disposable electrochemical sensors due to a high-volume production with economic, high reproducibility and practical feasibility. It can fabricate by printing an ink on different substrate like ceramic and many types of plastics. For the screen printing technique, the woven mesh of screen located cover the substrate with an ink on it. Then, the squeegee is moved across the screen stencil. While the squeegee is moved, it pushes the ink into the open area of stencil. After that, the surplus ink on screen mesh is removed. Then, the printed substrate needs to be dried. This method is shown in

Figure 2.7

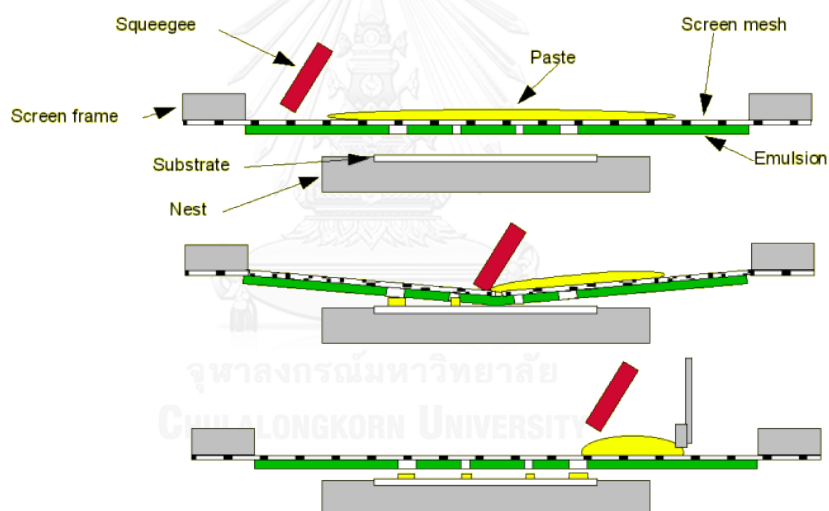


Figure 2.7 Schematic of screen-printing method for fabricating electrochemical sensors.

For the screen-printed electrode, the different inks are performed. Working electrode is commonly fabricated by carbon ink or modification of carbon i.e. graphene, carbon nanotube etc. In addition, silver/silver chloride is often used for fabricating conductive tracks. Thanks to its simplicity, cheap cost, high volume of production, small size and disposable use, the screen-

printed electrochemical sensor has mostly used for pharmaceutical analysis, drug control, clinical and environmental analysis.

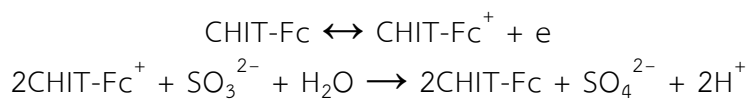
2.4 Target analytes

2.4.1 Sulfite

Sulfite is commonly used as a broad-spectrum preservative and antioxidant in food and beverage industries for preventing oxidation, inhibiting bacterial growth and controlling enzymatic reaction during production and storage. Despite many advantages, the content of sulfite in both food and beverage should be strictly limited due to its potential toxicity and harmful effect on hypersensitive people. The United States Food and Drug administration (USFDA) has required labeling on food containing more than 10 mg/kg of sulfite or beverage containing more than 10 mg/L of sulfite. Hence, it is a crucial to develop the rapid and sensitive sensors that have high accuracy and precision for the determination of sulfite in those products in order to control the product quality.

Garcia, T. and co-workers [36] developed sulfite sensor using iron hexacyanoferrate film modified electrode. The modified electrode showed a reversible response ascribed to a redox response of iron atom on modified electrode. This modified showed an electrocatalytic activity toward sulfite and given a LOD of 80 μM with $R^2 = 0.999$. In addition, this system is successfully used for determination of sulfite in wine samples validated by iodometric method.

Zhou, H and his colleagues [37] fabricated sulfite sensor based on multi-walled carbon nanotube/ferrocene branched chitosan (MTCNT/CHIT-Fc). The results exhibited a good electrocatalytic activity toward oxidation of sulfite. The results obtained from cyclic voltammetry showed that ferrocene-branched chitosan provided an electrocatalytic activity for sulfite detection. The catalytic process could be express below;



2.4.2 Diclofenac

Diclofenac (DCF) is a non-steroidal anti-inflammatory drug (NSAID) used to reduce inflammatory and to relieve pain from acute injury or chronic disease such as arthritis. DCF is widely used throughout the world for human as well as veterinary purposes. Although DCF has a great advantages and extensive use, it has been found that it is harmful effects on many organisms such as increase in blood pressure, stroke and extensive hepatic metabolism. Furthermore, the ubiquity of DCF in the environment impairs the fish health and water quality because DCF is poor degradation and cannot be removed completely. Hence, the development of a sensitive, simple, rapid, precise and inexpensive sensor for determination of DCF in pharmaceutical preparation and clinical application is very necessary. There are several analytical methods reported for determination of DCF such as chromatography, liquid chromatography, capillary electrophoresis, spectrophotometry and gas chromatography-mass spectrometry. In general, these methods have long time analysis, expensive cost and sophisticated instruments. Conversely, electrochemical technique is rapid, simple, and cheap. This is an alternative tool for determination of diclofenac.

Goodarzian and co-worker [38] developed electrochemical sensor for diclofenac detection using ionic liquid multi-walled carbon nanotubes. This proposed system showed a great improvement for electrode process of diclofenac comparing with conventional carbon paste electrode. This sensor provides limit of detection of 0.09 μM with linear range of 0.3-750 μM .

CHAPTER III

EXPERIMENTAL

For this chapter, it gives the information of chemicals, materials, instrument, prepared procedures, electrochemical detection and sample preparation.

3.1 Chemicals and reagents

- Chloroplatinic acid (Sigma Aldrich, St. Louis, MO)
- Diclofenac (Merck, Darmstadi, Germany)
- Disodium hydrogen phosphate (Na_2HPO_4) (Sigma Aldrich, St. Louis, MO)
- Graphene oxide (GO) (graphene supermarket, NY, USA)
- Graphene (G) powder (skyspring nanomaterials, Inc., Houston, TX, USA)
- Polyvinylpyrrolidone (PVP; MW = 10,000) (Sigma Aldrich, St. Louis, MO, USA)
- Potassium ferricyanide ($\text{K}_3[\text{Fe}(\text{CN})_6]$) (Sigma Aldrich, St. Louis, MO, USA)
- Potassium ferrocyanide ($\text{K}_4[\text{Fe}(\text{CN})_6]$) (Sigma Aldrich, St. Louis, MO, USA)
- Potassium hydrogen phosphate (KH_2PO_4) (Sigma Aldrich, St. Louis, MO, USA)
- Sodium sulfite (Sigma Aldrich, St. Louis, MO, USA)
- Carbon ink (Gwen group, Torfaen, UK)
- Carbon graphene ink (Gwen group, Torfaen, UK)
- Silver/silver chloride ink (Gwen group, Torfaen, UK)

3.2 Apparatus

- CHI 1240B electrochemical analyzer (CH instruments, Inc., USA)
- Scanning electrode microscope (SEM) (JSM-6400; Japan Electron Optic Laboratory Co., Ltd., Japan)
- Scanning electron microscope (SU3500; Hitachi Co., Ltd., Japan)
- Transmission electron microscope (TEM) (JEM-2100; Japan Electron Optic

Laboratory Co., Ltd., Japan)

- Screen frame (Chaiyaboon Co., Bangkok Thailand)

3.3 Fabrication of electrode

The screen-printed carbon electrode patterns were designed by Adobe Illustrator. The designed electrode is 4 cm long and 2 cm high. The diameter of working is 4 mm. the reference electrode and conductive is silver/silver chloride. The working electrode and counter electrode is carbon. The designed electrode configuration is shown in Figure 3.1.

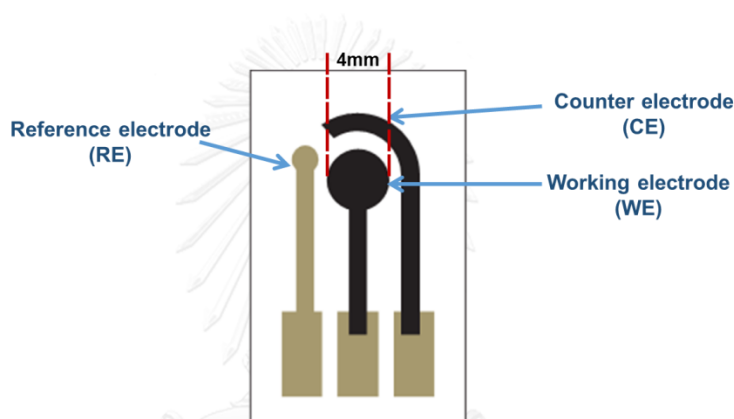


Figure 3.1 Three electrode configuration.

The three electrodes were fabricated by an in-house screen printing technique. The method is described briefly (Figure 3.2). Firstly, the silver/silver chloride ink was screened on polyvinylchloride (PVC) substrate as the reference electrode (RE) and conductive pads. Secondly, the screened PVC substrate was heated up at 50 °C for 1 hour in an oven. Thirdly, the carbon ink was screened on the same PVC substrate as working electrode (WE) and counter electrode (CE). Finally, the screened PVC was heated up 50°C for 1 hour in an oven and 48 electrodes are given for one PVC.

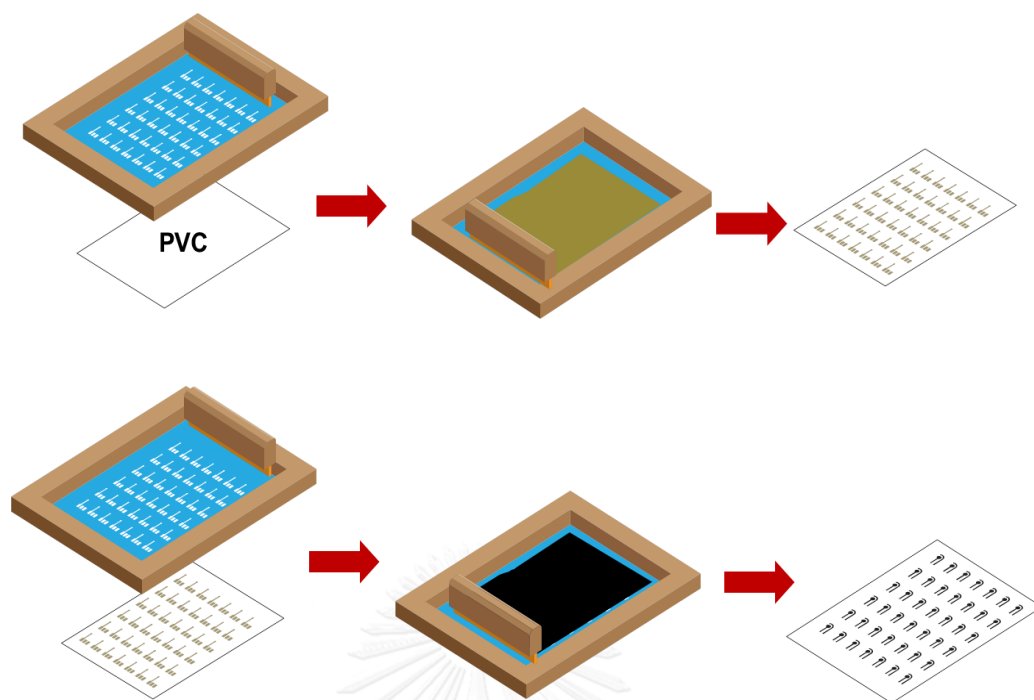


Figure 3.2 Scheme of in-house screen-printing electrode process.

3.4 Electrode modification for sulfite detection

3.4.1 Preparation of stock solution

All solutions were prepared using Milli-Q water (18.2 M Ω cm).

3.4.1.1 Preparation of phosphate buffer solution (PB) pH 7.0

Disodium hydrogen phosphate (2.0833 g) and potassium dihydrogen phosphate (1.4051 g) were dissolved in 250 mL of Milli-Q water. The pH of prepared solution was adjusted to 7.0 by using 0.1 M sodium hydroxide or 0.1 M hydrochloric acid. The PB solution was used as supporting electrolyte.

3.4.1.2 Preparation of 0.5 M potassium chloride

9.31 g of KCl was dissolved in Milli-Q water.

3.4.1.3 Preparation of 1 mM ferri/ferrocyanide

16.46 mg ferricyanide and 21.12 mg ferrocyanide were dissolved in 100 mL of 0.5 M KCl. Then the prepared solution was diluted 5 times in KCl.

3.4.1.4 Preparation of graphene/polyvinylpyrrolidone solution

To prepare the graphene/polyvinylpyrrolidone, 5 mg of graphene was dispersed in 5 ml of ethanol containing 1.5 mg of PVP as a stabilizer. The as-prepared mixture was sonicated for 3 hours at room temperature the as-prepared solution denoted as G/PVP solution and the G/PVP solution was kept for electrode modification.

3.4.1.5 Preparation of sulfite

1 mM of sulfite was prepared by dissolving x mg of sulfite in phosphate solution pH 7.0.

3.4.2 Modification of electrode surface

To modify electrode surface, the as-prepared graphene/polyvinylpyrrolidone (1 μ L) was dropped on the working electrode surface. The electrode was heated at 50 °C for 3 minutes, denoted as G/PVP modified electrode.

3.4.3 Optimization of electrode modification

The important factors effecting on electrochemical detection for sulfite detection including graphene concentration and polyvinylpyrrolidone concentration were studied.

3.4.3.1 Effect of graphene concentration on modified electrode

The effect of graphene loading was studied by varying graphene concentration (0.5, 1.0, 1.5, 2.0 and 2.5 mg/ml). The modified electrode was used for sulfite detection.

3.4.3.2 Effect of polyvinylpyrrolidone on modified electrode

The effect of polyvinylpyrrolidone was investigated by varying the percentage of polyvinylpyrrolidone in range of 0.01-0.05%

3.4.4 Characterization of modified electrode

3.4.4.1 Surface morphology characterization

The surface morphology of unmodified electrode, and G/PVP modified electrode were characterized by scanning electron microscopy (SEM). Furthermore, the G/PVP nanocomposite was characterized by transmission electron microscopy (TEM).

3.4.4.2 Electrochemical characterization

To control all electrochemical measurements, a potentiostat (CHI 1240B, CH Instruments, Inc., USA) was used. The electrochemical behavior of the modified electrode was investigated by cyclic voltammetry (CV) using standard ferri/ferrocyanide in KCl and the potential was scanned from -0.5-1.0 V. For sulfite detection, cyclic voltammetry was performed in a potential range of 0-1.0 V at a scan rate 50 mV/s

3.5 Electrode modification using simultaneously reduced graphene oxide/platinum for diclofenac detection

3.5.1 Preparation of stock solution

All solutions were prepared using Milli-Q water (18.2 M Ω cm).

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3.5.1.4 Preparation of graphene/platinum solution

2 mg ml⁻¹ of GO was dispersed in PB solution and then 0.2 mM of H₂PtCl₆ was added at ratio of 1:1 to GO solution. After that, the as-prepared solution was physically mixed by ultra-sonication for 2 hours. The homogenous suspension of GO and Pt was obtained.

3.5.1.5 Preparation of diclofenac

1 mM of diclofenac was freshly prepared by dissolving x mg of diclofenac in 10 mL of 0.1 M phosphate buffer solution (pH 7.0).

3.5.2 Modification of electrode surface

100 µL of as-prepared GO/Pt mixture solution was dropped cover on screen printed electrode. After that, the simultaneous reduction of graphene oxide and Pt was carried out by cyclic voltammetry in a potential range of -1.5–0.3 V. (vs Ag/AgCl) at the scan rate of 50 mV/s for 5 cycles. The electrochemically reduced graphene oxide/platinum modified electrode was obtained for further experiment.

3.5.3 Optimization of electrode modification

For electrode surface modification by electrochemically reduced GO/Pt, the factors affecting the performance of RGO/Pt modified electrode were investigated and optimized using cyclic voltammetry (CV) and the potential was scanned from -0.5-1.0 V for standard ferri/ferrocyanide and 0-0.6V for diclofenac.

3.5.3.1 Effect of grapene oxide concentration

To study the effect of GO, the amount of GO in GO/Pt solution was investigated within the concentrations of 0.5, 1.0, 2.0, 3.0 4.0 mg ml⁻¹. After that, the simultaneous reduction of graphene oxide and Pt was carried out by cyclic voltammetry in a potential range of -1.5–0.3 V. (vs Ag/AgCl) at the scan rate of 50 mV/s for 5 cycles.

3.5.3.2 Effect of chloroplatinic concentration

The concentration of H₂PtCl₆ in GO/Pt solution used to modified electrode surface was investigated in the range from 0.05 to 0.4 mM. After that, the simultaneous reduction of graphene oxide and Pt was carried out by cyclic voltammetry in a potential range of -1.5 – 0.3 V. (vs Ag/AgCl) at the scan rate of 50 mV/s for 5 cycles.

3.5.3.3 Effect of number of scan

The effect of number of scan was studied by cyclic voltammetry with various number of scan (1, 3, 5, 7 and 9 cycles) with scan rate of 50 mV/s.

3.5.3.4 Effect of scan rate

The effect of scan rate was studied by cyclic voltammetry with various scan rates from 50-200 mV/s for 5 cycles.

3.5.4 Characterization of modified electrode

3.5.4.1 Surface morphology characterization

The surface morphologies of unmodified and modified electrode were studied by scanning electron microscopy (SEM).

3.5.4.2 Electrochemical characterization

For electrochemical characterization, the cyclic voltammetry was performed using 1 mM standard ferri/ferrocyanide in 0.1 M KCl solution. The potentials scanned from -0.5-1.0 V with scan rate 100 mV/s for 2 cycles. Furthermore, Differential pulse voltammetry (DPV) was employed for determination of diclofenac. The potentials were scanned from 0-0.6 V.

3.5.4.3 Optimization of parameters of differential pulse voltammetry

To obtain a highest chemical sensitivity of this electrochemical system for diclofenac, the parameters of differential pulse voltammetry including step potential and amplitude were optimized using 100 μ M of DCF in 0.1 M PB solution (pH 7.0) on RGO/Pt electrode. The optimum condition was chosen from high current intensity compromise with sharp peak. For step potential investigation, the differential pulse voltammetry was performed using 100 μ M diclofenac with various step potential in range of 4-20 mV. The Amplitude was also varied in the range of 20-120 mV.

3.6 Analytical performances of GO/Pt modified electrode

3.6.1 Calibration plot

The various concentrations of diclofenac were prepared by diluting 1 mM of diclofenac in phosphate buffer (pH 7.0). The differential pulse voltammetry was performed with optimum condition. Then, current

responses were plotted versus the concentrations of diclofenac to obtain the linearity for diclofenac detection.

3.6.2 Limit of detection (LOD)

The limit of detection was obtained by calculating from the formula of $LOD = 3S_b/m$ where S_b and m are a standard deviation of the blank and a slope of the calibration curve, respectively.

3.6.3 Stability and reproducibility

The stability and reproducibility of the modified electrode were evaluated by measuring the current response of fixed concentration of DCF at 50 μ M in PB solution (pH 7.0). To investigate the stability of modified electrode, the modified electrodes were kept in desiccator for a week. The reproducibility of modified electrode also investigated by measuring on five different modified electrodes prepared under the same conditions. The percentage of relative standard deviation (%RSD) was calculated by formula below;

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

3.6.4 Interference

The selectivity of modified electrode was investigated by determination of 50 μ M DCF in the presence of different concentrations of interferences. The change in peak current of DCF before and after adding different interferences were measured. Tolerance limit is defined as the maximum concentration of interferences that cause an error $\pm 5\%$ for DCF detection.

3.7 Real sample analysis

3.7.1 Real sample preparation

Human urine sample was collected from 10 healthy human volunteers who are male and female. After collection, the urine samples

were centrifuged at 1600 rpm for 20 minutes. Then the supernatant was kept for further experiment. A standard addition method was used to detect DCF in human urine samples. The standard solutions of DCF were added at 1:1 ratio to undiluted human urine samples. Then the samples were analyzed on RGO/Pt modified electrode using differential pulse voltammetry.

3.7.2 Recovery

For real sample analysis, the percentage of recovery was used to evaluate the applicability of proposed system by standard addition method. The %recovery was calculated by equation below;

$$\% \text{recovery} = \frac{\text{found concentration of spike sample}}{\text{known concentration of spike sample}} \times 100$$

CHAPTER IV

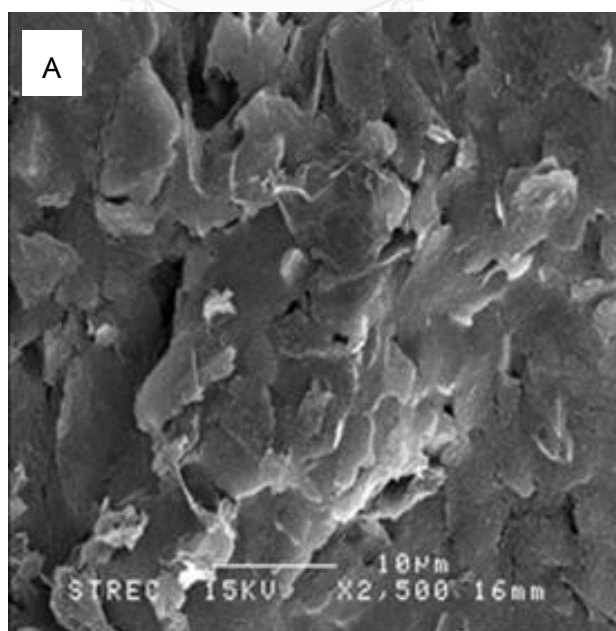
RESULTS AND DISCUSSION

For this chapter, the results including surface morphology characterization, optimization of electrode modification and the electrochemical performances of the developed sensor were discussed.

4.1 Electrode modification for sulfite detection

4.1.1 Surface morphology characterization

The surface morphology of G/PVP modified electrode was characterized by scanning electron microscopy (SEM) and the dispersion of graphene was also characterization by transmission electron microscopy (TEM). As shown in Figure 4.1A, an SEM image shows the presence of homogeneous graphene layer on the electrode surface. According to Figure 4.1B, a TEM image of G/PVP illustrates a well dispersion of graphene without severe agglomeration along with its electron diffraction pattern (Figure 4.1C), which is well matched with the previous report [39].



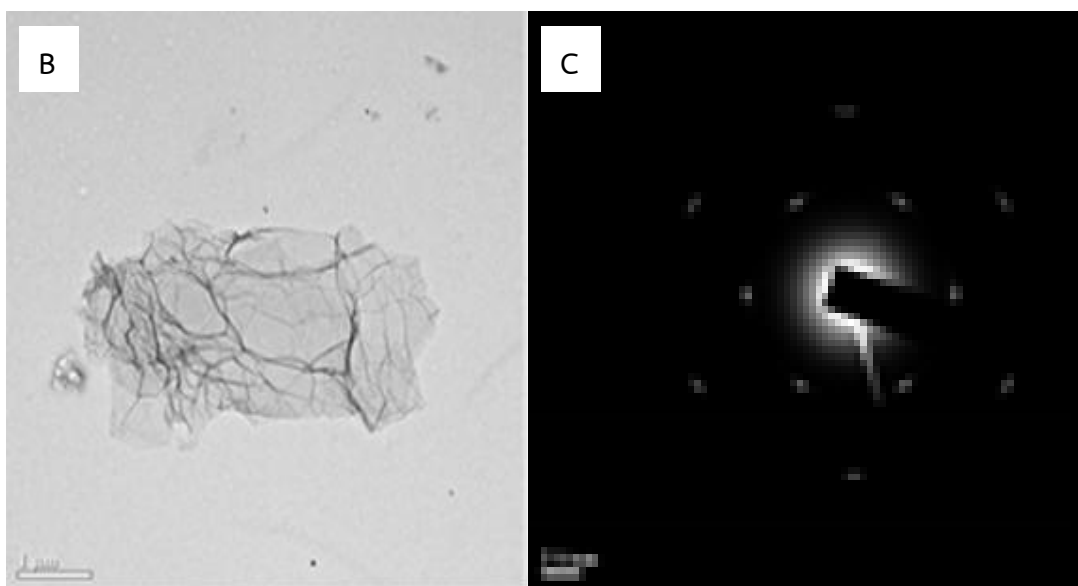


Figure 4.1 (A) an SEM image of G/PVP modified electrode (B) A TEM image of G/PVP and electron diffraction pattern of dispersed graphene (C).

4.1.2 Electrochemical characterization

Then the electrochemical performances of G/PVP modified electrodes were investigated by using cyclic voltammetry (CV), using 1 mM standard ferri/ferrocyanide redox couple.

As shown in Figure 4.2, the results exhibited the well-defined anodic and cathodic peaks of ferri/ferrocyanide. Comparing with an unmodified electrode (green line), graphene modified electrode (red line) showed the higher current response indicating that graphene can increase electrochemical sensitivity of the system. Moreover, when the results were compared with graphene modified electrode, G/PVP modified electrode (blue line) illustrated the higher current response verifying that PVP can improve the dispersion of graphene. This results showed the potentially high sensitivity of G/PVP modified electrode for electrochemical sensor.

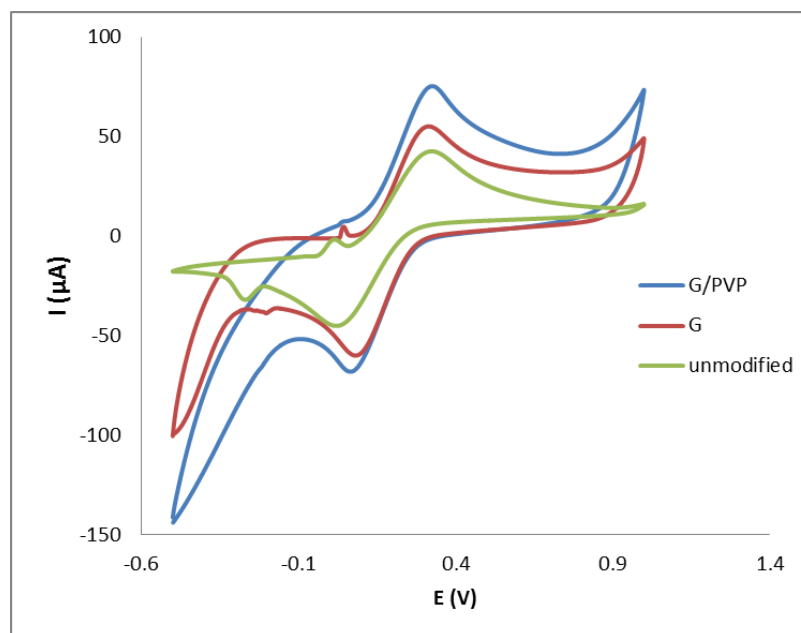


Figure 4. 2 Cyclic voltammograms of 1 mM ferri/ferrocyanide in 0.1 M KCl with scan rate of 100 mV/s measured on unmodified electrode, G modified electrode and G/PVP modified electrode.

4.1.3 Optimization of electrode modification

To get a sensitive electrochemical sensor for sulfite detection, the important parameters affecting the modified electrode were investigated. The optimized condition was chosen from the highest peak current and well-defined peak shape.

4.1.3.1 Effect of graphene loading

Initially, the effect of G loading was studied by using cyclic voltammetry measuring the current response of 2mM sulfite with a scan rate 100 mV/s. Graphene loading was varied in a range from 0.5-2.5 mg/ml. According to Figure 4.3, the current response increased when the graphene loading is increased from 0.5-1.0 mg/ml. then, the current response decreased after graphene loading is higher than 1.0 mg/ml. This is possibly caused by the self-agglomeration of high concentration of graphene. So, the result showed that 1 mg/ml graphene provides the highest current response

signal for sulfite detection and 1.0 mg/ml of graphene was used for all further experiments.

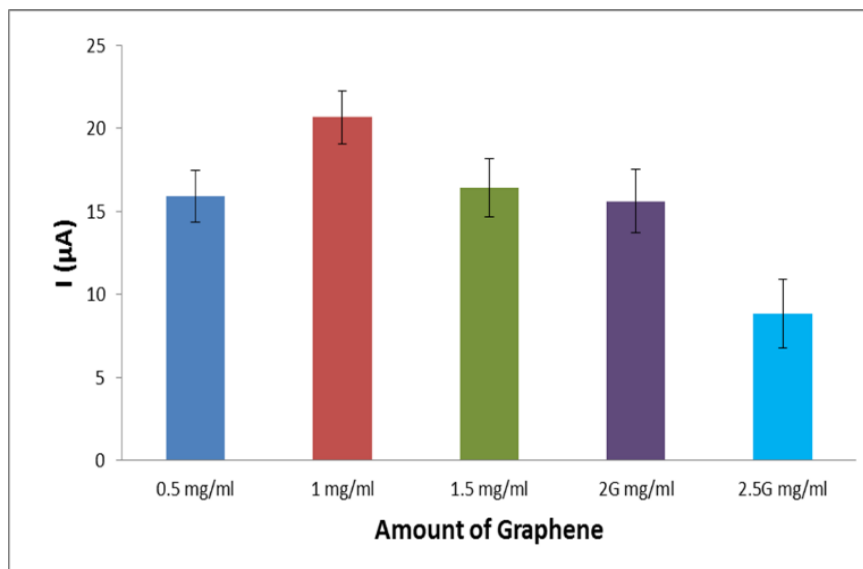


Figure 4.3 Histogram of current responses obtained from CVs in 2 mM sulfite with scan rate 100 mV/s measured on G/PVP modified electrode with varied graphene concentration.

4.1.3.2 Effect of percentage of polyvinylpyrrolidone loading

Then, the %PVP loading was varied from 0.01-0.05%. As shown in Figure 4.4, the increase of PVP from 0.01 to 0.03% is possibly caused the increase in current responses. Moreover, it showed that adding 0.03% of PVP provides the highest current response signal towards sulfite detection. Thus, 0.03% of PVP was an optimized for next experiments.

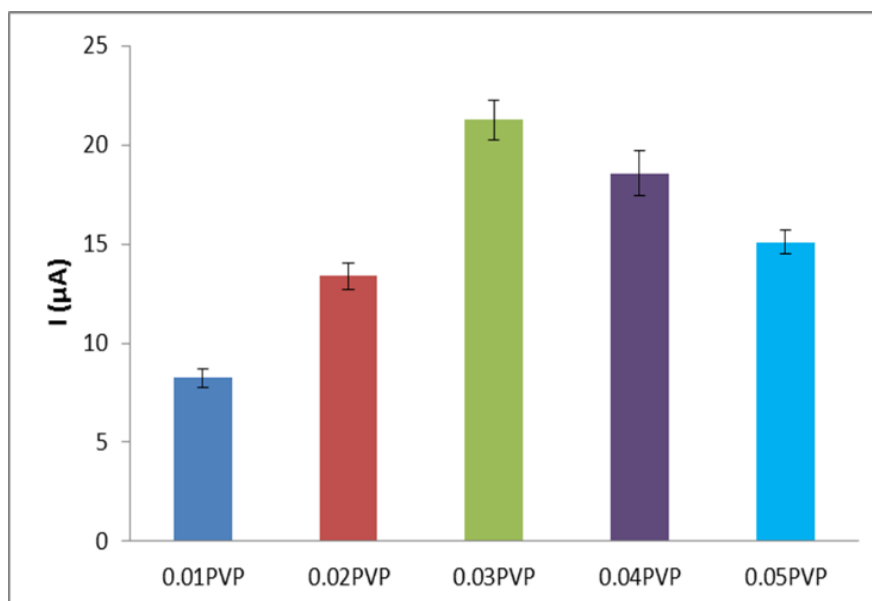


Figure 4.4 Histogram of current responses obtained from CVs in 2 mM sulfite with scan rate 100 mV/s measured on G/PVP modified electrode with varied %PVP.

4.1.4 Sulfite detection

For the electrochemical determination of sulfite, cyclic voltammetry was employed by using 2mM of sulfite with a scan rate 100 mV/s measured on different electrodes including unmodified electrode, graphene modified electrode and G/PVP modified electrode.

Figure 4.5 showed the cyclic voltammograms of 2 mM sulfite measured on different electrodes; unmodified electrode (red line), graphene modified electrode (blue line) and G/PVP modified electrode (green line). According to the cyclic voltammograms, there was no current response for sulfite on unmodified electrode, while graphene modified electrode displayed the oxidation peak of sulfite at a potential of +0.5 V. Among all, the peak current response obtained from G/PVP modified electrode showed the highest current response for sulfite indicating that adding PVP in this system can prevent the agglomeration of G sheet leading to increased electrochemical sensitivity of this system.

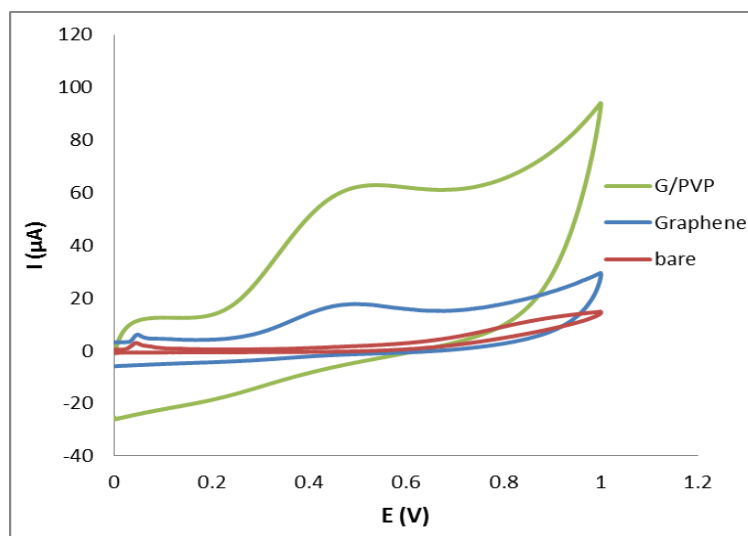


Figure 4.5 Cyclic voltammograms of 2.0 mM sulfite with scan rate of 100 mV/s measured on unmodified electrode (red line), graphene modified electrode (blue line) and G/PVP modified electrode (green line).

For the determination of sulfite, as shown in Figure 4.6, the current response for sulfite decreased when time consumption analysis increased. Because the sulfite is unstable, it can decompose leading to lose electrochemical response signal. This problem hinders the detection of sulfite. So the objective of this study is altered by focusing another analyte instead of sulfite. Afterwards, diclofenac is selected as a target analyte because it is commonly used throughout the world for anti-inflammatory disease and it is harmful for human organism such as hepatic metabolism.

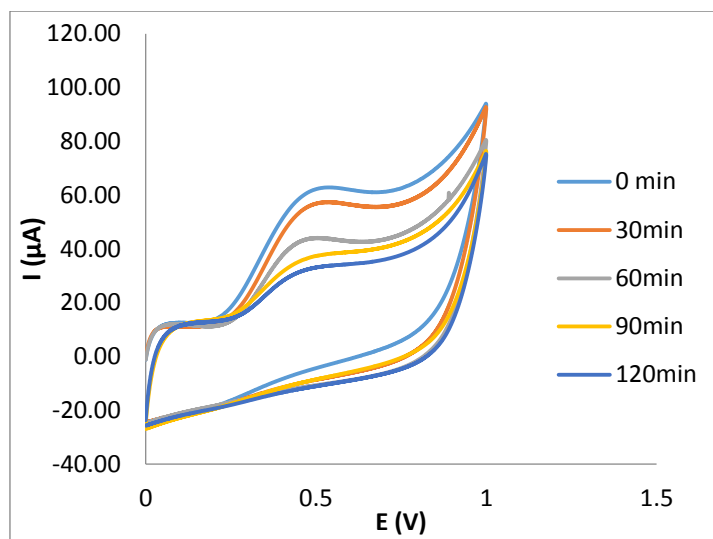


Figure 4.6 Cyclic voltammograms of 2.0 mM sulfite with scan rate of 100 mV/s measured by various time consumption for analysis (0-120 minutes).

4.2 Electrode modification using simultaneously reduced graphene oxide/platinum for diclofenac detection

4.2.1 Electrode characterization

4.2.1.1 Surface morphology characterization

To prove the successful of simultaneous electrochemical reduced GO/Pt on the working electrode surface, the morphology of both unmodified electrode and modified electrode were characterized by scanning electron microscopy (SEM). As shown in Figure 4.7A, this image showed a surface morphology of an unmodified electrode displaying a graphene flakes contaminated with carbon ink. Comparing with RGO/Pt modified electrode (Figure 4.7B), a SEM image showed a thin film of RGO decorated with platinum nanoparticles. According to Figure 4.7C, a SEM image of RGO/Pt modified electrode with 20000x magnification showed a flower-like morphology consisting of a bunch of Pt nanorods with the appearance of flower. Moreover, the results from energy dispersive X-ray spectroscopy (EDX) showed that the weight content of C, Pt and Cl is 87.65%, 11.55% and 0.80%, respectively and atomic content of C, Pt and Cl is 98.89, 0.80 and 0.31,

respectively

(Figure 4.7D). These results confirm that RGO/Pt was successfully deposited on electrode surface by in-situ one pot preparation of electrochemically reduced GO/pt.

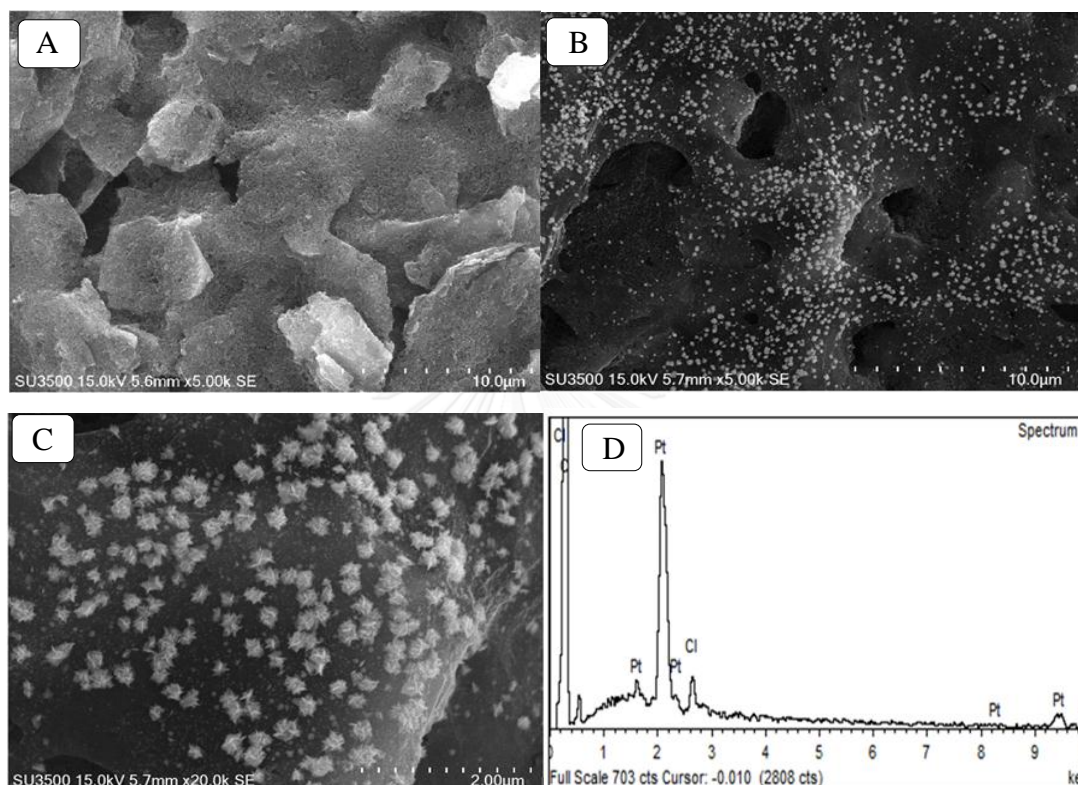


Figure 4.7 SEM images of unmodified electrode (A), RGO/PtNPs modified electrode (B), RGO/PtNPs modified electrode with 20000x magnification (C); EDX spectra of RGO/PtNPs modified electrode (D).

4.2.1.2 Electrochemical characterization of modified electrode

To investigate the electrode performance, cyclic voltammetry was employed by using 1 mM standard ferri/ferrocyanide redox couple in 0.1 M KCl solution. According to Figure 4.8, this figure displayed the cyclic voltammogram of 1 mM ferri/ferrocyanide measured on different electrodes including: unmodified electrode (red line), RGO modified

electrode (green line), Pt modified electrode (violet line) and RGO/Pt modified electrode (blue line). This cyclic voltammograms exhibited the well-defined peaks of anodic and cathodic current responses. As shown in Figure 4.8, the unmodified electrode provided anodic and cathodic peak of ferri/ferrocyanide with lowest current response signal and broad peak shape (red line). Comparing with unmodified electrode, both RGO modified electrode (green line) and Pt modified electrode (violet line) provided the higher current response signal. Those results indicated that RGO and Pt increase the electrochemical conductivity leading to high sensitivity of this sensing system. Then, RGO/Pt modified electrode showed the highest current response compared with other electrodes. This result can explain that synergistic effect of graphene and platinum provide a higher electrochemical sensitivity for this sensor.

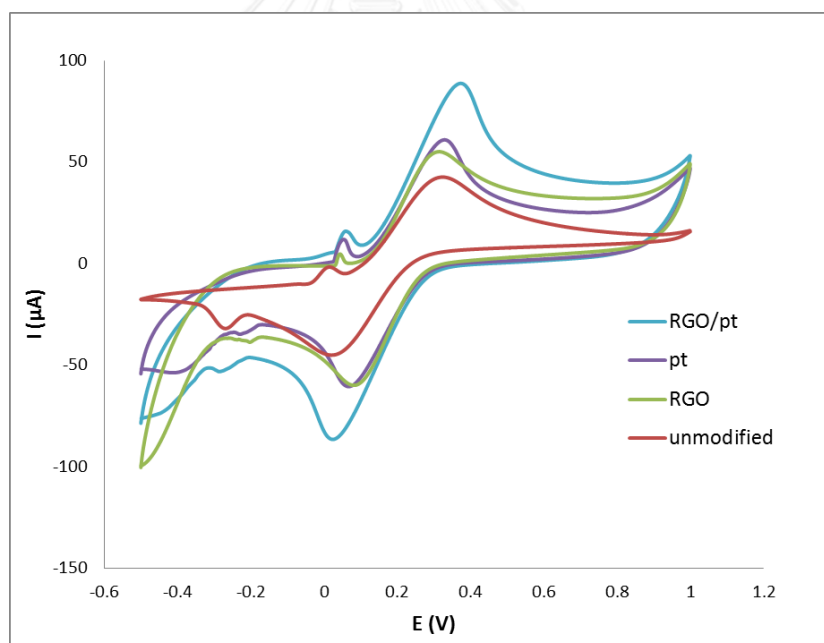


Figure 4.8 Cyclic voltammograms of 1 mM ferri/ferrocyanide in 0.1 M KCl on different electrodes; unmodified electrode (red line), RGO modified electrode (green line), Pt modified electrode (violet line) and RGO/PtNPs modified electrode (blue line).

The diffusion control of RGO/pt modified electrode was examined by cyclic voltammetry of 1 mM standard ferri/ferrocyanide with varied scan rate. Afterwards, the current responses were plotted as a function of square root of scan rates. According to Randle-Sevcik equation, diffusion controlled process, the linear relation between obtained currents and applied square root of scan rates was obtained. To study diffusion control of this proposed platform, cyclic voltammetry were scanned with different scan rates varied from 25-200 mV/s. measured on RGO/Pt modified electrode. The results (Figure 4.9) showed that anodic and cathodic peaks were directly proportional to a square root of scan rate and showed the linearity obtained from plotted current versus square root of scan rate.

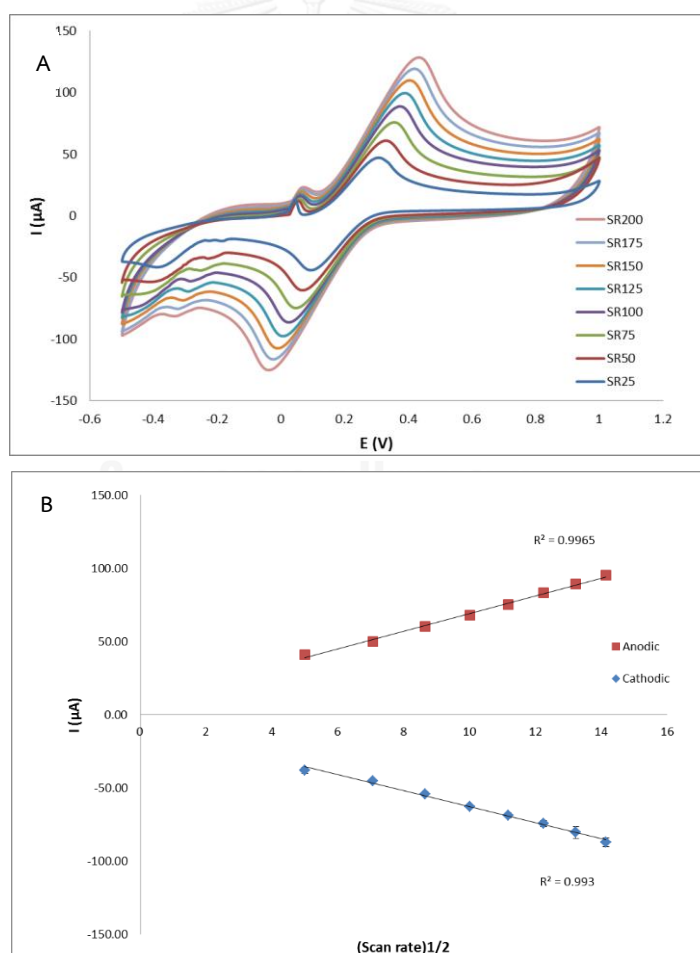


Figure 4.9 Cyclic voltammograms of 1 mM ferri/ferrocyanide in 0.1 M KCl solution measured on RGO/Pt modified electrode with different scan rates (A)

and a relation between anodic and cathodic current of ferri/ferrocyanide versus a function of square root of scan rates.

4.2.2 Optimization of electrode modification for diclofenac detection

For the electrode modification by electrochemically reduced GO/Pt, the factors affecting the performance of RGO/Pt modified electrode were investigated and optimized by cyclic voltammetry of 100 μ M. diclofenac in 0.1 M PB pH 7.0.

4.2.2.1 Effect of graphene oxide concentration

First of all, to study the effect of GO, the amount of GO in GO/Pt solution was investigated within a concentration range of 0.5, 1.0, 2.0, 3.0 4.0 mg/ml. According to Figure 4.10 A, the anodic current response was significantly increased when the concentration of GO increased from 0.5-1.0 mg/ml showing that GO could heighten the electrochemical sensitivity of the modified electrode. Obviously, adding 1.0 mg/ml of GO provides the highest anodic current response. However, the anodic current responses decreased when the amount of GO loading was in a range of 1.0 to 4.0 mg/ml due to a high based line of RGO at potential at 0.5 V. This may be caused by the self-agglomeration of RGO at the high concentration. Hence, 1.0 mg/ml of GO was selected as an optimum concentration.

4.2.2.2 Effect of chloroplatinic acid concentration

After that, the concentration of H_2PtCl_6 in GO/Pt solution used to modify the electrode surface was investigated in a range from 0.05 to 0.4 mM. As shown in Figure 4.10B, the anodic current response increased when the concentration of H_2PtCl_6 increased from 0.05 to 0.1 mM indicating that platinum deposited on the electrode can enhance the sensitivity of modified electrode. After that, the highest anodic current response was obtained at 0.1 mM of H_2PtCl_6 . Conversely, the current response was substantially decreased when the concentration of H_2PtCl_6 was higher than 0.1 mM since

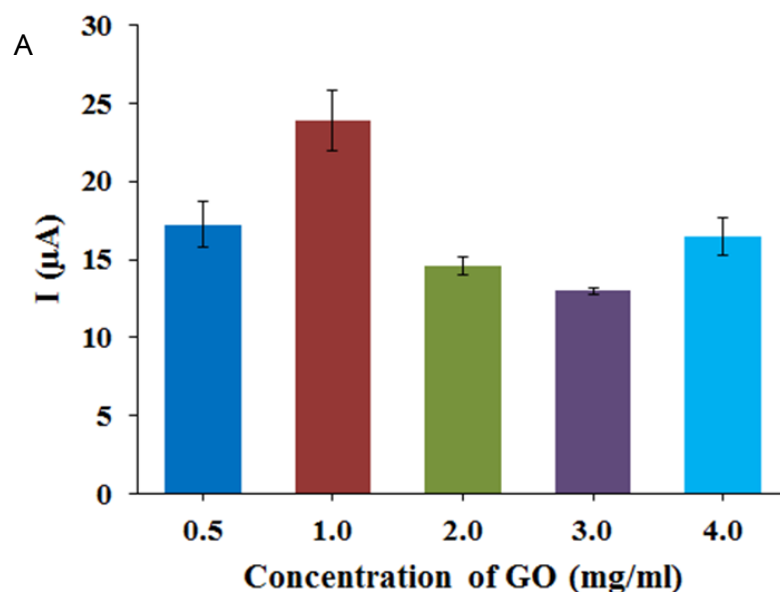
the platinum coated on the electrode was a bulk Pt instead of Pt nanoparticles. Thus, 0.1 mM of H_2PtCl_6 was selected as an optimum concentration in all further experiments.

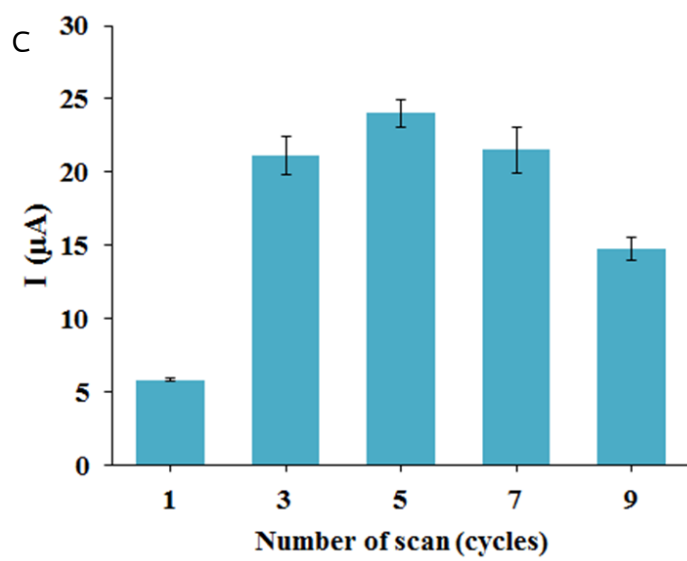
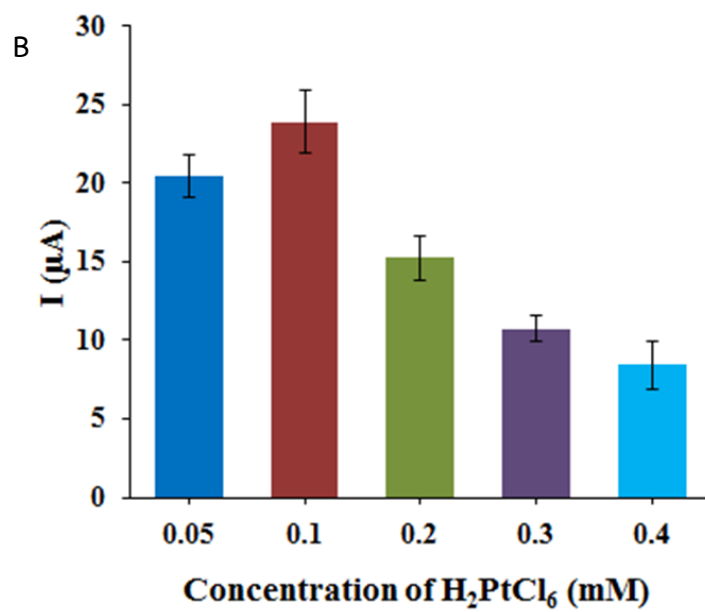
4.2.2.3 Effect of number of cycles

Next, an effect of number of scan for an electrochemical reduction process was also investigated. The number of scans was optimized by varying a number of scan in a range of 1-9 cycles. The results (Figure 4.10C) showed that 5 cycles of electrochemically reduced RGO/Pt provided the highest current response signal for diclofenac detection. Thus, 5 number of cycles was used for electrochemically reduced RGO/Pt for all further experiments.

4.2.2.4 Effect of number of scan rate

Finally, a scan rate of reducing process was scan in a range of 50-250 mV/s. The results exhibited that reducing GO/Pt with a scan rate of 50 mV/s provided the highest current response (Figure 4.10D). Therefore, a scan rate of 50 mV/s was selected for all further experiments.





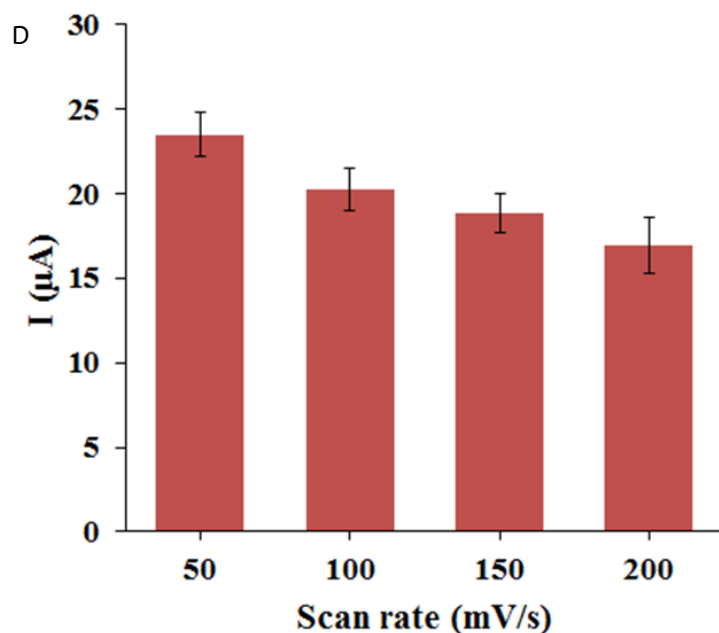
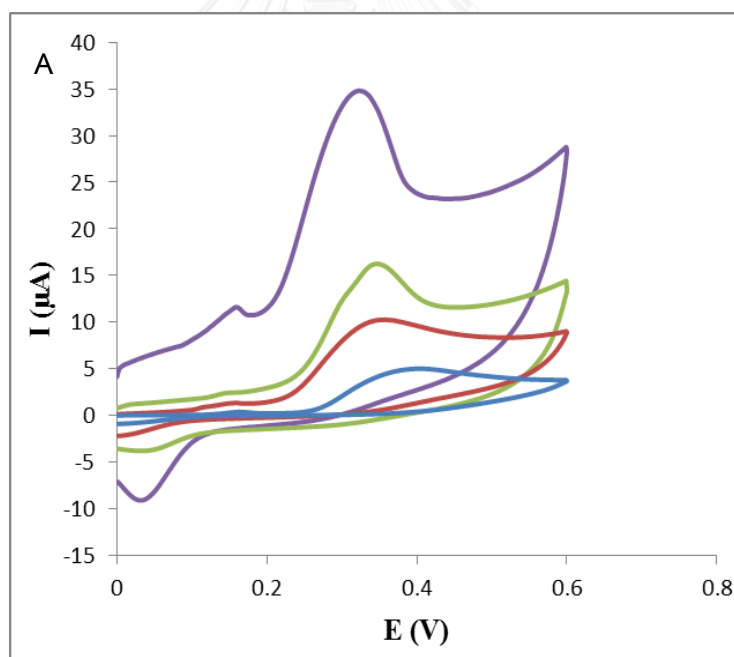


Figure 4.10 The effect of concentration of graphene oxide (A), concentration of H_2PtCl_6 (B), number of scan (C) and scan rate (D) using cyclic voltammetry of DCF 100 μM in 0.1 M PB solution pH 7.0 measured on RGO/Pt modified electrode.

4.2.3 Electrochemical behaviors of modified electrodes for diclofenac detection

To study the electrochemical performances of different electrodes for DCF detection, 100 μM of DCF in 0.1 M PB solution (pH 7.0) was performed by using cyclic voltammetry with a scan rate of 100 mV/s. As shown in Figure 4.11, the cyclic voltammograms showed the one oxidation peak current of DCF measured on different electrodes: unmodified electrode (blue line), Pt modified electrode (red line), G modified electrode (green line) and RGO/Pt modified electrode (violet line), respectively. All the peak currents were also integrated and plotted in to the bar graph as seen in Figure 4.11B. According to the cyclic voltammogram obtained from unmodified electrode, it showed a broad oxidation peak of DCF with a low current response. Comparing with an unmodified electrode, the current signal

obtained from platinum modified electrode was 1.5 times higher indicating the high electrocatalytic activity of Pt and a negative peak was observed when Pt were deposited on the electrode surface. For the cyclic voltammogram measured on RGO modified electrode, it provided a well-defined peak with higher current response signal than unmodified electrode for 3 times indicating that G sheet can enhance the electrochemical sensitivity due to a good electrochemical property of G. For the current response obtained from RGO/Pt modified electrode, it showed a well-defined peak and highest current response signal which was higher than an unmodified electrode for 5 times. The significantly raised current response can be explained by the synergistic effect of RGO and Pt. Hence, it is justified that RGO/Pt electrode is an efficient electrochemical sensor for DCF determination.



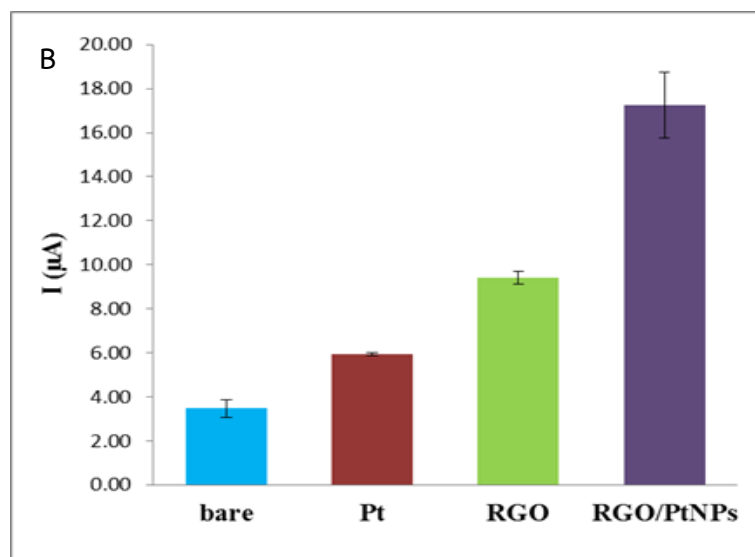


Figure 4.11 Cyclic voltammograms of 100 μM DCF in 0.1 M PB solution pH 7.0 (A) measure on unmodified electrode (blue line), pt modified electrode (red line), RGO modified electrode (green line) and RGO/Pt modified electrode (violet line) and histograms of peak currents measured on different electrode (B).

4.2.4 Optimization of parameters of differential pulse voltammetry for determination of diclofenac

The voltammetric behavior of DCF was further study by using differential pulse voltammetry (DPV). To obtain the highest electrochemical sensitivity of this system for DCF, the parameters of DPV detection including step potential and amplitude were optimized using 100 μM of DCF in 0.1 PB solution (pH 7.0) on RGO/Pt electrode. The optimum condition was chosen from highest current intensity compromising with a sharp peak. Start with an effect of step potential, DPV was scan with various step potential in a range of 4-20 mV. As shown in Figure 4.12A, the oxidation peak currents of DCF were found to increase with the increase of step potential up to 16 mV. At 16 mV of step potential, the peak current was high intensity with sharp peak, while 20 mV of step potential also provided a high current response but the peak shape was broad. Thus, 16 mV was chosen as an optimum step

potential for this system. The effect of amplitude was then investigated in a range of 20-120 mV. As seen in Figure 4.12B, the current response increased when increasing the amplitude. At a 100 mV of amplitude, the current response was slightly constant and sharper peak compared with 120 mV. Thus 100 mV of amplitude was selected to be an optimum condition for all next experiments.

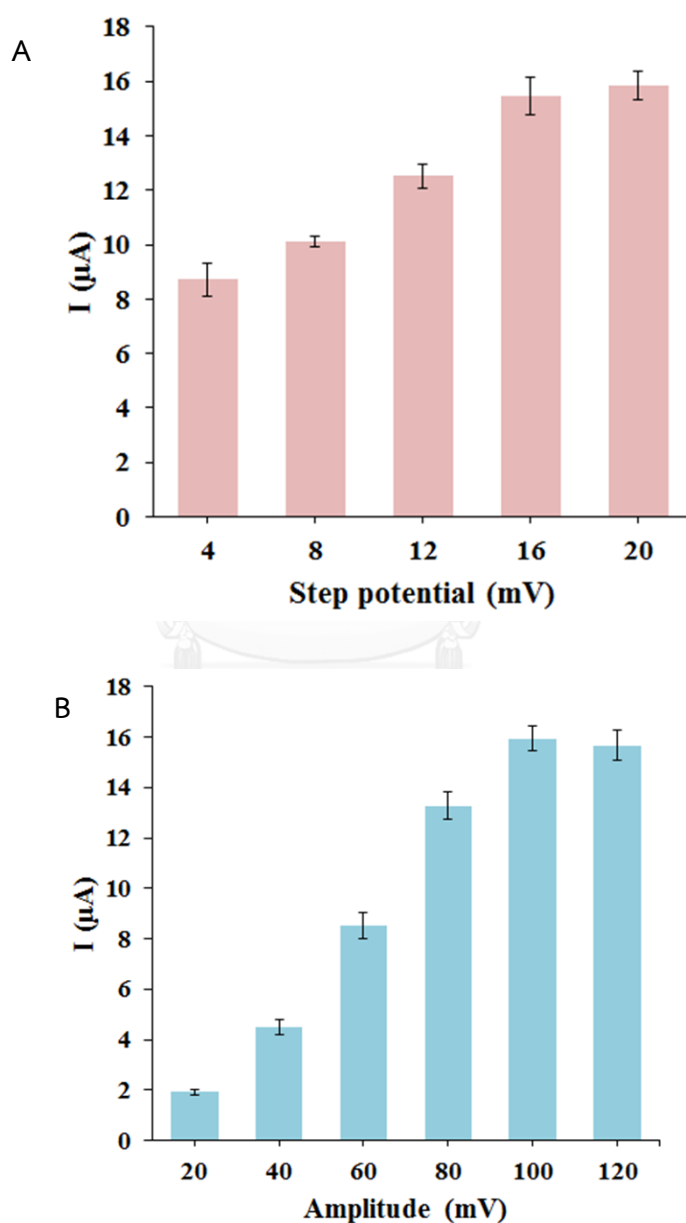
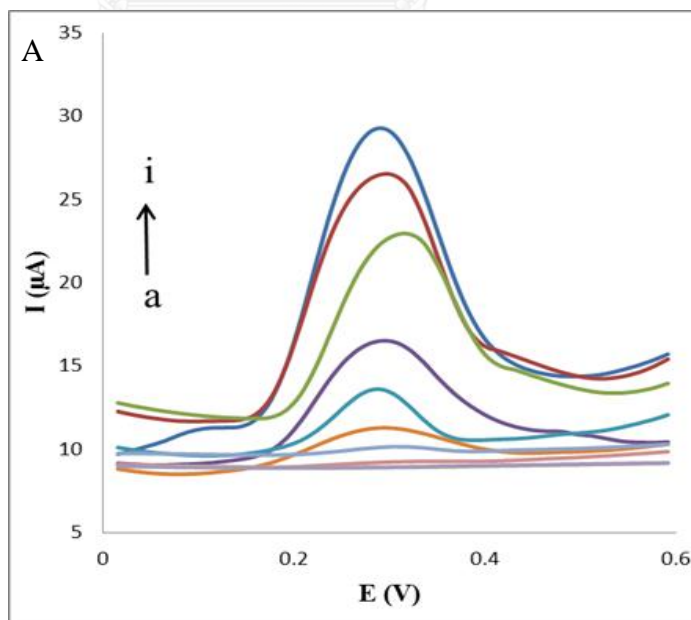


Figure 4.12 Histograms of peak current obtained from DPV of 100 μM of DCF in 0.1 M PB solution pH 7.0 with various step potential(A) and various amplitude (B).

4.2.5 Analytical performances of RGO/Pt modified electrode for diclofenac detection

Under the optimized conditions, the analytical performances of RGO/Pt modified electrode was investigated using DPV. As shown in Figure 4.13, the current response increased as a function of DCF concentration. The calibration curve was obtained by the plot of current intensity versus concentration and it was a linear in a range of 0.1-100 μM of DCF with a correlation coefficient (R^2) of 0.9992. The limit of detection (LOD), defined as $\text{LOD} = 3S_b/m$, where S_b and m are a standard deviation of the blank and a slope of the calibration curve, respectively, was found to be 0.04 μM for RGO/Pt modified electrode. The comparison of this proposed system and the previous reports for DCF determination was shown in Table 1.



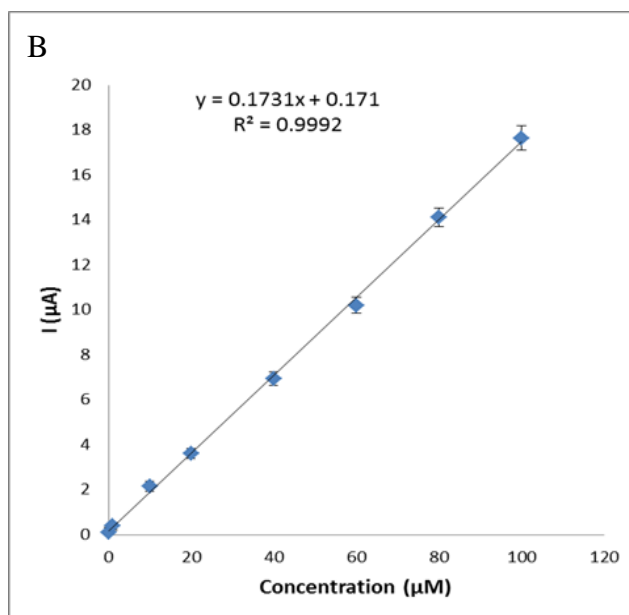


Figure 4.13 Differential pulse voltammograms of diclofenac in a concentration range of 0.1 – 100 μM (a-i) in 0.1 M PB solution pH 7.0 (A) and a calibration plot of DCF concentration versus current signal (B).

Table 1. Comparison between the proposed system with other modified electrode for diclofenac detection.

Modified electrode	Method	Linear range (μM)	LOD (μM)	ref
AuNPs/MWCNT/GCE	SWV	0.03-200	0.02	[40]
MCNT-IL	DPV	0.05-50	0.02	[41]
Aptasensor	EIS	0-5,10-1000	0.27	[42]
MWCT/CuOH ₂	DPV	0.18-119	0.04	[43]
TCPE	DPV	10-140	3.28	[44]
Cu-doped zeolite	DPV	3-15	0.3	[45]
MWCNTs-surfactant	LSV	0.17-2.5	0.08	[46]
Ni(OH) ₂ – Ni electrode	Amp	196-2650	31.7	[47]
IL-CNTEP	SWV	0.3-750	0.09	[38]
GO-COOH/GCE	LSV	1.2-400	0.09	[48]
RGO/PtNPs	DPV	0.1-100	0.04	This work

4.2.6 Stability and reproducibility

The stability and reproducibility of the modified electrode were evaluated by measuring the current response of a constant concentration of DCF (50 μM) in PB solution (pH 7.0). To investigate the stability of modified electrode, the modified electrodes were kept in a desiccator for a week. A minimal decrease of current response was observed with a % relative standard deviation (RSD) of 3.71% (n=3). It can verify the good stability of this modified electrode. The reproducibility of modified electrode was also investigated by measuring on five different modified electrodes prepared under the same conditions. The % RSD was found to be 4.37% which is an acceptable reproducibility. These results demonstrated the good stability and reproducibility of the modified electrodes.

4.2.7 Interference study

The selectivity of modified electrode was investigated by determination of 50 μM DCF in the presence of different concentrations of interferences. The change in peak current response signal of DCF before and after adding different interferences were measured. Tolerance limit is defined as the maximum concentration of interferences that cause an error $\pm 5\%$ for DCF detection. The results were given in Table 2. showed that the common ions such as Na^+ , Mg^{2+} , Cl^- and SO_4^{2-} do not interfere with DCF determination. For others interferences in biological sample, glucose, citric acid and oxalic acid had no effect of interference but histidine interfered at 10 fold excess over DCF. These results suggest that this proposed method might show a good selectivity towards DCF detection in urine sample.

Table 2. Interference study for DCF determination under the optimized conditions.

Interferences	Tolerance limit
Na^+ , Mg^{2+} , Cl^- , SO_4^{2-}	1000
Glucose	200
Citric acid	80
Oxalic acid	60
Histidine	10

4.2.8 Real sample analysis

To evaluate the performance of this system, RGO/Pt modified electrode was used for the determination of DCF in human urine samples by using a standard addition method. The human urine samples were collected and centrifuged at 1600 rpm for 20 min. Then the supernatants were kept for further analysis. The different concentrations of DCF were added at 1:1 ratio to undiluted human urine samples. The analytical results are summarized in Table 3. The recovery ranged from 101.67% to 104.43% and the acceptable %RSD (n=3) was below 7%. These results verified that this system is suitable for DCF detection in real human urine samples.

Table 3. Determination of diclofenac in human urine samples.

Sample	Concentration of diclofenac		Recovery (%)	RSD (%)
	Added (μM)	Found (μM)		
Human urine sample	2	2.03 ± 0.11	101.67	5.16
	20	20.44 ± 0.99	102.18	4.83
	40	41.77 ± 1.88	104.43	4.51

CHAPTER V

CONCLUSIONS

5.1 Conclusions

5.1.1 Electrode modification using graphene/polyvinylpyrrolidone nanocomposites for sulfite detection

G/PVP nanocomposites were successfully prepared and used to modify the working electrode surface for the determination of sulfite. Comparing with an unmodified electrode, G/PVP nanocomposites modified electrode showed higher electrochemical sensitivity towards sulfite detection. However, sulfite is unstable and easily decomposed, the electrochemical response signal substantially decreases after 1 hour of preparation. So, this target analyte is not further suitable for our developed system.

5.1.2 Electrode modification using simultaneously reduced graphene oxide/platinum for diclofenac detection

The electrochemical sensor for the determination of diclofenac was fabricated by using electrochemically reduced in-situ GO/Pt. This purposed system showed a high sensitivity towards diclofenac detection. The surface morphology of modified electrode was uniformed. Furthermore, all factors affecting to the sensitivity of diclofenac detection were systematically evaluated and optimized. Under the optimized conditions, RGO/Pt modified electrode provided a low LOD value of 0.04 μM with a wide linear range of 0.1-100 μM for diclofenac. Ultimately, this system was successfully applied for the diclofenac determination in human urine samples and a recovery range of 101.67-104.43% was obtained.

5.2 Suggestion work

This system based on RGO/Pt modified electrode might be very useful for other target analytes, which have similar chemical structure to diclofinac. The use of other metallic nanoparticles such as gold and palladium along with RGO might provide different selectivities for other target analytes of interest.



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APPENDIX

จุฬาลงกรณ์มหาวิทยาลัย
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VITA

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Proceeding

Kritthana Kimuam, Orawon Chailapalkul, Nattaya Ngamrojanavanich,

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