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**NANYANG
TECHNOLOGICAL
UNIVERSITY**

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2016

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Conferences

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Nomenclatures

Ag/AgCl	silver/silver chloride electrode
ASV	anodic stripping voltammetry
Bi	bismuth
Cd ²⁺	cadmium(II) ion
C/O	carbon to oxygen ratio
CPE	carbon paste electrode
Cu ²⁺	copper(II) ion
CV	cyclic voltammetry
CVD	chemical vapour deposition
DC	direct current
DLC	diamond like carbon
DME	dropping mercury electrode
DPV	differential pulse voltammetry
EDS	energy-dispersive X-ray spectroscopy
EIS	electrochemical impedance spectroscopy
FE-SEM	field emission scanning electron microscopy
GC	glassy carbon
GCE	glassy carbon electrode
GCS	glassy carbon sheet
GO	graphene oxide
GO-N	graphene oxide-Nafion suspension
Hg	mercury
HMDE	hanging mercury drop electrode
HOPG	highly order pyrolytic graphite
$i_{p,f}$	forward current peak
$i_{p,r}$	reverse current peak
KCl	potassium chloride
LOD	limit of detection
MFE	mercury film electrode

Na-ABS	sodium acetate buffer solution
NMC	nitrogen doped microporous carbon
NPs	nanoparticles
Pb ²⁺	lead(II) ion
ppb	part per billions
Pt	platinum
PtNPS	platinum nanoparticles
PT	polyimide tape
Pt-RGO	reduced graphene oxide decorated with platinum nanoparticles
R _{et}	electron-transfer resistance
RGO	reduced graphene oxide
RGO-N/Si	reduced graphene oxide-Nafion coated on Silicon wafer
RS	Raman spectroscopy
RSD	relative standard deviation
SCE	saturated calomel electrode
SHE	standard hydrogen electrode
S/N	signal to noise ratio
Sn	tin
Sn ²⁺ -GO	mixture of graphene oxide and Tin ions
Sn-RGO	reduced graphene oxide decorated with tin nanoparticles
SnNPS	tin nanoparticles
SWV	square wave voltammetry
SWASV	square wave anodic stripping voltammetry
XPS	x-ray photoelectron spectroscopy

Abstract

Water is essential to keep us to survive, but improper and careless treatment of the wastewater from industrialization may cause the release of heavy metals, such as Lead (Pb), Cadmium (Cd) and Copper (Cu) to water supply. These heavy metals may disrupt the supply chains and human health if the food and water were overdosed with heavy metals. Hence, detection of trace heavy metals in water is important for human health. The electroanalysis method was used with newly developed graphene-based working electrodes for the detection of trace heavy metals in aqueous solutions in this research due to their inexpensiveness, portability and sensitivity.

In this project, two methods were used in the fabrication of the graphene-based electrodes. The first was an electrochemical method, with which drop-cast graphene oxide (GO) was electrochemically reduced to reduced graphene oxide (RGO). The electrochemical method was selected because it is simple and economic. In order to enhance the sensitivity of the electrodes, the electrochemically reduced RGO (ERGO) electrodes were decorated with tin nanoparticles (SnNPs). The second method was thermal reduction, where the drop-cast GO was thermally reduced to RGO at a high temperature. The thermal reduction method was chosen because it could potentially achieve large scale production. In addition, the thermally reduced RGO was further decorated with platinum (Pt) NPs to improve its sensitivity and selectivity towards the analytes (Cd^{2+} , Pb^{2+} and Cu^{2+}) in electroanalysis.

In the characterization of the fabricated electrodes, several instruments, such as x-ray photoelectron spectroscopy (XPS), Raman spectroscopy, field emission scanning electron microscopy (FE-SEM) and energy dispersive x-ray spectroscopy (EDS) were

been used. XPS was employed to investigate the reduction percentage of RGO by studying the carbon to oxygen (C/O) ratio. Raman spectroscopy was used to determine the graphitic structure of RGO by studying the D/G intensity ratio (I_D/I_G). FE-SEM was used to study the surface microstructure of the RGO based electrodes, while EDS was used to verify the elemental composition of metal NPs decorated RGO.

In order to study the electrochemical behaviour of the electrodes, cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were performed. The surface reversibility of the RGO based electrodes could be identified through CV, while the electron-transfer resistance (R_{et}) at the interface between electrodes and electrolytes could be determined through EIS. After that, several parameters, such as pH of buffer solution, pre-concentration potential and pre-concentration time, were systematically investigated to optimize the working conditions of the RGO-based electrodes during electroanalysis. Furthermore, the reproducibility, repeatability and storage stability of the prepared electrodes were also analyzed.

After that, square wave anodic stripping voltammetry (SWASV) was performed to detect the analytes in aqueous solutions. Calibrations of the RGO-based electrodes were carried out to investigate the performance of the electrodes in electroanalysis, in terms of sensitivity, stability and selectivity. In addition, the limit of detection (LOD) of the analytes obtained from the electrodes was determined. Finally, real sample application was performed to justify the feasibility of the prepared RGO-based electrodes.

Chapter 1: Introduction

1.1 Background

Industrialization processes such as mining, plumbing, metal processing and refining operations produce a certain amount of heavy metal waste. If these heavy metals are not properly treated, they would be drained to the environment and bring the negative impacts to human, animals and plants. As a matter of fact, metal pollutants cannot be generated automatically by nature or completely eliminated. Instead, they will transfer from one source to others [1]. Hence, a proper treatment should be taken care of to prevent these metals from reaching to human. The most harmful heavy metals to human, include lead (Pb), cadmium (Cd) and copper (Cu) [2]. Moreover, a trace amount of them is enough to affect human's health.

In the past few years, there are a few industrial pollution cases that tremendously affected the residents. It was reported that in Fengxiang County, China, about 851 out of 1016 children were diagnosed to have unusual high levels of Pb in their blood [3]. According to the news, the poisoning was due to the pollution from a nearby smelter and irregular health screening to the residents. As an excessive amount of Pb may stunt children's growth and development of brain, the parents violently protested the government's slow response to the incident. On the other hand, another metal poisoning incident happened in Hunan Province, China, in which about 509 out of 2888 local residents were found to have excessively high levels of Cd in their urine. The pollution was reported to be originated from a nearby chemical company site [3].

Recently, tap water in Flint, Michigan, USA was seriously Pb-tainted and become toxic. The crisis began with switching the source of their drinking water from Lake Huron to Flint River in April 2014. The tap water was tasted bad and looked brown initially, but later was discovered to have increased levels of Pb in children's blood in the fall of 2015. According to lawsuit, the crisis was because the state government did not treat the Flint River water without an anti-corrosive agent, eroding the iron water mains. In addition, 50 % of the service lines to homes in Flint were constructed with Pb, and hence, lead began to leach into the water supply [4-6].

In general, Pb does not bioaccumulate. Therefore, it will not increase its concentration in food chains [1]. However, consumption of water that contains Pb will lead to malfunction of central nervous system, haemoglobin synthesis, anemia and kidney damage [1]. Cd is able to interact with calcium metabolism and causes hypocalcaemia and inhibiting of calcium uptake in water [1]. Besides, Cd is carcinogenic and able to accumulate mainly in kidney and liver and lead to kidney dysfunction [2]. Although human is able to handle a relatively larger amount of Cu, excess Cu could cause an adverse effect to health. Excessive and long term exposure of Cu may lead to irritation of nose, eyes and mouth. It may also cause headache, vomiting and the worst outcome can result in Wilson disease [7]. Hence, trace heavy metal detection is necessary to make sure the health safety of human.

There are several methods to detect trace heavy metals: atomic absorption spectrometry (AAS), graphite furnace atomic absorption spectrometry (GFAAS), electrothermal atomic absorption spectrometry (ETAAS), inductively coupled plasma-optical emission spectrometry (ICP-OES), inductively coupled plasma mass spectrometry

(ICP-MS) and anodic stripping voltammetry (ASV) [8]. In recent years, ICP-MS has been widely employed in water quality screening due to its high sensitivity. However, it suffers a few constraints, in which it requires the collection, preservation and transportation of water samples before analysis at an off-site location. This leads to high time consumption and high infrastructure cost for the analysis.

The basic considerations for the method selection are the detection limit required, the accuracy and precision required, the repeatability of the detection, the turnaround time desired and cost effectiveness. Among the methods, ASV is relatively inexpensive with low electrical power consumption, low maintenance and simple operation. To summarise, ASV is one of the most sensitive, which can reach to nM or ppb limit of detection, convenient and cost effective trace heavy metal detection method. In addition, the most interesting feature of ASV is its portability and real-time processability, in which the detection can be performed on-site and on-time. However, ASV suffers the low selectivity, whereby simultaneous detection of metals with approaching oxidation potentials can cause the overlapping of their voltammograms [8]. The selectivity of ASV is mainly due to the working electrode applied. Hence, the working electrode has to be improved to make the precise simultaneous detection of trace heavy metals possible.

Over the past few decades, the inert metals, especially mercury (Hg), have been widely employed as working electrode material for trace heavy metal detection due to its sensitivity and self-renewing ability. However, due to the potential poisonous of Hg, carbon (C) based materials, such as glassy carbon, highly order pyrolytic graphite (HOPG), and carbon paste, i.e. mixture of graphite powder and binder, replaced Hg to be working electrode materials. In order to enhance the analytical performances of the

carbon-based electrodes, bismuth (Bi) was used as modification materials, either through *in situ* or *ex situ* coating. Recently, nanomaterial-modified electrodes are getting popular as they provide higher sensitivity and selectivity. For example, gold nanoparticles on single-walled carbon nanotube (AuNP-SWCNT) film electrode can enhance the trace heavy metal detection of Pb^{2+} and Cu^{2+} with the detection limit of nM levels [9], whereas Bi/polyaniline/graphene electrode can give an ultralow detection limit of 0.33 nM (0.068 ppb) Pb^{2+} [10].

On the other hand, graphene has been investigated as being used for trace heavy metal sensor fabrication recently, due to its fast electrons transferred and high surface area to react with the metal ions. However, graphene always suffers from stacking of layers during sample preparation. As a result, nanoparticles (NPs) are usually used to prevent the agglomeration of graphene sheets, improving the consistency of the electroanalysis responses. There are several methods to synthesize graphene, including chemical vapour deposition, thermal decomposition of silicon carbide (SiC), mechanical exfoliation of bulk graphite, solid state carbon diffusion and chemical exfoliation of graphite. The methods employed rely on the substrates used, yield scalability, equipment availability and cost effectiveness.

In this project, the method of chemical exfoliation of graphite was focused as graphene oxide (GO) can be versatile decorated with metal particles, especially platinum (Pt) and tin (Sn), to enhance the sensitivity and selectivity of the sensors. GO was used to disperse the metal NPs to prevent agglomeration. At the same time, the presences of metal NPs also prevent reduced graphene oxide (RGO) sheets from aggregation after reduction. In addition, metal NPs could increase the overall electrons transfer rate. Electrochemical

method was employed for the reduction because it is simple and low cost. Furthermore, GO could be versatility associated with metal NPs before deposition and reduction. Nevertheless, the production scale of electrochemical method is low, in which one electrode could be prepared once. Hence, thermal reduction was applied for the preparation of electrode to achieve large scale production. However, it involves high vacuum and high temperature, which could incur high operating cost. After that, the ready RGO-based electrodes were analysed with field emission scanning electron microscopy (FE-SEM), energy dispersive x-ray spectroscopy (EDS), x-ray photoelectron spectroscopy (XPS) and Raman spectroscopy. Finally, the effectiveness of the RGO-based sensor was analysed by means of electrochemical workstation.

1.2 Challenges

There are several challenges in the designs of the working electrode applied in the determination of trace heavy metals. From the past until recent, the surface passivation of the electrode always limits its performance in the detection. The surface active species in a solution could poison the sensor. This means that the species could foul with the active sites of the electrode, reducing the active sites for the analytes to react. As a result, the sensitivity of the electrode drops.

In order to efficiently synthesize graphene, chemical vapour deposition (CVD), thermal decomposition of SiC and solid-state carbon diffusion are usually employed. However, these methods require specific substrates, especially metal substrates, such as Cu and Ni during synthesis. In order to transfer the graphene that grew on the metal substrate to a designed substrate, the metal can be dry-etched or wet-etched to leave the free floating graphene layers [11]. However, the transfer process might cause defects such

as wrinkle formation, impurities and graphene tearing [12]. Furthermore, these methods are not feasible to functionalize graphene with metal nanoparticles.

Agglomeration of metal NPs always occurred in a solution, leading to the poor effectiveness of NPs in enhancing the sensitivity of the electrode. Hence, surfactants, such as sodium dodecyl sulphate (SDS) and sodium citrate, are always used to prevent metal NPs from aggregation. However, these surfactants are usually non-conductive, reducing the efficiency of electrochemical deposition and electrochemical reduction. In contrast, stacking of RGO sheets after reduction always causes the RGO to lose its characteristics and replaced with graphite characteristics.

Hydrogen evolution, which may occur at negative potential, can reduce the sensitivity of an electrode. The bubbles created could hinder the analytes from being reacted with the surface of the electrode. In addition, hydrogen evolution shrinks the potential window, reducing the species of analytes to be detected. As a result, the selectivity of the electrode may also be affected. In addition, hydrogen evolution may corrode the surface of electrode, lowering the repeatability and stability of the electroanalysis performance of the electrode.

1.3 Objectives

This project aims to develop graphene-based electrodes decorated with metal NPs and to study their structural characteristics and performance in electrochemical analysis of trace heavy metals. The objectives are:

- To optimize the fabrication parameters of graphene-based electrodes via electrochemical reduction and thermal reduction methods;

- To enhance the sensitivity and stability of graphene-based electrodes in simultaneous detection of several trace heavy metals at nM or ppb concentration level;
- To study the role of Nafion in the thermal reduction of GO;
- To investigate the effect of metal NPs on the electrochemical response of the prepared electrodes;

1.4 Scope

To achieve the objectives, the surface morphology, structure and composition of the graphene-based electrodes will be studied:

- To optimize the reduction conditions of GO to RGO via electrochemical and thermal reduction;
- To decorate the RGO by using metal nanoparticles and optimize the concentration of metal NPs applied;
- To characterize the graphene-based electrodes via FE-SEM, XPS, EDS and Raman spectroscopy;
- To study the formation mechanisms of RGO and metal NPs;
- To simultaneously detect several trace heavy metals, such as Pb, Cd and Cu in aqueous solutions via square wave anodic stripping voltammetry (SWASV) using the functionalized graphene-based electrodes;
- To investigate the electroanalytical performance of the functionalized graphene-based electrodes via CV and EIS.

1.5 Novelty

This thesis describes a novel approach to fabricate functionalized graphene-based electrodes without involving post-transferring. In the past, most of the preparation methods of RGO, such as chemical vapour deposition (CVD) and chemical reduction require a transfer of RGO sheets from one substrate to another. The transferring process may cause tearing and poor adhesion to the new substrate. In contrast, the RGO sheets were drop-cast and electrochemically reduced in this study. Although two-step preparation was involved in electrochemical reduction of GO, the functionalized RGO layers can fully cover the substrates. The formation mechanism of the functionalized RGO was discussed to give a new insight to the reader.

In order for large scale production of the electrodes, thermal reduction was also employed. Through the fabrication process, polyfluorocarbon from Nafion was discovered to have healing and binding effects on RGO during the reduction process. The samples were thoroughly investigated using XPS and a mechanism of the effect of Nafion to RGO was proposed.

In the electroanalysis of trace heavy metals Cd^{2+} , Pb^{2+} and Cu^{2+} , the Pb-Cu alloy is always formed during electrodeposition step. The Pb-Cu could affect the stability of the fabricated electrode and compete for the active sites with the rest of the ions, especially Cd^{2+} . As a result, the standard deviation of the fabricated electrode is large, while the sensitivity of the prepared electrode drops. In this project, PtNPs were found out to be able to reduce the formation of the Pb-Cu, which produced higher stability and sensitivity to the electrochemical detection.

1.6 Contributions

There were two main fabrication methods of electrodes involved in the project, which are electrochemical method and thermal reduction method. Both methods possess advantages and disadvantages in the preparation, such as processing time, cost, reproducibility and effectiveness in GO reduction. In the thesis, the electrochemical reduction and thermal reduction of GO were evaluated and compared.

The presence of metal NPs in RGO was again proved to prevent agglomeration of metal NPs and stacking of RGO sheets. Furthermore, the effect of the metal NPs was identified through electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV). From the EIS and CV graphs, it was discovered that the metal NPs played a catalytic role in the electroanalysis of the trace heavy metals, thus increasing the sensitivity towards the analytes and suppressing the interfering effects in the electroanalysis. In addition, the synergistic effect of RGO and metal NPs enhanced electron kinetic transfer rate, which increased the sensitivity in the detection. As a result, the limit of detection (LOD) achieved was very low, which was 0.1 nM (0.01 ppb) to nM (0.1 ppb) concentration levels in detection of Cd^{2+} , Pb^{2+} and Cu^{2+} ions.

The working conditions of the prepared electrodes were optimized before they were applied in the detection. It was found that the graphene based electrodes worked the best with the preconcentration potential of about -1 V, preconcentration time of about 180 s and in the aqueous solution of around pH 5.

1.7 Organization of Thesis

The thesis is arranged into 7 chapters. Chapter 1 discusses the overview of electroanalysis, challenges, objectives, scopes and novelty of the project. Chapter 2 reviews the

background and state-of-arts of electroanalysis and electroanalytical techniques, the development of materials applied in working electrodes, the properties of graphene and GO, the synthesis and reduction of GO, functionalization of GO and its application in graphene-based sensors. Chapter 3 describes the experimental procedures, including preparation, characterization methods, electroanalysis and real sample application, of the graphene-based electrodes. Chapter 4 studies the important fabrication parameters, formation mechanism and electroanalysis performance of the electrochemically reduced Sn-RGO electrodes. Chapter 5 illustrates the critical fabrication parameters, the role of Nafion in the contribution of healing effects on the RGO and the electroanalysis performance of thermally reduced RGO. Chapter 6 discusses the formation mechanism and electroanalysis performance of PtNPs decorated RGO (Pt-RGO) electrodes, as well as comparing the performances of the electrodes used in this study with the reported electrodes. Chapter 7 concludes the project by summarizing the results and discussion of chapter 4 to chapter 6, justifying the strengths and weaknesses of the fabricated electrodes, as well as the limitations of the proposed methods.

Chapter 2: Literature Review

2.1 Background of Electrochemistry

Electrochemistry is the studies on the inter-conversion between chemical energy and electrical energy. It involves the reactions of electrons transferred between electrodes (anode and cathode) in an electrolyte (molten metallic solution, ionic solution) [13]. There are two main reactions in electrochemistry, which are galvanic process and electrolytic process. Electrolytic process is a process whereby external energy (electrical energy) is applied to drive the chemical reaction. In contrast, galvanic process is a process whereby the chemical reaction involved causes the transferred of electrons, which produces electricity [13].

Redox reaction involves oxidation and reduction. Oxidation is a reaction whereby an atom, molecule or ion losses one or more electrons and causes an increase in its oxidation number. For example, sodium is oxidized to sodium ion by releasing one electron ($\text{Na} \rightarrow \text{Na}^+ + \text{e}^-$). In contrast, reduction is a reaction whereby an atom, molecule or ion gains one or more electron and causes a decrease of its oxidation number. For example, aluminum ion is reduced to aluminum atom by absorbing three electrons ($\text{Al}^{3+} + 3\text{e}^- \rightarrow \text{Al}$).

Electrochemistry is crucial in several areas of science and technology, which are

- Electroanalytical processes, such as detection of trace heavy metal ions in water
- Semiconductor fabrication processes
- Corrosion processes
- Battery and fuel cell processes

2.2 Overview of Electroanalysis

Electroanalysis is used to study the inter-conversion between electricity and chemistry, where electrical quantities, such as current, potential and charge were measured upon the chemical reactions. It has been widely applied in pharmaceutical analysis, industrial and environmental monitoring. The two main divisions of electroanalysis techniques are bulk method and interfacial method. Bulk method involves the measurement of a property in a solution, such as the conductivity of the solution, whereby the conductivity is proportional to the total concentration of dissolved ions [14]. On the other hand, interfacial method measures the electrical signals occurring at the electrode-solution interface.

Then, interfacial method is further classified to static technique and dynamic technique. In static technique, there is no current flow through the electrodes, hence, the concentration of the analytes remained unchanged. However, in dynamic technique, current will flow through the system, and hence, the concentration of the analytes changed due to redox reactions. Dynamic electrochemistry involves the study of the electron transfer reactions between electrodes and reactant molecules in a solution phase. There are several parameters that affect the electrode reaction, which are the electrode potential, the reactivity (ease of oxidation or reduction) of the solution species, the nature of the electrode surface, rate of transport material between the electrode and bulk solution, the structure of the interfacial region over which the electron transfer occurs [15].

After that, dynamic technique is further categorized to controlled-potential technique and controlled-current technique. In controlled-potential technique (potentiostatic technique), the electrical potential is applied to activate an electron transfer reaction, followed by the measurement of the resultant current, whereas in controlled-

current technique, a current is applied to the electrodes, followed by the measurement of the potential established. The strengths of controlled-potential techniques are high sensitivity and selectivity toward electroactive species, a wide range of applicable electrodes, portable and inexpensive devices. In addition, the potentiostatic technique is divided to the fixed potential and variable potential [16]. The genealogical chart of the electrochemical techniques is exhibited in Fig. 2-1.

Fig. 2-1: Genealogical chart of the electrochemical techniques [16].

Potentiometry is performed in zero-current condition, whereby high input impedance is applied to prevent the drastic ohmic potential drop to the total difference of potential during measuring the voltage across working electrode and reference electrode [17]. It is a two-electrode system, formed by sensing electrode and reference electrode.

The main strengths of potentiometry are highly selective and quantitative. For example, an ideal ion-selective electrode (ISE), its membrane could only allow the uptake of only one specific ion into it. However, it is not suitable to apply in simultaneous detection of several analytes.

There are three main forms of dynamic electroanalysis, which are coulometry, voltammetry and amperometry. Coulometric methods are based on an exhaustive electroanalysis of the analyte, in which the analyte is quantitatively oxidized or reduced at the working electrode. The coulometry is used to determine the number of electrons flowed in the reaction. In voltammetry, a time-dependent potential is applied to an electrochemical cell containing the analytes, then the current induced by the redox reaction of the analytes is collected and plotted on a voltammogram. The voltammogram is able to provide quantitative and qualitative information of the analytes in the redox reactions. Hence, voltammetry is suitable for simultaneous detection of several analytes. In amperometry, current is measured as a function of time while maintaining a constant potential. Since the potential is not scanned, voltammogram cannot be obtained. As a result, amperometry is only suitable for individual analysis.

2.3 Electrochemical Reactions

In electroanalytical experiment, a controlled potential is supplied to accomplish the transfer of electron during the redox process of the analytes. The reaction can only occur if potential applied is enough to make the electron transfer thermodynamically or kinetically favorable. The equilibrium potential required can be calculated by using Nernst equation, as shown in equation (2-1) [18].

$$E_{red} = E_{red}^0 - \frac{RT}{zF} \ln \frac{a_{red}}{a_{ox}} \quad (2-1)$$

Where,

- E_{red} is the half-cell reduction potential
- E_{red}^0 is the standard half-cell reduction potential
- R is the universal gas constant: $R = 8.314 \text{ J K}^{-1} \text{ mol}^{-1}$
- T is the absolute temperature
- a is the chemical activity for the relevant species, where a_{red} is the reductant and a_{ox} is the oxidant
- F is the Faraday constant, which is the number of coulombs per mole of electrons, whereby $F=9.648 \times 10^4 \text{ C mol}^{-1}$
- z is the number of moles of electrons transferred in the cell reaction or half-reaction

Nevertheless, the Nernst equation is only applicable if there is no net current flow through the electrodes. Once there is a current flow, the ions at the electrode surface will react and the activity will change. As a result, overpotential will occur.

As a result of the change of the oxidation state of the electroactive species, a current, which is faradaic current built across the electrode interface. The faradaic current is directly proportional to the rate of the redox reaction, and the resulting plot is displayed in voltammogram. Apart from faradaic current, the total current displayed in the voltammogram also includes faradaic current of the blank solution and non-faradaic current, where non-faradaic background current is due to the charging current of double layer.

A simple pathway of the electrochemical reactions is the mass transport of the analytes to the electrode surface, and then there is an electron transferred across the electrode-solution interface, followed by the transport of the product back to the bulk solution. The net rate of the reaction may be limited by either mass transport of reactants or the rate of electrode transfer, which are usually determined by the operating potential, electrode material, electrolyte and mode of mass transport (diffusion, convection and migration). For a more complex reaction, additional chemical and surface processes may be involved to precede the electron transferred.

2.4 Electrical Double Layer (EDL)

The EDL is the structure whereby the charged particles or oriented dipoles formed at every electrode interface when the material is exposed to fluid. There are two parallel layers of charges surrounding the electrode, as shown in Fig. 2-2. The first layer is known as compact layer (Helmholz inner and outer layers), consisted of ions with opposite charged corresponding to the electrode adsorbed onto the electrode. The charges in this layer are strongly held by the electrode. The second layer is known as diffuse layer, which composed of free ions that attracted to the compact layer through Coulomb force. The diffuse layer is loosely attached on the electrode and extended to bulk solution. The charging process of double layer is non-faradaic because the electrons are not transferred across the electrode-solution interface. In addition, it causes the background current, which reduces the detectability of the electrode [16].

Fig. 2-2: (a) Schematic diagram of the EDL and (b) The potential across the EDL [16]

2.5 Types of Voltammetry

Voltammetry is used to investigate the half-cell reactivity of analytes. In the experiment, the potential is varied to induce the redox reactions of the analytes. The resulting current will be recorded and displayed in voltammogram. The shape of the curves in voltammogram illustrates the species of the analytes, the reactivity of the electrode towards the analytes and the nature of the electrochemical reactions, which are reversible, quasi-reversible and irreversible. The curves could be affected by speed of the potential applied (scan rate) and the mode of mass transport. There are several types of voltammetry, such as CV, square wave voltammetry (SWV), linear sweep voltammetry (LSV), anodic stripping voltammetry (ASV) and cathodic voltammetry (CaV). Each technique has its own advantages and limits, depending on its application.

2.5.1 Cyclic Voltammetry

CV is the most commonly used voltammetry techniques in electrochemical analysis. It is able to provide the information of the thermodynamics of redox processes, adsorption processes and the kinetics of electron transfer reactions by referring the current peak of the CV curve. The shape of the CV curve depends on the reversibility of the A/B redox couple, as shown in Fig. 2-3. In a typical CV scan, the potential is increased from E_1 to E_2 linearly followed by the reversed scan, whereby the potential is decreased from E_2 to E_1 , as shown in Fig. 2-4. The potential E_2 is selected so that the potential interval ($E_2 - E_1$) includes a reduction and oxidation process of interest. As potential is increased negatively from E_1 (0.5 V) to E_2 (-0.5 V), the reduction of A to B occurred.

Fig. 2-3: CV for (a) irreversible, (b) reversible and (c) quasi-irreversible electron transfer reactions [15]

Fig. 2-4: A schematic diagram of potential waveform employed to the working electrode in the CV experiment [19]

As presented in Fig. 2-5, for the forward scan, initially, at relatively positive potential side, no current flows because the potential is not negative enough to reduce A to B. However, as increasing potential become more negative, the current rises when the electrochemical rate constant becomes large enough to drive the reaction. After reaching the peak value, the current decreases accordingly, even the potential is increasing continuously towards negative. It is due to the concentration of species A is reducing after reaching the maximum point.

Fig. 2-5: Oxidation and reduction reactions during a CV scan [19]

For the reverse scan, whereby E_2 is decreased back to E_1 , at relatively negative potentials, no current flows because the potential is not positive enough to oxidize B back to A. However, at higher positive potential value, the current rises in the opposite direction when the electrochemical rate constant becomes large enough to drive the reaction. After reaching the minimum point of the curve, the current is decreasing as the concentration of B decreases. The current stop at a point, typically at zero (depends on the reversibility of the reaction) when all B has been oxidized back to A [19]. For a reversible couple, the heights of the forward ($i_{p,f}$) and reverse current peaks ($i_{p,r}$) are at the same magnitude and the both peaks are separated by a potential is about 59 mV at 25 °C, regardless of scan rate, as shown in Fig. 2-6.

However, for an irreversible couple, when B is reconverted back into A, an appreciable overpotential is required and the reverse peak appears at potentials much more negative than E_1 of reversible couple. Moreover, $i_{p,r}$ is also slightly lower than that of the $i_{p,f}$, which is possibly due to the formation of B in the forward scan, which might

diffuse into bulk solution and cause re-oxidation failed. This fact could be prevented by increasing the scan rate by making the electrode to be very oxidizing to drive the reaction. For reversible scan, the peak current is linearly proportional to scan rate power of $1/2$ ($i_p \propto v^{1/2}$), while it does not apply to irreversible or quasi-irreversible couple [15].

Fig. 2-6: CV for a reversible electron transfer reaction [15]

In this project, CV is used to investigate the mechanism of the redox reactions. For an example, if the reaction is quasi-reversible, the peak ratio $i_{p,r} / i_{p,f}$ will be either larger or smaller than unity because chemical competition for the electrochemical reactant product or reactant. If the ratio is smaller than unity, it implies that an EC mechanism has occurred, as shown in equation (2-2), in which the product R is chemically transformed into product Z. Hence, for the reverse scan, there is lesser product R to be electrochemically oxidized back to the reactant O. Furthermore, by reducing the scan rate, quasi-reversible reaction could be approached to reversible reaction. Hence, various scan rates could be performed and the scan rate that exhibited the peak ratio with nearer to unity and smaller peaks potential separation will be selected for subsequence experiments.

Moreover, CV can be applied to evaluate the interfacial behavior of electroactive compounds [16]. In most cases, both reactant and product participated in an adsorption-desorption process. By studying the CV curves, a progressive increase of the cathodic and anodic peak currents could be observed. This implies that gradual adsorptive accumulation of electroactive compounds on the electrode surface.

2.5.2 Pulse Voltammetry

As compared to LSV, whereby the current measured while the potential is swept linearly in time, pulse voltammetric technique could significantly lower the detection limits. This is because after the potential stepped with a short duration, the charging current decays exponentially to negligible value, while faradaic current decays slower [16]. As a result, the ratio between the faradaic and non-faradaic currents is increasing, and hence lower concentration level, as low as 10^{-8} could be quantified. There are several pulse voltammetric techniques, such as normal pulse voltammetry, differential pulse voltammetry (DPV) and SWV. Each of them possesses different excitation waveform and current sampling regime. Among the techniques, SWV contributes the highest sensitivity and shortest analysis duration due to the fast scan rate in SWV.

2.5.3 Anodic Stripping Voltammetry

ASV is an extremely sensitive voltammetry technique in the detection of trace heavy metals [20, 21]. The remarkably sensitivity of ASV is mainly due to the involvement of an effective preconcentration step that generates a favorable signal to noise ratio. The preconcentration step can greatly lower the detection limits by two or three orders of magnitude as compared to voltammetry without this step. In addition, ASV could measure four to six metals simultaneously in various concentrations. There are two main steps

involving in ASV, which are preconcentration step and stripping step, as presented in Fig. 2-7.

Firstly, the preconcentration step, which is also known as accumulation step, is carried out, whereby a relatively negative potential to the potential of the analytes is applied on the working electrode, so that the analytes of interest, such as Pb^{2+} , Cu^{2+} and Cd^{2+} are electrodeposited on the working electrode. For example, $\text{Cu}^{2+}(\text{aq}) + 2\text{e}^- \rightarrow \text{Cu}(\text{electrode})$. After that, the potential from the working electrode is swept to induce Faradaic loss of the deposited analytes from the electrode [19]. For example, $\text{Cu}(\text{electrode}) \rightarrow \text{Cu}^{2+}(\text{aq}) + 2\text{e}^-$. The current induced during the stripping step will be recorded and produced a characteristic peak that allows for quantification of the target trace ion. Each peak in the current signal at specific potential refers to the oxidation of species/trace ions.

In some cases, the electrodeposition of the analytes ion during preconcentration step may not be uniform, which will affect the current readings. Moreover, the ‘incomplete stripping’, in which the analytes still remain on the surface of working electrode may affect the next measurement. Hence, before any measurement, the working electrode needs to be cleaned by relatively positive potential under magnetic stirring, to remove attached analytes from the surface of working electrode. In order to ensure uniform deposited surface by the analyte ions, stirring is also performed during preconcentration step followed by quite time (stirring stops) to allow the deposition and solution to reach its steady state.

The stripping step can be further categorized into linear, staircase, square wave and pulse. ASV combining with square-wave waveform (SWASV) appears to be the best features of these methods. It is because SWASV technique gives a short analysis time and offers comparable or better sensitivity and differentiation against charging current background than that of other techniques [22].

Fig. 2-7: Waveform for preconcentration step and stripping step in ASV.

2.6 Electrochemical Cell Instrumentation

In order to conduct voltammetry test, potentiostat is performed to identify the analyte in the solution. It consists of three electrodes, which are listed from section 2.6.1 to 2.6.3 as follows:

2.6.1 Working Electrode

Working electrode possesses the interface that reaction of interest is occurring [19]. It can be referred as anode or cathode depending on the location of the reaction. The common

working electrodes are inert metals, such as gold (Au), platinum (Pt) or silver (Ag) and inert carbon, such as glassy carbon or pyrolytic carbon.

2.6.2 Reference Electrode

An applied potential is a quantity that could not be directly measured, but it is measured relative to that reference point. Hence, a reference electrode is required to work as a standard potential for a controlled-potential measurement. In electroanalysis measurement, reference electrode is used in conjunction with working electrode and counter electrode. Reference electrode has to be independent on the electrolyte and the reactions on electrodes, meaning it has to be reversible. In addition, a reference electrode should possess the ability to reach stable equilibrium potential quickly, mainly achieved by the ‘inbuilt’ constant buffered or saturated concentration that carried out the redox reaction to reach the equilibrium potential [19]. An ideal reference electrode should be stable and reproducible even there is a small current passing through. Furthermore, it should exhibit a constant potential at constant temperature throughout the experiment.

The common reference electrodes are standard hydrogen electrode (SHE), silver/silver chloride (Ag/AgCl) electrode and saturated calomel electrode (SCE). SHE is not recommended for laboratories practice because it is hard to prepare due to the difficulty to contain hydrogen gas. As compared to SCE, Ag/AgCl is inexpensive, thermally stable and nontoxic. However, evaporation of potassium chloride solution (KCl) in Ag/AgCl may change the potential shift to the electrode, which will not be an issue to SCE. Hence, saturated KCl is suggested for Ag/AgCl [23].

2.6.3 Counter Electrode

Counter electrode, also known as auxiliary electrode, plays the role to pass the induced current through the working electrode and prevent any current flow between working electrode and reference electrode [19]. The common counter electrode is platinum electrode.

2.6.4 Working Principle of Potentiostat

Potentiostat imposes a fixed potential between the working electrode and reference electrode. As shown in the eq. (2-3), any changes in potential, E are reflected as changes in working electrode due to negligible current drawn through the reference electrode.

$$E = (\phi_M - \phi_S)_{\text{working}} - (\phi_M - \phi_S)_{\text{reference}} \quad (2-3)$$

where ϕ_M is the potential of the electrode, and ϕ_S is the potential of solution

The imposition of potential difference on the working electrode-solution interface will lead a current to flow, whereby the current changes can be used to investigate the electrochemical reaction between working electrode and the solution. To prevent any changes of potential between working electrode and reference electrode due to the current flow, the counter electrode is applied to pass through the same amount of the induced current to flow through the working electrode [19].

2.7 Working Electrode Requirements

The potential difference applied between reference electrode and working electrode will drive the electrochemical reaction at the surface of the working electrode. The current produced from the chemical reaction at the working electrode will be balanced by counter

electrode to prevent any current change between working electrode and reference electrode. The current measured is mainly proportional to the concentration of the analytes. However, in some cases, due to the poor performance of working electrode, faradic noise which is due to unwanted redox reaction, working electrode material itself and solvent delivery system may interrupt the faradic current and hence affect the final detection results.

An optimal working electrode is determined by its

- Potential window, which is confined by the potentials at reduction and oxidation of the solvent take place. A wide potential window is favourable as more analytes, e.g., Pb^{2+} , Cu^{2+} , Cd^{2+} , can be detected simultaneously. The potential window is affected by the solution pH applied and amount of dissolved oxygen. Hydrogen evolution also affects the potential window.
- Involvement of the electrode in the redox reactions, i.e. inertness of the electrode. The electrode may take part in the electrolytic reactions or may be oxidized by the analytes. In principle, the working electrode material should not react with the solvent and supporting electrolyte, or else interferences, such as overlapping peaks that can cause poor resolution, may occur on the results obtained.
- Kinetics of electron transfer reactions, whereby the rate of electrons transferred determines the reactions. Kinetically fast reaction working electrode possesses reversible reactions. However, kinetically slow reaction working electrode tends to be irreversible to the reactions. As a result, overpotentials are required to accelerate the reactions, which will cause noise and poorer limit of detection. Moreover, irreversibly adsorbed substances remaining on the electrode surface

will eventually affect the next measurement. Hence, proper cleaning and polishing are necessary to reuse these kinds of electrode.

2.8 Working Electrode Materials

Basically, there are three main types of working electrode, as shown from section 2.8.1 to 2.8.3 as follows:

2.8.1 Metal

Noble metals, such as platinum, gold and silver electrode were the popular materials used in working electrode. Platinum and gold electrodes are commonly employed in electrochemical analyses because they are chemically stable and easily obtained, and hence they can be manufactured to electrodes conveniently. In addition, they offer favourable electron transfer kinetics and wide anodic potential range [24]. However, low hydrogen overvoltage restricts their cathodic potential window. In addition, the tendency of the formation of surface oxide and adsorption of hydrogen atoms also lead to high background currents in a wide potential window [24]. These absorbed films will change the electron kinetic transfer rate of the electrode, causing irreversible reactions. Hence, before electrochemical measurement, pulse potential is applied to the electrode to remove these layers.

As compared to platinum and gold, mercury becomes an attractive selection for trace heavy metals detection due to its wide cathodic potential. This is because mercury possesses extraordinary high overvoltage of hydrogen evolution [25]. However, the oxidation of mercury restricts its anodic limit, which could be accomplished by platinum and gold. In general, it possesses highly reproducible, renewable and smooth surface,

which makes it suitable for electroanalysis. There are various types of mercury electrodes, including dropping mercury electrode (DME), hanging mercury drop electrode (HMDE) and the mercury film electrode (MFE). The most attractive advantage of DME and HMDE is its self-renewing ability, in which uncontaminated and uniform surface of new mercury drop can be used for each measurement by dropping the used mercury drop [25]. However, the toxicity of mercury has constrained its usage. Although the MFE has comparably less toxic due to less mercury applied, it does not have a pure mercury surface but consisted of oxide films. Hence, it suffers lower hydrogen voltage and hence higher background currents [16].

2.8.2 Carbon Based Materials

Carbon based materials, such as glassy carbon electrode (GCE), carbon paste electrode (CPE) and diamond like carbon (DLC) are mostly used in working electrode nowadays as they can be easily obtained and processed. Carbon materials usually possess wide potential window as compared to platinum and mercury, as shown in Fig. 2-8. Moreover, they are comparative chemical inert, low cost and suit for various sensing applications, such as heavy metals, organic and inorganics detections [16, 25]. Although most carbon materials share the basic six-member aromatic ring and sp^2 structure, they differ in the relative density of the edge and basal planes as their surfaces, in which this relative density affects the electron transfer and adsorption rate [16].

Fig. 2-8: Potential window of carbon, mercury and platinum in various solutions [16].

Graphite involves layers of hexagonally arranged carbon atoms in a planar condensed ring system with each carbon are covalent with sp^2 hybridization, as shown in Fig. 2-9. The layers, known graphene layers, are stacked parallel to each other in a three-dimensional structure, where the layers are bonded by weak van der Waals force [26]. Pure graphite is generally a poor electrode material due to its high porosity that will lead to high background current [27]. As a result, pure graphite does not have practical application in electroanalysis.

Fig. 2-9: Hexagonal unit cell structure of graphite [28].

On the other hand, glassy carbon (GC) is carefully prepared by annealing various polymers, especially polyacrylonitrile at the temperature of 1000-3000 °C in an inert atmosphere. Usually, the carbonization is performed until fully elimination of oxygen, hydrogen and nitrogen, remaining carbon in the structure [16, 29]. The structure of GC consists of thin, tangled ribbons of cross-linked graphite-like sheets, as shown in Fig. 2-10. As compared to pure graphite, GC possesses higher density and small pore size. In general, it has excellent mechanical and electrical properties and chemical inertness [16]. Hence, it is suitable to work as working electrode because GCE has wide potential window and relatively reproducible performance. However, pretreatment is required before electroanalysis. This is mainly because the surface of GCE may be covered by impurities, such as organic and inorganic compounds, which could severely interrupt the electrons transfer in GCE.

Fig. 2-10: Structural model of GC [30].

The usual surface pretreatment involves polishing with alumina slurries with particles size of 0.05 μm , and then sonicating in deionized (DI) water [31, 32]. Besides the traditional polishing method, the surface impurities could also be removed through vacuum and heat treatments, laser activation and electrochemical pretreatment [29]. Generally, the electron transfer rate is higher at the edge plane than at the basal plane [33]. Hence, by increasing the exposure of the fresh edge carbon through pretreatment, the electron transfer rate could be enhanced. Furthermore, some redox reactions, such as $\text{Fe}^{3+}/\text{Fe}^{2+}$ were improved through the catalytic effect of surface oxides, and hence electrochemical oxidation process is suggested [29].

Apart from GCE, CPE has also become an alternative due to its several advantages. CPE consists of polycrystalline graphite powder mixed with either insulating organic liquid, such as paraffin oil or conducting liquid, like Nafion or ionic liquid, working as binder [24]. The composition of graphite powder and binder could heavily affect the electroanalytic performance. The binder could decrease the electron transfer rate and contribute to background current. However, the graphite is too brittle for electroanalysis

with binder. The main advantage of CPE is the paste is renewable without extensive polishing. Secondly, the basal and edge plane of the graphite powder are randomly exposed, which makes the CPE electrochemical reactive. Moreover, it possesses high signal to noise ratio due to its microstructure and geometrical structure [29]. Similarly to GCE, it has wide potential window that could accommodate more types of analytes.

DLC is another choice carbon material that could be functioned as working electrode for electroanalysis. DLC is prepared at low pressure by physical vapour deposition [34]. DLC is generally hard and wear resistive, but it is not conductive enough for electrochemical purposes. Hence, DLC is usually doped with boron or nitrogen to increase its conductivity [16]. It was reported that boron-doped DLC possesses several attractive properties, including wide potential window, high overvoltage for hydrogen evolution, low and stable background currents, good electrochemical reactivity without any pretreatment and extreme hardness [35, 36].

Although GCE, CPE and DLC provide several advantages in electroanalysis, they suffer a few challenges. Firstly, GCE required pretreatment, which is quite inconvenient and time consuming. Furthermore, the resulting surface after polishing is generally quite complex [31]. Besides, its sensitivity could only reach at μM concentration level, which may not be low enough for some analysis, especially drinking water and baby food products. Hence, in the last decade, GCE is modified with various nanomaterials to enhance its sensitivity and selectivity [37, 38]. CPE is comparably poorer in electrocatalytic reactions. It also suffers from interference with adsorbed particles, and it is less stable in some solvent [29]. Although it is inexpensive, easy to prepare, renewable, it is more suitable for lab purpose than for industry usage. Doped DLC owns a few attractive properties, but it

comparably hard to be modified with other nanomaterials, such as graphene and metal nanoparticles, which scaling down its commercial values in electroanalysis.

2.8.3 Modification with Electrochemical Methods

Electroplating is one of the interesting and cost-effective methods performed in electrode coating. It could produce uniform and thickness controlled surface. However, a workpiece with sharp corners should be avoided to prevent charge concentration that causing thicker coating than to other areas. Electroplating, also known electrodeposition is a process whereby electrical current is used to reduce the dissolved metal cations or to oxidize the dissolved anions so that they can be coated on an electrode. Metal deposition on metal is used for decorative, protective or enhances the specific properties of a surface [39]. Before electrodeposition, surface pretreatment of the electrode is necessary to remove contaminants, such as dust, films and oxide layers. These contaminants could interfere the bonding, leading to poor adhesion between the surface and the coating. The surface treatment operation usually involves surface cleaning, surface treatment and rinsing [39].

Basically, there are two types of metal electrodeposition performed to modify the surface of working electrode, which are *in situ* electrodeposition and *ex situ* electrodeposition. Both methods have their own strengths and weaknesses. For *ex situ* electrodeposition, it is easier to use, in which the pre-electroplated electrode can be used directly in electroanalysis. Unlike *in situ* electrodeposition, the catalytic cations are required to be added into the solution (medium to be analyzed) before electroanalysis. However, in some cases, *in situ* electroanalysis possesses better analysis capability than that of *ex situ* prepared electrode.

In trace heavy metal electroanalysis, besides of the inert metal and carbon based electrodes, the thin film electrodes are usually coated with bismuth to improve the performance in electroanalysis. Bismuth electrode provides comparable electroanalytical capability as mercury electrode. However, bismuth possesses some features that make it a material of choice in electrochemistry field for sometimes. Compared to mercury electrode, bismuth is more environmental friendly with negligible toxicity. Moreover, it works well in both faradic and non-faradic measurements, insensitive to the presence of oxygen and couples well with inert electrode, such as platinum, gold and silver [40]. Bismuth electrode is able to detect most of the metals and inorganic compounds, as shown in Fig. 2-11.

H																	He	
Li	Be											B	C	N	O	F	Ne	
Na	Mg											Al	Si	P	S	Cl	Ar	
K	Ca	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br	Kr	
Rb	Sr	Y	Zr	Nb	Mo	Te	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I	Xe	
Cs	Ba	*	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At	Rn	
Fr	Ra	**	Unq	Unp	Unh	Uns	Uno	Une										
			*	La	Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu
			**	Ac	Th	Pa	U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No	Lr

Fig. 2-11: Periodic table of elements showing the elements detected by Bi film electrode as shaded [40]

For *ex situ* electrodeposition of bismuth, the glassy carbon electrode (GCE), which worked as working electrode was immersed in the bismuth plating solution and a potential at -1.2 V was applied under stirring condition and then bismuth film was formed. Then, pre-deposition of trace heavy metals at -1.4 V was applied under stirring, followed by 10 s

of 30 s quite time for stabilisation. SWASV swept was applied from -1.4 V to -0.4 V, followed by cleaning of residual metals at -0.4 V [41]. For *in situ* electrodeposition of bismuth, GCE was immersed in a solution that was consisted of Bi^{3+} ($500 \mu\text{g L}^{-1}$) and trace heavy metals (e.g. Cd^{2+} , Zn^{2+} and Pb^{2+}) and then a potential at -1.4 V was applied under stirring condition. Then, a quiet time of 10 s was applied, followed by SWASV swept from -1.4 V to 0 V. The current induced was recorded. After that, 30 s at 0 V under stirring was applied to clean the residual metals and bismuth from the GCE [41]. In general, both plating methods provided comparable performance in reproducibility and sensitivity. For the bismuth film electrode plated *in situ*, the detection limit achieved were 1.77 nM for Cd and Pb and 10 nM for zinc (Zn) [41].

2.8.4 Modification with Biomaterials

In the last decade, biosensing of heavy metals has been improved in the specificity, selectivity, sensitivity and repeatability [42-44]. In general, heavy metals could catalyst or inhibit the biosensing system during the interaction with biomolecules [45]. In the analytical setup of biosensors, biosensing devices consist of biosensing probe and a physicochemical transducer [42, 46]. Bioprobe is mainly work for specificity, while the sensitivity of the sensor is depending on the selection of transducer [47]. There are several choices for bioprobe, including whole cells, enzymes, antibodies, proteins, peptides, nucleic acids, DNAzymes, phytochelations, while the choices of transducers include of capacitive, potentiometric, impedimetric, amperometric, optic, conductometric and square wave voltammetry [45].

For protein and peptides, the physiochemical change involves conformational change of the protein structure. Upon the introduction of metal ions, the methallothioneins

undergoes folding process, as shown in Fig. 2-12. As a result, diffuse layers on the gold electrode are thickening, altering the capacitance of the transducers. This sensing mechanism is able to achieve detection limits of 1 nM of Cu^{2+} , Cd^{2+} , Zn^{2+} and Hg^{2+} [46]. Apart from the protein, enzyme has been widely applied in bioprobes due to its simplicity, relatively fast process and high sensitivity. The presence of metal ions binds with enzyme, and inhibits its activity [48]. Then, the suppression of the enzyme activity induces signals to the transducers [49]. There are various enzymes, such as glucose oxidase, alcohol oxidase and invertase have been used for biosensing of heavy metals. For example, immobilized glucose oxidase in a manganese dioxide modified carbon paste electrode is able to detect Hg^{2+} at 1 nM [50].

Fig. 2-12: Schematic diagram of the conformational change of protein upon the introduction of metal ions [46].

As compared to other materials, biomaterials offer high selectivity, cost effectiveness and the possibility in *in situ* analysis. However, in the current state, biomaterials are facing several challenges, such as long analysis duration, tedious in preparation and purification of proteins and enzymes, and the degradation of the biomaterials. Furthermore, multiple enzymes are required to generate measurable product,

which reduce its feasibility in environmental heavy metals sensing. The needs for cofactor or coenzymes to enhance the performances of biosensors have induced higher manufacturing cost and complicated the purification process. Moreover, simultaneous detection of several metal ions could only be achieved through multi-detection array. However, majority of the multi-detection array platforms were not stable, because it is challenging to accommodate optimal conditions for various enzymes on a single platform [51]. Hence, further researches and efforts are required to improve the application of biosensors in heavy metal ions detection.

2.8.5 Nanostructured Materials

Nanostructured materials own a one dimensional size ranged from 1-100 nm [52]. They are attracting more attention in recent years as they possess some properties that are absent in macroscopic materials. These properties include quantum effect, surface effect and specific surface area [25], which enhances the analytes detection abilities. These properties have been widely employed in various areas, such as biomedical, electronics and sensors. The nanostructured materials that are broadly researched in the last decade include carbon nanotubes (CNT), graphene, metal nanoparticles and metal oxide nanoparticles.

In general, CNT can be synthesized by laser ablation or chemical vapor deposition (CVD). CNT is categorized into multi-walled CNT (MWCNT), with diameters of less than 30 nm and single-walled CNT (SWCNT), with diameter of less than 2 nm [24], as shown in Fig. 2-13. The main CNT properties as a chemical sensors are fast electron transfer rate, high electrocatalytic activity, high surface area, good mechanical strength [53] and high mass sensitivity, which will increase mass transport rate and reduce the

solution resistance. Recent research reports showed that modification of SWCNT thin films with metal NPs such as gold, platinum and silver enhances its electrocatalytic activity [9]. The gold nanoparticles on SWCNT (AuNP-SWCNT) film electrode enhance the trace heavy metal detection of Pb^{2+} and Cu^{2+} with the LOD of 2.64 nM ($R^2=0.984$) and 9.65 nM ($R^2=0.991$) respectively with long term storage stability [9].

Fig. 2-13: Conceptual diagram of (A) SWCNT and (B) MWCNT [54].

Besides of CNT, graphene is emerged as another option of electrochemical sensor as well. Graphene consists of a flat monolayer of sp^2 -bonded C atoms that densely packed into a 2D honeycomb lattice, which has high surface area for reactions [55]. It possesses high electrical conductivity, high thermal conductivity and chemically inertness property [56]. Hence, besides of becoming superb electronic material, it can be one of the noble materials for biological, chemical and trace heavy metal detection sensors. In [10], it also has been demonstrated that the detection limit of graphene can be down to 7 nM for Pb^{2+} ; while modification of graphene thin film with both conductive PANI porous layers and Bi

NPs to form Bi/PANI/graphene electrodes gives an ultralow detection limit of 0.33 nM Pb^{2+} [10].

Furthermore, application of metal nanoparticles in the modification of electrode surface becomes an innovative approach in electrochemical sensing and electrocatalysis. The main reasons of medication using metal nanoparticles are their large surface-to-volume ratio, increasing the specific binding sites on the surface. As a result, more reactants could bind with the active sites to enhance the electrochemical reactions. In addition, the nanoparticles possess unique catalytic effects over their bulk counterparts. Their high catalytic activities could increase the rate of redox reactions. For example, the high electrocatalytic activity of platinum nanoparticles (PtNPs) in graphene-PtNPs towards the redox reactions of Arsenic (III) has greatly lowered the detection limit of the As(III) in electrochemical detection [57].

As compared with other modifications, electrode surface modified with nanostructured materials can tremendously alter the surface properties, which can significantly enhancing the sensitivity and selectivity of the working electrodes towards the analytes. This is because of the large surface-to-volume ratio of the nanostructured materials that could provide more surface active site for redox reactions. Furthermore, the high electrocatalytic activities of the nanostructured materials could promote faster redox reactions, which may shorten overall processing time. The stability of the nanostructured materials, especially CNT, graphene and metal nanoparticles is usually reliable, and hence it could be stored for later usage. By combining carbon nanomaterials with metal nanoparticles, the potential window could be widened by taking the advantages of the wide cathodic window of carbon materials, despite the poor cathodic window of bulk metal

materials. More importantly, electrode that modified with nanostructured materials has better advantages than others because it could simultaneously detect several heavy metals ions, with high selectivity and sensitivity. For example, graphene thin film electrode could simultaneously detect Cd^{2+} , Pb^{2+} and Cu^{2+} with high sensitivity and selectivity [58].

2.9 Graphene

Graphene is an allotrope of carbon in 2D form. The carbon atoms are tightly packed in hexagonal pattern [55]. The atoms are sp^2 –hybridized that forming three in-plane σ -bonds per atom. Each atom in the lattice possesses a π orbital, contributing to a delocalized network of electrons. Graphene can be stacked up to form a graphite, where van der Waals force is holding each layer of graphene to form the three-dimensional structure, as shown in Fig. 2-14. Furthermore, graphene can also be rolled to form one-dimensional carbon nanotubes (CNT) [59].

Fig. 2-14: Schematic diagrams of (a) graphene and (b) graphite [60].

Graphene possess various exceptional properties, such as high electrical conductivity, high tensile strength, high thermal conductivity and chemically inertness to

react with the analytes [56]. Owing to the 2D crystal lattice, graphene obtains extraordinary electronic properties [61-63], where its carrier mobility could exceed $200000 \text{ cm}^2/\text{Vs}$ [64]. Moreover, graphene owns ambipolarity properties, where the charge carriers could be adjusted between holes and electrons by supplying gate bias. Hence, graphene is highly advantageous in semiconductor applications. The ambipolarity also allows graphene to withdraw or donate electrons, leading to “chemical gating” of the material. In addition, the 2D structure of graphene contributes to high surface area to volume ratio, providing more active sites for analytes [59]. As a result, graphene has been widely developed as chemical or biosensors in the last decade due to its high conductivity and sensitivity.

Since 2004, when the first boom of graphene discovery, several methods have been developed to synthesize graphene, either in single-layer, bilayer, a few-layer or multi-layer. Although graphene is useful in various applications, it faces some challenges in the fabrication. The main concerns in the synthesis of graphene are quality of the prepared graphene and scalability of production. The overall quality of the crystal lattice affects the performances in electrochemical sensing, mechanical properties and electronic devices. High yield synthesis is desirable for industrial purposes, which lowers the production cost and time. There are two main approaches in the graphene synthesis, which are top-down and bottom-up approaches. The top-down method requires the exfoliation from the bulk materials, while bottom-up method involves the growing of graphene from precursors. Each fabrication method possesses advantageous and disadvantages, as discussed as below.

2.9.1 Synthesis of Graphene

There are several methods to fabricate graphene: mechanical exfoliation of bulk graphite, chemical exfoliation of graphite, thermal decomposition of SiC, chemical vapour deposition and solid state carbon diffusion. Each method has its own strengths and weaknesses depending on different applications.

a. Mechanical Exfoliation of Bulk Graphite

Single-layer graphene (SLG) and few-layer graphene (FLG) obtained through mechanical exfoliation were first done by the 2010 Nobel Prize in Physics winners, Andre Geim and Konstantin Novoselov. They successfully separated 1-carbon atom thick graphene sheets. In this method, scotch tape is used to peel off the thin layer of graphite [65], especially highly oriented pyrolytic graphite (HOPG). The main task of mechanical exfoliation is to overcome the van der Waals force between first and second layer, without disturbing the subsequent layers. Although this method is able to produce high quality and nanometer level graphene, it is unable to generate scalable area of graphene, low yield and labour intensive [66].

b. Thermal Decomposition of SiC

In order to achieve scalable yield of graphene for industrial purposes, thermal decomposition of SiC has been designed and explored. In this method, graphene was grown through the thermal decomposition of Si on the (0001) surface plane of single crystal of hexagonal-SiC (6H-SiC) [67]. When 6H-SiC was heated to 1250 °C to 1450 °C, for 1 to 20 min under etching of H₂, graphene sheets were formed [68]. During thermal decomposition, Si atoms desorbed and the remaining C atoms on the surface arranged themselves to form epitaxial graphene layers [69, 70]. The reactor pressure and gas

atmosphere environment were able to change the kinetics of graphene formation, structure and properties of graphene [12, 71].

Although this method looks attractive and possesses the possibility to attain industrial applications, the high cost of the SiC wafer and the high temperature and ultra-high vacuum of the process became the main disadvantages [72]. Furthermore, the challenges of the thickness control of graphene layers and reproduction of large area of graphene are yet to be solved [68]. Hence, in order to gain the ability to effectively control the number of layers growth, future research are required to understand the mechanism of the growth process. Nevertheless, this method is more suitable in semiconductor industry because functionalization of the graphene for the application in electrochemistry or chemistry may not be convenient.

c. Chemical Vapor Deposition (CVD)

In this method, the substrate, usually transition metals, such as Ruthenium (Ru) [73], Iridium (Ir) [74], Nickel (Ni) [75], Pt [76], Cobalt (Co) [77] and Cu [78] which was worked as catalyst, was exposed to gaseous environment, usually the gas mixture of hydrogen (H_2), methane (CH_4) and Ar. Then, the substrate was heated to about 1000 °C in this gaseous environment [11]. Upon heating, the CH_4 decomposed on the surface and diffused into the substrate, while the by-product, which was hydrogen evaporates. Upon cooling, the carbon component was segregated from the surface and formed the graphene layers. Fig. 2-15 illustrates the example of the growth mechanism of graphene on Cu foil, whereby the Cu oxide is reduced, followed by the development of Cu grains upon high temperature annealing. After that, CH_4 or H_2 decomposed on Cu substrate and form

graphene islands. Then, graphene domains were coalesced to form a film during cooling process.

Fig. 2-15: Schematic diagram showing the formation of graphene on Cu substrate [60].

Ni was always used to catalyst the CVD process but it has some limitations. By utilizing Ni as catalyst, the obtained single or few layered graphene were not homogeneous, which might be due to the difficulty of control over the number of graphene layers [79]. This difficulty might be due the segregation of carbon component from Ni-C happens too fast. However, by changing the catalyst to Cu, the thickness could be controlled easier and better [80].

In order to transfer the graphene grown on the metal substrate to a desirable substrate, the metal can be dry-etched or wet-etched to leave the free floating graphene layers [11]. However, the transfer process might cause defects such as wrinkles, impurities and graphene tearing [12]. Although CVD graphene may possess comparable electrical transport properties as high quality exfoliated graphene flakes, the control of process parameters imposes a challenge to obtain good quality graphene. Moreover, the

fabrication process is expensive due to the high energy consumption and the transfer process of the graphene to another substrate remains complicated.

d. Solid-state Carbon Diffusion

Solid-state carbon diffusion works similarly as the CVD method. The difference is that carbon was sputter-deposited on the metal substrate before annealing. The silicon wafer was deposited by amorphous carbon (a-C) followed by Nickel (Ni). During thermal processing (800 °C to 1100 °C), the carbon diffused into the metal substrate. Then, upon rapid cooling, the carbon component segregated to the metal surface and form graphene layers [81-83]. The graphene formed by this method usually possess better controlled thickness of graphene due to the ability to control the thickness of sputtered carbon layers. However, this method is not feasible for the functionalization of graphene.

e. Reduction of Graphene Oxide

Mass production of graphene-based materials is always in demand in several fields, such as biomedical, chemistry and fuel cells. Upon the booming of graphene in 2004, graphene oxide (GO), which was reported over a century has re-emerged as an intense research. This is because GO can be reduced to reduced graphene oxide (RGO) in large scale production. There are slightly differences in the properties of graphene and RGO. For example, supreme graphene possesses no defect in the sp^2 layer, while RGO consists of voids left by the oxide groups upon reduction. Nevertheless, RGO could be easily functionalized with various inorganic and organic compounds, which is vital in sensing applications.

2.10 Graphene oxide (GO)

GO consists of graphene and oxygen functional groups, which are epoxide (-O-), hydroxyl (-OH), carbonyl (-C=O) and carboxyl (-COOH) [84-86], as shown in Fig. 2-16. Epoxide and hydroxyl are the major components of GO, they are located on the basal plane of GO whereas the minor component of GO, carbonyl and carboxyl are distributed at the edges of GO [84-89]. Furthermore, epoxide groups of GO are located at the inner part of an aromatic domain of GO as well as at the edges of an aromatic domain of GO [90], which often determine the reactivity of graphene nanosheets [91]. GO is amphiphilic due to the presence of graphene, which is hydrophobic and oxygen functional groups, which is hydrophilic [92]. Hence, GO can be functionalized with other organic and inorganic chemicals to form composites.

Fig. 2-16: Proposed schematic diagram of GO structure [66].

GO is an electrical insulator, hence, it should be reduced to RGO to restore its electrical conductivity before it can be used as electrically conducting materials [93, 94]. There are several methods that can be applied to reduce GO, including chemical reduction, thermal reduction, plasma-assisted reduction and electrochemically reduction. Upon reduction, GO will be converted to RGO, which is more hydrophobic than GO itself.

Hence, recent report claimed that functionalized GO can be applied on self-cleaning coatings electronic devices [95] or windows [96].

2.10.1 Synthesis of GO

In general, GO could be obtained through exfoliation from graphite oxide, while graphite oxide could be produced through the oxidation of graphite [97]. There are three popular methods to prepare graphite oxide in recent years, which are Brodie method, Staudenmaier method and Hummers method. Brodie method was the earliest method, whereby Ceylon graphite was oxidized by potassium chlorate and fuming nitric acid. This method was later replaced by other methods due to its dependency on strong oxidizing mixture and the carbon to oxygen ratio (C:O) obtained is 5.51 where the ideal ratio supposed to be between 2.1 to 2.9.

The Staudenmaier-Hofmann-Hamdi method was developed to synthesize graphite oxide, whereby potassium chlorate was added into a mixture of concentrated sulfuric acid, concentrated (63%) nitric acid and graphite. The C:O obtained was improved to 2.89. However, the long processing time and the possibility of explosion if potassium chlorate was not used in control have set a limit to this method. Hummers method replaced the Staudenmaier-Hofmann-Hamdi method, where graphite was mixed with water-free concentrated sulfuric acid, sodium nitrate and potassium permanganate. This method required less than two hours and was much safe than the two other methods. The C:O obtained was also comparable to the Staudenmaier-Hofmann-Hamdi method, which was 2.25.

Graphite oxide prepared by Hummers method is yellowish. Ultrasonication is usually applied to graphite oxide before electrochemical deposition to ensure it is well

disperse in aqueous suspension. After a proper ultrasonication of graphite oxide for about 1 h, the exfoliation will result stable dispersions of thin GO sheets in solution [98]. If there is irreversible agglomerate of GO and colour changing from brown to black after the well-dispersed, it showed that reduction of GO sheet has occurred [99].

In order to exfoliate graphite oxide to GO, there are several solvents can be used. From the experiments, graphite oxide can be easily dispersed to achieve full exfoliation in N,N-dimethylformamide (DMF), N-methyl-2-pyrrolidone (NMP), tetrahydrofuran (THF), ethylene glycol (EG) [100] and water, as shown in Fig. 2-17. The exfoliation can be achieved because the hydrophobicity of GO leads the solvents to readily intercalate between the layers and disperse them into single layers [92]. This process is usually assisted by mechanical stirring or ultrasonication to enhance the bonds breaking between the carbon layers [92]. However, sonication period will strongly affect the lateral size of GO layers [101], which eventually affects the quality of RGO after reduction. Longer sonication will create smaller lateral size of GO, which reduces coverage on electrode surface [101]. Hence, shorter sonication duration, such as 1 h is usually preferred. Moreover, partially exfoliation may cause the overlapping or stacking of GO layers, which may reduce its transparency but increase its conductivity [101].

Fig. 2-17: Chemical exfoliation of GO in several solvents [102]

2.10.2 Reduction of GO

GO is unable to be applied in electroanalysis because it is non-conductive. As a result, GO is required to be reduced to RGO, which is electrical conductive for electrochemical reactions. Upon reduction, graphitic structure of GO will be restored, with the formation of new sp^2 clusters, leading to greater connectivity among original graphitic domains [103]. Hence, electrons are able to transport through the RGO sheets. There are a few ways to reduce GO, including chemical reduction, electroreduction and thermal reduction. Each method carries pros and cons, as discussed as follow.

Chemical reduction of GO is one of the traditional method, in which reductants, such as dimethylhydrazine [99, 104, 105], hydrazine [105], hydroquinone [106] and sodium borohydride ($NaBH_4$) [107] can be used to reduce GO to RGO or graphene. These reductants are usually toxic, carcinogenic and hazardous, but it is the most direct reduction method. In addition, chemical reduction can be achieved at room temperature or

moderate heating, without using complex equipment and environment. Hydrazine is the most popular reductant as it is compatible with water [108] and can reduce the suspended GO effectively. However, researchers discovered that sodium borohydride (NaBH_4) can reduce GO more effectively than hydrazine [109].

In recent years, chemical reduction method is getting replaced by other methods, such as, thermal reduction, solvothermal reduction and electrochemical reduction. It is due to the several weaknesses of chemical reduction, such as, low electrical conductance for electronic applications [110] and difficulty to obtain pure graphene [111]. For example, the C-O component in GO was changed to the C-N component after hydrazine treatment [101]. Furthermore, chemical reduction may be selective to certain groups depending on the reducing agent employed. For example, NaBH_4 can effectively reduce carbonyl group but poorly reduce epoxide, carboxylic and hydroxyl groups [103]. Although it seems that chemical reduction has several disadvantages, it is still applied industrially because of the ability for inexpensive and mass production [99, 107].

To further enhance the C/O ratio of RGO, solvothermal reduction is an alternative. Solvothermal reduction is carried out in a sealed container, where solvents such as butanol or N,N-dimethylacetamide (DMAc), instead of water, is added into the GO mixture and then heated in an oil-bath under the protection of nitrogen gas [112]. The sealed environment can bring the temperature of the solvent to well above its boiling point by increasing its pressure upon heating [113]. For example, a solvothermal treatment of GO was performed at 180 °C for 12 hr, using DMAc as a solvent, with a small amount of hydrazine as a reducing agent. The obtained C/O ratio of RGO was higher than the reduction produced by hydrazine at normal pressure. However, the electrical conductivity

of the RGO remained poor because of the nitrogen compounds contamination by hydrazine [114].

In thermal reduction, the GO film is usually heated under high vacuum condition at the heating rate of 5 °C/min [115] or 10 °C/min [101] and then cooled naturally, usually overnight. Slow heating is preferred to prevent film gasification and ripping due to rapidly expanding trapped water [116]. During heating, evaporation of excessive water in the film significantly reduces the thickness of the film [117]. In addition, the high temperature causes the oxygen functional groups attached on GO sheets decompose into gases. As a result, high pressure is created within the stacked layers [102], leading to exfoliation of layers. Meanwhile, GO is reduced to RGO, in which the decomposed oxygen functional groups release from the GO layers.

During removal of oxide groups and water, the lattice relaxation occurred [115] and caused the contact area of RGO with the substrate reduced [118], weakening the adhesion between RGO sheets and the substrate. Furthermore, wrinkles tend to be form and distortion of carbon plane is induced. The structural damage of RGO sheets affects the electrons transport in the layers, reducing the electrical conductivity [119]. It was reported that the electrical conductivity of RGO obtained through this strategy is much lower than the perfect graphene, implying the weak efficiency in GO reduction and poor restoration on the electronic structure [103].

Hence, instead of vacuum condition, thermal reduction could be carried out with chemical reductant. The chemical reductants can help to react with the residual oxygen atmosphere, lowering the reduction temperatures. In addition, carbon-based chemical

reductants can induce healing effect to RGO sheet, whereby the carbon-source repaired the voids leaving by the decomposed oxygen functional groups [103]. As a result, lattice structure could be improved. For example, hydrazine monohydrate vapor at 150 Pa was pumped in to the furnace [101]. The thermal reduction of GO with hydrazine monohydrate has resulted in a drastic increase of conductivity at a lower temperature than that of pure thermal reduction. Moreover, the conductivity achieved by hydrazine annealing after cooling is about 45 times higher than the conductivity of RGO obtained by vacuum annealing [101]. However, this method may cause the contamination (C=N groups) to RGO by the reductants. Annealing in ethanol vapor atmosphere was proposed to prevent the introduction of C=N groups to the RGO and also enhanced the GO reduction. In this method, the OH radicals decomposed from ethanol molecules reacted with oxygen functional groups of GO and then removed them [110]. However, effective reduction of GO can only occurs at 900 °C.

Electrochemical method is a promising approach to remove oxygen functional groups from GO. Electrochemical reduction of GO can be performed in a electrochemical cell using buffer solution at room temperature, without dangerous chemical reagents and elimination of by-products [103]. Moreover, simultaneous film deposition and reduction of GO could be achieved through electrochemical reduction, while mechanical deposition such as dip-coat, spray-coat, spin-coat and drop-cast on substrates is always required for pre- or post- thermal reduction and chemical reduction. Although mechanical deposition are one of the fastest and best methods to coat the GO on non-conducting thin film, GO thin film prepared in these methods usually result in multilayer aggregated and crumpled sheets. This may be due to uncontrolled capillary flow and de-wetting during evaporation,

which eventually leads to the formation of wrinkles [120]. In addition, these methods can seldom achieve well connected GO film [101].

There are several techniques for electrochemical reduction of GO, which can be carried out using constant potential, CV techniques and electrophoretic deposition. The specific features of each technique are discussed as follow.

- *Electroreduction using constant potential deposition* [108]

This is a two-step method. Firstly, a GO suspension was mixed with NaCl electrolyte. Then, the solution was stirred and bubbled with nitrogen gas to protect against critical oxidation [121]. After that, a pre-reduction step was performed to oxidize the dissolved oxygen. Next, the partial reduced RGO was deposited on the working electrode when a constant cathodic potential was applied to the system. Lastly, the GO modified electrode was immersed into the NaCl solution to further reduce the RGO.

The report claimed that the electrolyte conductivity can affect the quality of the GO deposition and reduction. In electrolyte, the anionic groups of GO will undergo partial dissociation, forming a layer with negative charges bound to the particles surface. This layer will contribute to double layer effect, but it stabilizes the suspended particles from agglomerating. By increasing the concentration of electrolyte (higher electrolyte conductivity), the thickness of this double layer will decrease, destabilizing the suspension and then forming lumpy agglomerate. However, in very dilute system (low electrolyte conductivity), the double layer is too thick for electrochemical reaction. According the report, the optimum electrolyte conductivity range for the reduction of 0.5 mg/mL GO was found to be 4 to 25 mS/cm.

Apart from the conductivity of the electrolyte, pH is a concern for optimum electrodeposition of GO. The pH can affect the deprotonation and protonation of functional groups of GO, determining the dispersion stability. In general, the electrodeposition can be performed in wide range of pH, which is from 1.5 to 12.5, whereby the suspension destabilized when the pH beyond the limit. Furthermore, at acidic region, GO and H^+ compete each other for electrochemical reduction. Electrochemical reduction of H^+ forms hydrogen bubbles, hindering the GO particles to deposit on the electrode. The report claimed that the deposits with higher cathodic potentials can be performed at neutral solution as compared to basic and acidic solution.

Since the deposition is almost constant at a given potential, the thickness of the film can be easily manipulated by adjusting the deposition duration. Furthermore, the C/O ratio obtained from this method ranges from 6.0 to 8.0, which is higher than that of chemically reduced graphene oxide employing hydrazine, with the range of 3.6 to 6.4. As compared to electrochemical reductions of pre-applied GO, the RGO films electrodeposited from suspension contain less oxygen. Nevertheless, this method requires two steps, which may not be convenient. Moreover, it is not suitable for large scale production.

- *Electroreduction using CV method* [122, 123]

In this method, electrodeposition was processed in phosphate buffer solution (PBS, pH 9.18, 0.067 M) containing 1.0 mg/ml GO, with stirring for 25 cycles under CV sweeping from 0.5 to -1.8V at 50mV/s. The solution was deoxygenated with nitrogen gas before electrodeposition. From the CV curve, the peak III represented to the irreversible

electroreduction of GO [122] , with the continuous increased of the peak currents with continuous CV scans of the CV curve, as shown in Fig. 2-18. This indicates that GO was successfully reduced to rGO, and adhered onto the electrode surface [124]. In the CV curve, there were three peaks obtained. The anodic peak I and cathodic peak II represented the redox reaction of some electrochemically active oxygen-containing groups on graphene oxide that are too stable to be reduced through CV method [122, 125]. The cathodic current peak II represented to the irreversible electroreduction of GO [122, 126].

Fig. 2-18 : CV curves of electroreduction of GO [122]

The reported method is a one-step electrodeposition of graphene directly from GO dispersions. Hence, it is a convenient and fast process. In addition, the increased thickness of the graphene film can be easily and indirectly determined through the CV curve. As the CV curve attributes the redox reaction of the process, the reduction mechanism of GO can be understood better. As a result, the optimum quality of the deposited RGO could be controlled by adjusting working parameters, such as scan rate, potential ranges and number of cycles. Yet, this method is more suitably for lab application.

2.10.3 Functionalized Graphene Sensors

Carbon based materials have been broadly employed as electrode materials in analytical and industrial electrochemistry because they provided wide potential window, allowing more analytes to be detected. Furthermore, they are generally electrochemically inert, in which they do not react with the analytes that could affect the accuracy of the detection. In addition, they are low cost, and hence suitable for mass production. However, their sensitivities are comparably low and their limit of detection (LOD) sometime unable to reach the requirements set by Environmental Protection Agency [127]. As a result, graphene becomes a boom in chemistry research since the boom in 2004. Graphene is an ideal material for sensing electrode because it has large surface-to-volume, which providing more active sites for the analytes. Moreover, it is highly electrical conductive, allowing faster kinetic electron-transfer [127]. Hence, higher sensitivity and lower LOD could be achieved in sensing.

Nafion-graphene (Nafion-G) composite has been used in electroanalysis on Pb^{2+} and Cd^{2+} . It has improved sensitivity and reducing interference in sensing, which is mainly due to the synergistic effect of graphene and Nafion [128]. The high cation exchange capacity of Nafion has also contributed in the high sensitivity of detection. However, it could easily suffer from the irreversible agglomerates of graphene sheets, leading to inconsistent measurements. Hence, graphene sheets could be incorporated with NPs to minimize the aggregation problems [127]. In fact, graphene can be considered as an ideal substrate for the dispersion of nanoparticles, especially novel metal nanoparticles, such as AuNP, AgNP, PtNP and PdNP due to its large surface area per mass unit, excellent conductivity and its high mechanical strength. The presence of graphene also

serves as supports to help to disperse the nanoparticles [55] and prevent them from agglomeration. The SEM micrograph of graphene-AuNPs composite is illustrated in Fig. 2-19.

Fig. 2-19: SEM micrographs of graphene-AuNPs with magnifications of (a) 22000x and (b) 100000x [129].

In most cases, GO is applied to prepare graphene-metal particle nanocomposites because oxygen functional groups in GO, which are negatively charged serves as precursors for the anchoring of nanometals cations through electrostatic forces [92]. Hence, the presence of oxygen functional groups at GO is necessary for the nucleation of nanometals. After the growth of nanometal on GO, this nanometal-GO compound is reduced through thermal reduction or chemical reduction to become graphene-nanometal composite. The RGO or graphene possesses high conductivity, which helps the electrons to transfer between charge carriers [55] and the electrochemistry system. NPs or nanometals always serve as surface particles [55]for electrocatalytic redox reactions.

Besides noble metal nanoparticles, graphene or graphene oxide is also reacting with metal oxide nanoparticles, such as tin oxide (SnO_2), manganese oxide (Mn_3O_4), cobalt oxide (Co_3O_4) and titanium oxide (TiO_2) to form graphene-metal oxide

nanocomposites [55]. Most of the graphene-metal oxide nanocomposites are used in fuel cell but there is no research to apply them in trace heavy metal detection. However, they have the potential to become the sensor because RGO can serve as a good conductive pathway for the charge carriers (metal oxide nanoparticles) to react with the heavy metal ions.

A report showed that RGO and bismuth (Bi) nanocomposite was formed chemically [130]. Then, the RGO/Bi nanocomposites were dispersed in methanol and drop-casted on a carbon paste electrode. Then, RGO/Bi nanocomposite-carbon paste electrode was electrochemically analyzed with trace heavy metal. The differential pulse anodic stripping voltammetry was used to determine the trace heavy metal solution separately and the detection limit at different deposition potential for Cd^{2+} , Pb^{2+} , Zn^{2+} and Cu^{2+} obtained were 0.0249 μM , 2.65 nM, 0.26 μM and 0.409 μM [130]. Although the sensitivity of the modified electrode might not be very impressive, it solved the overlapping problem of Cu and Bi. With the assistance of RGO, the modified nanocomposite electrode was able to allow copper peak appears before the Bi oxidation peak. Besides bismuth, there are a wide range of metals, such as Au and Ag [92] or metal oxide, such as tin oxide (SnO_2), manganese oxide (Mn_3O_4), cobalt oxide (Co_3O_4) and titanium oxide (TiO_2) [53] that can be functionalized with graphene to form nanocomposite. Table 2-1 summarises the analytical performance of modified electrodes for Cd^{2+} , Pb^{2+} and Cu^{2+} .

Table 2-1: Comparison of the analytical performance of modified electrodes for trace heavy metal detection.

Electrode	Technique	LOD of Cd^{2+} (nM)	LOD of Pb^{2+} (nM)	LOD of Cu^{2+} (nM)	Ref.
RGO/Bi nanocomposite	DPASV	24.9	2.65	409	[131]

CNT thread	OSWSV	1.9	1.5	0.27	[132]
Montmorillonite-calcium modified carbon paste electrode	SWASV	4.8	1.48	11.8	[133]
Screen printed electrode modified with mercury nano-droplets	SWASV	12	23	20	[134]
Nafion/Bi/NMC/GCE	DPASV	13.34	0.24	NA	[135]
Antimony film CPE	SWASV	7.12	0.97	NA	[136]
Sn/GNPs/gold microelectrode	SWSV	NA	14.48	31.47	[137]
Sn CPE	SWASV	10	NA	NA	[138]
Bi/PANI/GCE	SWASV	1.1	16.5	NA	[139]
Nafion-coated bismuth-film/GCE	SWASV	0.9	1.93	NA	[140]
Single-walled CNT (SWCNT)-Nafion film/GCE	LSV	4	NA	NA	[141]
Nafion/Bi/NMC/GCE	DPASV	13.34	0.24	NA	[135]
Hg/graphene nanocomposite electrode	SWASV	0.71	0.34	1.10	[142]
Hg/graphene nanocomposite electrode	DPASV	0.04	NA	NA	[143]
Nafion-graphene nanocomposite electrode	SWASV	0.80	0.63	NA	[144]
Bi/Nafion-graphene composite film electrode	DPASV	0.18	0.10	NA	[128]
Hydroxyapatite modified platinum electrode	SWV	88.5	NA	NA	[145]
Mercury plated platinum	SWASV	2.67	0.48	7.87	[146]

microelectrode array					
Kaolin modified platinum electrode	SWV	5.9	NA	NA	[147]
PtNPs incorporated CPE	ASV	NA	NA	3.9	[148]
Hydroxyapatite modified platinum electrode	SWV	NA	20.1	NA	[149]
Bismuth nanoparticle modified boron doped diamond	SWASV	20.46	9.17	NA	[150]
Gold Nanoparticles-Modified Screen-Printed Electrode	LSASV	NA	6.27	NA	[151]
Glutathione stabilized silver nanoparticles (GSH-AgNPs) modified screen printed electrode	CV	NA	49.63	NA	[152]
Antimony nanoparticle modified boron doped diamond (Sb-BDD) electrode	LSASV	339	124	NA	[153]
SnO₂/Reduced Graphene Oxide Nanocomposite	SWASV	0.10	0.18	0.23	[154]

Chapter 3: Experimental details

3.1 Overview

In this chapter, the experimental details were discussed. The preparation procedures and materials used for Sn-RGO electrodes and Pt-RGO electrodes were different. Electro-reduction method was applied in the fabrication of Sn-RGO electrodes, while thermal reduction was employed in the preparation of Pt-RGO electrodes. After that, both types of electrodes were used in the electroanalysis of Cd^{2+} , Pb^{2+} and Cu^{2+} in sodium acetate buffer solutions (Na-ABS), followed by real application in tap water samples.

3.2 Materials and Chemicals

All chemicals employed in this work were purchased from Sigma-Aldrich with analytical grade and used without further purification. Graphite flakes, sodium nitrate (NaNO_3), concentrated sulfuric acid (H_2SO_4), potassium chloride (KMnO_4), concentrated hydrochloric acid (HCl) and hydrogen peroxide solution (H_2O_2) were purchased for the preparation of GO.

For the preparation of Sn-RGO electrodes, a solution containing 0.05 M of tin ions (Sn^{2+}) was prepared by mixing tin (II) chloride dehydrate ($\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$) with 1 M of HCl . Then, a glassy carbon plate was purchased from HTW Hochttemperatur-Werkstoffe GmbH, Germany. A solution containing 0.25 M of sodium chloride (NaCl) was prepared by mixing 7.31 g of sodium chloride powder with 500 ml of deionized (DI) water. A solution containing 0.25 M of HCl and 0.25 M of sodium hydroxide solution (NaOH) was prepared by diluting concentrated HCl and NaOH .

In the preparation of Pt-RGO electrodes, a solution containing 8 wt. % of chloroplatinic acid (H_2PtCl_6) was diluted into 1 wt. %, while a 5 wt.% Nafion solution was used directly. Meanwhile, p-Si (100) wafers (boron doped, resistivity: 0.01-0.02 Ω cm, thickness: ~ 525 μm) were purchased from Analytical Technologies Pte Ltd, Singapore.

For the study of electrochemical behavior, stock solutions of potassium ferricyanide trihydrate ($\text{K}_3\text{Fe}(\text{CN})_6 \cdot 3\text{H}_2\text{O}$) and potassium ferrocyanide trihydrate ($\text{K}_4\text{Fe}(\text{CN})_6 \cdot 3\text{H}_2\text{O}$) were diluted using DI water to a concentration of 5 mM each. For electrochemical analysis, sodium acetate buffer solutions (Na-ABS) with varying pH were prepared by mixing different amounts of sodium acetate (CH_3COONa) and glacial acetic acid (CH_3COOH), as illustrated in Table 3-1. Stock solutions containing Cd^{2+} , Pb^{2+} and Cu^{2+} of 0.05 M, 0.1 mM and 1 μM each were prepared by mixing cadmium (II) nitrate, lead (II) nitrate and copper (II) nitrate with DI water.

Table 3-1: Quantities of CH_3COONa and CH_3COOH required in the preparation of Na-ABS solutions with different pH.

pH of 0.1 M Na-ABS	3	4	5	6
CH_3COONa (g)	0.072	0.619	2.624	3.881
CH_3COOH (ml)	2.81	2.46	1.03	0.15
DI water (ml)	500	500	500	500

3.3 Synthesis of GO

GO was synthesized using modified Hummer's method. The synthesis process is demonstrated in Fig. 3-1. Firstly, 92 ml of concentrated H_2SO_4 was mixed with 2 g of

flake graphite and 2 g of NaNO_3 in a 1000 ml flask. Next, the mixture was continuously stirred for 4 hrs in an ice bath with a temperature below 5 °C. After that, 12 g of KMnO_4 was added slowly into the suspension with continuous stirring. Meanwhile, the temperature of the mixture was kept below 15 °C to prevent explosion. Next, the ice bath was removed and the mixture in the flask was heated to 35 °C with continuous stirring for 1 hr. Then, 184 ml of DI water was added into the mixture, and the mixture was stirred well and heated up to 95 °C, with no boiling allowed.

After 2 hrs, the heater was switched off and the mixture was allowed to cool to room temperature (~23°C). Next, the mixture was diluted with 400 ml of DI water and stirred for 1 hr. To terminate the reaction, 40 ml of 30% H_2O_2 solution was added into the mixture, followed by 1 hr of continuous stirring. After that, the obtained GO suspension was washed by centrifugation with 5% of HCl and then DI water. The GO gel was vacuum dried at 60 °C overnight to obtain GO powder. The collected GO powder could be either stored as powder or dissolved into a suspension with DI water.

Fig. 3-1: Schematic diagram of the synthesis procedure of GO

3.4 Preparation of Samples

(a) Preparation of Sn-RGO Electrodes

The glassy carbon plate was cut into 1 cm x 3 cm glassy carbon sheets (GCSs) using a diamond blade cutting machine. The bare GCS was polished, followed by washing with acetone, ethanol and DI water for 10 min each sequentially. After that, the GCS was covered by a polyimide tape with an only 1 cm diameter circular area of each GCS surface exposed. Then, 30 μl of 0.5 mg ml^{-1} GO was drop-cast on the exposed area of GCS. Next, GO/GCS was dried in a vacuum chamber at room temperature. Vacuum drying was preferred than natural one because vacuum drying could increase the drying

rate. Faster evaporation could prevent the concentration process that occurred upon slow solvent loss [155], which could lead to an uneven GO film thickness.

Electrochemical reduction of GO was performed using electrochemical potentiostat (Gamry Interface 1000, Gamry Instruments, USA) with a platinum mesh as the counter electrode, silver/silver chloride (Ag/AgCl) as the reference electrode and the GO/GCS as the working electrode. The three electrodes were dipped into an electrolyte, which was 0.25 M of NaCl, HCl or NaOH. Next, a constant potential at -1.2 V was applied to the system for 10 min, with continuous stirring. The applied potential and time were manipulated in the range of -1 V to -1.6 V and 5 min to 20 min, respectively, to obtain optimum potential and time for the reduction.

In order to prepare Sn-RGO electrodes, 0.05 M of Sn^{2+} was dropped into 0.5 mg ml^{-1} GO, with the Sn^{2+} :GO weight ratios of 0.05, 0.1 and 0.3. Then, the mixtures were sonicate-bathed for 10 min. After that, 30 μl of Sn^{2+} -GO suspension was drop cast on the polished GCS, followed by vacuum drying. Next, the dried Sn^{2+} -GO/GCS samples were electrochemically reduced in a 0.25 M NaCl solution at -1.4 V for 15 min to form the Sn-RGO/GCS samples, followed by rinsing with DI water and drying with compressed air. The preparation procedure is schematically shown in Fig. 3-2.

Fig. 3-2: Schematic diagram showing preparation procedure of Sn-RGO/GCS electrodes.

(b) Preparation of Pt-RGO

The p-Si (100) wafers were cut into 1.5 cm × 1.5 cm sheets using a diamond cutter. The cut Si substrates were ultrasonically washed subsequently with acetone, ethanol and DI water for 10 min each, and then dried with compressed air. Each Si substrate was covered by a polyimide tape with 1 cm diameter circular area exposed. Meanwhile, the 0.5 mg ml⁻¹ of GO mixed with 0.05 wt.% of Nafion was drop-cast on the Si substrates, followed by vacuum drying. Then, the polyimide tape was peeled off before annealing process. The GO-Nafion cast Si (GO-N/Si) samples were thermally annealed in a high vacuum furnace (PTF - three-zone tube furnace, Lenton, UK). The annealing temperature and time were adjusted in the range of 150 °C to 900 °C and 15 min to 6 hrs, respectively. In addition, the concentration of Nafion was also adjusted in the range of 0.01 wt. % to 1 wt. %. (0.01, 0.05, 0.1, 0.5). The preparation procedure is schematically shown in Fig. 3-3. In the preparation of Pt-RGO electrodes, the similar procedure was repeated, with the GO-Nafion mixture replaced by GO-Nafion-H₂PtCl₆. The H₂PtCl₆: GO weight ratios of 0.1, 0.5, 1 and 2 were investigated.

Fig. 3-3: Schematic diagram showing preparation procedure of thermally annealed RGO.

In the heating chamber of the high vacuum furnace, the pressure was lowered to 10^{-7} bar. Fig. 3-4 illustrates the temperature profile performed during thermal annealing of GO. Initially, the samples placed on an alumina combustion crucible were heated up at a rate of $5\text{ }^{\circ}\text{C}/\text{min}$. Once the heating temperature was achieved, for example $150\text{ }^{\circ}\text{C}$, a dwelling stage took place, in which the temperature was maintained throughout the stage. After that, the samples were cooled down to the room temperature. The cooling rate was rather slow, which usually took up to about 6 hrs. Next, the chamber was vented slowly to the atmosphere pressure and then the samples were collected.

Fig. 3-4: Temperature profile of thermal treatment in high vacuum furnace.

3.5 Characterization of Samples

The surface morphology of the samples were measured using SEM and FESEM (JEOL, JSM 7600F, JEOL Ltd., Tokyo, Japan) at 2 kV and 5 kV. EDS was used to simultaneously identify the elemental compositions of materials imaged from SEM or FESEM. Then, the structure of the samples was analyzed by using Raman spectroscopy (Renishaw 1000, Renishaw, UK) with a 633 nm laser. A spectral range of 1000 to 4000 cm^{-1} was used to investigate the graphitic structures of the samples. Furthermore, XPS was applied for the GO reduction characterization as the measurements can prove the reduction of GO. In this project, the carbon and oxygen percentages of the RGO and the chemical composition of the samples were determined by XPS (AXIS Ultra DLD, Kratos Analytical Ltd, UK) using mono Al $K\alpha$ radiation. Wide scan and narrow scan were both performed using XPS. The software, Casa was used to analyze the XPS results.

3.6 Electrochemical Analysis

Electrochemical analysis was performed by using electrochemical potentiostat with platinum mesh as the counter electrode, silver/silver chloride (Ag/AgCl) as the reference electrode and the prepared samples as the working electrode. The electrochemical behaviour of the prepared electrodes was studied using CV and EIS, while the performance of the electrodes in detection of trace heavy metals was studied using SWASV.

To study surface reversibility of electrodes, CV was performed from -0.2 V to 0.6 V with a scan rate of 0.05 V s^{-1} on a solution containing 5 mM of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ containing 0.1 M of KCl. In the EIS investigation, Nyquist plots were performed in $[\text{Fe}(\text{CN})_6]^{3-/4-}$ containing 0.1 M KCl over the frequency range of 0.05 to 100000 Hz.

In the SWASV measurements, the RGO-based working electrodes were immersed into the 0.1 M ABS (pH 5.0) containing Cd^{2+} , Pb^{2+} and Cu^{2+} of predetermined concentrations. After that, a preconcentration potential of -1 V was applied for 180 s with continuous stirring of 400 rpm. Mechanical stirring is employed to reduce the thickness of double layers that could decrease the efficiency of deposition. Next, the magnetic stirrer was turned off and 30 s of quiet time was applied to stabilize the solutions. After that, anodic sweeping was employed from -1.1 V to 0.2 V with a scan rate of 0.1 V s^{-1} . For repetitive measurements, the electrode surfaces were cleaned after each test with continuous stirring of 400 rpm at 0.2 V for 180 s to remove the residuals of heavy metals that may still attach on the electrode surfaces.

In the study of the real sample application, tap water samples were collected and adjusted to the optimum pH obtained from the above experiments, for instance pH 5.0,

using glacial acetic acid and sodium acetate, followed by SWASV measurements using the prepared electrodes. In the recovery tests, Cd^{2+} , Pb^{2+} and Cu^{2+} ions with pre-determined quantities were added into the tap water samples. Then, the SWASV measurements were performed and the voltammograms were recorded and analyzed.

Chapter 4: Reduced Graphene Oxide Decorated with Tin Nanoparticles through Electrochemical Reduction

4.1 Overview

From the literature review, it is shown that metal NPs on RGO or graphene sheets could bring catalytic effects to a redox reaction. In the past, most of graphene-metal nanocomposites were usually prepared chemically, in which toxic chemicals like hydrazine are involved. In order to synthesis graphene films in a simple and green way, electrochemical methods were explored. In 2010, it was reported that GO can be electrochemically reduced and deposited on GCE at the same time through CV [122]. Later, Liu's group reported that graphene-Au electrode has been prepared through a similar method. The prepared electrode was applied in the electroanalysis of uric acid, dopamine and ascorbic acid. The results illustrated the sensitivity enhancement of the modified electrode [129]. Although this fabrication method is convenient, fast and environmentally friendly, it cannot fully cover the surface of the substrate. Hence, the exposed substrate will react with the analytes concerned. As a result, the sensitivity of the substrate, which is usually lower than the surface coating, will reduce the overall sensitivity and stability of the electrodes. In addition, it limits the possibility of the application of various substrates, especially non-conductive substrates and the materials that could induce irreversible redox reactions with the analytes.

Since the last decade, tin (Sn) film electrodes have been reported for the usage in the determination of divalent chromium, Cd and Zinc ions [156, 157]. Sn film electrodes are attractive because they are stable, non-toxic and inexpensive. The sensitivity of Sn film electrodes is around 10 nM that is fairly low [157]. Recently, SnNPs encapsulated in

porous multichannel carbon microtubes using a single-nozzle electrospinning technique [158] and Sn nanoparticles encapsulated in nitrogen-doped graphene sheets through thermal reduction [159] significantly enhance the Li-based batteries. Furthermore, SnO₂ NPs gas sensor was reported to perform highly sensitive in the detection of low concentration of ethanol vapour [160]. SnO₂ NPs employed in graphene based supercapacitor can improve the performances tremendously [161]. Nevertheless, most of the current studies seldom focus on the application of SnNPs on simultaneous and selective electroanalysis in trace heavy metals.

In this chapter, the method employed in the deposition of RGO has been modified to drop-cast method in order to make sure the full coverage of RGO on the substrate. Vacuum dried of the drop-cast was performed to speed up the drying rate, preventing the concentration process that may lead to non-uniform thickness. Then, the electrochemical reduction was carried out to reduce GO to RGO. Furthermore, the RGO was decorated with SnNPs to enhance the sensitivity in the determination of trace heavy metals. XPS was performed to study the effectiveness of the electrochemical reduction of GO. FE-SEM was performed to study the morphology of the Sn-RGO, while RS was used to investigate the reduction of GO and the interaction between RGO and SnNPs. Then, the electrochemical behaviours, such as surface reversibility and resistivity of the Sn-RGO were investigated through CV and EIS. Finally, the Sn-RGO was used in the sensing of trace Cd²⁺, Pb²⁺ and Cu²⁺ in Na-ABS using SWASV under optimized conditions. Tap water sample was also performed to explore the feasibility of the Sn-RGO in real samples application.

4.2 CV Study on the Electrochemical Reduction of GO

CV of electrochemical reduction of GO was performed to determine the onset potential where irreversible reduction of GO occurred. There are three peaks can be observed from Fig. 4-1. The anodic peak A and the cathodic peak B represents the redox pair of some electrochemically active oxygen functional groups in GO that are too stable to be reduced in this set-up. The peak C attributes the irreversible electrochemical reduction of GO, in which the effective potential for electrochemical reduction of GO occurred at around -1 V. The resulted RGO is hydrophobic, and hence it is insoluble in the solution, but attach firmly on the surface of the electrode.

Fig. 4-1: CVs of electrochemical reduction of GO on GCE.

For the subsequent experiments, drop-cast method is employed to make sure the surface of GCE is fully covered by RGO. By providing potential of -1 V, which is the onset potential for the irreversible electrochemical reduction, GO sheets that in direct contact with the electrode receive the electrons and then reduce to RGO. The resulted RGO is insoluble, and hence it can attach on the surface of the electrode. After that, the subsequent GO sheets that direct in contact with the resulted RGO layer were reduced and attached on the RGO layer, as illustrated in Fig. 4-2. The irreversible reduction process will continue until the GO sheets were completely reduced to RGO.

Fig. 4-2: Schematic diagram of electrochemical reduction steps of drop-cast GO.

4.3 Template Design for The Electrochemical Reduction of GO on GCE

In the preparation of RGO electrode, GO was drop-cast on the exposed circular area of GCS, preventing other area of GCS involved the redox reaction. However, when Sn^{2+} -GO dried on GCS, it shrunk, leaving small-scattered area of GCS exposed, as shown in Fig. 4-3. When electrochemical reduction was applied, this scattered area of GCE directly reacted with NaCl solution, leading to hydrogen evolution. The hydrogen gas bubbles

formed spread the circumference of the RGO, resulting delamination of RGO from GCS substrate. As a result, the template was re-designed by sticking another layer of polyimide tape (PT) on top of dried GO, with 6 mm diameter circular area of dried GO exposed. With the new design, the GO could be completely reduced to RGO, without delamination of RGO from GSC.

Fig. 4-3: GCE template re-design steps.

4.4 pH of Electrolyte Used in Electrochemical Reduction

The pH of electrolyte could severely affect the effectiveness in electrochemical reduction. The three electrolytes which are highly alkaline, acidic and neutral were used in the investigation. Table 4-1 presents the C/O ratio of RGO obtained upon the electrochemical reduction in HCl, NaCl and NaOH. The results show that electrochemical reduction of GO in NaCl giving the highest C/O ratio, indicating the high effectiveness in reducing the oxygen functional groups. In contrast, electrochemical reduction of GO in HCl demonstrates lower C/O ratio. Although it was reported that protonation involved in the electrochemical reduction process [155], as shown in equation 4-1, which could be facilitated at low pH, hydrogen evolution potential was shifted positively at the same time. As a result, competition of redox reactions between GO and H_2O reduce the rate of GO

reduction. In addition, RGO was partially peeled off from GCS substrate due to the vigorous hydrogen gas bubbles. At high pH, C/O ratio obtained is the lowest, suggesting the poorest reduction process. This may be due to the cathodic peak potentials of GO electro-reduction shifted negatively when pH increased, lowering the rate of reduction [162].

Where a , b and c are constants.

Table 4-1: XPS C/O ratio obtained from electrochemical reduction of GO with different electrolytes.

Electrolyte used in electro-reduction of GO	C/O ratio
HCl	3.92
NaCl	4.60
NaOH	3.10

4.5 Effect of Electrochemical Reduction Potential and Duration of RGO

The potential applied for the electrochemical reduction is one of the crucial factors that determining the quality of the electrodes. In this investigation, the applied constant potentials were adjusted from -1 V to -1.6 V. Fig. 4-4 presents that the C/O ratio is increasing upon the negatively increased of the constant potential. This is because more electrons were supplied for the reduction process as the potential was negatively increased. However, at higher reductive potential, which is -1.6 V, the relative standard deviation (RSD) of the results is larger, reflecting the instability of the reproducibility. This means that 5 batches of RGO reduced at -1.6 V produced a wide range of difference in the C/O

tests. This may be due to the hydrogen evolution that fluctuating the electrochemical reduction process.

Fig. 4-4: XPS C/O ratios obtained upon the electrochemical reduction of GO at various potentials, for 10 min.

Fig. 4-5 demonstrates the XPS C 1s spectra of the GO and RGO upon the electrochemical reduction at -1.4 V for 10 min. The figure clearly shows that the intensities of C-O and C=O-H groups dropped significantly with about 33.9% and 71% reduced, respectively. However, the intensity of C=O groups increased by about 41% after electrochemical reduction, which may be due to the reduction of C=O-H groups. The overall reduction of C=O groups was about 5%, which is rather low. The above results indicate that the electrochemical reduction is more effective on C-O groups than C=O

groups. In order to fully reduce the other oxygen functional groups, higher reductive potential is required. However, cathodic potential more than -1.4 V leads to hydrogen evolution, resulting poorer reduction stability.

Fig. 4-5: XPS C 1s spectra of (a) GO and (b) RGO upon electrochemical reduction at -1.4 V for 15 min.

Fig. 4-6 illustrates the peak current responses of the RGO that were reduced at different potentials towards the electroanalysis of 50 nM (10.36 ppb) of Pb^{2+} in 0.1 M Na-ABS (pH5). The SWASV peak currents increase as the potentials are increased negatively, reflecting the restoration of graphitic structure of the electrodes promotes the electrons kinetic transfer. However, as the cathodic potential is increased to -1.6 V, the SWASV peak current drops. Although electrochemical reduction of GO at -1.6 V produces slightly higher C/O ratio, the high reductive potential causes hydrogen evolution. The hydrogen gas bubbles lead to the RGO film peeled off from GCS substrate, leaving the GCS substrate, which has lower sensitivity in trace heavy metals detection, exposed to the solution that containing the analytes. Hence, the electrochemical reduction potential of GO for the subsequent studies was set at -1.4 V.

Fig. 4-6: SWASV responses of 50 nM Pb^{2+} in 0.1 M Na-ABS (pH 5) using RGO/GCSs at varying potentials for 180 s.

Apart from applied potential, the duration for the electrochemical reduction is another important factor that may influence the quality of the prepared electrodes. Fig. 4-7 exhibits the XPS C/O ratio of RGO that was reduced at -1.2 V at varying timings. In general, the longer time spent for the electrochemical reduction, more GO were able to participate in the redox reactions and be reduced. However, the results indicate that there is slight increment of the C/O ratio for the RGO electrochemical reduced for 5 min to 15 min. After that, the improvement is almost insignificant. Hence, electrochemical reduction of RGO was fixed at 15 min for the following investigations.

Fig. 4-7: XPS C/O ratio obtained upon the electrochemical reduction of GO using RGO/GCS at -1.2 V with varying timing.

4.6 Sn-RGO Modified Electrodes

In order to enhance the sensitivity and selectivity of the electrodes in the simultaneous detection of trace heavy metals, RGO was further modified with SnNPs. In the preparation, GO suspension was mixed Sn^{2+} ions. Then, the mixture was drop-cast on GCS, followed by vacuum dried. After that, electrochemical reduction was performed in the optimized conditions, which are electrochemical reduced in NaCl solution, with the applied constant cathodic potential at -1.4 V for 15 min. In this case, *ex situ* electrochemical reduction of SnNPs was performed because SnCl_2 cannot dissolve at neutral pH, which could eventually fail the *in situ* electrochemical process. Furthermore, *ex situ* process is more convenient for the subsequent experiments, in which the prepared Sn-RGO/GCS electrode can be used to carry out SWASV directly. In addition, *ex situ* process caused no wastage in the usage of SnCl_2 as compared to *in situ* process.

Fig. 4-8 exhibits the SWASV responses of Cd^{2+} , Pb^{2+} and Cu^{2+} simultaneously on the bare GCS, RGO/GCS and Sn-RGO/GCS electrodes. The preconditioning process is carried out at -1 V for 150 s in the 0.1 M Na-ABS (pH 5) solution containing 50 nM of Cd^{2+} (5.62 ppb), Pb^{2+} (10.36 ppb) and Cu^{2+} (3.18 ppb) without deaeration. There are very weak peaks measured with the bare GCS and RGO/GCS electrodes, but higher current peaks for the three target metal ions measured with the Sn-RGO/GCS. The three peaks corresponding to Cd^{2+} , Pb^{2+} and Cu^{2+} can be identified at the potentials of -0.79 V, -0.58 V and -0.09 V, respectively. The enhanced signals from the Sn-RGO/GCS may be due to the strong absorptive ability towards the three metal ions and the increased total surface area of the electrode. In addition, it may be because of the intercalation between SnNPs

and RGO preventing the RGO from agglomeration and hence improving the electrical conductivity of the RGO-Sn/GCS.

Fig. 4-8: SWASV responses of bare GCS, RGO/GCS and Sn-RGO/GCS to Cd^{2+} , Pb^{2+} and Cu^{2+} in 0.1 M Na-ABS (pH 5) containing Cd^{2+} , Pb^{2+} and Cu^{2+} of 50 nM each

4.7 Confirmation of the Formation of Sn-RGO

After confirmed the feasibility of the Sn-RGO in the electroanalysis of simultaneous detection of Cd^{2+} , Pb^{2+} and Cu^{2+} , the Sn-RGO samples were further improved and characterized by FE-SEM and RS. Fig. 4-9 (a) presents the FE-SEM micrographs of the Sn-RGO/GCS, where the SnNPs are uniformly distributed on the RGO sheets. However, it could be noticed that some area of the Sn-RGO/GCS are less deposited by SnNPs. Hence, the micrograph was zoom-in at a higher magnification power.

Fig. 4-9 (b) demonstrates that the size of SnNPs is quite consistent, with the average particle size of about 50 nm. The small size and uniform distribution of SnNPs is

crucial to optimize its nano-effect and increase the active sites to analytes. The wrinkled surface of the RGO increases the total surface area for analytes to react. Moreover, it could be observed that the wrinkled area of RGO sheets are tend to be deposited by SnNPs, as compared to the flat area of RGO sheets. This reflects that the wrinkled surface could help to ‘capture’ and retain the deposition of SnNPs. Meanwhile, the SnNPs prevent the agglomeration of RGO sheets during electrochemical reduction.

Fig. 4-9: FE-SEM micrographs of G-Sn/GCS ($\text{Sn}^{2+}:\text{GO} = 0.1$) at two different magnifications

Raman spectroscopy is a powerful tool for characterization of carbon products due to their high Raman intensities of conjugated and double C=C bonds [163]. A spectral range of 1000 to 4000 cm^{-1} was used to investigate the graphitic structures of the GO/GCS, Sn^{2+} -GO/GCS and RGO/GCS. Fig. 4-10 illustrates the Raman spectra of the GO/GCS, Sn^{2+} -GO/GCS and Sn-RGO/GCS. The spectra of the Sn^{2+} -GO/GCS and Sn-RGO/GCS consist of three prominent characteristic peaks that are D band ($\sim 1350 \text{ cm}^{-1}$), G band ($\sim 1582 \text{ cm}^{-1}$) and 2D band ($\sim 2700 \text{ cm}^{-1}$). The D band represents structural defects, while the G band indicates sp^2 hybridized carbon-based materials. The degree of structural defects could be obtained from the D/G intensity ratio (I_D/I_G) [164]. The 2D band is the second-order of the D band, which is very sensitive to the stacking order of the graphene sheets [165]. Usually, single layer graphene and graphite possess a strong 2D peak. However, the presence of the stacking order between graphene planes due to strong interactions between adjacent planes leads to a change in the 2D band from one peak to two peaks [166], as shown in Fig. 4-10.

Fig. 4-10: Raman Spectra of GO/GCS, Sn^{2+} -GO/GCS and Sn-RGO/GCS

Table 4-2 shows that the I_D/I_G ratio of the GO slightly increases from about 1.07 to 1.14 after the addition of Sn^{2+} , implying the increased structural defects due to the mildly reduced GO to partially reduced GO (PRGO). After electrochemical reduction, I_D/I_G of the Sn^{2+} -GO/GCS further increases from about 1.14 to 1.69, indicating the further increased structural defects due to the intercalation of the SnNPs in the RGO matrix. Furthermore, the D and G bands are getting narrower from the GO/GCS, Sn^{2+} -GO/GCS to the Sn-RGO/GCS, as presented in Table 4-2, proving the restoration of the graphitic structure [166]. The recovery of the graphitic structure is important for the smooth electrons transfer during redox reactions or electroanalysis.

Table 4-2: I_D/I_G ratios and D and G band widths of Raman spectra measured from GO/GCS, Sn^{2+} -GO/GCS and G-Sn/GCS

Sample	I_D/I_G	D band width (cm^{-1})	G band width (cm^{-1})
GO/GCS	1.07	123.0	88.6

Sn ²⁺ -GO/GCS	1.14	114.2	75.7
Sn-RGO/GCS	1.69	86.6	73.8

4.8 Proposed Mechanism of the Formation of Sn-RGO

When Sn²⁺ is added into the GO suspension, redox reactions will take place. Sn²⁺, as a reducing agent, will partially reduce GO to PRGO, while Sn²⁺ will be oxidized to Sn⁴⁺, as shown in equation 4-2. As PRGO is mildly reduced, it may still maintain its amphiphilic properties. In addition, HCl, a by-product, will lower the pH and the PRGO dispersion will remain stable at this lowered pH level, as shown in Table 4-3. Hence, PRGO could still disperse uniformly in the solution without agglomeration as shown in Fig. 4-11. At the same time, Sn⁴⁺ are drawn towards GO' by electrostatic interactions. When electrochemical reduction is performed, PRGO will be fully reduced to RGO. Further, Sn⁴⁺ will react with PRGO and be reduced to G-Sn as shown in equation 4-3.

Table 4-3: pH of the solutions

Solution	pH
0.5 mg ml ⁻¹ GO with the addition of 10 µl of 1.6 M HCl	3.02
DI water with the addition of 10 µl of SnCl ₂ .2H ₂ O that was dissolved in 1.6 M HCl (Sn ²⁺ :GO = 0.1) before mixing with GO	2.82

0.5 mg ml ⁻¹ GO with the addition of 10 µl of SnCl ₂ .2H ₂ O that was dissolved in 1.6 M HCl (Sn ²⁺ :GO = 0.1)	2.65
DI water with the addition of 10 µl of SnCl ₂ .2H ₂ O that was dissolved in 1.6 M HCl (Sn ²⁺ :GO = 0.3) before mixing with GO	2.66
0.5 mg ml ⁻¹ GO with the addition of 10 µl of SnCl ₂ .2H ₂ O that was dissolved in 1.6 M HCl (Sn ²⁺ :GO = 0.3)	2.34

Fig. 4-11: Schematic illustration of the electrochemical reduction mechanism of Sn-RGO nanocomposite

4.9 Optimization of the Preparation Process of Sn-RGO

During the preparation of the Sn-RGO/GCS, it was noticed that the concentration of Sn²⁺ applied in the GO suspension is a vital factor that could affect the performances of the electrodes. Hence, the concentration ratio of Sn²⁺: GO was carefully investigated. In this study, the weight ratio of Sn²⁺:GO was adjusted to 0.05, 0.1 and 0.3. Fig. 4-12 shows the effect of Sn²⁺:GO ratio on the detection of Pb²⁺. The preconditioning process is carried out at -1 V for 150 s in the 0.1 M Na-ABS (pH 5) solution containing 50 nM of Pb²⁺ without deaeration. From the graph, the Sn-RGO/GCS electrode prepared with the

Sn^{2+} :GO weight ratio of 0.1, shows a distinct and strong peak of Pb^{2+} . In contrast, the electrodes fabricated using the Sn^{2+} :GO weight ratio of 0.05 and 0.3 present weaker stripping peak currents of Pb^{2+} . The poor performance of the Sn-RGO/GCS electrode with the weight ratio of 0.05 may be due to too little SnNPs form in the RGO sheets, as shown in Fig. 4-13. Meanwhile, for the prepared electrode with weight ratio of 0.3, the weak stripping peak current is suspected to be due to poor reduction of both RGO and Sn^{2+} .

Fig. 4-12: SWASV responses of Sn-RGO/GCS with varying Sn^{2+} :GO ratios to Pb^{2+} in 0.1 M Na-ABS (pH 5) containing 50 nM of Pb^{2+}

Fig. 4-13: SEM micrograph of Sn-RGO/GCS with the Sn:GO weight ratio of 0.05.

When the concentration of Sn^{2+} is increased, the GO is further reduced to the RGO. The Sn^{2+} -GO mixture with the weight ratio of 0.1 and 0.3 each was drop-cast and dried on GCS to investigate the study. The I_D/I_G of Sn^{2+} :GO ratio of 0.3 is about 1.396, while I_D/I_G of Sn^{2+} :GO ratio of 0.1 is about 1.1, as shown in Fig. 4-14, indicating that a higher concentration of Sn^{2+} leads to further reduction. As the RGO is hydrophobic, it will not disperse uniformly in the solution. In addition, the by-product HCl lowers the pH of the solution, as illustrated in Table 4-3, causing the destabilization of the GO suspension, followed by the aggregation of the GO sheets [131]. Hence, the aggregated GO sheets can be only partially reduced to the RGO, where certain areas of the sample surface remain the brownish GO, as shown in Fig. 4-15.

Fig. 4-14: Raman spectra of Sn-GO nanocomposites with Sn^{2+} :GO ratios of 0.1 and 0.3 before electrochemical reduction

Fig. 4-15: Optical micrograph of Sn-RGO/GCS with Sn^{2+} :GO weight ratio of 0.3 after electrochemical reduction

In addition, I_D/I_G of Sn^{2+} :GO ratio of 0.3 after electrochemical reduction is about 1.47, which is lower than that of Sn^{2+} :GO ratio of 0.1, as shown in Fig. 4-16, implying a less effectiveness in electrochemical reduction, which may be affected by the aggregated GO sheets. The agglomerated GO sheets reduce the exposed surface area of GO surface for the reduction process.

Fig. 4-16: Raman spectra of Sn-RGO nanocomposites with Sn^{2+} :GO ratios of 0.1 and 0.3 after electrochemical reduction

4.10 Electrochemical Behaviour of Sn-RGO/GCS

The electrochemical behaviour of the modified electrodes was examined by using CV and EIS. The CV responses of the Sn-GO, bare GCS, RGO and Sn-RGO, as illustrated in Fig. 4-17, were performed using 5 mM of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ and 0.1 M KNO_3 within a frequency range of 0.01 to 10^5 Hz. From the graph, Sn-GO demonstrates an undefined curve, reflecting an irreversible process. GO possesses several functional groups that could block

the electrons transferred [154], inhibiting the redox reactions. As compared to the bare GCS, RGO and Sn-RGO exhibited the well-defined oxidative and reductive peaks with higher currents. The higher peak currents obtained indicate more electrons-transfer involved in the redox reactions. In addition, Sn-RGO shows the smallest peak potential separation, which is about 0.162 V among the rest. This implies the high electron transfer rate that may be due to the enhancement of the electrocatalytic activities of RGO by SnNPs.

Fig. 4-17: CV responses of Sn-GO, GCS, RGO and Sn-RGO in 5 mM of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ and 0.1 M KNO_3 .

The impedance measured from EIS was plotted in Nyquist plot, as shown in Fig. 4-18. In Nyquist plot, the diameter of the semicircle represents the electron-transfer resistance (R_{et}) at the interface between electrodes and electrolytes during electrochemical

reaction [167]. In the graph, the bare GCS gave the highest R_{et} , which is 105 Ω , followed by RGO with 82 Ω , while Sn-RGO demonstrated the lowest R_{et} , which is 60 Ω . The low electron transfer resistance of RGO reflects the restoration of the graphitic structure upon electrochemical reduction. By co-depositing with SnNPs, the R_{et} is further reduced. This suggests the synergistic effect between SnNPs and RGO that could promote the interfacial charge transfer. Furthermore, SnNPs also increase the total active sites for the redox reactions.

Fig. 4-18: EIS responses of Sn-GO, GCS, RGO and Sn-RGO in 5 mM of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ and 0.1 M KNO_3 .

4.11 Optimization of Electroanalysis Parameters

In the electrochemical detection of trace heavy metals, the electrodes, which are Sn-RGO/GCS (working electrode), platinum mesh (counter electrode) and Ag/AgCl

(reference electrode) were dipped into Na-ABS (electrolyte) containing pre-determined Cd^{2+} , Pb^{2+} and Cu^{2+} (analytes). After that, preconcentration potential was applied to reduce the analytes, as shown in equation 4-4. The reduced analytes were deposited on the surface of the Sn-RGO/GCS. Then, SWASV was performed to re-oxidize the deposited analytes. The electrons released during the redox reactions were recorded as current profile in voltammograms.

In electroanalysis process, the selection of a proper pH value of the electrolyte (Na-ABS) that containing the trace heavy metals could heavily affect the performance of the working electrode. Fig. 4-19 shows the performance of the Sn-RGO/GCS on the detection of Cd^{2+} , Pb^{2+} and Cu^{2+} at different pH levels. From pH 3-5, the SWASV responses on the detection of Cd^{2+} , Pb^{2+} and Cu^{2+} are enhanced. It is attributed to hydrogen evolution that reduces the electrode surface reactivity, which occur at a low pH. On the other hand, when pH is increased to about 6, the SWASV intensity is slightly reduced possibly due to the hydrolysis of metal ions which could happen when pH is increased. Hence, pH 5 is chosen as an optimum pH value.

Fig. 4-19: Effect of pH solutions on stripping peak current measured at the actual potentials of -0.81 V, -0.54 V and -0.07 V, respectively, using Sn-RGO/GCS in 0.1 M Na-ABS containing 50 nM of Cd^{2+} , Pb^{2+} and Cu^{2+} each, with preconcentration potential of -1 V and preconcentration time of 150 s.

The effect of the preconcentration potential on the detection of trace Cd^{2+} , Pb^{2+} and Cu^{2+} is studied in the range of -0.8 V to -1.2 V. As the potential is more negative, more analytes are reduced. Hence, the SWASV current is increasing, as shown in Fig. 4-20. However, after the optimal point, which is -1 V, the current intensity drops. This reflects the onset of hydrogen evolution that deteriorates the surface activity of the working electrode. As a result, -1 V is selected as an optimal preconcentration potential.

Fig. 4-20: Effect of preconcentration potential on stripping peak current measured at the actual potentials of -0.81 V, -0.54 V and -0.07 V, respectively, using Sn-RGO/GCS in 0.1 M Na-ABS (pH 5) containing 50 nM of Cd^{2+} , Pb^{2+} and Cu^{2+} each, with preconcentration time of 150 s.

Fig. 4-21 exhibits the SWASV current responses to Cd^{2+} , Pb^{2+} and Cu^{2+} in the range of preconcentration time of 60 s to 240 s. As expected, when the preconcentration time is increased, the current intensity is enhanced. However, a prolonged preconcentration time tends to cause the surface saturation at a high metal ion concentration. Furthermore, as the preconcentration time is increased up to 180 s, the standard deviation of the current, especially the SWASV response to Pb^{2+} , is higher. Hence, to compromise between high sensitivity, low LOD, high stability and high reproducibility, 150 s is selected as an optimal preconcentration time.

Fig. 4-21: Effect of preconcentration time on stripping peak current measured at the actual potentials of -0.81 V, -0.54 V and -0.07 V, respectively, using Sn-RGO/GCS in 0.1 M Na-ABS (pH 5) containing 50 nM of Cd²⁺, Pb²⁺ and Cu²⁺ each, with preconcentration potential of -1 V.

4.12 Calibration Curves Measured by Sn-RGO/GCS toward Cd²⁺, Pb²⁺ and Cu²⁺

Fig. 4-22 (a-c) illustrate the SWASV responses measured with the Sn-RGO/GCS to Cd²⁺, Pb²⁺ and Cu²⁺ individually with the concentrations varying from 10 nM to 100 nM in the 0.1 M ABS (pH 5) at -1 V for 150 s. The peaks obtained are well-defined and show good linear relationships with the concentrations of Cd²⁺, Pb²⁺ and Cu²⁺, respectively, as presented in Fig. 4-22 (d). The linear regression equation for Cd²⁺, Pb²⁺ and Cu²⁺ can be expressed as equation 4-5.

$$i = 289.16[\text{Cd}^{2+}] - 0.3279 \quad (\mu\text{A}) \quad (4-5a)$$

$$i = 367.73[\text{Pb}^{2+}] - 3.6869 \quad (\mu\text{A}) \quad (4-5b)$$

$$i = 145.36[\text{Cu}^{2+}] - 0.0752 \quad (\mu\text{A}) \quad (4-5c)$$

where i is the anodic stripping peak current in μA , while $[\text{Cd}^{2+}]$, $[\text{Pb}^{2+}]$ and $[\text{Cu}^{2+}]$ refer to the concentration of Cd^{2+} , Pb^{2+} and Cu^{2+} in μM . The regression coefficients of the above equations are 0.9967, 0.9955 and 0.9983, respectively, reflecting the good matches of the equations with the experimental results. The LOD is calculated using the equation 4-6.

$$C = 3S_{bl}/S \quad (\mu\text{M}) \quad (4-6)$$

where C is the concentration, S_{bl} is the standard deviation of blank measurements and S refers to the sensitivity of the calibration curves [35. 36]. From the calculation, the LOD of Cd^{2+} , Pb^{2+} and Cu^{2+} obtained are 0.63 nM (0.07 ppb), 0.60 nM (0.12 ppb) and 0.52 nM (0.03 ppb), respectively, with RSD of about 6.1%, 7.4% and 2.5%, respectively. The LODs attained are considered very low as compared to World Health Organization (WHO) standard. However, in reality, the analyte does not appear individually in a solution. Hence, simultaneous electroanalysis of the three analytes has to be performed to further calibrate the prepared electrode.

Fig. 4-22: (a-c) Stripping voltammograms of electrochemically reduced Sn-RGO/GCS in individual analyses of (a) Cd^{2+} , (b) Pb^{2+} and (c) Cu^{2+} over a concentration range of 10 nM to 100 nM each in 0.1 M Na-ABS of pH 5 at -1 V for 150 s, and (d) their respective calibration curves

Fig. 4-23 (a) demonstrates the SWASV responses measured with the Sn-RGO/GCS for the simultaneous detection of Cd^{2+} , Pb^{2+} and Cu^{2+} with the concentrations varying from 10 nM to 100 nM in the 0.1 M ABS (pH 5) at -1 V for 150 s. The stripping

peak current shows good linear relationships with respect to the concentrations of Cd^{2+} , Pb^{2+} and Cu^{2+} , as shown in Fig. 4-23 (b). The linear regression equations for Cd^{2+} , Pb^{2+} and Cu^{2+} are expressed as:

$$i = 43.572[\text{Cd}^{2+}] - 0.2321 \quad (\mu\text{A}) \quad (4-7a)$$

$$i = 173[\text{Pb}^{2+}] + 2.0638 \quad (\mu\text{A}) \quad (4-7b)$$

$$i = 86.217[\text{Cu}^{2+}] + 1.5109 \quad (\mu\text{A}) \quad (4-7c)$$

The regression coefficients of the three equations are 0.9967, 0.9967 and 0.9952, respectively. However, compared to the SWASV results from the individual trace metals, the sensitivities of these three simultaneous analytes are decreased significantly, as summarized in Table 4-4. The drop of sensitivities is mainly due to the competition of the analytes towards the limited active sites on the electrode. Moreover, there are two current peaks observed at about -0.46 V and -0.05 V, which may be due to the formation of alloys such as Pb-Cu [168] and Cd-Cu [169] during the deposition stage, which has suppressed the intensities of the Cd^{2+} , Pb^{2+} and Cu^{2+} peaks. Nevertheless, the peak current of Cd^{2+} remains low, which may be due to its poorer absorptivity on the electrode surface, as compared to Pb^{2+} and Cu^{2+} . The LODs of Cd^{2+} , Pb^{2+} and Cu^{2+} obtained are about 7.56 nM (0.85 ppb), 6.77 nM (1.40 ppb) and 5.62 nM (0.36 ppb), with comparably high RSDs of about 11%, 39% and 16%, respectively. This reflects that the formation of the alloys severely affects the stability and reproducibility of the electrode.

Fig. 4-23: (a) Stripping voltammograms of Sn-RGO/GCS in simultaneous analysis of Cd^{2+} , Pb^{2+} and Cu^{2+} over a concentration range of 10 nM to 100 nM each in 0.1 M Na-ABS of pH 5 at -1 V for 150 s, and (b) their respective calibration curves

Table 4-4: Comparison of sensing performance during individual and simultaneous SWASV measurements

Analyte	Type of Detection	LOD (nM)	Sensitivity ($\mu\text{A}\mu\text{M}^{-1}$)
Cd^{2+}	Individual	0.63	289.16
	Simultaneous	7.56	43.572
Pb^{2+}	Individual	0.60	367.73
	Simultaneous	6.77	173
Cu^{2+}	Individual	0.52	145.36
	Simultaneous	5.62	86.22

As it was suspected that Cu^{2+} may be the main factor that leads to the formation of the alloys, simultaneous SWASV of Pb^{2+} and Cd^{2+} without the addition of Cu^{2+} was performed. As illustrated in Fig. 4-24, there are no peaks formed at -0.46 V and -0.05 V, suggesting a negative effect of Cu^{2+} in the simultaneous electroanalysis. Furthermore, this also reflects the inability of the GCS, RGO/GCS and Sn-RGO/GCS electrodes in preventing the formation of alloys. As Cu^{2+} is one of the important analytes and pollutants in water, it could not be omitted from the experiments. Hence, the selectivity of the electrodes has to be improved to suppress the formation of these alloys.

Fig. 4-24: Stripping voltammogram of Sn-RGO/GCS in simultaneous analysis of Cd^{2+} and Pb^{2+} of 150 nM each in 0.1 M ABS of pH 5 at -1 V for 150 s

4.13 Reproducibility, Repeatability and Stability of Sn-RGO

The reproducibility was also investigated with six Sn-RGO/GCS electrodes in the electroanalysis of 50 nM of Cd^{2+} , Pb^{2+} and Cu^{2+} , respectively, under the optimized conditions. The obtained results demonstrate a good reproducibility of the electrodes, as shown in Fig. 4-25, with the RSDs of 9 %, 10.2 % and 8.4 % for the peak current responses of Cd^{2+} , Pb^{2+} and Cu^{2+} , respectively. This reflects the systematic of the fabrication process that produces the high reproducibility performances.

Fig. 4-25: SWASV responses of six fabricated Sn-RGO electrodes.

Furthermore, the repeatability of the Sn-RGO/GCS electrode was examined for 6 tests under optimized conditions. The SWASV peak currents of Cd^{2+} , Pb^{2+} and Cu^{2+} were recorded as depicted in Fig. 4-26. The peak currents perform consistently for the first 4 tests, but drop after the subsequent tests. In order to identify if the instability of the electrode is due to RGO or SnNPs, the repeatability of RGO was carried out. The SWASV responses of RGO demonstrate a good repeatability, with RSD of 7.1 %, 7.4 % and 6.3 % for Cd^{2+} , Pb^{2+} and Cu^{2+} , respectively. Hence, this implies that SnNPs may suffer from passivation that may be due to the irreversible redox reactions with the analytes. As a result, the active sites of SnNPs were blocked and result the sensitivity of the electrode dropped.

Moreover, the Sn-RGO/GCS electrodes confirmed a long-term stability with the RSD of 8.5 %, 8.5 %, and 9.9 % in the SWASV responses of Cd^{2+} , Pb^{2+} and Cu^{2+} ,

respectively between the freshly prepared electrodes and the electrodes stored for 10 days after the preparation. This indicates that Sn-RGO is able to perform normally if it was stored in dried box environment.

Fig. 4-26: SWASV responses of six repetitive tests on Sn-RGO/GCS electrode.

4.14 Comparison of Sn-RGO with Other Existing Electrodes

The obtained LODs using the Sn-RGO/GCS developed in this work are compared with the previously reported results measured using other electrodes modified with carbon nanotubes (CNT), nitrogen doped microporous carbon (NMC), gold nanoparticles (GNPs), RGO, bismuth (BI) and polyaniline (PANI) with the summary of the comparison illustrated in Table 4-5. From the table, the LODs of Cd^{2+} and Pb^{2+} from our work are the lowest though the LOD of Cu^{2+} is slightly higher than the CNT thread electrode. Hence, the Sn-RGO/GCS has been proved to be an effective working electrode for heavy metal detection.

Table 4-5: Comparison of the analytical performance of modified electrodes for determination of trace heavy metals

Electrode	Technique	LOD of Cd^{2+} (nM)	LOD of Pb^{2+} (nM)	LOD of Cu^{2+} (nM)	Ref.
RGO/Bi nanocomposite	DPASV	24.9	2.65	409	[131]
CNT thread	OSWSV	1.9	1.5	0.27	[132]
Montmorillonite- calcium modified carbon paste electrode	SWASV	4.8	1.48	11.8	[133]
Screen printed electrode modified with mercury nano- droplets	SWASV	12	23	20	[134]
Nafion/Bi/NMC/GCE	DPASV	13.34	0.24	NA	[135]
Antimony film CPE	SWASV	7.12	0.97	NA	[136]
Sn/GNPs/gold microelectrode	SWSV	NA	14.48	31.47	[137]
Sn CPE	SWASV	10	NA	NA	[138]
Bi/PANI/GCE	SWASV	1.1	16.5	NA	[139]
Sn-RGO/GCS (this work)	SWASV	0.63	0.60	0.52	

4.15 Real Sample Application

Furthermore, in order to investigate the feasibility of the proposed method in real sample application, the proposed electrode was used for the simultaneous detection of the three targeted metals in tap water samples. From the SWASV measurements on the tap water samples using the Sn-RGO/GCS developed in this work, no stripping peaks of the targeted metals are observed. However, after adding Cd^{2+} , Pb^{2+} and Cu^{2+} of two different concentrations each (i.e. 35 nM and 70 nM) into the tap water samples, obvious stripping peaks are observed. The results of the recovery tests summarized in Table 4-6 indicate that the Sn-RGO/GCS provides an excellent platform for the simultaneous detection of trace heavy metals in real samples.

Table 4-6: SWASV results measured from tap water samples using G-Sn/GCS for simultaneous determination of Cd^{2+} , Pb^{2+} and Cu^{2+} ions

Sample	Target metals			
Tap water		Added (nM)	Measured (nM)	Recovery (%)
	Cd^{2+}	35	35.6	102.2
		70	68.9	98.4
	Pb^{2+}	35	35.8	101.8
		70	70.5	100.1
	Cu^{2+}	35	33.4	97.2
		70	69.1	98.7

4.16 Summary

In this chapter, the Sn-RGO/GCS electrodes were developed through the electrochemical reduction of the Sn^{2+} -GO mixture that was drop-cast on GCS. In the characterization of the electrodes, FE-SEM presented a uniform distribution of SnNPs over the RGO sheets. XPS demonstrated a high C/O ratio obtained from the electrochemically reduced RGO, confirming the effectiveness of the electrochemical method. RS analysis illustrated increment in I_D/I_G and I_{2D}/I_{D+G} ratios after introducing SnNPs into RGO, reflecting a good intercalation of the SnNPs in the RGO matrix, and the strong interactions between adjacent RGO-SnNPs sheets.

In the fabrication of Sn-RGO, the electrochemical reduction process that was performed in the electrolyte with neutral pH (e.g. NaCl) demonstrated the highest C/O ratio. The low pH electrolyte induced the hydrogen evolution easily, while the high pH electrolyte led to cathodic peak potentials of GO electrochemical reduction shifted negatively when pH increased, lowering the rate of reduction. The optimum reduction potential and duration were -1.4 V and 15 min, respectively. Furthermore, a low weight Sn^{2+} :GO weight ratio was preferred to prevent the aggregation of GO that can possibly disrupt the reduction process of the GO. The Sn-RGO/GCS showed good reproducibility and storage stability, but poor repeatability after the 4th test, which may be due to the passivation of SnNPs in Sn-RGO/GCS.

In the analysis of electrochemical behaviour, the Sn-RGO/GCS presented much higher peak current intensity and lower R_{et} than the bare GCS, RGO/GCS and Sn/GCS. The modified Sn-RGO/GCS electrodes presented a highly linear behavior for the

voltammetric stripping peak current measurements and a high sensitivity for the detection of Cd^{2+} , Pb^{2+} and Cu^{2+} individually and simultaneously. The individual detection showed a good stability with the LODs of Cd^{2+} , Pb^{2+} and Cu^{2+} of about 0.63 nM (0.07 ppb), 0.60 nM (0.12 ppb) and 0.52 nM (0.03 ppb), respectively. However, the stability of the simultaneous detection was relatively lower due to the formation of some alloys, such as Cd-Cu and Pb-Cu. As a result, the LODs of Cd^{2+} , Pb^{2+} and Cu^{2+} were reduced to about 7.56 nM (0.85 ppb), 6.77 nM (1.40 ppb) and 5.62 nM (0.36 ppb), respectively, in the simultaneous detection. Nevertheless, the obtained LODs above mentioned were still very well below the values stipulated by the WHO [166-168]. In addition, the Sn-RGO/GCS demonstrated good recoveries of the targeted metals in tap water samples, proving that the Sn-RGO/GCS developed in this work could be a promising sensing device for the simultaneous electroanalysis of trace heavy metals in real samples including environmental and biological samples.

Chapter 5: Reduced Graphene Oxide Electrode upon Incorporation of Nafion through Thermal Reduction

5.1 Overview

According to chapter 4, it could be seen that the electrochemical reduction method has several advantages, including low production cost and simple operation. However, it suffers a few drawbacks, such as comparably low C/O ratio of the produced RGO that can eventually affect the sensing ability, and could prevent from large scale production. Large scale production is necessary to lower the processing time and maintain the high reproducibility of the sensing electrodes. Hence, in order to attain mass production, thermal reduction of GO is a reliable and systematic alternative. However, in the current stage, the delamination of RGO from the substrates always occurs during thermal annealing process, which is because of different thermal expansions of the RGO sheets and the substrates during heating. Meanwhile, it has been reported that Nafion has binding effect [144, 170-174], which may become a substituent to overcome the peeling effect.

Although several papers have reported that Nafion could be applied to enhance the sensitivity of working electrodes in trace heavy metals detection, the preparation methods have only been limited to the evaporation of Nafion. No publication has been found to discuss the thermal reduction of GO that involves Nafion. Furthermore, most of the reports have claimed that the sulfonate groups in Nafion could promote the cation-exchange in redox reactions [140, 141, 175-177]. However, other functional groups in Nafion, especially polyfluorocarbon, have seldom been reported. Hence, in this work, GO was mixed with Nafion to form a stable GO-Nafion suspension (GO-N). The GO-N was drop-cast on Si wafer substrates, followed by thermal annealing. The optimization

parameters, including the concentration ratio of GO to Nafion (GO:N), and thermal annealing temperature and duration of the reduced graphene oxide-Nafion coated Si (RGO-N/Si), were systematically investigated. In electrochemical analysis, the RGO-N/Si was used as a working electrode to simultaneously detect Cd^{2+} , Pb^{2+} and Cu^{2+} , followed by real sample recovery tests. A mechanism of the healing effect induced by the polyfluorocarbon in Nafion was proposed to provide a clear understanding to the reader.

5.2 Effect of Annealing Temperature on RGO-N

Thermal annealing temperature of the GO-N/Si affects the quality of the RGO-N/Si, which eventually affects the sensitivity of the electrodes. The FE-SEM micrographs in Fig. 5-1 show the surface morphologies of the RGO-N/Si after thermal annealing at 750 °C and 900 °C. With the concentration of 0.05 wt.% Nafion in the solution mixed with 0.5 mg ml^{-1} of GO, good intact, smooth and no delamination of the RGO-N sheets on the Si substrate occurs after 750 °C (or temperature below) annealing as shown in Fig. 5-1(a-c). However, a serious peeling occurs on the RGO-N/Si annealed at 900 °C, as demonstrated in Fig. 5-1(d). As a result, this leads the exposure of the Si substrate surface, thus eventually reducing the overall electrical conductivity and stability of the RGO-N/Si sample in electroanalysis.

Fig. 5-1: FE-SEM micrographs of annealed RGO-N/Si at (a) 300 °C, (b) 600 °C, (c) 750 °C and (d) 900 °C

The XPS measurements could provide a direct proof of the reduction of the GO-N after thermal annealing. The 4 peaks shown in Fig. 5-2(a) measured from the GO-N are originated from the components: C-C (~284.8 eV), C-O (~286.2 eV), C=O (~287.8 eV) and O-C=O (~289.0 eV) [164]. After 2 hrs of annealing at 750 °C under the vacuum condition, the intensity of C-C peak is increased from about 33.9% to 59.2%, while the C-O, C=O and O-C=O peaks decrease tremendously, as shown in Fig. 5-2(b).

Fig. 5-2: XPS C 1s spectra of (a) GO-N and (b) RGO-N

From Table 5-1, the atomic percentage of C 1s increases while the atomic percentage of O 1s in the samples decreases when the annealing temperature is increased,

indicating higher contents of oxygen functional groups are reduced at higher temperatures. The C/O ratio increases from about 2.06 to 20.96 for the GO-N/Si and RGO-N/Si (750 °C), respectively. The atomic percentage of sulfonate groups decreases with increasing the annealing temperature, and these groups are completely decomposed after annealing at 300 °C and above. As a result, the advantage of the sulfonate groups, which could facilitate the cation-exchange [140, 141, 175-177], is eliminated when the annealing temperature exceeds 300 °C. However, to achieve more effective reduction of the GO-N, a high annealing temperature is required. In addition, the atomic percentage of fluoride groups also decreases when the annealing temperature increases. It is suspected that the polyfluorocarbon groups have played a role of a binder in the RGO-N. Hence, as the amount of polyfluorocarbon groups drops, the RGO-N tends to delaminate as shown in Fig. 5-1(b).

Table 5-1: XPS survey scan data of GO-N and RGO-N annealed at different temperatures

Sample	C 1s (at. %)	O 1s (at. %)	F 1s (at. %)	S 2p (at. %)
GO-N	61.16	29.68	8.52	0.63
RGO-N (300 °C)	74.61	16.65	8.10	0.64
RGO-N (450 °C)	83.62	10.27	6.12	0
RGO-N (600 °C)	90.53	5.65	3.83	0
RGO-N (750 °C)	92.59	4.42	3.00	0
RGO-N (900 °C)	95.75	3.87	0.39	0

In the investigation on the performances of RGO-N annealed at varying temperatures, SWASV was also carried out. When the annealing temperature of the RGO-

N/Si is increased from 150 °C to 900 °C, the SWASV current of Cd^{2+} increases, as presented in Fig. 5-3, which is due to more oxygen functional groups in the RGO have been reduced, thus enhancing the electrical conductivity of the samples. However, when the RGO-N/Si is heated to 900 °C, most of the polyfluorocarbon compounds that bind the RGO have been eliminated. Hence, the delamination of the RGO-N from the Si substrate (900 °C) causes the exposure of the non-conductive Si surface during the detection. As a result, the SWASV current drops.

Fig. 5-3: SWASV responses to 100 nM of Cd^{2+} using RGO-N/Si annealed at temperatures from 300 °C to 900 °C.

5.3 Effect of Annealing Duration on RGO-N

Similarly to electrochemical reduction of GO, the time spent for thermal reduction may also influence the electroanalysis performances of the RGO-N/Si electrodes. In general, the longer the thermal annealing duration, the higher C/O ratio of RGO is obtained due to

more oxygen functional groups can be removed from GO. Fig. 5-4 illustrates the XPS C/O ratio of RGO-N annealed at 750 °C in the range of duration from 15 min to 6 hrs. The differences of the C/O ratio are unexpectedly small. However, it may not reflect the insignificant influences by the duration of thermal reduction.

In fact, the small changes may be due to the slow cooling rate of the high vacuum furnace. For example, after the RGO-N samples have been annealed for 15 min, the samples will take more than 6 hrs to cool down to room temperature before the samples can be removed from the furnace. As a result, during the cooling process, the samples may still undergo reduction due to the low cooling rate from a high temperature. Although 2 hrs of thermal annealing has only slightly increased the C/O ratio, it was still selected for the subsequent experiments to ensure the more complete reduction of GO.

Fig. 5-4: C/O ratios of the samples annealed at 750 °C with varying duration.

5.4 Effect of Nafion Concentration

After the investigation on the effect of temperature towards the samples, it is believed that polyfluorocarbon of Nafion may possess the binder function. As a result, the concentration of Nafion was adjusted to 0.01 %, 0.05 %, 0.1 % and 0.5 % to study the effect of the concentration of Nafion. After that, the same amount of GO, which is 0.5 mg ml⁻¹ was mixed with the adjusted concentration of Nafion. The GO-N mixture was drop-cast on Si substrate, followed by thermal annealing at 750 °C for 2 hrs.

From the Fig. 5-5, RGO-N with 0.05 % of Nafion demonstrates the highest peak current, followed by 0.1 %, 0.5 % and 0.01 %. When the concentration of Nafion is only 0.01 % in the GO mixture, severe delamination of RGO occurred, causing most of non-conductive Si substrate exposed during electroanalysis. However, when the concentration of Nafion was increased to 0.5 %, encapsulation effect of Nafion prevents poor reduction of GO [178], leading to low C/O ratio, which is 17.77. The poor reduction of GO decreases the electron transferred of the electrode, reducing the sensitivity of the electrode in the electroanalysis of Cd²⁺.

Fig. 5-5: SWASV responses to 100 nM of Cd^{2+} using RGO-N/Si annealed at temperatures at 750 °C for 2 hrs with different concentrations of Nafion.

5.5 Proposed Mechanism on the Healing Effect of Nafion on RGO

GO consists of graphene and oxygen functional groups, which are hydroxyl, carbonyl, epoxide and carboxyl groups [85, 86] as shown in Fig. 5-6. When heating temperature is increased to 200 °C or above, the oxygen functional groups will be removed [179]. The removal of the oxide groups creates voids in the RGO structure, causing higher defects that could be proven by the I_D/I_G ratio determined from the Raman spectroscopy measurement. By adding Nafion into the GO, the healing effect of the RGO could be achieved. At a heating temperature above 300 °C, the sulfonate groups in the Nafion will be decomposed, leaving polyfluorocarbon behind, as confirmed by the XPS results. The polyfluorocarbon reacts with the RGO and forms the RGO-N that has lower defects as proposed in the schematic diagram illustrated in Fig. 5-6. This means that the polyfluorocarbon will fill the voids that were created during the release of oxygen

functional groups. Furthermore, the polyfluorocarbon is suspected to possess a binder effect, which prevents the delamination of the RGO-N from the Si substrate.

Fig. 5-6: Schematic diagram of the proposed mechanism on the healing effect of Nafion on thermal reduction of GO

5.6 Optimization of The Electroanalysis Parameters of RGO-N

It is crucial to select a correct pH value to optimize the working conditions of the working electrodes. Similarly as Sn-RGO, at low pH, hydrogen evolution, which could reduce the sensitivity of the electrodes, could easily occur. Hence, by increasing the pH from 3.0 to 5.0, the SWASV current increases accordingly as shown in Fig. 5-7. However, the SWASV current drops at pH 6.0, which may be due to the hydrolysis of the metal ions

and the formation of the hydroxide compounds that reduce the concentrations of the metal ions in the solutions.

Fig. 5-7: SWASV responses of RGO-N/Si electrode to Cd²⁺, Pb²⁺ and Cu²⁺ in 0.1 M Na-ABS containing Cd²⁺, Pb²⁺ and Cu²⁺ of 100 nM each with pH 3 to 6.

Preconcentration potential and time are among the most important pre-conditioning factors. From Fig. 5-8, the peak currents of the three analytes increase as the preconcentration cathodic potential is increased from -0.8 V to -1.3 V. This is because a higher cathodic potential is able to reduce more analytes at a fix duration. However, the increment rate of the current drops with the increased cathodic potential from -1.1 V to -1.3 V, which may be due to the surface saturation of the working electrode. As compared to Sn-RGO, there is no hydrogen evolution occurred even the cathodic potential applied

was as high as -1.3 V. This may suggest that the SnNPs narrow the potential window, leading fewer analytes to be detected.

Fig. 5-8: SWASV responses of RGO-N/Si electrode to Cd²⁺, Pb²⁺ and Cu²⁺ in 0.1 M Na-ABS (pH 5) containing Cd²⁺, Pb²⁺ and Cu²⁺ of 100 nM each with preconcentration potentials of -0.8 V to -1.3 V and preconcentration time of 180 s.

At a constant preconcentration potential, the stripping peak current increases when the preconcentration time is increased from 60 s to 300 s, as shown in Fig. 5-9. In order to prevent a quick surface saturation of the working electrode at high concentration with more than one analyte, which can result in a poor sensitivity of the electrode, preconcentration potential of -1.1 V and preconcentration time of 180 s are selected for the subsequent investigations.

Fig. 5-9: SWASV responses of RGO-N/Si electrode to Cd^{2+} , Pb^{2+} and Cu^{2+} in 0.1 M Na-ABS (pH 5) containing Cd^{2+} , Pb^{2+} and Cu^{2+} of 100 nM each with preconcentration time of 60 s to 300 s and preconcentration potential of -1.1 V.

5.7 Calibration Curves Measured by RGO-N/Si Electrode toward Cd^{2+} , Pb^{2+} and Cu^{2+}

Fig. 5-10 (a-c) exhibits the SWASV responses of the RGO-N/Si toward Cd^{2+} , Pb^{2+} and Cu^{2+} individually with respect to their respective concentrations from 50 nM to 300 nM in the 0.1 M ABS (pH 5) at -1.1 V for 180 s. Good linear relationships between the well-defined and strong stripping currents and the concentrations of Cd^{2+} , Pb^{2+} and Cu^{2+} are determined from the three respective peaks (Fig. 5-10 (d)). The linear regression equations for Cd^{2+} , Pb^{2+} and Cu^{2+} are expressed as in equation 5-1.

$$i = 42.322[\text{Cd}^{2+}] - 0.1473 \quad (\mu\text{A}) \quad (5-1a)$$

$$i = 137.38[\text{Pb}^{2+}] - 0.172 \quad (\mu\text{A}) \quad (5-1b)$$

$$i = 70.34[\text{Cu}^{2+}] - 0.0964 \quad (\mu\text{A}) \quad (5-1c)$$

where i is the anodic stripping peak current in μA , while $[\text{Cd}^{2+}]$, $[\text{Pb}^{2+}]$ and $[\text{Cu}^{2+}]$ refer to the concentration of Cd^{2+} , Pb^{2+} and Cu^{2+} in μM . The regression coefficients of the above equations are 0.9969, 0.9996 and 0.9983, respectively, implying the good matches of the equations with the experimental results.

The LODs of Cd^{2+} , Pb^{2+} and Cu^{2+} obtained are 1.69 nM (0.19 ppb), 0.39 nM (0.08 ppb) and 2.16 nM (0.14 ppb) ($S/N=3$), with RSDs of 2.4 %, 1.8% and 5.1 %, respectively. Although the LODs achieved are higher than that of the Sn-RGO/GCS electrode, the RSDs of RGO-N/Si electrodes are lower than that of the Sn-RGO/GCS electrode, suggesting the higher stability of RGO-N/Si electrode than Sn-RGO/GCS electrode. The high stability of RGO-N/Si electrode demonstrates that the electrode do not incur fouling with the analytes or the compounds in the solution after performing redox reactions. This also means that the analytes could be easily detached from the surface of the electrode when sweeping applied, leaving the ‘clean’ surface of the electrode.

Fig. 5-10: : (a-c) SWASV responses of RGO-N/Si in individual analyses of Cd^{2+} , Pb^{2+} and Cu^{2+} over a concentration range of 50 nM to 300 nM each in 0.1 M ABS of pH 5 at -1.1 V for 180 s, and (d) their respective calibration curves

In the simultaneous determination of Cd^{2+} , Pb^{2+} and Cu^{2+} , the SWASV responses of the RGO-N/Si present obvious and well-defined stripping current peaks with good linear relationships with the concentrations of the three analytes as shown in Fig. 5-11. The linear regression equations for Cd^{2+} , Pb^{2+} and Cu^{2+} are illustrated as equation 5-2.

$$i = 5.7663[\text{Cd}^{2+}] - 0.0975 \quad (\mu\text{A}) \quad (5-2a)$$

$$i = 127.51[\text{Pb}^{2+}] - 0.564 \quad (\mu\text{A}) \quad (5-2b)$$

$$i = 40.691[\text{Cu}^{2+}] - 0.188 \quad (\mu\text{A}) \quad (5-2c)$$

The regression coefficients of the above equations are 0.9941, 0.9945 and 0.9787, respectively. Similarly to the Sn-RGO/GCS electrode, compared to the SWASV results measured from the individual trace metals, the sensitivity of the RGO-N/Si electrode to these three simultaneous analytes is significantly lower as presented in Table 5-2. This is due to the limited active sites on the electrode surface that is only able to accommodate a certain amount of analytes. Furthermore, the formation of an alloy, Pd-Cu (-0.46 V), during the deposition stage has suppressed the stripping current intensities of the three analytes [180]. As a result, the obtained LODs for Cd^{2+} , Pb^{2+} and Cu^{2+} are higher, which are about 21.6 nM (2.43 ppb), 9 nM (1.87 ppb) and 17.5 nM (1.11 ppb), respectively. Among the three analytes, Cd^{2+} exhibits the lowest stripping peak current, implying its poor absorptivity on the electrode surface. As compared to Sn-RGO/GCS electrode, the alloy peak Cd-Cu (-0.05 V) is less obvious for RGO-N/Si electrode. The possible reason may be the poorer absorptivity of RGO-N towards the analytes than that of Sn-RGO. Hence, the overall peak current of Cd-Cu was also lowered.

Fig. 5-11: (a) SWASV responses of RGO-N/Si in simultaneous analyses of Cd^{2+} , Pb^{2+} and Cu^{2+} over a concentration range of 50 nM to 300 nM each in 0.1 M ABS of pH 5 at -1.1 V for 180 s, and (b) their respective calibration curves

Table 5-2: Comparison of sensing performance during individual and simultaneous SWASV measurements

Analyte	Type of Detection	LOD (nM)	Sensitivity ($\mu\text{A}\mu\text{M}^{-1}$)
Cd^{2+}	Individual	1.69	42.32
	Simultaneous	21.6	5.77
Pb^{2+}	Individual	0.39	137.38
	Simultaneous	9.00	127.51
Cu^{2+}	Individual	2.16	70.34
	Simultaneous	17.5	40.69

5.8 Reproducibility, Repeatability and Stability of RGO-N

The reproducibility is also investigated with six RGO-N/Si electrodes in the electroanalysis of 100 nM of Cd^{2+} , Pb^{2+} and Cu^{2+} , respectively, under the optimized conditions. The obtained results, as presented in Fig. 5-12, demonstrate a good reproducibility of the electrodes with the RSDs of 4.7%, 4.4% and 3.7% for the peak current responses of Cd^{2+} , Pb^{2+} and Cu^{2+} , respectively. As compared to Sn-RGO in the chapter 4, RGO-N demonstrated lower RSDs because RGO-N was produced in large scale

and the parameters are easier to be controlled. Instead, Sn-RGO/GCS electrode was prepared one by one, in which the way of the electrodes were positioned may be slightly tilted during the removing and replacing process. Moreover, the uneven drop-cast surface may affect the charge distribution, and further affect the reduction process. In contrary, the non-uniform drop-cast surface will become more uniform after the thermal reduction due to the re-distribution and re-bonding of the particles.

Fig. 5-12: SWASV responses of six fabricated RGO-N electrodes.

The repeatability of RGO-N electrode is also studied through the electrochemical determination of nM of Cd²⁺, Pb²⁺ and Cu²⁺, respectively. As compared to Sn-RGO/GCS electrodes, the results, as shown in Fig. 5-13, illustrate a good repeatability of the electrodes with the RSDs of 5.8%, 5.6% and 5.9% for the peak current responses of Cd²⁺, Pb²⁺ and Cu²⁺, respectively. This reflects that although SnNPs can enhance the sensitivity,

it induced poor repeatability of the Sn-RGO/GCS electrodes, which may be due to the passivation of SnNPs upon several times of redox reactions. The passivation of SnNPs will eventually deteriorate the performance of the Sn-RGO/GCS electrodes. Hence, either a less likely poison-able nanoparticles could be employed to alleviate the instability of the electrodes or a disposable electrodes need to be introduced. Meanwhile, the prepared electrodes have confirmed a long-term stability with the RSDs of 5.6%, 5.3%, and 5.0% in the SWASV responses of Cd^{2+} , Pb^{2+} and Cu^{2+} , respectively, between the freshly prepared electrodes and the electrodes stored for 10 days after the preparation.

Fig. 5-13: SWASV responses of six repeatative tests on RGO-N electrode.

5.9 Comparison of Current Fabrication Method of RGO-N with Other Existing Methods

Table 5-3 compares the LOD results of Cd^{2+} , Pb^{2+} and Cu^{2+} obtained from this work with those reported in the literature in terms of materials and fabrication methods of electrodes, from which the RGO-N/Si electrode used in this study presents lower LODs, indicating a higher sensitivity. Although the RGO-N/Si shows a higher LOD of Pb^{2+} than the Nafion/Bi/NMC/GCE and a higher LOD of Cd^{2+} than both Nafion-graphene and Bi/Nafion-graphene nanocomposite electrodes, the RGO-N/Si electrode is able to detect Cu^{2+} that cannot be sensed by the both Nafion-graphene and Bi/Nafion-graphene nanocomposite electrodes. The Hg/graphene nanocomposite electrode provides the lowest LODs of the three analytes, but the toxicity of mercury may limit its applications. Furthermore, most of the reported electrodes are not meant for large scale production.

Table 5-3: Comparison of LODs of Cd^{2+} , Pb^{2+} and Cu^{2+} obtained from this work with those reported in the literature in terms of materials and fabrication methods of electrodes

Electrode	Summary of fabrication	Technique	LOD of Cd^{2+} (nM)	LOD of Pb^{2+} (nM)	LOD of Cu^{2+} (nM)	Ref.
Nafion-coated bismuth-film/GCE	Nafion was drop-cast on polished GCE, followed by curing by a hot air stream. The modified GCE was <i>in situ</i> and <i>ex situ</i> plated by Bi.	SWASV	0.9	1.93	NA	[140]
Single-	Mixture of SWCNT-Nafion	LSV	4	NA	NA	[141]

walled CNT (SWCNT)-Nafion film/GCE	was drop-cast on polished GCE, followed by evaporation.					
Nafion/Bi/NMC/GCE	Thermally produced nitrogen doped microporous carbon (NMC) was mixed with Nafion, followed by drop-casting on polished GCE and evaporation. Then, <i>in situ</i> Bi plating was performed.	DPASV	13.34	0.24	NA	[135]
Hg/graphene nanocomposite electrode	Mixture of graphene solution and Nafion-isopropyl alcohol was drop-cast on GCE, followed by <i>in situ</i> mercury (Hg) plating during electroanalysis.	SWASV	0.71	0.34	1.10	[142]
Hg/graphene nanocomposite electrode	Mixture of graphene solution and Nafion-isopropyl alcohol was drop-cast on GCE, followed by Hg plating on the modified	DPASV	0.04	NA	NA	[143]

	GCE					
Nafion-graphene nanocomposite electrode	Pre-treated pencil graphite electrode was immersed into Nafion-graphene solution for 5 min, followed by air drying.	SWASV	0.80	0.63	NA	[144]
Bi/Nafion-graphene composite film electrode	Graphene-Nafion mixture was coated on GCE, followed by solvent evaporation under an infrared lamp. Then, in situ Bi plating was performed during electroanalysis.	DPASV	0.18	0.10	NA	[128]
(this work)	Mixture of GO and Nafion was drop-cast on Si, followed by thermal annealing.	SWASV	1.69	0.39	2.16	

5.10 Real Sample Application

In order to demonstrate the performance of the RGO-N/Si electrodes in real sample analysis, the fabricated RGO-N/Si electrodes are employed for simultaneous detection of Cd^{2+} , Pb^{2+} and Cu^{2+} in tap water samples. The results of the recovery tests obtained from the RGO-N/Si electrode, as shown in Table 5-4, illustrate the deviation from the actual

results of less than 5 %. This indicates the promising performance in the determination of trace heavy metals the in real samples.

Table 5-4: Recovery test results from simultaneous detection of Cd^{2+} , Pb^{2+} and Cu^{2+} in tap water samples

Sample	Target metals			
		Added (nM)	Measured (nM)	Recovery (%)
Tap water	Cd^{2+}	150	156	104.0
		250	260	104.0
	Pb^{2+}	150	153	102.0
		250	247	98.8
	Cu^{2+}	150	145	96.7
		250	245	98.0

5.11 Summary

In this chapter, the RGO-N thin film electrodes coated on the Si substrates (RGO-N/Si) were successfully developed through thermal reduction of GO-N. The effectiveness of the GO reduction was proven by XPS analysis, in which the C/O ratio was increased from about 2.06 to 20.96 for the GO-N and RGO-N (750 °C), respectively. RS analysis demonstrated that the RGO-N had a lower I_D/I_G ratio than the RGO, suggesting the nanostructure restoration induced by the polyfluorocarbon in the Nafion.

In the fabrication of RGO-N/Si, the optimum thermal annealing temperature was 750 °C. The RGO-N/Si samples that were annealed at temperatures below 750 °C showed lower C/O ratios, while above 750 °C, the RGO sheets peeled off the substrates, exposing

the non-electrochemically conductive Si, thus leading to a poor performance of the electrodes in electroanalysis. For the RGO-N/Si annealed at 900 °C, the XPS results demonstrated a very low percentage of polyfluorocarbon, suggesting that the polyfluorocarbon in the Nafion was able to prevent the delamination of the RGO-N layers from the Si substrates. In the process, the thermal annealing duration (dwelling time) did not bring a significant effect on the RGO-N/Si. It was deduced that a slow cooling rate of the high temperature annealed RGO-N/Si caused on-going reduction during the cooling process. Hence, the effect of the thermal annealing duration was difficult to determine.

In the SWASV measurements for the detection of Cd^{2+} , Pb^{2+} and Cu^{2+} individually and simultaneously, the RGO-N/Si electrodes exhibited a high sensitivity and a highly linear behavior in terms of relationship between stripping peaks current and trace metal concentration, with the optimum preconcentration potential of -1.1 V, preconcentration time of 180 s and pH 5.0. Furthermore, the RGO-N/Si electrodes presented good reproducibility, repeatability and storage stability. In the real application investigation, the RGO-N/Si electrode proved good recoveries of the target trace heavy metals in the tap water samples, indicating a high potential of the RGO-N/Si electrodes in the electroanalysis of real samples.

Chapter 6: Reduced Graphene Oxide-Nafion Decorated with Platinum Nanoparticles through Thermal Reduction

6.1 Overview

From Chapter 5, the delamination of RGO from the substrate has been overcome by adding Nafion during thermal reduction. In addition, the fabricated RGO-N/Si electrode possesses high sensitivity and selectivity, with LODs of 1.69 nM, 0.39 nM and 2.16 nM for Cd^{2+} , Pb^{2+} and Cu^{2+} , respectively. However, the alloy of Pb-Cu that formed at the potential of about -0.46 V during SWASV, has yet to be avoided, as presented in Fig. 6-1. The alloy could cause the error in detection and high RSD to the results. A common method to alleviate the alloy is to use a complexing agent, such as gallium ions to form Cu-Ga and thus suppress the formation of alloys with copper [181, 182]. However, this method is not feasible to apply in our model because Cu^{2+} is one of the targeted analytes in the solution.

Fig. 6-1: SWASV response to Cd^{2+} , Pb^{2+} and Cu^{2+} , measured by using RGO-N/Si electrode.

It has been reported that different metals or surface morphology have different tendency to form alloys or intermetallic compounds. For example, Cu-Zn intermetallic compounds are less prone to form on HMDE than mercury film electrode (MFE) [183]. Furthermore, the formation of Cu-As (copper-arsenic) intermetallic complex is more pronounced on macro gold electrode than gold nanoparticle modified GCE [184]. Nevertheless, the exact reason on the suppression of the formation of alloys is still not clear.

Platinum nanoparticles (PtNPs) have also been widely employed in fuel cell [185, 186] and biosensing [187-189] due to their high surface area to volume ratio, biocompatibility, and high electrical conductivity and high catalytic activity [190]. In addition, PtNPs have also been used as modified in working electrode for the voltammetry determination of arsenic [57, 191] and Cu^{2+} [148]. It has been reported that PtNPs modified boron doped diamond microelectrodes [192] and PtNPs modified GCE [191] have overcome the alloy interference in arsenic detection. In contrast, the application of PtNPs in sensing of Pb and Cd has seldom been discussed.

In this work, RGO was functionalized with PtNPs and drop cast on Si substrate, followed by thermal annealing at high temperature to produce Pt-RGO/Si. As compared to most the synthesis method of PtNPs/RGO [188, 190, 193], our method is able to mass produce Pt-RGO with simple procedure. A mechanism of the formation of Pt-RGO through thermal reduction was proposed. The prepared Pt-RGO/Si electrode was then applied in the ASV simultaneous determination of Cd, Pb and Cu. The results confirmed the high sensitivity and selectivity of the Pt-RGO/Si in trace heavy metal detection.

6.2 Confirmation of the Formation of Pt-RGO

The FE-SEM micrograph of Pt-RGO, as shown in Fig. 6-2, exhibited the uniformly distributed PtNPs over the RGO sheets. The size of the PtNPs is generally quite consistent, with the average diameter size of 20 nm. However, there are a few particles with around diameter size of 100 nm are visible. These particles are the PtCl_2 or PtCl_4 , which did not fully decompose during the thermal annealing process. The wrinkled surface of the RGO sheets could also be observed, in which these wrinkled surface could increase the total surface area of the Pt-RGO electrode. In addition, the RGO sheets have prevented the PtNPs from agglomeration, increasing the total active site to the analytes during electroanalysis.

Fig. 6-2: FE-SEM micrograph of Pt-RGO (top view)

Fig. 6-3 demonstrates the high resolution of the cross-sectional FE-SEM micrograph of Pt-RGO. The thickness of the Pt-RGO deposit on the Si substrate is about 100 nm. Moreover, most of PtNPs are surrounded by RGO sheets, prevents the aggregation of PtNPs from forming a big islands, as well as preventing the RGO from agglomeration. There is no clear boundary from one layer to the subsequent layers, because of the concurring process of thermal decomposition of both H_2PtCl_6 and GO sheets. There are some porous area could be noticed which may be created during thermal decomposition. The porous area could increase the total surface area of the electrode, enhancing the overall sensitivity in the trace heavy metals analysis.

Fig. 6-3: FE-SEM micrograph of Pt-RGO (cross-sectional view)

Similarly to the previous samples, Raman spectroscopy was also used to characterize the changes of conjugated and double $\text{C}=\text{C}$ bonds in the samples. There are two prominent characteristic peaks, which are D band (1350 cm^{-1}) and G band (1580 cm^{-1})

¹), as shown in the spectra of the three samples in Fig. 6-4. In general, D band is induced by structural defects, while G band proves the existence of sp^2 hybridized carbon-based compounds. The D/G peak intensity ratio (I_D/I_G) indicates the degree of structural defects. Fig. 6-4 illustrates the RS curves of the three samples, showing the changes in the intensity of D peak and G peak upon thermal reduction. The width of the D band and G band are also decreased after thermal reduction, reflecting the restoration of the graphitic structure of RGO sheets.

Fig. 6-4: Raman spectra of Pt-GO, RGO-N and Pt-RGO.

As presented in Table 6-1, the I_D/I_G of Pt-GO significantly increases from 1.04 to 1.52 after annealing process, indicating reduction of GO to RGO occurred. Furthermore, as compared to RGO-N with I_D/I_G of 1.18, the I_D/I_G of RGO-Pt is higher, which is 1.52.

This implies the successful intercalation of PtNPs, which could induce higher structural defects to the RGO matrix.

Table 6-1: I_D/I_G ratios of Raman spectra measured from Pt-GO, RGO-N and Pt-RGO

Samples	I_D/I_G
Pt-GO	1.04
RGO-N	1.18
Pt-RGO	1.52

6.3 Effect of H_2PtCl_6 :GO Ratio

In order to optimize the detection ability of the Pt-RGO/Si electrode, the weight ratio of PtNPs and RGO was investigated. The mixtures of Pt-GO were prepared in the Pt:GO ratio of 0.1, 0.5, 1 and 2, followed by drop-cast and annealing at 750 °C for 2 hrs. Fig. 6-5 exhibits the SWASV responses of the Pt-RGO/Si electrodes with different Pt-GO ratio towards 100 nM Pb^{2+} in 0.1 Na-ABS (pH 5). It could be noticed that the peak currents increased when the samples with Pt-GO ratio increased from 0.1 to 0.5. However, the peak currents begin to drop when the samples with Pt-GO ratio were further increased to 1 and 2. Moreover, the standard deviations of the SWASV responses of the samples with Pt-GO ratio of 1 and 2 are higher, indicating the poor stability of the samples. When Pt:GO ratio was increased, PtNPs tend to aggregate and form large islands, as shown in Fig. 6-6. As a result, the catalytic function of nanoparticles lost, and replaced with the micro-effect of Pt, which has poorer detection ability.

Fig. 6-5: SWASV responses of the Pt-RGO electrodes with different Pt-GO ratio towards 100 nM Pb^{2+} in 0.1 Na-ABS (pH 5).

Fig. 6-6: FE-SEM micrograph of Pt-RGO with the H_2PtCl_6 : GO weight ratio of 2.

6.4 Proposed Mechanism of the Formation of Pt-RGO

Fig. 6-7 presents the schematic illustration of the thermal process mechanism of Pt-RGO nanocomposite. When H_2PtCl_6 solution was added into GO suspension, PtCl_4^- were uniformly distributed in GO suspension. At low temperature heating, H_2PtCl_6 and GO suspension will be firstly dried, in which the excess water that trapped in the compounds will be removed through evaporation. Meanwhile, the H_2PtCl_6 will be decomposed to PtCl_4 [164] and release the by-product HCl , as shown in equation 6-1. After that, annealing process was continuing carried out to 750°C . PtCl_4 , with a larger diameter size will decompose to PtNPs, with about average diameter size of 20 nm, as described in equation 6-2. Meanwhile, GO will be reduced to RGO. Subsequently, the PtNPs will react with RGO during annealing process and form Pt-RGO.

Fig. 6-7: Schematic illustration of the thermal reduction mechanism of Pt-RGO nanocomposite

6.5 Electrochemical Behaviours of RGO-N and Pt-RGO

Similarly to Sn-RGO, the electrochemical behaviours of the modified electrodes were studied by CV and EIS. The CV responses of bare GCE, Pt-GO/Si, RGO-N/Si and Pt-RGO/Si electrodes, as shown in Fig. 6-8, was investigated using 5 mM of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ and 0.1 M KNO_3 . Pt-GO/Si electrode illustrates an undefined curve, implying the irreversible process of oxygen functional groups of GO and platinum chloride. Moreover, GO possess several functional groups that could block the electrons transferred [154], causing low current intensity. As compared to bare GCE, RGO-N/Si electrode and Pt-RGO/Si electrode exhibit the well-defined peaks and higher currents. In addition, Pt-RGO/Si electrode shows the smallest peak potential separation, indicating the high electron transfer rate that may be due to the enhancement of the electrocatalytic activities of RGO by PtNPs.

Fig. 6-8: CV responses of GCE, Pt-GO/Si, RGO-N/Si and Pt-RGO/Si electrodes in 5 mM of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ and 0.1 M KNO_3 .

In Nyquist plot, the diameter of the semicircle represents the electron-transfer resistance (R_{et}) at the interface between electrodes and electrolytes during electrochemical reaction [167]. From the Fig. 6-9, the bare GCE gives the highest R_{et} , which is about 100 Ω , followed by RGO-N/Si electrode ($\sim 60 \Omega$) and Pt-RGO/Si electrode ($\sim 40 \Omega$). This suggests the low electron transfer resistance of RGO. In addition, the R_{et} is further reduced by adding PtNPs into the RGO matrix. This implies the synergistic effect between PtNPs and RGO could promote the interfacial charge transfer of Pt-RGO/Si electrode. Moreover, the intercalation between PtNPs and RGO sheets prevents the RGO sheets from agglomeration. As a result, it confirms the large active surface area and high electrical conductivity of RGO and PtNPs.

Furthermore, as compared to electrochemically reduced RGO/GCS electrode ($\sim 86 \Omega$) in the chapter 4, the RGO-N/Si electrode obtained demonstrates lower R_{et} , reflecting a higher electrical conductivity of RGO-N/Si electrode. This may indicate that the thermal reduction is more effective in removing oxygen functional groups from GO than that of electrochemical reduction. Meanwhile, the R_{et} is further reduced by 33 % after the addition of PtNPs into the RGO matrix. In contrast, the R_{et} has lower reduction rate, which is 27 % after the addition of SnNPs into the RGO matrix. This may imply that the PtNPs could either promote a higher electron transfer rate or induces a higher total surface area to the Pt-RGO/Si electrode.

Fig. 6-9: EIS responses of GCE, Pt-GO/Si, RGO-N/Si and Pt-RGO/Si electrodes in 5 mM of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ and 0.1 M KNO_3 .

6.6 Optimization of Electroanalysis Parameters

Preconcentration potential, preconcentration time and pH value are among the vital preconditioning factors. Fig. 6-10 presents the peak currents of Cd^{2+} , Pb^{2+} and Cu^{2+} detected in the range of -0.9 V to -1.3 V. In general, a higher cathodic potential induces more analytes to be reduced. However, unlike RGO-N/Si electrode, the peak currents measured by Pt-RGO/Si electrode do not increase accordingly when the cathodic potentials were increased from -0.9 V to -1.3 V. Instead, the peak currents increases as the cathodic potential was increased from -0.9 V to -1.1 V, but the peak currents reduces significantly when the potential was increased from -1.1 V to -1.3 V, indicating the onset of hydrogen evolution that deteriorates the surface activity of the electrode. Therefore, the analytes are less likely to be deposited on the surface during preconditioning steps. Hence, the analytes

involved in SWASV sweeping reduces, contributing fewer electrons released to the system. From the SWASV responses of the Pt-RGO/Si electrode, it indirectly reflects that the addition of PtNPs lower the overpotential of hydrogen evolution. Nevertheless, the involvement of PtNPs in Pt-RGO/Si electrode produced higher peak currents than RGO-N before the onset of hydrogen evolution, suggesting the higher activity of Pt-RGO.

Fig. 6-10: SWASV responses of Pt-RGO/Si electrode to Cd^{2+} , Pb^{2+} and Cu^{2+} in 0.1 M Na-ABS (pH 5) containing Cd^{2+} , Pb^{2+} and Cu^{2+} of 100 nM each with preconcentration time of 180s and preconcentration potentials varied from -0.8 V to -1.3 V.

Fig. 6-11 shows the peak currents of Cd^{2+} , Pb^{2+} and Cu^{2+} detected in the duration from 60 s to 300 s. As the preconcentration time increased, more analytes were reduced and hence produced high peak currents. However, saturation of the peak currents started at 240 s. Hence, 180 s of preconcentration time was selected for the subsequent

experiments. The trend of the results is similar as the results obtained from RGO-N/Si electrode, but higher peak currents were recorded for Pt-RGO/Si electrode.

Fig. 6-11: SWASV responses of Pt-RGO/Si electrode to Cd²⁺, Pb²⁺ and Cu²⁺ in 0.1 M Na-ABS (pH 5) containing Cd²⁺, Pb²⁺ and Cu²⁺ of 100 nM each with preconcentration potential of -1.1 V and preconcentration time varied from 60 s to 300 s.

Fig. 6-12 demonstrates the peak currents of the three analytes in the pH range of 3 to 6. At low pH, hydrogen evolution that could deteriorate the surface activity of the electrode tends to occur. Hence, at low pH 3, the peak currents obtained are low, with high standard deviation. However, at high pH, formation of hydroxide compounds easily happened, reducing the number of free metal ions. As compared to RGO-N/Si electrode and Sn-RGO/GCS electrode (chapter 4), the trends of the results are similar, indicating pH 5 of the Na-ABS is the most suitable pH for the working environment of RGO based electrodes.

Fig. 6-12: SWASV responses of Pt-RGO/Si electrode to Cd²⁺, Pb²⁺ and Cu²⁺ in 0.1 M Na-ABS containing Cd²⁺, Pb²⁺ and Cu²⁺ of 100 nM each with preconcentration potential of -1.1 V, preconcentration time of 180 s and pH varied from 3 to 6.

6.7 Calibration Curves Measured by Pt-RGO toward Cd²⁺, Pb²⁺ and Cu²⁺

Fig. 6-13 (a-c) present the SWASV responses of the Pt-RGO/Si electrode to Cd²⁺, Pb²⁺ and Cu²⁺ individually with the concentration varying from 50 nM to 300 nM in the 0.1 Na-ABS (pH 5) at -1.1 V for 180 s. The three well-defined peaks with strong stripping currents show good linear relationship with the concentration of Cd²⁺, Pb²⁺ and Cu²⁺, respectively (Fig. 6-13(d)). The linear regression equation for Cd²⁺, Pb²⁺ and Cu²⁺ can be expressed as in equation 6-3.

$$I = 88.217[\text{Cd}^{2+}] - 0.8258 \quad (\mu\text{A}) \quad (6-3a)$$

$$I = 202.31[\text{Pb}^{2+}] - 0.4551 \quad (\mu\text{A}) \quad (6-3b)$$

$$I = 117.31[\text{Cu}^{2+}] - 0.0667 \quad (\mu\text{A}) \quad (6-3c)$$

where i is the anodic stripping peak current in μA , while $[\text{Cd}^{2+}]$, $[\text{Pb}^{2+}]$ and $[\text{Cu}^{2+}]$ refer to the concentration of Cd^{2+} , Pb^{2+} and Cu^{2+} in μM . The linear regression coefficient of Cd^{2+} , Pb^{2+} and Cu^{2+} are 0.9992, 0.9987 and 0.9994, respectively. The LOD of Cd^{2+} is 0.87 nM (0.098 ppb) with the relative standard deviation of 2.6 %. The LOD of Pb^{2+} is 0.55 nM (0.114 ppb) with the relative standard deviation of 3.7 %, while the LOD of Cu^{2+} is 1.69 nM (0.107 ppb) (S/N=3) with the relative standard deviation of 6.6 %, respectively.

As presented in Fig. 6-13(d), the stripping peak currents to the concentration responses of $[\text{Pb}^{2+}]$ gives the steepest straight line, followed by $[\text{Cu}^{2+}]$ and $[\text{Cd}^{2+}]$. This indicates the Pt-RGO/Si electrode demonstrating the highest sensitivity detection towards Pb^{2+} , which may due to the strongest absorptivity of Pt-RGO towards Pb^{2+} during pre-conditioning, as compared to the other two analytes.

Fig. 6-13: (a-c) Stripping voltammograms of electrochemically reduced Pt-RGO/Si electrode in individual analyses of Cd^{2+} , Pb^{2+} and Cu^{2+} over a concentration range of 50 nM to 300 nM each in 0.1 M Na-ABS (pH 5) at -1.1 V for 180 s, and (d) their respective calibration curves.

In simultaneous determination of Cd^{2+} , Pb^{2+} and Cu^{2+} , the SWASV responses of the Pt-RGO/Si electrode illustrate well-defined peaks with good linear relationships with the concentration of the three metals ion, as presented in Fig. 6-14. The linear regression equations of Cd^{2+} , Pb^{2+} and Cu^{2+} can be expressed as in equation 6-4.

$$I = 20.326[\text{Cd}^{2+}] - 0.2797 \quad (\mu\text{A}) \quad (6-4a)$$

$$I = 174.33[\text{Pb}^{2+}] - 0.4488 \quad (\mu\text{A}) \quad (6-4b)$$

$$I = 75.914[\text{Cu}^{2+}] - 0.1305 \quad (\mu\text{A}) \quad (6-4c)$$

The correlation coefficient of Cd^{2+} , Pb^{2+} and Cu^{2+} are 0.9934, 0.9950 and 0.9959, respectively. The LODs for Cd^{2+} , Pb^{2+} and Cu^{2+} are 6.08 nM (0.683 ppb), 0.93 nM (0.193 ppb) and 2.88 nM (0.183 ppb), with the RSDs of 4.1 %, 5.4 % and 7.3 %, respectively. As compared to individual electroanalysis, the sensitivity of the electrode towards the three analytes dropped, as summarized in Table 6-2. This suggests the limited active sites of the electrodes that only able to support certain concentration of the total analytes.

In general, the standard deviation of the individual and simultaneous analysis is quite small. This may be due to low formation rate of alloys, which is Pd-Cu that could severely affect the stability of the electroanalysis, as occurred in RGO-N/Si electrode as discussed in chapter 5. As a result, lower LOD could be achieved. Nevertheless, the sensitivity towards Cd^{2+} is the lowest among the three analytes, implying to its poor absorptivity on the electrode surface.

Fig. 6-14: (a) Stripping voltammograms of electrochemically reduced Pt-RGO/Si electrode in simultaneous analyses of Cd^{2+} , Pb^{2+} and Cu^{2+} over a concentration range of 50 nM to 300 nM each in 0.1 M Na-ABS (pH 5) at -1.1 V for 180 s, and (b) their respective calibration curves.

Table 6-2: Comparison of sensing performance during individual and simultaneous SWASV measurements

Analyte	Type of Detection	LOD (nM)	Sensitivity ($\mu\text{A}\mu\text{M}^{-1}$)
Cd²⁺	Individual	0.87	88.22
	Simultaneous	6.08	20.33
Pb²⁺	Individual	0.55	202.31
	Simultaneous	0.93	174.33
Cu²⁺	Individual	1.69	117.31
	Simultaneous	2.88	75.91

6.8 Reproducibility, Repeatability and Storage Stability of Pt-RGO

The reproducibility of Pt-RGO/Si electrode was investigated with six Pt-RGO/Si electrodes in the electroanalysis of 100 nM of Cd²⁺, Pb²⁺ and Cu²⁺, respectively, under the optimized conditions. The obtained results, as presented in Fig. 6-15, illustrate a good reproducibility of the electrodes with RSDs of 6.4%, 5.7% and 7.3% for the peak current responses of Cd²⁺, Pb²⁺ and Cu²⁺, respectively. The reproducibility of Pt-RGO/Si electrode is comparable with RGO-N/Si electrode, but with a slightly higher RSDs. This is because the concurrence processes, which are the thermal reduction of RGO and the decomposition of the H₂PtCl₆, may induce higher deviation in the production. Meanwhile, the prepared electrodes have confirmed a long-term stability with the RSDs of 6.5%, 6.4%, and 7.1% in the SWASV responses of Cd²⁺, Pb²⁺ and Cu²⁺, respectively, between the freshly prepared electrodes and the electrodes stored for 10 days after the preparation.

Fig. 6-15: SWASV responses of 6 Pt-RGO/Si samples.

In addition, the repeatability of the Pt-RGO/Si electrode was examined with 6 tests under optimized conditions. The SWASV peak currents of Cd²⁺, Pb²⁺ and Cu²⁺ were recorded as depicted in Fig. 6-16. As compared to RGO-N/Si electrode and Sn-RGO/GCS electrode, Pt-RGO/Si electrode possesses the poorest repeatability. From the Fig. 6-16, the RSD of the first three tests is low, but the fourth test onwards, the peak currents of the analytes demonstrate significant reducing trend. The decrease of the sensitivity may be due to the poisoning of the PtNPs, leading to irreversible reactions. Although the porous structure of the Pt-RGO/Si electrode could induce a higher surface area for detection, it may cause the entrapment of the analytes, leading to the poor detachment of the analytes from the Pt-RGO surface. However, the high catalytic activity of PtNPs could alleviate the problem related to alloys due to their high sensitivity to the analytes, and thus suppressing the formation of the Pb-Cu. Hence, there are pros and cons of employing the PtNPs in the Pt-RGO. Nevertheless, the design of the electrode was meant for disposable.

Fig. 6-16: SWASV responses of 6 repetitive tests of Pt-RGO/Si electrode.

6.9 Comparison of Electrodes

SWASV is widely employed in trace heavy metals electroanalysis due to its high sensitivity and cost effectiveness [58]. Fig. 6-17 presents the SWASV responses of Cd²⁺, Pb²⁺ and Cu²⁺ simultaneously on bare GCE, RGO/Si electrode, RGO-N/Si electrode and Pt-RGO/Si electrode. There are three peaks in the graph, corresponding to Cd²⁺, Pb²⁺ and Cu²⁺, at the potentials of -0.8 V, -0.5 V and -0.1 V, respectively. Based on the graph, Pt-RGO/Si electrode demonstrates the highest current intensity, indicating its high sensitivity and conductivity. This may be due to the strong absorptivity towards the three metal ions, high surface area and electrical conductivity of RGO and PtNPs. Furthermore, as compared to RGO-N/Si electrode, the alloy, Pb-Cu (-0.4 V) peak was not obvious for Pt-RGO/Si electrode. This suggests the strong electrocatalytic activity of PtNPs that promotes the redox reaction of the analytes, suppressing the formation of the alloys.

RGO/Si electrode performs poor SWASV responses, mainly due to the delamination of RGO sheets that expose the non-conductive Si substrate.

Fig. 6-17: SWASV responses of Cd^{2+} , Pb^{2+} and Cu^{2+} on bare GCE, RGO/Si electrode, RGO-N/Si electrode and Pt-RGO/Si electrode in 0.1 M Na-ABS (pH 5) containing 100 nM of Cd^{2+} , Pb^{2+} and Cu^{2+} .

In addition, Pt-RGO/Si electrode was compared with other existing electrodes in the previous reports. Table 6-3 discusses the LODs of Cd^{2+} , Pb^{2+} and Cu^{2+} measured by platinum based electrodes, PtNPs modified electrode and other metal NPs modified electrodes. The Pt-RGO/Si electrode achieved the lowest LODs among the previous reports, except to SnO_2/RGO nanocomposite electrode, suggesting the effectiveness of Pt-RGO/Si electrode in trace heavy metals detection. Although SnO_2/RGO nanocomposite electrode possesses lowest LODs, the fabrication method is not feasible to large scale production.

Table 6-3: Comparison of the analytical performance of modified electrodes for trace heavy metal detection.

Electrode	Technique	LOD of Cd²⁺ (nM)	LOD of Pb²⁺ (nM)	LOD of Cu²⁺ (nM)	Ref.
Hydroxyapatite modified platinum electrode	SWV	88.5	NA	NA	[145]
Mercury plated platinum microelectrode array	SWASV	2.67	0.48	7.87	[146]
Kaolin modified platinum electrode	SWV	5.9	NA	NA	[147]
PtNPs incorporated CPE	ASV	NA	NA	3.9	[148]
Hydroxyapatite modified platinum electrode	SWV	NA	20.1	NA	[149]
Bismuth nanoparticle modified boron doped	SWASV	20.46	9.17	NA	[150]

diamond					
Gold Nanoparticles-Modified Screen-Printed Electrode	LSASV	NA	6.27	NA	[151]
Glutathione stabilized silver nanoparticles (GSH-AgNPs) modified screen printed electrode	CV	NA	49.63	NA	[152]
Antimony nanoparticle modified boron doped diamond (Sb-BDD) electrode	LSASV	339	124	NA	[153]
SnO₂/Reduced Graphene Oxide Nanocomposite	SWASV	0.10	0.18	0.23	[154]
(This work)	SWASV	1.69	0.55	0.87	

6.10 Real Sample Application

Furthermore, in order to investigate the performance of the Pt-RGO/Si electrode in real sample analysis, the fabricated electrode was evaluated and applied for simultaneous detection of Cd^{2+} , Pb^{2+} and Cu^{2+} in tap water samples. The results of the recovery tests obtained from the Pt-RGO/Si electrode, as shown in Table 6-4, proves the promising performance of the Pt-RGO/Si electrode in the determination of trace heavy metals in real samples.

Table 6-4: SWASV results measured from tap water samples using Pt-RGO/Si electrode for simultaneous determination of Cd^{2+} , Pb^{2+} and Cu^{2+} ions.

Sample	Target metals			
		Added (nM)	Measured (nM)	Recovery (%)
Tap water	Cd^{2+}	100	100.3	100.3
		200	193.3	96.7
	Pb^{2+}	100	98.6	98.6
		200	205.3	102.7
	Cu^{2+}	100	98.4	98.4
		200	207.7	103.8

6.11 Summary

In this chapter, the Pt-RGO/Si electrodes were successfully developed through thermal annealing process. In fact, the fabrication process was similar as used for the RGO-N/Si, with the only difference of the addition of H_2PtCl_6 into the GO-N mixture before thermal reduction. The significant increment of I_D/I_G in the RS investigation suggested a good

intercalation between the PtNPs and RGO sheets. The FE-SEM micrographs also illustrated the uniform distribution of PtNPs in the RGO-N sheets.

In the fabrication process, the weight ratio of $\text{H}_2\text{PtCl}_6\text{:GO-N}$ was a crucial factor for highly sensitive Pt-RGO/Si electrodes. When the ratio was lower than 0.5, the PtNPs were not enough to bring about a significant effect, while the ratio was higher than 1, the PtNPs tended to coagulate and form larger islands, losing their nano-effect [194]. From the process, it was found that the ratio of 0.5 gave the best performance in electroanalysis. Furthermore, the fabricated Pt-RGO/Si electrodes demonstrated good reproducibility and storage stability, but with a poor repeatability after the 3rd test, indicating the poisoning of the PtNPs. In addition, the porous structure of the Pt-RGO/Si electrodes may cause the entrapment of the analytes from easily detached from the surfaces of electrodes.

The prepared Pt-RGO/Si electrodes were further characterized through electrochemical methods. Both CV and EIS results indicated a synergistic effect between PtNPs and RGO, which promoted a high electron transfer rate. In the both individual and simultaneous detection of Cd^{2+} , Pb^{2+} and Cu^{2+} , the modified Pt-RGO/Si electrodes presented highly linear behaviour for the SWASV peak current measurements and high sensitivity. The LODs achieved from the individual detection of Cd^{2+} , Pb^{2+} and Cu^{2+} were about 1.69 nM (0.19 ppb), 0.55 nM (0.08 ppb) and 0.87 nM (0.14 ppb), respectively. However, the LODs from the simultaneous detection of Cd^{2+} , Pb^{2+} and Cu^{2+} were increased to about 6.08 nM (0.68 ppb), 0.93 nM (0.19 ppb) and 2.88 nM (0.18 ppb), respectively, suggesting the limited active sites of the electrodes.

Nevertheless, the standard deviations for both individual and simultaneous detections remained almost the same, which was below 8 %, implying a good stability of the electrodes. The good stability of the electrodes was mainly due to the less formation of alloys that could otherwise severely affect the stability and sensitivity of the electrodes. The strong electrocatalytic activity of PtNPs could promote the redox reactions of the analytes, and hence suppress the formation of alloys (Pb-Cu). In the real sample application, the Pt-RGO/Si electrodes showed good recoveries of the target metals in the tap water samples, proving their feasibility in real sample application.

Chapter 7: Conclusions

In the project, graphene based electrodes decorated with SnNPs and PtNPs were prepared for simultaneous determination of trace heavy metals in aqueous solutions. In general, RGO was employed in the electrodes because it had high electrical conductivity, inertness to the analytes and large surface area. Meanwhile, metal nanoparticles were used because they could prevent the RGO sheets from agglomeration, and enhance the electron transfer rate in the electrodes. Successfully, the prepared electrodes possessed high sensitivity and stability in the electroanalysis of Cd^{2+} , Pb^{2+} and Cu^{2+} . In addition, real sample application was performed to prove the feasibility of the electrodes. The electrodes presented good recoveries in the tap water electroanalysis.

Two methods, namely electrochemical reduction and thermal reduction, were employed to reduce GO to RGO in this study. Based on the C/O ratios determined from XPS, the thermally reduced GO showed higher C/O ratios, leading to higher electron transfer rates, as proved by using EIS and CV measurements. As a result, the thermally reduced RGO exhibited higher sensitivity and selectivity in the electroanalysis of trace heavy metals. As the thermally reduced RGO was prepared in batches, while electrochemically reduced GO was fabricated in pieces, thermal reduction could achieve large scale production, saving production time and cost. As the RGO electrodes were produced at large scale, the reproducibility rate was higher, eliminating the detection errors. However, as compared to the electrochemical method, the thermal reduction involved a more complex operating process, including high temperature and high vacuum. In contrast, the electrochemical method only required a power supply or a potentiostat to reduce the GO that was drop cast on the GCS.

In the electrochemical reduction, the RGO electrodes were further modified with SnNPs to increase their sensitivity and selectivity. A good intercalation of SnNPs and RGO matrix was verified by Raman spectroscopy, while the uniform distribution of SnNPs in the RGO matrix was investigated by FE-SEM. By decorating RGO with SnNPs, the stacking of the RGO sheets could be prevented, resulting larger total surface area for redox reactions. Moreover, according to the EIS and CV results, the SnNPs could also increase the electron transfer rate, improving the sensitivity of the electrodes. The optimized Sn-RGO/GCS electrodes demonstrated higher signal to noise ratios as compared to the GCS and RGO/GCS electrodes. Furthermore, it showed lower LODs of Cd^{2+} , Pb^{2+} and Cu^{2+} as compared to the reported electrodes in the literature. However, when Sn:GO weight ratio became too high, the reduction of GO would be affected, which was because when Sn^{2+} was added into GO suspension, GO was partially reduced, while Sn^{2+} was oxidized to Sn^{4+} . The by-product, such as HCl lowered the pH of the mixture, causing the agglomeration of the partially reduced GO and GO suspension. As a result, the electrochemical reduction of GO became less effective.

In the thermal reduction of GO, Nafion was added into the GO suspension, followed by the drop-casting on Si substrates. Without the Nafion added, the thermally reduced RGO layers easily peeled off from the Si substrates, exposing the relatively non-conductive Si surfaces to the electroanalysis. Hence, the SWASV responses to the analytes became poor. By adding Nafion, the delamination problem was alleviated, improving the sensitivity of the RGO-N/Si electrodes. According to the XPS and Raman spectroscopy results, the polyfluorocarbon in Nafion played the binding and self-healing

roles, preventing the delamination of the RGO sheets from the substrates and improving the restoration of graphitic structure in the RGO.

Based on the SWASV results obtained using the GCE, Sn-RGO/GCS electrodes and RGO-N/Si electrodes, the alloy, Pb-Cu existed. The alloys were usually formed during the pre-conditioning, which could severely affect the stability of the detection. Hence, the RGO-N/Si electrodes were decorated with PtNPs. Based on the results obtained from EIS and CV, the Pt-RGO/Si electrodes demonstrated the highest electron transfer rate and lowest electron resistance among the Sn-RGO/GCS electrodes, RGO-N/Si electrodes and GCE. As a result, in the SWASV responses, the Pt-RGO/Si electrodes showed the relatively highest peak current intensities of Cd^{2+} , Pb^{2+} and Cu^{2+} , with much lower relative standard deviations. Furthermore, the peak current intensity of alloy, Pb-Cu was suppressed, which could be due to the increase of the sensitivity of the Pt-RGO/Si electrodes towards the three analytes, hence suppressing the formation of Pb-Cu alloy. However, when the weight ratio of Pt:GO surpassed the optimum value, the PtNPs tended to coagulate and form larger islands. Hence, the characteristic of the nano-effect of Pt was lost, leaving the micro-effect of Pt, which led to much lower sensitivity and selectivity to the analytes. The optimized Pt-RGO/Si electrodes exhibited much lower LODs of the three analytes as compared to the reported electrodes in the literature. However, as compared to the RGO/GCS, Sn-RGO/GCS and RGO-N/Si electrodes, the fabricated Pt-RGO/Si electrodes suffered a poor repeatability in electroanalysis, which was because the PtNPs were easily poisoned by the analytes and the compounds in the solutions. Nevertheless, the prepared Pt-RGO/Si electrodes were meant for disposable application.

Limitations and recommendations

There are several limitations in the fabrication, sensing and results justification on the prepared electrodes. In the thermal reduction of RGO-N and Pt-RGO, the duration of the thermal annealing may be crucial. However, in the experiment, the C/O ratio of the RGO did not illustrate significant changes. The main reason behind was the slow cooling process, which took about 6 hrs to cool from 750 °C to the room temperature, which meant that the RGO was still undergoing the reduction process even in the cooling process. As a result, it was difficult to justify the optimum annealing duration (dwelling time) of the process, which may be important for industrial production. Hence, in order to improve the justification, rapid cooling process could be introduced for further studies.

The drop-cast method was applied because it was simple, economic and able to achieve a full surface coverage. However, the drop-cast method induced uneven surface layer thicknesses. In the experiment, vacuum drying was employed to increase the drying rate, preventing the concentration process that could lead to uneven film thickness. In the electrochemical reduction of Sn-RGO/GCS electrodes, the thickness of the layers varied by about 100 nm. The uneven surface thickness could affect the accuracy and stability of the electroanalysis. In contrast, the film thickness only varied by about 10 nm in the thermally reduced Pt-RGO/Si electrodes, which meant that thermal annealing could redistribute the surface compounds and flatten the films. Hence, in order to obtain a more even film thickness, a spin coating or spraying method could be applied, or post processing with thermal annealing could help to improve the thickness uniformity.

It has been reported that solution-phase and electrolyte molecules can diffuse through the nanoparticles to the base material, leading to a possible inaccurate measurement on the electron transfer rate of coated materials [195]. This means that the CV surface reversibility test may not bring an accurate conclusion to justify if a high electron transfer rate was due to the coated materials or the base materials. Hence, in order to clearly investigate the mechanism, different substrates, especially non-conductive substrates could be used to replace the conductive base materials. Nevertheless, the peak current intensity achieved by the Sn-RGO electrodes (with GCE as a substrate) was 50 times higher than that of bare GCE, indicating the insignificant contribution of the bare GCE. For the thermally reduced Pt-RGO, the substrates employed were a relatively non-electrochemically conductive Si. As a result, whether the solution-phase analytes diffused to the base material or not would not significantly affect the CV results. However, the question of “whether the high electron transfer rate was due to the metal nanoparticles or the effect of increased surface area after the decoration of metal nanoparticles” is still open for debate. Hence, in future work, Brunauer–Emmett–Teller (BET) analysis could be carried out to measure the total surface area of the electrode.

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Appendix 1: Standard Redox Potentials of Some Common Heavy Metals at 25 °C

Standard Reduction Potentials in Aqueous Solution at 25°C	
Acidic Solution	E° (V)
$\text{F}_2(\text{g}) + 2 \text{e}^- \longrightarrow 2 \text{F}^-(\text{aq})$	+2.87
$\text{Co}^{3+}(\text{aq}) + \text{e}^- \longrightarrow \text{Co}^{2+}(\text{aq})$	+1.82
$\text{Pb}^{4+}(\text{aq}) + 2 \text{e}^- \longrightarrow \text{Pb}^{2+}(\text{aq})$	+1.8
$\text{H}_2\text{O}_2(\text{aq}) + 2 \text{H}^+(\text{aq}) + 2 \text{e}^- \longrightarrow 2 \text{H}_2\text{O}$	+1.77
$\text{NiO}_2(\text{s}) + 4 \text{H}^+(\text{aq}) + 2 \text{e}^- \longrightarrow \text{Ni}^{2+}(\text{aq}) + 2 \text{H}_2\text{O}$	+1.7
$\text{PbO}_2(\text{s}) + \text{SO}_4^{2-}(\text{aq}) + 4 \text{H}^+(\text{aq}) + 2 \text{e}^- \longrightarrow \text{PbSO}_4(\text{s}) + 2 \text{H}_2\text{O}$	+1.685
$\text{Au}^+(\text{aq}) + \text{e}^- \longrightarrow \text{Au}(\text{s})$	+1.68
$2 \text{HClO}(\text{aq}) + 2 \text{H}^+(\text{aq}) + 2 \text{e}^- \longrightarrow \text{Cl}_2(\text{g}) + 2 \text{H}_2\text{O}$	+1.63
$\text{Ce}^{4+}(\text{aq}) + \text{e}^- \longrightarrow \text{Ce}^{3+}(\text{aq})$	+1.61
$\text{NaBiO}_3(\text{s}) + 6 \text{H}^+(\text{aq}) + 2 \text{e}^- \longrightarrow \text{Bi}^{3+}(\text{aq}) + \text{Na}^+(\text{aq}) + 3 \text{H}_2\text{O}$	+~1.6
$\text{MnO}_4^-(\text{aq}) + 8 \text{H}^+(\text{aq}) + 5 \text{e}^- \longrightarrow \text{Mn}^{2+}(\text{aq}) + 4 \text{H}_2\text{O}$	+1.51
$\text{Au}^{3+}(\text{aq}) + 3 \text{e}^- \longrightarrow \text{Au}(\text{s})$	+1.5
$\text{ClO}_3^-(\text{aq}) + 6 \text{H}^+(\text{aq}) + 5 \text{e}^- \longrightarrow 1/2 \text{Cl}_2(\text{g}) + 3 \text{H}_2\text{O}$	+1.47
$\text{BrO}_3^- + 6 \text{H}^+(\text{aq}) + 6 \text{e}^- \longrightarrow \text{Br}^-(\text{aq}) + 3 \text{H}_2\text{O}$	+1.44
$\text{Cl}_2(\text{g}) + 2 \text{e}^- \longrightarrow 2 \text{Cl}^-(\text{aq})$	+1.358
$\text{Cr}_2\text{O}_7^{2-} + 14 \text{H}^+(\text{aq}) + 6 \text{e}^- \longrightarrow 2 \text{Cr}^{3+}(\text{aq}) + 7 \text{H}_2\text{O}$	+1.33
$\text{N}_2\text{H}_5^+(\text{aq}) + 3 \text{H}^+(\text{aq}) + 2 \text{e}^- \longrightarrow 2 \text{NH}_4^+(\text{aq})$	+1.24
$\text{MnO}_2(\text{s}) + 4 \text{H}^+(\text{aq}) + 2 \text{e}^- \longrightarrow \text{Mn}^{2+}(\text{aq}) + 2 \text{H}_2\text{O}$	+1.23
$\text{O}_2(\text{g}) + 4 \text{H}^+(\text{aq}) + 4 \text{e}^- \longrightarrow 2 \text{H}_2\text{O}$	+1.229
$\text{Pt}^{2+}(\text{aq}) + 2 \text{e}^- \longrightarrow \text{Pt}(\text{s})$	+1.2
$\text{IO}_3^-(\text{aq}) + 6 \text{H}^+(\text{aq}) + 5 \text{e}^- \longrightarrow 1/2 \text{I}_2(\text{aq}) + 3 \text{H}_2\text{O}$	+1.195
$\text{ClO}_4^-(\text{aq}) + 2 \text{H}^+(\text{aq}) + 2 \text{e}^- \longrightarrow \text{ClO}_3^-(\text{aq}) + \text{H}_2\text{O}$	+1.19
$\text{Br}_2(\text{l}) + 2 \text{e}^- \longrightarrow 2 \text{Br}^-(\text{aq})$	+1.066
$\text{AuCl}_4^- + 3 \text{e}^- \longrightarrow \text{Au}(\text{s}) + 4 \text{Cl}^-(\text{aq})$	+1
$\text{Pd}^{2+}(\text{aq}) + 2 \text{e}^- \longrightarrow \text{Pd}(\text{s})$	+0.987
$\text{NO}_3^-(\text{aq}) + 4 \text{H}^+(\text{aq}) + 3 \text{e}^- \longrightarrow \text{NO}(\text{g}) + 2 \text{H}_2\text{O}$	+0.96
$\text{NO}_3^-(\text{aq}) + 3 \text{H}^+(\text{aq}) + 2 \text{e}^- \longrightarrow \text{HNO}_2(\text{aq}) + \text{H}_2\text{O}$	+0.94
$2 \text{Hg}^{2+}(\text{aq}) + 2 \text{e}^- \longrightarrow \text{Hg}_2^{2+}(\text{aq})$	+0.92
$\text{Hg}_2^{2+}(\text{aq}) + 2 \text{e}^- \longrightarrow \text{Hg}(\text{l})$	+0.855
$\text{Ag}^+(\text{aq}) + \text{e}^- \longrightarrow \text{Ag}(\text{s})$	+0.7994
$\text{Hg}_2^{2+}(\text{aq}) + 2 \text{e}^- \longrightarrow 2 \text{Hg}(\text{l})$	+0.789
$\text{Fe}^{3+}(\text{aq}) + \text{e}^- \longrightarrow \text{Fe}^{2+}(\text{aq})$	+0.771
$\text{SbCl}_6^-(\text{aq}) + 2 \text{e}^- \longrightarrow \text{SbCl}_4^-(\text{aq}) + 2 \text{Cl}^-(\text{aq})$	+0.75
$[\text{PtCl}_4]^{2-}(\text{aq}) + 2 \text{e}^- \longrightarrow \text{Pt}(\text{s}) + 4 \text{Cl}^-(\text{aq})$	+0.73
$\text{O}_2(\text{g}) + 2 \text{H}^+(\text{aq}) + 2 \text{e}^- \longrightarrow \text{H}_2\text{O}_2(\text{aq})$	+0.682
$[\text{PtCl}_6]^{2-}(\text{aq}) + 2 \text{e}^- \longrightarrow [\text{PtCl}_4]^{2-}(\text{aq}) + 2 \text{Cl}^-(\text{aq})$	+0.68
$\text{H}_3\text{AsO}_4(\text{aq}) + 2 \text{H}^+(\text{aq}) + 2 \text{e}^- \longrightarrow \text{H}_3\text{AsO}_3(\text{aq}) + \text{H}_2\text{O}$	+0.58

$\text{I}_2(\text{s}) + 2 \text{e}^- \longrightarrow 2 \text{I}^-(\text{aq})$	+0.535
$\text{TeO}_2(\text{s}) + 4 \text{H}^+(\text{aq}) + 4 \text{e}^- \longrightarrow \text{Te}(\text{s}) + 2 \text{H}_2\text{O}$	+0.529
$\text{Cu}^+(\text{aq}) + \text{e}^- \longrightarrow \text{Cu}(\text{s})$	+0.521
$[\text{RhCl}_6]^{3-}(\text{aq}) + 3 \text{e}^- \longrightarrow \text{Rh}(\text{s}) + 6 \text{Cl}^-(\text{aq})$	+0.44
$\text{Cu}^{2+}(\text{aq}) + 2 \text{e}^- \longrightarrow \text{Cu}(\text{s})$	+0.337
$\text{HgCl}_2(\text{s}) + 2 \text{e}^- \longrightarrow 2 \text{Hg}(\text{l}) + 2 \text{Cl}^-(\text{aq})$	+0.27
$\text{AgCl}(\text{s}) + \text{e}^- \longrightarrow \text{Ag}(\text{s}) + \text{Cl}^-(\text{aq})$	+0.222
$\text{SO}_4^{2-}(\text{aq}) + 4 \text{H}^+(\text{aq}) + 2 \text{e}^- \longrightarrow \text{SO}_2(\text{g}) + 2 \text{H}_2\text{O}$	+0.2
$\text{SO}_4^{2-}(\text{aq}) + 4 \text{H}^+(\text{aq}) + 2 \text{e}^- \longrightarrow \text{H}_2\text{SO}_3(\text{g}) + \text{H}_2\text{O}$	+0.17
$\text{Cu}^{2+}(\text{aq}) + \text{e}^- \longrightarrow \text{Cu}^+(\text{aq})$	+0.153
$\text{Sn}^{4+}(\text{aq}) + 2 \text{e}^- \longrightarrow \text{Sn}^{2+}(\text{aq})$	+0.15
$\text{S}(\text{s}) + 2 \text{H}^+(\text{aq}) + 2 \text{e}^- \longrightarrow \text{H}_2\text{S}(\text{aq})$	+0.14
$\text{AgBr}(\text{s}) + \text{e}^- \longrightarrow \text{Ag}(\text{s}) + \text{Br}^-(\text{aq})$	+0.0713
$2 \text{H}^+(\text{aq}) + 2 \text{e}^- \longrightarrow \text{H}_2(\text{g})$ (reference electrode)	0
$\text{N}_2\text{O}(\text{g}) + 6 \text{H}^+(\text{aq}) + \text{H}_2\text{O} + 4 \text{e}^- \longrightarrow 2 \text{NH}_3\text{OH}^+(\text{aq})$	-0.05
$\text{Pb}^{2+}(\text{aq}) + 2 \text{e}^- \longrightarrow \text{Pb}(\text{s})$	-0.126
$\text{Sn}^{2+}(\text{aq}) + 2 \text{e}^- \longrightarrow \text{Sn}(\text{s})$	-0.14
$\text{AgI}(\text{s}) + \text{e}^- \longrightarrow \text{Ag}(\text{s}) + \text{I}^-(\text{aq})$	-0.15
$[\text{SnF}_6]^{2-}(\text{aq}) + 4 \text{e}^- \longrightarrow \text{Sn}(\text{s}) + 6 \text{F}^-(\text{aq})$	-0.25
$\text{Ni}^{2+}(\text{aq}) + 2 \text{e}^- \longrightarrow \text{Ni}(\text{s})$	-0.25
$\text{Co}^{2+}(\text{aq}) + 2 \text{e}^- \longrightarrow \text{Co}(\text{s})$	-0.28
$\text{Tl}^+(\text{aq}) + \text{e}^- \longrightarrow \text{Tl}(\text{s})$	-0.34
$\text{PbSO}_4(\text{s}) + 2 \text{e}^- \longrightarrow \text{Pb}(\text{s}) + \text{SO}_4^{2-}(\text{aq})$	-0.356
$\text{Se}(\text{s}) + 2 \text{H}^+(\text{aq}) + 2 \text{e}^- \longrightarrow \text{H}_2\text{Se}(\text{aq})$	-0.4
$\text{Cd}^{2+}(\text{aq}) + 2 \text{e}^- \longrightarrow \text{Cd}(\text{s})$	-0.403
$\text{Cr}^{3+}(\text{aq}) + \text{e}^- \longrightarrow \text{Cr}^{2+}(\text{aq})$	-0.41
$\text{Fe}^{2+}(\text{aq}) + 2 \text{e}^- \longrightarrow \text{Fe}(\text{s})$	-0.44
$2 \text{CO}_2(\text{g}) + 2 \text{H}^+(\text{aq}) + 2 \text{e}^- \longrightarrow (\text{COOH})_2(\text{aq})$	-0.49
$\text{Ga}^{3+}(\text{aq}) + 3 \text{e}^- \longrightarrow \text{Ga}(\text{s})$	-0.53
$\text{HgS}(\text{s}) + 2 \text{H}^+(\text{aq}) + 2 \text{e}^- \longrightarrow \text{Hg}(\text{l}) + \text{H}_2\text{S}(\text{g})$	-0.72
$\text{Cr}^{3+}(\text{aq}) + 3 \text{e}^- \longrightarrow \text{Cr}(\text{s})$	-0.74
$\text{Zn}^{2+}(\text{aq}) + 2 \text{e}^- \longrightarrow \text{Zn}(\text{s})$	-0.763
$2 \text{H}_2\text{O}(\text{l}) + 2 \text{e}^- \longrightarrow \text{H}_2(\text{g}) + 2 \text{OH}^-(\text{aq})$	-0.8277
$\text{Cr}^{2+}(\text{aq}) + 2 \text{e}^- \longrightarrow \text{Cr}(\text{s})$	-0.91
$\text{Mn}^{2+}(\text{aq}) + 2 \text{e}^- \longrightarrow \text{Mn}(\text{s})$	-1.18
$\text{V}^{2+}(\text{aq}) + 2 \text{e}^- \longrightarrow \text{V}(\text{s})$	-1.18
$\text{Zr}^{4+}(\text{aq}) + 4 \text{e}^- \longrightarrow \text{Zr}(\text{s})$	-1.53
$\text{Al}^{3+}(\text{aq}) + 3 \text{e}^- \longrightarrow \text{Al}(\text{s})$	-1.66
$\text{H}_2(\text{g}) + 2 \text{e}^- \longrightarrow 2 \text{H}^-(\text{aq})$	-2.25
$\text{Mg}^{2+}(\text{aq}) + 2 \text{e}^- \longrightarrow \text{Mg}(\text{s})$	-2.37
$\text{Na}^+(\text{aq}) + \text{e}^- \longrightarrow \text{Na}(\text{s})$	-2.714
$\text{Ca}^{2+}(\text{aq}) + 2 \text{e}^- \longrightarrow \text{Ca}(\text{s})$	-2.87
$\text{Sr}^{2+}(\text{aq}) + 2 \text{e}^- \longrightarrow \text{Sr}(\text{s})$	-2.89
$\text{Ba}^{2+}(\text{aq}) + 2 \text{e}^- \longrightarrow \text{Ba}(\text{s})$	-2.9
$\text{Rb}^+(\text{aq}) + \text{e}^- \longrightarrow \text{Rb}(\text{s})$	-2.925

$\text{K}^+(\text{aq}) + \text{e}^- \longrightarrow \text{K}(\text{s})$	-2.925
$\text{Li}^+(\text{aq}) + \text{e}^- \longrightarrow \text{Li}(\text{s})$	-3.045
Basic Solution	
$\text{ClO}^-(\text{aq}) + \text{H}_2\text{O} + 2 \text{e}^- \longrightarrow \text{Cl}^-(\text{aq}) + 2 \text{OH}^-(\text{aq})$	0.89
$\text{OOH}^-(\text{aq}) + \text{H}_2\text{O} + 2 \text{e}^- \longrightarrow 3 \text{OH}^-(\text{aq})$	0.88
$2 \text{NH}_2\text{OH}(\text{aq}) + 2 \text{e}^- \longrightarrow \text{N}_2\text{H}_4(\text{aq}) + 2 \text{OH}^-(\text{aq})$	0.74
$\text{ClO}_3^-(\text{aq}) + 3 \text{H}_2\text{O} + 6 \text{e}^- \longrightarrow \text{Cl}^-(\text{aq}) + 6 \text{OH}^-(\text{aq})$	0.62
$\text{MnO}_4^-(\text{aq}) + 2 \text{H}_2\text{O} + 3 \text{e}^- \longrightarrow \text{MnO}_2(\text{s}) + 4 \text{OH}^-(\text{aq})$	0.588
$\text{MnO}_4^-(\text{aq}) + \text{e}^- \longrightarrow \text{MnO}_4^{2-}(\text{aq})$	0.564
$\text{NiO}_2(\text{s}) + 2 \text{H}_2\text{O} + 2 \text{e}^- \longrightarrow \text{Ni}(\text{OH})_2(\text{s}) + 2 \text{OH}^-(\text{aq})$	0.49
$\text{Ag}_2\text{CrO}_4(\text{s}) + 2 \text{e}^- \longrightarrow 2 \text{Ag}(\text{s}) + \text{CrO}_4^{2-}(\text{aq})$	0.446
$\text{O}_2(\text{g}) + 2 \text{H}_2\text{O} + 4 \text{e}^- \longrightarrow 4 \text{OH}^-(\text{aq})$	0.4
$\text{ClO}_4^-(\text{aq}) + \text{H}_2\text{O} + 2 \text{e}^- \longrightarrow \text{ClO}_3^-(\text{aq}) + 2 \text{OH}^-(\text{aq})$	0.36
$\text{Ag}_2\text{O}(\text{s}) + \text{H}_2\text{O} + 2 \text{e}^- \longrightarrow 2 \text{Ag}(\text{s}) + 2 \text{OH}^-(\text{aq})$	0.34
$2 \text{NO}_2^-(\text{aq}) + 3 \text{H}_2\text{O} + 4 \text{e}^- \longrightarrow \text{N}_2\text{O}(\text{g}) + 6 \text{OH}^-(\text{aq})$	0.15
$\text{N}_2\text{H}_4(\text{aq}) + 2 \text{H}_2\text{O} + 2 \text{e}^- \longrightarrow 2 \text{NH}_3(\text{aq}) + 2 \text{OH}^-(\text{aq})$	0.1
$[\text{Co}(\text{NH}_3)_6]^{3+}(\text{aq}) + \text{e}^- \longrightarrow [\text{Co}(\text{NH}_3)_6]^{2+}(\text{aq})$	0.1
$\text{HgO}(\text{s}) + \text{H}_2\text{O} + 2 \text{e}^- \longrightarrow \text{Hg}(\text{l}) + 2 \text{OH}^-(\text{aq})$	0.0984
$\text{O}_2(\text{g}) + \text{H}_2\text{O} + 2 \text{e}^- \longrightarrow \text{OOH}^-(\text{aq}) + \text{OH}^-(\text{aq})$	0.076
$\text{NO}_3^-(\text{aq}) + \text{H}_2\text{O} + 2 \text{e}^- \longrightarrow \text{NO}_2^-(\text{aq}) + 2 \text{OH}^-(\text{aq})$	0.01
$\text{MnO}_2(\text{s}) + 2 \text{H}_2\text{O} + 2 \text{e}^- \longrightarrow \text{Mn}(\text{OH})_2(\text{s}) + 2 \text{OH}^-(\text{aq})$	-0.05
$\text{CrO}_4^{2-}(\text{aq}) + 4 \text{H}_2\text{O} + 3 \text{e}^- \longrightarrow \text{Cr}(\text{OH})_3(\text{s}) + 5 \text{OH}^-(\text{aq})$	-0.12
$\text{Cu}(\text{OH})_2(\text{s}) + 2 \text{e}^- \longrightarrow \text{Cu}(\text{s}) + 2 \text{OH}^-(\text{aq})$	-0.36
$\text{Fe}(\text{OH})_3(\text{s}) + \text{e}^- \longrightarrow \text{Fe}(\text{OH})_2(\text{s}) + \text{OH}^-(\text{aq})$	-0.56
$2 \text{H}_2\text{O} + 2 \text{e}^- \longrightarrow \text{H}_2(\text{g}) + 2 \text{OH}^-(\text{aq})$	-0.8277
$2 \text{NO}_3^-(\text{aq}) + 2 \text{H}_2\text{O} + 2 \text{e}^- \longrightarrow \text{N}_2\text{O}_4(\text{g}) + 4 \text{OH}^-(\text{aq})$	-0.85
$\text{Fe}(\text{OH})_2(\text{s}) + 2 \text{e}^- \longrightarrow \text{Fe}(\text{s}) + 2 \text{OH}^-(\text{aq})$	-0.877
$\text{SO}_4^{2-}(\text{aq}) + \text{H}_2\text{O} + 2 \text{e}^- \longrightarrow \text{SO}_3^{2-}(\text{aq}) + 2 \text{OH}^-(\text{aq})$	-0.93
$\text{N}_2(\text{g}) + 4 \text{H}_2\text{O} + 4 \text{e}^- \longrightarrow \text{N}_2\text{H}_4(\text{aq}) + 4 \text{OH}^-(\text{aq})$	-1.15
$[\text{Zn}(\text{OH})_4]^{2-}(\text{aq}) + 2 \text{e}^- \longrightarrow \text{Zn}(\text{s}) + 4 \text{OH}^-(\text{aq})$	-1.22
$\text{Zn}(\text{OH})_2(\text{s}) + 2 \text{e}^- \longrightarrow \text{Zn}(\text{s}) + 2 \text{OH}^-(\text{aq})$	-1.245
$[\text{Zn}(\text{CN})_4]^{2-}(\text{aq}) + 2 \text{e}^- \longrightarrow \text{Zn}(\text{s}) + 4 \text{CN}^-(\text{aq})$	-1.26
$\text{Cr}(\text{OH})_3(\text{s}) + 3 \text{e}^- \longrightarrow \text{Cr}(\text{s}) + 3 \text{OH}^-(\text{aq})$	-1.3
$\text{SiO}_3^{2-}(\text{aq}) + 3 \text{H}_2\text{O} + 4 \text{e}^- \longrightarrow \text{Si}(\text{s}) + 6 \text{OH}^-(\text{aq})$	-1.7

Appendix 2: Potentials of Common Reference Electrodes

Reference Electrode	E vs. SCE	E vs. NHE
Saturated Calomel (SCE, sat'd KCl)	0	0.241
Silver/Silver Chloride (Ag/AgCl, sat'd KCl)	-0.042	0.198
Mercury/Mercurous Sulfate (Hg/Hg ₂ SO ₄ , saturated K ₂ SO ₄)	0.41	0.654
Ag/AgNO ₃ (0.01 M) in MeCN Ag/AgNO ₃ (0.1 M) in MeCN	0.3 (vs aq SCE) 0.36 (vs ag SCE)	NA
Normal Hydrogen Electrode (NHE)	-0.241	0