Electrochemical detection of environmental pollutants and food contaminants

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**NANYANG
TECHNOLOGICAL
UNIVERSITY**

**ELECTROCHEMICAL DETECTION OF
ENVIRONMENTAL POLLUTANTS AND FOOD
CONTAMINANTS**

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SCHOOL OF PHYSICAL & MATHEMATICAL SCIENCES

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ELECTROCHEMICAL DETECTION OF ENVIRONMENTAL POLLUTANTS AND FOOD CONTAMINANTS

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“My success can only come from Allah. In Him I trust, and unto Him I look”

– Al- Qur’an 11:88

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In this chapter, the use of modified 3D printed metal electrodes for the detection of nitroaromatic and phenolic compounds were investigated.

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In this chapter, a summary of the findings in this thesis and future perspectives in the area of research were described.

Appendices

Summary

With a rapidly changing global environment, the evolution of techniques and methodologies has to keep up with advancement in technology. Electrochemistry has been a useful and effective technique in sensing applications. The versatility of electrochemical techniques, coupled with emerging new materials and technology, has enabled numerous methodologies to be developed for the detection of environmental pollutants and food contaminants with enhanced sensitivity and selectivity. In this thesis, graphite and transition metal dichalcogenides (TMDs) were identified as the nanomaterials to provide an alternative material for electrochemical sensing applications. The simultaneous detection of several mycotoxins on edge plane pyrolytic graphite (EPPG) was successfully demonstrated with high degree of selectivity and sensitivity. The limitations of TMDs in electrochemical applications were studied after which the performance was optimized for the fabrication of an enzyme biosensor system for the detection of organophosphate pesticide with high sensitivity and low limits of detection. The incorporation of new technology and ideas, such as 3D printing and single particle detection, with electrochemical techniques were demonstrated. With the use of screen-printed electrodes, the detection of defined monodispersed silver nanoparticles and lipid liposomes were successfully demonstrated using impact electrochemistry. 3D printed metal electrodes modified with gold have proven to be a promising technique for the detection of nitroaromatic and phenolic pollutants which outperformed that of conventional glassy carbon electrode. The proof-of-concept applications open up limitless possibilities for the potential advancements in the use of nanomaterials and emerging technology for electrochemical sensing applications.

List of Publications

1. **Muhammad Zafir Mohamad Nasir**, Martin Pumera. Simultaneous Detection of Zearalenone and Citrinin by Voltammetry on Edge Plane Pyrolytic Graphite Electrode. *Electroanalysis* **2014**, 26, 1901.
2. **Muhammad Zafir Mohamad Nasir**, Martin Pumera. Simultaneous Anodic and Cathodic Voltammetric Detection of Patulin and Ochratoxin A on Well-Defined Carbon Electrodes. *Electroanalysis*. **2014**, 27, 924.
3. **Muhammad Zafir Mohamad Nasir**, Zdenek Sofer, Adriano Ambrosi, Martin Pumera. A Limited Anodic and Cathodic Potential Window of MoS₂: Limitations in Electrochemical Applications. *Nanoscale* **2015**, 7, 3126.
4. **Muhammad Zafir Mohamad Nasir**, Zdenek Sofer, Martin Pumera. Effect of Electrolyte pH on the Inherent Electrochemistry of Layered Transition-Metal Dichalcogenides (MoS₂, MoSe₂, WS₂, WSe₂). *ChemElectroChem* **2015**, 2, 1713.
Highlighted as “Very Important” paper
5. **Muhammad Zafir Mohamad Nasir**, Martin Pumera. Impact Electrochemistry on Screen-Printed Electrodes for the Detection of Monodispersed Silver Nanoparticles of Sizes 10 – 107 nm. *PhysChemChemPhys* **2016**, 18, 28183.
6. **Muhammad Zafir Mohamad Nasir**, Carmen C. Mayorga-Martinez, Zdenek Sofer, Martin Pumera. Two-Dimensional 1T-Phase Transition Metal Dichalcogenides as nano-Carriers to Enhance and Stabilise Enzyme Activity for Electrochemical Pesticide Detection. *ACS Nano* **2017**, 11, 5774.
7. Cavin Tan, **Muhammad Zafir Mohamad Nasir**, Adriano Ambrosi, Martin Pumera. 3D Printed Electrodes for the Detection of Nitroaromatic Explosives and Nerve Agents. *Anal. Chem.* **2017**, 89, 8995.

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8. Tay Siew Cheng, **Muhammad Zafir Mohamad Nasir**, Adriano Ambrosi, Martin Pumera. 3D-Printed Metal Electrodes Electrochemical Detection of Phenols. *Appl. Mater. Today* **2017**, 9, 212.
 9. Qi Dong, **Muhammad Zafir Mohamad Nasir**, Martin Pumera. Semi-Conducting Single-Walled Carbon Nanotubes are Detrimental When Compared to Metallic Single-Walled Carbon Nanotubes for Electrochemical Applications. *Phys.Chem.Chem.Phys.* **2017**, 19, 27320.
 10. **Muhammad Zafir Mohamad Nasir**, Joshua A. Jackman, Nam-Joon Cho, Adriano Ambrosi, Martin Pumera. Detection of Amphipathic Viral Peptide on Screen-Printed Electrodes by Liposome Rupture Impact Voltammetry. *Anal. Chem.* **2017**, 89, 11753.

Thesis Organisation

This thesis is organized into **nine (9) Chapters**. The objectives of this thesis are presented in **Chapter 1**. **Chapter 2** will provide an introduction to the different detection techniques of environmental pollutants and food contaminants. Two-dimensional (2D) layered materials will also be introduced with the various reported electrochemical applications. In-depth discussions on previously reported works with regard to 2D layered materials in electrochemical sensors and biosensors, impact electrochemistry and electrochemical applications of 3D printing are included. A collection of research towards the objectives established in **Chapter 1** will be provided in **Chapters 3-9**.

In detail, **Chapter 3** will demonstrate the simultaneous detection of mycotoxins on well-defined electrodes. **Chapter 4** will highlight the limited potential window of MoS₂ for electrochemical sensing applications. **Chapter 5** will compare the effect of pH on the inherent anodic peaks of different TMDs. **Chapter 6** will demonstrate the use of WS₂ as a transducer in an inhibition-based enzyme biosensor. **Chapter 7** will show the use of impact electrochemistry towards detection of monodispersed silver nanoparticles and demonstrate the detection of lipid liposomes using rupture impact electrochemistry. **Chapter 8** will illustrate the use of 3D printing technology in electrochemical sensing of environmental pollutants. A perspective of the work presented in this thesis will be presented and summarised in **Chapter 9**.

The published version of the results in **Chapters 3-8** are shown in the **Appendices**.

List of Abbreviations

μL	Microlitre
$^{\circ}\text{C}$	Degree Centigrade
cm^{-1}	Inverse Centimeter
AChE	Acetylcholinesterase
ACN	Acetonitrile
ATCh	Acetylthiocholine
BDD	Boron-Doped Diamond
BPPG	Basal Plane Pyrolytic Graphite
BSA	Bovine Saline Albumin
CIT	Citrinin
CNT	Carbon Nanotube
CV	Cyclic Voltammetry
DOPC	1,2-dioleoly- <i>sn</i> -glycero-3-phosphocholine
DPV	Differential Pulse Voltammetry
EPPG	Edge Plane Pyrolytic Graphite
GA	Glutaraldehyde
GC	Glassy Carbon
hr	Hour (time)
LOD	Limit of Detection
LOQ	Limit of Quantification
min	Minute (time)
mL	Millilitre
mM	Millimolar
mm	Millimeter
nM	Nanomolar
nm	Nanometer
OTA	Ochratoxin A
PAT	Patulin
PBS	Phosphate Buffer Saline
RSD	Relative Standard Deviation
s^{-1}	Inverse second
SAED	Selected Area Electron Diffraction
SEM	Scanning Electron Microscopy
SPE	Screen-Printed Electrode
STEM	Scanning Transmission Electron Microscopy
t-BuLi	tert-butyllithium
TEM	Transition Electron Microscopy
TMD	Transition Metal Dichalcogenide
XPS	X-ray Photoelectron Spectroscopy
ZEA	Zearalenone

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Chapter 1

Objectives of thesis

Electrochemical detection of biological compounds is widely used in medical, industrial and environmental applications. The electrochemical detection of food contaminants and environmental pollutants has gained widespread interest among researchers due to the urgent need to regulate and control the negative side effects such as environmental pollution and spread of epidemic diseases.

Mycotoxins are toxic compounds often found in the food stock that are not properly stored. When consumed, these toxins can have negative side effects to human health such as liver failure, respiratory complications, and even death if consumed in lethal amounts. Organophosphate pesticides (OPs) inhibit acetylcholinesterase (AChE) found in the nervous system resulting in a neurotoxic response in target pests. However, these pesticides can also be highly toxic to non-targeted species if found at high dosages. Other forms of pollutants and contaminants include heavy metals, nanoparticles and microorganisms. These pollutants are highly dangerous when they accumulate in human body causing negative health implications. This was observed in Japan where the consumption of fish contaminated with mercury from industrial wastewater dumped into the sea resulted in paralysis, loss of hearing and even death at the extreme case. As such, it is of paramount importance to have effective point-of-care on-site detection devices to allow authorised personnels to take immediate action required if necessary. Electrochemistry also offers the portability for miniaturized sensor for detection of such compounds.

The use of carbon-based materials, such as graphene, as electrode materials for electrochemical sensing and biosensing applications has been widely reported. There is a need for alternative materials which could potentially outperform and improve

research in this direction to improve current technology. Recently, a new group of layered materials have been garnering interest and attention, which are transition metal dichalcogenides (TMDs).

In order to develop better electrochemical devices, research focus has to be geared into improving current methodologies and practices. Innovation in the electrode materials materials used, electrochemical methodologies and fabrication process of electrochemical apparatus has to be looked into. As such, the need for more efficient and sensitive methodologies has led to the three objectives for the thesis as illustrated in **Figure 1.1**. The objectives aim to address and improve the areas of research on electrochemical sensing techniques currently available. Background knowledge and review leading to these aims would be highlighted in **Chapter 2**.

Objective 1: Two-dimensional layered materials for the electrochemical detection of environmental pollutants and food contaminants.

Electrochemistry provides a useful and quick tool for the detection of environmental pollutants and food contaminants. Layered materials, such as graphene and transition metal dichalcogenides (TMDs), are found to exhibit excellent electrical, chemical, and physical properties which make them attractive for sensing and biosensing applications. While numerous different carbon-based electrodes have been able to detect single mycotoxins, there are very little studies performed on the possibility of detecting different mycotoxins simultaneously electrochemically which is of great puzzle. We wish to address this question by attempting to detect different

mycotoxins simultaneously using well-defined electrodes and study any possible interactions between them that might affect the accuracy and separation of the electrochemical signals obtained. **Chapter 3** will illustrate the possibility for simultaneous detection of mycotoxins on well-defined electrodes and analyse any interactions that might take place.

Besides that, current research has predominantly focused on using carbon-based materials for electrochemical applications such as sensing and capacitors. We will look into an emerging class of layered material with similar properties to graphene, which is transition metal dichalcogenides (TMDs). TMDs have been touted as good materials with diverse possible applications such as energy storage and sensing platform. However, despite the advantageous properties, there is insufficient information available on the limitations of the material with respect to application as a sensing platform and performance with varying experimental conditions. These limitations have to be taken into consideration in the construction of electrochemical devices. The analytical limitations and performance of TMDs will be elaborated in **Chapters 4 and 5**.

Having understood the limitations and analytical characteristics of the TMD materials, herein, we aim to optimise the use of TMDs to provide a more sensitive platform for biosensing applications, more specifically in the enzymatic detection of organophosphate pesticides (OPs). The developed technique should have profound impact on research towards portable sensors for pesticide detection. For this research direction, we will be using enzymes as the bioreceptor for pesticide detection. In the biosensor system, an enzyme called acetylcholinesterase will be immobilised onto TMD material and analysed for its sensitivity and reproducibility. Real samples studies will

also be performed to test the selectivity of the biosensor system. The application of TMDs as a transducer platform for inhibition-based enzymatic pesticide detection will be demonstrated in **Chapter 6**.

Objective 2: Impact electrochemistry on screen-printed technology for detection of single particle entities

The detection of nanoparticles involves the use of sophisticated machinery with tedious sample preparation steps and long processing time such as spectroscopy. As such, current methodologies are inadequate for rapid detection of nanoparticle pollutants for on-site point-of-care detection. An alternative approach has to be developed and researchers have recently explored a technique called ‘impact electrochemistry’. Impact electrochemistry provides the capability to detect single nanoparticles at very low concentrations and small volumes. At a fixed set potential, upon collision of a nanoparticle onto the electrode surface, it will undergo an electrochemical reaction which will result in an electrochemical signal generated which can then be analysed. This technique will allow for the monitoring of single particle reaction kinetics and potentially detect specific analytes in real samples analysis.

In this thesis, we wish to explore the possibility of an alternative point-of-care detection method with the use of screen-printed electrodes to demonstrate on-site detection of silver nanoparticles in solution. **Chapter 7** will illustrate the detection and analysis of variable sized monodispersed silver nanoparticles on screen-printed electrodes.

Furthermore, the possibility for detecting 'soft' particles or membrane-bound structures such as vesicles will be advantageous for the preventive detection of infectious bacteria or harmful unicellular organisms in real samples analysis. Numerous works has been reported on the possibility of detecting 'soft' particles with the aid of mediator compounds loaded into them. Despite that, there are no reports on the direct detection of such 'soft' particles. Hitherto, the possibility of detecting a lipid vesicle upon induced rupture by an amphipathic α -helix (AH) peptide will be explored. The lipid vesicle will serve as a model 'soft' particle. Again, the use of screen-printed electrode will be employed to provide a proof-of-concept for the on-site detection of such particles which will be demonstrated in **Chapter 7**.

Objective 3: Application of 3D printing technology in electrochemical sensing

The use of tools can be traced back to the beginning of mankind and represents milestones in human evolution. Throughout the centuries, many machines and devices have been developed to help shape the world. An ever changing global landscape has led to greater and more complex tools to be developed for specialised functions. The ability to craft tools for specialised functions economically and rapidly would be highly beneficial.

3D printing affords for the customization and special fabrication of devices. The versatility of this technique can be explored and employed for sophisticated procedures and manufacture of point-of-care detection devices at low cost with little resources required. In electrochemistry, it opens up possibilities for specialised electrodes to be

designed for complex procedures and applications. 3D printing of customised electrodes will be investigated and compared with conventional glassy carbon electrode. Proof-of-concept applications of 3D printed metal electrodes for the direct detection of nitroaromatic and phenolic environmental pollutants will be demonstrated and elaborated in **Chapter 8**.

Figure 1.1 Summary of the challenges and objectives of this project

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Chapter 2

Introduction and literature review

2.1 *Environmental pollutants and food contaminants*

2.2 *Detection methodologies and techniques*

2.3 *Electrochemical sensors and biosensors*

2.4 *Two-dimensional layered materials*

2.5 *Impact electrochemistry*

2.6 *Three-dimensional (3D) printing*

2.1 Environmental pollutants and food contaminants

Food and water are essential parts of human sustenance in the world and any threat to the global food supply will have adverse effects on human lives. Such threats can come in the forms of spoilage by naturally occurring and man-made contaminants. Food contaminants such as mycotoxins are known to cause spoilage to food crops while high doses of pesticides and nanoparticles can be toxic and even cause death. Taking into consideration the harmful effects brought about by such chemicals and substances, numerous international and regional agencies have pledged to limit and control the amount of pollution taking place by legislating tough penalties and issuing severe punishments for offenders who do not abide by the strict regulations imposed.

Mycotoxins are a group of compounds that are harmful to humans when consumed. They are secondary toxins produced by fungi¹ and the consumption of these toxins has been known to cause serious health problems and even death.² Mycotoxins are known to be the cause of cardiac beriberi or “yellow rice disease” in Japan.³ Ochratoxin A (OTA) has been categorised as potentially carcinogenic to humans (Group 2B) by the International Agency for Research on Cancer (IARC).⁴ Mycotoxins are usually found in food which is stored in conditions favourable towards the growth of fungi. They are neither contagious nor infectious. However, they can occur in herd-wide basis. A small dose of mycotoxins (usually in the micrograms scale) is sufficient to exert its effect on humans and animals. As such, most regulatory agencies have permitted the maximum amount of mycotoxins to as low as parts per billion (ppb) level.^{1,5,6}

Figure 2.1 Chemical structures of (A) zearalenone (ZEA), (B) ochratoxin A (OTA), (C) citrinin (CIT), and (D) patulin (PAT).

Pesticides have also been identified as poisonous chemical agents. To meet the ever increasing demand for food in an ever expanding global population, farming techniques have evolved accordingly to improve crop yield and reduce spoilage. The need has catalysed and revolutionised farming practices to increase the global food supply and crop yield. Farming productivity has improved with greater pest control and use of agricultural chemicals such as pesticides. These practices have increased crop yield and reduce crop loss by invasive pests. There are several different categories of pesticides such as carbamates, organophosphates, pyrethroids and organochlorides. Organophosphate pesticides (OPs) constitute a large proportion of pesticides that are commercially available.⁷ Examples of OPs are fenitrothion and paraoxon. OPs function by binding irreversibly to nerve receptors in the nervous systems of pests which results in the over-stimulation of neurotransmitter acetylcholine and eventually leads to death. Acetylcholine is an important chemical neurotransmitter which transmits electrical

signals along the nervous system to all parts of the body.⁸ At the end of the signal transmission, acetylcholine gets hydrolysed to inactive choline and acetate (**Figure 2.2A**). OPs inhibit acetylcholinesterase enzyme (AChE) function of regulating acetylcholine in nerve tissues. It is an irreversible inhibitor which binds strongly to the enzyme and prevents hydrolysis of acetylcholine at the active site (**Figure 2.2B**).⁹ The formation of a stable enzyme-pesticide complex blocks the active site of acetylcholinesterase which prevents acetylcholine from binding. As such, excess acetylcholine is transmitted along the nervous system which results in overstimulation and could lead to severe negative health effects.¹⁰

Figure 2.2 (A) The enzymatic breakdown of acetylcholine by acetylcholinesterase, and (B) the irreversible inhibition effect of organophosphate pesticide (paraoxon) on acetylcholinesterase.

The widespread use of pesticides, namely organophosphorus pesticides (OPs), has caused several health and environmental issues due to improper practice and excessive use. Besides that, since OPs are soluble in water, excess use has led to seepage

of OPs into water bodies which pose a threat to aquatic life and contaminate groundwater.¹¹ The effects of OPs are also comparable to known nerve agents, such as sarin and VX. These agents were used as chemical weapons in global conflicts such as World War I¹² and Persian Gulf War (Iran-Iraq War).¹³ Hence, besides its use as pesticide, OPs can also be developed into dangerous chemical warfare agents.^{14,15} For example, paraoxon was once used during apartheid South Africa during a covert chemical and biological weapons operation known as Project Coast.¹⁶ The amount of OPs in food and environment is closely monitored due to their adverse health effects and high toxicity. Therefore, there is the increased need for sensitive and fast detection of OPs.

Nanoparticles can also affect human health and bodily functions negatively. The advancements in nanotechnology have sparked the development of numerous nanoparticles which can perform several specialised functions efficiently. Examples of nanoparticles include titanium dioxide (TiO₂) and silver nanoparticles. Silver nanoparticles are known antimicrobial agents with varied applications which includes clothing, washing devices and cosmetics.^{17,18} However, the widespread and diversified use of nanoparticles can potentially affect human organs¹⁹ due to the ease of transport in the circulatory and respiratory system.²⁰ These nanoparticles are also able to enter the human body through the skin without the need to be ingested or inhaled due to the small size.²¹ The accumulation of nanoparticles in human body poses a threat to human health and can potentially cause severe negative effects.²² With such diversified applications, these nanoparticles can easily be washed off and contaminate the environment.

The accumulation of such toxic chemicals and substances will generate even dire

negative effects on human health upon consumption leading to widespread epidemic and health risks. Numerous global, regional and domestic health organisations have identified pesticides and mycotoxins as harmful substances to humans due to their toxic nature and negative impacts to human health. It is thus necessary to track levels of pollutants and contaminants in our food supply and environment to prevent food spoilage and deter harmful diseases. Henceforth, there is an increased need for a fast and efficient detection technology of food and environmental contaminants for on-site detection to ensure that the food and water is safe for consumption.

2.2 Detection methodologies and techniques

2.2.1 Analytical detection of OPs and mycotoxins

Ever since the commercialisation and widespread use of OPs, many analytical methods have been developed to detect these compounds^{11,23,24}, such as gas chromatography (GC), high performance liquid chromatography (HPLC) and enzyme-linked immunosorbent assay (ELISA). Similar techniques have also been developed for the detection of mycotoxins in food samples.²⁵⁻²⁷ Such analytical techniques prove to be efficient in the detection of OPs and mycotoxins with low limits of detection. Besides that, chromatographic techniques allow for the sensitive identification of specific compounds from complex mixtures based on the different elution times and masses through the optimization of experimental conditions. However, they pose several disadvantages such as long processing time, use of sophisticated instruments, tedious sample preparation procedures and possibility of sample degradation during the analysis process.²⁸ Recently, immunoassay-based techniques

have been attracting attention due to the high specificity that is afforded with the use of biological elements such as enzymes, antibodies and immunoglobins.²⁹⁻³¹ Despite that, these biological elements have small tolerance window and are unstable in harsh experimental conditions. The highly selective nature of enzymes and antibodies to specific pesticides or mycotoxins might also be disadvantageous with extensive sample preparation procedures and long analysis time required. Spectroscopic techniques may provide an alternative for mycotoxins and OPs detection with high sensitivity and specificity. However, such techniques are limited by matrix effect and the need for expensive, bulky machinery. This severely limits its use for rapid on-site point-of-care detection of real samples. As such, alternative methodologies have to be explored.

2.2.2 Analytical detection of nanoparticles

The detection of nanoparticles has often relied on microscopic and spectroscopic techniques with the use of sophisticated, bulky instruments that require specialised training and elaborate sample preparation methods for accurate analysis. Such techniques pose several limitations especially so in microscopy. The elaborate sample preparation steps required may compromise the integrity of the samples and affect accuracy of analysis. The samples prepared might contain agglomerates and undesired side reactions might occur.³² Detection of liquid-based samples is also not possible with microscopy which requires samples to be dry in the vacuum environment.

To overcome this limitation, liquid-based detection is available with dynamic light scattering (DLS) technique which provides a distribution of sizes of nanoparticles in solutions. Analysis is done *in situ* with the help of a laser source. However, the

resolution of the technique is limited by the laser and particles which are too small may not be detected. Besides that, the technique is unable to discern between individual particles and agglomerates which further limits its sensitivity. The presence of impurities such as dust particles can also affect the detection process.³² Advances in technology have led to more techniques available for the detection of nanoparticles with the use of optical analysis³³⁻³⁵ and analytical disc ultracentrifugation (ADC) techniques for accurate detection of nanoparticles.^{36,37} ADC is a high-resolution particle sizing technique governed by Stokes' law to estimate particle sizes of unknown distribution in a set centrifugal field. It measures the sedimentation time of the particles in a fluid of known viscosity and density.³⁸ As the particles' sedimentation rate is proportional to the square of their diameter, the resolution of the analytical disc centrifuge is adequate to separate particles with very small size differences. However, calibration needs to be performed before each run with a solution of known viscosity and standard of particle size distribution. Moreover, long analysis time and expensive instrumentation is still required.

2.2.3 Electrochemical detection techniques

Field detection of pollutants and contaminants are essential in order to reduce health risks and reduce environmental damage. However, the analytical techniques described above are not suitable for on-site field detection. Considering the limitations and challenges associated with the use of analytical techniques described above, researchers have explored the possibility of using electrochemical techniques with the development of electrochemical sensors. Electrochemistry provides the capability to

simplify analytical detection of OPs, mycotoxins and nanoparticles.

The development of electrochemical sensors is limited by and relies on the electrochemical redox properties of the target analytes. The incorporation of nanomaterials and biological elements to electrochemical sensors has the capability to improve selectivity and sensitivity towards detection of OPs and mycotoxins. Lately, progress has been reported on the successful detection of single nanoparticle collisions by electrochemistry which has been termed as ‘impact electrochemistry’.³⁹ It relies on the transfer of electrons when nanoparticles oxidise or reduce upon interaction with an electrode surface set at a fixed potential. A detailed analysis of such electrochemical techniques and innovations will be further outlined in the preceding sections.

2.3.2.1 Advantages and disadvantages of electrochemical techniques

It should be duly noted that the use of electrochemical techniques require the target analytes to be electroactive which limits its applications towards detection of non electroactive targets. In comparison to chromatographic techniques, the use of electrochemical techniques may not accurately distinguish compounds with similar electroactive groups, such as stereoisomers, due to the similar electrochemical reaction taking place.

However, despite the disadvantages stated above, it should be noted that electrochemical detection techniques do confer advantages over other techniques available. The use of electrochemical techniques requires simple sample preparation procedures which do not significantly compromise the integrity of samples and can potentially decrease analysis time. Reaction kinetics and mechanisms can be accurately

analysed with minimal sample required. It also opens up possibilities for development of portable electrochemical prototypes which can aid relevant authorities for efficient on-site detection of contaminants and pollutants in environmental and biological samples analysis. This device can also potentially be used to simultaneously detect multiple environmental and food contaminants efficiently with high degree of selectivity.

2.3 Electrochemical sensors and biosensors

An electrochemical reaction involves the transfer of electrons between the reacting species and electrode surface which generates an electrochemical signal. An electrochemical sensor is able to detect target analytes directly using their electroactive properties or indirectly from the electrochemical responses of electroactive mediators and electrocatalysts.

2.3.1 Electrochemical detection techniques

Electrochemical sensors can be categorised as either amperometric, voltammetric or conductometric sensors depending on the detection mode used.^{40,41}

Figure 2.4 provides a summary of the different electrochemical detection techniques discussed with a comparison on the different working principles involved in each technique.

2.3.1.1 Amperometric sensors

Amperometric sensors measure the current response of an electrochemical reaction at an applied potential. The applied potential serves as the driving force for the electrochemical reaction to take place. It constitutes the majority of sensors commercially available. The change in current measured is a result of the change in concentration of reacting species present in the solution. There are two modes of operation for amperometric sensors which are amperometry-based and voltammetry-based. Amperometry refers to measuring a current response at a fixed applied potential. Alternatively, when the current response is measured at a set range of potentials, it is referred to as voltammetry.

In amperometry, the reaction is held at a fixed constant potential at the working electrode where current response is measured over a period of time.⁴² The current response correlates to the concentration of reacting analyte present in the sample. Amperometry is a useful technique which allows for selective detection as specific potentials are applied corresponding to specific electrochemical reactions. Furthermore, it has negligible charging current which greatly enhances the signal obtained and reduces interferences from background signal thus further improving limits of detection. As such, amperometric methods are widely used in the development of sensors.

In the case of voltammetry, potential is swept over a determined range and the current response is measured. A typical voltammetric response shows a peak current value in the voltammogram where the peak height is proportional to the bulk concentration of the reacting analyte present (**Figure 2.3**).⁴³ Voltammetry is a versatile and useful technique which allows for the analysis of samples at low concentrations.

Examples of voltammetric techniques include cyclic voltammetry, differential pulsed voltammetry, polarography, stripping voltammetry, and square wave voltammetry.

Figure 2.3 A typical current-potential graph obtained for cyclic voltammetry. E_{pc} corresponds to the peak current obtained at cathodic potential; E_{pa} corresponds to peak current at anodic potential.

2.3.1.2 Conductometric sensors

Conductometric sensors measure the changes in electrical conductivity of an analyte or medium. Unlike amperometric sensors, conductometric sensors are generally non-selective. However, recent research in electrode surface modification has made conductometric sensors to be selective. Modification of the electrode surface with biological elements such as enzymes has allowed for the analysis of enzymatic reactions on conductometric sensors by measuring the change in concentration of reacting analyte in the solution. The progress of conductometric sensors has evolved electrochemical biosensors with different biological elements immobilised to improve the sensitivity and function.

2.3.1.3 Potentiometric sensors

Potentiometric sensors function by measuring the potential or charge accumulated at the working electrode at negligible current flow.^{44,45} The response signal is measured between the working and reference electrode and is governed by the Nernst equation which correlates the potential to logarithm of the concentration of the analyte. The signal generated allows the study of ion activities in the sample.⁴⁶ Potentiometric sensors are generally designed to react selectively with the use of ion selective electrodes where the difference in potential across the electrode is measured. The widely used potentiometric sensor is the pH glass electrode.

Figure 2.4 Comparison between the types of electrochemical detection techniques with their working principles.

2.3.2 Electrochemical biosensors

Recent advances and development of electrochemical sensors have incorporated the use of nanomaterials and biological elements in an integrated approach.⁴⁷ These developments take place hand-in-hand which aims to improve the performance and durability of electrochemical sensors. Correspondingly, it has also led to the development of biological-based electrochemical sensors which have been termed as electrochemical biosensors. According to the definition set by International Union of Pure and Applied Chemistry (IUPAC),⁴⁸ electrochemical biosensors can be defined as 'self-contained integrated devices capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element retained in direct spatial contact with an electrochemical transduction element.'⁴⁹

Electrochemical biosensors have varied applications in medical, environmental and commercial use for detection of target analytes with high selectivity and sensitivity. The biological mediators play different roles in catalysis or indirect detection of electrochemical processes. Examples of biosensors are enzymatic biosensors, antibody-antigen based biosensors and DNA receptors. These biosensor systems make use of the naturally-occurring biological functions of the biological components in the detection mode. They are generally highly versatile which can be tailor-made for selective or simultaneous detection with the use of small sample volumes.⁵⁰

An electrochemical biosensor comprises of three general components which are (1) the bioreceptor, (2) signal transducer, and (3) signal processor (**Figure 2.5**). A bioreceptor is the biological element of the biosensor which controls the biological reaction of the system. Examples of commonly used bioreceptors are enzymes,

antibodies and nucleic acid bases. It is immobilised onto a platform known as the transducer which enhances the electrochemical signal in the presence of the target analyte. Transducer signals include changes in pH, oxidation or reduction of electroactive products, and temperature changes. The signals generated can be measured and analysed electrochemically, optically or calorimetrically. In the case of electrochemical biosensors, the transducer is an electroactive substance which can be measured by using potentiometric, amperometric or conductometric techniques. A biosensor is usually selective due to the specific binding affinities and interactions between the bioreceptor and target analyte. Besides that, the biosensor has to be able to generate accurate and reproducible results rapidly over a broad range of study.

Figure 2.5 Schematic illustration on the different components of an electrochemical biosensor system.

2.3.3 Types of electrochemical biosensors

Electrochemical biosensors can be divided based on the type of bioreceptor. There are two general types of electrochemical biosensors which are biocatalyst-based, and affinity-based biosensors.⁴³

2.3.3.1 Affinity-based biosensors

In affinity-based biosensors, the target analytes binds to the bioreceptors which induces a change in surrounding environment and generates an electrochemical signal. Bioreceptors used for such system includes antibodies and nuclei acids. **Figure 2.6** shows an example of affinity-based biosensor for the detection of mycotoxins using antibodies as the bioreceptor. The mycotoxin and mycotoxin-horse radish peroxidase (mycotoxin-HRP) conjugate has same affinity to the antibody and competes for binding. The binding of mycotoxin-HRP conjugate with antibody activates HRP which results in the catalysis of hydroquinone to electroactive benzoquinone in the presence of hydrogen peroxide. The formation of benzoquinone can be detected electrochemically. The signal generated is thus proportional to the activity of HRP which is inversely proportional to the concentration on mycotoxin present. However, when mycotoxin binds with the antibody, due to the absence of HRP, no electrochemical reaction is induced and hence no electrochemical signal is detected. As such, it can then be deduced that the concentration of mycotoxin in the sample is high.

Figure 2.6 Antibody as bioreceptor in affinity-based biosensor system for the detection of mycotoxin. The binding of mycotoxin-HRP conjugate generates an electrochemical signal while no signal is generated when mycotoxin binds.

2.3.3.2 Biocatalyst-based biosensors

Biocatalyst-based biosensors rely on bioreceptors which can catalyse reactions such as enzymes and cells. The response signal is generated based on the rate of formation of product, disappearance of analyte or inhibition effect. The first reported biocatalyst-based biosensor was in 1962 by Clark and Lyons for the monitoring of glucose levels in blood.⁵¹ It comprised of an enzyme glucose oxidase which binds specifically to glucose and oxidises it in the presence of oxygen. Therefore, glucose concentration was proportional to the decrease in oxygen concentration.⁵² It is also an example of an amperometric-based biosensor. Enzyme biocatalyst-based biosensors are more common due to its reusability, high turnover number and specificity due to the binding model of enzymes.

Rapid detection and analysis of pesticides in environmental and biological

samples is of utmost importance for appropriate measures to be implemented. As described previously, OPs are harmful neurotoxic compounds that are commonly used as nerve agents and pesticides. Due to their high toxicity, rapid detection of OPs has been a topic of interest. **Figure. 2.2** shows an enzyme commonly used for the detection of OPs. OPs bind irreversibly to the enzymes and inhibit their function. The signal output generated is thus inversely proportional to the concentration of pesticide present in the sample. This biosensor functions on an inhibition-based principle which will be discussed in detail in the next section.

2.3.4 Inhibition-based enzyme biosensor

Inhibition-based enzyme biosensor measures the degree of inhibition by comparing response signals before and after the biosensor is introduced to the target analyte. The target analyte will usually bind irreversibly to the active site of the enzymes which blocks the mediators from binding. In order to have a quantifiable basis of comparison on the extent of inhibition, the inhibition percentage (I%) is determined as illustrated in **eq 2.1**. A high I% value would indicate greater inhibition of enzymes which conversely relates to higher concentration of target analyte present.

$$I (\%) = \frac{[(R_b - R_a)]}{R_b} \times 100 \quad (2.1)$$

where R_b is the signal response before inhibition and R_a is the signal response after inhibition.

The fabrication of inhibition-based enzyme biosensor for the detection of OPs uses cholinesterase enzyme. There are two types of cholinesterase which are acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE).¹¹ AChE is primarily found

in red blood cells and neuron synapses while BuChE is primarily found in blood plasma. Different cholinesterase-based biosensor systems have been reported depending on the mode of detection which are amperometric,^{53,54} potentiometric,⁵⁵ and conductometric.⁵⁶

Improvements in inhibition-based enzyme biosensors performance have focused on lowering limits of detection, expanding detection range, enhancing sensitivity and improving enzyme immobilisation. Modification of signal transducer platforms have been of particular interest in aims of improving conductivity and increasing surface area for greater loading of enzymes. New nanomaterials and their composites have been studied with aims of improving the biosensor response signals and immobilisation of enzymes. Direction of research in transducer materials over the past decade has placed emphasis on carbon-based materials such as carbon nanotubes^{53,57} graphene,^{58,59} carbon black^{60,61} and their modified composites.^{62,63} These nanomaterials have significantly improved performance of enzyme-based biosensor systems with good stability and reproducibility. However, a paradigm shift has to be taken and other layered materials have to be explored which can potentially further improve the performance of biosensor systems. An emerging class of two-dimensional layered materials, which shifts away from carbon-based materials, is transition metal dichalcogenides (TMDs). TMDs have been found to exhibit excellent electrical conductivity and versatility which is comparable to graphene. In-depth discussion of TMDs can be found in the following section.

For the scope of this thesis, we will be focusing on amperometric inhibition-based AChE biosensor system. **Chapter 6** will outline the development of an

inhibition-based enzyme biosensor with the use of transition metal dichalcogenides, more specifically tungsten disulphide (WS_2), for the detection of OPs.

2.4 Two-dimensional layered materials

Two-dimensional (2D) materials, sometimes described as single-layered materials, are widely studied due to their unique physical and chemical properties which allows for numerous potential applications. Two different types of materials will be introduced which are (1) graphene, and (2) transition metal dichalcogenides.

2.4.1 Graphene

Graphene is a well-known carbon material with widespread application such as lubricants, refractory material in factories and pencils brought about by its excellent electrical, chemical and physical properties.⁶⁴ It consists of a single layer of sp^2 hybridised carbon atoms arranged in a honeycomb-like structure as illustrated in **Figure 2.7**. The structure was first theorised and proposed by P. R. Wallace in 1946 in an attempt to understand electronic properties of graphite.⁶⁵ It was not until 2004 where the first successful isolation and characterisation of graphene was reported by Konstantin Novoselov and Andre Geim by mechanical exfoliation of graphite using adhesive tape.⁶⁶ This discovery led to them receiving the Nobel Prize in Physics for the “ground-breaking experiments regarding the two-dimensional material graphene”.⁶⁷ Soon after, it sparked widespread research into studying the chemical and physical properties of graphene bringing new insights on its excellent electronic, mechanical, thermal and chemical properties.⁶⁸⁻⁷¹

Figure 2.7 Atomic structure of a single layer of sp^2 hybridised carbon atoms representing a single graphene sheet.

When several layers of graphene sheets are stacked parallel over one another, they form the three-dimensional structure known as graphite.⁷² It is defined by IUPAC as ‘an allotropic form of the element carbon consisting of layers of hexagonally arranged carbon atoms in a planar condensed ring system (**Figure 2.8**).⁷³ In their natural form, graphite can exist in various forms which are vein, flake and amorphous graphite.⁷² They differ in their crystallinity which affects the electrical and physical performance. Recently, pencil graphite modified with multi-walled carbon nanotubes have been used as an electrode for the detection of diclofenac sodium (DIC), an anti-inflammatory drug.⁷⁴ The fabricated electrode was able to detect low concentration of DIC in real samples with low limits of detection over wide concentration range.

Figure 2.8 Structure of graphite with alternating graphene layers arranges in parallel manner. Reproduced from ref. 72.

Highly ordered pyrolytic graphite (HOPG) specifically refers to graphite with graphene sheets stacked in a highly ordered manner. HOPG comprises of two distinct regions which are the basal and edge planes (**Figure 2.9**). The basal plane refers to the surface of HOPG which lies parallel to the stacked graphene layers while the edge plane is the surface perpendicular to the stacked graphene sheets. The two planes differ greatly in their electrochemical performance with the edge plane being the more reactive region.⁷⁵⁻⁷⁷ The difference in the performances can be attributed to the difference in the degree of defects present between the edge and basal plane.⁷⁸

Figure 2.9 Illustration of basal vs. edge plane of graphite. Reproduced from ref. 75.

Boero and co-workers compared the performance of edge (EPPG) and basal plane (BPPG) of HOPG for the detection of β -Nicotinamide adenine dinucleotide (NAD^+) and its reduced derivative (NADH). They concluded that EPPG gave better response due to the defects present at the edge surface and presence of oxygen-containing groups, as determined by XPS analysis. These factors facilitated electron transfer and adsorption of the target analyte on EPPG which makes it more electroactive as compared to BPPG.⁷⁹ The voltammograms obtained with EPPG gave defined peaks in the presence of NADH (Figure 2.10).

Figure 2.10 Cyclic voltammograms of (A) EPPG, and (B) BPPG (a) before, and (b) after immersed in 5 mM NADH solution for 1 hr. Condition: Phosphate buffer of 50 mM, pH 7.4. Reproduced from ref. 79.

However, recently, Unwin and co-workers refuted the traditional understanding on the inert nature of BPPG.⁸⁰ From their works, they deduced that pristine basal plane provided an electroactive area for electron transfer process which is contrary to traditional long-standing understandings.

Nevertheless, the different graphitic electrodes have proven to be effective which makes them suitable for use in various electrochemical applications such as supercapacitors and sensing platforms.⁸¹ The performance of EPPG, BPPG and other

well-defined electrodes will be compared and further analysed for the multiple detection of different mycotoxins in **Chapter 3**.

2.4.2 Transition metal dichalcogenides

The extensive research conducted on graphene and its analogues has renewed interest on research of other 2D materials which has led to the resurgence of interest in transitional metal dichalcogenides (TMDs).⁸² TMDs are a group of two-dimensional materials, similar to graphene, of chemical formula MX_2 , where M is a transition metal (Mo, W) with X being the chalcogen atom (S, Se). The transition metal atom layer is sandwiched between two chalcogen atom layers.^{83,84} The most widely studied TMD material is molybdenum disulphide (MoS_2).

Periodic Table of the Elements																			

Figure 2.11 Highlighted elements show the elements that can form layered transition metal dichalcogenides.

The structure of a single-layer TMD material has either an octahedral or trigonal prismatic coordination phase (**Figure 2.12**). TMDs are able to form three different types of polymorphs exhibiting different stacking sequence governed by their symmetries.

They are (1) 1T which has a single layer of tetragonal symmetry, (2) 2H which has two layers with hexagonal symmetry, and (3) 3R which comprises of three layers with rhombohedral symmetry (**Figure 2.12**). Mixtures of polymorphic structures arise in multi-layered TMDs as each individual layer can possess either coordination phases. The different coordination structures and stacking sequence in multi-layered TMDs exhibit different electronic, physical and chemical properties. TMDs occur mainly in bulk with 2H symmetry and limited applications due to its semi-conducting nature. The properties alter when the bulk TMDs are exfoliated to form few or single layers. Chemical exfoliation techniques with organolithium (butyllithium, *tert*-butyllithium) have been the most widely adopted exfoliation technique. Lithium ions will move between the TMD sheets which draws them apart thus resulting in exfoliation.⁸⁵ The Li-intercalation of TMDs are widely used as a ‘top-down’ approach for obtaining exfoliated TMD sheets with superior electronic properties.^{82,86,87} With the organolithium exfoliation to few or single layer, the electronic properties of TMDs are altered due to the change in symmetry from 2H to 1T.⁸⁸ The exfoliated TMD sheets exhibit metal-like behaviours which are different from the semi-conducting bulk precursors. Consequently, the superior performance of exfoliated TMDs can be used for electrochemical application, more specifically in sensing and biosensing applications.

Figure 2.12 Metal coordination and stacking sequence of the respective TMD unit cell. Metal coordination with octahedral arrangement yields a tetragonal symmetry (1T). A trigonal prismatic arrangement can give either hexagonal symmetry (2H) or rhombohedral symmetry (3R). Reproduced from ref. 84.

2.4.2.1 Transition metal dichalcogenides for electrochemical sensing

TMDs have been reported to be effective for use in numerous applications mainly in energy storage devices, such as lithium-ion batteries and capacitors, and electrochemical sensing applications. For the purpose of this thesis, the electrochemical sensing applications of TMDs will be highlighted and discussed.

Molybdenum disulphide (MoS_2) is the most widely studied TMD presently. Zhang and co-workers obtained single layer MoS_2 sheets by electrochemical reduction which was demonstrated to use for glucose sensing and selective sensing of dopamine in the presence of ascorbic and uric acid by voltammetry.⁸⁹ Subsequently, Xia and co-workers

successfully immobilised a biological element, horse radish peroxidase (HRP), onto MoS₂ nanosheets via electrostatic attraction for the electrocatalytic reduction of H₂O₂. The synthesized MoS₂ sheets were negatively charged which forms strong interactions with HRP.⁹⁰ More recently, Pumera and co-workers explored and demonstrated the use of other TMD materials in electrochemical sensing and biosensing application. 1T-phase WS₂ was demonstrated to be an excellent transducer in a heme-based H₂O₂ biosensor by providing a large surface area conductive platform.⁹¹ Besides that, MoSe₂ nanoparticles were found to be efficient labels in a magneto-immunoassay system for the detection of rabbit immunoglobulin G (IgG) protein with good selectivity and low limits of detection.⁹²

Despite the extensive research conducted on alternative synthetic strategies and device fabrication, it is surprising to note that fundamental understandings on the physical and chemical properties of TMD materials are not widely analysed or studied. It is especially crucial to have a sound understanding of the intrinsic properties of TMDs before prototypes and operational devices can be constructed with good efficiency. The topic of interest that has to be mentioned is the study of the inherent behaviour TMDs possess. The term 'inherent electrochemistry' is coined to describe the intrinsic behaviour of the material when subjected to an electrochemical reaction. It is brought about by redox reactions which the materials undergo at specific anodic or cathodic potentials independent of analyte used. Each material will have their inherent electrochemistry due to the electrochemical reactions of functional groups present. For example, graphene oxides, analogues of TMDs, were found to undergo reduction at cathodic potentials which will limit its use at high cathodic potentials (**Figure 2.13**).⁹³

The study of inherent electrochemistry of TMDs is of importance to prevent negative results where signal response from analytes obtained gets masked.

Figure 2.13 Cyclic voltammograms of four different graphene oxides (GO) in blank 20 mM borate buffer. Inherent reduction peak observed at cathodic potentials for all 4 GO materials in comparison to bare GC (dotted line). Reproduced from ref. 93.

Chorkendorff and co-workers reported the negative effect of the inherent oxidation of MoS_2 towards hydrogen evolution reaction (HER) electrocatalysis.⁹⁴ The material was observed to undergo an irreversible oxidation at anodic potentials which decreases the HER activity observed (**Figure 2.14**). Through XPS analysis, it was determined that MoS_2 was oxidised to MoO_3 in blank buffer.

Figure 2.14 Cyclic voltammograms showing the inherent electrochemical activity of MoS_2 . Condition: 0.5 M H_2SO_4 with N_2 purging. Inset shows formation of irreversible anodic peak with a decrease in peak in the second sweep. Reproduced from ref. 94.

Besides that, the exfoliation of various TMD materials with different organolithium compounds does not have an adverse effect on the inherent electrochemistry of the materials and it was shown to still persist. Anodic peaks can be seen forming at the first voltammetric scan for both bulk and exfoliated TMDs.⁸⁵ The peak diminishes significantly on the subsequent scans which illustrates the irreversible nature of the oxidation of the respective TMD materials (**Figure 2.15**). On the other hand, despite the limitations it imposes, inherent electrochemistry can be put to positive use and application. Pumera and co-workers have taken advantage of the inherent oxidation of MoS_2 as labels for the detection of DNA hybridization.⁹⁵

Figure 2.15 Cyclic voltammograms showing the inherent electrochemical activity of various TMD materials in bulk and after exfoliation with methyllithium (MeLi), and butyllithium BuLi). Reproduced from ref. 85.

A comprehensive study on the limitations of MoS₂ is still lacking. In order for MoS₂ to be an excellent material for electrochemical sensing applications, it has to be stable and inert over large potential window. The inherent electrochemistries of other TMDs are also not well documented and explored. The electrochemical behaviours of different TMD materials are expected to be different attributed to the different stabilities, reacting species involved and oxidation products as illustrated by Pourbaix diagrams.⁹⁶ Buffer conditions, such as pH and temperature, will also influence the reactions. Thus, it is crucial to optimise buffer conditions for optimal performance of TMD materials. This is especially important to optimise the capability of TMDs for future sensing and biosensing applications. As such, this thesis aims to provide some insights

into the working range and limitations of MoS₂ as a sensing platform which will be analysed in **Chapter 4**. The inherent electrochemistries of other TMD materials such as MoSe₂, WS₂, and WSe₂ at variable pH buffer conditions will also be discussed in detail in **Chapter 5**.

With a good and comprehensive understanding of the inherent electrochemistry of TMD materials, it can be used to optimal use in the construction of electrochemical biosensor system for real samples analysis which will be further demonstrated in **Chapter 6**.

2.5 Impact electrochemistry

Advancement in nanotechnology and nanofabrication techniques has enhanced the performance of nanomaterials. Nanomaterials have found numerous applications ranging from commercial, industrial, healthcare and scientific research. A commonly used nanomaterial is silver nanoparticle or 'nanosilver'. Silver nanoparticles can be found in many applications such as clothing, cosmetics, electronic devices and food due to its durability and strong antimicrobial property. The widespread use of silver nanoparticles could result in leaching into the environment and groundwater due to runoff thus resulting in contamination. Besides that, the accumulation of silver nanoparticles has been found to have severe toxicological effects on human health. The accumulation of nanoparticles has potential to cause severe negative effects to human health and the environment.¹⁹ As such, there is the urgent need to monitor and control the amount of nanoparticles.

The ability to detect and analyse single particle reactions has always been the

desired aim of researchers. This will enable the accurate understanding of reaction kinetics and particles interactions. As previously discussed, current methods available to detect nanoparticles involve the use of spectroscopic and microscopic techniques such as UV-Vis spectroscopy, fluorescence spectroscopy and electron microscopy. The use of such techniques requires elaborate sample preparation procedures and use of specialised equipment which are often expensive. Besides that, the sample preparation steps, which usually involve drying steps, may compromise the features of the nanoparticles involved which will affect the reliability of measurements obtained. Dispersive light scattering (DLS) and analytical disc ultracentrifugation (ADC) can be used to try counter these limitations. It enables for accurate sizing and distribution of nanoparticles *in situ* in solution. These techniques have proven to be more reliable and accurate with respect to spectroscopic techniques mentioned above. However, the accuracy and reproducibility is greatly dependent on the handling capabilities of the operator. Besides that, these techniques are unable to distinguish monodispersed particles from agglomerates which may compromise the results obtained. Calibration with known standards also needs to be performed each time before analysis which is time consuming.

A more robust alternative approach is required for more accurate and sensitive detection of nanoparticles. Recently, the use of electrochemistry has been explored to provide dynamic and real time results for *in situ* analysis. 'Impact electrochemistry' is an upcoming technique which has enabled for the detection of nanoparticles and reactions at low concentrations and volumes.^{32,97} The use of chronoamperometry has been explored with promising results. When a nanoparticle strikes onto a charged electrode

surface set at a desired potential, the interaction will initiate a redox reaction which involves electron transfer. A spike signal will be obtained with each successful particle collision reaction. From the signal obtained, the area under a spike signal can be calculated which corresponds to the total amount of charge transferred during the reaction based on the modified Faraday's law of electrolysis (**eq 2.2**). Conversely, the number of reacting particles can be determined.

$$m = \frac{QM}{Fz} \quad (2.2)$$

where m is mass of nanoparticle, Q is total charge (C), F is Faraday constant, M is molar mass, z is the number of electrons involved in the impact electrochemistry process.

2.5.1 Faradaic vs. non-faradaic process

Two different impact electrochemistry processes can occur which are classified as faradaic and non-faradaic process (**Figure 2.17**).

In a faradaic impact electrochemistry process, direct transfer of electrons is involved between the reacting nanoparticles and electrode surface. The particles come into contact with the electrode surface and an electrochemical redox reaction occurs if it is kinetically feasible. Relevant information can be obtained from the spike signal generated by applying the modified Faraday's equation on electrolysis (**eq 2.3**) such as the size of the nanoparticle (**Figure 2.16**). This is of the assumption that the nanoparticles are monodispersed and exist as defined spheres. Further reaction kinetics can also be inferred from the results obtained such as mass flux. Faradaic impact electrochemistry process usually involves particles which have defined shape termed as 'hard' particles due to their rigid structure and shape.

$$r = \sqrt[3]{\frac{3QAr}{4F\pi\rho}} \quad (2.3)$$

where r is radius, ρ is density, Ar is atomic mass and m is mass of nanoparticle, Q is total charge (C), F is Faraday constant, M is molar mass, z is the number of electrons involved in the impact electrochemistry process.

Figure 2.16 Illustration of faradaic impact electrochemistry working principle. A nanoparticle gets oxidised or reduced upon impact with electrode surface. A spike signal is produced in chronoamperometry. Analysis of impact results gives the size distribution of nanoparticles.

Conversely, a non-faradaic or double-layer impact electrochemistry process occurs as a result of the transfer of charges at the electrical double layer due to capacitance effect. No apparent chemical reaction is observed during this process and the signal obtained is a result of the charged electrode surface being perturbed by nanoparticles near it. This transfer of charges, instead of electrons, results in the signal generated. Particles that undergo such a process are generally electrochemically inert and not easily oxidised or reduced. This can be due to their stability or having complex undefined structure. Since these nanoparticles do not have a rigid and defined structure, they are labelled as 'soft' particles.

Figure 2.17 Comparison between non-faradaic and faradaic impact electrochemistry processes.

2.5.2 Progress in impact electrochemistry

The first reported experimental observations of impact electrochemistry was by Micka in the 1950s and 1960s with the use of polarography⁹⁸ for the detection of metals,⁹⁹ and metal compounds.^{100,101} Zutic and co-workers made similar observations with the use of organic droplets on mercury electrode using polarography.¹⁰² The works were further explored by Heyrovsky with his observations with colloidal tin oxide (SnO_2)¹⁰³ and titanium oxide (TiO_2).¹⁰⁴ From his findings, he concluded that the voltammograms obtained were a summation of individual signals of the dispersed nanoparticles which overlapped and superimposed onto one another resulting in the final signal observed (**Figure 2.18**).¹⁰⁵

Figure 2.18 Voltammetric curves for colloidal (A) SnO_2 , and (B) TiO_2 . Dotted curves are representative voltammograms obtained from individual particle reaction which will superimpose to form the resultant voltammetric curves. Reproduced from ref. 105.

Following the findings by Heyrovsky, Bard provided detailed experimental findings for the electrochemical detection of single molecule.¹⁰⁶ Subsequently, Scholz and co-workers demonstrated a similar technique to detect adhesion activity of liposomes made from lecithin molecules using mercury electrode (**Figure 2.19B**).¹⁰⁷ From the signal obtained, the number of lecithin molecules which makes up the liposomes was determined. This was followed up with the detection of montmorillonite particles by adhesion events.¹⁰⁸ A comprehensive study on the kinetics of the adhesion events was subsequently performed.¹⁰⁹

Figure 2.19 (A) Schematic illustration on the adhesion mechanism of lecithin liposomes upon contact with static mercury drop electrode. (B) Chronoamperometric measurements of adhesion process signals obtained. Conditions: 2.2 g L^{-1} liposome suspension concentration, phosphate buffer pH 7.4, potential of 0 V vs. Ag/AgCl. Reproduced from ref. 107.

Moving away from mercury electrodes, significant progress was made into developing impact electrochemistry techniques by Compton and co-workers.¹¹⁰⁻¹¹² This catalysed the progress and research on nano-impacts with focus on sizing of nanoparticles,¹¹³⁻¹¹⁵ improving sensitivity of detection techniques,¹¹⁶⁻¹¹⁹ and indirect detection of nanoparticles using electrocatalytic mediators.¹²⁰⁻¹²² Recently the direction

for impact electrochemistry is moving away from the detection of defined nanoparticles and is shifting towards detection of nanomaterials such as layered nanosheets^{123,124} and ‘soft’ particles such as nanodroplets,¹²⁵ bacteria,¹²⁶ and liposomes.^{127,128} The possibility of detecting single enzyme catalytic activity was also explored^{129,130} with some differing theoretical understandings and interpretations.¹³¹⁻¹³³

The field of impact electrochemistry may seem limitless as researchers constantly push the boundaries for single entity detection.¹³⁴ However, detecting reactions at the nano scale pose several limitations which are sometimes overlooked. As such, one has to understand the limitations and challenges present before designing and performing impact electrochemistry investigations.

2.5.1 Experimental Challenges and Limitations

The detection of nanoparticles at the small scale poses numerous problems and limitations due to the small nature of the compounds.

In a macro perspective, there are several challenges faced in experimental procedure such as background noise from the buffer and electrical signal, spontaneous agglomeration or aggregation of the nanoparticles before reaction and the lack of mobility of nanoparticles. These challenges mentioned affect the accuracy and detection capabilities of impact electrochemistry. Improvements in experimental procedures have been investigated to improve the sensitivity and detection limits of nano-impact technique. To reduce noise from electrical interferences, experiments can be performed in a Faraday’s cage. Besides that, the size of the electrode has to be small. The use of ultramicroelectrodes have aided in reducing the effect of background noise

and electrical signal thus amplifying the signal from nano-impact events. Recently, an array of microelectrodes has been used with significantly lower detection limits.¹³⁵ In order to reduce the effect of agglomeration or aggregation; capping agents have been used on the nanoparticles. The surfaces of nanoparticles are functionalised with stable and non-electroactive compounds which repel the nanoparticles by preventing direct contact. For example, silver nanoparticles have been known to have citrate-capping to prevent their aggregation and remain monodispersed.¹³⁶ It is important to have the particles monodispersed for accurate size determination. The last perimeter is buffer used. Buffer systems are crucial in any impact electrochemistry experiment as it affects motion of the nanoparticles and prevents nanoparticles aggregation. In the example of silver nanoparticles, buffer with high concentration of chloride ions is not ideal as it promotes the formation of insoluble silver chloride salt upon the oxidation of silver nanoparticles. The presence of insoluble silver chloride in the solution will affect the motion of the silver nanoparticles and block the electrode surface causing interference.

Recently, research has been geared to understanding the dynamics and kinetics of nanoparticle interactions in the nano perspective. This allows researchers to analyse the motion and behaviour of the nanoparticles to understand real-time reaction kinetics and particle interactions.¹³⁷⁻¹³⁹ The information generated will provide better understanding of reactions at the nano scale and potentially overcome the limitations of impact electrochemistry. With their studies on silver nanoparticles, Kanoufi and co-workers discovered that not every nano-impact event results in an electrochemical signal due to insufficient contact time between the nanoparticles and electrode surface, insufficient exposure of the reacting nanoparticles and not every nanoparticle is

kinetically active.^{140,141} Besides that, the surface of the electrode might not be uniformly electroactive. Thus, there is the possibility of nanoparticles landing on the non-electroactive site of the electrode which does not generate any signals.

Figure 2.20 (Left) Schematic of experimental set-up. An electrochemical fluidic device coupled to a CCD camera with silver nanoparticles impact activity taking place on gold ultramicroelectrode (UME). (Right) Green light represents the scattered light signal from a single Ag nanoparticle upon landing on the UME. The disappearance of the scattered light correlates with the appearance of the brown line which is attributed to it being fully oxidised. Reproduced from ref. 141.

Whilst extensive research has focused on well-defined or ‘hard’ particles, lately, studies are being conducted on the detecting particles with no defined shape which are membrane-bound known as ‘soft’ particles. Due to their fluid structure, several challenges arise in detecting these particles. The direct detection of ‘soft’ nanoparticles is complex due to the fluid nature of the particles. The complex nature of the membranes formed makes it difficult for cells and liposomes to be detected. Compton and co-workers had demonstrated the direct detection of single micelles made of cetyltrimethylammonium bromide (CTAB).¹⁴² The micelles were detected by the

oxidation of the bromide anions at the membrane of the micelles. The indirect detection of 'soft' particles has been preferred where the particle will be filled with electroactive mediators or labels. The mediators or labels usually catalyse known reactions such as hydrogen evolution or hydrazine oxidation. With a known volume of electroactive compounds in the 'soft' particles, and by analysing the secondary reactions taking place, the 'soft' particles can be detected and quantified through mathematical calculations (**Figure 2.21**). Ideally, direct detection of 'soft' particles is more favourable as it involves faster preparation and analysis time.

Figure 2.21 Illustration on the working process for the indirect detection of 'soft' particles with electroactive label loaded by indirect faradaic impact electrochemistry process.

One method that can be used is to rupture the 'soft' particle upon impact on the electrode surface and detect it via a non-faradaic process. This is analogous to the detection of lecithin liposomes by Scholz using mercury electrode¹⁰⁷ and oil droplets by Zutic using polarography.¹⁰² The rupturing of the liposomes will induce a change in double layer capacitance on the electrode surface and generate electrochemical signals which can be analysed and quantified. However, the challenge to rupture liposomes remains. We set forth to ascertain the detection of 'soft' particles by rupture impact electrochemistry using a lipid liposome model which will be elaborated in **Chapter 7**.

Figure 2.22 Summary of ‘soft’ particles detection by impact electrochemistry.

2.6 Three-dimensional (3D) printing

3D printing, also known as additive manufacturing, can be defined as the process of fabricating a three-dimensional object based on a software-designed template in a layer-by-layer formation process.¹⁴³ The advent of 3D printing has moved away from traditional processes of constructing objects and allowed for the assembly of complex devices and specialized equipment with high precision. In additive manufacturing, with the aid of computer-aided design software, the 3D design is sectioned into layers after which the object will be printed in the individual layer stacked over one another. The first known and reported 3D printed object was in 1981 by Hideo Kodama with the fabrication of three-dimensional plastic polymer models cured under ultraviolet (UV) light.¹⁴⁴ The plastic model was formed by successively solidifying and curing layers of polymer under UV light exposure. Following this, the first commercially available system was developed by Charles W. Hull in 1984. The system adopted similar strategy developed by Kodama which became known as stereolithography (SLA). Hull further

introduced the STL file format widely used today which links the digitally designed object to 3D printing devices.

Over the years, several 3D printing methodologies have been developed which can be categorised as extrusion, powder-based, light polymerisation and lamination.¹⁴⁵ They differ in the materials used and methods of binding. A summary of the various 3D printing techniques is shown in **Table 2.1**.

Table 2.1 Summary of 3D printing methodologies.

Process	Techniques	Materials
Extrusion	Fused deposition modelling (FDM) Direct ink writing (DIW) Composite filament fabrication (CFF)	Thermoplastics, rubber, metals Ceramic, metals, and their composites Nylon and its composites
Powder-based	Selective laser sintering (SLS) Electron beam melting (EBM) Selective laser melting (SLM) Direct metal laser sintering (DMLS) Directed energy deposition Selective heat sintering (SHS)	Thermoplastics, metals, ceramic Metals Metals Metals Metals Thermoplastic
Light polymerisation	Stereolithography (SLA) Material jetting Digital light processing (DLP) Continuous liquid interface printing (CLIP) 2 photon polymerisation (2PP)	Photopolymers Photopolymers Photopolymers Photopolymers UV-curable resins
Lamination	Laminated object manufacturing (LOM)	Paper, metals, plastics as laminated sheets

3D printing has many advantages and benefits in manufacturing and mass production in comparison to traditionally-available techniques.¹⁴⁶ With the rise of 3D printing, customisable items can be easily and cheaply fabricated requiring lesser time and use of material. 3D printing has found numerous applications in microfluidics

development especially so for point-of-care diagnostics¹⁴⁷ such as blood samples analysis,^{148,149} detection of virus,¹⁵⁰ miniaturised automated biomedical applications,^{151,152} and cell toxicity analysis.¹⁵³

With such versatility in its applications and capabilities, the incorporation of 3D printing with electrochemistry will be an interesting paradigm which can potentially open up greater possibilities for complex research which require specialised prototype devices.

2.6.1 3D Printing Applications in Electrochemistry

The integration of 3D printing with electrochemistry has the possibility to open up limitless potential applications in research and prototype development. The ability to fabricate electrodes for complex and specific functions at low cost is an attractive factor that can be explored. With the ability to fabricate customised electrodes and electrochemical devices, it provides an alternative from the use of carbon-based electrodes widely used today. The possibility and ability to tailor-make electrochemical set-ups have the potential to improve experimental procedures and accuracies of results obtained. Electrochemical methods are not only limited to either amperometry or voltammetry. It can be further extended to other methods which include impedance, potentiometry and piezoelectricity.¹⁵⁴

Recently, works on 3D printing for electrochemical applications have shown promising outcomes. 3D printing has been advantageous in the fabrication of microfluidic devices for the electrochemical detection of heavy metals¹⁵⁵ and glucose biosensing devices¹⁵⁶ with low limits of detection in real samples. The ability to fabricate

specialised channels in the microfluidic system makes 3D printing an appealing tool for microfluidic design. Besides applications in microfluidics, 3D printing has been applied for energy related applications. Izumi and co-workers fabricated a $\text{Li}_4\text{Ti}_5\text{O}_{12}$ electrode by 3D printing with high aspect ratio for application in lithium-ion battery (**Figure 2.23A**).¹⁵⁷ Li and co-workers fabricated graphene aerogel microlattices for supercapacitors application (**Figure 2.23B**). The fabricated aerogel has shown to have high capacitive retention and power densities comparable to other reported devices.¹⁵⁸ These examples demonstrate the possibilities of fabricating unique devices with specially designed structures with comparable performance to conventional devices reported.

Figure 2.23 (A) Microscopic image of 3D printed $\text{Li}_4\text{Ti}_5\text{O}_{12}$ electrode by Izumi and co-workers as cathode in Li-ion battery. Reproduced from ref. 157. (B) 3D printed graphene-graphene nanoplatelets aerogel microlattice printed by Li and co-workers as supercapacitor. Reproduced from ref. 158.

For the interest of this thesis, we will be further exploring the use and application of 3D printing in electrochemical sensing applications. Electrodes can be easily designed and printed for specialised applications in electrochemical sensing. Pumera and co-workers have demonstrated the varied possible applications of helical-shaped 3D printed stainless steel electrodes (**Figure 2.24**) for sensing and biosensing applications. Modification of the helical stainless steel electrodes with IrO_2

has demonstrated good catalytic properties in oxygen reduction reactions in alkaline solutions.¹⁵⁹ Similar fabricated electrodes were then electrodeposited with gold and used as a transducer platform in the biosensing for the detection of DNA hybridisation.¹⁶⁰ The performance of the electrodes was enhanced by the metallic backbone of the stainless steel electrodes. More recently, a self-contained electrochemical device was fabricated comprising of electrodes and electrochemical cell. This self-contained prototype was demonstrated to be a proof-of-concept device capable of water splitting reactions with good performance and wide potential for future applications.¹⁶¹

Figure 2.24 (A) Schematic diagrams of helical 3D printed stainless steel electrode design used by Pumera and co-workers for sensing and biosensing applications. (B) Dimensions of 3D printed electrode used. Reproduced from ref. 159.

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Chapter 3

Simultaneous Detection of Mycotoxins by Voltammetry

3.1 *Introduction*

3.2 *Experimental*

3.3 *Discussion*

3.3.1 *Simultaneous electrochemical detection of zearalenone and citrinin on edge plane pyrolytic graphite electrode*

3.3.2 *Simultaneous anodic and cathodic detection of patulin and ochratoxin A on well-defined carbon electrodes*

3.4 *Conclusion*

The results in this chapter were published in the following manuscripts:

Muhammad Zafir Mohamad Nasir, Martin Pumera. Mycotoxins: Simultaneous Detection of Zearalenone and Citrinin by Voltammetry on Edge Plane Pyrolytic Graphite Electrode. *Electroanalysis* **2014**, 26, 1901-1904.

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Muhammad Zafir Mohamad Nasir, Martin Pumera. Simultaneous Anodic and Cathodic Voltammetric Detection of Patulin and Ochratoxin A on Well-defined Carbon Electrode. *Electroanalysis* **2014**, 27, 924-928.

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Summary of Project

Mycotoxins are toxic compounds found in food and feedstock. It is of paramount importance to establish proper analytical techniques for efficient point-of-care detection of such compounds for food quality control. Presently, research has been focused on the single detection of mycotoxins with modified sensor and biosensor platforms. In this study, we show that edge-plane pyrolytic electrode offers excellent selectivity and sensitivity towards simultaneous detection of zearalenone (ZEA) and citrinin (CIT).

We also investigate here the possibility to simultaneously detect two other important mycotoxins with distinctive cathodic and anodic electrochemical signals. Here, we compare well-defined surfaces for electrochemical detection of patulin (PAT) and ochratoxin A (OTA) on edge plane pyrolytic graphite and glassy carbon electrodes. Patulin shows well-defined peak at -1.5 V, deeply in cathodic region; ochratoxin A is in contrary oxidizable at potential +0.9 V. We show that edge-plane pyrolytic graphite provides more sensitive response over glassy carbon electrode. It is clear that high density of the edges on graphite is highly beneficial to the voltammetric response of these mycotoxins. The developed technique should have profound impact on research towards portable sensors of mycotoxins.

3.1 Introduction

Mycotoxins are secondary metabolites produced by fungi.¹ The presence of mycotoxins in food and animal feed supply has been a growing problem in many countries due to the negative health effects they cause.² Regulations have been put forward by international agencies such as Joint Research Centre (JRC) of the European Union (EU),^{3,4} Food and Agriculture Organisation (FAO)⁵ and World Health Organisation (WHO)⁶ of the United Nations to control the levels of mycotoxins in food supply. It has been discovered that one fungal species may produce several different mycotoxins.⁷ Hence there is a need for the development of a simultaneous detection method of mycotoxins. Patulin and Ochratoxin A are two mycotoxins produced by similar species of fungi and usually found present together in food and animal feed.

Ochratoxin A (OTA) is a commonly found and well-studied mycotoxin. It is produced by the *Aspergillus* and *Penicillium* fungi. OTA is a main contaminant in cereals, grains and beans.⁸ It is a known nephrotoxin which targets the liver and kidneys of humans.⁸ It is also described to be a possible carcinogen by the International Agency of Research for Cancer (IARC) and is classified as a Group 2B carcinogen.⁹ One prevalent disease caused by consumption of OTA is Balkan endemic nephropathy which plagues countries in the Balkan Peninsula.¹⁰ Patulin (PAT) is produced by *Aspergillus*, *Byssosclamyces* and *Penicillium* fungi¹¹ and commonly found in fruits. It is heat-stable and soluble in water which facilitates the contamination of apple products such as juices and ciders. PAT is known to cause apples to rot and form blue mold.¹² Extensive chromatographic studies have been conducted on the detection and quantification of PAT in apple juice.^{13,14} PAT is found to have negative effects on mice which caused

decrease mortality and suppression of respiratory burst.¹⁵ A study conducted in 1986 showed PAT to undergo an electrochemical reduction process,¹⁶ which is uncommon, as most known mycotoxins undergo electrochemical oxidation.

Scheme 3.1 Chemical structures of (A) ochratoxin A (OTA) and (B) patulin (PAT).

Zearalenone (ZEA) and citrinin (CIT) are two other examples of mycotoxins found in food substances. ZEA is a mycotoxin mainly produced by *Fusarium* species and is usually found in cereal crops such as wheat and barley. A study in 1988 showed that ZEA was found to have contaminated cereals in many countries which brought about an urgent need for effective detection methods then.¹⁷ ZEA is found to mimic the properties of oestrogen and may result in infertility and abortion.¹⁵ CIT has been identified as a mycotoxin and it was first isolated from *Penicillium citrinum* in 1931.¹⁸ CIT has a distinct yellow colour and can usually be found in grains and milk. It is known to be a nephrotoxin which affects the functioning of kidneys¹⁵ and is usually present with ochratoxin.⁸

Scheme 3.2 Chemical structures of (A) zearalenone (ZEA) and (B) citrinin (CIT).

Effective and efficient detection methodologies are crucial to ensure safety of the global food supply. Several techniques have been developed to detect and analyse mycotoxins in food substances such as thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), gas chromatography coupled with mass spectroscopy (GC-MS) and ELISA.¹⁹⁻²⁴ Lab-on-chip devices²⁵⁻²⁷ have also been developed to improve food analysis. Voltammetric techniques have recently been adopted as an alternative analytical technique to study the redox mechanism and behaviour of mycotoxins.^{28,29} These voltammetric techniques require shorter processing time and are more time efficient compared to the conventional techniques employed. Previous studies have been conducted where only one type of mycotoxin was detected by the various analytical techniques. However, to our knowledge, no studies have been conducted on the use of voltammetry for the simultaneous detection of mycotoxins. Simultaneous determination of OTA and PAT has been carried out with HPLC though.³⁰ Henceforth, we set our research objectives towards using voltammetry for the simultaneous detection of mycotoxins and investigate if the mycotoxins will interact with one another and affect the final voltammetric signals obtained.

Our studies provide an approach on the use of voltammetry for simultaneous detection of mycotoxins, mainly using cyclic (CV) and differential pulse voltammetry (DPV).

3.2 Experimental

3.2.1 Chemicals and apparatus

Zearalenone (ZEA), citrinin (CIT), patulin (PAT), ochratoxin A (OTA), potassium phosphate dibasic, sodium phosphate monobasic, sodium chloride, potassium chloride and perchloric acid (HClO_4) were obtained from Sigma-Aldrich (Singapore). Acetonitrile (ACN) was obtained from Merck (Singapore). Deionised water of conductivity $18.5 \text{ M}\Omega \text{ cm}$ was used.

Stock solutions of ZEA, CIT, PAT and OTA were prepared in ACN and stored in the dark at 4°C . Working solutions were prepared daily by adding suitable amounts of stock solutions to 20% ACN + 80% 1 M HClO_4 mixture solution for ZEA and CIT, while 20% ACN + 80% PBS buffer mixture solution for PAT and OTA which serves as the supporting electrolyte.

The boron-doped diamond (BDD) electrode, with diameter of 3 mm, was obtained from Windsor Scientific and used as received. Glassy carbon (GC) electrode, with a diameter of 3 mm, was obtained from CH Instruments (Texas, USA). Edge-plane pyrolytic graphite (EPPG) and basal-plane pyrolytic graphite (BPPG) electrodes, with diameters of 3 mm, were obtained from Autolab (Netherlands). The EPPG, GC and BDD electrodes surfaces were renewed by polishing with alumina particles (0.05 μm) on a cloth. The BPPG electrode surface was renewed by pressing the surface onto a cello tape to remove the top few layers of graphene. It was repeated several times before rinsing the surface with acetone to remove any residual adhesive glue from the cello tape.³¹ Ag/AgCl reference electrode and platinum (Pt) counter electrode were purchased from Autolab.

3.2.2 Voltammetric measurements

Voltammetric measurements were conducted using μ Autolab Type III electrochemical analyser (Eco Chemie, Netherlands) controlled by a NOVA 1.8 software (Eco Chemie) at room temperature by using a three-electrode configuration. Cyclic voltammetry (CV) experiment was performed at a scan rate of 0.1 V s^{-1} three runs, with three scans per run. Differential pulse voltammetry (DPV) experiment was performed with amplitude pulse of 25 mV, pulse width of 50 ms and scan rate of 10 mV s^{-1} .

3.3 Discussion

3.3.1 Simultaneous electrochemical detection of zearalenone and citrinin on edge plane pyrolytic graphite electrode

Cyclic voltammetry (CV) was performed on the mixture solution and results are shown in **Figure 3.1**. Two distinct oxidation peaks at 0.95 V for citrinin (CIT) and at 1.15 V for zearalenone (ZEA) were observed. No peaks were observed at the reverse scan which suggests that the oxidation process is chemically irreversible for both mycotoxins under the experimental conditions. The most apparent signals observed were at the EPPG electrode. The oxidation peaks obtained, which corresponded to ZEA, were very distinct while the oxidation peaks for CIT exhibited slight peaks. Despite its wide potential window,^{32,33} the CV obtained from BDD showed only one broad peak which corresponded to ZEA, while no observable peak could be assigned to CIT.

Figure 3.1 Cyclic voltammograms of CIT and ZEA mixture in 20% ACN + 80% HClO₄ supporting electrolyte solution with GC, EPPG, BPPG and BDD electrodes. ($c^*_{CIT} = 27.3 \mu\text{M}$, $c^*_{ZEA} = 29.8 \mu\text{M}$, scan rate = 0.1 V s^{-1})

Since CV is not an analytical technique due to its limited sensitivity, we moved to differential pulse voltammetry (DPV) to simultaneously determine the mycotoxins in the mixture solution. The pronounced oxidation peaks (0.95 V for CIT and 1.15 V for ZEA) in DPV are shown in **Figure 3.2**. Comparing the four electrodes used, EPPG produced the greatest peak heights for both CIT and ZEA. The DPV observed using BDD showed a very small and broad peak for ZEA and no visible peaks could be assigned to CIT.

From the voltammograms obtained in **Figures 3.1 and 3.2**, it was concluded that BDD is not an effective material to be used for mycotoxin detection and is not a better alternative to graphitic materials. EPPG gave distinct oxidation peaks with the best separation and peak height of all four electrodes tested. The reactive edge plane sites contributed to the high sensitivity of the EPPG coupled resulting in its low detection limit and overpotential.³⁴ Therefore,

EPPG was chosen as the electrode of choice for the simultaneous detection experiment.

Figure 3.2 Differential pulse voltammograms of CIT and ZEA mixture in 20% ACN + 80% HClO₄ supporting electrolyte solution with GC, EPPG, BPPG and BDD electrodes. ($c^*_{CIT} = 27.3 \mu\text{M}$, $c^*_{ZEA} = 29.8 \mu\text{M}$).

We set our attention on the simultaneous determination of mycotoxins at different concentrations. We kept one mycotoxin concentration constant while varying the concentration of the other mycotoxin. DPV was then conducted to determine the detection limit of the mycotoxins in the mixtures. **Figure 3.3** shows the DPV obtained at a constant CIT concentration of 27.3 μM with variable concentration of ZEA from 0.209 μM to 17.96 μM . It was observed that the peak heights corresponding to ZEA decreased with the decreasing concentration of ZEA. The concentration dependency of signal from ZEA had a slope of 0.0339 $\mu\text{A M}^{-1}$ with $R = 0.9637$.

Figure 3.3 Differential pulse voltammogram of CIT and ZEA mixture in 20% ACN + 80% HClO₄ supporting electrolyte solution with variable ZEA concentrations (0.209 μM to 17.96 μM) with EPPG electrode. ($c_{CIT} = 27.3 \mu\text{M}$).

The experiment was repeated by keeping the concentration of ZEA constant at 29.8 μM while varying the concentration of CIT from 7.988 μM to 99.4 μM. A similar trend was also observed for this experiment as illustrated in **Figure 3.4**. However, no peaks were visible at CIT concentrations less than 7.988 μM. The concentration dependency of signal from CIT had a slope of $0.0121 \mu\text{A M}^{-1}$ with $R = 0.9991$.

In summary, we have demonstrated that voltammetry can be used for the simultaneous detection of two priority mycotoxins zearalenone and citrinin where two distinct oxidation peaks were obtained and easily distinguishable. EPPG is an effective material which can be used in mycotoxin detection for point-of-care detection.

Figure 3.4 Differential pulse voltammogram of CIT and ZEA mixture with variable CIT concentrations (7.988 μM to 99.4 μM) in 20% ACN + 80% HClO_4 supporting electrolyte solution with EPPG electrode. ($c_{\text{ZEA}} = 29.8 \mu\text{M}$).

3.3.2 Simultaneous anodic and cathodic detection of patulin and ochratoxin A on well-defined carbon electrodes

We compared here the utility of well-defined electrode surfaces, such as edge-plane pyrolytic graphite (EPPG) and glassy carbon (GC) electrodes for simultaneous voltammetric determination of the priority mycotoxins. Cyclic voltammetry (CV) was performed on the mixture solution. **Figure 3.5A** shows one anodic peak at about 0.9 V corresponding to ochratoxin A (OTA) and one cathodic peak at about -1.3 V corresponding to patulin (PAT) for the forward scan. The reverse scan shows similar observations as shown in **Figure 3.5B**. A sharp cathodic peak was obtained at about -0.7 V which corresponded to the oxygen reaction with the phosphate buffer solution.

Comparing the voltammograms obtained from GC and EPPG, it was observed that the peaks correlate with one another. From **Figure 3.5**, it can be deduced that the

oxidation of OTA and reduction of PAT are electrochemically irreversible reactions and their redox products do not interact with one another. This is evident by the similar peak positions and shapes for both scans. The reduction peak obtained for PAT was very distinct while the oxidation peak for OTA was less distinct.

Figure 3.5 Cyclic voltammograms of (A) forward and (B) reverse scan of OTA and PAT mixture in 20% ACN + 80% phosphate buffer solution ($c_{PBS} = 50$ mM, pH = 7.2) with GC and EPPG electrodes. ($c_{PAT} = 0.1$ mM, $c_{OTA} = 0.05$ mM, scan rate = 0.05 V s⁻¹)

We then moved to differential pulse voltammetry (DPV) to determine the mixture solution due to its increased sensitivity. The distinct anodic and cathodic peaks (0.85 V for OTA and -1.3 V for PAT) in DPV were obtained as shown in **Figure 3.6**. GC and EPPG voltammograms showed similar peak separation patterns and comparable peak heights. However, the cathodic peak corresponding to PAT is more distinct in GC as compared to EPPG.

Figure 3.6 Differential pulse voltammograms of OTA and PAT mixture in 20% ACN + 80% phosphate buffer solution ($c_{PBS} = 50 \text{ mM}$, $\text{pH} = 7.2$) with GC and EPPG electrodes. ($c_{PAT} = 0.1 \text{ mM}$, $c_{OTA} = 0.05 \text{ mM}$)

We subsequently focused our attention on the simultaneous determination of mycotoxins at different concentrations by keeping one mycotoxin concentration constant while varying the other. DPV was conducted to also determine the detection limit of the mycotoxins in the mixture. The experiment was carried out under N_2 purging to eliminate any side reactions with oxygen and obtain a voltammogram of higher resolution by eliminating the unwanted peaks. A peak centred at -0.8 V was removed and can be attributed to oxygen reaction with the buffer.

Figure 3.7 shows the DPV obtained at a constant PAT concentration of 0.1 mM with variable concentration of OTA from $1 \text{ }\mu\text{M}$ to $50 \text{ }\mu\text{M}$. It was observed that the peak heights corresponding to OTA decreased with decreasing concentration of OTA. No visible peaks were observed at OTA concentrations less than $2 \text{ }\mu\text{M}$ for both electrode materials. A linear calibration graph with $R = 0.996$ and $R = 0.995$ was obtained for GC

and EPPG respectively. Both electrode materials displayed very similar electrochemical trends. The slope for calibration graph of GC is $1.17 \times 10^{-8} \mu\text{A } \mu\text{M}^{-1}$ while that of EPPG is $1.16 \times 10^{-8} \mu\text{A } \mu\text{M}^{-1}$.

Figure 3.7 Differential pulse voltammograms of OTA and PAT mixture in 20% ACN + 80% phosphate buffer solution ($C_{PBS} = 50 \text{ mM}$, $\text{pH} = 7.2$) with (A) GC and (B) EPPG electrodes with variable OTA concentrations (1 μM to 50 μM). ($C_{PAT} = 0.1 \text{ mM}$)

The experiment was then repeated by keeping the concentration of OTA constant at 0.05 mM while varying the concentration of PAT from 3 μM to 150 μM . A similar trend was observed as illustrated in **Figure 3.8**. No peaks were visible at PAT concentrations less than 20 μM for GC while EPPG showed no visible peaks at PAT concentrations less than 40 μM . A linear calibration graph with $R = 0.964$ and $R = 0.991$ was obtained for GC and EPPG respectively. Both electrode materials exhibited similar electrochemical trends. The slope for calibration graph of GC is $4.29 \times 10^{-9} \mu\text{A } \mu\text{M}^{-1}$ while EPPG is $4.22 \times 10^{-9} \mu\text{A } \mu\text{M}^{-1}$.

Figure 3.8 Differential pulse voltammograms of OTA and PAT mixture in 20% ACN + 80% phosphate buffer solution ($C_{PAT} = 50 \text{ mM}$, $\text{pH} = 7.2$) with (A) GC and (B) EPPG electrodes with variable PAT concentrations (3 μM to 150 μM). ($C_{OTA} = 0.05 \text{ mM}$).

From **Figures 3.7A** and **3.8A**, it was observed that the voltammograms obtained from GC showed a broad peak centred at -0.1 V despite conducting the experiments under N_2 purging. Smaller peaks were observed for voltammograms from EPPG as observed in **Figures 3.3B** and **3.4B**. The broad peak may potentially overlap with signals from other mycotoxins thus giving negative results as compared to EPPG. The voltammograms obtained using EPPG gave more apparent signals with lesser background signal as compared to GC. The calibration graphs obtained also showed better linearity for EPPG and the results are more reproducible.

The lowest concentrations of target mycotoxins detected by EPPG were higher as compared to the standards set in the Codex standards³⁵ and FAO limits⁵ set for both OTA and PAT. From our experiments, lowest concentration detectable experimentally for OTA was $2 \mu\text{M}$ (1.38 ppm) which was higher than the maximum legal amount determined (5 ppb). As for PAT, our experimental limit was at 10.5 ppm which was also higher than the legal limits (50 ppb). Further studies have to be conducted to lower the limit of

detection for more sensitive sensing studies.

We have demonstrated that voltammetry can be used for the simultaneous detection of two target mycotoxins, patulin and ochratoxin A, where one anodic and cathodic peak was obtained and easily distinguishable. From the results obtained, EPPG is a more effective material than GC in mycotoxins detection due to its better linearity and resolution of voltammogram.

3.4 Conclusion

Direct detection of mycotoxins by cyclic voltammetry was conducted on several well-defined electrode surfaces. Here we showed that edge-plane pyrolytic graphite (EPPG) offered excellent selectivity and sensitivity towards simultaneous detection of zearalenone and citrinin. We also compared the electrochemical detection of patulin and ochratoxin A on edge-plane pyrolytic graphite and glassy carbon electrodes. Patulin got reduced and showed well-defined peak at ~ -1.5 V, deeply in the cathodic region while ochratoxin A, in contrary, got oxidised at potential $+0.9$ V. We investigate here the possibility to simultaneously detect two important mycotoxins with distinctive cathodic and anodic signals to identify any difference in final signal generated. We also showed that edge-plane pyrolytic graphite provides more sensitive response over glassy carbon electrode.

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Chapter 4

Limitations of Molybdenum Disulfide (MoS_2) in Electrochemical Applications

4.1 Introduction

4.2 Experimental

4.3 Discussion

4.3.1 Limited potential window of MoS_2 : Limitations in electrochemical applications

4.4 Conclusion

The results in this chapter were published in the following manuscript:

Muhammad Zafir Mohamad Nasir, Zdenek Sofer, Adriano Ambrosi, Martin Pumera. A Limited Anodic and Cathodic Potential Window of MoS₂: Limitations in Electrochemical Applications. *Nanoscale* **2015**, 7, 3126-3129.

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The thesis author was the primary investigator and author of this paper.

Summary of Project

Molybdenum disulphide (MoS_2) has been touted as a good material with diverse possible applications such as an energy storage and sensing platform. However, we demonstrate here the limitation of MoS_2 as an analytical sensing platform due to the limited potential window in both the anodic and cathodic regions attributed to the inherent electrochemistry (oxidation of Mo^{4+} to Mo^{6+}) and the catalytic hydrogen evolution reaction due to H_3O^+ reduction on the MoS_2 surface, respectively. The electrochemical window of MoS_2 lies in the region of ~ -0.6 V to $+0.7$ V (vs. AgCl). We show that such a limited working potential window characteristic of MoS_2 precludes the detection of important analytes such as nitroaromatic explosives, pesticides and mycotoxins which are instead detectable on carbon surfaces. The limited potential window of MoS_2 has to be taken into consideration in the construction of electroanalytical devices based on MoS_2 .

4.1 Introduction

Transition metal dichalcogenides (TMDs) have gained increasing interest among scientists recently^{1,2} due to their special features and possible future applications.^{3,4} TMDs have layered structures, similar to graphite, held by weak Van der Waal's forces of attraction between each layer.⁵ TMDs exhibit desired properties for many applications such as lubricants,⁶ capacitors,^{3,7,8} energy storage devices^{3,9} and sensing platforms.^{10,11} TMDs are prepared in a similar fashion to graphene due to the similarities of their layered structures. Mechanical exfoliation, such as lithium intercalation^{12,13} and ultrasonication,¹⁴ is one of the common methods of producing single-layered TMD films.

The electrochemical window is the potential range in which the electrode is stable which is limited by the inherent electrochemistry (change in electrode surface properties) of the electrode material and the redox behaviour (decomposition) of the solvent. Some examples include the dissolving of mercury from mercury electrodes into the solution at potentials greater than 0 V (vs. saturated calomel electrode) and the oxidation of Au electrodes at high anodic potentials.¹⁵ We have previously shown the limitation of graphene oxides at cathodic potentials as a result of the electroactive oxygen-containing groups present.¹⁶

Here we demonstrate the limitation of the electrochemical working window of molybdenum disulfide both in the anodic and cathodic regions, which significantly limits the detection of oxidation and reduction processes of important analytes of interest. This is largely due to the inherent electrochemical activity of molybdenum disulfide in the anodic region and catalytic hydrogen evolution in the cathodic region.

4.2 Experimental

4.2.1 Materials and apparatus

Zearalenone (ZEA), uric acid (UA), ascorbic acid (AA), sodium tetraborate decahydrate, 2-(N-morpholino)ethanesulfonic acid (MES) monohydrate, paraoxon, perchloric acid, potassium phosphate dibasic, sodium phosphate monobasic, sodium chloride and potassium chloride were obtained from Sigma-Aldrich (Singapore). 2-Nitrotoluene (2-NT) was obtained from Alfa Aesar (Ward Hill, MA). Acetonitrile (ACN) was obtained from Merck (Singapore). MoS₂ bulk powder (<2 μ m) and tert-butyllithium (1.7 M in pentane) were obtained from Sigma-Aldrich (Czech Republic). Hexane was obtained from Lach-Ner, Czech Republic. Deionised water of conductivity 18.2 M Ω cm (at 25 °C) was used.

Stock solutions of ZEA and paraoxon were prepared in ACN. 10 mM stock solutions of uric acid and ascorbic acid were prepared in PBS buffer. 20 mM 2-nitrotoluene stock solution was prepared in borate buffer. All stock solutions were stored at 4 °C.

Platinum (Pt) counter electrode, Ag/AgCl reference electrode and glassy carbon (GC) electrode, with a diameter of 3 mm, was obtained from CH Instruments (Texas, USA). The GC electrode surface was renewed by polishing with alumina particles (0.05 mm) on a cloth.

4.2.2 Voltammetric measurements

Voltammetric measurements were conducted using a μ Autolab Type III electrochemical analyser (Eco Chemie, Netherlands) controlled by a NOVA 1.10 software (Eco Chemie) at room temperature (25 °C) by using a three-electrode configuration. Cyclic voltammetry (CV) experiment was performed at a scan rate of 100 mV s⁻¹ three runs, with three scans per run. The buffers used were purged with nitrogen gas for 15 min before each measurement was taken.

4.2.3 Preparation of exfoliated MoS₂

3 g of bulk MoS₂ powder was suspended in 20 mL of t-BuLi. The suspension was stirred for 72 hr at 25 °C under an argon atmosphere in order to intercalate the MoS₂ with Li. The Li-intercalated material was separated by suction filtration under an argon atmosphere and washed thoroughly with hexane (dried over Na). Repeated centrifugation (10000 g) was carried out after placing the separated Li-intercalated material in water (100 mL). The exfoliated MoS₂ was eventually dried in a vacuum oven at 50 °C for 48 hr before use. It was later dispersed in water at a concentration of 1 mg mL⁻¹ and ultrasonicated for 30 min. 1 μ L of the colloidal suspension was drop-coated onto the glassy carbon electrode surface and left to dry at room temperature.

4.3 Discussion

4.3.1 Limited potential window of MoS₂: Limitations in electrochemical applications.

We investigated the potential window of molybdenum disulfide (MoS₂) using cyclic voltammetry. We first looked into the anodic region of the potential window followed by the cathodic region. A voltammetric scan was carried out in blank buffer which resulted in the oxidation of MoS₂ starting at +0.6 V and peaking at +1.0 V.^{17–19} This is the result of oxidation of Mo⁴⁺ to Mo⁶⁺.¹⁶ Subsequent scans with analytes were carried out. Important biomarkers, ascorbic acid (AA) and uric acid (UA) were selected as analytes of interest to investigate whether an inherent oxidation peak of MoS₂ interferes with their electrochemical signal. **Figure 4.1** shows the voltammograms obtained for ascorbic acid and uric acid scanned with MoS₂-modified GC. The voltammograms obtained showed oxidation peaks corresponding to the target biomarkers (+0.45 V) which were easily distinguishable and did not overlap with the oxidation peak of MoS₂. In this scenario, MoS₂ exhibits excellent properties as a sensing platform. One can note that the shoulder in the voltammograms of ascorbic and uric acids at about +1.0 V corresponds to the oxidation of MoS₂.

Figure 4.1 Cyclic voltammograms of blank buffer, 10 mM ascorbic acid (AA), and 10 mM uric acid (UA) with MoS₂-modified GC in phosphate buffer (50 mM, pH 7.2). Buffer solution purged with N₂ for 15 minutes before each measurement. (Scan rate = 100 mV s⁻¹).

However, not all analytes are oxidised at low potentials. Important phenolic compounds such as mycotoxins can be oxidised only at higher potentials.²⁰ Therefore, the experiment was repeated using another target sample which is zearalenone (ZEA). ZEA is a common mycotoxin found in food. A voltammetric scan was initially carried out with MoS₂-modified GC in a blank buffer which showed a sharp peak at +1.0 V (**Figure 4.2A**), similar to that observed in **Figure 4.1**. From the scan with bare GC (**Figure 4.2B**), the oxidation peak of ZEA is expected at +1.15 V. However, when the experiment was repeated using MoS₂-modified GC, the oxidation peak for ZEA overlaps with the oxidation peak from MoS₂ and is masked by it (**Figure 4.2A**). A very broad peak was produced which makes it difficult to distinguish the peak from oxidation of ZEA. This does not make MoS₂ suitable as a sensing platform for mycotoxins in food analysis. Most mycotoxins have phenolic structures.²¹ The oxidation potential of most phenolic

compounds are expected beyond the potentials of +0.7 V.^{22–24} This range appears to overlap with the inherent oxidation peak of MoS₂ in the different types of buffers used. It is therefore not possible to utilise MoS₂ as a screening platform for rapid detection of mycotoxins in food supply. It is clear that MoS₂ exhibits a limited anodic potential window due to its inherent electrochemistry at about +0.7 V and at higher potentials.

Figure 4.2 Cyclic voltammograms of 20 μM mycotoxin zearalenone (ZEA) in 20% ACN + 80% 1 M HClO₄ buffer with (A) MoS₂-modified GC and (B) bare GC electrode. Buffer solution purged with N₂ for 15 minutes before each measurement. (Scan rate = 100 mV s⁻¹).

It was observed that changing from a neutral phosphate buffer to a more acidic buffer (20% ACN + 80% HClO₄) did not shift the position of the peak for the blank scans significantly. This might suggest that the oxidation of MoS₂ is not greatly dependent on the pH of the buffer used and the inherent oxidation of the material is pH insensitive.²⁵ We also investigated the suitability of MoS₂ surfaces in the cathodic region by choosing two classes of compounds relevant for environmental analysis which are nitroaromatic explosives and pesticides, more specifically 2-nitrotoluene (2-NT)²⁶ and paraoxon.²⁷

Figure 4.3 shows the voltammograms obtained for the reduction of 2-NT and paraoxon

with bare GC and MoS₂-modified GC. From the voltammetric scans of the respective blank buffers, no observable peaks were observed on the GC electrode. However, hydrogen evolution reaction can be observed at about -1.2 V on GC electrode (**Figure 4.3A**) and at much lower potentials (~ -0.6 V) in the case of MoS₂ (**Figure 4.3B**). **Figure 4.3A** shows distinguishable reduction peaks for 2-NT and paraoxon on bare GC as they are reduced at -0.66 V and -0.8 V, respectively, while the cathodic electrochemical window of GC is up to -1.2 V. MoS₂ is known to be catalytic to hydrogen evolution reaction in aqueous solutions. The hydrogen evolution is a limiting factor for the potential window in the cathodic region in general. We see that it is pronounced in MoS₂ (**Figure 4.3B**). One can note in **Figure 4.3B** that the hydrogen evolution reaction is indeed taking place as early as at ~ -0.6 V (pH 5.0) while on the GC electrode the hydrogen evolution starts at ~ -1.2 V. Clearly, MoS₂ shows a limited MoS₂ potential window as a result of early hydrogen evolution.

Figure 4.3 Cyclic voltammograms of 10 mM paraoxon in MES buffer (20 mM, pH 5.0) and 20 mM 2-nitrotoluene (2-NT) in borate buffer (20 mM, pH 9.3) with (A) bare GC and (B) MoS₂-modified GC. Buffer solution purged with N₂ for 15 minutes before each measurement. (Scan rate = 100 mV s⁻¹).

The usability of MoS₂ as an electrode material for sensing is also limited at the cathodic region as demonstrated. The anodic region of the MoS₂ potential window is greatly restricted by the inherent oxidation of the material at about +0.7 V while the cathodic potential window is limited at ~ -0.6 or ~ -0.9 V, depending on the pH used. It is of interest to compare the electrochemical window of MoS₂ to other layered materials. For an example, electrochemically reduced graphene shows a window of -1.6 to $+1.2$ V,^{16,28} fluorographite -1.0 to $+1.1$ V²⁹ due to proton reduction and water oxidation, respectively. It should be noted that while the ability of MoS₂ to act as a catalyst for hydrogen evolution is unwanted for analytical applications, it is highly useful for 'clean energy' applications.³⁰

4.4 Conclusion

We have demonstrated that the electrochemical window of MoS₂ is limited in both the anodic and cathodic regions. This is a result of the oxidation of MoS₂ at anodic potentials and the electrocatalytic hydrogen evolution at cathodic potentials. As a result, it limits the possible electrochemical sensing applications of MoS₂ only to those analytes with redox potentials between ~ -0.6 (due to electrocatalytic hydrogen evolution from H₃O⁺ present in the solvent) and +0.7 V (due to oxidation of the electrode material itself, from Mo⁴⁺ to Mo⁶⁺) (vs. AgCl). This has a profound effect on the sensing applications as a large amount of molecules are reduced at lower potentials or oxidised at higher potentials than at the potential window of MoS₂.

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Chapter 5

Effect of Electrolyte pH on the Inherent Electrochemistry of Layered Transition Metal Dichalcogenides (TMDs)

5.1 *Introduction*

5.2 *Experimental*

5.3 *Discussion*

5.3.1 *Effect of pH on the inherent electrochemistry of MoS₂, MoSe₂, WS₂ and WSe₂.*

5.4 *Conclusion*

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Summary of Project

TMDs are found to undergo inherent oxidation at anodic potentials which limits its capabilities and performance in sensing applications. The inherent anodic peaks can be influenced by several factors of the buffer system such as pH, temperature and electrolyte used. This in turn affects the morphology of the materials and their redox mechanisms.

We set forth to investigate the changes in the inherent anodic peaks of different transition metal dichalcogenides (TMDs), namely MoS_2 , MoSe_2 , WS_2 , WSe_2 , at different electrolyte pH. Changing the pH of the electrolyte was found to influence and affect the inherent oxidation of TMDs, more noticeably the peak position. This could be attributed to the different reaction mechanisms and stabilities at different pHs. An increase in the electrolyte pH from 0 to 8 shows a linear decrease in the peak potentials of the inherent oxidative peaks. However, small changes in peak potentials were observed at low alkaline pH levels (from 8 to 11). Beyond pH 11, the emergence of an additional peak at low potentials, apart from the inherent oxidative peak, was observed for most of the TMD materials studied. The insight into pH dependence of the oxidation of TMD materials is of paramount importance for their electrochemical applications.

5.1 Introduction

The research on two-dimensional (2D) layered compounds has gained increased interest recently. Such examples include graphene and its derivatives. Increasingly, a new class of layered materials known as transition metal dichalcogenides (TMDs) are attracting significant research interest^{1,2} as they are found to have exhibited great physical and chemical properties.³ These properties allow for the potential applications of TMDs as capacitors,^{4,5} lubricants,^{6,7} energy storage devices^{3,8,9} and sensing platforms.^{10,11}

However, despite these improved properties, TMDs hold several limitations in their applications. There is the need to study the fundamental properties of TMDs before devices can be made so as to maximise its operational capabilities. One such limitation of TMDs can be observed by the inherent electrochemistry of these materials.^{12,13} The term inherent electrochemistry refers to the changes in the properties of a material when it is used as an electrode surface. Such materials undergo redox reactions at specific applied potentials which cause a change in their physical and chemical compositions. These changes can have negative effects on its uses especially in sensing and biosensing applications. Molybdenum disulphide (MoS_2) was found to have a limited electrochemical potential window due to the oxidation of MoS_2 at anodic potentials (+0.7 V vs. Ag/AgCl) and electrocatalytic hydrogen evolution at cathodic potentials (-0.6 V vs. Ag/AgCl).¹⁴ This limits the variety of analytes that it can detect when used as a sensing platform. The inherent oxidation of MoS_2 has been attributed to the oxidation of the molybdenum ion (Mo^{4+}) which results in the formation of MoO_3 (Mo^{6+}), SO_4^{2-} and S^{2-} at high potentials.^{15,16}

These inherent oxidative peaks have been found to be influenced by several factors of the buffer systems such as pH, temperature and electrolyte used. These conditions affect the morphology of the materials and their redox mechanisms. It is of utmost importance to study the changing behaviours of these materials to variable factors to optimise experimental conditions and obtain accurate results. This is especially so important in application in point-of-care detection.^{17,18} pH is an important variable in buffer systems which can be easily manipulated to optimise experimental conditions as compared to other factors. The electrode surface has to be relatively stable to such changes and not affect the voltammetric signals.

To our knowledge, the effect of pH on inherent electrochemical oxidation of TMDs has not been widely studied. Besides that, the most widely studied TMD material is MoS₂ while there is a dearth of information on the inherent electrochemistry of other TMDs such as MoSe₂, WS₂ and WSe₂. Therefore, we set our sights to investigate and compare the effect of changing pH on the inherent electrochemical oxidation of the four mentioned materials.

5.2 Experimental

5.2.1 Materials and apparatus

Sodium hydroxide, perchloric acid, potassium phosphate dibasic, sodium phosphate monobasic, sodium chloride and potassium chloride were obtained from Sigma-Aldrich (Singapore). MoS₂, WS₂, MoSe₂ and WSe₂ bulk powder (<2 µm) and tert-butyllithium (1.7 M in pentane) were obtained from Sigma-Aldrich (Czech Republic). Hexane was obtained from Lach-Ner (Czech Republic). Deionised water of conductivity

18.5 M Ω cm (at 25 °C) was used. Platinum (Pt) counter electrode, Ag/AgCl reference electrode and glassy carbon (GC) electrode with a diameter of 3 mm, was obtained from CH Instruments (Texas, USA). The GC electrode surface was renewed by polishing with alumina particles (0.05 mm) on a cloth.

5.2.2 Preparation of exfoliated TMDs

3 g of respective bulk powder was suspended in 20 mL of 1.7 M tert-butyllithium. The suspension was stirred for 72 hr at 25 °C under an argon atmosphere in order to intercalate with Li. The Li-intercalated material was separated by suction filtration under an argon atmosphere and washed thoroughly with hexane (dried over sodium). Repeated centrifugation (18 000 g) was carried out after placing the separated Li-intercalated material in water (100 mL). The exfoliated material was dried in a vacuum oven at 50 °C for 48 hr before use. It was later dispersed in water at a concentration of 5 mg mL⁻¹ and ultrasonicated for 30 min. 1 μ L of the suspension was drop-coated onto the glassy carbon electrode surface and left to dry at room temperature.

5.2.3 Electrochemical Measurement

Voltammetric measurements were conducted using a μ Autolab Type III electrochemical analyser (Eco Chemie, Netherlands) controlled by a NOVA 1.10 software (Eco Chemie) at room temperature by using a three-electrode configuration. Cyclic voltammetry (CV) experiment was performed at a scan rate of 100 mV s⁻¹ three cycles, with three scans per cycle. The buffers used were purged with nitrogen gas for 20 minutes before each measurement was taken.

5.3 Discussion

5.3.1 Effect of pH on the inherent electrochemistry of MoS₂, MoSe₂, WS₂ and WSe₂

We investigated here the electrochemical behaviour of transition metal dichalcogenides (TMDs), namely MoS₂, MoSe₂, WS₂ and WSe₂; using cyclic voltammetry at buffers of different pH ranging from 0 to 13 (**Figure 5.1**). The anodic regions were studied where the inherent peaks of the various TMD materials were observed. Peak potential was used to analyse the changes in behaviours of the inherent electrochemistry for the different TMDs.

The anodic peaks do not seem to be affected by the varying pH and are visibly distinguishable.¹⁹ Upon scanning in the anodic direction (0 – 1.6 V), distinct anodic peaks were observed for the different TMDs at positive potentials. For the same cycle, the peaks disappeared with subsequent scans which can be attributed to the irreversible oxidation of the materials. There is possibly a passivation of the oxidised products on the electrode surface which blocks the underlying layers from having contact with the electrolytes.¹³ From **Figure 5.1**, it was observed that the voltammograms obtained from MoS₂ were broad while the voltammograms for the other TMD materials showed sharper peaks. The peak shape for MoS₂ gets broader with increasing pH of the electrolyte (**Figure 5.1A**). However, the peak shapes of the other TMD materials remained relatively constant and seemed unaffected by the increase in pH of the electrolyte (**Figures 5.1B to 5.1D**). The sharp peaks obtained suggest that the oxidation process is surface bound and is not influenced by diffusion. The anodic electrochemistry is surface confined on the electrode itself. The anodic electrochemistry of MoS₂ may

require the involvement of other species in the electrolyte which could have resulted in the broader peaks obtained.

The findings of the anodic behaviour of TMDs can be categorised into two regions which are the acidic (pH 0 to 7) and alkaline (pH 8 to 13) regions where different observations are made. In the acidic region, with the exception of WS₂, the TMD materials showed a shift in peak positions as pH increases from 0 to 7. The peaks shift to less positive potentials with increasing pH of the electrolyte. However, the peak positions remained relatively constant at low alkaline region (pH 8 to 10). Additionally, more than one apparent peak appeared at high alkaline pH levels (beyond pH 10). The additional peaks could be attributed to the formation of intermediate oxidation state products of the various TMDs with the influence of high hydroxide ion (OH⁻) concentrations due to the high alkaline pH used.²⁰ An interesting observation was made for WS₂ where the anodic peaks appear more diagonal as the pH of the buffer increases and returns upright at high alkaline pH (**Figure 5.1C**). This trend is very distinct and unlike that of other TMD materials used.

Figure 5.1 Cyclic voltammograms of (A) MoS₂, (B) MoSe₂, (C) WS₂, and (D) WSe₂-modified GC in phosphate buffers of variable pH (from 0 to 13). Buffer solutions were purged with N₂ for 20 minutes before each measurement. ($C_{PBS} = 50$ mM, scan rate = 100 mV s^{-1})

To obtain a better understanding on the changing trends, a dependence of peak potential was plotted against pH of buffer used. It allows for a visual representation for

the analysis of the changes to the different materials with the different pH. **Figure 5.2** shows the dependences obtained from the different TMD materials. It was noted that most of the materials showed similar trends with increasing pH of the buffer systems with the exception of WS₂. A downward linear trend with R values of 0.984, 0.963 and 0.985 were obtained for MoS₂, MoSe₂ and WSe₂ respectively at acidic region (pH 0 to 7). At low alkaline pH, the peak potential changes are relatively small before an apparent drop was then observed at high alkaline pH levels. This can be attributed to different reaction oxidative products forming at high alkaline pH levels. As for WS₂, it was noted that the peak position saw an increase with pH increase from 0 to 2 but subsequently remained relatively constant (**Figure 5.2C**).

From the modified Nernst equation, the proton-coupled electron transfer (PCET) mechanism can be understood. The gradients of the potential-pH dependence graphs can be compared to the integer values in modified Nernst equation and number of protons and electrons can be determined.²¹ From **Figure 5.2**, the gradients of the slopes at the acidic pH region (0.14 to 7.82) for MoS₂, MoSe₂ and WSe₂ were 0.028, 0.0274 and 0.0385 respectively. The values for molybdenum-based dichalcogenides were approximately half of 0.059 which corresponds to a two-electron, one-proton PCET mechanism. This corresponds to previous studies which showed the oxidation of molybdenum from Mo⁴⁺ to Mo⁶⁺. The acidic region provides the protons which are then able to interact with the TMDs resulting in the oxidation of the electrode surface to give the anodic peak in the voltammograms. However, the value for WSe₂ is significantly different. This suggests a different reaction mechanism unlike that of molybdenum.

Figure 5.2 Peak position calibration plots of (A) MoS₂, (B) MoSe₂, (C) WS₂, and (D) WSe₂-modified GC at phosphate buffers of variable pH (from 0 to 13). Black plots correspond to peak potentials of inherent oxidation of respective TMD materials, red and green plots correspond to the peak potentials of shifted inherent oxidation and the emergence of additional oxidative peaks at pH 11.86 and 12.56 respectively.

Potential-pH diagrams, better known as Pourbaix diagrams,²² can be used to provide an illustration of the changes in the states of the phases at different equilibrium potentials. **Figure 5.3** shows the expected changes in the states of WS₂ and MoS₂ with variable pH (vs. NHE).²³

In the case of WS₂, it shows limited variations at different electrolyte pH (**Figure 5.2C**). The similarity in the anodic peaks of WS₂ at variable pH could have been a result of the formation of WO₄²⁻ at region beyond pH 3. This could have attributed to the

distinct peak shape for WS_2 . Tungsten undergoes oxidation which results in the increase in oxidation state from +4 to +6.

In comparison, the equilibrium potential of MoS_2 decreases as pH increases (**Figure 5.3B**). As such, the corresponding oxidation potential gets lower with increasing pH. This trend is similar to experimental results which saw a shift to less positive potentials for MoS_2 inherent anodic peaks (**Figure 5.2A**). There is also a possibility of forming variable species at the different pH. The formation of different oxidised products and varying stabilities could have resulted in the broadening of the anodic inherent peak as the pH of electrolyte increases due to the differing properties of the new products. Molybdenum generally undergoes oxidation from an oxidation state of +4 to +6 for beyond pH 5.

Figure 5.3 Pourbaix diagrams representing thermodynamic stabilities (vs. NHE) of (A) tungsten-oxygen-hydrogen-sulphur (W-O-H-S) system, and (B) molybdenum-sulphur-oxygen-hydrogen (Mo-S-O-H) system. Reproduced with permission from ref. 23.

We next set out to analyse the changes in peak heights of the materials with the changes in pH of the buffers used as they may have applications in electron analysis.²⁴ From **Figure 5.4**, there appears to be no distinct trends to the changes in peak heights with variable pH for all TMD materials analysed. WS₂ showed the greatest peak heights over the pH range analysed (**Figure 5.4C**). Tungsten-based dichalcogenides showed relatively stable peak heights throughout the pH range analysed. However, molybdenum-based dichalcogenides showed a decreasing trend, with MoSe₂ exhibiting an additional increasing trend beyond pH 6. The relative stability of peak heights for tungsten-based dichalcogenides could probably be attributed to the formation of similar oxidised species where reaction kinetics are expected to be the similar. However, for molybdenum-based dichalcogenides, due to the formation of different oxidised products, different reaction kinetics take place which corresponds to the different peak heights observed at different pH ranges.

Figure 5.4 Plots for peak heights of (A) MoS₂, (B) MoSe₂, (C) WS₂, and (D) WSe₂-modified GC at phosphate buffers of variable pH (from 0 to 13). Black plots correspond to peak heights of inherent oxidation of respective TMD materials, red and green plots correspond to peak heights of the shifted inherent peak potential and the additional oxidative peaks at pH 11.86 and 12.56 respectively.

5.4 Conclusion

We have demonstrated the changing behaviour of the inherent oxidation of the TMD materials at different pH of the buffer systems. MoS₂ and MoSe₂ were found to undergo oxidation from +4 to +6 states for pH range of 0 to 8 corresponding to a two-electron, one-proton PCET mechanism. The peak position remains almost stable up to pH 11 and shows a major shift beyond that. WS₂ showed almost similar peak position and relative stability over pH ranges of 2 to 12. Moreover, tungsten-based dichalcogenides exhibited relative kinetics stability due to almost consistent peak heights as compared to molybdenum-based dichalcogenides.

Peak height analysis can be used to investigate the number of electrochemically available catalytic site for heterogeneous electron transfer (HET) to take place. By monitoring the changes in the peak heights, charge transfer kinetics can be analysed. Besides that, it can also be used as a potential label for bioassays and bio recognition events when TMDs are used as labels.²⁴ By doing so, the identity of various biological molecules can be identified and applied in point-of-care detection devices.

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Chapter 6

Two-Dimensional 1T-Phase Metal Dichalcogenides as Nano-Carriers to Enhance and Stabilise Enzyme Activity for Electrochemical Pesticide Detection

6.1 *Introduction*

6.2 *Experimental*

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6.3.3 *Electrochemical analysis of pesticide biosensor system*

6.3.4 *Optimisation of parameters of pesticide biosensor system*

6.3.5 *Analytical response and real sample analysis*

6.4 *Conclusion*

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Summary of Project

Single or few layers lithium-exfoliated transition metal dichalcogenides (TMDs) are found to exist predominantly in the conducting metallic 1T-polymorph which makes it desirable for numerous applications due to its large surface area, good electrical conductivity and enhanced electrocatalytic capabilities. We demonstrated the use of tert-butyllithium (t-BuLi) exfoliated TMDs (MoS_2 , MoSe_2 , WS_2 , WSe_2) as a platform for the indirect electrochemical detection of an organophosphate pesticide, fenitrothion, *via* a model enzymatic inhibition pathway. All four reported materials enhanced the response of the enzymatic biosensor in comparison to the corresponding biosensor in absence of TMDs. However, 1T-phase WS_2 serves to be the best material and we proved that it serves as an excellent transducer for enhancing electron transfer in a robust model enzyme-based inhibition assay system using cross-linking immobilisation with glutaraldehyde. The reported system showed a broad fenitrothion concentration range (1 – 1000 nM) with an excellent linearity ($R = 0.987$). Moreover, the system displayed high sensitivity with low limit of detection (2.86 nM) obtained which far exceeds the required limit set by Food and Agriculture Organisation (FAO) of the United Nations (UN). The feasibility of the proposed system in real samples was demonstrated in apple juice samples with good recoveries of 80.2% and 80.3% obtained at 10 and 1000 nM fenitrothion respectively.

6.1 Introduction

Transition metal dichalcogenides (TMDs) are a new class of layered materials, analogous to graphene, which have gained significant interest among scientists^{1,2} due to their good physical and chemical properties.³⁻⁸ Exfoliation of the TMDs into single or few layer sheets was discovered to enhance the chemical properties of TMDs as a result of the increased available surface area and tuning of their electronic properties.⁹ This allows for potential applications in capacitors,^{4,10} batteries,^{4,9} vapour sensing,¹¹ and biosensing applications.¹²⁻¹⁴ Different exfoliation procedures have been reported with chemical exfoliation being the most commonly practiced. Recently, it was discovered that exfoliation with tert-butyllithium (t-BuLi) produced TMD sheets with a high composition of metallic 1T-phase structures.¹⁵ This metal-like property could be advantageous in constructing an analytical device^{11,16} which utilises the metal-like nature of 1T-phase TMDs in enhancing sensitivity for analytical detection.

The increase in global population has generated a need to increase the global food supply which has led to more research performed to increase crop productivity and yield. Such developments have evolved commercial farming techniques with greater emphasis placed on crop management and pest control. Organophosphorus pesticides have been developed and widely used to counter the loss of crop yield from pests' invasions. Pesticides are also commonly used for household uses. Lately, there is also an increased need for detection of phosphorus compounds due to their use in chemical weapons such as sarin.¹⁷ With such widespread and diversified use, there is the potential for excess pesticides to leech into the environment which could lead to contamination. Numerous global and governmental agencies have invested heavily in

enforcing high safety standards in food produce. Organophosphate pesticide is an example of a harmful chemical closely monitored as it can cause harmful effects to human physiology and health¹⁸ if the residues are present in huge quantities. Fenitrothion is a commonly used organophosphate pesticide in agriculture for pest control. It binds irreversibly to the active sites of acetylcholinesterase (AChE) found on the synaptic membranes on nerve cells along the nervous system¹⁹ which inhibits the enzyme and renders it inactive. This prevents the breakdown of neurotransmitter acetylcholine which leads to its accumulation in the nerve junctions thus resulting in over-stimulation in the nervous system. Long term exposure could lead to severe health problems in human population,²⁰ and even death.²¹ It is also well-absorbed from numerous routes such as ingestion, respiratory tract and even through the skin.²² As such, it is of utmost importance to develop an effective technique for accurate detection and quantification of pesticides in environmental and biological samples.

The development of electrochemical biosensors has mainly focused on the modification of electrodes with carbon-based materials, such as graphene^{23,24} and carbon nanotubes.²⁵⁻²⁷ These materials act as transducers in improving sensitivity and amplifying the response generated. More recently, nanoparticles²⁸ have been incorporated to further improve the accuracies and limits of detection. The use of other layered materials such as TMDs in enzymatic biosensors for the detection of pesticides is still unexplored and literature is lacking. TMDs would be a more favourable and attractive alternative in the fabrication of biosensors due to its lower cytotoxicity²⁹ and greater tunability as compared to graphene and other carbon-based materials. Besides that, the metallicity of exfoliated TMD is expected to enhance the response signal of

biosensor assay systems. TMDs have recently been used as labels in immunoassays,¹⁶ quencher in genosensing systems^{30,31} as well as a platform to enhance the electrochemical signal in DNA and H₂O₂ sensing.^{32,33} From our search, there have been no reports on the use of TMDs in enzymatic biosensor applications for pesticide detection. As such, we set forth to investigate the effectiveness of 1T-phase exfoliated TMDs as a platform for the detection of fenitrothion in the robust enzymatic-based inhibition assay as a model system. We adopted an acetylcholinesterase inhibition assay model as a basis of comparison to investigate the effectiveness and durability of the exfoliated TMD for such application. Henceforth, we aim to develop a biosensor based on immobilisation of AChE on 1T-phase t-BuLi exfoliated TMD.

6.2 Experimental

6.2.1 Materials and apparatus

Potassium chloride, potassium hexacyanoferrate (II) trihydrate, potassium hexacyanoferrate (III), bovine serum albumin (BSA), acetylthiocholine iodide (ATChI), acetylcholinesterase (AChE) from electric eel, glutaraldehyde (GA, 50%), fenitrothion, ascorbic acid, phenol, and phosphate buffer saline (PBS) in tablets were purchased from Sigma-Aldrich (Singapore). High quality water, purified in a Milli-Q system (Millipore, MA, USA) with a resistivity of 18 MΩ cm was used. Biosan TS-100 (Latvia) thermoshaker was used to stir the samples during the inhibition process at 37 °C with shaking at 400 rpm.

Platinum (Pt) counter electrode, Ag/AgCl reference electrode and glassy carbon (GC) electrode, with a diameter of 3 mm, was obtained from CH Instruments (Texas, USA).

6.2.2 Electrochemical measurements

Electrochemical measurements were conducted using a μ Autolab Type III electrochemical analyser (Eco Chemie, The Netherlands) controlled by NOVA 1.9 software (Eco Chemie) at room temperature (25 °C) by using a three-electrode configuration. Cyclic voltammetry (CV) experiment was performed at a scan rate of 100 mV s⁻¹. All chronoamperometry measurements were recorded at 10 ms intervals in 0.01 M PBS. A magnetic stirrer was used to assist convection.

6.2.3 Characterization

X-ray photoelectron spectroscopy (XPS) was carried out with a Phoibos 100 spectrometer with a monochromatic Mg X-ray source (SPECS, Germany) at 1254 eV. Transmission electron microscopy (TEM) and high resolution transmission electron microscopy (HR-TEM) was carried out with EFTEM JEOL 2200 FS microscope (JEOL, Japan) with acceleration voltage of 200 eV. Raman spectroscopy was carried out using confocal micro-Raman LabRam HR instrument (HORIBA Scientific) in backscattering geometry with CCD detector, 514.5 nm argon excitation laser, and an 100x objective lens mounted to an Olympus optical microscope. The spectra range used was from 100 to 1000 cm⁻¹. Calibration was carried out before measurements were taken using an internal silicon reference at 520 cm⁻¹ with peak position resolution of less than 1 cm⁻¹. Scanning electron microscopy (SEM) was performed with JEOL 7600F-SEM (JEOL, Japan). SEM was performed on a silicon wafer operating at 2 kV.

6.2.4 Preparation of exfoliated transition metal dichalcogenides

3 g of respective TMDs bulk powder was suspended in 20 mL of 1.7 M tert-butyllithium (t-BuLi). Subsequently, it was stirred for 72 hr at 25 °C under argon atmosphere to intercalate with Li. The intercalated material was separated by suction filtration under argon atmosphere and washed thoroughly with hexane (dried over sodium). The separated Li-intercalated material was placed in water (100 mL) before repeated centrifugation (18 000 g) was carried out. The exfoliated material was dried in a vacuum oven at 50 °C for 48 hr before use. It was later dispersed in water and ultrasonicated for 60 min to obtain a well-dispersed suspension.

6.2.5 Preparation of Biosensor System

AChE (1 mg in 50 µL) and 5% BSA were prepared in 0.01 M PBS. 3 µL suspension of t-BuLi exfoliated TMDs of desired concentrations were drop-coated on the glassy carbon (GC) electrode surface and left to dry at room temperature. 0.25% of glutaraldehyde (GA) was prepared in ultrapure water and 5 µL of the solution was coated onto the GC/TMD surface. 2 µL of AChE in 5% BSA (1:1) was the next coated onto the modified surface. The electrodes were oven dried at 35 °C in between steps for 30 min.

6.2.6 Real Samples Preparation

Fresh apples were bought from a local supermarket and blended with 0.01 M PBS. The blend was filtered several times to remove any insoluble and larger particles which may affect the measurements. The fresh juice was obtained as filtrate.

6.3 Discussion

6.3.1 Fabrication of pesticide biosensor system

Figure 6.1 Illustration on the construction of the proposed pesticide biosensor: (A) Loading of glassy carbon electrode with t-BuLi exfoliated 1T-phase TMDs, (B) conjugation with glutaraldehyde (GA) layer, (C) immobilisation of acetylcholinesterase-bovine saline albumin mixture (AChE-BSA) on the modified surface, (D) the enzymatic hydrolysis of acetylthiocholine with the subsequent electrochemical oxidation of thiocholine produced, and (E) the inhibition effect of fenitrothion on AChE.

The model enzymatic biosensor was fabricated as described in experimental section and schematically illustrated in **Figure 6.1**. In summary, the respective t-BuLi exfoliated TMDs was first deposited onto glassy carbon (GC) electrode followed by the deposition of glutaraldehyde (GA) layer before the final immobilisation of

acetylcholinesterase-bovine saline albumin (AChE-BSA) mixture. Glutaraldehyde was used as it serves as a cross-linker between the protein groups in AChE and t-BuLi exfoliated TMD to hold the enzymes in place as well as provide structural stability to the biosensor system. AChE is the enzyme of choice due to its specificity, high turnover number and catalytic power.¹⁹ It is a hydrolase enzyme which catalyses the breakdown of neurotransmitter acetylcholine to choline and acetic acid (**eq 6.1**). Choline formed is an electroactive compound which can be oxidised and detected electrochemically (**eq 6.2**). The presence of oxidised choline in the solution will be detected by voltammetry and chronoamperometry. BSA serves as a blocking agent to prevent non-specific binding onto the enzyme molecule.^{34,35} It will also ensure the optimum interaction of substrate with the active site of AChE and amplify the response generated. Organophosphate pesticides are irreversible inhibitors which form strong covalent bonds between the phosphate groups of the pesticide and the hydroxyl groups within the serine residues in the active site of the enzyme. This results in the formation of a stable enzyme-pesticide complex where the enzyme gets phosphorylated and inhibited. With the binding of fenitrothion to the active site of AChE, it forms a barrier at the active site of the enzyme which prevents the binding of acetylcholine (**eq 6.3**). Thus, the amount of acetylcholine catalysed decreases based on the concentrations of fenitrothion present.

6.3.2 Characterisation of transition metal dichalcogenides

Characterisation was first carried out to better understand the synergistic interactions, chemical composition and morphologies of the materials. It will also affirm the metallicity of the respective t-BuLi exfoliated TMDs. X-ray photoelectron spectroscopy (XPS) was performed to analyse the surface elemental composition and bonding interactions involved in the respective exfoliated TMDs. **Figure 6.2** shows high resolution spectrums of deconvoluted peaks for Mo 3d and W 4f modes for the respective tert-butyllithium (t-BuLi) exfoliated TMDs. The $3d_{5/2}$ and $3d_{3/2}$ bonding modes were analysed for molybdenum. Upon analysis of the high-resolution spectrums for the molybdenum-based exfoliated TMDs, three distinct pairs of peaks were observed corresponding to the 2H and 1T polymorphs as well as the Mo (VI) oxidised state. MoSe₂ showed a significant presence of 1T polymorph from the higher intensity of the respective 1T peaks with a small contribution of the semi-conducting 2H polymorph (**Figure 6.2B**). However, deconvolution of the Mo 3d spectrum for t-BuLi exfoliated MoS₂ showed a predominance of the semi-conducting 2H polymorph, as denoted by the greater intensities of the various 2H peaks (**Figure 6.2A**). The XPS results would suggest a lesser extent of exfoliation for MoS₂ in comparison to that of MoSe₂. This would also suggest that the t-BuLi exfoliated MoS₂ will be a less sensitive transducer material. Peaks present at high binding energies (greater than 230 eV) suggest the presence of oxidised products of molybdenum (Mo⁶⁺) which exists in rather significant proportions. In the case of tungsten-based TMDs, the $4f_{7/2}$ and $4f_{5/2}$ bonding modes were analysed. Three distinct pairs of peaks were also noted for tungsten-based exfoliated TMDs. On the contrary, both t-BuLi exfoliated WS₂ and WSe₂ showed dominance of metallic 1T

polymorph over the semi-conducting 2H polymorph in the structures after exfoliation (**Figure 6.2C and 6.2D**). It was interesting to note that tungsten-based TMDs showed a greater proportion of 1T-phase than the molybdenum counterparts with more intense peaks observed. There is also a significant percentage of the oxidised form of tungsten (W^{6+}) as evident from the peaks present at higher binding energies (greater than 35 eV). From the XPS spectrums, it was evident that the molybdenum-based TMDs have lower efficiency of conversion from 2H polymorph to 1T polymorph upon exfoliation as previously reported.¹⁵

Figure 6.2 High-resolution X-ray photoelectron spectroscopy (XPS) spectrums of Mo 3d and W 4f modes for tert-butyllithium (t-BuLi) exfoliated (A) MoS₂, (B) MoSe₂, (C) WS₂, and (D) WSe₂.

Raman spectroscopy was next carried out as it is able to observe the extent of exfoliation of the respective TMDs with great sensitivity. The differences in the features of the peaks at the respective vibrational modes changes with varying layer thickness.³⁶ Comparison between the respective bulk^{37,38} and t-BuLi exfoliated TMDs were carried out to observe any such deviations. The in-plane E_{2g}^1 and out-of-plane A_{1g} vibrational modes were analysed in the Raman spectrums for all t-BuLi exfoliated TMDs as they are Raman active modes,³⁹ consistent with the D_{6h} point group of the respective bulk TMDs.⁴⁰ For both MoS_2 and $MoSe_2$ (**Figure 6.3A and 6.3B**), exfoliation with t-BuLi resulted in the broadening and softening of the E_{2g}^1 and A_{1g} bands in comparison to the bulk counterparts. The A_{1g} band appears at a higher frequency than the E_{2g}^1 band for $MoSe_2$, as compared to MoS_2 , which correlates with previous observations.^{41,42} The broadening of the A_{1g} band in $MoSe_2$ is more distinct as compared to the E_{2g}^1 band which remains almost unchanged. This correlates with previous findings where the E_{2g}^1 band remains unchanged and unaffected. The broadening effect observed can be attributed to phonon confinement effect and the decrease in lattice size.^{43,44} The Raman spectrum for WS_2 was next studied. A visible indication on the extent of exfoliation will be the change in $I_{A_{1g}}/I_{E_{2g}^1}$ ratio which will increase with decreasing WS_2 thickness.⁴⁵ From **Figure 6.3C**, the intensity of E_{2g}^1 band increased in comparison to that from the bulk material which correlates to an increase in the observed $I_{A_{1g}}/I_{E_{2g}^1}$ ratio. Hence, it can be inferred that t-BuLi has successfully intercalated and exfoliated bulk WS_2 . Lastly, for WSe_2 , the E_{2g}^1 and A_{1g} bands are degenerate and would result in the formation of a single peak in the bulk material. The splitting of the degenerate peaks becomes pronounced with the exfoliation of the bulk material.⁴⁶ **Figure 6.3D** shows the presence

of two small peaks closely located at about 250 and 253 cm^{-1} which overlaps as a single broad peak. It can thus be postulated from the splitting of the degenerate peaks that WSe_2 was exfoliated by t-BuLi. From the Raman spectroscopy and XPS studies, it suggests that the lithium exfoliation of tungsten-based TMDs appears to be more effective and efficient in comparison to their molybdenum counterparts. From the respective characterisation studies conducted, it can be concluded that the t-BuLi exfoliated TMDs exhibit metal-like characteristics and exist predominantly in the 1T polymorph which will aid in the response amplification for the model enzymatic inhibition biosensor assay.

Figure 6.3 Raman spectrums for t-BuLi exfoliated (A) MoS_2 , (B) MoSe_2 , (C) WS_2 , and (D) WSe_2 .

6.3.3 Electrochemical analysis of pesticide biosensor system

It is of utmost interest to evaluate the performances of the different t-BuLi exfoliated 1T-TMDs (MoS_2 , MoSe_2 , WS_2 , WSe_2) to monitor any differences in sensitivity and response based on their different inherent properties. We next set forth to investigate the feasibility of the proposed biosensor system for the detection of fenitrothion. Chronoamperometric measurements were carried out to analyse the response of the pesticide biosensing system fabricated using the different t-BuLi exfoliated TMDs through successive injection of acetylthiocholine. All four materials showed enhanced biosensor performance as compared to biosensor without TMDs (green line of **Figure 6.4**). MoSe_2 and WSe_2 showed almost similar responses which were lower than MoS_2 and WS_2 . It was interesting to note that MoS_2 outperformed both MoSe_2 and WSe_2 despite being predominantly in the 2H polymorph. A possible reason for the rather sensitive response could be the successful exfoliation of MoS_2 into few layered sheets. 1T- WSe_2 displayed the lowest sensitivity which is contrary to the results from characterisation studies. This anomaly could suggest the smaller extent of exfoliation of WSe_2 and its existence in multiple layers thus providing a lower surface area. As such, the metal-like properties are compromised and the sensitivity is reduced. The greater sensitivity of WS_2 in comparison to WSe_2 would also suggest successful exfoliation of the bulk material as evident from the greater separation of the E_{2g}^1 and A_{1g} vibrational modes for WS_2 (**Figure 6.3C**). It was also interesting to note that sulfur-based dichalcogenides performed better than their selenide-based counterparts. Moreover, 1T-phase WS_2 is postulated to provide also increased density of catalytic active sites that for the 1T-Phase materials have on both the basal plane and edges.⁴⁷⁻⁵⁰

All of these capabilities of 1T-phase facilitate the electrochemical oxidation of thiocholine in the proposed biosensor (**Figure 6.1D and eq 6.2**). From the studies performed, it can be deduced that 1T-WS₂ is the most suitable TMD for further electrochemical characterisation of the biosensor.

Initial studies with 1T-WS₂ modified biosensor using cyclic voltammetry showed a small anodic peak at 0.1 V in the presence of acetylthiocholine iodide (**Figure 6.4**). However, a significant enhanced current intensity was observed in the CV of the biosensor in presence of acetylthiocholine iodide (red line) in comparison with the biosensor without analyte (black line). From the voltammogram obtained, it was decided that a potential of 0.1 V would be used as the applied potential for further chronoamperometric studies. This potential was chosen as it is relatively low and away from the anodic potential of possible interference signals such as polyphenols, flavonoids and vitamins (**Figure 6.5**). Other potentials were also tested (data not shown) and the best response was observed with 0.1 V.

The metallic nature and increased density of catalytic active sites of 1T-WS₂ enhanced the current response signal from the oxidation of thiocholine produced from the enzymatic catalysis of acetylthiocholine and thus the oxidation potential gets shifted to a lower anodic potential as shown in **Figure 6.4**. A similar observation on the shift in oxidation potential of thiocholine in pesticide based on nanomaterials was previously reported for carbon nanotubes,⁵¹ composites of polyaniline/carbon nanotubes,^{52,53} and Au nanoparticles dispersed in carbon nanotubes.⁵⁴

Figure 6.4 Cyclic voltammogram of 1T-WS₂ modified biosensor with blank 0.01 M PBS (black line) and presence of acetylthiocholine iodide in 0.01 M PBS (red line).

Having ascertained that the enzymatic biosensor assay system is feasible, we proceed to study any effects of interferences on the proposed system. Chronoamperometric analysis was performed at 0.1 V with Fe²⁺, Cu²⁺, ascorbic acid, and phenol (**Figure 6.5**). The compounds were chosen as they are commonly found in most variants of fruits and the respective concentrations reflect closely their physiological concentrations.⁵⁵ From **Figure 6.5**, it was determined that the mentioned compounds do not interfere significantly with the oxidation signal of acetylthiocholine (current change < 7%) which validates the use of low working potential. Utilising this information, we next set forth to carry out chronoamperometry for subsequent procedures at 0.1 V.

Figure 6.5 Chronoamperometric measurements at 100 mV showing the effects of interference analytes on 1T-WS₂ modified biosensor system in 0.01 M PBS. Arrows indicate the point of injection of respective compounds. Concentration of acetylthiocholine (ATCh) and phenol = 500 μM, ascorbic acid = 260 μM, Fe²⁺ = 1.2 ppm, Cu²⁺ = 0.27 ppm.

Figure 6.6 Chronoamperometric measurements with successive injection of ATCh on different t-BuLi exfoliated TMD modified biosensor in 0.01 M PBS. Arrows indicate the point of injection of ATCh. (Concentration of ATCh = 500 μM)

Figure 6.7 Scanning electron microscopy (SEM) images of (A) WS₂, and (B) WS₂/GA/AChE. SEM images were taken using backscattered electrons mode. Scale as indicated in the respective images.

The extent of exfoliation of the 1T-WS₂ was further analysed with TEM, selected area electron diffraction (SAED) and HR-TEM (**Figure 6.8**). From the TEM image, exfoliation of the materials is evident with the presence of few layer sheets of about one micrometre observed. SEM image also shows similar observations with few layers observed for 1T-WS₂ (**Figure 6.7A**).

SAED pattern showed hexagonal symmetry of WS₂. HR-TEM image further validates the 1T-phase of WS₂ from the hexagonal arrangement of atoms which corresponds to 1T-phase arrangement. These findings validate the results obtained from XPS and Raman spectroscopy in validating the 1T polymorph and extent of exfoliation of WS₂ used in the model enzymatic biosensor.

Figure 6.8 Transmission electron microscopy (TEM) image with selected area electron diffraction (SAED) pattern (insert) of WS_2 . High resolution TEM (HR-TEM) images show dominant presence of 1T-phase (red hexagon). Scale as indicated in the images respectively.

Chronoamperometry was performed to study such postulations and the chronoamperometric responses were observed with successive injection of acetylthiocholine to the different modified layers. From **Figure 6.11**, there was no visible current response for measurements carried out on bare glassy carbon. Modification of the glassy carbon electrode with WS_2 resulted in a small increase in the current response upon injection of acetylthiocholine. The signal generated could be from the oxidation of small quantities of iodide counter ions present with acetylthiocholine used^{56,57} (**Figures 6.9 and 6.10**).

Figure 6.9 Cyclic voltammogram of acetylthiocholine iodide on bare glassy carbon (black line) and 1T-WS₂ modified biosensor system (red line) in 0.01 M PBS.

Figure 6.10 Cyclic voltammogram of potassium iodide on bare glassy carbon (black line) and 1T-WS₂ modified biosensor system (red line) in 0.01 M PBS.

GC/WS₂/GA and GC/WS₂/BSA showed low chronoamperometric responses which validates the absence of the catalytic activity of enzyme. The importance of enzyme AChE is evident with an enhanced response obtained after injection of acetylthiocholine. The significant increase in response upon the immobilisation of AChE

can be attributed to the high turnover number and strong catalytic power^{19,58} of the enzyme which was found to catalyse about 10^4 molecules per second.⁵⁹ With more acetylthiocholine being catalysed, the amount of thiocholine present greatly increased and thus more thiocholine gets oxidised in return. An enhanced response was further obtained upon the introduction of BSA to AChE. This could correlate to more electroactive thiocholine produced and lesser acetylthiocholine lost from non-specific binding of the AChE. The combined effect of the metal-like property and enhanced catalytic activity of t-BuLi exfoliated 1T-WS₂ enables more efficient electro-oxidation of thiocholine and greater amount of enzymes to be immobilised to get better sensitivity of the model enzymatic inhibition biosensor. As such, the exfoliated material aids in improving the sensitivity and response signal of this system. The chronoamperometric measurements carried out validates the importance of the different layers in the proposed biosensor and their successful immobilisation in the preparation steps. It further proves the robustness and stability of the biosensor system that was used as a model assay to evaluate the electrocatalytic effect of different TMDs exfoliated by t-butyllithium.

Figure 6.11 Chronoamperometric measurements with successive injection of ATCh on each modified layer of the proposed biosensor system in 0.01 M PBS. Arrows indicate the point of injection of ATCh. (Concentration of ATCh = 500 μ M)

Figure 6.12 Cyclic voltammogram showing the inhibition effect of fenitrothion on 1T-WS₂ modified biosensor system.

The inhibition effect of fenitrothion on the enzymatic biosensor system was next studied. Initial cyclic voltammetry (CV) scans on the modified electrodes were performed. It was noted that the anodic peak diminishes in the presence of fenitrothion

(Figure 6.12) with a corresponding decrease in current intensity of the CV after inhibition with fenitrothion.

Figure 6.13 Chronoamperometric measurements showing the inhibition effect of fenitrothion on 1T-WS₂ modified biosensor system in 0.01 M PBS. Black arrow indicates the point of injection of ATCh and blue arrow indicates the drop in current response observed after inhibition with fenitrothion. (Concentration of ATCh = 500 μM)

Chronoamperometric studies were subsequently performed to analyse the inhibition effect of fenitrothion with the proposed biosensor system (**Figure 6.13**). In the absence of fenitrothion, injection of acetylthiocholine saw a sharp increase in current signal which gradually becomes steady. The current response obtained after inhibition with fenitrothion (500 μM) showed a decrease in current response by about 0.05 μA. The drop in current signal obtained is indicative of less thiocholine being oxidised which corroborates to a decrease in the amount of acetylthiocholine catalysed by AChE. Fenitrothion was successful in binding irreversibly to the active site of AChE which hinders the catalysis of acetylthiocholine and suppressed the signal obtained. From

these initial observations, it can be concluded that the biosensor is responsive to the presence of fenitrothion as evident from the decrease in response current.

6.3.4 Optimisation of parameters of pesticide biosensor system

We next set forth to study various experimental parameters to optimise the performance of the biosensor. As mentioned previously, AChE gets phosphorylated when fenitrothion forms a covalent bond with the hydroxyl group of the serine residues in the active site thus inhibiting the enzyme. The response generated from the electrochemical oxidation of thiocholine thus decreases with increasing exposure to fenitrothion. Using this concept, the degree of inhibition can be measured based on the change in thiocholine signal response before and after fenitrothion inhibition. The inhibition percentage (I%) was calculated as a means to evaluate the extent of inhibition before (I_b) and after incubating (I_a) with fenitrothion (**eq 6.4**) to analyse the inhibition activity of fenitrothion on AChE. A higher I% would be favourable as it indicates greater inhibition of enzymes which correlates to better performance of the biosensor.

$$I (\%) = \frac{[I_b - I_a]}{I_b} \times 100 \quad (6.4)$$

The first parameter of interest is acetylthiocholine concentration. Acetylthiocholine serves as the substrate for enzymatic catalysis. A substrate concentration which is higher than the turnover number of the enzyme could slow down the response time due to saturation at the active site of the enzymes. However, substrate concentrations which are too low could generate weak or negative response. It is thus important to choose a suitable concentration to ensure optimum working

capacity of the enzymes. Three different acetylthiocholine concentrations were studied which were 50, 100 and 250 μM . **Figure 6.14A** shows I% for the different acetylthiocholine concentrations which increases with increasing concentration. Acetylthiocholine concentration of 50 μM produced negative I% which suggests insufficient substrate present to interact with the enzymes. Acetylthiocholine concentration of 250 μM showed greater I% as compared to a concentration of 100 μM and was thus selected as the working concentration for the experiment.

We next set forth to optimise the amount of 1T-WS₂ to be deposited onto glassy carbon electrode. 1T-WS₂ serves as the transducer for electron transfer and it is important to ensure optimum loading of the material onto the electrode surface. Three different WS₂ concentrations were studied which were 1, 5, and 10 mg mL^{-1} . From **Figure 6.14B**, the optimum concentration to be deposited is 1 mg mL^{-1} and it was the only concentration with positive I%. Higher concentrations of WS₂ (5 and 10 mg mL^{-1}) gave negative I% which might suggest saturation or passivation of the electrode surface. Besides that, the higher concentrations might have caused an excess of WS₂ to be deposited which could have created a barrier and hindered electron transfer. As such, WS₂ concentration of 1 mg mL^{-1} was chosen as the optimum concentration to be loaded onto the electrode surface.

The last variable optimised is pesticide inhibition time. A lower response will be obtained when the pesticide binds irreversibly to the active site of the enzyme. It is thus important to optimise this variable to allow sufficient time for fenitrothion to bind to the active site of the enzymes to optimise I% results. However, the time domain should also not be too long for on-site analysis. Three different inhibition times were chosen which

were 5, 15 and 30 min. From **Figure 6.14C**, inhibition times of 5 and 15 min showed almost similar I% results. It was also interesting to observe that the longest inhibition time of 30 min showed the lowest I% result. The long inhibition time might have saturated the enzyme as it reaches its turnover rate. The enzymes could also have detached from the modified surface during the long time duration. 5 min inhibition time was chosen due to its better reproducibility with the smallest error bar obtained. The shorter time duration was also taken into consideration as an important criteria to be an efficient on-site, point-of-care analytical device.

Figure 6.14 Optimisation of (A) ATCh concentration, (B) WS_2 concentration, and (C) fenitrothion inhibition time.

6.3.5 Analytical response and real sample analysis

The analytical response of the model biosensor system with the optimised parameters was then examined using chronoamperometry with injection of acetylthiocholine into stirred 0.01 M PBS (**Figure 6.15**). Increasing the fenitrothion concentration is predicted to generate greater inhibition of AChE which will correlate to decrease catalytic activity and hence generate a greater I% value. **Figure 6.16A** shows the linear plot for fenitrothion concentrations between 1 - 1000 nM for the proposed biosensor system with $R = 0.987$. The limit of detection (LOD) was calculated by multiplying the quotient of the standard deviation of the chronoamperometric current of the lowest concentration of pesticide and gradient for the calibration graph by 3 and limit of quantification (LOQ) was obtained by multiplying the same quotient described previously by 10.⁶⁰⁻⁶⁴ The system has a low LOD of 2.86 nM and LOQ of 9.54 nM. A new modified electrode has to be used for each experiment after exposure to fenitrothion due to the irreversible binding of the pesticide to the enzymes. The error bars obtained are hence a result of same day repeatability of measurements having relative standard deviation (RSD) of less than 10% ($n = 4$) for each concentration. The RSD is the average of 4 different concentrations where each concentration was measured 4 times. When applying the one-way ANOVA statistical analysis with a confidence interval of 0.95, the mean values for the inhibition percentages were different, $p = 0.00019$. The good linearity and analytical performance of the biosensor system makes it promising for potential applications.

Figure 6.15 Chronoamperometric measurements at fenitrothion concentrations of (A) 1 nM, (B) 10 nM, (C) 100 nM, and (D) 1000 nM in 0.01 M PBS and 250 μ M ATCh. Black arrow indicates the point of injection of ATCh and blue arrow indicates the drop in current response observed after inhibition with fenitrothion.

Recovery experiments were then carried out to demonstrate the working capabilities of the proposed biosensor with real samples where interfering species were present. Recovery percentages were calculated as shown in **eq 6.5**. Recovery percentages of 80.2% and 80.3% were obtained at fenitrothion concentrations of 10 and 1000 μ M respectively in fresh apple juice (**Figure 6.16B**). The results obtained suggest that the proposed biosensor system is feasible and responsive for detecting low concentrations of fenitrothion in real samples. The system also showed good reproducibility and analytical performance in detecting fenitrothion with apple juice.

The model biosensor system, where t-BuLi exfoliated 1T-WS₂ was used as an active electrocatalytic platform with enhanced analytical, serves as a promising platform which can be extended towards the development of future TMD-based enzymatic biosensors for environmental and biomedical applications.

$$R (\%) = \left(\frac{[Fenitrothion]_{buffer}}{[Fenitrothion]_{real\ sample}} \right) \times 100 \quad (6.5)$$

Figure 6.16 (A) Calibration plot showing the inhibition of biosensor vs. fenitrothion concentration. (B) Recoveries with fresh apple juice for pesticide concentrations of 10 and 1000 nM.

Moreover, the use of 1T-WS₂ has proven to outperform most carbon-based materials and provide a more sensitive alternative compared to previous works reported. Our proposed biosensor system exhibited excellent analytical performance with respect to detection limits and range in comparison to other works previously reported for fenitrothion detection (**Table 6.1**). The analytical performance with t-BuLi exfoliated 1T-WS₂ is also comparable to the use of dispersed graphene oxide by Liu *et. al.*⁶⁵ with similar detection range and magnitude in the limit of detection (LOD) observed.

Table 6.1 Comparison of different systems for the detection of organophosphorus pesticides.

Analytical System	Scanning Method	Linear Range (nM)	LOD (nM)	Reference
SPE/CNT/CHO/AChE	Chronoamperometry	1 – 200 000	50	[25]
Electrochemically pre-treated GC	Voltammetry	0.4 - 50	78	[66]
GC/1T-WS ₂ /GA/AChE-BSA	Chronoamperometry	1 - 1000	2.86	This work
GC/GO/Fenitrothion	Voltammetry	3.6 - 1443	0.36	[65]

One advantage of our proposed system also is its selectivity brought about by the immobilisation of AChE and low working potential (0.1 V) used. The high selectivity of AChE towards binding with organophosphate pesticides makes it attractive as a detection method for our target analytes. The use of low potential also reduces interference signals from other analytes present in real samples analysis. Besides that, the time taken for fenitrothion detection is rather short (5 min) with great reproducibility and linearity. The LOD obtained is 2.86 nM which corresponds to 0.743 ppb. This value obtained is significantly lower than the maximum allowed quantity set by various environmental agencies such as Food and Agriculture Organisation (FAO) of the United Nations (10 ppm)⁶⁷ and Agri-Food and Veterinary (AVA) of Singapore (2 ppm).⁶⁸

6.4 Conclusion

A model biosensor based on the immobilisation of AChE on t-BuLi exfoliated 1T-phase WS₂ has been developed for the effective detection of organophosphate pesticide, fenitrothion. Moreover, it was demonstrated that the t-BuLi exfoliated metallic 1T-phase WS₂ serves as a conductive transducer platform with increased density of catalytic sites for the efficient electrochemical oxidation of thiocholine which enhances the response obtained. The proposed biosensor showed good linearity with a low limit of detection (2.86 nM). Good recovery percentages were also obtained in real sample analysis with apple juice. With these findings, it also shows conceptually the possible application of TMDs in the area of biosensors and varied possibilities for the development of future TMD-based biosensors for on-site, point-of-care environmental and biological samples analysis.

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Chapter 7

Applications of Impact Electrochemistry for Nanoparticles Detection

7.1 Introduction

7.2 Experimental

7.3 Discussion

7.3.1 Electrochemical detection of variable sized monodispersed Ag nanoparticles by impact electrochemistry

7.3.2 Detection of amphiphatic viral peptide by rupture impact voltammetry

7.4 Conclusion

The results in this chapter were published in the following manuscript:

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Muhammad Zafir Mohamad Nasir, Joshua A. Jackman, Nam-Joon Cho, Adriano Ambrosi, Martin Pumera. Detection of Amphipathic Viral Peptide on Screen-Printed Electrodes by Liposome Rupture Voltammetry. *Anal. Chem.* **2017**, *89*, 11753-11757.

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The thesis author was the primary investigator and author of these papers.

Summary of Project

Impact electrochemistry provides a useful alternative technique in the detection of silver nanoparticles in solutions. The combined use of impact electrochemistry on screen-printed electrodes (SPEs) for the successful detection of silver nanoparticles provides an avenue for future on-site, point-of-care detection devices to be made for environmental, medicinal and biological uses. Here we discuss the use of screen-printed electrodes for the detection of well-defined monodispersed silver nanoparticles of the sizes 10, 20, 40, 80, and 107 nm.

Detection of infectious viruses and disease biomarkers are of utmost importance in clinical screening for effective identification and treatment of diseases. We demonstrate here the use of liposome rupture voltammetry for the qualitative detection of model amphipathic viral peptide on screen-printed electrode (SPE). This novel, proof-of-concept method was proposed for the quick and reliable detection of virus by non-faradaic liposome rupture voltammetry with the aid of 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) liposomes. This provides an avenue for the development of future on-site, point-of-care detection devices for medical and biological applications.

7.1 Introduction

Impact electrochemistry is an emerging field which aims to study and detect individual particles at the nano-scale with greater accuracy. The bombardment of the nanoparticles onto the electrode surface at pre-set potentials will generate electrochemical signals which can then be processed and analysed.^{1,2} Upon the striking of a particle on an electrode surface at a fixed potential, it undergoes redox reactions with the exchange of electrons which corresponds to a transfer of charge. This charge transfer correspondingly leads to a “spike” formed as the electrochemical signal which is proportional to the number of electrons transferred. The first measurements on the colloidal impacting particles were reported by Micka using polarography in the 1950s.³⁻⁵ Such similar observations were later reported by Heyrovsky where he studied the dispersions of colloidal SnO₂ and TiO₂.⁶⁻⁹ Lately, advances in impact electrochemistry techniques have enabled for the detection and quantification of various nanoparticles directly¹⁰⁻¹⁵ or indirectly.¹⁶⁻²⁰ More recently, impact electrochemistry has been explored to detect individual nanoparticles to greater accuracies.^{21,22} This technique is useful for future application as it requires minimal sample volume and a simple set-up for operation. These factors are indeed favourable for future on-site detection devices.

Silver nanoparticle, sometimes known as “nanosilver”, is a robust and well-studied material with varied applications due to its anti-microbial properties.^{23,24} Lately, silver nanoparticles have found applications in washing machines, clothing, cosmetics and lotions. With the widespread use of silver nanoparticles, there is the potential for these nanoparticles to leech into our environment especially during

washing and cleaning up process. The accumulation of silver nanoparticles poses a potential hazard to human health^{25,26} and there is the need to be able to identify and remove them from the environment.²⁷⁻²⁹ Recently, there has been increasing interest and research carried out to find methods to detect silver nanoparticles. Several methods include microfluidics,³⁰ direct voltammetry³¹ and multi-techniques approach.^{32,33} However, these techniques require specialised instrumentation and extensive training to operate the devices. As such, they may not be ideal for on-site, point-of-care detection of silver nanoparticles in real samples. A different approach has to be considered which can provide a more viable and effective mode for the detection of silver nanoparticles. One potential technique that has been identified is by applying impact electrochemistry.

Recently, there has been an increasing interest and research conducted on the study of different colloidal systems and nanoparticles.³⁴⁻³⁶ More recently, “soft” particles such as cells,³⁷ liposomes,³⁸⁻⁴¹ vesicles,⁴² micelles,¹⁹ and nano-droplets^{43,44} have been studied to analyse cell exocytosis events and show potential applications in targeted cargo delivery. The signals generated from these “soft” particles could be from a faradaic process from the redox reactions brought about by mediators loaded into the particles or a non-faradaic process as a result of charge transfer brought about by capacitive effect of the unloaded particles. Zutic *et. al.* showed the interactions between phytoplankton,⁴⁵ oil droplets,⁴⁶ and phospholipid vesicles⁴⁷ at mercury electrode which generated capacitive signals due to the adhesion of the substances onto the electrode surface upon rupture or adhesion. Several similar techniques have also been developed which allows for the study of cell content⁴⁸ and exocytosis events⁴⁹ such as patch amperometry^{50,51} and electrochemical cytometry.⁵²⁻⁵⁵ The use of microfluidics with such

techniques were successfully demonstrated with high accuracies and selectivity.^{56,57}

Cho and co-workers discovered that a synthetic peptide corresponding to the amphipathic α -helix (AH) of the N-terminus of the NS5A protein of HCV⁵⁸ is able to cause lipid vesicle rupture by destabilising the lipid bilayer of the vesicle,⁵⁹ resulting in the formation of planar bilayers onto solid surfaces such as gold and titanium dioxide as verified by atomic force microscopy, localized surface plasmon resonance, and other surface-sensitive measurement techniques.⁶⁰⁻⁶² By making use of the rupturing capabilities of the AH peptide, we aim to establish a new methodology for the qualitative detection of the model viral peptide in a rapid and efficient approach in comparison to techniques currently available such as fluorescence spectroscopy and quartz crystal microbalance-dissipation (QCM-D).⁶³ This technique may be useful for future application as it non-evasive, requires minimal sample and a simple set-up. These factors are highly desirable for future on-site detection devices.

Another effective concept that can be explored for use as electrode surface is the use of screen-printed technology. Screen-printing has been well researched and studied as it provides a more economical and efficient alternative over carbon fibres or microelectrodes. The use of screen-printed technology has enabled the development of portable screening devices such as the blood glucose sensor. In electrochemistry, screen-printed electrodes (SPEs) have garnered great interest in their applications due to the ease of electrodes design, combination, and manipulation.⁶⁴ SPEs have also long been known to be a useful disposable electrode for effective on-site detection. Recent developments and advances in the manufacture of SPEs will enable for cheaper and more economical instrumentations to be developed in the future.

Acknowledging the advantages of both impact electrochemistry and the use of SPEs, we set forth to investigate using SPEs as an economical and convenient platform for the detection of monodispersed silver nanoparticles. By using SPEs, we aim to provide a proof-of-concept for the fast and easy detection of nanoparticles for future applications in environmental, medicinal, and biological studies. The combination of both efficient techniques will enable for fast and easier detection of silver nanoparticles and detection of AH peptide with this technique. It is of importance to be able to characterize the silver nanoparticles according to their sizes as nanoparticles which are small enough will be able to penetrate through the membranes of cells and blood vessels and get accumulated in vital organs. This work can potentially be further extrapolated and applied as a sensing platform for the detection of agents which cause cell lysis or vesicle ruptures as well.

7.2 Experimental

7.2.1 Materials and apparatus

Potassium chloride, potassium phosphate dibasic, sodium phosphate monobasic and sodium chloride were obtained from Sigma-Aldrich (Singapore). Trisodium citrate dehydrate was obtained from Alfa Aesar (Singapore). Colloidal nanoXact silver nanoparticles (0.02 mg mL^{-1} , citrate-capping) of sizes 10, 20, 40, 80, and 107 nm were purchased from Nanocomposix (USA). High quality water, purified in a Milli-Q system (Millipore, MA, USA) with a resistivity of $18 \text{ M}\Omega \text{ cm}$ was used. The AH peptide (>95% purity) was produced using solid-phase peptide synthesis by Anaspec Corporation (San Jose, CA, USA) with a molecular weight of 3284.5 Da. It has amino acid sequence as such:

H-Ser-Gly-Ser-Trp-Leu-Arg-Asp-Val-Trp-Ile-Cys-Thr-Val-Leu-Thr-Asp-Phe-Lys-Thr-Trp-Leu-Gln-Ser-Lys-Leu-Asp-Tyr-Lys-Asp-NH₂

Screen-printed electrodes (SPEs) of 3 mm working electrode diameter, with an Ag/AgCl surface as reference electrode and a platinum counter electrode surrounding the working electrode surface, were obtained from Zensor (Taiwan). Platinum (Pt) counter electrode, Ag/AgCl reference electrode and glassy carbon (GC) electrode, with a diameter of 3 mm, was obtained from CH Instruments (Texas, USA). The GC electrode surface was renewed by polishing with alumina particles on a cloth.

7.2.2 Electrochemical measurements

Electrochemical measurements were conducted using a μ Autolab Type III electrochemical analyser (Eco Chemie, The Netherlands) controlled by NOVA 1.9 software (Eco Chemie) at room temperature (25 °C) by using a three-electrode configuration. Cyclic voltammetry (CV) experiment was performed at a scan rate of 100 mV s⁻¹. Chronoamperometry measurements were recorded at 10 ms intervals.

7.2.3 Characterisation

Scanning electron microscopy (SEM) and scanning transmission electron microscopy (STEM) were carried with Joel 7600F-SEM (Jeol, Japan). SEM was performed on a silicon wafer operating at 2 kV with a working distance of 5 mm to analyse the morphology of the various silver nanoparticles dispersed in supporting buffer. STEM was performed at an accelerating voltage of 30 kV on lacey carbon copper grids (Ted Pella, USA) using a 0.1 mg mL⁻¹ dispersion.

7.2.4 Liposomes preparation

DOPC liposomes were prepared at a nominal concentration of 5 mg/mL in PBS buffer by the thin-film hydration and extrusion method, and a MiniExtruder apparatus (Avanti Polar Lipids, Alabaster, AL, USA) was used to repeatedly pass the liposomes through a track-etched polycarbonate membrane containing 50 nm diameter pores. The average diameter of the extruded liposomes was around 75 nm diameter, as determined by dynamic light scattering.

7.2.5 Procedure

The SPE was positioned in a horizontal manner before 35 μ L of the silver nanoparticle mixture or liposome solution was dropped onto the electrode surface. This helps to ensure that the impacting of the nanoparticles is additionally assisted by gravity besides Brownian motion. All chronoamperometry measurements were recorded at 10 s intervals in 10 mM citrate + 90 mM KCl supporting buffer for Ag nanoparticles.³⁵ Chronoamperometry scans were performed for duration of 50 s to detect all possible impacting of the nanoparticles onto the electrode surface. Chronoamperometric scans were performed for 1000 s before AH peptide or PBS was introduced. Movement around the set-up was minimized during the scans to ensure signals generated were not a result of external vibrations.

7.3 Discussion

7.3.1 Electrochemical detection of variable sized monodispersed Ag nanoparticles by impact electrochemistry

Scanning transmission electron microscopy (STEM) was carried out to ascertain the average sizes and spherical nature of the silver nanoparticles. As shown in **Figure 7.1**, the silver nanoparticles are spherical in shape and the actual sizes relate closely to the expected sizes indicated by the manufacturer. As such, the nanoparticles obtained can be used for further impact electrochemistry experiments.

Cyclic voltammetry (CV) was then carried out with a screen-printed electrode on colloidal monodispersed silver nanoparticles dissolved in citrate/KCl supporting buffer⁶⁵ to determine the oxidation potential of the silver nanoparticles of the various sizes. **Figure 7.2** shows the typical cyclic voltammograms of the various sizes of silver nanoparticles of interest. From the voltammograms obtained, it was noted that the nanoparticles undergo oxidation reaction at positive potentials followed by a reduction on the opposite scan. Most of the silver nanoparticles undergo electrochemical oxidation at potential of $\sim +0.07$ V and a subsequent electrochemical reduction of the generated silver ions at ~ -0.1 V. There are slight variations in the oxidation potentials of the different sizes of silver nanoparticles which correlates to our previous study conducted.⁶⁶ From the voltammetric experiments, a standing potential of $+0.15$ V (vs. Ag/AgCl) was chosen as the standing potential for chronoamperometric experiments as it is beyond the oxidation potentials of nanoparticles of all sizes. This standing potential was chosen to ensure that any silver nanoparticles impacting on the electrode surface has a high chance of being oxidised during the impact electrochemistry experiments.

Figure 7.1 Scanning transmission electron microscopy (STEM) images of (A) 107 nm, (B) 80 nm, (C) 40 nm, (D) 20 nm, and (E) 10 nm silver nanoparticles. Scales as indicated in the respective images.

Figure 7.2 Cyclic voltammograms (CVs) of silver nanoparticle of sizes 107, 80, 40, 20, and 10 nm for a screen-printed electrode in 10 mM citrate + 90 mM KCl supporting buffer with scan rate = 100 mV s^{-1} (vs. Ag/AgCl).

As observed from **Figure 7.2**, silver undergoes oxidation at positive potential with the release of an electron during the process (**eq 7.1**). The electron released will subsequently generate an electrochemical signal in chronoamperometry. At a high enough potential, the silver nanoparticle undergoes oxidation upon striking onto the electrode surface. The charge transferred will generate a “spike” which corresponds to a signal in the chronoamperometry. The nanoparticles are assumed to impact on the electrode surface in a stochastic manner by Brownian motion and assisted by gravity.

Chronoamperometry was first carried out on the blank supporting buffer to ensure that background signal from the supporting buffer does not interfere in the quantification process. The average spike height is calculated. This average value acts as a reference point and baseline for subsequent quantification calculations where spikes

that are twice that of this value are considered for quantification. It is to ensure that the signals identified in subsequent experiments are correctly chosen. Chronoamperometry was carried out on the SPE at a standing potential of +0.15 V (vs. Ag/AgCl) to observe and analyse the impacting of the silver nanoparticles as seen in **Figure 7.3**. A significant difference in chronoamperometric signals were observed for the chronoamperometry measurements between the blank supporting buffer (red lines) and solutions with silver nanoparticles (black lines). It was also noted that the frequency and heights of the spikes decreases with the decreasing size of silver nanoparticles.

The signals obtained from the various chronoamperometric measurements were collated and analysed accordingly. Assuming a perfect sphere for each nanoparticle⁶⁷ as confirmed by electron microscopy (**Figure 7.1**), a modified Faraday's equation on electrolysis (**eq 7.2**) was applied to correlate the electrochemical signal obtained with the various nanoparticle sizes. The area under each "spike" corresponds to the amount of charge transferred per impact event. Understanding this relationship, the radius can be calculated from **eq 7.2** to ascertain the average sizes of the impacting nanoparticles. This will also be useful in studying the extent of agglomeration of the nanoparticles in the supporting buffer. The results obtained were summarised in **Table 7.1**.

$$r = \sqrt[3]{\frac{3QAr}{4F\pi\rho}} \quad (7.2)$$

where Q is charge, A_r is atomic mass, F is Faraday's constant and ρ is density of the nanoparticle.

Figure 7.3 Chronoamperometric measurements on silver nanoparticles of sizes (A) 107 nm, (B) 80 nm, (C) 40 nm, (D) 20 nm, and (E) 10 nm in citrate/KCl supporting buffer at +0.15 V (vs. Ag/AgCl). Red lines correspond to blank buffer; black lines correspond to silver nanoparticles in buffer. Inserts show zoomed-in view from 10 s to 20 s.

From **Table 7.1**, it was observed that the average number of nanoparticles involved in the impact per “spike” increases drastically as the real sizes of the nanoparticles (measured by electron microscopy) decrease. It was also interesting to note that the experimentally determined radii of the nanoparticles were significantly

larger than the values obtained by electron microscopy (and stated by manufacturer) which suggest the formation of large clusters. This is expected with the introduction of chloride ions in high ratio in the supporting buffer. Chloride is known to bind strongly with silver which promotes the formation of agglomerates and aggregates as validated by the experimental results. Studies have shown the possibility to distinguish impact events between agglomerates and aggregates.⁶⁸ However, due to the limitations of our system; we were unable to distinguish such differences. However, these results obtained were insufficient to analyse the movement and diffusion of the nanoparticles onto the electrode surface.

Table 7.1 Calculations and quantification of nanoparticles per impact obtained from chronoamperometry.

Particle Diameter (nm) ^a	Average charge (C)	Calculated radii (nm) ^b	Average no of nanoparticles per "spike" ^c
107	2.63×10^{-11}	155.4	5
80	2.19×10^{-11}	141.2	9
40	2.00×10^{-11}	144.4	67
20	3.05×10^{-11}	165.6	813
10	1.11×10^{-11}	120.2	2364

^a Based on electron microscopy and manufacturer information

^b Based on chronoamperometric signals

^c Derived from (a) and (b)

The diffusion coefficients (D_0) and mass flux (J) were determined by applying Stokes-Einstein equation⁶⁹ (**eq 7.3**) and Fick's first law of diffusion⁷⁰ (**eq 7.4**) respectively to have an understanding of the flow of the nanoparticles above the electrode surface.

By examining these variables, it would provide an understanding on the behaviour of the nanoparticles which may influence the impacting onto the electrode surface. The diffusion coefficients were theoretically determined by applying **eq 7.3**. With the assumption that the working electrode surface of the SPE corresponds to that of its geometric area, an additional function, $f(\tau)$, was introduced into **eq 7.4** to facilitate the calculation of the mass flux with the subsequent introduction of the definition for τ as approximated by Szabo *et. al*^{71,72}. As such, the mass flux values were calculated based on this approximate.⁷³ The results obtained from the calculations are summarised in **Table 7.2**.

$$D_0 = \frac{k_B T}{6\pi\eta r} \quad (7.3)$$

where k_b is the Boltzmann constant, T is temperature, η is viscosity and r is radius of nanoparticle.

$$J = - \frac{D_0 C_0}{f(\tau)} \quad (7.4)$$

$$f(\tau) = 0.7854 + 0.8862\tau^{-0.5} + 0.2146e^{-0.7823\tau^{-0.5}}, \quad \tau = \frac{4D_0 t}{r_0^2}$$

where r_0 is radius of electrode, t is duration of the chronoamperometric measurement, C_0 is the bulk concentration of the silver nanoparticles.

From **Table 7.2**, it was observed that a very low percentage of successful impacts were obtained which correlates to a relatively low frequency of successful collision when comparing the mass flux and diffusion coefficient values. Several reasons for this possibility could be attributed to the limitations in the nature of the SPE, and the interaction between the silver nanoparticles in the supporting buffer.

Table 7.2 Calculated values of the diffusion coefficient and mass flux to determine the average number of silver nanoparticles expected at the diffusion layer at average impact duration.

Particle Size (nm) ^a	Diffusion Coefficient of Nanoparticles ($\text{m}^2 \text{s}^{-1}$)	Mass flux of nanoparticles ($\text{mol m}^{-2} \text{s}^{-1}$)	Average no. of nanoparticles <u>present</u> per “spike” ^b	Average no of nanoparticles <u>oxidised</u> per “spike”
107	4.58×10^{-12}	9.581×10^{-15}	1537	5
80	6.13×10^{-12}	1.478×10^{-14}	2453	9
40	1.23×10^{-11}	4.145×10^{-14}	5909	67
20	2.45×10^{-11}	1.159×10^{-13}	24297	813
10	4.90×10^{-11}	3.225×10^{-13}	37746	2364

^a Based on electron microscopy and manufacturer information

^b Based on theoretical particle flux

The morphology and the electroactive properties of the working electrode surface of the SPE were examined. Scanning electron microscopy (SEM) was carried out to understand the surface morphology and nature of the working electrode surface. From **Figure 7.4**, it was observed that the surface of the SPE working electrode is not planar and forms “wrinkles”. The electrode surface appears to be layered and uneven. There is also presence of additional nanostructures which were confirmed to be that of the polymer coating on the working electrode surface. As a result of this “wrinkled” surface, the SPE might not be homogeneously electroactive and not all the nanoparticles which collide with the electrode surface will get oxidised.

Figure 7.4 Scanning electron microscopy (SEM) images of the morphology of the bare screen-printed electrodes (SPE) at different magnifications (A) 8000x, and (B) 1000x. Scale as indicated in the respective images.

We proceed to investigate the electroactive properties of the working electrode by cyclic voltammetry (CV).⁷⁴ This study will provide a better understanding on the actual area on the working electrode that facilitates electrochemical reactions from taking place. Wide scan rates between 1000 to 10 mV s^{-1} were carried out on ferro/ferricyanide redox. Increasing scan rates showed an increase in peak currents (i_p) with a corresponding increase in peak-to-peak separation as predicted.

The Randle-Sevcik equation (**eq 7.5**) was applied as it allows for the correlation between the i_p and square root of the scan rate ($v^{1/2}$) based on the semi-infinite diffusion model.^{75,76} The dependence of i_p against scan rate (v) was then studied as shown in **Figure 7.5**. By doing so, the actual electroactive surface area of the SPE working electrode surface can be determined.

$$i_p = 0.446FAC \left(\frac{FDv}{RT} \right)^{\frac{1}{2}} \quad (7.5)$$

From **Figure 7.5**, the experimental i_p values obtained were significantly smaller than the theoretically calculated i_p values. This indicates a negative deviation from the expected electrochemistry of the working electrode surface and a smaller actual electroactive area on the SPE. The electroactive area of the SPE does not exceed the geometric area of the working electrode itself. The gradient of the plots in **Figure 7.5** allows for the calculation of the electroactive area of the electrode surface which was found to be 0.01673 cm^2 . This is significantly lower than the theoretical area based on the geometric calculations of the SPE working electrode of 0.07069 cm^2 . This is contrary to **Figure 7.4** where the electroactive surface area was expected to be higher due to the layered and “wrinkled” nature of the electrode surface. As such, not all the impact events would have resulted in an electrochemical signal⁷⁷ being generated due to a significant portion of the electrode surface not being electroactive. Nanoparticles would have bounced off the electrode surface without any electrochemical oxidation from taking place.

Figure 7.5 Randle-Sevcik plots of theoretical graph (dash line) and experimentally-determined peak currents (red line) on screen-printed electrode (SPE).

The use of SPE itself has some inherent limitations in detecting silver nanoparticles of small sizes. The average background charge (noise) obtained from the supporting buffer is about 2.68×10^{-12} C. As observed from **Table 7.1**, the theoretical charges calculated for individual nanoparticles smaller than 100 nm are lower than the average background charge from the supporting buffer. This serves as a challenge for the quantification of the sizes of the different silver nanoparticles. Despite that, this limitation opens up possibilities for future research work in optimising the performance and architecture of SPEs for such detection technique. Examples of optimisation could be the pre-polishing surface,⁷⁸ surface modification of the electrode, changing the electrode design, and further optimisation of the supporting buffer.⁶⁷

Having examined the electrode surface, it is also useful to understand the aggregation behaviour of the nanoparticles which could have also attributed to the low percentage of successful impact events. As mentioned above, the introduction of chloride ions into the supporting buffer promotes the agglomeration of silver nanoparticles, thus forming agglomerates of particles of larger sizes. Hence, the expected diffusion coefficients are slower which translates to a lower percentage of particles impacting the electrode surface and getting oxidised per unit time. Within the agglomerates, the bonding between the individual nanoparticles gets weaker with the increasing size of the silver nanoparticle size. As such, there is also the possibility that the agglomerates may not undergo complete oxidation upon impacting with the electrode surface where only a few nanoparticles in the agglomerate gets oxidised while the others break away to the bulk solution.⁴⁹ This could have also resulted in the lower

than expected values and frequency of impact. This correlates with the results for silver nanoparticles of size 107 nm which shows the smallest percentage of spike signals of all the different sizes. The presence of chloride ions could have also promoted the formation of insoluble AgCl when it reacts with the silver ions released.^{77,79} The insoluble salt could potentially block the electroactive surface of the electrode which would reduce the electroactive area available thus, attributing to the low frequency of successful impacts.

7.3.2 Detection of amphiphatic viral peptide by rupture impact voltammetry

From previous studies performed, Cho and co-workers discovered that the liposomes will adhere and form a single layer on the surface over a period of time.⁸⁰ Upon introduction of amphipathic α -helix (AH) peptide, it perturbs and destabilises the lipids of the liposomes which results in deformation and a rupture activity.⁸¹ The lipids will form a bilayer on the electrode surface after rupturing.⁸² **Figure 7.6** shows an illustration of the rupture and spreading of liposomes upon introduction of AH peptide.

Figure 7.6 Illustration on the rupture and spreading of DOPC liposomes on screen printed electrode (SPE) surface: (A) migration and (B) adhesion of liposomes onto electrode surface, (C) rupturing of liposomes in the presence of AH peptide, and (D) the formation of lipid bilayer upon rupture. Figures are not drawn to scale.

Cyclic voltammetry (CV) was first performed with screen-printed electrode (SPE) as preliminary study to identify any inherent electrochemical reactions of the DOPC liposomes (**Figure 7.7**). This is to ensure that the signals obtained during chronoamperometry measurements will be a result of the AH peptide rupturing the DOPC liposomes and not from the inherent oxidation/reduction of the DOPC lipids themselves. The signal obtained will be a result of non-faradaic charge transfer upon the formation of the lipid bilayer on the electrode surface upon rupture by AH peptide. In a non-faradaic process, upon the formation of the lipid bilayer on the electrode surface,

charge transfer takes place as a result of a change in capacitance. As such, a signal output will be detected. A background scan with blank phosphate buffer (PBS) was first performed (red line) in the absence of DOPC liposomes. The DOPC liposomes were then drop-coated onto the SPE surface and left to adhere before CV was performed in phosphate buffer (black line). Upon comparing the voltammograms from blank buffer and liposomes-modified SPE, the voltammograms obtained were very similar in their shapes and features. There was an absence of additional peaks in the voltammogram for the liposome-modified SPE which signified the absence of any oxidation/reduction reactions taking place. It can thus be deduced that the liposomes are not electroactive due to the absence of additional peaks in the voltammograms obtained. Any signals obtained during chronoamperometry should come from non-faradaic capacitive charge transfer upon formation of the lipid bilayer on the electrode surface and not from the oxidation/reduction of the DOPC lipids of the liposomes (**Figure 7.6D**). As such, the spikes obtained will be from the rupturing of liposomes in the presence of AH peptide. An applied potential of +0.35 V was chosen for subsequent chronoamperometry experiment.

Figure 7.7 Cyclic voltammograms of blank PBS (red line) and DOPC liposomes modified glassy carbon electrode in PBS (black line).

We next proceed to investigate the effect of AH peptide on the rupture events. The DOPC liposomes were filled with ferro/ferricyanide solution and subsequently drop-coated onto the electrode surface for adhesion to take place. AH peptide was then injected and allowed to interact with the immobilised liposomes for 20 min. The rupturing of the liposomes will result in the leaking of ferro/ferricyanide solution into the buffer and will be detected by voltammetry. The response signals were subsequently analyse with cyclic voltammetry (not shown). The signal response percentage (R%) was calculated to compare the effects before (R_b) and 20 min after (R_a) introduction of AH peptide (**eq 7.6**). A higher percentage would be more favourable which would suggest better rupture activity taking place and possibly greater signal in chronoamperometry.

$$R (\%) = \frac{[(R_b - R_a)]}{R_b} \times 100 \quad (7.6)$$

Two different AH peptide concentrations were studied which were 26 and 100 μM . The introduction of AH peptide at both concentrations gave positive signal response as compared to the absence of AH peptide in the system which gave a negative R% value (**Figure 7.8**). The importance of AH peptide in the system is apparent from this observation where it induces liposome rupture events. AH concentration of 26 μM was chosen for the preceding experiments as it gave the optimum R% and requires minimal peptide for the induced liposome rupturing to occur. This is an importance parameter of consideration for effective detection through liposome rupture voltammetry.

Figure 7.8 Effect of variable AH peptide concentration on the change in signal response.

The analytical performance was subsequently examined using chronoamperometry with injection of 26 μM AH peptide or PBS as control. The stability of the liposomes at the applied potential was analysed over a period of 1000 s. The stabilisation process was performed to ascertain the stability of the liposomes at the applied potentials and it does not rupture spontaneously. This process also allows for the background signal to be determined which will be used for further quantification

steps. From **Figure 7.9A**, minimal spikes were formed during the stabilisation period apart from the signal generated by electrical background noise. The average background spike height (δ) was calculated during the 1000 s stabilisation period before the introduction of AH peptide. This value serves as a reference for quantification calculations where only spikes with heights twice this value will be considered. This will also ensure the correct identification of signals in the measurements obtained. Chronoamperometry was carried out on the SPE at a standing potential of +0.35 V (vs. Ag/AgCl) to observe the rupturing of the liposomes (**Figure 7.9A**). PBS was injected as a control. From **Figure 7.9A**, a significant difference in chronoamperometric signals were observed upon injection of PBS and AH peptide. Spikes with higher frequencies and intensities were obtained in the presence of AH peptide while no apparent spikes were formed when PBS was injected. The experiment was repeated three times and similar observations were made (not shown). As previously mentioned, the spikes obtained were a result of non-faradaic rupture voltammetry process. The formation of the lipid bilayer onto the electrode surface upon rupture of the DOPC liposomes results in the transfer of charge between the electrode surface and lipid bilayer. The results obtained demonstrate the ability of AH peptide to rupture the adhered liposomes and the events can be detected by rupture voltammetry. The spikes obtained from the chronoamperometric measurements were then collated and analysed accordingly. Only spikes with amplitudes twice that of the background signal (δ) were considered and were categorized according to their amplitudes (**Figure 7.9B**). From the distribution, it shows that more than half of the spikes obtained had amplitudes twice that of the background noise.

Figure 7.9 (A) Chronoamperometric measurements of the signals obtained. Insert shows enlarged view of spikes obtained. The scans are offset for clarity. Arrows indicate the point of injection of PBS and AH peptide accordingly. (B) Summary of spike count of liposome ruptures upon addition of AH peptide.

In addition to adding AH peptide to induce liposome rupture, it is also possible to trigger liposome rupture by other means such as incorporating divalent cations⁸³ or changing solution pH.⁸⁴ The general measurement principles of liposome rupture voltammetry provide a robust method to detect liposome rupture events across such possibilities, with broad potential for diagnostic and sensing applications.

7.4 Conclusion

We have demonstrated the use of screen-printed electrodes (SPEs) for the detection of monodispersed silver nanoparticles. It has the potential to be an effective on-site, point-of-care detection device for preliminary detection of silver in real samples. It can potentially be used for analysis of environmental and biological samples. Despite that, there are several limitations of SPEs as a tool for the quantification and characterisation of silver nanoparticles. These limitations open avenues for future research that can be conducted to optimise the SPE design to enhance its performance

to enable the quantification and characterisation of the different silver nanoparticles. We also demonstrated a novel, proof-of-concept application of rupture impact electrochemistry with the aid of AH peptide. It has the potential to be an effective on-site, point-of-care detection device for preliminary detection of viral peptides in biological samples. It may prove to be more effective and requires lesser processing procedures as compared to the conventional methods presently available. It could also be extended for the detection of other biological targets such as bacteria and unicellular organisms. More research will have to be conducted to analyse the detection limits and quantification of liposome rupture events.

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Chapter 8

Electrochemical Applications of 3D Printed Electrodes for Environmental Sensing Applications

8.1 *Introduction*

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Summary of Work

3D-printing has proven to be a versatile and useful technology for specialised applications in industry and scientific research. We demonstrate its potential use towards the electrochemical detection of environmental contaminants in aqueous solution via cyclic and differential pulse voltammetry, namely nitroaromatic and phenolic compounds. Stainless steel electrodes were fabricated by 3D-printing and the surface was electroplated with gold. The electrochemical performance of the 3D-printed electrodes were compared against the conventionally employed glassy carbon electrode (GCE) and proved to be more sensitive towards the detection of three nitroaromatic compounds. The 3D printed gold-plated electrode produced distinct oxidation peaks for phenolic compounds at lower anodic potentials as compared to GCE. The fabricated electrode was stable at anodic potentials and the inherent electrochemistry of the electrode did not interfere with the electrochemical signals obtained. The detection of these compounds is of utmost importance in military and forensic applications. 3D-printing of customisable electrodes provides a viable alternative to traditional electrodes for the analysis of samples with electrochemical methods. Thus, we have demonstrated a potential application of 3D printing technology for point-of-care detection of phenolic compounds in environmental samples.

8.1 Introduction

The advent of 3D-printing technology has garnered interest among researchers and is gaining popularity^{1,2} brought about from its ability for rapid in-lab prototyping of customisable devices.³ The ability and autonomy to fabricate uniquely-shaped devices at low costs renders 3D-printing an attractive technology which has the potential to accelerate processes and be more economical. Recent advances in 3D printing technology have expanded its capabilities into research and medical uses.² Applications for 3D printing includes the fabrication of human organs,⁴ customized bone prosthetics,⁵ reactionware for chemical synthesis,⁶ supercapacitors,⁷ and buildings construction.⁸ As this technology becomes more established, portable 3D printing systems become more affordable, thus expanding its use into research labs and even homes. This opens up limitless opportunities for the application of 3D printing technology in environmental remediation and other analytical applications. In the field of electrochemistry, it will be beneficial to fabricate customisable electrodes for specific functions and studies with control over the morphology, sizes and performance of the electrodes.¹ In a previous work, helical-shaped electrodes of various sizes were designed and printed to showcase the versatility of selective laser melting (SLM) method and its ability to accommodate unconventional and relatively complex design.⁹ Such use of 3D-printed electrodes can be extended towards the fabrication of sensing devices for environmental samples analysis.

Pollution is a rising cause of concern brought about by an increasing global population and rapid industrialization. Waste generated from industries and households have reciprocal effects on the environment if mismanaged and would hence limit access

to clean water. Among the broad number of water pollutants, phenols and its substituents are highly toxic and can cause harm to human health.^{10,11} It is used as a raw material in industries for the manufacture of plastics and dyes. Phenol can also be found as a component of cosmetics and pesticides, as well as antiseptic in pharmaceutical products.¹² Consequently, it is commonplace to find phenol discarded into water bodies as industrial waste. As a result of its high toxicity, both the United States Environmental Protection Agency (EPA) and the European Union (EU) list phenol as a priority pollutant. Phenolic compounds are also found in drugs and pharmaceuticals. One of the most widely used and popular analgesic drugs is paracetamol which degrades hydrolytically to form *p*-aminophenol (*p*-AP).¹³ *p*-AP is known to have nephrotoxic and teratogenic effects.¹⁴ As such, numerous international and governmental agencies have closely monitored the concentration of *p*-AP in pharmaceuticals.¹⁵ *p*-AP is also used as a raw material in the production of dyes, rubber, petroleum, and in photography.¹⁶ Hence, large amounts of *p*-AP may be released into the environment, causing serious threat to aquatic life. Therefore, there is a need for an efficient and reliable analytical technique for its detection.

Nitroaromatic compounds are found to be harmful to living organisms and are used as precursors for the manufacture of explosive devices. Nitroaromatic pesticides are developed and widely used for household uses and in farms for pest control. Fenitrothion (FT) is a commonly used nitroaromatic organophosphate pesticide. It acts as an acetylcholinesterase inhibitor¹⁷ by binding irreversible to nerve cells¹⁸ and has been classified as toxic, particularly to aquatic life, by the United States Environmental Protection Agency (US EPA) and World Health Organisation (WHO).¹⁹ Therefore,

pesticide levels in water bodies have to be closely monitored, even at trace levels.²⁰ Besides that, such pesticides can be improvised and be developed as nerve agents.²¹ 2,4-dinitrotoluene (DNT) and 2,4,6-trinitrotoluene (TNT) are commonly found nitroaromatic compounds in explosives. The detection of DNT has gained interest due to its presence in TNT-based explosives as a by-product from the incomplete nitration of toluene.²² Trace level detection of DNT and TNT may provide governmental agencies and security personnel with rapid initial detection of concealed explosives in potential terrorist attacks and wartime situations. Detection of nitroaromatic compounds may also be useful in forensic applications or investigating possible sources of pollution from poorly maintained weapons and ammunition manufacturing or storage facilities. As such, it is of great importance to develop a technique for accurate detection of such compounds. These nitroaromatic compounds can be electrochemically detected from the reduction of the nitro (-NO₂) groups around the aromatic ring.²³

Research on the trace level detection of these compounds has primarily focused on various analytical techniques such as microfluidic devices,^{24,25} high performance liquid chromatography (HPLC),²⁶ colorimetric detection,²⁷ gas chromatography (GC),^{28,29} capillary zone electrophoresis,^{30,31} and micellar electrokinetic chromatography.³² Despite their good performance, these techniques require expensive, sophisticated instrumentation with specialized training and long processing times for analysis. As such, they are unsuitable for rapid, on-site point-of-care detection of environmental samples. 3D printing technology coupled with voltammetric techniques has been proven to be a viable alternative for rapid and sensitive detection of analytes.^{1,9} Surface modification of electrodes with conductive transducer platform such as graphene,³³ mesoporous

carbon,^{34,35} carbon black,³⁶ carbon nanotubes,^{37,38} and nanoparticles,³⁹ have also been studied. We set forth to investigate the feasibility of using 3D-printed electrodes for voltammetric detections of nitroaromatic and phenolic compounds. As a proof-of-concept, bare and gold-plated 3D-printed stainless steel helical electrodes will be used to detect three nitroaromatic environmental pollutants; fenitrothion (FT), 2,4-dinitrotoluene (DNT), and 2,4,6-trinitrotoluene (TNT); and two phenolic compounds; phenol and *p*-aminophenol (*p*-AP). Their performances will be compared to conventional glassy carbon electrode (GCE).

Surface modification of 3D-printed electrode with gold has been proven to be an efficient tool in biosensing for the detection of DNA,⁴⁰ and heavy metals.⁴¹ Despite that, the performance and working potential of the fabricated electrodes might be limited by the inherent oxidation of the material deposited on the 3D-printed electrode surface. Hence, we set forth to investigate the feasibility and stability of using 3D printed gold-plated electrode towards the anodic detection of phenolic compounds.

8.2 Experimental

8.2.1 Materials and apparatus

2,4,6-Trinitrotoluene (TNT) was obtained from Accustandard. Fenitrothion (FT), 2,4-dinitrotoluene (DNT), phenol, *p*-aminophenol (*p*-AP), potassium phosphate dibasic, sodium phosphate monobasic, sodium chloride, potassium chloride, nitric acid and potassium hydroxide were obtained from Sigma-Aldrich (Singapore). Hydrogen peroxide was obtained from Alfa-Aesar (Singapore). Acetonitrile (ACN) was obtained from Merck (Singapore). Deionised (Milli-Q) water (25 °C) with conductivity of 18.2 MΩ cm was

used.

Stock solutions of FT and DNT were prepared in CAN while stock solutions of *p*-AP and phenol were prepared in phosphate buffer. The respective stock solutions were stored in the refrigerator at 4 °C. TNT was used as obtained. Solutions used for analysis were freshly prepared when required by addition of aliquots of the stock solutions to a mixture of 1 mL ACN and 4 mL phosphate buffered saline (PBS) pH 7.2, purged using nitrogen for 10 min.

3D-printed stainless steel electrodes were fabricated as previously reported³ using selective laser melting (SLM).

Glassy carbon electrode (GCE), with a diameter of 3 mm, Ag/AgCl reference electrode and platinum (Pt) counter electrode were obtained from CH Instruments (Texas, USA). A piece of platinum foil was used as counter electrode with 3D-printed electrodes to ensure a comparable surface area for electron flow.

8.2.2 Electrochemical measurements

All electrochemical measurements were carried out using an Autolab PGSTAT101 potentiostat (Metrohm Autolab B. V., Utrecht, The Netherlands) connected to a personal computer and controlled using NOVA version 1.10 at room temperature (25 °C), using a 3-electrode configuration. Cyclic voltammetry (CV) experiments were performed at scan rates of 100 mV s⁻¹ and differential pulse voltammetry (DPV) experiments were performed at amplitude pulse of 25 mV, pulse width of 50 ms and scan rate of 10 mV s⁻¹ unless otherwise stated.

8.2.3 Fabrication of 3D-printed electrode

Helical stainless-steel 3D printed electrodes were fabricated as previously reported.^{9,40} Electrode design was first designed using sketch-up 3D modelling open-source software. Metal 3D printing was carried out with a ConceptLaser printer (Concept Laser GmbH, Germany) using selective laser melting (SLM) technique. A focused, high-energy laser beam fuses and link metallic article deposited in powder form on a printing stage according to the design in a layer by layer fashion.

8.2.4 Electroplating of 3D-printed electrode with gold

Gold (Au) electroplating was performed as previously reported.⁴ Briefly, the stainless steel electrode was immersed into a commercial gold plating solution and the electroplating was carried out applying a current of -20 mA for 90 min while stirring in the presence of a Ag/AgCl reference electrode and a platinum counter electrode.

8.2.5 Electrode surface cleaning

GCE was polished using alumina slurry (0.05 μm) on a polishing pad. The bare 3D-printed stainless-steel electrode surface was cleaned by immersing in 0.5 M nitric acid. Cyclic voltammetry was then carried out at a potential range between -1 to 1 V for 10 cycles to remove any impurities. It was subsequently rinsed with deionised water. The gold-plated 3D electrode cleaning method was adapted and modified from a previous study.⁴² In summary, the electrodes were first soaked for 10 min in a solution of 50 mM KOH and 25% w/w H_2O_2 before rinsing with deionised water. Cyclic voltammetry was subsequently performed in 50 mM KOH for 10 cycles at a potential range between 0 to -0.9 V (for nitroaromatic compounds) or 0 V to 0.8 V (for phenolic compounds) at a

scan rate of 100 mV s^{-1} . It was then washed with deionised water.

For experiments with nitroaromatic compounds, the electrode was then placed in a solution containing 20% ACN and 80% PBS, and cycled for another 10 cycles in the same potential range or until a stable signal was achieved. Without further rinsing, it was then used for measurement.

8.2.6 Characterisation

Scanning electron microscopy (SEM) was performed using JEOL 7600F-SEM (JEOL, Japan) operating at 15 kV. A section of the 3D printed gold-plated electrode was placed onto carbon tape held on a SEM holder. Energy dispersive X-ray spectroscopy (EDX) data was obtained with an Oxford instrument and analysed using Aztec software.

8.3 Discussion

8.3.1 Characterisation studies on gold-plated 3D printed electrodes

Figure 8.1A provides a simplified illustration on the fabrication process of 3D printed steel electrode. Briefly, the electrode was first designed using modelling software before it was metal printed by selective laser melting (SLM) process.

Characterisation was first performed using scanning electron microscopy coupled with energy dispersive X-ray spectroscopy (SEM-EDX) to better understand the elemental composition and surface morphology of the 3D-printed stainless steel electrodes before and after gold-plating.

From the SEM images (**Figures 8.1B and 8.1C**), it is observed that selective laser melting (SLM) fabrication process resulted in the formation of a rough surface consisting

of sporadic spherical-shaped structures with diameters of 10-30 μm . From the EDX map analysis, the main components of the stainless steel electrode were found to be iron and nickel which is consistent with the composition of stainless steel (**Figures 8.1D and 8.1E**).

Figure 8.1 (A) Scheme illustrating the fabrication of 3D printed steel electrode. Scanning electron microscopy (SEM) images of 3D-printed stainless steel using a 5.0 kV electron source at (B) 50x and (C) 500x magnification. Energy dispersive X-ray (EDX) spectroscopy map showing (D) nickel and (E) iron distribution. Scale bars correspond to 100 μm (B) and 10 μm (C).

Figure 8.2 (A) Scheme illustrating the gold electroplating process of 3D printed steel electrode. Scanning electron microscopy (SEM) images of gold-plated 3D-printed stainless steel electrode using a 5.0 kV electron source at (B) 30x, (C) 500x and (D) 2000x, magnification. Energy dispersive X-ray (EDX) spectroscopy map showing (E) nickel, (F) iron, and (G) gold distribution. Scale bars correspond to 100 μm (B) and 10 μm (C and D).

The surface morphology of the 3D-printed electrode was again analysed after electroplating with gold (**Figure 8.2A**). It is observed that the electrode surface is coated with a layer of uneven microstructures (**Figures 8.2B to 8.2D**), about 3-5 μm with several edge features (**Figure 8.2D**). This layer significantly increased the surface area of the electrode, allowing for greater current flow. The exposed edges may also play a role in catalysing reduction reactions. EDX map analysis confirmed that gold was the predominant surface element. The signals obtained for iron and nickel (**Figures 8.2E and**

8.2F) were less intense as compared to **Figures 8.1D** and **8.1E** which suggests a homogeneous gold layer forming on the electrode surface.

8.3.2 3D printed metal electrodes for detection of nitroaromatic compounds

Having established and affirmed the successful deposition of gold onto the 3D-printed electrodes, we next set forth to investigate the feasibility and working capability of the fabricated electrodes. The reduction of one nitro group in the nitroaromatic compound undergoes a $4\text{ e}^-/4\text{ H}^+$ reaction mechanism which results in the formation of hydroxylamine (NHOH) functionality. This electrochemical step will result in the formation of a cathodic peak in voltammetry. Correspondingly, increasing the number of nitro groups in the aromatic compound would lead to the formation of more cathodic peaks observed. The positions of the cathodic peaks were also found to be influenced by the position of the nitro groups (*ortho*-, *meta*-, *para*-). Cyclic voltammetry (CV) was firstly employed to study the electrochemical behaviours of fenitrothion (FT), 2,4-dinitrotoluene (DNT) and 2,4,6-trinitrotoluene (TNT). This was then followed by the use of differential pulse voltammetry (DPV) for quantitative analysis. Experiments were performed using 3D-printed electrodes (bare and gold-plated) and glassy carbon electrode (GCE) for comparison.

Fenitrothion (FT)

Figure 8.3A shows the voltammograms obtained with GCE. A cathodic peak was observed at -0.76 V which corresponds to the irreversible reduction of nitro (-NO₂)

group into hydroxylamine (-NHOH). The anodic and cathodic peak pair at about -0.2 V and 0 V respectively corresponds to the reversible redox pair of -NHOH to a nitroso group (-NO).⁴³ Having established the redox potentials of FT with GCE, we next investigated the electrochemical capabilities of the bare 3D-printed stainless steel electrode. From **Figure 8.3B**, no apparent cathodic peaks were observed within the same potential window, even at high concentrations of FT. This would suggest that stainless steel might not be ideal for direct use for the electrochemical reduction of nitroaromatic compounds. The modification of the bare stainless steel electrode with gold enabled the formation of a broad cathodic peak which increases in peak height with a corresponding increase in FT concentration (**Figure 8.3C**). The peak potentials obtained ranged between -0.4 V to -0.5 V which is a significant shift obtained with glassy carbon. The cathodic current signals were about nine times higher than that with glassy carbon electrode. This could be attributed to the significant increase in surface area of the working electrode which corresponds to higher rate of charge transfer.

DPV was next performed to compare the performance and sensitivities of the different electrodes. From **Figure 8.3D**, distinct peaks were observed with GCE which centred at about -0.7 V. However, no visible peaks were observed with bare 3D-printed electrode (**Figure 8.3E**), which was a similar observation to the CV scans carried out (**Figure 8.3B**). The gold-plated 3D electrode produced intense cathodic peaks centred at about -0.75 V, similarly to GCE (**Figure 8.3F**). This observation corroborates with the formation of the cathodic peaks in CV (**Figure 8.3C**) which shows the electrochemical reduction of FT by gold on the 3D-printed electrode. The intensity of the peaks obtained with the gold-plated 3D-printed electrodes were more intense and of significantly higher

magnitudes. This could be attributed to the synergistic effect of the conductivity of the stainless steel electrode and the electrocatalytic capability of the gold surface.

Figure 8.3 Cyclic voltammograms of FT with (A) glassy carbon electrode, (B) bare and (C) gold-plated 3D stainless steel electrode. Differential pulse voltammograms on (D) glassy carbon, (E) bare, and (F) gold-plated 3D stainless steel electrode. Conditions are as described in the experimental section.

Calibration plots (**Figure 8.4**) were then generated from DPV measurements based on the current density signals at different FT concentrations. Geometrical surface area was taken into consideration (GCE, 7 mm²; gold-plated, 15 mm²). The gradient obtained for glassy carbon electrode was 0.471 mA/cm²μM with R = 0.9545 while gold-plated 3D-printed stainless steel electrode generated a gradient of 2.1 mA/cm²μM with R = 0.997. It was determined that the gold-plated 3D electrode is about 4.5 times more sensitive for the electrochemical detection of FT than GCE brought about by a steeper gradient observed for the calibration plot. Gold-plated 3D-printed stainless steel electrode was also determined to have a broader detection range for FT.

Figure 8.4 Calibration plots for bare glassy carbon (black line) and gold-plated 3D-printed electrode (red line) with variable concentrations of fenitrothion.

2,4-Dinitrotoluene (DNT)

From our previous findings,¹¹ it was determined that the reduction of each nitro group in TNT occurs at specific reduction potentials resulting in the formation of three cathodic peaks with voltammetry. This can also be applied for the reduction of DNT

where two reduction peaks would be observed. Two distinct cathodic peaks would be expected from the distinct reduction of the *ortho*- and *para*- substituted nitro groups in DNT.²²

Cyclic voltammetry was performed with all 3 electrodes as shown in **Figure 8.5**. As predicted, two irreversible reductive peaks (-0.64 V and -0.82 V) were observed with GCE (**Figure 8.5A**). The redox peak pair previously observed in FT was also present, suggesting that nitroaromatic compounds generally undergo a similar reaction pathway. It was interesting to note that the voltammograms obtained using bare 3D electrode presented an inherent electrochemical behaviour due to the redox processes of its main components such as Fe, Cr and Ni (**Figure 8.5C**). However, no apparent peaks from the reduction of DNT were observed, similar to that with FT. This supports previous observed results where stainless steel is not an ideal material for electrochemical detection of nitroaromatic compounds. A broad cathodic peak was observed between -0.3 V to -0.8 V with gold-plated 3D electrode (**Figure 8.5B**). Differential pulse voltammetry also performed to analyse the cathodic peaks with greater accuracy. The stainless-steel electrode was deemed to be of limited use for this detection and thus no DPV was performed with it. Two distinct peaks were observed with GCE (**Figure 8.5D**) which was similar to the observation made with CV. It is interesting to note that the reduction of *ortho*- nitro group (-0.60 V) showed current peak heights about two times higher than the reduction of *para*- nitro group (-0.77 V). The DPVs obtained using the gold-plated 3D electrode was significantly different than that with GCE. From **Figure 8.5E**, the reduction of the *ortho*- group does not occur coherently at a specific potential. Thus, the reduction signal of the nitro group in *para*- position was chosen to plot the

calibration plots.

Figure 8.5 Cyclic voltammograms of DNT with (A) glassy carbon, (B) gold-plated and (C) bare 3D stainless steel electrode. Differential pulse voltammograms of DNT at (D) glassy carbon, and (E) gold-plated 3D stainless steel electrode. Conditions are as described in the experimental section.

From the calibrations plots obtained (**Figure 8.6**), the gradient of the slope for glassy carbon electrode was $0.1175 \text{ mA/cm}^2\mu\text{M}$ with $R = 0.996$ while the gradient for gold-plated 3D- printed stainless steel electrode was $0.4197 \text{ mA/cm}^2\mu\text{M}$ with $R = 0.997$. Taking into consideration the geometrical surface area of the electrodes, it was found that the gold-plated 3D electrode was about 3.6 times more sensitive towards DNT than the GCE with a similar linearity range between $60 \mu\text{M}$ and $220 \mu\text{M}$ compared to 1 to $200 \mu\text{M}$ ⁴⁴ obtained for GCE.

Figure 8.6 Calibration plots for bare glassy carbon (black line) and gold-plated 3D-printed electrode (red line) with variable concentrations of DNT.

2,4,6-Trinitrotoluene (TNT)

From previous studies conducted,^{23,45} three distinct cathodic peaks would be formed for TNT brought about by the electrochemical reduction of the three different nitro groups in TNT. The first nitro group in TNT to be reduced is the *ortho*- nitro with respect to the methyl group. This is followed by the reduction of the next *ortho*- nitro. The formation of the last cathodic peak at the highest potential is a result of the reduction of the nitro group in the *para*- position with respect to the methyl group in the aromatic compound probably due to the kinetic effect of the *ortho*- substituted groups.²³ Theoretically, the methyl group acts as an electron donating group which causes the *ortho*- nitro groups on the ring to experience a slightly higher partial negative charge than the group at the *para*-nitro group, thus requiring less energy for the first reduction step. This might account for preferential reduction at *ortho*- over the *para*- nitro group.

Cyclic voltammetry was performed on all three electrodes and their voltammograms are shown in **Figure 8.7**. As predicted, three irreversible cathodic peaks (-0.532 V, -0.722 V and -0.935 V) were observed for glassy carbon electrode (**Figure 8.7A**). No visible peaks were again observed using bare 3D-printed electrode. **Figure 8.7B** shows the voltammograms for TNT with gold-plated 3D electrode. A very broad peak was observed between -0.25 V to -0.8 V. The bare 3D-printed stainless steel electrode showed no apparent peak formation.

Figure 8.7 Cyclic voltammograms of TNT on (A) glassy carbon, and (B) gold-plated 3D stainless steel electrode. Differential pulse voltammograms with (C) glassy carbon, and (D) gold-plated 3D stainless steel electrode. Conditions are as described in the experimental section.

DPV measurements were then carried out with GCE and gold-plated 3D electrodes. From **Figure 8.7C**, DPV with GCE showed three well-resolved peaks corresponding to the reduction of two *ortho*- (-0.46 V and -0.63 V) and one *para*- nitro group (-0.80 V). Three peaks (-0.37 V, -0.51 V, -0.69 V) were also observed with gold-plated 3D electrode (**Figure 8.7D**). The peaks from the electrochemical reduction of *ortho*- nitro groups starts to overlap and form a broad peak as concentration of TNT increases. Hence, the only characteristic peak that could be used for analysis was the *para*- nitro group reduction peak.

Calibration graphs were subsequently plotted based on the peak heights for the electrochemical reduction of *para*- nitro group with both GCE and gold-plated 3D electrode (**Figure 8.8**). From the plots obtained, the gradient for glassy carbon electrode was 0.176 mA/cm²μM with R = 0.996) and gold-plated 3D-printed stainless steel electrode was 0.244 mA/cm²μM with R = 0.998. The calibration plots show that the gold-plated 3D electrode has sensitivity about 1.4 times greater than GCE with a linearity range between 220 and 400 μM.

Figure 8.8 Calibration plots for bare glassy carbon (black line) and gold-plated 3D-printed electrode (red line) with variable concentrations of TNT.

Based on the electrochemical analysis of the three different nitroaromatic compounds, it is observed that 3D-printing can be a reliable technique in the fabrication of customisable electrodes for environmental analysis. The stainless steel backbone of the 3D-printed electrode provides a conductive platform for the transfer of charges during the electrochemical process which can easily undergo surface modification to confer a greater surface area and specific catalytic properties for a more sensitive

response signal. Here, the Au film electroplated onto the steel electrodes enables a sensitive quantification of nitroaromatic compounds by electro-reduction of the nitro groups. The analytical performance is comparable and even superior to that obtained with the GC electrode. In terms of material, we show that surface modified steel metal electrodes fabricated through 3D-printing, have great potential to be adopted for electroanalytical applications which are dominated by carbon based electrodes.⁴⁶⁻⁴⁸ The well-established screen-printing fabrication technology offer currently the possibility to produce disposable electrodes (both carbon and metal-based) at very low price. 3D-printing is at its very early stage of development promising significantly reduced production costs in the near future with the important advantage over screen-printing methods of infinite design and shape possibilities to adapt easily to specific applications.^{2,49} In particular, the possibility to combine during the same 3D-printing process several different materials will ultimately enable the fabrication of complete functional devices able to provide features such as liquid handling, sensing and electronic read-out, altogether.

8.3.2 3D printed metal electrodes electrochemical detection of phenols

We next set forth to investigate the durability and stability of the fabricated electrodes towards the anodic detection of phenolic compounds. Cyclic voltammetry (CV) was first performed on a solution of 100 μ M phenol in 100 mM PBS (pH = 7.2) with GC electrode. Phenol undergoes electro-oxidation to form phenoxy radicals which then leads to the formation of benzoquinones.⁵⁰⁻⁵² From **Figure 8.9A**, a single anodic peak (1a) was observed at +654 mV with GC electrode. There was no corresponding cathodic peak

in the reverse scan, suggesting that the oxidation of phenol to a phenoxy radical is irreversible. The appearances of peaks 2c (+342 mV) and 3c (+248 mV) correspond to the reductions of *ortho*-quinone to catechol and *para*-quinone to hydroquinone respectively. Two additional anodic peaks (peak 2a at +373 mV and peak 3a at +276 mV) formed at the second scan which suggests that the reduction of catechol and hydroquinone is reversible. The calculated peak-to-peak separation (ΔE_p) between peaks 2a and 2c is 31 mV while ΔE_p between peaks 3a and 3c is 28 mV. The values obtained are similar to the theoretical value of 30 mV for a two-electron reversible reaction (eq 8.1) which supports the conclusion that the reaction is reversible.

$$\Delta E_p = |E_{pa} - E_{pc}| = \frac{0.0592}{n} \quad (8.1)$$

where E_{pa} and E_{pc} are the anodic and cathodic peak potentials respectively, and n is the number of electrons transferred in the reaction.

CV was then performed with 3D printed gold-plated electrode (**Figure 8.9B**) and the anodic peak shifted to a lower potential (+598 mV). This could be attributed to the better electron transfer kinetics of the gold surface⁵³ and electrical conductivity of the stainless steel backbone. The two redox peak pairs (peaks 2 and 3, **Figure 8.9A**) were not visible with 3D printed gold-plated electrode. This could be due to the formation of a multilayer film on the gold surface where the phenoxy radicals generated underwent intramolecular rearrangements and adhered to gold thus causing passivation of the electrode surface.^{54,55} The gold surface could have facilitated polymerization reaction that consumed the phenoxy radicals. No additional peaks were observed which suggests no inherent oxidation of the fabricated electrode towards the anodic detection of phenol. This suggests the stability of the 3D printed gold-plated electrode and its ability

to detect phenol. Anodic peak from first scans were only taken into consideration for further investigations with phenol.

Figure 8.9 Cyclic voltammograms of 100 μM phenol in 100 mM PBS (pH = 7.2) with (A) glassy carbon and (B) 3D printed gold-plated electrode. Scan rate = 100 mV s^{-1} .

Figure 8.10 Cyclic voltammograms of variable scan rates (0.01 V s^{-1} to 0.25 V s^{-1}) 100 μM phenol in 100 mM PBS (pH = 7.2) with (A) GC and (B) 3D gold-plated electrode.

The effect of scan rate on the electrochemical behaviour of phenol was next studied. The anodic peak heights of phenol increased with a corresponding increase in scan rate for both electrodes (**Figure 8.10**). There was also a notable shift in peak potential towards the positive direction with the increase in scan rate, which is

characteristic of irreversible systems.⁵⁶

A plot of peak current against square root of scan rate (**Figure 8.11**) yields a linear graph of gradient $11.2 \mu\text{A} (\text{V s}^{-1})^{-1/2}$ and $R = 0.979$ on GC electrode, and a slope of $35.1 \mu\text{A} (\text{V s}^{-1})^{-1/2}$ and $R = 0.956$ for 3D printed gold-plated electrode. From the Randles-Sevcik equation (**eq 8.2**), i_p is proportional to $\sqrt{v}$. As such, oxidation of phenol is determined to be diffusion controlled which corroborates with previous studies.⁵⁷

$$i_p = (2.69 \times 10^5) n^{3/2} A C \sqrt{D v} \quad (8.2)$$

where i_p is the peak current (A), n is the number of electrons involved in the reaction, A is the electrode area (cm^2), D is the diffusion coefficient of analyte ($\text{cm}^2 \text{s}^{-1}$), v is scan rate (V s^{-1}), and C is analyte concentration (mol cm^{-3}).

Figure 8.11 Plot of oxidation peak current against square root at variable scan rate (0.01 V s^{-1} to 0.25 V s^{-1}) for $100 \mu\text{M}$ phenol in 100 mM PBS ($\text{pH} = 7.2$).

We next set forth to investigate the effect of phenol concentration on the electrochemical behaviour on the two electrodes. Phenol concentration was varied from $50 \mu\text{M}$ to $175 \mu\text{M}$. From **Figure 8.12A**, it was observed that the anodic peak shifts to less

positive values with a corresponding increase in phenol concentration with GC electrode. However, an opposite trend was observed with 3D printed gold-plated electrode where the anodic peak shifts to more positive potentials as phenol concentration is increased (**Figure 8.12B**). Despite that, both electrodes exhibit similar trend where the peak current increases with increasing phenol concentration. DPV was next performed to understand the differences in the performance and sensitivities of the electrodes. From **Figures 8.12C and 8.12D**, voltammetric current signal increased with increasing phenol concentration for both electrodes. From **Figure 8.12C**, the anodic peaks on GC shifted to less positive potentials with increasing phenol concentrations. However, the anodic peaks remained constant with increasing phenol concentration for 3D printed gold-plated electrode (**Figure 8.12D**). The peaks obtained with GC electrode were also sharper while the peaks were broader with the 3D printed gold-plated electrode.

Figure 8.12 Cyclic voltammograms of variable phenol concentrations (50 μM to 175 μM) with (A) glassy carbon and (B) 3D printed gold-plated electrode. Scan rate = 100 mV s⁻¹. Differential pulse voltammograms of variable phenol concentrations (50 μM to 175 μM) with (C) glassy carbon and (D) 3D printed gold-plated electrode (Pulse amplitude = 25 mV, pulse width = 50 ms, scan rate = 10 mV s⁻¹).

A calibration graph of peak current density against concentration was then plotted (**Figure 8.13**). It was interesting to note that the linear plots for both electrodes gave almost similar results. GC electrode gave a slope of $1.70 \times 10^{-3} \mu\text{A mm}^{-2} \mu\text{M}^{-1}$ with $R = 0.998$ while 3D printed gold-plated electrode gave a slope of $1.39 \times 10^{-3} \mu\text{A mm}^{-2} \mu\text{M}^{-1}$ with $R = 0.997$. Despite the enhanced electrical conductivity of gold, there was no significant difference observed in the performance of 3D printed gold-plated electrode

and GC electrode for the detection of phenol. This could be attributed to the fouling of the 3D printed gold-plated electrode by the formation of an organic film on its surface which could have affected its electrocatalytic activity.³¹ However, it demonstrates the feasibility of using modified 3D-printed electrode for the detection of phenol with comparable sensitivities with conventionally used GC electrode. Further surface modifications can be explored which might be required for improved sensitivity of phenol with 3D printed electrodes.

Figure 8.13 Plot of peak current density against variable phenol concentrations (50 μM to 175 μM) in 100 mM PBS (pH = 7.2) with glassy carbon (black line) and 3D printed gold-plated electrode (red line).

Cyclic voltammetry was next performed on *p*-AP with GC and 3D printed gold-plated electrode (**Figure 8.14**). A pair of anodic and cathodic peak was observed for both electrodes which shows that the electrochemical reaction of *p*-AP is reversible.^{58,59} The anodic peak for *p*-AP shifted to less positive potential while the cathodic peak shifted to more positive potential with 3D printed gold-plated electrode as compared to

GC electrode. The 3D printed gold-plated electrode gave a smaller ΔE_p of 82 mV in comparison to GC electrode ($\Delta E_p = 142$ mV). It was also observed that the magnitudes for the peak heights differ significantly between the two electrodes. 3D printed gold-plated electrode gave sharp and distinct peaks while the peaks obtained with GC electrode were broader and lower intensity. This observation is opposite to the results obtained for phenol (**Figure 9.9**).

Figure 8.14 Cyclic voltammograms of 1.0 mM *p*-AP in 100 mM PBS (pH = 7.2) with glassy carbon (black line) and 3D printed gold-plated (red line) electrode. Scan rate = 100 mV s⁻¹.

The effect of scan rate on the electrochemical reaction of *p*-AP was next investigated. **Figure 8.15** shows the cyclic voltammograms for GC and 3D printed gold-plated electrodes at different scan rates. The peak heights on both electrodes were observed to increase with increasing scan rate (**Figure 8.15**). From **Figure 8.15A**, no significant change in peak positions were observed for GC electrode. However, 3D printed gold-plated electrode saw a shift for the anodic peak to more positive potentials

and more negative potentials for the cathodic peak (**Figure 8.15B**). This correlates to an almost similar ΔE_p for the GC electrode but an increasing ΔE_p for the 3D printed gold-plated electrode with increasing scan rate.

Figure 8.15 Cyclic voltammograms of variable scan rates (0.01 V s^{-1} to 0.25 V s^{-1}) on a solution of 1.0 mM *p*-aminophenol in 100 mM PBS ($\text{pH} = 7.2$) with (A) GC and (B) 3D gold-plated electrode.

A plot of peak current against square root of scan rate demonstrates the proportionality between the two functions (**Figure 8.16**). The plot for the anodic peaks with GC electrode gave a gradient of $102 \mu\text{A (V s}^{-1})^{-1/2}$ and $R = 0.994$ while that for 3D printed gold-plated electrode gave a gradient of $1.16 \times 10^3 \mu\text{A (V s}^{-1})^{-1/2}$ with $R = 0.993$. The cathodic peak currents gave a slope of $-110 \mu\text{A (V s}^{-1})^{-1/2}$ and $R = -1$ with GC electrode while a slope of $-1.44 \times 10^3 \mu\text{A (V s}^{-1})^{-1/2}$ and $R = -0.997$ was obtained for 3D printed gold-plated electrode. Therefore, from the Randles-Sevik equation (**eq 8.2**), *p*-AP reaction kinetics was determined to be diffusion-controlled.^{60,61}

Figure 8.16 Plot of redox peak currents against square root at variable scan rates (0.01 V s^{-1} to 0.25 V s^{-1}) for 1.0 mM *p*-aminophenol in 100 mM PBS (pH = 7.2) with glassy carbon (black and red lines) and 3D printed gold-plated electrode (blue and green lines).

The concentration of *p*-AP was increased from 0.5 mM to 3.0 mM and studied using CV and DPV (**Figure 8.17**). The anodic and cathodic peak heights increased for both electrodes as concentration of *p*-AP was increased (**Figure 8.17A and 8.17B**). The anodic peak shifted to more positive potential while the cathodic peak shifted to more negative potentials with increasing concentration of *p*-AP for GC electrode (**Figure 8.17A**). However, both cathodic and anodic peaks shifted to more positive potentials for the 3D printed gold-plated electrode (**Figure 8.17B**). DPV was next performed with both electrodes to compare their sensitivities and performance (**Figure 8.17C and 8.17D**). The increase in *p*-AP concentration led to the corresponding increase in the anodic peak height for both electrodes. An additional peak was observed ($\sim +200$ mV) on DPV for both electrodes which was not observable from CV. This could be from the formation of

intermediate products in the oxidation reaction.^{62,63} The peak height of the additional peak increases as the concentration of *p*-AP was increased accordingly.

Figure 8.17 Cyclic voltammograms of variable *p*-AP concentrations (0.5 mM to 3.0 mM) with (A) glassy carbon and (B) 3D printed gold-plated electrode. Scan rate = 100 mV s⁻¹. Differential pulse voltammograms of variable *p*-AP concentrations (0.50 mM to 3.0 mM) with (C) glassy carbon and (D) 3D printed gold-plated electrode (Pulse amplitude = 25 mV, pulse width = 50 ms, scan rate = 10 mV s⁻¹).

A calibration graph of peak current density against *p*-AP concentration was plotted for both electrodes (**Figure 8.18**). The calibration plot for GC electrode gave a gradient of 2.80 μAmm⁻² mM⁻¹ and R = 0.999 while the 3D printed gold-plated electrode gave a gradient of 5.12 μAmm⁻² mM⁻¹ and R = 0.984. The 3D printed gold-plated

electrode displayed improved sensitivity of about 1.8 times in the detection of *p*-AP.

Figure 8.18 Plot of peak current density against variable *p*-aminophenol concentrations (0.50 mM to 3.0 mM) in 100 mM PBS (pH = 7.2) with glassy carbon (black line) and 3D printed gold-plated electrodes (red line).

Studies on the simultaneous detection of these two phenolic compounds were next carried out on GC and 3D printed gold-plated electrodes using DPV. The concentration of one analyte was kept constant while the other was varied. The concentration of *p*-AP was kept constant at 1.0 mM while the concentration of phenol was varied from 50 μM to 175 μM. From **Figure 8.19**, simultaneous detection of the two compounds did not seem to affect the concentration dependency of signal from *p*-AP as peak current increased with increasing phenol concentration. The peak shifted slightly to less positive potentials with GC electrode (**Figure 8.19A**) while there was no apparent shift in peak position with 3D printed gold-plated electrode as concentration of phenol was increased (**Figure 8.19B**).

Figure 8.19 Differential pulse voltammograms of phenol and *p*-AP mixture with variable phenol concentrations (50 μM to 175 μM) in 100 mM PBS (pH = 7.2) with (A) glassy carbon and (B) 3D printed gold-plated electrode. Inserts show zoomed in phenol oxidation range. ($c_{p\text{-AP}} = 1.0 \text{ mM}$, pulse amplitude = 25 mV, pulse width = 50 ms, scan rate = 10 mV s^{-1})

A calibration graph of peak current density against concentration was then plotted. From **Figure 8.20**, GC electrode calibration plot gave a gradient of $2.50 \times 10^{-3} \mu\text{A mm}^{-2} \mu\text{M}^{-1}$ and $R = 0.999$ while 3D printed gold-plated electrode gave a gradient of $2.42 \times 10^{-3} \mu\text{A mm}^{-2} \mu\text{M}^{-1}$ and $R = 0.993$. There was no significant difference in sensitivity between GC and 3D printed gold-plated electrode and their performance was comparable.

Figure 8.20 Plot of peak current density against variable phenol concentrations (50 μM to 175 μM) in 100 mM PBS (pH = 7.2) with glassy carbon (black line) and 3D printed gold-plated electrode (red line).

The concentration of phenol was then kept constant at 100 μM while the concentration of *p*-AP was varied from 0.5 mM to 3.0 mM. From **Figure 8.21**, the increase in *p*-AP concentration led to the proportional increase in peak heights for both electrodes. However, the peak heights for phenol decreased significantly with increasing *p*-AP concentration for GC electrode despite keeping the phenol concentration unchanged (**Figure 8.21A**). Phenol could have undergone a side reaction with *p*-AP which was now present in excess causing some phenol to react. This became more apparent as concentration of *p*-AP increased which causes the phenol peak height to decrease correspondingly. However, this observation was not apparent with 3D printed gold-plated electrode where the anodic peak height of phenol remained almost constant with increasing concentrations of *p*-AP (**Figure 8.21B**). Phenol might have formed an organic film on the gold surface²⁹ and as such, this occurrence was observed

at each concentration of *p*-AP investigated. Thus, the peak current obtained did not change with increasing *p*-AP concentration.

Figure 8.21 Differential pulse voltammogram of phenol and *p*-AP mixture with variable *p*-AP concentrations (0.50 mM to 3.0 mM) in 100 mM PBS (pH = 7.2) with (A) glassy carbon and (B) 3D printed gold-plated electrode. Inserts show zoomed in phenol oxidation range. ($c_{\text{phenol}} = 100 \mu\text{M}$, pulse amplitude = 25 mV, pulse width = 50 ms, scan rate = 10 mV s^{-1})

A calibration graph of peak current density against variable concentrations of *p*-AP was plotted (**Figure 8.22**). The calibration for GC electrode gave a gradient of $3.27 \mu\text{A mm}^{-2} \text{ mM}^{-1}$ and $R = 0.997$ while 3D printed gold-plated electrode gave a gradient of $4.92 \mu\text{A mm}^{-2} \text{ mM}^{-1}$ with $R = 0.989$. The 3D printed gold-plated electrode presented a noticeable increase in sensitivity which was 1.5 times greater than GC electrode.

Figure 8.22 Plot of peak current density against variable *p*-AP concentrations (0.50 mM to 3.0 mM) in 100 mM PBS (pH = 7.2) with glassy carbon (black line) and 3D printed gold-plated electrodes (red line).

The detection of phenolic compounds is enhanced with 3D printed gold-plated electrode when compared with GC electrode. The detection of *p*-AP saw an almost doubled sensitivity with the 3D printed gold-plated electrode with amplified signals of higher magnitudes. The stainless steel backbone of the 3D printed electrode provides a conductive platform for more efficient electron transfer which facilitated and enhanced the electrochemical response signal for *p*-AP. However, the detection of phenol is slightly limited as a result of the strong adhesion of phenol onto gold²⁹ which caused passivation of the 3D printed gold-plated electrode surface. Despite that, the performance of the 3D printed gold-plated electrode was comparable to GC electrode for the detection of phenol and its possible application is demonstrated. The 3D printed gold-plated electrode was also stable at anodic working potentials with no interference signals obtained for the detection of the phenolic compounds. This could be attributed

to the relatively low anodic potentials of the compounds as compared to the oxidation potential of gold. As such, the detection of the compounds of interest is not hindered. Despite that, this limitation would pose a hindrance for detecting compounds with higher anodic potentials due to the formation of gold oxide from the oxidation of gold.

8.4 Conclusion

We have demonstrated a proof-of-concept for the fabrication of helical 3D-printed stainless-steel electrode modified with gold for the detection of nitroaromatic and phenolic compounds where distinguishable peaks were obtained for each compound. 3D-printing provides a convenient technology for fabricating customisable electrodes economically with a high degree of control over their morphologies and composition. The performance of the gold-plated 3D-printed stainless steel electrode outperformed that of glassy carbon electrode for all target analytes, displaying in general higher sensitivities and broader analyte detection range. However, despite the abovementioned advantages, the 3D printed gold-plated electrode has limitations in detecting phenol. This is attributed to the formation of an organic film on the gold surface.^{52,53} Furthermore, compounds with high oxidation potentials might not be accurately analysed due to the oxidation of gold which would influence the rate of reactions. Despite that, we have demonstrated a proof-of-concept for the successful application of modified 3D-printed electrodes for the detection of phenolic and nitroaromatic compounds. Surface functionalised 3D-printed metal electrodes are potential alternatives to conventionally used electrodes for the electrochemical detection of environmental and biological samples.

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Chapter 9

Conclusion and Epilogue

9.1 *Conclusion*

9.2 *Epilogue*

9.1 Conclusion

This thesis provided several outlooks and perspectives on the electrochemical detection of environmental pollutants and food contaminant. Electrochemistry has proven to be a versatile technique in developing various methodologies for the detection of environmental pollutants and food contaminants as illustrated in **Chapters 3 to 8**. As our capabilities to design materials at the microscale and nanoscale levels continue to improve, there is growing interest in developing functional platforms that actively respond to dynamic environmental factors. As such three different research directions were outlined in the thesis which investigated on (1) new electrode materials, (2) alternative electrochemical methodology and (3) customised electrode design.

In **Part 1**, electrode materials and their performance were investigated namely pyrolytic graphite and transition metal dichalcogenides (TMDs). Their potential applications in sensing and biosensing applications were also illustrated in **Chapters 3 to 6**. Edge plane pyrolytic graphite (EPPG) was found to be an excellent electrode amongst the well-defined electrodes for simultaneous detection of multiple mycotoxins which produced distinguishable and well-defined peaks as illustrated in **Chapter 3**. We also demonstrated the proof-of-concept for detecting more than one mycotoxin where the signals do not interfere with one another. **Chapters 4 and 5** highlighted the limitations of transition metal dichalcogenides with regards to their potential window, inherent electrochemistry and the changes to these properties with variable buffer pH. These TMD materials exhibit anodic peaks at positive potentials which may affect the sensitive detection of analytes in the region. These anodic peaks are found to be affected by the pH of buffer used due to the formation of different oxidative products at different pH

ranges. With these findings, it enabled us to have a better understanding on the potential limitations and work around the limited potential range of these mentioned materials. Using the information gathered, an enzyme biosensor using WS_2 as the transducer platform for the detection of organophosphate pesticide was fabricated as illustrated in **Chapter 6**. The biosensor system displayed low limits of detection, comparable performance with previous reports and good selectivity in real samples analysis.

Following that, alternative methodologies with the use of impact electrochemistry were demonstrated in **Part 2** for the detection of nanoparticles with high sensitivities. The application of ‘impact electrochemistry’ was explored for the detection of silver nanoparticles and lipid liposomes. Well-defined nanoparticles (‘hard’ particles) and ‘soft’ nanoparticles were proven to be successfully detected using chronoamperometry on screen-printed electrodes. We also demonstrated the use of screen-printed electrodes as an alternative form the commonly used ultramicroelectrodes. The findings were highlighted in **Chapter 7**.

Finally, **Part 3** provided insights on the potential use of 3D printing coupled with electrochemical techniques for the detection and quantification of environmental pollutants, namely nitroaromatic and phenolic compounds. In this part, a helical stainless steel electrode was fabricated and its surface modified with gold to improve the detection capabilities. They displayed enhanced performance when compared with conventional glassy carbon electrode which is discussed in **Chapter 8**.

9.2 Epilogue

Change and innovation has always been the backbone in scientific research with the quest to discover better materials and methodologies to improve human capabilities. Significant progress has evolved significantly ever since the Stone Age. As we develop and enter a new age in nanotechnology, the possibilities to the development of electrochemical tools and methodologies are limitless. The need for sensitive sensors and biosensors is increasingly important in these ever changing global threats for applications in forensic science, security control, environmental remediation and medical applications.

Interest in research of layered materials has been ignited ever since the successful isolation of graphene by Andre Geim and Konstantin Novoselov. With the great interest in synthesis of new graphene analogues, it is also important to have a sound understanding of the fundamental properties of these materials besides development of new generation technologies. Transition metal dichalcogenides is a promising class of layered materials with good electrical sensitivity, physical and chemical properties.

The incorporation of new technology with electrochemistry has greatly improved the robustness of research with the ability to fabricate customizable prototypes for specialized electrochemical applications. 3D printing is an up-coming tool which can be incorporated and allows great control of the potential fabrication of electrochemical cells and apparatus. This new approach has the potential to expand the capabilities for more sophisticated research which were previously limited by instrumental limitations.

The ability to incorporate new ideas, tools, technology and materials is what catalyze scientific endeavor and breakthroughs in scientific research.

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Appendices

Mycotoxins: Simultaneous Detection of Zearalenone and Citrinin by Voltammetry on Edge Plane Pyrolytic Graphite Electrode

Muhammad Zafir Mohamad Nasir^[a] and Martin Pumera^{*[a]}

Abstract: Mycotoxins are highly toxic compounds often found in the food. It is of paramount importance to have analytical technique for point-of-care on-spot detection for authorised personnel to immediately take the action required. Electrochemistry offers the portability for mini-

aturized sensor of mycotoxins. Here we show that edge-plane pyrolytic electrode offers excellent selectivity and sensitivity towards simultaneous detection of zearalenone and citrinin. This will have a great impact for point-of-care mycotoxin detection.

Keywords: Mycotoxins • Citrinin • Zearalenone • Simultaneous detection • Cyclic voltammetry • Differential pulse voltammetry

1 Introduction

Mycotoxins are secondary metabolites produced by fungi [1] and the consumption of these substances has been known to cause cancer and even death [2]. Recently, strict guidelines and measures have been issued by organisations such as the Joint Research Centre (JRC) of the European Union (EU) [3,4] and Food and Agriculture Organisation (FAO) of the United Nations (UN) [5] to limit the amount of mycotoxins present in food and animal feed.

Zearalenone (ZEA) and citrinin (CIT) are two examples of mycotoxins found in food substances. ZEA (Scheme 1a) is a mycotoxin mainly produced by *Fusarium* species. ZEA is usually found in cereal crops such as wheat and barley. A study in 1988 showed that ZEA was found to have contaminated cereals in many countries which brought about an urgent need for effective detection methods then [6]. ZEA is found to mimic the properties of oestrogen and may result in infertility and abortion [7]. CIT (Scheme 1b) has been identified as a mycotoxin and it was first isolated from *Penicillium citrinum* [8]. CIT has a distinct yellow colour and can usually be found in grains and milk. It is known to be a nephrotoxin which affects the functioning of kidneys [7] and is usually present with ochratoxin [9].

Effective and efficient detection methodologies are crucial to ensure safety of the global food supply. Several techniques have been developed to detect and analyse mycotoxins in food substances such as TLC, HPLC, GC-MS, ELISA [10–14] and Lab-on-a-chip devices [15–17]. Recently, voltammetric techniques have been adopted to study the redox mechanism and behaviour of mycotoxins [18,19]. These voltammetric techniques are faster and require shorter processing time compared to the conventional techniques. To our knowledge, no studies have been conducted in using voltammetric techniques for simultaneous detection of mycotoxins. Therefore, our

Scheme 1. Chemical structure of (a) zearalenone (ZEA) and (b) citrinin (CIT).

paper provides an approach on the use of voltammetry for simultaneous point-of-care detection of mycotoxins, mainly using cyclic (CV) and differential pulse voltammetry (DPV). We investigate here detection of mycotoxins on well-defined electrodes such as edge-plane pyrolytic

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graphite (EPPG), basal plane pyrolytic graphite (BPPG) and boron-doped diamond (BDD).

We investigate here the utility of well-defined electrode surfaces, such as edge-plane pyrolytic graphite (EPPG), basal plane pyrolytic graphite (BPPG), glassy carbon (GC) and boron-doped diamond (BDD) electrodes for simultaneous voltammetric determination of the priority mycotoxins.

2 Experimental

2.1 Chemicals

ZEA, CIT and perchloric acid (HClO_4) were obtained from Sigma-Aldrich (Singapore). Acetonitrile (ACN) was obtained from Merck (Singapore). Deionised water of conductivity $18.2 \text{ M}\Omega \text{ cm}$ was used.

Stock solutions of ZEA and CIT were prepared in ACN and stored in the dark at 4°C . Working solutions were prepared daily by adding suitable amounts of stock solutions to 20% ACN + 80% 1 M HClO_4 mixture solution which serves as the supporting electrolyte.

The boron-doped diamond (BDD) electrode, with diameter of 3 mm, was obtained from Windsor Scientific and used as received. Glassy carbon (GC) electrode, with a diameter of 3 mm, was obtained from CH Instruments (Texas, USA). Edge-plane pyrolytic graphite (EPPG) and basal-plane pyrolytic graphite (BPPG) electrodes, with diameters of 3 mm, were obtained from Autolab (The Netherlands). The EPPG, GC and BDD electrodes surfaces were renewed by polishing with alumina particles (0.05 μm) on a cloth. The BPPG electrode surface was renewed by pressing the surface onto a cello tape to remove the top few layers of graphene. It was repeated several times before rinsing the surface with acetone to remove any residual adhesive glue from the cello tape [20]. Ag/AgCl reference electrode and platinum (Pt) counter electrode were purchased from Autolab.

2.2 Electrochemical Apparatus

Voltammetric measurements were conducted using $\mu\text{Autolab}$ Type III electrochemical analyser (Eco Chemie, The Netherlands) controlled by a NOVA 1.8 software (Eco Chemie) at room temperature by using a three-electrode configuration. Cyclic voltammetry (CV) experiment was performed at a scan rate of 0.1 V s^{-1} three runs, with three scans per run. Differential pulse voltammetry (DPV) experiment was performed with amplitude pulse of 25 mV, pulse width of 50 ms and scan rate of 10 mV s^{-1} .

3 Results and Discussion

Cyclic voltammetry (CV) was performed on the mixture solution and results are shown in Figure 1. Two distinct oxidation peaks at 0.95 V for citrinin (CIT) and at 1.15 V for zearalenone (ZEA) were observed. No peaks were

Fig. 1. Cyclic voltammograms of CIT and ZEA mixture in 20% ACN + 80% HClO_4 supporting electrolyte solution with GC, EPPG, BPPG and BDD electrodes. ($c_{\text{CIT}}^* = 27.3 \mu\text{M}$, $c_{\text{ZEA}}^* = 29.8 \mu\text{M}$, scan rate = 0.1 V s^{-1}).

observed at the reverse scan which suggests that the oxidation process is chemically irreversible for both mycotoxins under the experimental conditions. The most apparent signals observed were at the EPPG electrode. The oxidation peaks obtained, which corresponded to ZEA, were very distinct while the oxidation peaks for CIT exhibited slight peaks. Despite its wide potential window [21,22], the CV obtained from BDD showed only one broad peak which corresponded to ZEA, while no observable peak could be assigned to CIT.

Since CV is not an analytical technique due to its limited sensitivity, we moved to differential pulse voltammetry (DPV) to simultaneously determine the mycotoxins in the mixture solution. The pronounced oxidation peaks (0.95 V for CIT and 1.15 V for ZEA) in DPV are shown

Fig. 2. Differential pulse voltammograms of CIT and ZEA mixture in 20% ACN + 80% HClO_4 supporting electrolyte solution with GC, EPPG, BPPG and BDD electrodes. ($c_{\text{CIT}}^* = 27.3 \mu\text{M}$, $c_{\text{ZEA}}^* = 29.8 \mu\text{M}$, pulse amplitude = 25 mV, pulse width = 50 ms, scan rate = 10 mV s^{-1}).

Fig. 3. Differential pulse voltammogram of CIT and ZEA mixture in 20% ACN+80% HClO_4 supporting electrolyte solution with variable ZEA concentrations (0.209 μM to 17.96 μM) with EPPG electrode. ($c_{\text{CIT}}^* = 27.3 \mu\text{M}$, pulse amplitude = 25 mV, pulse width = 50 ms, scan rate = 10 mV s^{-1}).

Fig. 4. Differential pulse voltammogram of CIT and ZEA mixture with variable CIT concentrations (7.988 μM to 99.4 μM) in 20% ACN+80% HClO_4 supporting electrolyte solution with EPPG electrode. ($c_{\text{ZEA}}^* = 29.8 \mu\text{M}$, pulse amplitude = 25 mV, pulse width = 50 ms, scan rate = 10 mV s^{-1}).

in Figure 2. Comparing the four electrodes used, EPPG produced the greatest peak heights for both CIT and ZEA. The DPV observed using BDD showed a very small and broad peak for ZEA and no visible peaks could be assigned to CIT.

From the voltammograms obtained in Figures 1 and 2, it was concluded that BDD is not an effective material to be used for mycotoxin detection and is not a better alternative to graphitic materials. EPPG gave distinct oxidation peaks with the best separation and peak height of all four electrodes tested. The reactive edge plane sites contributed to the high sensitivity of the EPPG coupled resulting in its low detection limit and overpotential [23]. Therefore, EPPG was chosen as the electrode of choice for the simultaneous detection experiment.

We set our attention on the simultaneous determination of mycotoxins at different concentrations. We kept one mycotoxin concentration constant while varying the concentration of the other mycotoxin. DPV was then conducted to determine the detection limit of the mycotoxins in the mixtures.

Figure 3 shows the DPV obtained at a constant CIT concentration of 27.3 μM with variable concentration of ZEA from 0.209 μM to 17.96 μM . It was observed that the peak heights corresponding to ZEA decreased with the decreasing concentration of ZEA. The concentration dependency of signal from ZEA had a slope of $0.0339 \mu\text{A M}^{-1}$ with $R = 0.9637$.

The experiment was repeated by keeping the concentration of ZEA constant at 29.8 μM while varying the concentration of CIT from 7.988 μM to 99.4 μM . A similar trend was also observed for this experiment as illustrated in Figure 4. However, no peaks were visible at CIT concentrations less than 7.988 μM . The concentration dependency of signal from CIT had a slope of $0.0121 \mu\text{A M}^{-1}$ with $R = 0.9991$.

In summary, we have demonstrated that voltammetry can be used for the simultaneous detection of two priority mycotoxins zearalenone and citrinin where two distinct oxidation peaks were obtained and easily distinguishable. EPPG is an effective material which can be used in mycotoxin detection for point-of-care detection. However, one should note that for efficient electrochemical detection of the mycotoxins in the real samples the target analyte discrimination should be provided. This is our future work aim, following immunoassay or imprinted polymer routes. Integration of the electrodes into the microfluidic format is on the way.

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Simultaneous Anodic and Cathodic Voltammetric Detection of Patulin and Ochratoxin A on Well-Defined Carbon Electrodes

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Dedicated to Professor R. G. Compton on the Occasion of his 60th Birthday

Abstract: Detection of mycotoxins is paramount importance for food quality control. Here we compare well-defined surfaces for electrochemical detection of patulin and ochratoxin A on edge plane pyrolytic graphite and glassy carbon electrodes. Patulin shows well-defined peak at ~ -1.5 V, deeply in cathodic region; ochratoxin A is in contrary oxidizable at potential $\sim +0.9$ V. We investigate here the possibility to simultaneously detect two impor-

tant mycotoxins with distinctive cathodic and anodic signals. We show that edge-plane pyrolytic graphite provides more sensitive response over glassy carbon electrode. It is clear that high density of the edges on graphite is highly beneficial to the voltammetric response of these mycotoxins. The developed technique should have profound impact on research towards portable sensors of mycotoxins.

Keywords: Mycotoxins • Ochratoxin A • Patulin • Simultaneous detection • Voltammetry • Differential Pulse Voltammetry • Edge plane pyrolytic graphite • Glassy carbon

1 Introduction

Mycotoxins are poisonous secondary metabolites produced by fungi [1]. The presence of mycotoxins in food and animal feed supply has been a growing problem in many countries due to the negative health effects they cause [2]. Regulations have been put forward by international agencies such as Food and Agriculture Organisation (FAO) [3] and World Health Organisation (WHO) [4] of the United Nations to control the levels of mycotoxins in food supply.

It has been discovered that one fungal species may produce several mycotoxins [5]. Hence there is a need for the development of a simultaneous detection method of mycotoxins. The mycotoxins of interest are patulin and ochratoxin A. Both mycotoxins are produced by similar species of fungi and usually found present together in food and animal feed.

Ochratoxin A (OTA) is a commonly found and well-studied mycotoxin. It is produced by the *Aspergillus* and *Penicillium* fungi. It has a structure as shown in Scheme 1a. OTA is a main contaminant in cereals, grains and beans [6]. It is a known nephrotoxin which targets the liver and kidneys of humans [6]. It is also described to be a possible carcinogen by the International Agency of Research for Cancer (IARC) and is classified as a Group 2B carcinogen [7]. One prevalent disease caused by consumption of OTA is Balkan endemic nephropathy which plagues countries in the Balkan Peninsula [8].

Patulin (PAT) is produced by *Aspergillus*, *Byssoschlamys* and *Penicillium* fungi [9]. Scheme 1b shows the chemical structure of PAT. It is commonly found in fruits.

Scheme 1. Chemical structures of (a) ochratoxin A (OTA) and (b) patulin (PAT)

It is heat-stable and soluble in water which aids in the contamination of apple products such as juices and ciders. PAT is known to cause apples to rot and form blue mold [10]. Extensive chromatographic studies have been conducted on the detection and quantification of PAT in apple juice [11,12]. PAT is found to have negative effects on mice which caused decrease mortality and suppression of respiratory burst [13]. A study conducted in 1986

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showed PAT to undergo an electrochemical reduction process [14], which is uncommon, as most known mycotoxins undergo electrochemical oxidation.

There is a need for effective and efficient detection methods for the determination of mycotoxins in food supply. Several chromatographic techniques such as TLC, HPLC and GC-MS [15–20] have been developed to detect and analyse these mycotoxins. Recently, lab-on-a-chip devices [21–23] have been developed to improve food analysis. Voltammetry has recently been used as an alternative analytical technique to study the redox mechanism and behaviour of mycotoxins [24,25,28]. These techniques are faster and more time efficient as compared to the conventional chromatographic techniques. Simultaneous determination of OTA and PAT has been carried out with HPLC [26]. However, to our knowledge, no studies have been conducted on the use of voltammetry for the simultaneous detection of these mycotoxins. In this paper, we aim to show an alternative and more efficient method for the simultaneous detection of OTA and PAT with the use of voltammetry, mainly using cyclic (CV) and differential pulse voltammetry (DPV). Two well-defined surfaces are used which are edge-plane pyrolytic graphite (EPPG) and glassy carbon (GC).

2 Experimental

2.1 Materials and Apparatus

Patulin (PAT), ochratoxin A (OTA), potassium phosphate dibasic, sodium phosphate monobasic, sodium chloride, potassium chloride were obtained from Sigma-Aldrich (Singapore). Acetonitrile (ACN) was obtained from Merck (Singapore). Deionised water of conductivity 18.5 M Ω cm was used.

Stock solutions of PAT and OTA were prepared in ACN and stored in the dark at 4 °C. Working solutions were prepared daily by adding suitable amounts of stock solutions to 20% ACN + 80% PBS buffer mixture solution which serves as the supporting electrolyte.

Glassy carbon (GC) electrode, with a diameter of 3 mm, was obtained from CH Instruments (Texas, USA). Edge-plane pyrolytic graphite (EPPG), with diameter of 3 mm, was obtained from Autolab (The Netherlands). The EPPG and GC electrodes surfaces were renewed by polishing with alumina particles (0.05 mm) on a cloth. Ag/AgCl reference electrode and platinum (Pt) counter electrode were purchased from Autolab.

2.2 Electrochemical Measurement

Voltammetric measurements were conducted using μ Autolab Type III electrochemical analyser (Eco Chemie, The Netherlands) controlled by a NOVA 1.8 software (Eco Chemie) at room temperature by using a three-electrode configuration. Cyclic voltammetry (CV) experiment was performed at a scan rate of 0.05 V s⁻¹ three runs, with three scans per run. Differential pulse voltammetry

(DPV) experiment was performed amplitude pulse of 25 mV, pulse width of 50 ms and scan rate of 10 mV s⁻¹.

3 Results and Discussion

We compared here the utility of well-defined electrode surfaces, such as edge-plane pyrolytic graphite (EPPG) and glassy carbon (GC) electrodes for simultaneous voltammetric determination of the priority mycotoxins. Cyclic voltammetry (CV) was performed on the mixture solution. Figure 1a shows one anodic peak at about 0.9 V corresponding to ochratoxin A (OTA) and one cathodic peak at about -1.3 V corresponding to patulin (PAT) for the forward scan. The reverse scan shows similar observations as shown in Figure 1b. A sharp cathodic peak was

Fig. 1. Cyclic voltammograms of (a) forward scan and (b) reverse scan of OTA and PAT mixture in 20% ACN + 80% phosphate buffer solution ($c_{\text{PBS}}^* = 50$ mM, pH 7.2) with GC and EPPG electrodes. ($c_{\text{PAT}}^* = 0.1$ mM, $c_{\text{OTA}}^* = 0.05$ mM, scan rate = 0.05 V s⁻¹)

Fig. 2. Differential pulse voltammograms of OTA and PAT mixture in 20% ACN+80% phosphate buffer solution ($c_{\text{PBS}}^* = 50 \text{ mM}$, pH 7.2) with GC and EPPG electrodes. ($c_{\text{PAT}}^* = 0.1 \text{ mM}$, $c_{\text{OTA}}^* = 0.05 \text{ mM}$, pulse amplitude = 25 mV, pulse width = 50 ms, scan rate = 10 mV s^{-1})

obtained at about -0.7 V which corresponded to the oxygen reaction with the phosphate buffer solution.

Comparing the voltammograms obtained from GC and EPPG, it was observed that the peaks correlate with one another. From Figures 1a and b, it can be deduced that the oxidation of OTA and reduction of PAT are electrochemically irreversible reactions and their redox products do not interact with one another. This is evident by the similar peak positions and shapes for both scans. The reduction peak obtained for PAT was very distinct while the oxidation peak for OTA was less distinct.

We then moved to differential pulse voltammetry (DPV) to determine the mixture solution due to its increased sensitivity. The distinct anodic and cathodic peaks (0.85 V for OTA and -1.3 V for PAT) in DPV were obtained as shown in Figure 2. GC and EPPG voltammograms showed similar peak separation patterns and comparable peak heights. However, the cathodic peak corresponding to PAT is more distinct in GC as compared to EPPG.

We subsequently focused our attention on the simultaneous determination of mycotoxins at different concentrations by keeping one mycotoxin concentration constant while varying the other. DPV was conducted to also determine the detection limit of the mycotoxins in the mixture. The experiment was carried out under N_2 purging to eliminate any side reactions with oxygen and obtain a voltammogram of higher resolution by eliminating the unwanted peaks. A peak centred at -0.8 V was removed and can be attributed to oxygen reaction with the buffer.

Figure 3 shows the DPV obtained at a constant PAT concentration of 0.1 mM with variable concentration of OTA from $1 \text{ }\mu\text{M}$ to $50 \text{ }\mu\text{M}$. It was observed that the peak heights corresponding to OTA decreased with decreasing

Fig. 3. Differential pulse voltammograms of OTA and PAT mixture in 20% ACN+80% phosphate buffer solution ($c_{\text{PBS}}^* = 50 \text{ mM}$, pH 7.2) with (a) GC and (b) EPPG electrodes with variable OTA concentrations ($1 \text{ }\mu\text{M}$ to $50 \text{ }\mu\text{M}$). ($c_{\text{PAT}}^* = 0.1 \text{ mM}$, pulse amplitude = 25 mV, pulse width = 50 ms, scan rate = 10 mV s^{-1}).

concentration of OTA. No visible peaks were observed at OTA concentrations less than $2 \text{ }\mu\text{M}$ for both electrode materials. A linear calibration graph with $R = 0.996$ and $R = 0.995$ was obtained for GC and EPPG respectively. Both electrode materials displayed very similar electrochemical trends. The slope for calibration graph of GC is $1.17 \times 10^{-8} \text{ }\mu\text{A }\mu\text{M}^{-1}$ while that of EPPG is $1.16 \times 10^{-8} \text{ }\mu\text{A }\mu\text{M}^{-1}$.

The experiment was then repeated by keeping the concentration of OTA constant at 0.05 mM while varying the concentration of PAT from $3 \text{ }\mu\text{M}$ to $150 \text{ }\mu\text{M}$. A similar trend was observed as illustrated in Figure 4. No peaks were visible at PAT concentrations less than $20 \text{ }\mu\text{M}$ for GC while EPPG showed no visible peaks at PAT concen-

Fig. 4. Differential pulse voltammograms of OTA and PAT mixture in 20% ACN + 80% phosphate buffer solution ($c_{\text{PBS}}^* = 50 \text{ mM}$, $\text{pH} = 7.2$) with (a) GC and (b) EPPG electrodes with variable PAT concentrations ($3 \mu\text{M}$ to $150 \mu\text{M}$). ($c_{\text{OTA}}^* = 0.05 \text{ mM}$, pulse amplitude = 25 mV , pulse width = 50 ms , scan rate = 10 mV s^{-1}).

trations less than $40 \mu\text{M}$. A linear calibration graph with $R = 0.964$ and $R = 0.991$ was obtained for GC and EPPG respectively. Both electrode materials exhibited similar electrochemical trends. The slope for calibration graph of GC is $4.29 \times 10^{-9} \mu\text{A} \mu\text{M}^{-1}$ while EPPG is $4.22 \times 10^{-9} \mu\text{A} \mu\text{M}^{-1}$.

From Figures 3a and 4a, it was observed that the voltammograms obtained from GC showed a broad peak centred at -0.1 V despite conducting the experiments under N_2 purging. Smaller peaks were observed for voltammograms from EPPG as observed in Figures 3b and 4b. The broad peak may potentially overlap with signals from other mycotoxins thus giving negative results as compared to EPPG. The voltammograms obtained using

EPPG gave more apparent signals with lesser background signal as compared to GC. The calibration graphs obtained also showed better linearity for EPPG and the results are more reproducible.

The lowest concentrations of target mycotoxins detected by EPPG were higher as compared to the standards set in the Codex standards [27] and FAO limits [3] set for both OTA and PAT. From our experiments, lowest concentration detectable experimentally for OTA was $2 \mu\text{M}$ (1.38 ppm) which was higher than the maximum legal amount determined (5 ppb). As for PAT, our experimental limit was at 10.5 ppm which was also higher than the legal limits (50 ppb). Further studies have to be conducted to lower the limit of detection for more sensitive sensing studies.

4 Conclusions

We have demonstrated that voltammetry can be used for the simultaneous detection of two target mycotoxins, patulin and ochratoxin A, where one anodic and cathodic peak was obtained, easily distinguishable. From the results obtained, EPPG is a more effective material than GC in mycotoxins detection due to its better linearity of the voltammogram.

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A limited anodic and cathodic potential window of MoS₂: limitations in electrochemical applications†

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Molybdenum disulphide has been touted as a good material with diverse possible applications such as an energy storage and sensing platform. However, we demonstrate here the limitation of MoS₂ as an analytical sensing platform due to the limited potential window in both the anodic and cathodic regions attributed to the inherent electrochemistry (oxidation of Mo⁴⁺ to Mo⁶⁺) and the catalytic hydrogen evolution reaction due to H₃O⁺ reduction on the MoS₂ surface, respectively. The electrochemical window of MoS₂ lies in the region of ~ -0.6 V to $+0.7$ V (vs. AgCl). We show that such a limited working potential window characteristic of MoS₂ precludes the detection of important analytes such as nitroaromatic explosives, pesticides and mycotoxins which are instead detectable on carbon surfaces. The limited potential window of MoS₂ has to be taken into consideration in the construction of electroanalytical devices based on MoS₂.

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1. Introduction

Transition metal dichalcogenides (TMDs) have gained increasing interest among scientists recently^{1,2} due to their special features and possible future applications.^{3,4} TMDs have layered structures, similar to graphite, held by weak Van der Waal's forces of attraction between each layer.⁵ TMDs exhibit desired properties for many applications such as lubricants,⁶ capacitors,^{3,7,8} energy storage devices^{3,9} and sensing platforms.^{10,11}

TMDs are prepared in a similar fashion to graphene due to the similarities of their layered structures. Mechanical exfoliation, such as lithium intercalation^{12,13} and ultrasonication,¹⁴ is one of the common methods of producing single-layered TMD films.

The electrochemical window is the potential range in which the electrode is stable which is limited by the inherent electrochemistry (change in electrode surface properties) of the electrode material and the redox behaviour (decomposition) of the solvent. Some examples include the dissolving of mercury from mercury electrodes into the solution at potentials greater than 0 V (vs. saturated calomel electrode) and the oxidation of Au electrodes at high anodic potentials.¹⁵

We have previously shown the limitation of graphene oxides at cathodic potentials as a result of the electroactive oxygen-containing groups present.¹⁶

Here we demonstrate the limitation of the electrochemical working window of molybdenum disulphide both in the anodic and cathodic regions, which significantly limits the detection of oxidation and reduction processes of important analytes of interest. This is largely due to the inherent electrochemical activity of molybdenum disulphide in the anodic region and catalytic hydrogen evolution in the cathodic region.

2. Experimental

Zearalenone (ZEA), uric acid (UA), ascorbic acid (AA), sodium tetraborate decahydrate, 2-(*N*-morpholino)ethanesulfonic acid (MES) monohydrate, paraoxon, perchloric acid, potassium phosphate dibasic, sodium phosphate monobasic, sodium chloride and potassium chloride were obtained from Sigma-Aldrich (Singapore). 2-Nitrotoluene (2-NT) was obtained from Alfa Aesar (Ward Hill, MA). Acetonitrile (ACN) was obtained from Merck (Singapore). MoS₂ bulk powder (<2 μ m) and *tert*-butyllithium (1.7 M in pentane) were obtained from Sigma-Aldrich, Czech Republic. Hexane was obtained from Lach-Ner, Czech Republic. Deionised water of conductivity 18.2 M Ω cm (at 25 $^{\circ}$ C) was used.

Stock solutions of ZEA and paraoxon were prepared in ACN. 10 mM stock solutions of uric acid and ascorbic acid were

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prepared in PBS buffer. 20 mM 2-nitrotoluene stock solution was prepared in borate buffer. All stock solutions were stored at 4 °C.

3 g of bulk MoS₂ powder was suspended in 20 mL of *t*-BuLi. The suspension was stirred for 72 hours at 25 °C under an argon atmosphere in order to intercalate the MoS₂ with Li. The Li-intercalated material was separated by suction filtration under an argon atmosphere and washed thoroughly with hexane (dried over Na). Repeated centrifugation (10 000g) was carried out after placing the separated Li-intercalated material in water (100 mL). The exfoliated MoS₂ was eventually dried in a vacuum oven at 50 °C for 48 hours before use. It was later dispersed in water at a concentration of 1 mg mL⁻¹ and ultrasonicated for 30 minutes. The exfoliated MoS₂ was examined by XPS (see Fig. S1, ESI†); according to XPS survey scan, it contained Mo and S but not lithium (note that the detection limit of XPS is ~0.1%). 1 µL of the colloidal suspension was drop-coated onto the glassy carbon electrode surface and left to dry at room temperature. The buffers used were purged with nitrogen gas for 15 minutes before each measurement was taken.

Pt, Ag/AgCl and glassy carbon (GC) electrodes (diameter of 3 mm) were obtained from CH Instruments (Texas, USA). Voltammetric measurements were conducted using a µAutolab Type III electrochemical analyser (Eco Chemie, The Netherlands) controlled by a NOVA 1.10 software (Eco Chemie) at room temperature by using a three-electrode configuration. The electrochemical measurements were performed at room temperature (25 ± 2 °C).

3. Results and discussion

We investigate here the potential window of molybdenum disulphide (MoS₂) using cyclic voltammetry. We first looked into the anodic region of the potential window followed by the cathodic region.

A voltammetric scan was carried out in blank buffer which resulted in the oxidation of MoS₂ starting at +0.6 V and peaking at +1.0 V.^{17–19} This is the result of oxidation of Mo⁴⁺ to Mo⁶⁺.¹⁶ Subsequent scans with analytes were carried out. Important biomarkers, ascorbic acid (AA) and uric acid (UA), were selected as analytes of interest to investigate whether an inherent oxidation peak of MoS₂ interferes with their electrochemical signal. Fig. 1 shows the voltammograms obtained for ascorbic acid and uric acid scanned with MoS₂-modified GC. The voltammograms obtained showed oxidation peaks corresponding to the target biomarkers (+0.45 V) which were easily distinguishable and did not overlap with the oxidation peak of MoS₂. In this scenario, MoS₂ exhibits excellent properties as a sensing platform. One can note that the shoulder in the voltammograms of ascorbic and uric acids at about +1.0 V corresponds to the oxidation of MoS₂.

However, not all analytes are oxidised at low potentials. Important phenolic compounds such as mycotoxins can be oxidised only at higher potentials.²⁰ Therefore, the experiment was repeated using another target sample which is zearalenone

Fig. 1 Cyclic voltammograms of a blank buffer, 10 mM ascorbic acid (AA), and 10 mM uric acid (UA) with MoS₂-modified GC in phosphate buffer (50 mM, pH 7.2). Buffer solution purged with N₂ for 15 minutes before each measurement, scan rate 0.1 V s⁻¹.

(ZEA). ZEA is a common mycotoxin found in food. A voltammetric scan was initially carried out with MoS₂-modified GC in a blank buffer which showed a sharp peak at +1.0 V (Fig. 2A), similar to that observed in Fig. 1. From the scan with bare GC (Fig. 2B), the oxidation peak of ZEA is expected at +1.15 V. However, when the experiment was repeated using MoS₂-modified GC, the oxidation peak for ZEA overlaps with the oxidation peak from MoS₂ and is masked by it (Fig. 2A). A very broad peak was produced which makes it difficult to distinguish the peak from oxidation of ZEA. This does not make MoS₂ suitable as a sensing platform for mycotoxins in food analysis. Most mycotoxins have phenolic structures.²¹ The oxidation potential of most phenolic compounds are expected beyond the potentials of +0.7 V.^{22–24} This range appears to overlap with the inherent oxidation peak of MoS₂ in the different types of buffers used. It is therefore not possible to utilise MoS₂ as a screening platform for rapid detection of mycotoxins in food supply. It is clear that MoS₂ exhibits a limited anodic potential window due to its inherent electrochemistry at about +0.7 V and at higher potentials.

It was observed that changing from a neutral phosphate buffer to a more acidic buffer (20% ACN + 80% HClO₄) did not shift the position of the peak for the blank scans significantly. This might suggest that the oxidation of MoS₂ is not greatly dependent on the pH of the buffer used and the inherent oxidation of the material is pH insensitive.²⁵

We also investigated the suitability of MoS₂ surfaces in the cathodic region by choosing two classes of compounds relevant for environmental analysis which are nitroaromatic explosives and pesticides, more specifically 2-nitrotoluene (2-NT)²⁶ and paraoxon.²⁷ Fig. 3 shows the voltammograms obtained for the reduction of 2-NT and paraoxon in bare GC and MoS₂-modified GC. From the voltammetric scans of the blank buffers, no observable peaks were observed on the GC

Fig. 2 Cyclic voltammograms of 20 μM mycotoxin zearalenone (ZEA) in 80% 1 M HClO_4 (pH 0.15) + 20% ACN buffer with (A) MoS_2 -modified GC and (B) bare GC. Buffer solution purged with N_2 for 15 minutes before each measurement, scan rate 0.1 V s^{-1} .

Fig. 3 Cyclic voltammograms of 10 mM paraoxon in MES buffer (20 mM, pH 5.0) and 20 mM 2-nitrotoluene (2-NT) in borate buffer (20 mM, pH 9.3) with (A) bare GC and (B) MoS_2 -modified GC. Buffer solution purged with N_2 for 15 minutes before each measurement, scan rate 0.1 V s^{-1} .

electrode. However, the hydrogen evolution reaction can be observed at about -1.2 V on the GC electrode and at much lower potentials ($\sim -0.6 \text{ V}$) in the case of MoS_2 . Fig. 3A shows distinguishable reduction peaks for 2-NT and paraoxon on bare GC as they are reduced at -0.66 V and -0.8 V , respectively, while the cathodic electrochemical window of GC is up to -1.2 V . MoS_2 is known to be catalytic to the hydrogen evolution reaction in aqueous solutions. The hydrogen evolution is a limiting factor for the potential window in the cathodic region in general. We see that it is pronounced in MoS_2 (Fig. 3B). One can note in Fig. 3B that the hydrogen evolution reaction is indeed taking place as early as at $\sim -0.6 \text{ V}$ (pH 5.0) while on the GC electrode the hydrogen evolution starts at $\sim -1.2 \text{ V}$. Clearly, MoS_2 shows a limited MoS_2 potential window as a result of early hydrogen evolution.

The usability of MoS_2 as an electrode material for sensing is also limited at the cathodic region as demonstrated. The anodic region of the MoS_2 potential window is greatly

restricted by the inherent oxidation of the material at about $+0.7 \text{ V}$ while the cathodic potential window is limited at ~ -0.6 or $\sim -0.9 \text{ V}$, depending on the pH used. It is of interest to compare the electrochemical window of MoS_2 to other layered materials. For an example, electrochemically reduced graphene shows a window of -1.6 to $+1.2 \text{ V}$,^{16,28} fluorographite -1.0 to $+1.1 \text{ V}$ ²⁹ due to proton reduction and water oxidation, respectively. It should be noted that while the ability of MoS_2 to act as a catalyst for hydrogen evolution is unwanted for analytical applications, it is highly useful for “clean energy” applications.³⁰

4. Conclusion

We have demonstrated that the electrochemical window of MoS_2 is limited in both the anodic and cathodic regions. This is a result of the oxidation of MoS_2 at anodic potentials and

the electrocatalytic hydrogen evolution at cathodic potentials. As a result, it limits the possible electrochemical sensing applications of MoS₂ only to those analytes with redox potentials between ~ -0.6 (due to electrocatalytic hydrogen evolution from H₃O⁺ present in the solvent) and $+0.7$ V (due to oxidation of the electrode material itself, from Mo⁴⁺ to Mo⁶⁺) (vs. AgCl); this has a profound effect on the sensing applications as a large amount of molecules are reducible at lower potentials or oxidizable at higher potentials than at the potential window of MoS₂.

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VIP Very Important Paper

Effect of Electrolyte pH on the Inherent Electrochemistry of Layered Transition-Metal Dichalcogenides (MoS₂, MoSe₂, WS₂, WSe₂)Muhammad Zafir Mohamad Nasir,^[a] Zdeněk Sofer,^[b] and Martin Pumera^{*[a]}

We set forth to investigate the changes in the inherent oxidative peaks of different transition-metal dichalcogenides (TMDs), namely MoS₂, MoSe₂, WS₂, and WSe₂, at different electrolyte pH values. Changing the pH of the electrolyte was found to influence and affect the inherent oxidation of TMDs, more noticeably the peak position. This could be attributed to the different reaction mechanisms and stability at the different pH values. An increase in the electrolyte pH from 0 to 8 shows a linear

decrease in the peak potentials of the inherent oxidative peaks. However, small changes in peak potentials were observed at low alkaline pH levels (from 8 to 11). Beyond pH 11, the emergence of an additional peak at low potentials, apart from the inherent oxidative peak, was observed for most of the TMD materials studied. This insight into the pH dependence of the oxidation of TMD materials is of paramount importance for their electrochemical applications.

1. Introduction

Research on two-dimensional (2D) layered compounds has gained increasing interest recently. Such examples include graphene and its derivatives. Increasingly, a new class of layered materials known as transition-metal dichalcogenides (TMDs) are attracting significant research interest,^[1,2] as they are found to exhibit great physical and chemical properties.^[3] These properties allow for potential applications of TMDs as capacitors,^[4,5] lubricants,^[6,7] energy-storage devices,^[3,8,9] and sensing platforms.^[10,11]

However, despite these improved properties, TMDs hold several limitations in their applications. There is the need to study the fundamental properties of TMDs before devices can be made, so as to maximize their operational capabilities. One such limitation of TMDs can be observed by the inherent electrochemistry of these materials.^[12,13] The term inherent electrochemistry refers to the changes in the properties of a material when it is used as an electrode surface. Such materials undergo redox reactions at specific applied potentials, which cause a change in their physical and chemical compositions. These changes can have negative effects on their uses, especially in sensing and biosensing applications. Molybdenum disulfide (MoS₂) was found to have a limited electrochemical potential

window, owing to the oxidation of MoS₂ at anodic potentials (+0.7 V vs. Ag/AgCl) and electrocatalytic hydrogen evolution at cathodic potentials (−0.6 V vs. Ag/AgCl).^[14] This limits the variety of analytes that it can detect when used as a sensing platform. The inherent oxidation of MoS₂ has been attributed to the oxidation of the molybdenum ion (Mo⁴⁺), which results in the formation of MoO₃ (Mo⁶⁺), SO₄^{2−}, and S^{2−} at high potentials.^[15,16]

These inherent oxidative peaks have been found to be influenced by several factors of the buffer system such as pH, temperature, and electrolyte used. These conditions affect the morphology of the electrode materials and their redox mechanisms. It is of utmost importance to study the changing behaviors of materials to variable factors to optimize experimental conditions and obtain accurate results. This is especially important in applications in point-of-care detection.^[17,18] The pH value is an important variable in buffer systems, which can be easily manipulated to optimize experimental conditions, as compared to other factors. The electrode surface has to be relatively stable to such changes and not affect the voltammetric signals.

To the best of our knowledge, the effect of pH on inherent electrochemical oxidation of TMDs has not been widely studied. Besides that, the most widely studied TMD material is MoS₂, but there is a dearth of information on the inherent electrochemistry of other TMDs such as MoSe₂, WS₂, and WSe₂. Therefore, we set our sights to investigate and compare the effect of changing pH on the inherent electrochemical oxidation of these four mentioned materials.

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Experimental Methods

Materials

Sodium hydroxide, perchloric acid, potassium phosphate dibasic, sodium phosphate monobasic, sodium chloride, and potassium chloride were obtained from Sigma–Aldrich (Singapore). MoS_2 , WS_2 , MoSe_2 , and WSe_2 bulk powder ($< 2 \mu\text{m}$) and *tert*-butyllithium (1.7 M in pentane) were obtained from Sigma–Aldrich (Czech Republic). Hexane was obtained from Lach-Ner, Czech Republic. De-ionized water of conductivity $18.5 \text{ M}\Omega \text{ cm}$ (at 25°C) was used.

Preparation of Exfoliated TMDs

The respective bulk powder (3 g) was suspended in 1.7 M *tert*-butyllithium (20 mL). The suspension was stirred for 72 h at 25°C under an argon atmosphere to intercalate with Li. The Li-intercalated material was separated by suction filtration under an argon atmosphere and washed thoroughly with hexane (dried over sodium). Repeated centrifugation (18000 g) was carried out after placing the separated Li-intercalated material in water (100 mL). The exfoliated material was dried in a vacuum oven at 50°C for 48 h before use.

It was later dispersed in water at a concentration of 5 mg mL^{-1} and ultrasonicated for 30 min. The suspension (1 μL) was then drop-coated onto the glassy carbon electrode surface and left to dry at room temperature. The buffers used were purged with nitrogen gas for 20 min before each measurement was taken.

Electrochemical Measurement

Pt, Ag/AgCl, and glassy carbon (GC) electrodes (diameter of 3 mm) were obtained from CH Instruments (Texas, USA). Voltammetric measurements were conducted by using a $\mu\text{Autolab Type III}$ electrochemical analyzer (Eco Chemie, The Netherlands) controlled by NOVA 1.10 software (Eco Chemie) at room temperature by using a three-electrode configuration. Cyclic voltammetry (CV) experiments were performed at a scan rate of 0.1 V s^{-1} over three cycles, with three scans per cycle.

2. Results and Discussion

We investigated the electrochemical behavior of transition-metal dichalcogenides (TMDs), namely MoS_2 , MoSe_2 , WS_2 , and WSe_2 , using CV in buffers of different pH values ranging from 0 to 13. The anodic region was studied where the inherent peaks of the various TMD materials were observed. Peak potentials were used to analyze the changes in behaviors of the inherent electrochemistry for the different TMDs.

The anodic peaks do not seem to be affected by the varying pH and are visibly distinguishable.^[19] Upon scanning in the anodic direction (0–1.6 V), distinct anodic peaks were observed for the different TMDs at positive potentials. For the same cycle, the peaks disappeared with subsequent scans, which can be attributed to the irreversible oxidation of the materials. There is possibly a passivation of the oxidized products on the electrode surface, which blocks the underlying layers from having contact with the electrolytes.^[13] From Figure 1, it was observed that the voltammograms obtained from MoS_2 were broad, whereas the voltammograms for the other TMD materi-

als showed sharper peaks. The peak shape for MoS_2 gets broader with increasing pH of the electrolyte. However, the peak shapes of the other TMD materials remained relatively constant and seemed unaffected by the increase in pH of the electrolyte. The sharp peaks obtained suggest that the oxidation process is a surface-bound process and is not influenced by diffusion. The anodic electrochemistry is surface-confined on the electrode itself. The anodic electrochemistry of MoS_2 may require the involvement of other species in the electrolyte, which could have resulted in the broader peaks obtained.

The findings of the anodic behavior of TMDs can be categorized into two regions, which are the acidic (pH 0 to 7) and alkaline (pH 8 to 13) regions, where different observations are made. In the acidic region, with the exception of WS_2 , the TMD materials showed a shift in peak positions as the pH increases from 0 to 7. The peaks shift to less positive potentials with increasing pH of the electrolyte. However, the peak positions remained relatively constant in the low alkaline region (pH 8 to 10). Additionally, more than one apparent peak appeared at high alkaline pH levels (beyond pH 10). The additional peaks could be attributed to the formation of intermediate oxidation state products of the various TMDs with the influence of high hydroxide ion (OH^-) concentrations, owing to the high alkaline pH used.^[20] An interesting observation was made for WS_2 , for which the anodic peaks appear more diagonal as the pH of the buffer increases and return upright at high alkaline pH value. This trend is very distinct and unlike that of other TMD materials used.

To obtain a better understanding of the changing trends, the dependence of the peak potential was plotted against the pH of the buffers used. It allows for a visual representation for the analysis of the changes to the different materials with the different pH. Figure 2 shows the dependences obtained from the different TMD materials. It was noted that most of the materials showed similar trends with increasing pH values of the buffer systems, with the exception of WS_2 . A downward linear trend with *R* values of 0.984, 0.963, and 0.985 was obtained for MoS_2 , MoSe_2 , and WSe_2 respectively, in the acidic region (pH 0–7). At low alkaline pH, the peak potential changes were relatively small before an apparent drop was then observed at high alkaline pH levels. This can be attributed to different oxidative reaction products forming at high alkaline pH levels. As for WS_2 , it was noted that the peak position saw an increase as the pH increased from 0 to 2, but subsequently remained relatively constant.

From the modified Nernst equation, the proton-coupled electron transfer (PCET) mechanism can be understood. The gradients of the potential–pH dependence graphs can be compared to the integer values in the modified Nernst equation and the number of protons and electrons can be determined.^[21] From Figure 2, the gradients of the slopes in the acidic pH region (0.14 to 7.82) for MoS_2 , MoSe_2 , and WSe_2 were 0.028, 0.0274, and 0.0385, respectively. The values for molybdenum-based dichalcogenides were approximately half of 0.059, corresponding to a two-electron, one-proton PCET mechanism. This corresponds to previous studies, which showed the oxidation of molybdenum from Mo^{4+} to Mo^{6+} . The acidic region

Figure 1. Cyclic voltammograms of A) MoS₂-modified, B) MoSe₂-modified, C) WS₂-modified, and D) WSe₂-modified GC in phosphate buffers of variable pH (from 0 to 13). Buffer solutions were purged with N₂ for 20 min before each measurement (phosphate buffer concentration = 50 mM, scan rate = 0.1 V s⁻¹).

provides the protons that are then able to interact with the TMDs, resulting in the oxidation of the electrode surface to give the anodic peak in the voltammograms. However, the

value for WSe₂ is significantly different. This suggests a different reaction mechanism, unlike that of molybdenum.

Figure 2. Peak position calibration plots of A) MoS₂-modified, B) MoSe₂-modified, C) WS₂-modified, and D) WSe₂-modified GC in phosphate buffers of variable pH (from 0 to 13). Black plots correspond to peak potentials of inherent oxidation of respective TMD materials, whereas red and green plots correspond to the peak potentials of shifted inherent oxidation and the emergence of additional oxidative peaks at pH 11.86 and 12.56, respectively.

Potential–pH diagrams, better known as Pourbaix diagrams,^[22] can be used to provide an illustration of the changes in the states of the phases at different equilibrium potentials. Figure S1 shows the expected changes in the states of WS₂ and MoS₂ with variable pH (vs. NHE).^[23]

WS₂ shows limited variations at different electrolyte pH values. The similarity in the anodic peaks of WS₂ at variable pH values could be a result of the formation of WO₄^{2−} in the region beyond pH 3. This could have attributed to the distinct peak shape for WS₂. Tungsten undergoes oxidation, which results in the increase in oxidation state from +4 to +6.

In comparison, the equilibrium potential of MoS₂ decreases as pH increases (Figure S1B). As such, the corresponding oxidation potential gets lower with increasing pH. This trend is similar to experimental results, which saw a shift to less positive potentials for MoS₂ inherent anodic peaks. There is also the possibility of forming variable species at the different pH values. The formation of different oxidized products and varying stabilities could have resulted in the broadening of the anodic inherent peak as the pH of the electrolyte increased, owing to the differing properties of the new products. Molybdenum generally undergoes oxidation from an oxidation state of +4 to +6 beyond pH 5.

We next set out to analyze the changes in peak heights of the materials with the changes in pH of the buffers used, as they may have applications in electron analysis.^[24] From Figure 3, there appears to be no distinct trends in the changes in peak heights with variable pH values for all TMD materials analyzed. WS₂ showed the greatest peak heights over the pH range analyzed. Tungsten-based dichalcogenides showed relatively stable peak heights throughout the pH range analyzed. However, molybdenum-based dichalcogenides showed a decreasing trend, with MoSe₂ exhibiting an additional increasing trend beyond pH 6. The relative stability of peak heights for tungsten-based dichalcogenides could probably be attributed to the formation of similar oxidized species, where the reaction kinetics are expected to be the similar. However, for molybdenum-based dichalcogenides, owing to the formation of different oxidized products, different reaction kinetics take place, corresponding to the different peak heights observed in different pH ranges.

3. Conclusions

We have demonstrated the changing behavior of the inherent oxidation of TMD materials in buffer systems with different pH values. MoS₂ and MoSe₂ were found to undergo oxidation

Figure 3. Plots for peak heights of A) MoS₂-modified, B) MoSe₂-modified, C) WS₂-modified, and D) WSe₂-modified GC in phosphate buffers of variable pH (from 0 to 13). Black plots correspond to peak heights of inherent oxidation of respective TMD materials, whereas red and green plots correspond to peak heights of the shifted inherent peak potential and the additional oxidative peaks at pH 11.86 and 12.56, respectively.

from +4 to +6 states for the pH range of 0–8, corresponding to a two-electron, one-proton PCET mechanism. The peak position remains almost stable up to pH 11 and shows a major shift beyond that. WS₂ showed an almost similar peak position and relative stability over the pH range of 2–12. Moreover, tungsten-based dichalcogenides exhibited relative kinetics stability, owing to almost consistent peak heights, as compared to molybdenum-based dichalcogenides.

Peak-height analysis can be used to investigate the number of electrochemically available catalytic sites for heterogeneous electron transfer (HET) to take place. By monitoring the changes in the peak heights, charge-transfer kinetics can be analyzed. Besides that, it can also be used as a potential label for bioassays and biorecognition events when TMDs are used as labels.^[24] By doing so, the identity of various biological molecules can be identified and applied in point-of-care detection devices.

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Impact electrochemistry on screen-printed electrodes for the detection of monodispersed silver nanoparticles of sizes 10–107 nm

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Impact electrochemistry provides a useful alternative technique for the detection of silver nanoparticles in solutions. The combined use of impact electrochemistry on screen-printed electrodes (SPEs) for the successful detection of silver nanoparticles provides an avenue for future on-site, point-of-care detection devices to be made for environmental, medicinal and biological uses. Here we discuss the use of screen-printed electrodes for the detection of well-defined monodispersed silver nanoparticles of sizes 10, 20, 40, 80, and 107 nm.

Introduction

Silver nanoparticles, sometimes known as “nanosilver”, are robust and well-studied materials with varied applications due to their anti-microbial properties.^{1,2} Lately, silver nanoparticles have found applications in washing machines, clothing, cosmetics and lotions. With the widespread use of silver nanoparticles, there is the potential for these nanoparticles to leach into our environment especially during washing and cleaning up processes. The accumulation of silver nanoparticles poses a potential hazard to human health^{3,4} and there is the need to be able to identify and remove them from the environment.^{5–7} Recently, there has been increasing interest and research carried out to find methods to detect silver nanoparticles. Several methods include microfluidics,⁸ direct voltammetry⁹ and multi-technique approaches.^{10,11} However, these techniques require specialised instrumentation and extensive training to operate the devices. As such, they may not be ideal for on-site, point-of-care detection of silver nanoparticles in real samples. A different approach has to be considered which can provide a more viable and effective mode for the detection of silver nanoparticles. One potential technique that has been identified is the application of impact electrochemistry.

Impact electrochemistry is an emerging field which aims to study and detect individual particles in the nano-scale range with greater accuracy. The bombardment of the nanoparticles onto the electrode surface at pre-set potentials will generate electrochemical signals which can then be processed and analysed.^{12,13} Upon the striking of a particle on an electrode surface at a fixed potential, it undergoes redox reactions with the exchange of

electrons, which corresponds to a transfer of charge. This charge transfer correspondingly leads to the formation of a “spike” as the electrochemical signal, which is proportional to the number of electrons transferred. The first measurements on the colloidal impacting particles were reported by Micka using polarography in the 1950s.^{14–16} Such similar observations were later reported by Heyrovsky where he studied the dispersions of colloidal SnO₂ and TiO₂.^{17–20} Lately, advances in impact electrochemistry techniques have enabled the detection and quantification of various nanoparticles directly^{21–26} or indirectly.^{27–31} More recently, impact electrochemistry has been explored to detect individual nanoparticles with greater accuracy.^{32,33} This technique is useful for future applications as it requires minimal sample volume and a simple set-up for operation. These factors are indeed favourable for future on-site detection devices.

Another effective concept that can be explored for use as an electrode surface is the use of screen-printed technology. Screen-printing has been well researched and studied as it provides a more economical and efficient alternative to carbon fibres or microelectrodes. The use of screen-printed technology has enabled the development of portable screening devices such as blood glucose sensors. In electrochemistry, screen-printed electrodes (SPEs) have garnered great interest in their applications due to the ease of electrode design, combination, and manipulation.³⁴ SPEs have also long been known as useful disposable electrodes for effective on-site detection. Recent developments and advances in the manufacture of SPEs will enable for the development of cheaper and more economical instrumentations in the future.

Acknowledging the advantages of both impact electrochemistry and the use of SPEs, we set forth to investigate using SPEs as economical and convenient platforms for the detection of monodispersed silver nanoparticles. By using SPEs, we aim to

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provide a proof-of-concept for the fast and easy detection of silver nanoparticles for future applications in environmental, medicinal, and biological studies. The combination of both efficient techniques will enable for fast and easier detection of silver nanoparticles in real samples. Additionally, we aim to study the possibility of quantifying and characterising silver nanoparticles based on their different sizes using this technique. We base our investigation on monodispersed Ag NPs with well-defined sizes of 10, 20, 40, 80 and 107 nm. It is of importance to be able to characterise silver nanoparticles according to their sizes as nanoparticles, which are small enough, will be able to penetrate through the membranes of cells and blood vessels and become accumulated in vital organs.

Experimental

Materials and apparatus

Potassium chloride was obtained from Sigma-Aldrich (Singapore). Trisodium citrate dehydrate was obtained from Alfa Aesar (Singapore). Colloidal nanoXact silver nanoparticles (0.02 mg mL^{-1} , citrate-capping) of sizes 10, 20, 40, 80, and 107 nm were purchased from Nanocomposix (USA). High quality water, purified in a Milli-Q system (Millipore, MA, USA) with a resistivity of $18 \text{ M}\Omega \text{ cm}$, was used. Three-E screen-printed electrodes (SPEs) of 3 mm working electrode diameter were obtained from Zensor (Taiwan).

Electrochemical measurements

Chronoamperometric measurements were conducted using a μ Autolab Type III electrochemical analyser (Eco Chemie, the Netherlands) controlled using NOVA 1.10 software (Eco Chemie) at room temperature (25°C). Voltammetric experiments were performed using a 3-electrode system on the SPE, with an Ag/AgCl surface as the reference electrode and a platinum counter electrode surrounding the working electrode surface.

Characterisation

Scanning electron microscopy (SEM) and scanning transmission electron microscopy (STEM) were carried out using Joel 7600F-SEM (Jeol, Japan). SEM was performed on a silicon wafer operating at 2 kV with a working distance of 5 mm to analyse the morphology of the various silver nanoparticles dispersed in the supporting buffer. STEM was performed at an accelerating voltage of 30 kV on lacey carbon copper grids (Ted Pella, USA) using a 0.1 mg mL^{-1} dispersion.

Procedure

The SPE was positioned in a horizontal manner before $35 \mu\text{L}$ of the silver nanoparticle mixture was dropped onto the electrode surface. This helps to ensure that the impacting of the nanoparticles is additionally assisted by gravity besides Brownian motion. All chronoamperometry measurements were recorded at 10 ms intervals in 10 mM citrate + 90 mM KCl supporting buffer.³⁵ Chronoamperometry scans were performed for a duration of 50 s to detect all possible impacting of the nanoparticles onto the electrode surface. For accurate data collection, movement around

the set-up was minimized during the scans to ensure the signals generated were not a result of external vibrations. Chronoamperometric measurements were also carried out on a supporting buffer to obtain the background signal generated, which will provide the baseline for the quantification of the “spikes” generated.

Results and discussion

Scanning transmission electron microscopy (STEM) was carried out to ascertain the average sizes and spherical nature of the silver nanoparticles. As shown in Fig. 1, the silver nanoparticles are spherical in shape and the actual sizes relate closely to the expected sizes indicated by the manufacturer. As such, the nanoparticles obtained can be used for further impact electrochemistry experiments.

Cyclic voltammetry (CV) was then carried out using a glassy carbon macro electrode on colloidal monodispersed silver nanoparticles dissolved in citrate/KCl supporting buffer³⁵ to determine the oxidation potential of silver nanoparticles of various sizes. Fig. 2 shows the typical cyclic voltammograms of the various sizes of silver nanoparticles of interest. From the voltammograms obtained, it was noted that the nanoparticles undergo oxidation reactions at positive potentials followed by a reduction on the opposite scan. Most of the silver nanoparticles undergo electrochemical oxidation at a potential of $\sim +0.07 \text{ V}$ and a subsequent electrochemical reduction of the generated silver ions at $\sim -0.1 \text{ V}$. There are slight variations in the oxidation potentials of the different sizes of silver nanoparticles, which correlate with our previous study.³⁶ From the voltammetric experiments, a potential of $+0.15 \text{ V}$ (vs. Ag/AgCl) was chosen as the constant potential for chronoamperometric experiments as it is beyond the oxidation potentials of nanoparticles of all sizes. This constant potential was chosen to ensure that any silver nanoparticles impacting on the electrode surface have a high chance of being oxidised, thus producing an electrochemical signal during the impact electrochemistry experiments.

As observed from Fig. 2, silver undergoes oxidation at a positive potential with the release of an electron during the process (eqn (1)). The electron released will subsequently generate an

Fig. 1 Scanning transmission electron microscopy (STEM) images of (A) 107 nm, (B) 80 nm, (C) 40 nm, (D) 20 nm, and (E) 10 nm silver nanoparticles. Scale bars are $1 \mu\text{m}$ for (A) and (B), 100 nm for (C) and (D), and 10 nm for (E). Inserts show detailed view of nanoparticles with scale bar of 100 nm .

Fig. 2 Cyclic voltammograms (CVs) of silver nanoparticles of sizes 107, 80, 40, 20, and 10 nm for a glassy carbon electrode in 10 mM citrate + 90 mM KCl supporting buffer with a scan rate of 100 mV s^{-1} (vs. Ag/AgCl).

electrochemical signal in chronoamperometry. At a high enough potential, the silver nanoparticle undergoes oxidation upon striking onto the electrode surface. The charge transferred will generate a “spike” which corresponds to a signal in the chronoamperometry. The nanoparticles are assumed to impact on the electrode surface in a stochastic manner by Brownian motion and further assisted by gravity.

Chronoamperometry was first carried out on the blank supporting buffer to ensure that the background signal from the supporting buffer does not interfere with the quantification process. The average spike charge was calculated. This average value acts as a reference point and a baseline for subsequent quantification calculations where spikes that are twice this value are considered for quantification. This is to ensure that the signals identified in subsequent experiments are correctly chosen. Chronoamperometry was carried out on the SPE at a standing potential of $+0.15 \text{ V}$ (vs. Ag/AgCl) to observe and analyse the impacting of the silver nanoparticles as seen in Fig. 3. A significant difference in chronoamperometric signals were observed for the chronoamperometry measurements between the blank supporting buffer (red lines) and the solutions with silver nanoparticles (black lines). It was also noted that the frequency and heights of the spikes decrease with the decreasing size of silver nanoparticles.

The signals obtained from the various chronoamperometric measurements were collated and analysed accordingly. Assuming a perfect sphere for each nanoparticle,³⁷ as confirmed using electron microscopy (Fig. 1), a modified Faraday's equation on electrolysis (eqn (2)) was applied to correlate the electrochemical signal obtained with the various nanoparticle sizes. The area under each “spike” corresponds to the amount of charge transferred per impact event. Upon understanding this relationship, the radius can be calculated from eqn (2), below, to ascertain the average sizes of

Fig. 3 Chronoamperometric measurements on silver nanoparticles of sizes (A) 107 nm, (B) 80 nm, (C) 40 nm, (D) 20 nm, and (E) 10 nm in citrate/KCl supporting buffer at $+0.15 \text{ V}$ (vs. Ag/AgCl). Red lines correspond to the blank supporting buffer and black lines correspond to the silver nanoparticles in supporting buffer. Insets show the zoomed-in view from 10 s to 20 s.

the impacting nanoparticles. This will also be useful in studying the extent of agglomeration of the nanoparticles in the supporting buffer. The results obtained are summarised in Table 1.

$$r = \sqrt[3]{\frac{3QA_r}{4F\pi\rho}} \quad (2)$$

Table 1 Calculations and quantification of nanoparticles per impact obtained via chronoamperometry

Particle diameter ^a (nm)	Average charge (C)	Calculated radii ^b (nm)	Average no. of nanoparticles per “spike” ^c
107	2.63×10^{-11}	155.4	5
80	2.19×10^{-11}	141.2	9
40	2.00×10^{-11}	144.4	67
20	3.05×10^{-11}	165.6	813
10	1.11×10^{-11}	120.2	2364

^a Based on electron microscopy and manufacturer information. ^b Based on chronoamperometric signals. ^c Derived from (a) and (b).

where Q is charge, A_r is atomic mass, F is Faraday's constant and ρ is the density of the nanoparticle.

From Table 1, it is observed that the average number of nanoparticles involved in the impact per “spike” increases drastically as the real sizes of the nanoparticles (measured using electron microscopy) decrease. It is also interesting to note that the experimentally determined radii of the nanoparticles were significantly larger than the values obtained using electron microscopy (and stated by the manufacturer), which suggests the formation of large clusters. This is expected with the introduction of chloride ions in a high ratio in the supporting buffer. Chloride is known to bind strongly with silver, which promotes the formation of agglomerates and aggregates as validated by the experimental results. Studies have shown the possibility to distinguish impact events between agglomerates and aggregates.³⁸ However, due to the limitations of our system, we were unable to distinguish such differences. However, these obtained results were insufficient to analyse the movement and diffusion of the nanoparticles onto the electrode surface.

The diffusion coefficients (D_0) and mass flux (J) were determined by applying the Stokes–Einstein equation³⁹ (eqn (3)) and Fick's first law of diffusion⁴⁰ (eqn (4)) respectively to have an understanding of the flow of the nanoparticles above the electrode surface. By examining these variables, it provides an understanding on the behaviour of the nanoparticles, which may influence the impact onto the electrode surface. The diffusion coefficients were theoretically determined by applying eqn (3). With the assumption that the working electrode surface of the SPE corresponds to that of its geometric area, an additional function, $f(\tau)$, was introduced into eqn (4) to facilitate the calculation of the mass flux with the subsequent introduction of the definition for τ as approximated by Szabo *et al.*^{41,42}

As such, the mass flux values were calculated based on this approximate.⁴³ The results obtained from the calculations are summarised in Table 2.

$$D_0 = \frac{k_B T}{6\pi\eta r} \quad (3)$$

where k_B is the Boltzmann constant, T is temperature, η is viscosity and r is radius of nanoparticle.

$$J = -\frac{D_0 C_0}{f(\tau)} \quad (4)$$

$$f(\tau) = 0.7854 + 0.8862\tau^{-0.5} + 0.2146e^{-0.7823\tau^{-0.5}}, \quad \tau = \frac{4D_0 t}{r_0^2}$$

where r_0 is the radius of the electrode, t is duration of the chronoamperometric measurement, and C_0 is the bulk concentration of the silver nanoparticles.

From Table 2, it can be observed that a very low percentage of successful impacts was obtained, which correlates to a relatively low frequency of successful collisions when comparing the mass flux and diffusion coefficient values. Several reasons for this possibility could be attributed to the limitations in the nature of the SPE and the interaction between the silver nanoparticles in the supporting buffer.

The morphology and the electroactive properties of the working electrode surface of the SPE were examined. Scanning electron microscopy (SEM) was carried out to understand the surface morphology and nature of the working electrode surface. From Fig. 4, it was observed that the surface of the SPE working electrode is not planar and forms “wrinkles”. The electrode surface appears to be layered and uneven. The presence of additional nanostructures was also observed, which were confirmed to be those of the polymer coating on the working electrode surface. As a result of this “wrinkled” surface, the SPE might not be homogeneously electroactive and not all the nanoparticles which collide with the electrode surface will get oxidised.

We proceeded to investigate the electroactive properties of the working electrode using cyclic voltammetry (CV).⁴⁴ This study will provide a better understanding of the actual area on the working electrode that facilitates electrochemical reactions. Wide scan rates between 1000 and 10 mV s⁻¹ were carried out on the ferro/ferricyanide redox. Increasing scan rates showed an increase in peak currents (i_p) with a corresponding increase in peak-to-peak separation as predicted.

Table 2 Calculated values of diffusion coefficient and mass flux to determine the average number of silver nanoparticles expected at the diffusion layer at average impact duration

Particle size ^a (nm)	Diffusion coefficient of nanoparticles (m ² s ⁻¹)	Mass flux of nanoparticles (mol m ⁻² s ⁻¹)	Average no. of nanoparticles present per “spike” ^b	Average no. of nanoparticles oxidised per “spike”
107	4.58×10^{-12}	9.581×10^{-15}	1537	5
80	6.13×10^{-12}	1.478×10^{-14}	2453	9
40	1.23×10^{-11}	4.145×10^{-14}	5909	67
20	2.45×10^{-11}	1.159×10^{-13}	24 297	813
10	4.90×10^{-11}	3.225×10^{-13}	37 746	2364

^a Based on electron microscopy and manufacturer information. ^b Based on theoretical particle flux.

Fig. 4 Scanning electron microscopy (SEM) images of the morphology of the bare screen-printed electrodes (SPEs) at different magnifications: (A) 8000 $\times$ and (B) 1000 $\times$. Scale as indicated in the respective images.

The Randles–Sevcik equation (eqn (5)) was applied as it allows for the correlation between i_p and the square root of the scan rate ($\nu^{1/2}$) based on the semi-infinite diffusion model.^{45,46} The dependence of i_p on the scan rate (ν) was then studied as shown in Fig. 5. By doing so, the actual electroactive surface area of the SPE working electrode surface can be determined.

$$i_p = 0.446FAC \left(\frac{FD\nu}{RT} \right)^{1/2} \quad (5)$$

From Fig. 5, one can see that the experimental i_p values obtained were significantly smaller than the theoretically calculated i_p values. This indicates a negative deviation from the expected electrochemistry of the working electrode surface and a smaller actual electroactive area on the SPE. The electroactive area of the SPE does not exceed the geometric area of the working electrode itself. The gradient of the plots in Fig. 5 allows for the calculation of the electroactive area of the electrode surface, which was found to be 0.01673 cm². This is significantly lower than the theoretical area based on the geometric calculations of the SPE working electrode of 0.07069 cm². This is contrary to Fig. 4 where the electroactive surface area was expected to be higher due to the layered and “wrinkled” nature of the electrode surface. As such, not all the impact events would have

Fig. 5 Randles–Sevcik plots of theoretical graph (---) and experimentally determined peak currents (red line) of the screen-printed electrodes (SPEs).

resulted in an electrochemical signal⁴⁷ being generated due to a significant portion of the electrode surface not being electroactive. Nanoparticles would have bounced off the electrode surface without any electrochemical oxidation taking place.

The use of SPEs has some inherent limitations in the detection of silver nanoparticles of small sizes. The average background charge (noise) obtained from the supporting buffer is about 2.68×10^{-12} C. As observed from Table 1, the theoretical charges calculated for individual nanoparticles smaller than 100 nm are lower than the average background charge from the supporting buffer. This serves as a challenge for the quantification of the sizes of different silver nanoparticles. Despite this, this limitation opens up possibilities for future research in optimising the performance and architecture of SPEs for such detection techniques. Examples of optimisation could be the pre-polishing surface,⁴⁸ surface modification of the electrode, changing the electrode design, and further optimisation of the supporting buffer.³⁷

Having examined the electrode surface, it is also useful to understand the aggregation behaviour of the nanoparticles which could have also attributed to the low percentage of successful impact events. As mentioned above, the introduction of chloride ions into the supporting buffer promotes the agglomeration of silver nanoparticles, thus forming agglomerates of particles of larger sizes. Hence, the expected diffusion coefficients are slower, which translates to a lower percentage of particles impacting the electrode surface and getting oxidised per unit time. Within the agglomerates, the bonding between individual nanoparticles gets weaker with an increasing size of silver nanoparticle. As such, there is also the possibility that the agglomerates may not undergo complete oxidation upon impacting with the electrode surface where only a few nanoparticles in the agglomerate get oxidised while the others break away into the bulk solution.⁴⁹ This could have also resulted in the lower than expected values and frequency of impact. This correlates with the results for silver nanoparticles of size 107 nm, which show the smallest percentage of spike signals of all the different sizes. The presence of chloride ions could have also promoted the formation of insoluble AgCl when it reacts with the released silver ions.^{47,49} The insoluble salt could potentially block the electroactive surface of the electrode, which would reduce the electroactive area available thus attributing to the low frequency of successful impacts.

Conclusions

We have demonstrated the use of screen-printed electrodes (SPEs) for the detection of monodispersed silver nanoparticles. They have the potential to be an effective on-site, point-of-care detection device for the preliminary detection of silver in real samples. It can potentially be used for the analysis of environmental and biological samples. Despite this, there are several limitations of SPEs as a tool for the quantification and characterisation of silver nanoparticles. These limitations open avenues for future research that can be conducted to optimise SPE design to enhance its performance to enable the quantification and characterisation of different silver nanoparticles.

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Two-Dimensional 1T-Phase Transition Metal Dichalcogenides as Nanocarriers To Enhance and Stabilize Enzyme Activity for Electrochemical Pesticide Detection

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S Supporting Information

ABSTRACT: Single or few layers lithium-exfoliated transition metal dichalcogenides (TMDs) are found to exist predominantly in the conducting metallic 1T-polymorph, which makes it desirable for numerous applications due to its large surface area, good electrical conductivity, and enhanced electrocatalytic capabilities. We demonstrated the use of *tert*-butyllithium exfoliated TMDs (MoS₂, MoSe₂, WS₂, WSe₂) as a platform for the indirect electrochemical detection of an organophosphate pesticide, fenitrothion, via enzymatic inhibition pathway. All four reported materials enhanced the response of the enzymatic biosensor in comparison to the corresponding biosensor in the absence of TMDs. 1T-Phase WS₂ outperforms all other TMD materials, and we proved that it serves as an excellent transducer for enhancing electron transfer in a robust model enzyme-based inhibition assay system using cross-linking immobilization with glutaraldehyde. The reported system showed a broad fenitrothion concentration range (1–1000 nM) with an excellent linearity ($r = 0.987$). Moreover, the system displayed high sensitivity with low limit of detection (2.86 nM) obtained, which far exceeds the required limit set by Food and Agriculture Organisation (FAO) of the United Nations (UN). The feasibility of the proposed system in real samples was demonstrated in apple juice samples with good recoveries of 80.2% and 80.3% obtained at 10 and 1000 nM fenitrothion, respectively.

KEYWORDS: fenitrothion, organophosphate pesticide, acetylcholinesterase, 1T-phase, transition metal dichalcogenides, tungsten disulfide, enzyme biosensor

Transition metal dichalcogenides (TMDs) are a new class of layered materials, analogous to graphene, which have gained significant interest among scientists^{1,2} due to their good physical and chemical properties.^{3–8} Exfoliation of the TMDs into single or few layer sheets was discovered to enhance the chemical properties of TMDs as a result of the increased available surface area and tuning of their electronic properties.⁹ This allows for potential applications in capacitors,^{4,10} batteries,^{4,9} vapor sensing,¹¹ and biosensing applications.^{12–14} Different exfoliation procedures have been reported with chemical exfoliation being the most commonly practiced. Recently, it was discovered that exfoliation with *tert*-butyllithium (*t*-BuLi) produced TMD sheets with a high composition of metallic 1T-phase structures.¹⁵ This metal-like property could be advantageous in constructing an analytical

device,^{11,16} which utilizes the metal-like nature of 1T-phase TMDs in enhancing sensitivity for analytical detection.

The increase in global population has generated a need to increase the global food supply, which has led to more research performed to increase crop productivity and yield. Such developments have evolved commercial farming techniques with greater emphasis placed on crop management and pest control. Organophosphorus pesticides have been developed and widely used to counter the loss of crop yield from pests' invasions. Pesticides are also commonly used for household

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Figure 1. Illustration on the construction of the proposed pesticide biosensor: (A) loading of glassy carbon electrode with *t*-BuLi exfoliated 1T-phase TMDs, (B) conjugation with glutaraldehyde (GA) layer, (C) immobilization of acetylcholinesterase-bovine saline albumin mixture (AChE-BSA) on the modified surface, (D) the enzymatic hydrolysis of acetylthiocholine with the subsequent electrochemical oxidation of thiocholine produced, and (E) the inhibition effect of fenitrothion on AChE.

uses. Lately, there is also an increased need for detection of phosphorus compounds due to their use in chemical weapons such as sarin.¹⁷ With such widespread and diversified use, there is the potential for excess pesticides to leech into the environment, which could lead to contamination. Numerous global and governmental agencies have invested heavily in enforcing high safety standards in food produce. Organophosphate pesticide is an example of a harmful chemical closely monitored as it can cause harmful effects to human physiology and health¹⁸ if the residues are present in huge quantities. Fenitrothion is a commonly used organophosphate pesticide in agriculture for pest control. It binds irreversibly to the active sites of acetylcholinesterase (AChE) found on the synaptic membranes on nerve cells along the nervous system,¹⁹ which inhibits the enzyme and renders it inactive. This prevents the breakdown of neurotransmitter acetylcholine, which leads to its accumulation in the nerve junctions thus resulting in overstimulation in the nervous system. Long-term exposure could lead to severe health problems in human population²⁰ and even death.²¹ It is also well-absorbed from numerous routes such as ingestion, respiratory tract, and even through the skin.²² As such, it is of utmost importance to develop an effective technique for accurate detection and quantification of pesticides in environmental and biological samples.

The development of electrochemical biosensors has mainly focused on the modification of electrodes with carbon-based materials, such as graphene^{23,24} and carbon nanotubes.^{25–27} These materials act as transducers in improving sensitivity and amplifying the response generated. More recently, nanoparticles²⁸ have been incorporated to further improve the accuracies and limits of detection. The use of other layered materials such as TMDs in enzymatic biosensors for the detection of pesticides is still unexplored and literature is lacking. TMDs would be a more favorable and attractive

alternative in the fabrication of biosensors due to their lower cytotoxicity²⁹ and greater tunability as compared to graphene and other carbon-based materials. Besides that, the metallicity of exfoliated TMDs is expected to enhance the response signal of biosensor assay systems. TMDs have recently been used as labels in immunoassays,¹⁶ quencher in genosensing systems,^{30,31} and a platform to enhance the electrochemical signal in DNA and H₂O₂ sensing.^{32,33} From our search, there have been no reports on the use of TMDs in enzymatic biosensor applications for pesticide detection. As such, we set forth to investigate the effectiveness of 1T-phase exfoliated TMDs as a platform for the detection of fenitrothion in the robust enzymatic-based inhibition assay as a model system. We adopted an acetylcholinesterase inhibition assay model as a basis of comparison to investigate the effectiveness and durability of the exfoliated TMD for such application. Henceforth, we aim to develop a biosensor based on immobilization of AChE on 1T-phase *t*-BuLi exfoliated TMD.

RESULTS AND DISCUSSION

The enzymatic biosensor was fabricated as described in the [Experimental Section](#) and schematically illustrated in [Figure 1](#). In summary, the respective *t*-BuLi exfoliated TMD was first deposited onto a glassy carbon (GC) electrode followed by the deposition of a glutaraldehyde (GA) layer before the final immobilization of acetylcholinesterase–bovine saline albumin (AChE–BSA) mixture. Glutaraldehyde was used because it serves as a cross-linker between the protein groups in AChE and *t*-BuLi exfoliated TMD to hold the enzymes in place as well as provide structural stability to the biosensor system. AChE is the enzyme of choice due to its specificity, high turnover number, and catalytic power.¹⁹ It is a hydrolase enzyme that catalyzes the breakdown of neurotransmitter acetylcholine to

choline and acetic acid (eq 1). Choline formed is an electroactive compound that can be oxidized and detected

electrochemically (eq 2). The presence of oxidized choline in the solution will be detected by voltammetry and chronoamperometry. BSA serves as a blocking agent to prevent nonspecific binding onto the enzyme molecule.^{34,35} It will also ensure the optimum interaction of substrate with the active site of AChE and amplify the response generated. Organophosphate pesticides are irreversible inhibitors that form strong covalent bonds between the phosphate groups of the pesticide and the hydroxyl groups within the serine residues in the active site of the enzyme. This results in the formation of a stable enzyme–pesticide complex where the enzyme gets phosphorylated and inhibited. With the binding of fenitrothion to the active site of AChE, it forms a barrier at the active site of the enzyme, which prevents the binding of acetylcholine (eq 3). Thus, the amount of acetylcholine catalyzed decreases based on the concentrations of fenitrothion present.

Characterization was first carried out to better understand the synergistic interactions, chemical composition, and morphologies of the materials. It will also affirm the metallicity of the respective *t*-BuLi exfoliated TMDs. X-ray photoelectron spectroscopy (XPS) was performed to analyze the surface

elemental composition and bonding interactions involved in the respective exfoliated TMDs. Figure 2 shows high resolution spectra of deconvoluted peaks for Mo 3d and W 4f modes for the respective *tert*-butyllithium (*t*-BuLi) exfoliated TMDs. The 3d_{5/2} and 3d_{3/2} bonding modes were analyzed for molybdenum. Upon analysis of the high-resolution spectra for the molybdenum-based exfoliated TMDs, three distinct pairs of peaks were observed corresponding to the 2H and 1T polymorphs, as well as the Mo(VI) oxidized state. MoSe₂ showed a significant presence of 1T polymorph from the higher intensity of the respective 1T peaks with a small contribution of the semiconducting 2H polymorph (Figure 2B). However, deconvolution of the Mo 3d spectrum for *t*-BuLi exfoliated MoS₂ showed a predominance of the semiconducting 2H polymorph, as denoted by the greater intensities of the various 2H peaks (Figure 2A). The XPS results would suggest a lesser extent of exfoliation for MoS₂ in comparison to that of MoSe₂. This would also suggest that the *t*-BuLi exfoliated MoS₂ will be a less sensitive transducer material. Peaks present at high binding energies (greater than 230 eV) suggest the presence of oxidized products of molybdenum (Mo⁶⁺), which exists in rather significant proportions. In the case of tungsten-based TMDs, the 4f_{7/2} and 4f_{5/2} bonding modes were analyzed. Three distinct pairs of peaks were also noted for tungsten-based exfoliated TMDs. In contrast, both *t*-BuLi exfoliated WS₂ and WSe₂ showed dominance of metallic 1T polymorph over the semiconducting 2H polymorph in the structures after exfoliation (Figure 2C,D). It was interesting to note that tungsten-based TMDs showed a greater proportion of 1T-phase than the molybdenum counterparts with more intense peaks observed. There is also a significant percentage of the oxidized form of tungsten (W⁶⁺) as evident from the peaks present at higher binding energies (greater than 35 eV). From

Figure 2. High-resolution X-ray photoelectron spectroscopy (XPS) spectra of Mo 3d and W 4f modes for *tert*-butyllithium (*t*-BuLi) exfoliated (A) MoS₂, (B) MoSe₂, (C) WS₂, and (D) WSe₂.

Figure 3. Raman spectra for *t*-BuLi exfoliated (A) MoS₂, (B) MoSe₂, (C) WS₂, and (D) WSe₂.

the XPS spectra, it was evident that the molybdenum-based TMDs have lower efficiency of conversion from 2H polymorph to 1T polymorph upon exfoliation as previously reported.¹⁵ High-resolution XPS spectra of S 2p and Se 3d modes were also performed (see Figure S1).

Raman spectroscopy was next carried out as it is able to observe the extent of exfoliation of the respective TMDs with great sensitivity. The differences in the features of the peaks at the respective vibrational modes changes with varying layer thickness.³⁶ Comparison between the respective bulk^{37,38} and *t*-BuLi exfoliated TMDs was carried out to observe any such deviations. The in-plane E_{2g}¹ and out-of-plane A_{1g} vibrational modes were analyzed in the Raman spectra for all *t*-BuLi exfoliated TMDs as they are Raman active modes,³⁹ consistent with the D_{6h} point group of the respective bulk TMDs.⁴⁰ For both MoS₂ and MoSe₂ (Figure 3A,B), exfoliation with *t*-BuLi resulted in the broadening and softening of the E_{2g}¹ and A_{1g} bands in comparison to the bulk counterparts. The A_{1g} band appears at a higher frequency than the E_{2g}¹ band for MoSe₂, as compared to MoS₂, which correlates with previous observations.^{41,42} The broadening of the A_{1g} band in MoSe₂ is more distinct as compared to the E_{2g}¹ band, which remains almost unchanged. This correlates with previous findings where the E_{2g}¹ band remains unchanged and unaffected. The broadening effect observed can be attributed to phonon confinement effect and the decrease in lattice size.^{43,44} The Raman spectrum for WS₂ was next studied. A visible indication on the extent of exfoliation will be the change in I_{A_{1g}}/I_{E_{2g}¹} ratio, which will increase with decreasing WS₂ thickness.⁴⁵ From Figure 3C, the intensity of E_{2g}¹ band increased in comparison to that from the

bulk material, which correlates to an increase in the observed I_{A_{1g}}/I_{E_{2g}¹} ratio. Hence, it can be inferred that *t*-BuLi has successfully intercalated and exfoliated bulk WS₂. Lastly, for WSe₂, the E_{2g}¹ and A_{1g} bands are degenerate and would result in the formation of a single peak in the bulk material. The splitting of the degenerate peaks becomes pronounced with the exfoliation of the bulk material.⁴⁶ Figure 3D shows the presence of two small peaks closely located at about 250 and 253 cm⁻¹, which overlap as a single broad peak. It can thus be postulated from the splitting of the degenerate peaks that WSe₂ was exfoliated by *t*-BuLi. From the Raman spectroscopy and XPS studies, it suggests that the lithium exfoliation of tungsten-based TMDs appears to be more effective and efficient in comparison to their molybdenum counterparts. From the respective characterization studies conducted, it can be concluded that the *t*-BuLi exfoliated TMDs exhibit metal-like characteristics and exist predominantly in the 1T polymorph, which will aid in the response amplification for the model enzymatic inhibition biosensor assay.

It is of utmost interest to evaluate the performances of the different *t*-BuLi exfoliated 1T-TMDs (MoS₂, MoSe₂, WS₂, WSe₂) to monitor any differences in sensitivity and response based on their different inherent properties. We next set forth to investigate the feasibility of the proposed biosensor system for the detection of fenitrothion. Chronoamperometric measurements were carried out to analyze the response of the pesticide biosensing system fabricated using the different *t*-BuLi exfoliated TMDs through successive injection of acetylthiocholine. All four materials showed enhanced biosensor performance as compared to biosensor without TMDs

(green line of Figure 4). MoSe₂ and WSe₂ showed similar responses, which were lower than MoS₂ and WS₂. It was

Figure 4. Chronoamperometric measurements with successive injection of ATCh on different *t*-BuLi exfoliated TMD modified biosensor in 0.01 M PBS. Arrows indicate the point of injection of ATCh (concentration of ATCh = 500 μM).

interesting to note that MoS₂ outperformed both MoSe₂ and WSe₂ despite being predominantly in the 2H polymorph. A possible reason for the rather sensitive response could be the successful exfoliation of MoS₂ into few layered sheets. 1T-WSe₂ displayed the lowest sensitivity, which is contrary to the results from characterization studies. This anomaly could suggest the smaller extent of exfoliation of WSe₂ and its existence in multiple layers thus providing a lower surface area. As such, the metal-like properties are compromised and the sensitivity is reduced. The greater sensitivity of WS₂ in comparison to WSe₂ would also suggest successful exfoliation of the bulk material as evident from the greater separation of the E_{2g}¹ and A_{1g} vibrational modes for WS₂ (Figure 3C). It was also interesting to note that sulfur-based dichalcogenides performed better than their selenide-based counterparts. Moreover, 1T-phase WS₂ is demonstrated to provide also increased density of catalytic active sites that for the 1T-phase materials are on both the basal

plane and edges.^{47–50} All of these capabilities of 1T-phase facilitate the electrochemical oxidation of thiocholine in the proposed biosensor (Figure 1D and eq 2). From the studies performed, it can be deduced that 1T-WS₂ is the most suitable TMD for further electrochemical biosensor fabrication.

Initial studies with 1T-WS₂ modified biosensor using cyclic voltammetry showed a small anodic peak at 0.1 V in the presence of acetylthiocholine iodide (Figure S2). However, a significantly enhanced current intensity was observed in the CV of the biosensor in the presence of acetylthiocholine iodide (red line) in comparison with the biosensor without analyte (black line). From the voltammogram obtained, it was decided that a potential of 0.1 V would be used as the applied potential for further chronoamperometric studies. This potential was chosen as it is relatively low and away from the anodic potential of possible interference signals such as polyphenols, vitamins, and others (see Figure S3). Higher and lower potentials were also tested, and the best response was observed with 0.1 V.

The metallic nature and increased density of catalytic active sites of 1T-WS₂ enhanced the current response signal from the oxidation of thiocholine produced from the enzymatic catalysis of acetylthiocholine, and thus the oxidation potential gets shifted to a lower anodic potential as shown in Figure S2. A similar observation on the shift in oxidation potential of thiocholine in pesticide based on nanomaterials was previously reported for carbon nanotubes,⁵¹ composites of polyaniline/carbon nanotubes,^{52,53} and Au nanoparticles dispersed in carbon nanotubes.⁵⁴

Having ascertained that the enzymatic biosensor assay system is feasible, we proceed to study any effects of interferences on the proposed system. Chronoamperometric analysis was performed at 0.1 V with Fe²⁺, Cu²⁺, ascorbic acid, and phenol (Figure S3). The compounds were chosen as they are commonly found in most variants of fruits and the respective concentrations reflect closely their physiological concentrations.⁵⁵ From Figure S3, it was determined that the mentioned compounds do not interfere significantly with the oxidation signal of acetylthiocholine (current change <7%), which validates the use of low working potential. Utilizing this information, we next set forth to carry out chronoamperometry for subsequent procedures at 0.1 V.

The extent of exfoliation of the 1T-WS₂ was further analyzed with TEM, selected area electron diffraction (SAED), and HR-

Figure 5. Transmission electron microscopy (TEM) image with selected area electron diffraction (SAED) pattern (insert) of WS₂. High resolution TEM (HR-TEM) images show dominant presence of 1T-phase (red hexagon). Scale as indicated in the images, respectively.

TEM (Figure 5). From the TEM image, exfoliation of the materials is evident with the presence of few layer sheets of about 1 μm observed. SEM image also shows similar observations with few layers observed for 1T-WS₂ (Figure S4A). SAED pattern showed hexagonal symmetry of WS₂. HR-TEM image further validates the 1T-phase of WS₂ from the hexagonal arrangement of atoms, which corresponds to 1T-phase arrangement. These findings validate the results obtained from XPS and Raman spectroscopy in validating the 1T polymorph and extent of exfoliation of WS₂ used in the model enzymatic biosensor.

Chronoamperometry was performed, and the chronoamperometric responses were observed with successive injection of acetylthiocholine to the different modified layers. From Figure 6, there was no visible current response for measure-

Figure 6. Chronoamperometric measurements with successive injection of ATCh on each modified layer of the proposed biosensor system in 0.01 M PBS. Arrows indicate the point of injection of ATCh (Concentration of ATCh = 500 μM).

ments carried out on bare glassy carbon. Modification of the glassy carbon electrode with WS₂ resulted in a small increase in the current response upon injection of acetylthiocholine. The signal generated could be from the oxidation of small quantities of iodide counterions present with acetylthiocholine used^{56,57} (Figure S5 and S6). GC/WS₂/GA and GC/WS₂/BSA showed low chronoamperometric responses, which validates the absence of the catalytic activity of enzyme. The importance of enzyme AChE is evident with an enhanced response obtained after injection of acetylthiocholine. The significant increase in response upon the immobilization of AChE can be attributed to the high turnover number and strong catalytic power^{19,58} of the enzyme, which was found to catalyze about 10⁴ molecules per second.⁵⁹ With more acetylthiocholine being catalyzed, the amount of thiocholine present greatly increased, and thus more thiocholine gets oxidized in return. An enhanced response was further obtained upon the introduction of BSA to AChE. This could correlate to more electroactive thiocholine produced and lesser acetylthiocholine lost from nonspecific binding of the AChE. The combined effect of the metal-like property and enhanced catalytic activity of *t*-BuLi exfoliated 1T-WS₂ enables more efficient electro-oxidation of thiocholine and greater amount of enzymes to be immobilized to get better sensitivity of the model enzymatic inhibition biosensor. As

such, the exfoliated material aids in improving the sensitivity and response signal of this system. The chronoamperometric measurements carried out validates the importance of the different layers in the proposed biosensor and their successful immobilization in the preparation steps. It further proves the robustness and stability of the biosensor system that was used as a model assay to evaluate the electrocatalytic effect of different TMDs exfoliated by *t*-butyllithium.

The inhibition effect of fenitrothion on the enzymatic biosensor system was next studied. Initial cyclic voltammetry (CV) scans on the modified electrodes were performed. It was noted that the anodic peak diminishes in the presence of fenitrothion (Figure S7) with a corresponding decrease in current intensity of the CV after inhibition with fenitrothion. Chronoamperometric studies were subsequently performed to analyze the inhibition effect of fenitrothion with the proposed biosensor system (Figure 7). In the absence of fenitrothion,

Figure 7. Chronoamperometric measurements showing the inhibition effect of fenitrothion on 1T-WS₂ modified biosensor system in 0.01 M PBS. Black arrow indicates the point of injection of ATCh, and blue arrow indicates the drop in current response observed after inhibition with fenitrothion (concentration of ATCh = 500 μM).

injection of acetylthiocholine (500 μM) saw a sharp increase in current signal which gradually becomes steady. The current response obtained after inhibition with fenitrothion showed a decrease in current response by about 0.05 μA . The drop in current signal obtained is indicative of less thiocholine being oxidized, which corroborates a decrease in the amount of acetylthiocholine catalyzed by AChE. Fenitrothion was successful in binding irreversibly to the active site of AChE, which hindered the catalysis of acetylthiocholine and suppressed the signal obtained. From these initial observations, it can be concluded that the biosensor is responsive to the presence of fenitrothion as evident from the decrease in response current.

We next set forth to study various experimental parameters to optimize the performance of the biosensor. As mentioned previously, AChE gets phosphorylated when fenitrothion forms a covalent bond with the hydroxyl group of the serine residues in the active site thus inhibiting the enzyme. The response generated from the electrochemical oxidation of thiocholine thus decreases with increasing exposure to fenitrothion. Using

this concept, the degree of inhibition can be measured based on the change in thiocholine signal response before and after fenitrothion inhibition. The inhibition percentage ($I\%$) was calculated as a means to evaluate the extent of inhibition before (I_b) and after incubating (I_a) with fenitrothion (eq 4) to analyze the inhibition activity of fenitrothion on AChE. A higher $I\%$ would be favorable as it indicates greater inhibition of enzymes, which correlates to better performance of the biosensor.

$$I(\%) = \frac{[(I_b - I_a)]}{I_b} \times 100 \quad (4)$$

The first parameter of interest is acetylthiocholine concentration. Acetylthiocholine serves as the substrate for enzymatic catalysis. A substrate concentration that is higher than the turnover number of the enzyme could slow down the response time due to saturation at the active site of the enzymes. However, substrate concentrations that are too low could generate weak or negative response. It is thus important to choose a suitable concentration to ensure optimum working capacity of the enzymes. Three different acetylthiocholine concentrations (50, 100, and 250 μM) were studied. Figure 8A shows $I\%$ for the different acetylthiocholine concentrations, which increases with increasing concentration. Acetylthiocholine concentration of 50 μM produced negative $I\%$, which suggests insufficient substrate present to interact with the enzymes. Acetylthiocholine concentration of 250 μM showed greater $I\%$ as compared to a concentration of 100 μM and was thus selected as the working concentration for the experiment.

We next set forth to optimize the amount of 1T-WS₂ to be deposited onto glassy carbon electrode. 1T-WS₂ serves as the transducer for electron transfer, and it is important to ensure optimum loading of the material onto the electrode surface. Three different WS₂ concentrations (1, 5, and 10 mg mL^{-1}) were studied. From Figure 8B, the optimum concentration to be deposited is 1 mg mL^{-1} , and it was the only concentration with positive $I\%$. Higher concentrations of WS₂ (5 and 10 mg mL^{-1}) gave negative $I\%$, which might suggest saturation or passivation of the electrode surface. Besides that, the higher concentrations might have caused an excess of WS₂ to be deposited, which could have created a barrier and hindered electron transfer. As such, WS₂ concentration of 1 mg mL^{-1} was chosen as the optimum concentration to be loaded onto the electrode surface.

The last variable optimized is pesticide inhibition time. A lower response will be obtained when the pesticide binds irreversibly to the active site of the enzyme. It is thus important to optimize this variable to allow sufficient time for fenitrothion to bind to the active site of the enzymes to optimize $I\%$ results. However, the time domain should also not be too long for on-site analysis. Three different inhibition times (5, 15, and 30 min) were chosen. From Figure 8C, inhibition times of 5 and 15 min showed similar $I\%$ results. It was also interesting to observe that the longest inhibition time of 30 min showed the lowest $I\%$ result. The long inhibition time might have saturated the enzyme as it reaches its turnover rate. The enzymes could also have detached from the modified surface during the long time duration. Five minute inhibition time was chosen due to its better reproducibility with the smallest error bar obtained. The shorter time duration was also taken into consideration as an important criteria to be an efficient on-site, point-of-care analytical device.

Figure 8. Optimization of (A) ATCh concentration, (B) WS₂ concentration, and (C) fenitrothion inhibition time.

The analytical response of the model biosensor system with the optimized parameters was then examined using chronoamperometry with injection of acetylthiocholine into stirred 0.01 M PBS. Increasing the fenitrothion concentration is predicted to generate greater inhibition of AChE, which will correlate to decreased catalytic activity (see Figure 8) and hence generate a greater $I\%$ value. Figure 9A shows the linear plot for fenitrothion concentrations between 1 and 1000 nM for the proposed biosensor system with $r = 0.987$. The limit of detection (LOD) was calculated by multiplying the quotient of the standard deviation of the chronoamperometric current of the lowest concentration of pesticide and gradient for the calibration graph by 3, and limit of quantification (LOQ) was obtained by multiplying the same quotient described previously by 10.^{60–64} The system has a low LOD of 2.86 nM and LOQ of 9.54 nM. A new modified electrode has to be used for each experiment after exposure to fenitrothion due to the irreversible binding of the pesticide to the enzymes. The RSD is the average of 4 different concentrations where each concentration was measured 4 times. The error bars obtained are hence a result of same day repeatability of measurements having relative

Figure 9. (A) Calibration plot showing the inhibition of biosensor vs fenitrothion concentration. (B) Recoveries with fresh apple juice for pesticide concentrations of 10 and 1000 nM.

standard deviation (RSD) of $\sim 10\%$. When applying the one-way ANOVA statistical analysis with a confidence interval of 0.99, the mean values for the inhibition percentages were different with $p = 0.00019$. Moreover, the good linearity and analytical performance of the biosensor system makes it promising for potential applications.

Recovery experiments were then carried out to demonstrate the working capabilities of the proposed biosensor with real samples where interfering species were present. Recovery percentages were calculated as shown in eq 5. Recovery percentages of 80.2% and 80.3% were obtained at fenitrothion concentrations of 10 and 1000 μM , respectively, in fresh apple juice (Figure 9B). The results obtained suggest that the proposed biosensor system is feasible and responsive for detecting low concentrations of fenitrothion in real samples. The system also showed good reproducibility and analytical performance in detecting fenitrothion with apple juice. The model biosensor system, where *t*-BuLi exfoliated 1T-WS₂ was used as an active electrocatalytic platform with enhanced analytical performance, serves as a promising platform that can be extended toward the development of future TMD-based enzymatic biosensors for environmental and biomedical applications.

$$R(\%) = \left(\frac{[\text{fenitrothion}]_{\text{buffer}}}{[\text{fenitrothion}]_{\text{real-sample}}} \right) \times 100 \quad (5)$$

Moreover, the use of 1T-WS₂ has proven to outperform most carbon-based materials and provide a more sensitive alternative compared to previous works reported. Our proposed biosensor system exhibited excellent analytical performance with respect to detection limits and range in comparison to other works previously reported for fenitrothion detection (see Table S1). The analytical performance with *t*-BuLi exfoliated 1T-WS₂ is also comparable to the use of dispersed graphene oxide by Liu et al.⁶⁵ with similar detection range and magnitude in the limit of detection (LOD) observed. One advantage of our proposed system also is its selectivity brought about by the immobilization of AChE and low working potential (0.1 V) used. The high selectivity of AChE toward binding with organophosphate pesticides makes it attractive as a detection method for our target analytes. The use of low potential also reduces interference signals from other analytes present in real sample analysis. Besides that, the time taken for fenitrothion detection is rather short (5 min) with great reproducibility and linearity. The LOD obtained is 2.86 nM, which corresponds to 0.743

ppb. This value obtained is significantly lower than the maximum allowed quantity set by various environmental agencies such as Food and Agriculture Organisation (FAO) of the United Nations (10 ppm)⁶⁶ and Agri-Food and Veterinary (AVA) of Singapore (2 ppm).⁶⁷

CONCLUSION

A biosensor based on a robust enzymatic-based inhibition assay was used as a model system, where AChE and *t*-BuLi exfoliated 1T-phase WS₂ was immobilized onto a glassy carbon electrode. This system has been developed for the effective detection of organophosphate pesticide fenitrothion. Besides that, it was demonstrated that the *t*-BuLi exfoliated metallic 1T-phase WS₂ shows the best performance among the other group 6 TMDs exfoliated using the same method, such as MoS₂, MoSe₂, and WSe₂. 1T-Phase WS₂ serves as a conductive platform transducer with increased density of catalytic sites for the efficient electrochemical oxidation of thiocholine, which enhances the amperometric response. The proposed biosensor shows good linearity with a low limit of detection (2.86 nM). Good recovery percentages are also obtained in real sample analysis with apple juice. With these findings, it also shows conceptually the possible application of TMDs in the area of biosensors and varied possibilities for the development of future TMD-based biosensors for on-site, point-of-care environmental and biological sample analysis.

EXPERIMENTAL SECTION

Materials and Apparatus. Molybdenum disulfide, molybdenum diselenide, tungsten disulfide and tungsten diselenide were obtained from Alfa Aesar (Germany). Potassium chloride, potassium hexacyanoferrate(II) trihydrate, potassium hexacyanoferrate(III), bovine serum albumin (BSA), acetylthiocholine iodide (ATChI), acetylcholinesterase (AChE) from electric eel, glutaraldehyde (GA, 50%), fenitrothion, ascorbic acid, phenol, and phosphate buffer saline (PBS) in tablets were purchased from Sigma-Aldrich (Singapore). High quality water, purified in a Milli-Q system (Millipore, MA, USA) with a resistivity of 18 M Ω cm was used. Biosan TS-100 (Latvia) thermoshaker was used to stir the samples during the inhibition process at a temperature of 37 $^{\circ}\text{C}$ with shaking at 400 rpm.

Electrochemical Measurements. Electrochemical measurements were conducted using a μ Autolab Type III electrochemical analyzer (Eco Chemie, The Netherlands) controlled by NOVA 1.9 software (Eco Chemie) at room temperature (25 $^{\circ}\text{C}$) by using a three-electrode configuration. Platinum (Pt), Ag/AgCl, and GC electrodes (diameters of 3 mm) were obtained from CH Instruments (Texas, USA).

Cyclic voltammetry (CV) experiment was performed at a scan rate of 100 mV s⁻¹. All chronoamperometry measurements were recorded at 10 ms intervals in 0.01 M PBS. A magnetic stirrer was used to assist convection.

Characterization. X-ray photoelectron spectroscopy (XPS) was carried out with a Phoibos 100 spectrometer with a monochromatic Mg X-ray source (SPECS, Germany) at 1254 eV. Transmission electron microscopy (TEM) and high resolution transmission electron microscopy (HR-TEM) was carried out with EFTEM JEOL 2200 FS microscope (JEOL, Japan) with acceleration voltage of 200 keV. Raman spectroscopy was carried out using confocal micro-Raman LabRam HR instrument (HORIBA Scientific) in backscattering geometry with CCD detector, 514.5 nm argon excitation laser, and a 100× objective lens mounted to an Olympus optical microscope. The spectra range used was from 100 to 1000 cm⁻¹. Calibration was carried out before measurements were taken using an internal silicon reference at 520 cm⁻¹ with peak position resolution of less than 1 cm⁻¹. Scanning electron microscopy (SEM) was performed with JEOL 7600F-SEM (JEOL, Japan). SEM was performed on a silicon wafer operating at 2 kV.

Preparation of Exfoliated Transition Metal Dichalcogenides. Three grams of respective TMD bulk powder was suspended in 20 mL of 1.7 M *tert*-butyllithium (*t*-BuLi). Subsequently, it was stirred for 72 h at 25 °C under argon atmosphere to intercalate with Li. The intercalated material was separated by suction filtration under argon atmosphere and washed thoroughly with hexane (dried over sodium). The separated Li-intercalated material was placed in water (100 mL) before repeated centrifugation (18 000g) was carried out. The exfoliated material was dried in a vacuum oven at 50 °C for 48 h before use. It was later dispersed in water and ultrasonicated for 60 min to obtain a well-dispersed suspension.

Preparation of Biosensor System. AChE (1 mg in 50 μL) and 5% BSA were prepared in 0.01 M PBS. Three microliters of the suspension of *t*-BuLi exfoliated TMD of desired concentration was drop-coated on the glassy carbon (GC) electrode surface and left to dry at room temperature. Glutaraldehyde (GA), 0.25%, was prepared in ultrapure water, and 5 μL of the solution was coated onto the GC/TMD surface. Two microliters of AChE in 5% BSA (1:1) was next coated onto the modified surface. The electrodes were oven-dried at 35 °C between steps for 30 min.

Real Sample Preparation. Fresh apples were bought from a local supermarket and blended with 0.01 M PBS. The blend was filtered several times to remove any insoluble and larger particles, which may affect the measurements. The fresh juice was obtained as filtrate for further real sample analysis.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsnano.7b01364.

High-resolution X-ray photoelectron spectroscopy of the chalcogens, scanning electron microscopy of WS₂, cyclic voltammograms illustrating the effect of 1T-WS₂ on the model biosensor inhibition assay, acetylthiocholine iodide on the biosensor system, influence of iodide ions and inhibition effect of fenitrothion, chronoamperometric measurements of variable fenitrothion concentrations before and after inhibition, illustration of the influence of interference analytes on the biosensor system, and table of different analytical systems for fenitrothion detection (PDF)

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Notes

The authors declare no competing financial interest.

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3D Printed Electrodes for Detection of Nitroaromatic Explosives and Nerve Agents

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ABSTRACT: Three-dimensional (3D) printing has proven to be a versatile and useful technology for specialized applications in industry and also for scientific research. We demonstrate its potential use toward the electrochemical detection of nitroaromatic compounds 2,4,6-trinitrotoluene (TNT), 2,4-dinitrotoluene (DNT), and fenitrothion (FT). The detection of these compounds is of utmost importance in military and forensic applications. Stainless steel electrodes were fabricated by 3D printing, and the surface was electroplated with gold. The electrochemical performance of the 3D printed electrodes was compared to that of the conventionally employed glassy carbon electrode (GCE) and proved to be more sensitive toward the detection of all three nitroaromatic compounds. 3D printing of customizable electrodes provides a viable alternative to traditional electrodes for the analysis of samples with electrochemical methods.

The advent of three-dimensional (3D) printing technology has garnered interest among researchers and is gaining popularity^{1,2} brought about from its ability for rapid in-lab prototyping of customizable devices. The ability and autonomy to fabricate uniquely shaped devices at low costs renders 3D printing an attractive technology which has the potential to accelerate processes and be more economical. In the field of electrochemistry, it will be beneficial to fabricate customizable electrodes for specific functions and studies with control over the morphology, sizes, and performance of the electrodes.¹ In a previous work, helical-shaped electrodes of various sizes were designed and printed to showcase the versatility of the selective laser melting (SLM) method and its ability to accommodate unconventional and relatively complex designs. These electrodes have proven to be versatile and useful for various applications³ including DNA biosensing and environmental analysis.⁴ Such use of 3D printed electrodes can be extended toward the fabrication of sensing devices for the detection of other analytes with different electrochemical behavior.

Nitroaromatic compounds are found to be harmful to living organisms and are used as precursors for the manufacture of explosive devices. Nitroaromatic pesticides are developed and widely used for household uses and in farms for pest control. Fenitrothion (FT) is a commonly used nitroaromatic organophosphate pesticide. It acts as an acetylcholinesterase inhibitor⁵ by binding irreversibly to nerve cells⁶ and has been classified as toxic, particularly to aquatic life, by the United States Environmental Protection Agency (US EPA) and World Health Organization (WHO).⁷ Therefore, pesticide levels in water bodies have to be closely monitored, even at trace levels.⁸ Besides that, such pesticides can be improvised and developed

as nerve agents.⁹ 2,4-Dinitrotoluene (DNT) and 2,4,6-trinitrotoluene (TNT) are commonly found nitroaromatic compounds in explosives. Detection of DNT has gained interest due to its presence in TNT-based explosives as a by-product from the incomplete nitration of toluene.¹⁰ Trace level detection of DNT and TNT may provide governmental agencies and security personnel with rapid initial detection of concealed explosives in potential terrorist attacks and wartime situations. Detection of nitroaromatic compounds may also be useful in forensic applications or investigating possible sources of pollution from poorly maintained weapons and ammunition manufacturing or storage facilities. As such, it is of great importance to develop a technique for accurate detection of such compounds. These nitroaromatic compounds can be electrochemically detected from the reduction of the nitro ($-\text{NO}_2$) groups around the aromatic ring.¹¹

Research on the trace level detection of nitroaromatic compounds has primarily focused on lab-on-chip devices^{12,13} and modification of electrodes with conductive transducer platforms such as graphene,¹⁴ mesoporous carbon,^{15,16} carbon black,¹⁷ carbon nanotubes,^{18,19} and nanoparticles.²⁰ We aim to provide an alternative sensing tool fabricated by means of 3D printing technology for the detection of nitroaromatic compounds. We set forth to investigate the feasibility of using 3D printed electrodes for voltammetric detections of nitroaromatic explosives and improvised nerve agents. As a proof-of-concept, bare and gold-plated 3D printed stainless

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Figure 1. (A) Scheme illustrating the fabrication process of 3D printed steel electrode. SEM images of 3D printed stainless steel using a 5.0 kV electron source at (B) 50 $\times$ and (C) 500 $\times$ magnification. EDX spectroscopy map showing (D) nickel and (E) iron distribution. Scale bars correspond to 100 (B) and 10 μ m (C).

steel helical electrodes were used to detect three nitroaromatic environmental pollutants, FT, DNT, and TNT, and their performance was compared to that of the conventional glassy carbon electrode (GCE).

EXPERIMENTAL SECTION

Materials. TNT was obtained from Accustandard. FT, DNT, potassium phosphate dibasic, sodium phosphate monobasic, sodium chloride, potassium chloride, nitric acid, and potassium hydroxide were obtained from Sigma-Aldrich (Singapore). Hydrogen peroxide was obtained from Alfa-Aesar (Singapore). Acetonitrile (ACN) was obtained from Merck (Singapore). Deionized (Milli-Q) water with conductivity 18.2 M Ω cm was used. Stock solutions of FT and DNT were prepared in ACN and stored in the refrigerator at 4 $^{\circ}$ C. TNT was used as obtained. Solutions used for analysis were freshly prepared when required by addition of aliquots of the stock solutions to a mixture of 1 mL of ACN and 4 mL of phosphate buffered saline (PBS) pH 7.2, purged using nitrogen for 10 min.

3D printed stainless steel electrodes were fabricated as previously reported³ using selective laser melting (SLM). GCE with a diameter of 3 mm, Ag/AgCl reference electrode, and platinum (Pt) counter electrode were obtained from CH Instruments (Texas, US). A piece of platinum foil was used as counter electrode with 3D printed electrodes to ensure a comparable surface area for electron flow.

Apparatus. All electrochemical measurements were carried out using an Autolab PGSTAT101 potentiostat (Metrohm Autolab B. V., Utrecht, The Netherlands) connected to a personal computer and controlled using NOVA version 1.10 at room temperature (25 $^{\circ}$ C) using a 3-electrode configuration. Scanning electron microscopy coupled energy dispersive X-ray spectroscopy (SEM-EDX) was performed with a JEOL 7600F

Schottky field emission scanning electron microscope (JEOL, Japan).

Electrochemical Measurements. Cyclic voltammetry (CV) experiments were performed at scan rates of 100 mV s⁻¹, and differential pulse voltammetry (DPV) experiments were performed at amplitude pulse of 25 mV, pulse width of 50 ms, and scan rate of 10 mV s⁻¹ unless otherwise stated.

Electroplating of 3D-Printed Electrode with Gold. Gold (Au) electroplating was performed as previously reported.⁴ Briefly, the stainless steel electrode was immersed into a commercial gold plating solution, and the deposition was carried out applying a current of -20 mA for 90 min while stirring in the presence of a Ag/AgCl reference electrode and a platinum counter electrode.

Electrode Surface Cleaning. GCE was polished using alumina slurry (0.05 μ m) on a polishing pad. The bare 3D printed stainless steel electrode surface was cleaned by immersion in 0.5 M nitric acid. Cyclic voltammetry was then carried out at a potential range between -1 to 1 V for 10 cycles to remove any impurities. It was subsequently rinsed with deionized water. The gold-plated 3D electrode cleaning method was adapted and modified from a previous study.²¹ In summary, the electrodes were first soaked for 10 min in a solution of 50 mM KOH and 25% w/w H₂O₂ before rinsing with deionized water. Cyclic voltammetry was subsequently performed in 50 mM KOH at a potential range between 0 to -0.9 V for 10 cycles, followed by washing with deionized water. The electrode was then placed in a solution containing 20% ACN and 80% PBS and cycled for another 10 cycles in the same potential range or until a stable signal was achieved. Without further rinsing, it was then used for measurement.

Figure 2. (A) Scheme illustrating the gold electroplating process of 3D printed steel electrode. SEM images of gold-plated 3D printed stainless steel electrode using a 5.0 kV electron source at (B) 30×, (C) 500×, and (D) 2000× magnification. EDX spectroscopy map showing (E) nickel, (F) iron, and (G) gold distribution. Scale bars correspond to 100 (B) and 10 μm (C and D).

RESULTS AND DISCUSSION

Figure 1A provides a simplified illustration on the fabrication process of the 3D printed steel electrode. Briefly, the electrode was first designed using modeling software before it was metal printed by the SLM process. Characterization was first performed using SEM-EDX to better understand the elemental composition and surface morphology of the 3D printed stainless steel electrodes before and after gold-plating.

From the SEM images (Figures 1B and C), it is observed that SLM fabrication process resulted in the formation of a rough surface consisting of sporadic spherical-shaped structures with diameters of 10–30 μm . From the EDX map analysis, the main components of the stainless steel electrode were found to be iron and nickel, which is consistent with the composition of stainless steel (Figures 1D and E).

The surface morphology of the 3D printed electrode was again analyzed after electroplating with gold (Figure 2A). It was observed that the electrode surface is coated with a layer of uneven microstructures (Figures 2B–D), about 3–5 μm with several edge features (Figure 2D). This layer significantly increased the surface area of the electrode, allowing for greater current flow. The exposed edges may also play a role in catalyzing reduction reactions. EDX map analysis confirmed that gold was the predominant surface element. The signals obtained for iron and nickel (Figures 2E and F) were less intense as compared to Figures 1D and E, which suggests a homogeneous gold layer forming on the electrode surface.

Having established and affirmed the successful deposition of gold onto the 3D printed electrodes, we next set forth to investigate the feasibility and working capability of the fabricated electrodes. The reduction of one nitro group in the nitroaromatic compound undergoes a $4\text{e}^-/4\text{H}^+$ reaction mechanism which results in the formation of hydroxylamine (NHOH) functionality. This electrochemical step will result in the formation of a cathodic peak in voltammetry. Correspondingly, increasing the number of nitro groups in the aromatic compound would lead to the formation of more cathodic peaks

observed. The positions of the cathodic peaks were also found to be influenced by the position of the nitro groups (*ortho*-, *meta*-, *para*-). CV was first employed to study the electrochemical behaviors of FT, DNT, and TNT. This was then followed by the use of differential pulse voltammetry (DPV) for quantitative analysis. Experiments were performed using 3D printed electrodes (bare and gold-plated) and GCE for comparison.

Fenitrothion. Figure 3A shows the voltammograms obtained with GCE. A cathodic peak was observed at -0.76 V which corresponds to the irreversible reduction of nitro ($-\text{NO}_2$) group into hydroxylamine ($-\text{NHOH}$). The anodic and cathodic peak pair at about -0.2 and 0 V , respectively corresponding to the reversible redox pair of $-\text{NHOH}$ to a nitroso group ($-\text{NO}$).²² Having established the redox potentials of FT with GCE, we next investigated the electrochemical capabilities of the bare 3D printed stainless steel electrode. From Figure 3B, no apparent cathodic peaks were observed within the same potential window, even at high concentrations of FT. This would suggest that stainless steel might not be ideal for direct use for the electrochemical reduction of nitroaromatic compounds. The modification of the bare stainless steel electrode with gold enabled the formation of a broad cathodic peak which increases in peak height with a corresponding increase in FT concentration (Figure 3C). The peak potentials obtained ranged between -0.4 to -0.5 V , which is a significant shift obtained with glassy carbon. The cathodic current signals were about nine times higher than that with glassy carbon electrode. This could be attributed to the significant increase in surface area of the working electrode which corresponds to higher rate of charge transfer.

DPV was next performed to compare the performance and sensitivities of the different electrodes. From Figure 3D, distinct peaks were observed with GCE which centered at about -0.7 V . However, no visible peaks were observed with bare 3D printed electrode (Figure 3E), which was a similar observation to the CV scans carried out (Figure 3B). The gold-plated 3D electrode produced intense cathodic peaks centered at about

Figure 3. Cyclic voltammograms of FT with (A) glassy carbon electrode, (B) bare electrode, and (C) gold-plated 3D stainless steel electrode. Differential pulse voltammograms on (D) glassy carbon, (E) bare electrode, and (F) gold-plated 3D stainless steel electrode. Conditions are as described in the Experimental Section.

−0.75 V, similarly to GCE (Figure 3F). This observation corroborates with the formation of the cathodic peaks in CV (Figure 3C) which show the electrochemical reduction of FT by gold on the 3D printed electrode. The peaks obtained with the gold-plated 3D printed electrodes were more intense and of significantly higher magnitudes. This could be attributed to the synergistic effect of the conductivity of the stainless steel electrode and the electrocatalytic capability of the gold surface.

Calibration plots were then generated from DPV measurements based on the current density signals at different FT concentrations. Geometrical surface area was taken into consideration (GCE, 7 mm²; gold-plated, 15 mm²). The gradient obtained for glassy carbon electrode was 0.471 mA/cm²μM with $R = 0.9545$ while gold-plated 3D printed stainless steel electrode generated a gradient of 2.1 mA/cm²μM with $R = 0.997$. It was determined that the gold-plated 3D electrode is about 4.5 times more sensitive for the electrochemical detection of FT than GCE brought about by a steeper gradient observed for the calibration plot. Gold-plated 3D printed stainless steel

electrode was also determined to have a broader detection range for FT.

2,4-Dinitrotoluene. From our previous findings,¹¹ it was determined that the reduction of each nitro group in TNT occurs at specific reduction potentials resulting in the formation of three cathodic peaks with voltammetry. This can also be applied for the reduction of DNT, where two reduction peaks would be observed. Two distinct cathodic peaks would be expected from the distinct reduction of the *ortho*- and *para*-substituted nitro groups in DNT.¹⁰

Cyclic voltammetry was performed with all three electrodes as shown in Figure 4. As predicted, two irreversible reductive peaks (−0.64 and −0.82 V) were observed with GCE (Figure 4A). The redox peak pair previously observed in FT was also present, suggesting that nitroaromatic compounds generally undergo a similar reaction pathway. It was interesting to note that the voltammograms obtained using bare 3D electrode presented an inherent electrochemical behavior due to the redox processes of its main components such as Fe, Cr, and Ni (Figure 4C). However, no apparent peaks from the reduction

Figure 4. Cyclic voltammograms of DNT with (A) glassy carbon, (B) gold-plated electrode, and (C) bare 3D stainless steel electrode. Differential pulse voltammograms of DNT at (D) glassy carbon and (E) gold-plated 3D stainless steel electrode. Conditions and parameters are as described in the [Experimental Section](#).

of DNT were observed, similar to that with FT. This supports previous observed results where stainless steel is not an ideal material for electrochemical detection of nitroaromatic compounds. A very broad cathodic peak was observed between -0.3 to -0.8 V with gold-plated 3D electrode (Figure 4B). Differential pulse voltammetry was also performed to analyze the cathodic peaks with greater accuracy. The stainless steel electrode was deemed to be of limited use for this detection, and thus no DPV was performed with it. Two distinct peaks were observed with GCE (Figure 4D), which was similar to the observation made with CV. It is interesting to note that the reduction of *ortho*- nitro group (-0.60 V) showed current peak heights about two times higher than those of the reduction of *para*- nitro group (-0.77 V). The DPVs obtained using the gold-plated 3D electrode were significantly different than those with GCE. From Figure 4E, the reduction of the *ortho*- group does not occur coherently at a specific potential, and thus, only the reduction signal of the nitro group in the *para*- position was chosen to plot the calibration plots.

From the calibration plots obtained, the gradient of the slope for glassy carbon electrode was $0.1175 \text{ mA/cm}^2 \mu\text{M}$ with $R = 0.996$, while the gradient for gold-plated 3D printed stainless steel electrode was $0.4197 \text{ mA/cm}^2 \mu\text{M}$ with $R = 0.997$. Taking into consideration the geometrical surface area of the electrodes, it was found that the gold-plated 3D electrode was about 3.6 times more sensitive toward DNT than the GCE with a similar linearity range between 60 and $220 \mu\text{M}$ compared to 1 to $200 \mu\text{M}$ ²³ obtained for GCE.

2,4,6-Trinitrotoluene. From previous studies conducted,^{11,24} three distinct cathodic peaks would be formed for TNT brought about by the electrochemical reduction of the three different nitro groups in TNT. The first nitro group in TNT to be reduced is the *ortho*- nitro with respect to the methyl group. This is followed by the reduction of the next *ortho*- nitro. The formation of the last cathodic peak at the highest potential is a result of the reduction of the nitro group in the *para*- position with respect to the methyl group in the aromatic compound probably due to the kinetic effect of the *ortho*- substituted groups.¹¹ Theoretically, the methyl group

Figure 5. Cyclic voltammograms of TNT on (A) glassy carbon and (B) gold-plated 3D stainless steel electrode. Differential pulse voltammograms with (C) glassy carbon and (D) gold-plated 3D stainless steel electrode. Conditions are as described in the [Experimental Section](#).

acts as an electron donating group which causes the *ortho*-nitro groups on the ring to experience a slightly higher partial negative charge than the group at the *para*-nitro group, thus requiring less energy for the first reduction step. This might account for preferential reduction at *ortho*- over the *para*-nitro group. Cyclic voltammetry was performed on all three electrodes, and their voltammograms are shown in [Figure 5](#). As predicted, three irreversible cathodic peaks (-0.532 , -0.722 , and -0.935 V) were observed for the glassy carbon electrode ([Figure 5A](#)). No visible peaks were again observed using bare 3D printed electrode. [Figure 5B](#) shows the voltammograms for TNT with the gold-plated 3D electrode. A very broad peak was observed between -0.25 to -0.8 V. The bare 3D printed stainless steel electrode showed no apparent peak formation.

DPV measurements were then carried out with GCE and gold-plated 3D electrodes. From [Figure 5C](#), DPV with GCE showed three well-resolved peaks corresponding to the reduction of two *ortho*- (-0.46 and -0.63 V) and one *para*-nitro group (-0.80 V). Three peaks (-0.37 , -0.51 , and -0.69 V) were also observed with gold-plated 3D electrode ([Figure 5D](#)). The peaks from the electrochemical reduction of *ortho*-nitro groups start to overlap and form a broad peak as the concentration of TNT increases. Hence, the only characteristic peak that could be used for analysis was the *para*-nitro group reduction peak.

Calibration graphs were subsequently plotted based on the peak heights for the electrochemical reduction of *para*-nitro group with both GCE and gold-plated 3D electrode. From the plots obtained, the gradient for glassy carbon electrode was $0.176 \text{ mA/cm}^2 \mu\text{M}$ with $R = 0.996$, and that for the gold-plated 3D printed stainless steel electrode was $0.244 \text{ mA/cm}^2 \mu\text{M}$ with $R = 0.998$. The calibration plots show that the gold-plated 3D electrode has sensitivity about 1.4 times greater than that of GCE with a linearity range between 220 and $400 \mu\text{M}$.

On the basis of the electrochemical analysis of the three different nitroaromatic compounds, it is observed that 3D

printing can be a reliable technique in the fabrication of customizable electrodes for environmental analysis. The stainless steel backbone of the 3D printed electrode provides a conductive platform for the transfer of charges during the electrochemical process which can easily undergo surface modification to confer a greater surface area and specific catalytic properties for a more sensitive response signal. Here, the Au film electroplated onto the steel electrodes enables a sensitive quantification of nitroaromatic compounds by electroreduction of the nitro groups. The analytical performance is comparable and even superior to that obtained with the GC electrode. In terms of material, we show that surface modified steel metal electrodes fabricated through 3D printing have great potential to be adopted for electroanalytical applications which are dominated by carbon-based electrodes.^{25–27} The well-established screen-printing fabrication technology currently offers the possibility to produce disposable electrodes (both carbon and metal-based) at a very low price. 3D printing is at its very early stage of development promising significantly reduced production costs in the near future with the important advantage over screen-printing methods of infinite design and shape possibilities to adapt easily to specific applications.^{2,28} In particular, the possibility to combine during the same 3D printing process several different materials will ultimately enable the fabrication of complete functional devices able to provide features such as liquid handling, sensing, and electronic read-out all together.

CONCLUSION

We demonstrated a proof-of-concept for the fabrication of a helical 3D printed stainless steel electrode modified with gold for the detection of three nitroaromatic compounds. 3D printing provides a convenient technology for fabricating customizable electrodes economically and with a high degree of control over their morphologies and composition. The performance of the gold-plated 3D printed stainless steel

electrode outperformed that of the glassy carbon electrode for the analysis of TNT, DNT, and FT, displaying in general higher sensitivities and broader analyte detection range. Surface functionalized 3D printed metal electrodes are potential alternatives to conventionally used electrodes for the electrochemical detection of environmental and biological samples.

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Notes

The authors declare no competing financial interest.

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3D-printed metal electrodes for electrochemical detection of phenols

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ABSTRACT

3D printed metal electrode modified by means of electroplating methodologies was tested and compared with conventional glassy carbon (GC) electrode for the individual and simultaneous electrochemical detection of phenol and *p*-aminophenol (*p*-AP) in aqueous solution via cyclic and differential pulse voltammetry. The 3D printed gold-plated electrode produced two distinct oxidation peaks for phenol and *p*-AP at lower anodic potentials as compared to GC electrode. It also showed improved analytical performance with higher sensitivity for *p*-AP detection. The fabricated electrode was stable at anodic potentials and the inherent electrochemistry of the electrode did not interfere with the electrochemical signals obtained. Thus, we have demonstrated a potential application of 3D printing technology for detection of phenolic compounds in environmental samples.

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1. Introduction

Three-dimensional (3D) printing technology has been widely used in industries to produce engineering prototypes and customisable devices [1]. Recent advances in 3D printing technology have expanded its capabilities into research and medical uses [2]. Applications for 3D printing includes the fabrication of human organs [3], customized bone prosthetics [4], reactionware for chemical synthesis [5], supercapacitors [6], and buildings construction [7]. As this technology becomes more established, portable 3D printing systems become more affordable, thus expanding its use into research labs and even homes. This opens up limitless opportunities for the application of 3D printing technology in environmental remediation and other analytical applications.

Pollution is a rising cause of concern brought about by an increasing global population and rapid industrialization. Waste generated from industries and households have reciprocal effects on the environment if mismanaged and would hence limit access to clean water. Among the broad number of water pollutants, phenols and its substituents are highly toxic and can cause harm to human health [8,9]. It is used as a raw material in industries for the manufacture of plastics and dyes. Phenol can also be found as a component of cosmetics and pesticides, as well as antiseptic in pharmaceutical products [10]. Consequently, it is commonplace to find phenol discarded into water bodies as industrial waste. As a

result of its high toxicity, both the United States Environmental Protection Agency (EPA) and the European Union (EU) list phenol as a priority pollutant. Phenolic compounds are also found in drugs and pharmaceuticals. One of the most widely used and popular analgesic drugs is paracetamol which degrades hydrolytically to form *p*-aminophenol (*p*-AP) [11]. *p*-AP is known to have nephrotoxic and teratogenic effects [12]. As such, numerous international and governmental agencies have closely monitored the concentration of *p*-AP in pharmaceuticals [13]. *p*-AP is also used as a raw material in the production of dyes, rubber, petroleum, and in photography [14]. Hence, large amounts of *p*-AP may be released into the environment, causing serious threat to aquatic life. Therefore, there is a need for an efficient and reliable analytical technique for its detection.

The reliability and sensitivity of phenolic compounds determination is dependent on the type of analytical method used. Numerous works have been published for analysis and detection of phenolic compounds using various analytical techniques such as high performance liquid chromatography (HPLC) [15], colorimetric detection [16], gas chromatography [17,18], capillary zone electrophoresis [19,20], and micellar electrokinetic chromatography [21]. Despite their good performance, these techniques require expensive, sophisticated instrumentation with specialized training and long processing times for analysis. As such, they are unsuitable for rapid, on-site detection of phenols and its substituents. 3D printing technology coupled with voltammetric techniques has been proven to be a viable alternative for sensing applications [22,23]. Surface modification of 3D-printed electrode with gold has been proven to be an efficient tool in biosensing for the detection of

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DNA [24], heavy metals [25] and nitroaromatic explosives [26]. Despite that, the performance and working potential of the fabricated electrodes might be limited by the inherent oxidation of the material deposited on the 3D-printed electrode surface. Hence, we set forth to investigate the feasibility and stability of using 3D printed gold-plated electrode towards the anodic detection of phenolic compounds.

2. Experimental

2.1. Materials

Phenol, *p*-aminophenol (*p*-AP), potassium phosphate dibasic, sodium phosphate monobasic, sodium chloride and potassium chloride were obtained from Sigma–Aldrich (Singapore). Deionized (DI) water with resistivity of 18.2 MΩ cm was used. 50 mM stock solutions of *p*-AP and phenol were prepared in PBS and deionised water respectively. Both solutions were stored in darkness at 4 °C. Working solutions were made fresh daily by diluting appropriate amounts of stock solution for electrochemical measurements.

2.2. Electrochemical measurements

Voltammetric measurements were conducted at room temperature (25 °C) using a three-electrode configuration with a μAutolab Type III electrochemical analyser (Eco Chemie, The Netherlands) controlled by a NOVA 1.10 software (Eco Chemie). Ag/AgCl and glassy carbon (GC) electrode (diameters of 3 mm), were obtained from CH Instruments (Texas, USA). Platinum foil was used as the reference electrode.

Cyclic voltammetry (CV) experiments were performed at a scan rate of 100 mV s^{−1} unless otherwise stated. Differential pulse voltammetry (DPV) was performed at an amplitude pulse of 25 mV, pulse width of 50 ms, and scan rate of 10 mV s^{−1}.

2.3. Fabrication of 3D printed gold-plated electrode

Helical stainless-steel 3D printed electrodes were fabricated as previously reported [23,24]. Electrode design was first designed using sketch-up 3D modelling open-source software. Metal 3D printing was carried out with a ConceptLaser printer (Concept Laser GmbH, Germany) using selective laser melting (SLM) technique. A focused, high-energy laser beam fuses and link metallic article deposited in powder form on a printing stage according to the design in a layer by layer fashion.

Electroplating of gold was performed as previously reported [24]. In summary, the 3D printed stainless steel electrode was immersed in a commercial gold plating solution and electroplating was performed by applying current of −20 mA for 90 min with stirring in the presence of Ag/AgCl reference electrode and platinum counter electrode. Au thin layer of overage thickness of 300 nm was deposited.

2.4. Electrochemical cleaning of 3D printed gold-plated electrode

The following cleaning method was adapted from a previous study reported [27]. The electrode was first soaked in a solution of 50 mM KOH and 25% (v/v) H₂O₂ for 5 min before rinsing with deionised water. Cyclic voltammetry was then performed in 50 mM KOH for 10 cycles between 0 V and 0.8 V at a scan rate of 100 mV s^{−1}. Subsequently, the electrode was dried with N₂ gas before use.

2.5. Characterization

Scanning electron microscopy (SEM) was performed using JEOL 7600 F-SEM (JEOL, Japan) operating at 15 kV. A section of the 3D

printed gold-plated electrode was placed onto carbon tape held on a SEM holder. Energy dispersive X-ray spectroscopy (EDX) data was obtained with an Oxford instrument and analyzed using Aztec software.

3. Results and discussion

Characterization was first performed for the 3D printed gold-plated electrode using scanning electron microscopy coupled with energy dispersive X-ray (SEM-EDX) spectroscopy to understand the surface morphology and chemical composition of the electrode. Fig. 1A and B shows the metal electrode design as obtained from CAD software and the optical image of the 3D printed electrode after gold plating, respectively. SEM image and the corresponding element mapping of a section of the 3D printed electrode are shown in Fig. 1C–F. It was noted that the electrode surface appears rough with random arrangements of spherical structures observed. This could be attributed to the selective laser melting (SLM) fabrication technique used. The main elements of stainless steel, the material used to create the electrode, is iron and nickel as confirmed by EDX analysis. The elemental mapping analysis shows a uniform and homogeneous layer of gold distributed on the electrode surface as obtained by electroplating. The less intense signals from iron and nickel in the EDX analysis is also an indication of the successful coating of gold onto the 3D printed stainless steel electrode. As such, it can be concluded that the 3D printed stainless steel electrode has been successfully coated with gold during the electroplating process and can be used for subsequent electrochemical analysis.

Having ascertained the successful deposition of gold onto the 3D printed stainless steel electrode, we set forth to investigate the durability and stability of the fabricated electrodes towards anodic detection of phenolic compounds. Cyclic voltammetry (CV) was first performed on a solution of 100 μM phenol in 100 mM PBS (pH = 7.2) with GC electrode. Phenol undergoes electro-oxidation to form phenoxy radicals which then leads to the formation of benzoquinones [28–30]. From Fig. 2A, a single anodic peak (1a) was observed at +654 mV with GC electrode. There was no corresponding cathodic peak in the reverse scan, suggesting that the oxidation of phenol to a phenoxy radical is irreversible. The appearances of peaks 2c (+342 mV) and 3c (+248 mV) correspond to the reductions of *ortho*-quinone to catechol and *para*-quinone to hydroquinone respectively. Two additional anodic peaks (peak 2a at +373 mV and peak 3a at +276 mV) formed at the second scan which suggests that the reduction of catechol and hydroquinone is reversible. The calculated peak-to-peak separation (ΔE_p) between peaks 2a and 2c is 31 mV while ΔE_p between peaks 3a and 3c is 28 mV. The values obtained are similar to the theoretical value of 30 mV for a two-electron reversible reaction (Eq. (1)) which supports the conclusion that the reaction is reversible.

$$\Delta E_p = |E_{pa} - E_{pc}| = \frac{0.0592}{n} \quad (1)$$

where E_{pa} and E_{pc} are the anodic and cathodic peak potentials respectively, and n is the number of electrons transferred in the reaction.

CV was then performed with 3D printed gold-plated electrode (Fig. 2B) and the anodic peak shifted to a lower potential (+598 mV). This could be attributed to the better electron transfer kinetics of the gold surface [31] and electrical conductivity of the stainless steel backbone. The two redox peak pairs (peaks 2 and 3, Fig. 2A) were not visible with 3D printed gold-plated electrode. This could be due to the formation of a multilayer film on the gold surface where the phenoxy radicals generated underwent intramolecular rearrangements and adhered to gold thus causing passivation of the electrode surface [32,33]. The gold surface could have facilitated polymerization reaction that consumed the phenoxy radicals. No

Fig. 1. (A) Schematic of the electrode design by CAD software. (B) Optical image of the 3D printed electrode after Au electroplating. Scale bar corresponds to 1 cm. (C) SEM image of a section of the electrode with the corresponding element mapping analysis for (D) Au, (E) Fe and (F) Ni.

Fig. 2. Cyclic voltammograms of 100 μM phenol in 100 mM PBS (pH = 7.2) with (A) glassy carbon and (B) 3D printed gold-plated electrode. Scan rate = 100 mV s^{-1} .

additional peaks were observed which suggests no inherent oxidation of the fabricated electrode towards the anodic detection of phenol. This suggests the stability of the 3D printed gold-plated electrode and its ability to detect phenol. Anodic peak from first scans were only taken into consideration for further investigations with phenol.

The effect of scan rate on the electrochemical behaviour of phenol was next studied. The anodic peak heights of phenol increased with a corresponding increase in scan rate for both electrodes (Fig. S1). There was also a notable shift in peak potential towards the positive direction with the increase in scan rate, which is characteristic of irreversible systems [34]. A plot of peak current against square root of scan rate (Fig. 3) yields a linear graph of gradient $11.2 \mu\text{A}(\text{V s}^{-1})^{-1/2}$ and $R=0.979$ on GC electrode, and a slope of $35.1 \mu\text{A}(\text{V s}^{-1})^{-1/2}$ and $R=0.956$ for 3D printed gold-plated electrode. From the Randles–Sevcik equation (Eq. (2)), i_p is proportional to $\sqrt{v}$. As such, oxidation of phenol is determined to be diffusion controlled which corroborates with previous studies [35].

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

where i_p is the peak current (A), n is the number of electrons involved in the reaction, A is the electrode area (cm^2), D is the diffusion coefficient of analyte ($\text{cm}^2 \text{s}^{-1}$), v is scan rate (V s^{-1}), and C is analyte concentration (mol cm^{-3}).

We next set forth to investigate the effect of phenol concentration on the electrochemical behaviour on the two electrodes. Phenol concentration was varied from 50 μM to 175 μM . From Fig. 4A, it was observed that the anodic peak shifts to less positive values with a corresponding increase in phenol concentration with GC electrode. However, an opposite trend was observed with 3D printed gold-plated electrode where the anodic peak shifts to more positive potentials as phenol concentration is increased (Fig. 4B). Despite that, both electrodes exhibit similar trend where the peak current increases with increasing phenol concentration. DPV was next performed to understand the differences in the performance and sensitivities of the electrodes. From Fig. 4C and D, voltammetric current signal increased with increasing phenol concentration for both electrodes. From Fig. 4C, the anodic peaks on GC shifted to less positive potentials with increasing phenol concentrations. However, the anodic peaks remained constant with increasing phenol concentration for 3D printed gold-plated electrode

Fig. 3. Plot of oxidation peak current against square root at variable scan rate ($0.01\text{--}0.25\text{ V s}^{-1}$) for $100\text{ }\mu\text{M}$ phenol in 100 mM PBS ($\text{pH} = 7.2$).

(Fig. 4D). The peaks obtained with GC electrode were also sharper while the peaks were broader with the 3D printed gold-plated electrode.

A calibration graph of peak current density against concentration was then plotted (Fig. 5). It was interesting to note that the linear plots for both electrodes gave almost similar results. GC electrode gave a slope of $1.70 \times 10^{-3}\text{ }\mu\text{A mm}^{-2}\text{ }\mu\text{M}^{-1}$ with $R=0.998$ while 3D printed gold-plated electrode gave a slope of $1.39 \times 10^{-3}\text{ }\mu\text{A mm}^{-2}\text{ }\mu\text{M}^{-1}$ with $R=0.997$. Despite the enhanced

Fig. 5. Plot of peak current density against variable phenol concentrations ($50\text{--}175\text{ }\mu\text{M}$) in 100 mM PBS ($\text{pH} = 7.2$) with glassy carbon (black line) and 3D printed gold-plated electrode (red line). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

electrical conductivity of gold, there was no significant difference observed in the performance of 3D printed gold-plated electrode and GC electrode for the detection of phenol. This could be attributed to the fouling of the 3D printed gold-plated electrode by the formation of an organic film on its surface which could have affected its electrocatalytic activity [31]. However, it demonstrates the feasibility of using modified 3D-printed electrode

Fig. 4. Cyclic voltammograms of variable phenol concentrations ($50\text{--}175\text{ }\mu\text{M}$) with (A) glassy carbon and (B) 3D printed gold-plated electrode. Scan rate = 100 mV s^{-1} . Differential pulse voltammograms of variable phenol concentrations ($50\text{--}175\text{ }\mu\text{M}$) with (C) glassy carbon and (D) 3D printed gold-plated electrode (pulse amplitude = 25 mV , pulse width = 50 ms , scan rate = 10 mV s^{-1}).

Fig. 6. Cyclic voltammograms of 1.0 mM *p*-AP in 100 mM PBS (pH = 7.2) with glassy carbon (black line) and 3D printed gold-plated (red line) electrode. Scan rate = 100 mV s⁻¹. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

for the detection of phenol with comparable sensitivities with conventionally used GC electrode. Further surface modifications can be explored which might be required for improved sensitivity of phenol with 3D printed electrodes.

Cyclic voltammetry was next performed on *p*-AP with GC and 3D printed gold-plated electrode (Fig. 6). A pair of anodic and cathodic peak was observed for both electrodes which shows that the electrochemical reaction of *p*-AP is reversible [36,37]. The anodic peak for *p*-AP shifted to less positive potential while the cathodic peak shifted to more positive potential with 3D printed gold-plated electrode as compared to GC electrode. The 3D printed gold-plated electrode gave a smaller ΔE_p of 82 mV in comparison to GC electrode (ΔE_p = 142 mV). It was also observed that the magnitudes for the peak heights differ significantly between the two electrodes. 3D printed gold-plated electrode gave sharp and distinct peaks while the peaks obtained with GC electrode were broader and lower intensity. This observation is opposite to the results obtained for phenol (Fig. 2).

The effect of scan rate on the electrochemical reaction of *p*-AP was next investigated. Fig. S2 shows the cyclic voltammograms for GC and 3D printed gold-plated electrodes at different scan rates. The peak heights on both electrodes were observed to increase with increasing scan rate (Fig. S2). From Fig. S2A, no significant change in peak positions were observed for GC electrode. However, 3D printed gold-plated electrode saw a shift for the anodic peak to more positive potentials and more negative potentials for the cathodic peak (Fig. S2B). This correlates to an almost similar ΔE_p for the GC electrode but an increasing ΔE_p for the 3D printed gold-plated electrode with increasing scan rate. A plot of peak current against square root of scan rate demonstrates the proportionality between the two functions (Fig. 7). The plot for the anodic peaks with GC electrode gave a gradient of 102 $\mu\text{A} (\text{Vs}^{-1})^{-1/2}$ and $R = 0.994$ while that for 3D printed gold-plated electrode gave a gradient of $1.16 \times 10^3 \mu\text{A} (\text{Vs}^{-1})^{-1/2}$ with $R = 0.993$. The cathodic peak currents gave a slope of $-110 \mu\text{A} (\text{Vs}^{-1})^{-1/2}$ and $R = -1$ with GC electrode while a slope of $-1.44 \times 10^3 \mu\text{A} (\text{Vs}^{-1})^{-1/2}$ and $R = -0.997$ was obtained for 3D printed gold-plated electrode. Therefore, from the Randles-Sevcik equation (Eq. (2)), *p*-AP reaction kinetics was determined to be diffusion-controlled [38,39].

The concentration of *p*-AP was increased from 0.5 mM to 3.0 mM and studied using CV and DPV (Fig. 8). The anodic and cathodic peak heights increased for both electrodes as concentration of *p*-AP was increased (Fig. 8A and B). The anodic peak shifted to more positive potential while the cathodic peak shifted to more negative potentials with increasing concentration of *p*-AP for GC electrode

Fig. 7. Plot of redox peak currents against square root at variable scan rates (0.01–0.25 V s⁻¹) for 1.0 mM *p*-aminophenol in 100 mM PBS (pH = 7.2) with glassy carbon (black and red lines) and 3D printed gold-plated electrode (blue and green lines). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

(Fig. 8A). However, both cathodic and anodic peaks shifted to more positive potentials for the 3D printed gold-plated electrode (Fig. 8B). DPV was next performed with both electrodes to compare their sensitivities and performance (Fig. 8C and D). The increase in *p*-AP concentration led to the corresponding increase in the anodic peak height for both electrodes. An additional peak was observed ($\sim +200$ mV) on DPV for both electrodes which was not observable from CV. This could be from the formation of intermediate products in the oxidation reaction [40,41]. The peak height of the additional peak increases as the concentration of *p*-AP was increased accordingly.

A calibration graph of peak current density against *p*-AP concentration was plotted for both electrodes (Fig. 9). From Fig. 9, the calibration plot for GC electrode gave a gradient of $2.80 \mu\text{A mm}^{-2} \text{mM}^{-1}$ and $R = 0.999$ while the 3D printed gold-plated electrode gave a gradient of $5.12 \mu\text{A mm}^{-2} \text{mM}^{-1}$ and $R = 0.984$. The 3D printed gold-plated electrode displayed improved sensitivity of about 1.8 times in the detection of *p*-AP.

Studies on the simultaneous detection of these two phenolic compounds were next carried out on GC and 3D printed gold-plated electrodes using DPV. The concentration of one analyte was kept constant while the other was varied. The concentration of *p*-AP was kept constant at 1.0 mM while the concentration of phenol was varied from 50 μM to 175 μM . From Fig. 10, simultaneous detection of the two compounds did not seem to affect the concentration dependency of signal from *p*-AP as peak current increased with increasing phenol concentration. The peak shifted slightly to less positive potentials with GC electrode (Fig. 10A) while there was no apparent shift in peak position with 3D printed gold-plated electrode as concentration of phenol was increased (Fig. 10B).

A calibration graph of peak current density against concentration was then plotted. From Fig. 11, GC electrode calibration plot gave a gradient of $2.50 \times 10^{-3} \mu\text{A mm}^{-2} \mu\text{M}^{-1}$ and $R = 0.999$ while 3D printed gold-plated electrode gave a gradient of $2.42 \times 10^{-3} \mu\text{A mm}^{-2} \mu\text{M}^{-1}$ and $R = 0.993$. There was no significant difference in sensitivity between GC and 3D printed gold-plated electrode and their performance was comparable.

The concentration of phenol was then kept constant at 100 μM while the concentration of *p*-AP was varied from 0.5 mM to 3.0 mM. From Fig. 12, the increase in *p*-AP concentration led to the proportional increase in peak heights for both electrodes. However,

Fig. 8. Cyclic voltammograms of variable *p*-AP concentrations (0.5–3.0 mM) with (A) glassy carbon and (B) 3D printed gold-plated electrode. Scan rate = 100 mV s^{-1} . Differential pulse voltammograms of variable *p*-AP concentrations (0.50–3.0 mM) with (C) glassy carbon and (D) 3D printed gold-plated electrode (pulse amplitude = 25 mV , pulse width = 50 ms , scan rate = 10 mV s^{-1}).

Fig. 9. Plot of peak current density against variable *p*-aminophenol concentrations (0.50–3.0 mM) in 100 mM PBS (pH = 7.2) with glassy carbon (black line) and 3D printed gold-plated electrodes (red line). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

the peak heights for phenol decreased significantly with increasing *p*-AP concentration for GC electrode despite keeping the phenol concentration unchanged (Fig. 12A). Phenol could have undergone a side reaction with *p*-AP which was now present in excess causing some phenol to react. This became more apparent as concentration of *p*-AP increased which causes the phenol peak height to decrease correspondingly. However, this observation was not apparent with

3D printed gold-plated electrode where the anodic peak height of phenol remained almost constant with increasing concentrations of *p*-AP (Fig. 12B). Phenol might have formed an organic film on the gold surface²⁹ and as such, this occurrence was observed at each concentration of *p*-AP investigated. Thus, the peak current obtained did not change with increasing *p*-AP concentration.

A calibration graph of peak current density against variable concentrations of *p*-AP was plotted (Fig. 13). The calibration for GC electrode gave a gradient of $3.27 \mu\text{A mm}^{-2} \text{ mM}^{-1}$ and $R = 0.997$ while 3D printed gold-plated electrode gave a gradient of $4.92 \mu\text{A mm}^{-2} \text{ mM}^{-1}$ with $R = 0.989$. The 3D printed gold-plated electrode presented a noticeable increase in sensitivity which was 1.5 times greater than GC electrode.

From the electrochemical analysis performed, the detection of phenolic compounds is enhanced with 3D printed gold-plated electrode when compared with GC electrode. The detection of *p*-AP saw an almost doubled sensitivity with the 3D printed gold-plated electrode with amplified signals of higher magnitudes. The stainless steel backbone of the 3D printed electrode provides a conductive platform for more efficient electron transfer which facilitated and enhanced the electrochemical response signal for *p*-AP. However, the detection of phenol is slightly limited as a result of the strong adhesion of phenol onto gold [29] which caused passivation of the 3D printed gold-plated electrode surface. Despite that, the performance of the 3D printed gold-plated electrode was comparable to GC electrode for the detection of phenol and its possible application is demonstrated. The 3D printed gold-plated electrode was also stable at anodic working potentials with no interference sig-

Fig. 10. Differential pulse voltammograms of phenol and *p*-AP mixture with variable phenol concentrations (50–175 μM) in 100 mM PBS (pH = 7.2) with (A) glassy carbon and (B) 3D printed gold-plated electrode. Inserts show zoomed in phenol oxidation range ($c_{p\text{-AP}} = 1.0 \text{ mM}$, pulse amplitude = 25 mV, pulse width = 50 ms, scan rate = 10 mV s^{-1}).

Fig. 11. Plot of peak current density against variable phenol concentrations (50–175 μM) in 100 mM PBS (pH = 7.2) with glassy carbon (black line) and 3D printed gold-plated electrode (red line). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

nals obtained for the detection of the phenolic compounds. This could be attributed to the relatively low anodic potentials of the compounds as compared to the oxidation potential of gold. As such, the detection of the compounds of interest is not hindered.

Fig. 13. Plot of peak current density against variable *p*-AP concentrations (0.50–3.0 mM) in 100 mM PBS (pH = 7.2) with glassy carbon (black line) and 3D printed gold-plated electrodes (red line). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Despite that, this limitation would pose a hindrance for detecting compounds with higher anodic potentials as a result of the formation of gold oxide film brought about by the oxidation of gold.

Fig. 12. Differential pulse voltammogram of phenol and *p*-AP mixture with variable *p*-AP concentrations (0.50–3.0 mM) in 100 mM PBS (pH = 7.2) with (A) glassy carbon and (B) 3D printed gold-plated electrode. Inserts show zoomed in phenol oxidation range ($c_{\text{phenol}} = 100 \mu\text{M}$, pulse amplitude = 25 mV, pulse width = 50 ms, scan rate = 10 mV s^{-1}).

4. Conclusion

We have investigated the effectiveness of 3D printed gold-plated electrode for the individual and simultaneous electrochemical detection of phenol and *p*-AP in aqueous solutions. Two distinct and distinguishable oxidation peaks were obtained with 3D printed gold-plated electrode. Moreover, the electrochemical signal for both phenol and *p*-AP appeared at lower oxidation potentials as compared to glassy carbon electrode. The fabricated electrode was also more sensitive towards the detection of *p*-AP. However, despite the abovementioned advantages, the 3D printed gold-plated electrode has limitations in detecting phenol. This is attributed to the formation of an organic film on the gold surface [30,31]. Furthermore, compounds with high oxidation potentials might not be accurately analyzed due to the oxidation of gold which would influence the rate of reactions. Despite that, we have demonstrated a proof-of-concept for the successful application of modified 3D-printed electrodes for the detection of phenolic compounds. Surface functionalized 3D-printed metal electrodes can be potential alternatives to conventional electrodes for on-site point-of-care detection of environmental and biological samples.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.apmt.2017.07.005.

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