Two-dimensional materials and three-dimensional printing for electrochemical biosensing applications

Nasuha Rohaizad

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**TWO-DIMENSIONAL MATERIALS AND
THREE-DIMENSIONAL PRINTING FOR
ELECTROCHEMICAL BIOSENSING APPLICATIONS**

NASUHA BINTE ROHAIZAD
Interdisciplinary Graduate School
NTU Institute for Health Technologies

2020

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THREE-DIMENSIONAL PRINTING FOR
ELECTROCHEMICAL BIOSENSING APPLICATIONS**

NASUHA BINTE ROHAIZAD

**Interdisciplinary Graduate School
NTU Institute for Health Technologies**

A thesis submitted to the Nanyang Technological University in partial
fulfillment of the requirement for the degree of
Doctor of Philosophy

2020

Statement of Originality

I hereby certify that the work embodied in this thesis is the result of original research, is free of plagiarised materials, and has not been submitted for a higher degree to any other University or Institution.

30 December 2020

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Nasuha Binte Rohaizad

Supervisor Declaration Statement

I have reviewed the content and presentation style of this thesis and declare it is free of plagiarism and of sufficient grammatical clarity to be examined. To the best of my knowledge, the research and writing are those of the candidate except as acknowledged in the Author Attribution Statement. I confirm that the investigations were conducted in accord with the ethics policies and integrity standards of Nanyang Technological University and that the research data are presented honestly and without prejudice.

5 January 2021

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Date

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Richard David Webster

Authorship Attribution Statement

This thesis contains material from 5 papers published in the following peer-reviewed journals where I was the first author.

Part of the writing in Chapter 4 is published as Rohaizad, N.; Mayorga-Martinez, C. C.; Fojtů, M.; Latiff, N. M.; Pumera, M. Two-Dimensional Materials in Biomedical, Biosensing and Sensing Applications. *Chem. Soc. Rev.* **2021**, 50, 619-657. DOI: 10.1039/D0CS00150C

The contributions of the co-authors are as follows:

- I wrote the section on “electrochemical biosensors”, as well as formatted and revised the manuscript.
- Dr. Mayorga-Martinez provided the ideas leading to the review, wrote the section on “optical biosensors” and “sensors”, as well as revised the manuscript.
- Dr. Fojtů wrote the section on “therapeutic applications” and revised the manuscript.
- Dr. Naziah wrote the section on “toxicity” and revised the manuscript.
- Prof Pumera provided the project supervision, as well as revised the manuscript.

Chapter 5 is published as Rohaizad, N.; Mayorga-Martinez, C. C.; Sofer, Z.; Pumera, M. 1T-Phase Transition Metal Dichalcogenides (MoS₂, MoSe₂, WS₂, and WSe₂) with Fast Heterogeneous Electron Transfer: Application on Second-Generation Enzyme-Based Biosensor. *ACS Appl. Mater. Interfaces* **2017**, 9 (46), 40697–40706. DOI: 10.1021/acsami.7b13090.

The contributions of the co-authors are as follows:

- I was responsible for the investigation process, specifically conducting electrochemical and characterisation experiments (scanning electron microscopy, energy dispersive X-ray spectroscopy, X-ray photoelectron spectroscopy), as well as analysing the data collected.
- Dr. Mayorga-Martinez provided ideas and methodology, mentored the research process including data presentation, and revised the manuscript.

- Prof Sofer synthesised the materials, performed transmission electron microscopy as well as X-ray diffraction, and revised the manuscript.
- Prof Pumera provided the project direction and supervision, suggested the materials, and revised the manuscript.

Chapter 6 is published as Rohaizad, N.; Mayorga-Martinez, C. C.; Sofer, Z.; Webster, R. D.; Pumera, M. Niobium-Doped TiS₂: Formation of TiS₃ Nanobelts and Their Effects in Enzymatic Biosensors. *Biosens. Bioelectron.* **2020**, *155*, 112114–112120. DOI: 10.1016/j.bios.2020.112114.

The contributions of the co-authors are as follows:

- I was responsible for the investigation process, specifically conducting electrochemical and characterisation experiments (scanning electron microscopy, X-ray photoelectron spectroscopy), as well as analysing the data collected.
- Dr. Mayorga-Martinez provided ideas and methodology, mentored the research process including data presentation, and revised the manuscript.
- Prof Sofer synthesised the materials, performed Raman spectroscopy as well as X-ray diffraction, and revised the manuscript.
- Prof Webster supported the research activity planning and execution, and revised the manuscript.
- Prof Pumera provided the project direction together with supervision, and revised the manuscript.

Chapter 7 is published as Rohaizad, N.; Mayorga-Martinez, C. C.; Sofer, Z.; Webster, R. D.; Pumera, M. Layered Platinum Dichalcogenides (PtS₂, PtSe₂, PtTe₂) for Non-Enzymatic Electrochemical Sensor. *Appl. Mater. Today* **2020**, *19*, 100606–100614. DOI: 10.1016/j.apmt.2020.100606.

The contributions of the co-authors are as follows:

- I was responsible for the investigation process, specifically conducting electrochemical and characterisation experiments (scanning electron microscopy, energy dispersive X-ray spectroscopy, X-ray photoelectron

spectroscopy, Raman spectroscopy, atomic force microscopy), as well as analysing the data collected.

- Dr. Mayorga-Martinez provided ideas and methodology, mentored the research process including data presentation, and revised the manuscript.
- Prof Sofer synthesised the materials, performed transmission electron microscopy as well as X-ray diffraction, and revised the manuscript.
- Prof Webster supported the research activity planning and execution, and revised the manuscript.
- Prof Pumera provided the project direction together with supervision, and revised the manuscript.

Chapter 9 is published as Rohaizad, N.; Mayorga-Martinez, C. C.; Novotný, F.; Webster, R. D.; Pumera, M. 3D-Printed Ag/AgCl Pseudo-Reference Electrodes. *Electrochem. Commun.* **2019**, *103*, 104–108. DOI: 10.1016/j.elecom.2019.05.010.

The contributions of the co-authors are as follows:

- I was responsible for the investigation process, specifically conducting electrochemical and characterisation experiments (scanning electron microscopy, energy dispersive X-ray spectroscopy), as well as analysing the data collected.
- Dr. Novotný fabricated the electrodes using 3D printing.
- Prof Webster provided insights and suggestions pertaining electrochemical experiments, and revised the manuscript.
- Prof Pumera provided the project direction together with supervision, and revised the manuscript.

30 December 2020

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Date

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Nasuha Binte Rohaizad

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Table of Contents

Acknowledgements	i
Table of Contents	iii
Summary	iv
List of Publications	v
Chapter 1 Thesis Objectives & Organisation	1
Chapter 2 Introduction to Biosensors	5
Chapter 3 Background of Electrochemical Biosensors	16

Part I: Two-Dimensional Materials

Chapter 4 Background of Two-Dimensional Transition Metal Dichalcogenides..	30
Chapter 5 Phase Engineering of Group 6 Transition Metal Dichalcogenides (MoS ₂ , MoSe ₂ , WS ₂ , WSe ₂)	49
Chapter 6 Niobium-Doped Group 4 Titanium Disulfide (TiS ₂).....	78
Chapter 7 Electrochemical Pretreatment of Group 10 Transition Metal Dichalcogenides (PtS ₂ , PtSe ₂ , PtTe ₂) for Non-Enzymatic Sensing	108

Part II: Three-Dimensional Printing

Chapter 8 Background of Three-Dimensional (3D) Printing	136
Chapter 9 3D Printed Ag/AgCl Pseudo-Reference Electrodes	152
Chapter 10 Conclusion & Future Outlook.....	166

Summary

Two-dimensional (2D) materials and three-dimensional (3D) printing are promising technologies in advancing the development and commercialisation of electrochemical biosensors. 2D materials have been sought to enhance the sensitivity of biosensors due to their alluring large surface area that exposes more active sites as well as facilitates the immobilisation of biorecognition elements. This thesis aimed to delve into transition metal dichalcogenides (TMDs), a prominent class of 2D materials, for electrochemical biosensors. Different elemental compositions were scrutinised as unique properties were anticipated, specifically TMDs of group 4 (TiS_2), group 6 (MoS_2 , MoSe_2 , WS_2 , and WSe_2) and group 10 (PtS_2 , PtSe_2 and PtTe_2). Correlations between structures, properties as well as biosensing capabilities were elucidated from extensive characterisation data and electrochemical experiments. At the same time, strategies relevant to the compounds were simultaneously explored to enhance their conductivity. They were phase engineering, doping and electrochemical pretreatment. The superior material from each group of TMDs was developed into electrochemical biosensors with key analytical parameters established, prior to a comparison against other developed biosensors in literature. In parallel, this thesis aimed to leverage the advantages of 3D printing to fabricate a fundamental electrochemical component, the reference electrode. It was a straightforward process to 3D print the electrodes, subsequently modifying them to obtain Ag/AgCl pseudo-reference electrodes. Their electrochemical feasibility was studied in heterogeneous electron transfer reactions using different redox systems. With the advent of 3D printing, there are limitless opportunities for customisation of electrochemical devices as well as remote testing.

List of Publications

First Author Articles

1. **N. Rohaizad**, C. C. Mayorga-Martinez, M. Fojtů, N.M. Latiff, M. Pumera. Two-Dimensional Materials in Biomedical, Biosensing and Sensing Applications. *Chem. Soc. Rev.* **2021**, 50, 619-657. (Review)
2. **N. Rohaizad**, C. C. Mayorga-Martinez, Z. Sofer, R. D. Webster, M. Pumera. Layered Platinum Dichalcogenides (PtS₂, PtSe₂, PtTe₂) for Non-Enzymatic Electrochemical Sensor. *Appl. Mater. Today* **2020**, 19, 100606.
3. **N. Rohaizad**, C. C. Mayorga-Martinez, Z. Sofer, R. D. Webster, M. Pumera. Niobium-Doped TiS₂: Formation of TiS₃ Nanobelts and Their Effects in Enzymatic Biosensors. *Biosens. Bioelectron.* **2020**, 155, 112114.
4. **N. Rohaizad**, C. C. Mayorga-Martinez, F. Novotný, R. D. Webster, M. Pumera. 3D-Printed Ag/AgCl Pseudo-Reference Electrodes. *Electrochem. Commun.* **2019**, 103, 104–108.
5. **N. Rohaizad**, C. C. Mayorga-Martinez, Z. Sofer, M. Pumera. 1T-Phase Transition Metal Dichalcogenides (MoS₂, MoSe₂, WS₂, and WSe₂) with Fast Heterogeneous Electron Transfer: Application on Second-Generation Enzyme-Based Biosensor. *ACS Appl. Mater. Interfaces* **2017**, 9, 40697–40706.

Co-Author Articles

1. E. Rahmanian, C. C. Mayorga Martinez, **N. Rohaizad**, J. Luxa, Z. Sofer, M. Pumera. Structural Transition Induced by Niobium Doping in Layered Titanium Disulfide: The Impact on Electrocatalytic Performance. *Appl. Mater. Today* **2020**, 19, 100555.
2. N. F. Rosli, **N. Rohaizad**, J. Sturala. A. C. Fisher, R. D. Webster, M. Pumera. Siloxene, Germanane, and Methylgermanane: Functionalized 2D Materials of Group 14 for Electrochemical Applications. *Adv. Funct. Mater.* **2020**.
3. L. Kong, **N. Rohaizad**, M. Z. M. Nasir, J. Guan, M. Pumera. Micromotor-Assisted Human Serum Glucose Biosensing. *Anal. Chem.* **2019**, 91, 5660–5666.
4. N. F. Rosli, C. C. Mayorga-Martinez, N. M. Latiff, **N. Rohaizad**, Z. Sofer, A. C. Fisher, M. Pumera. Layered PtTe₂ Matches Electrocatalytic Performance of Pt/C for Oxygen Reduction Reaction with Significantly Lower Toxicity. *ACS Sustainable Chem. Eng.* **2018**, 6, 7432–7441.

Chapter 1

Thesis Objectives & Organisation

Electrochemical biosensors, with the primary function of detecting target analytes, are imperative devices serving a multitude of applications. Security, environmental monitoring, food safety and quality are some examples, although healthcare is undoubtedly the most significant application. An ageing population, increasing prevalence of chronic and infectious diseases, as well as patient self-advocacy, drive the demand for efficient healthcare where electrochemical biosensors are sought to fulfil. Combining the selectivity of biological recognition elements and sensitivity of electrochemical transducers, target analytes are accurately detected to attain key information.

This thesis aims to advance the development and commercialisation of electrochemical biosensors via two promising technologies, two-dimensional (2D) materials and three-dimensional (3D) printing. They each address different aspects, the former to enhance sensitivity while the latter facilitates fabrication of electrochemical biosensors. The thesis is consequently organised into two parts with their respective objectives. Prior to those parts, **Chapter 2** describes the general landscape of biosensors which includes a myriad of biological elements, transduction methods, and applications. Recognition mechanism of biological elements are particularly elaborated as they confer selectivity to the analytical devices. **Chapter 3** then provides a technical background on electrochemical biosensors. On top of the various techniques to immobilise biorecognition elements, a handful of electrochemical techniques are discussed to understand the translation of recognition events into measurable signals. Both chapters convey fundamental information on electrochemical biosensors, the overarching theme of this thesis.

Part I, 2D Materials. Materials of such size and dimensionality are attractive to electrochemical biosensors due to their high surface-to-volume ratio. Large surface area and more exposed active sites would enhance conductivity and immobilisation of biorecognition elements. An ultrasensitive biosensor is envisioned, capable of detecting low concentrations of

target analytes in non-invasive samples. Additionally, 2D materials possess remarkable mechanical strength and flexibility that are promising for wearable biosensors. The interest in these materials is highlighted in **Chapter 4**, along with information on transition metal dichalcogenides (TMDs). They are a prominent class of 2D materials and exhibit versatile chemistry.

The objective of Part I is to explore various elemental compositions of TMDs for the development of electrochemical biosensors. Characterisation and electrochemical experiments would be performed to elucidate structural, chemical as well as key electrochemical properties. At the same time, a handful of strategies are employed to enhance their performances as electrochemical biosensors. The material would then be incorporated into a biosensing electrode, with analytical parameters established for the target analyte before comparing against relevant biosensors in literature.

Chapter 5 presents the study on TMDs composed of group 6 transition metals (MoS_2 , MoSe_2 , WS_2 , WSe_2), which are prepared by *t*-BuLi intercalation and exfoliation to attain the metallic 1T phase, instead of the natural semiconducting 2H. The influence of transition metal and chalcogen on the conductive, metallic 1T phase is scrutinised together with their development as 2nd generation glucose biosensor. **Chapter 6** focuses on TiS_2 , a group 4 TMD, doped with varying levels of Nb. Implications on structure and electrochemical properties are analysed to better understand doping effects as well as optimise electrochemical biosensing capabilities. Lastly, group 10 TMDs (PtS_2 , PtSe_2 , PtTe_2) take centre stage in **Chapter 7** with their dramatic chalcogen dependence in bonding and conductivity. Active inherent electrochemistry of the material prompts electrochemical pretreatment as a strategy to modify chemical states. Overall, expanding the library of TMDs would unravel the different properties, strategies and performances for electrochemical biosensors.

Part II, 3D Printing. Manufacturing advancement is one of the key drivers towards successful commercialisation of electrochemical biosensors. The early revolution of home-use glucose metres is credited to the breakthrough of screen printing, as devices are mass manufactured at low cost. Today, 3D printing is the manufacturing method pursued to accelerate the pace of commercialisation. There are numerous advantages and possibilities especially on-demand, customised as well as rapid manufacturing. In addition, the gap between academia and industry is bridged because of economical printers and compatible materials. The integration of 3D printing with electrochemical biosensors is reviewed in **Chapter 8**.

The objective of Part II is to fabricate, using 3D printing, components of electrochemical biosensors. Recognising that reference electrodes are integral to every electrochemical system, **Chapter 9** describes the fabrication of an Ag/AgCl pseudo-reference electrode. Fused deposition modelling (FDM), one of the 3D printing techniques, entails affordable printers together with a straightforward design-to-object process. Therefore, it is an overall simple process to produce carbon conductive electrodes via FDM and modifying them into Ag/AgCl pseudo-reference electrode.

This thesis concludes with **Chapter 10**, to highlight key findings and provide future insights of 2D materials and 3D printing for electrochemical biosensors.

Chapter 2

Introduction to Biosensors

Glucose meters and pregnancy test kits are familiar examples of biosensors capable of providing information about one's medical condition. These devices are commonly found on shelves and conveniently purchased over the counter before their usage in a comfortable home setting.¹ Glucose levels can be quantified reliably via sampling of a tiny blood droplet obtained from a finger prick, or interstitial fluid extracted by the needle of an implanted sensor. Pregnancy, on the other hand, can be determined based on the human chorionic gonadotropin (hCG) biomarker in urine samples, even the duration of pregnancy can be estimated.² Such essential information is made accessible by biosensors to individuals, in a simple and fast manner.

These devices have transcended the pioneer conception of biosensor by Clark and Lyons, who described the coupling of enzymes with existing electrode systems to ease the detection of blood analytes.³ They proposed a layer of glucose oxidase enzyme trapped between membranes which glucose analytes have to diffuse through, before their recognition and conversion into gluconic acid by the enzyme. The decrease in pH or consumption of dissolved oxygen are respectively measured by pH or pO₂ electrodes, correlating to glucose levels. In addition to specificity and efficiency, enzymes bring along advantages of stability and usability for continuous measurements. The attractive concept of “enzyme electrode” was realised shortly after^{4,5}, and evolves into the biosensors we know today.

A biosensor, following the International Union of Pure and Applied Chemistry (IUPAC), is “a device that uses specific biochemical reactions mediated by isolated enzymes, immunosystems, tissues, organelles or whole cells to detect chemical compounds usually by electrical, thermal or optical signals”.^{6,7} At the same time, there is an IUPAC definition exclusively for electrochemical biosensors owing to its early inception and successful commercialisation. It is adapted, for a general description of biosensor, to a “self-contained integrated device capable of providing specific quantitative or semi-quantitative analytical

information using a biological recognition element which is retained in direct spatial contact with a transduction element”.⁸ Both definitions are fundamentally similar, yet the latter elegantly summarises the biosensor’s two main components as biological recognition element and transducer. Examples of each component as well as the typical operation of a biosensor are shown in Figure 2.1.

Figure 2.1. The biosensor is an integrated device of two main components, biological recognition element and transducer, with examples listed for each. The biorecognition element demonstrates specificity to the analyte of interest, inducing a change in physical or chemical properties measurable by the transducer. A reader subsequently processes the signal to produce an interpretable result for users. Figure adapted with permission from ref. 9, Copyright 2017 Elsevier.

The exquisite specificity of a biological recognition element, also referred as biorecognition element for short or bioreceptor, is desirable for analytical devices. It bestows a preferential response towards the target analyte amidst the presence of other species in a sample. There are various biorecognition elements as listed in Figure 2.1, notably enzymes and

antibodies. These are naturally occurring and isolated from living organisms, although there are artificial ones developed to achieve similar physiological interactions with analytes.¹⁰ Molecularly imprinted polymers (MIPs) are recognised as “plastic antibodies”, synthesised via polymerisation of functional monomers complexed with an imprinted template of the target molecule. Subsequent removal of the molecular template yields MIPs which mimic the shape and function of antibodies, with cavities to encapsulate analytes.^{11,12} In between, there are pseudo natural or semi-synthetic receptors that are artificially engineered using natural subunits, such as aptamers. They are single-stranded nucleic acids with commendable specificity. Systemic Evolution of Ligands by Exponential Enrichment (SELEX), a combinatorial selection process, is employed to finalise the sequence of nucleic acids that successfully and selectively binds to the target analyte.^{13,14}

Each biorecognition element has inherent characteristics, advantages and disadvantages that would influence the overall performance of the biosensor.^{10,15} Their distinct structures contain strategic binding sites that interact strongly and selectively towards target analytes due to the complementary size, shape or functionality.¹⁶⁻¹⁸ Successful recognition would prompt two possible mechanisms, either catalytic or affinity.^{8,19-21} In the catalytic mechanism, the biorecognition element facilitates a chemical conversion of the analyte to corresponding products. It can subsequently regenerate into its active form and continuously consume the analyte, because of their reversible interaction, to attain a reusable biosensor. Enzymes are the most established biorecognition element for such mechanism, further exemplified by the prevalent glucose meters described earlier. In contrast, the affinity mechanism involves formation of a complex between biorecognition element and analyte. The complex cannot regenerate the initial state for further consumption of the analyte, eventually reaching equilibrium and results in a non-reusable biosensor. Pregnancy test kits rely on this mechanism, particularly the representative antibody-antigen complex. Upon introduction of hCG present in

a positive urine sample, the target analyte would be specifically captured by an antibody conjugated to labels and together flow towards the test line where a second antibody is immobilised. The analyte is captured once more to form a sandwich complex.^{22,23}

The recognition event, depending on mechanism, induce changes in chemical or physical properties of the biorecognition element or its environment. These changes vary as a function of analyte concentration and are translated into measurable quantities by transducers, the second main component of biosensors. Among the different transduction methods, also presented in Figure 2.1, electrochemistry has dominated where charge distribution and transfer related to the recognition event are converted to voltage, current or resistance, all of which are quantifiable electrical signals. It is predicted to maintain 70% of the biosensors market, although optical is narrowing the gap with advancements in miniaturisation.^{24,25} Optically active compounds are involved in recognition schemes of spectrochemical techniques, including fluorescence and chemiluminescence, to absorb or emit light at a specific wavelength for target analyte quantification.²⁶ Alternatively, light interaction can monitor physical properties that are altered upon binding, namely refractive index in techniques like surface plasmon resonance (SPR).²⁷ The next significant transduction method is acoustic wave, relying on piezoelectric materials to interconvert mechanical and electrical energy while detecting changes in mass or interfacial properties attributed to the recognition event.^{28,29}

Biosensors, characterised by biorecognition element and transducer, encompass easy-to-use and portable devices as well as sophisticated and high-throughput laboratory instruments.³⁰ Not limited to glucose meters and pregnancy test kits, biosensors have been successfully developed and commercialised for a multitude of target analytes and applications.^{31,32} They are of medical utility in the detection of metabolites, other than glucose, and disease-related biomarkers.^{33,34} An established example is Abbott's i-STAT system, a handheld device with single-use cartridges, commonly used by medical staff at the patient's

bedside. The system is capable of quantifying various analytes, including lactate, urea and cardiac markers, without sending samples to a central laboratory in order to make timely decisions.³⁵ In parallel, there are threats posed by bacteria and virus (pathogens) together with their toxins, naturally occurring or deliberately released, and magnified by lowered barriers of international travel.³⁶ Their detection, achieved by biosensors and traditional bioanalytical techniques, would facilitate appropriate response to manage infectious diseases, such as human immunodeficiency viruses (HIV) and influenza.^{32,37} Pathogens and toxins persist beyond the healthcare industry, to environmental monitoring, food safety and quality, along with security, areas where biosensors are valued.^{32,38-43}

Granted their expanding significance in various applications and reputation as advantageous analytical devices, new or improved biosensors are continuously in demand. They are especially sought in healthcare, challenged by the ageing population as well as increasing prevalence of chronic and infectious diseases. Accurate diagnosis prior to treatment and prevention of disease are potentially attainable by biosensors, in addition to over-the-counter consumer products which advocate patient self-monitoring.³⁰ Current research efforts gravitate towards ultrasensitive biosensors that are capable of detecting target analytes at sub-picomolar concentrations, or even a single molecule.^{44,45} This would unearth opportunities in the detection of new biomarkers to provide fundamental insights into diseases, or established biomarkers at lower concentrations in non-invasive samples.^{46,47} Together with miniaturisation, biosensors with enhanced sensitivity while fulfilling analytical and cost requirements are promising for wearables and point-of-care diagnostics. Among key drivers in the advancement of biosensors are nanomaterials and printing technologies, further elaborated in subsequent chapters.

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Chapter 3

Background of Electrochemical Biosensors

3.1 Why Electrochemical Transducers?

Electrochemistry dominates the various transduction modes of biosensors, about 70% of the market, credited to its early inception and successful commercialisation.^{1,2} The seminal vision of integrating enzymes with electrodes by Clark and Lyons³ spurred tremendous research efforts towards its realisation. Enzyme electrodes, biocatalytic membrane electrodes and bioelectrodes were earlier terminologies of biosensors, emphasising the historical significance of electrochemical transducers.⁴ Although the field progresses to include other biorecognition elements and transducers, electrochemistry achieved breakthroughs that even engaged the world's major diagnostic companies. Mediated electrochemistry⁵ and advances in manufacturing enabled the mass production of self-testing strips, used with portable glucose meters. The successful commercialisation of a home-use device catapulted electrochemical biosensors ahead of other competing technologies.⁶

The steadfast efforts in electrochemical biosensors are fuelled by the transducer's attractive features and advantages over conventional analytical tools.^{7,8} The instrumentation is simple and inexpensive, yet capable of rapid and sensitive quantification. In addition to its easy usage, there can be minimal or no sample processing required since coloured or turbid fluids would not interfere with measurements. Even very small sample volumes can be used since detection effectively occurs at the electrode-solution interface instead of the bulk volume. Moreover, the feasibility of miniaturisation to manufacture robust and portable electrochemical biosensors broadens their applicability for decentralised testing.

In subsequent parts within this chapter, immobilisation and transduction techniques are described to respectively address how biorecognition elements are incorporated in the electrochemical detection system, as well as the translation of molecular recognition events into measurable signals.

3.2 Immobilisation of Biorecognition Elements

The definition of biosensors requires biorecognition elements and the transducer to be in intimate, direct spatial contact. Pertaining to electrochemical biosensors, biorecognition elements are immobilised on an electrode surface as both molecular recognition and transduction event occur at the electrode-solution interface. Immobilisation, generally referring to the confinement of biorecognition elements on solid support, is desirable to increase their stability.^{9,10} Compared to being mobile in solutions, they are less perturbed by the environment and more likely to maintain their native conformations, thereby avoiding denaturation.¹¹ For enzymes, immobilisation enables their recovery and continuous use as catalysts to optimise their activities, costs and environmental benefits.¹²⁻¹⁴

Immobilisation techniques herein are elaborated specifically to enzymes since they are the primary biorecognition element described in this thesis, nonetheless still relevant to others such as nucleic acids and aptamers.¹⁵ The main immobilisation techniques are non-covalent adsorption, covalent bonding, cross-linking, as well as entrapment. There are several considerations when choosing a technique, especially the surface properties of the enzyme and electrode.¹⁶ The choice of technique, moreover, greatly influences the analytical performance of biosensors as enzymes' properties are implicated.¹⁷ Certain techniques promote better stability of enzymes, by retaining them on the surface and not denatured, consequently expected to generate biosensors capable of accurate measurements, sensor-to-sensor reproducibility and longer operational lifetimes. Techniques also induce different orientation of enzymes, methodical or random, which determine the accessibility of active sites for target substrates along with sensitivity of the biosensor. It is therefore imperative to understand each of the immobilisation techniques, including their mechanistic details as well as advantages and disadvantages, before selecting one that is suitable for the intended development and application of biosensor.

Non-Covalent Adsorption. This is the simplest immobilisation technique where a solution of the enzyme is placed in contact with the electrode surface for an allocated duration or until the solvent evaporates, also termed as drop cast. There are three possible non-covalent interactions leading to the adsorption of the enzyme onto the electrode surface, depending on their surface properties.¹⁶ Among the three is hydrophobic interaction, which involves weak van der Waals' forces between large lipophilic surface areas of enzyme and hydrophobic electrode surface. Adsorption is induced by a gain in entropy as large number of water molecules are displaced from both surfaces. On the contrary, the surface of enzyme is typically dominated by hydrophilic amino acid residues. Hydrophilic surfaces of both enzyme and electrode would favour hydrogen bonding, the second non-covalent interaction for adsorption. Lastly, ionic interactions govern the adsorption of charged enzymes, conditioned following its isoelectric point and pH of the solution, onto oppositely charged electrode surface.

Non-covalent adsorption, as a whole, is a straightforward and low-cost immobilisation technique. With no chemical modifications necessary of enzyme or electrode surface, it is non-destructive for enzyme activity. However, active sites may be blocked from target substrates due to non-specific, random orientation of enzymes. Another apparent drawback is the weak and reversible non-covalent interactions provoking the desorption of enzymes, especially when subjected to changes in pH, ionic strength, and temperature.

Covalent Bonding. This immobilisation technique involves the formation of stable chemical bonds between functional groups of enzyme and electrode surface. The leveraged functionalities should not be essential for catalytic activity and can be chemically incorporated or naturally present, such as amino ($-NH_2$) and thiol ($-SH$) moieties of lysine and cysteine respectively.¹¹ They are required to be activated prior to immobilisation, using reagents like carbodiimide ($RN=C=NR$) in the popular reaction between amino groups of enzyme and carboxylic acids ($-COOH$) on electrode, or vice versa. Besides amino and carboxylic acids,

thiol is another functional group commonly employed.¹⁸ There is an obvious chemistry between thiol and gold substrates, hence thiol-containing enzymes can directly immobilise, or chemisorbed, onto gold electrodes. Alternatively, thiolated compounds can be directed to assemble on gold electrode and perform as linkers, with terminal moieties binding to enzymes of choice.¹⁵

The core advantage of covalent bonding for immobilisation is the increased stability of enzymes. Retention of enzymes, together with their conformational integrity, contributes to minimal desorption and denaturation. At the same time, enzymes may still be deactivated or denature because of chemical modifications and reactions in the immobilisation process. Furthermore, the process is complex and requires high volume of bioreagents despite low immobilisation yield.¹⁹

Cross-Linking. It is an extreme case of covalent bonding between an enzyme and a reagent to establish a three-dimensional network of intermolecular cross-linkages, where the electrode surface is redundant. Glutaraldehyde, a 5-carbon dialdehyde, is distinctly the most effective cross-linking agent. On top of its low cost and commercial availability, it is highly reactive even in mild conditions. The aldehyde rapidly reacts with amine, among various enzyme moieties, in ambient temperature, near neutral pH and within four hours or less to yield chemically and thermally stable crosslinks.²⁰ To involve an electrode surface, the techniques of non-covalent adsorption and cross-linking are performed successively. Drawbacks of the former technique are overcome as enzyme stabilisation and retention are attained.²¹

Overall, this technique capitalises on the stability of covalent bonds yet is simple and inexpensive. The intermolecular cross-linkages between reagent and enzyme result in a more rigid molecule, reliable in resisting conformational changes in the presence of denaturation factors. Stability of immobilised enzyme onto electrode surface is enhanced although loss of

enzyme activity is possible. The random immobilisation orientation may not expose active sites to target substrates and enzyme itself may be distorted during cross-linking.

Entrapment. An enzyme is physically trapped within a polymer matrix or membrane developed on the electrode surface, namely electropolymerized film or silica sol-gel. Initially, the electrode surface is exposed to a solution of monomer or low molecular weight polymer solution, mixed with enzyme, before polymerisation via a chemical reaction or applied potential. Immobilisation is achieved as the resulting matrix on the electrode surface traps the enzyme, also shielding it from the external environment that may compromise its structure, including mechanical shear and hydrophobic solvents.²² The pores of the matrix should be adequately small to restrict the enzyme from diffusing out, yet large enough to allow movement of substrates and products. Even with the critical control of sizes, the matrix would serve as diffusional barriers which slow down the recognition process, especially with increasing matrix thickness.¹⁹

3.3 Electrochemical Techniques

The principle of electrochemical biosensors is the recognition event between immobilised biomolecule and target analyte, which elicits changes in electrical properties of the sensing material or solution such as ionic strength, potential and electric current. Amperometry, voltammetry, potentiometry and impedance are detection techniques selected to measure those changes through their respective transduction mechanisms.²³

Amperometry, Voltammetry. Among the handful of electrochemical techniques, amperometry and voltammetry are prevalently employed in biosensors. The electrochemical setup consists of either two electrodes, a working and reference electrode, or three with an additional counter/auxiliary electrode. A suitable potential is applied to the electrodes to drive electron transfer reactions. On the surface of working electrode, electroactive species would be

oxidized or reduced where the consumption or production of electrons would generate a measurable change in current that correlates to the concentration of target analyte.

Amperometry and voltammetry are distinguished by their respective applied potential profiles. The former applies a constant potential while monitoring changes in current, arising from electron transfer reactions, with respect to time. Charging current, the current responsible for applying potential to the system, is negligible because of the fixed potential. This minimises background noise and relatively magnifies the current signal to unearth lower detection limits.⁷ Additionally, measurements can be performed in flow conditions or with stirring to accelerate mass transport to the electrode surface. The amperometric technique thereby offers sensitive and robust detection for decentralised testing. On the other hand, the potential applied in voltammetry techniques is swept over a predetermined range. Cyclic voltammetry (CV) is recognised as a “fingerprint” technique to obtain qualitative information regarding electrochemical processes. A quantifiable and sensitive response, contrastingly, can be acquired with other excitation profiles like square-wave voltammetry (SWV) and differential pulse voltammetry (DPV). Current is sampled as a function of voltage where a peak denotes an electron transfer reaction, and the magnitude is translated from the concentration of electroactive species in the solution.

There are several detection schemes of amperometric and voltammetric-based biosensors, depending on the mechanisms of molecular recognition. In the catalytic mechanism, mostly concerning enzymes as discussed in the previous chapter, electrons are directly transferred from target substrate to electrode via the active site of enzymes.²⁴ However, the active sites are usually difficult to access so detection is achieved by relying on other electroactive species present. Co-substrates, responsible for regenerating enzymes, as well as products and co-products would provide quantification of the target substrate based on their current responses, if they undergo oxidation or reduction. Another approach is incorporating

non-physiological redox molecules as mediators to rapidly regenerate the enzyme's active site while transferring electrons to the electrode. Moving on to the affinity mechanism, the formation of complex between biorecognition element and complementary analyte generally does not yield any current response due to redox-inert nature of the involved species. Electroactive species are therefore included in detection schemes, namely labels attached to target analyte or redox probes present in the solution. In the case of the latter, electron transfer would be enhanced or impeded by the complex on the electrode surface.

Impedance. Impedance expresses the opposition to the flow of alternating current (AC) in an electrical circuit. In addition to resistance, which is the only opposition to direct current (DC) flow, there are other electrical parameters against AC. Capacitance comes into play as AC can flow across a capacitor via alternating charging-discharging processes.²⁵ A capacitor is fundamentally composed of an insulator separating two metal plates which become oppositely charged upon an applied voltage. When an electrochemical system is subjected to a potential, charges on the electrode are balanced by ions of opposite charges accumulated in the nearby solution. This capacitor-like structure on the electrode-solution interface, known as the electrical double layer, paves another way for current to flow and consequently contributes to impedance.

Developed on AC circuits, electrochemical impedance spectroscopy (EIS) presents a powerful technique to attain information pertaining electrical parameters, including resistance and capacitance, as well as electrochemical processes. Measurements are initiated by a sinusoidal AC excitation signal over a frequency range, with a small amplitude between 2 to 10 mV.⁷ The resulting current, sinusoidal AC of the same frequency, differs nonetheless in amplitude and phase following impedance of the system.^{26,27} This acquired data subsequently undergoes mathematical processing to be modelled into equivalent circuits. They are

representations of resistors and capacitors to simulate the AC response in electrochemical systems, allowing the determination of their corresponding values.

EIS is indeed a valuable detection technique in biosensors, owing to its sensitivity towards changes at the electrochemical interface. As biorecognition elements are immobilised onto the electrode surface, binding event with target analytes would modulate the resistance or capacitance of the system.²⁸ Faradaic EIS, one of the two measurement modes, implicates the reversible electron transfer of a redox couple in solution. Biased at, or close to, its half-wave potential, the generated current would be perturbed by steric or electrostatic effects of the biorecognition element. Such effects are reported by charge transfer resistance (R_{ct}) from equivalent circuits, with varying values computed upon immobilisation and molecular recognition event. Conversely, redox couples along with their electrochemical reactions are absent in non-Faradaic EIS, which instead probes the interfacial capacitance. Immobilisation and molecular recognition event ensue changes at the electrode-solution interface, particularly charge distribution at the double layer, thereby influencing the value of double-layer capacitance (C_{dl}) parameter in equivalent circuits. An apparent advantage of EIS-based biosensors is the independency from mediators to access active sites, and labels to garner measurable responses. A label-free detection is possible and brings along advantages such as higher signal-to-noise ratio and lower assay cost.⁷ At the same time, non-specific binding or adsorption of solution components should be minimised.

Potentiometry. This electrochemical technique is distinctly characterised by the negligible, or ideally zero, current while measuring the potential difference between working and reference electrodes in solution. Since most potentiometric biosensors use ion-selective electrode as the working electrode, the potential difference is derived from activity of ions.^{29,30} Biorecognition elements that are immobilised on the ion-selective membrane would interact with target substrate, leading to the production of ions or changes in ion transport.²⁸ In turn, a

non-uniform distribution of electric charges develops at the membrane-solution interface and gives rise to the measurable potential difference.

An advantage of the potentiometry technique is its capability to detect redox-inert species under equilibrium conditions since applied potential, which drives electron transfer reactions, is not required. At the same time, other ions present in the solution may pose as interferences, especially at high concentrations where there is a more conspicuous effect of ionic strength. There would be significant deviations in measurements and sensor has to be recalibrated frequently because of the potential drift.²³

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PART I

TWO-DIMENSIONAL MATERIALS

Part I

Chapter 4

Background of Two-Dimensional Transition Metal Dichalcogenides

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The thesis author is the first author of the above paper.

4.1 Two-Dimensional Nanomaterials

The “nano” concept was first conveyed by Richard P. Feynman in 1959. His renowned talk titled “there’s plenty of room at the bottom” was an invitation to manipulate and control things at the atomic level that could consequently present a plethora of opportunities.¹ This concept was formally termed as “nanotechnology” by Eric Drexler in 1986 and attracted a broad range of disciplines working at a size range of 1 – 100 nm.^{2,3} Ever since, there has been an exponential growth of activities in this field worldwide, driven by the exciting applications and economic impacts of understanding new science.⁴

Nanomaterials have been garnering attention and studied intensively towards improving the performance of biosensors. Their small and quantum size effect as well as surface and interface effect remarkably improve the important performance indexes of biosensors.⁵ Modification of conventional electrodes with nanomaterials addresses the size mismatch between electronic transducer and biological elements, which significantly enhance biocompatibility and sensitivity.⁶ Meanwhile, the quantum size effects unearth catalytic activities not found in bulk structures, due to shifts in the valence band as well as oxidation potentials.

The burgeoning research on 2D materials began after graphene was isolated and its enthralling electronic properties were reported in 2004.⁷ 2D refer to materials with one dimension restricted to a single-atom layer, while in literature they include monolayer and few-layer nanomaterials.⁸ 2D materials not only possess favourable size characteristics, but also dimensionality and signature properties that will enhance the performance of biosensors. These planar materials flaunt a large surface-to-volume ratio thereby providing a platform for straightforward and easy functionalization with biological elements or chemical moieties.⁹ The optimal exposure would increase efficiency as well as sensitivity.¹⁰ Attributes of high

conductivity and chemical stability, depending on the degree of exfoliation, further results in desirable electrochemical properties like enhanced heterogeneous electron transfer (HET) and water electrolysis reactions. Together with biocompatibility, 2D materials are greatly sought after the development of high-performance electrochemical biosensors.^{11,12} While the enzyme offers selectivity in a biosensor, 2D materials work as transducers because of their electrical conductivity, biocompatibility and stability.

2D materials are classically represented by graphene since it has been pursued with great fervour, and is the first in its class to enter the market.^{13,14} A roadmap has been laid out, with ambitions that laboratories discoveries would transform into real applications and products.¹⁵ Following the reignited interest in graphene, transition metal dichalcogenides (TMDs) is another group of 2D materials that has been pursued. Its research has well-progressed and contemporarily riding the waves of printing¹⁶⁻¹⁹ and flexible²⁰⁻²³ technologies.

With a general formula of MX_2 , transition metal layers are sandwiched between two chalcogen layers (S, Se, Te) via covalent bonds and stacked together by weak van der Waals interactions. Interest in TMDs arises when they are discovered to possess versatile chemistry unlike graphene. In contrast to graphene which lacks the band gap, TMDs have sizeable bandgap.²⁴ The transition from an indirect to a direct bandgap occurs when single or few layers to TMDs are obtained. As a consequence of quantum confinement and surface effects, unique electrical and optical properties are attained. Another parameter, conductivity, can be changed between metallic and semi-conducting which consequently tune fluorescent and electrochemical performances.²⁵ Apart from dimensionality, one attractive aspect of TMDs is elemental composition. There are about 40 layered compounds in the library of TMDs²⁶, of varying transition metal or chalcogen component, showcasing diverse polymorphs, properties and versatile chemistry.^{27,28}

4.2 Structure of 2D TMDs

Transition metal (M) and chalcogen (X) atoms are held together by a predominant covalent bond, but not all configurations exist in layered structures. The periodic table in Figure 4.1 highlights the selected transition metals from group 4 –7 and 10, that will form layers with the chalcogens (X = S, Se, Te). There is also a handful of elements- group 9 metals and nickel that are partially highlighted since only some of the dichalcogenides are layered.²⁶ These compounds of specific elemental composition form layered sheets, possessing a general formula of MX_2 where the oxidation states of M and X are +4 and -2 respectively.

H	MX ₂ M = Transition metal X = Chalcogen																He
Li	Be											B	C	N	O	F	Ne
Na	Mg	3	4	5	6	7	8	9	10	11	12	Al	Si	P	S	Cl	Ar
K	Ca	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br	Kr
Rb	Sr	Y	Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I	Xe
Cs	Ba	La - Lu	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At	Rn
Fr	Ra	Ac - Lr	Rf	Db	Sg	Bh	Hs	Mt	Ds	Rg	Cn	Uut	Fl	Uup	Lv	Uus	Uuo

Figure 4.1. Highlighted transition metals (M) and chalcogen (X) elements which are found to exist in layered structures, with a general formula of MX_2 . Transition metals in group 9 and 10, except Pd and Pt, are partially highlighted as only some of the dichalcogenides are layered. Figure reproduced with permission from ref. 27, Copyright 2013 Springer Nature.

Unlike its graphite analogue, each TMD layer consist of three atomic layers (X-M-X) and could stack in different orientations via van der Waals forces. This calls for a more detailed description of the structure, polymorphs. The most commonly found polymorphs for TMDs are 1T, 2H and 3R, although only 1T and 1H are observed for monolayer TMDs. The digit represents the number of layers in the crystallographic unit cell while the letter denotes

tetragonal (T), hexagonal (H) or rhombohedral (R) symmetry. Other information that can be described by a given polymorph are stacking sequence, metal coordination as well as electronic structure. Figure 4.2 illustrates and summarises the three common polymorphs found in TMDs.

Figure 4.2. Common polymorphs observed for 2D TMDs- 1T, 2H and 3R. Figure reproduced with permission from ref. 29, Copyright 2015 American Chemical Society.

A closer look at the metal coordination, differentiating between octahedral and trigonal prismatic, is presented in Figure 4.3. They are formed accordingly when two chalcogenide layers stagger or stack directly above each other. The influential factors are the *d*-electron count of the metals as well as packing considerations, where the latter refers to size cavities for metals and sizes of the chalcogenides. Informed of the various possible structures, the preferred polymorph for a specific TMD is ultimately determined by the *d*-electron count of the transition metal. Table 4.1 captures the typical polymorphs for each transition metal component.

Figure 4.3. Two chalcogenide layers stagger or stack above each other, forming octahedral (left) and trigonal prismatic (right) holes respectively. Figure reproduced with permission from ref. 30, Copyright 2015 American Chemical Society.

Table 4.1. Typical metal coordination of various transition metals in layered structures, depending on their *d*-electron count.

Transition Metal	<i>d</i> -electron count	Metal coordination
Group 4: Ti, Hf, Zr	d^0	Octahedral
Group 5: V, Nb, Ta	d^1	Octahedral & trigonal prismatic
Group 6: Mo, W	d^2	Trigonal prismatic
Group 7: Tc, Re	d^3	Distorted octahedral
Group 10: Pd, Pt	d^6	Octahedral

4.3 Preparation of 2D TMDs

There are various established synthetic routes to prepare 2D TMDs, categorised as either top-down or bottom-up, and they each generates nanosheets of different characteristics. Therefore, it is crucial to consider the preparation method when studying a material due to the significant influence on key properties such as electrical conductivity, stability and high surface area. Top-down methods generally starts with bulk TMDs, then exfoliation to strategically overcome weak van der Waals forces between the layers. This is achieved through mechanical, chemical or liquid-based ultrasonic exfoliation.³¹ On the other hand, bottom-up approaches

yield TMDs from their respective precursors. They include chemical vapour deposition (CVD) and wet chemical methods.³²⁻³⁵

It is imperative to primarily discuss the preparation methods of 2D materials since their properties will be greatly influenced. Sonication-assisted liquid exfoliation is one of the top-down methods which scales down bulk material to single or few layers dispersed in solvents. Isopropyl alcohol (IPA), dimethylformamide (DMF), and N-methyl pyrrolidone (NMP) are amongst solvents that have been suggested for effective exfoliation.³⁶ Including ethanol (EtOH)/water, these four solvents are studied in the preparation of MoS₂ nanosheets towards a lactate electrochemical biosensor.³⁷ Lactate oxidase enzyme is drop casted following the layer of exfoliated materials on GC, to selectively detect lactate with hydroxymethylferrocene as mediator. Comparing lactate response in cyclic voltammograms, MoS₂ exfoliated in NMP solvent is asserted to have superior conductivity based on the highest electrochemical signal generated. An apparent explanation is the surface energy of NMP being close to MoS₂, therefore attaining quality MoS₂ nanosheets with enhanced conductivity.

The preparation method of interest is liquid exfoliation upon intercalation. Intercalation of TMDs has long been a topic of literature as there was a search for electron donating species that could insert between layers. Alkali metals were realised to be effective intercalating agents due to their very electropositive nature.^{38,39} Further refinement of the method was necessary to overcome drawbacks mainly caused by the high temperature. This was achieved by employing *n*-butyllithium (n-BuLi) in hexane at an ambient temperature, forming highly crystalline stoichiometric intercalated products while eliminating undesired side products.^{40,41} The breakthrough for the method was when Morrison and co-workers prepared single-layered TMDs.⁴² They took a step further by exfoliating lithium intercalated MoS₂ in water that generated hydrogen gas, which further expanded and separated the layers. The chemical reactions behind the method are as follows⁴³:

Apart from yielding 2D TMDs with remarkable properties, a phase transition is observed that is unique to the lithium intercalation method.²⁷ The electron donating nature of lithium induces an effective change in the d electron count, consequently destabilising the original 2H phase to form 1T phase.⁴⁴ Conductivity can be changed from metallic to semi-conducting which consequently tune electrochemical performances.⁴⁵ The metallic phase has been distinguished to exhibit excellent capacitive behaviour and enhanced electrocatalytic performance towards gas sensing as well as hydrogen evolution reaction.⁴⁶ The electronic structure corresponding to the phase transition is shown in Figure 4.4.

Figure 4.4. Electronic structures of 1T and 2H (or 3R) phases upon intercalation by a donor atom, typically alkali metals such as lithium. Figure reproduced with permission from ref. 47, Copyright 2011 American Chemical Society.

4.4 Relevant Properties for Biosensors

Inherent electrochemistry of materials is an important characteristic of consideration prior to their applications in electrochemical biosensors. Intrinsic reduction or oxidation of the material may either pose an interference or advantage to the target signal. Wu et al. reported the first biosensor based on MoS₂, who puts forth an irreversible electrochemical reduction of the nanomaterial in NaCl aqueous solution saturated with N₂.⁴⁸ Comparing MoS₂ and the electrochemically reduced MoS₂, signals arise for the latter when probed with redox couples such as [Fe(CN)₆]^{3-/4-} and [Ru(NH₃)₆]^{2+/3+}, implying efficient electron transfer and good conductivity. It is further modified with glucose oxidase (GOx), the biorecognition element, to analyse its sensing capabilities towards glucose. Recognizing the inherent electrochemistry of the material, electrochemical pretreatment presents a straightforward strategy for enhancing the conductivity of the material which resultingly improves the sensitivity of the biosensor.

MoS₂ nanosheets are revealed to be negatively charged through zeta potential measurements. Such property proves to be desirable in the construction of biosensor as positively charged enzymes can be immobilised onto the electrode surface. This is demonstrated for the detection of H₂O₂ where electrostatic interactions between MoS₂ and HRP stabilize the electrode system.⁴⁹ The biocompatibility of MoS₂ nanosheets is also assured, Fourier-transform infrared (FTIR) spectroscopy as well as cyclic voltammograms indicate that the assembled HRP maintain its native activity as it effectively reduces H₂O₂ with an aid of hydroquinone mediator.

The negative charges of MoS₂ can further be relied on to form nanocomposites with noble metal particles, namely gold nanoparticles (Au NPs). Above pH 7.4, the surface charges of Au NPs are positive therefore spontaneous as well as homogenous self-assembly can occur onto MoS₂ via electrostatic interactions.⁵⁰ The elemental presence of sulfur in MoS₂ also allows

bonding with Au NPs.⁵¹ While MoS₂ nanosheets intrinsically demonstrate fast electron transfer kinetics, MoS₂/Au NPs improve the dynamic range as well as sensitivity for glucose or cholesterol. Forming nanocomposites with Au NPs^{52,53} or Au nanorods⁵⁴ is a prevalent approach due to its established contribution in electrochemical biosensors, especially increasing electroactive surface area and conductivity. A strong synergistic effect of higher enzyme loading and facilitated electron transfer are consequently achieved. There are also other MoS₂ nanocomposites mixed of varying dimensionality- 0D graphene quantum dots (QDs),⁵⁵ 1D bimetallic alloy nanowires⁵⁶ and 2D graphene.⁵⁷

Apart from enhancing electrochemical signal transduction, 2D materials continue to fascinate us with another of their attribute in relation to nucleic acid-based biosensors. The physisorption of aromatic and conjugated compounds on the basal planes of MoS₂ inspired and led to the realization of contrasting affinity towards single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA).^{58,59} Higher affinity, therefore higher adsorption, are observed for ssDNA due to van der Waals' forces between the nucleobases and 2D materials. On the other hand, the nucleobases are buried between the densely negatively charged helical phosphate backbone for dsDNA, which accounts for the low affinity and weak adsorption.

A correlation between chemical compositions of TMDs and their differential affinity towards DNA were studied within group 6 TMDs (MoS₂, MoSe₂, WS₂, WSe₂).⁶⁰ Based on their interactions with hairpin DNA, greater physisorption occurs for Mo than W, Se than S components. Acknowledging that chemical composition plays a significant role in influencing a biosensor's performance, 2D materials offers a myriad of compounds varying from graphene, TMDs to pnictogens. Their properties can be exploited and further fabricated into electrochemical biosensors, like the papers discussed above. It is therefore promising to explore other 2D materials apart from group 6 TMDs (MoS₂, WS₂).

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Part I

Chapter 5

Phase Engineering of Group 6 Transition Metal Dichalcogenides
(MoS₂, MoSe₂, WS₂, WSe₂)

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The thesis author was the primary investigator and first author of the above paper.

5.1 Introduction

The interest in two-dimensional (2D) nanomaterials resurfaced after graphene along with its striking properties was discovered in 2004.¹ There is a broad range of layered bulk materials that can be exfoliated to obtain 2D structures or ultrathin sheets. Nonetheless, transition metal dichalcogenides (TMDs) have been the centre of attention following graphene. An archetypal compound of TMDs is MoS₂, prevalently investigated because of its establishment in several applications as well as natural occurrence.² Lithium intercalation exfoliation is amongst several other methods, namely micromechanical exfoliation,^{3,4} liquid-based direct exfoliation⁵ and chemical vapour deposition,⁶⁻⁸ to prepare single or few-layered nanosheets of TMDs. Owing to their size and dimensionality, a myriad of applications has been realised in optoelectronics,⁹ supercapacitors,¹⁰ (bio)sensors^{11,12} as well as lubrication of interfaces.¹³ Their commendable electrochemical properties, especially fast heterogeneous electron transfer (HET) capabilities, and tuneable band gap explains their dominance.^{14,15} In addition, they possess lower cytotoxicity compared to graphene rendering them suitable for biosensors.¹⁶ TMDs, with such fascinating attributes, have stood out as the budding material for biosensor development towards environment and biomedical applications.

The world biosensors market is dominated by glucose biosensors, estimated about 85%, because of the prevalence of diabetes in developed nations.¹⁷ At the same time, electrochemical biosensors are touted as a particularly promising transduction class of analytical devices due to their exceptional performance superior to conventional analytical tools.¹⁸ They generate quantitative and robust measurements based on simple and low-cost instrumentation, triggering the possibility of portability and miniaturisation towards the ideal biosensor.¹⁹ Integrating a biological recognition element, prominently glucose oxidase, electrochemical biosensor boasts a high degree of selectivity towards glucose. Needless to say, there are other analytical aspects such as sensitivity, and nanomaterials have been widely studied to enhance the performance of

biosensors. Recently, the exploration of 2D nanomaterials is rising in the biosensor field due to their favourable electrochemical properties.²⁰

The aim of this study is to investigate the potential of MoS₂ analogues towards development of a 2nd generation electrochemical glucose biosensor. Group 6 TMDs- MoSe₂, WS₂, WSe₂ together with MoS₂ are materials of interest prepared by *t*-BuLi intercalation and exfoliation. Their intriguing HET capabilities will be compared, and the superior pure TMD(s) is further developed into a biosensor without any doping or decoration with nanoparticles of noble metals.

5.2 Results and Discussion

Heterogeneous electron transfer (HET) rates of Group 6 TMDs (MoS₂, MoSe₂, WS₂ and WSe₂), chemically exfoliated using tert-butyllithium (*t*-BuLi) organic intercalant, are analysed towards developing a 2nd generation electrochemical glucose biosensor. Once the materials are prepared, various characterisation methods including transmission electron microscopy (TEM) and X-ray photoelectron spectroscopy (XPS) are relied on for elucidating the structure of the exfoliated compounds along with their properties. These TMDs are incorporated into the biosensor by drop cast onto bare glassy carbon electrode (GCE), before glucose oxidase (GOx) and glutaraldehyde (GTA) (Scheme 5.1). Their performances in detecting glucose are compared *via* oxidation signals of ferrocenemethanol (FcMeOH), the mediator. Analytical parameters of the glucose biosensor based on the superior performing TMD(s) are established thereafter.

Scheme 5.1. Schematic representation illustrating (a) exfoliation of bulk TMDs employing *t*-BuLi as intercalating agent; construction of biosensor electrode by drop casting (b) exfoliated TMDs onto bare GCE followed by (c) GOx then (d) crosslinking using GTA; lastly, (e) electrochemical detection mechanism of glucose.

The four Group 6 TMDs were characterised after their preparation by *t*-BuLi intercalation and exfoliation. TEM images exhibit distinct few-layer sheets with lateral dimensions up to a micron, also reflecting the varying extents of exfoliation of the materials (Figure 5.1A-D). High-resolution TEM (HR-TEM) and selective area electron diffraction (SAED) were additionally carried out to shed light on atomic arrangement. Both honeycomb and hexagonal lattices are observed, implying that both 2H and 1T phases are present accordingly (Figure 5.1E- H).²¹

Figure 5.1. (A) TEM micrographs with SAED (inset), and (E-H) HR-TEM of the *t*-BuLi based exfoliated WS_2 , WSe_2 , MoS_2 and MoSe_2 in order. Scale bar: TEM= $1\mu\text{m}$ and HR-TEM= 10 nm.

The existence of the 1T phase is a prominent feature of lithium intercalation method, where phase transition occurs.²² The electron donating nature of lithium induces an effective change in the d electron count of the host, consequently destabilising the naturally occurring 2H phase to form 1T phase.^{23,24} The crystal symmetry and electronic structures differ across phases, along with their properties.²⁵ 2H phase represents a hexagonal symmetry where layers of chalcogen atoms are stacked above each other, while the sandwiched transition metal adopts a trigonal prismatic coordination. In contrast, chalcogen layers are offset from each other to establish a tetragonal symmetry as well as an octahedral metal coordination in the 1T phase. The conductivity of 2H is semiconducting whereas 1T is metallic, in which the latter is recognised for its reactivity towards functionalisation^{26,27} as well as enhancing electrochemical properties.²⁸ Moreover, both edge and basal sites are active in 1T phase TMDs, further improving the electrocatalytic performance.^{29,30} With good conductivity, fast electron transfer kinetics and increased catalytic sites, these 1T-TMDs are anticipated to promote the signal transduction of an electrochemical biosensor.

Figure 5.2 displays the X-ray diffraction (XRD) data, shedding light on the extent of exfoliation and phase purity. A pronounced (00l) diffraction peak is observed for all exfoliated TMDs, implying a well-ordered stacking structure composed of few layers.^{31,32} With an exception of MoSe₂, the materials prepared are pure along with a high preferential orientation. The diffractogram of MoSe₂, on the contrary, reveals peaks that are indexed to the semiconducting 2H phase (ICSD No. 644346).^{33,34} This highlights the significant phase existence in MoSe₂.

Figure 5.2. X-ray diffraction (XRD) of (A) WS₂, (B) WSe₂, (C) MoS₂ and (D) MoSe₂ *t*-BuLi exfoliated TMDs.

Upon characterisation of the exfoliated 2D TMDs and realisation of their metallic and crystalline nature, the electrochemical performances of these materials were studied prior to the development of an electrochemical glucose biosensor. A study on their heterogeneous electron transfer (HET) capabilities was conducted by employing ferro/ferricyanide, a redox probe sensitive towards the surface of electrode materials.³⁵ HET rate is an imperative electrocatalytic parameter correlated to peak-to-peak separation (ΔE_p) where narrow separation between anodic and cathodic peaks translates to fast HET rate.³⁶ ΔE_p of the four materials were attained and ranked in ascending order: WSe₂ < WS₂ < MoSe₂ < MoS₂ < Bare GCE (Figure 5.3A). With the smallest ΔE_p , WSe₂ demonstrates the fastest HET rate and therefore offers the best electrocatalytic performance for sensing applications.

As FcMeOH is involved as the mediator to 2nd generation biosensors, HET capabilities of the materials were compared in an electrolyte consisting of FcMeOH dissolved in PBS. A slight deviation from the previous results were obtained based on ΔE_p : $\text{WSe}_2, \text{bare GCE} < \text{WS}_2 < \text{MoSe}_2 < \text{MoS}_2$ (Figure 5.3B). Nonetheless, the tungsten dichalcogenides outperformed their molybdenum counterparts with WSe_2 again demonstrated the best HET capability.

Figure 5.3. Cyclic voltammograms of the four prepared TMDs against bare GCE in (A) 5 mM $[\text{Fe}(\text{CN})_6]^{4-/3-}$ in 0.1 M KCl and (B) 2 mM FcMeOH in PBS (pH 7.2) for HET studies. Conditions: scan rate, 100 mV s^{-1} .

After determining the HET capabilities, the 2nd generation GOx/ *t*-BuLi- based TMDs glucose biosensor are implemented and evaluated. Figure 5.4 presents the cyclic voltammograms of the biosensor in 2mM FcMeOH in PBS (pH 7.2) and in the presence of 10 mM or 0.5 mM of glucose accordingly. The disparity in the glucose detection response is apparent among the materials.

Figure 5.4. (A) Cyclic voltammogram (10 mM glucose; scan rate, 100 mV s⁻¹) and (B) chronoamperometry data (0.5 mM glucose added every 100 seconds; potential, 0.1 V) of 2nd generation electrochemical glucose biosensor based on different 1T-TMDs. Conditions: electrolyte, FcMeOH (2 mM) in PBS (pH 7.2).

Comparing the performance of the glucose biosensor as a function of the TMDs, both WS₂ and WSe₂ similarly generated the highest response, followed by MoS₂ then MoSe₂. The superior performance of WX₂ (X= S, Se) is credited to their significant metallic 1T phase, the phase responsible for the commendable electrocatalytic activity. This is substantiated by high-resolution XPS, which reveals their surface elemental composition. Zooming in to the high-resolution W 4f spectra (Figure 5.5), peaks corresponding to W 4f_{7/2} and W 4f_{5/2} are observed. Further deconvolution of both metal and chalcogen spectra provides the phase composition, 2H and 1T,²⁹ in which the latter is dominant. Cross-referencing to relevant XPS data in literature, the metallic phase of WX₂ is higher in composition than MoX₂ for both X=S, Se.³⁷

Figure 5.5. High resolution X-ray photoelectron spectra of (A) W 4f and (B) S 2p regions of 1T-WS₂, (C) W 4f and (D) Se 3d regions of 1T-WSe₂.

One factor which influences the higher proportion of metallic 1T phase in WX₂ (X = S, Se) is the extent of exfoliation, which depends on the metal/chalcogen composition and intercalating agent.³⁸ Apparent in MoSe₂, earlier characterization data presented in this article shows the poor exfoliation of the material. The phase transition to 1T could be inefficient, therefore lacking in metallicity to promote the biosensor's performance. Another plausible contributing factor is the stability of the metallic 1T phase. It is to be put forth that 1T-WS₂ is more stable than MoS₂^{21,39} based on the transition temperatures and activation barrier for the 1T to 2H.^{40,41} Our results suggest that the comparison could extend beyond sulfides and be applied to selenides as well.

Figure 5.6. Energy dispersive X-ray spectra (EDS) of the *t*-BuLi based exfoliated TMDs, together with their respective elemental atomic percentage.

Energy dispersive X-ray spectroscopy (EDS), may also provide an explanation for the electrochemical performances. Apart from verifying the elemental composition of the materials, the elemental atomic percentages are acquired (Figure 5.6). The metal: chalcogen ratio (M:X) of WX₂ are closer or even exceeding 2, the ideal theoretical value for TMDs, unlike MoX₂. Henceforth, M:X ratio possibly influence electrochemical properties.

Overall, the various characterisation techniques- XRD, XPS, and EDS substantiate the superior electrochemical performance and HET capabilities of WS₂ and WSe₂. The dominance of 1T phase, together with a satisfactory metal:chalcogen ratio, are important attributes to possess, especially in the context of biosensors. Subsequently, the glucose biosensors are developed based on these two tungsten dichalcogenides.

The detection mechanism of glucose in this 2nd generation biosensor begins with an enzymatic breakdown, particularly at the flavin redox centre (FAD) of GOx (Equation 1). Instead of relying on O₂ as the cofactor to regenerate FAD-GOx, FcMeOH intervenes as the mediator (Equation 2). The electron transfer to the electrode surface is made more efficient since FcMeOH is sufficiently small to access the deeply buried flavin redox centre.^{42,43} Interconversion between ferrocene (FcMeOH) and ferrocenium ion (Fc⁺MeOH) eventually gives rise to an oxidation signal which correlates to glucose levels (Equation 3).⁴⁴ This signal is achieved more efficiently and at a lower potential due to the presence of TMDs.

TMDs

100 mV

The mechanism of the glucose biosensor is confirmed on the basis of WS₂ as a model. Two assays were performed, one in N₂ purged FcMeOH/ PBS buffer solution while the other in unpurged condition (Figure 5.7A). The same trend is observed for both conditions where anodic current signals increase significantly in the presence of glucose. This confirms the detection mechanism for the developed biosensor. However, the current signals are slightly lower in unpurged solution, indicating a possible competition with oxygen to regenerate the oxidised form of the redox centre of GOx. The signal is nonetheless from the oxidation of FcMeOH ion, performing its part as a mediator.

Figure 5.7. (A) Cyclic voltammogram comparing responses of WS₂-based biosensor in N₂ purged against unpurged conditions (25 mM glucose, scan rate, 100 mV s⁻¹) and (B) chronoamperometry data (0.5 mM glucose added every 100 seconds; potential, 0.1 V) of 2nd generation electrochemical glucose biosensor based on different electrode configurations. Conditions: electrolyte, FcMeOH (2 mM) in PBS (pH 7.2).

Moving on to optimise the electrochemical glucose biosensor, the concentration of 1T WX₂ drop casted onto bare GCE was varied. A 5.0 mg/mL suspension in water produces the best results (Figure 5.8). A lower concentration implies lesser amount of the catalytic material to enhance the signal, whereas too high a concentration of TMDs on electrode surface may block the signal transduction.

Figure 5.8. Chronoamperometry data of different concentrations of (A) WS₂ and (B) WSe₂ for the 2nd generation electrochemical glucose biosensor. 5 mM of glucose was added every 100 seconds. Conditions: electrolyte, FcMeOH (2 mM) in pH 7.2 PBS; potential, 0.1 V.

Another aspect optimised was the operating potential based on selectivity, to fulfil an analytical criterion of any developed biosensor. There are endogenous compounds present in blood, at concentrations 30 times lower than glucose,⁴⁴ such as L-ascorbic acid (AA), dopamine hydrochloride (DA) and uric acid (UA). They pose as interferences to the measurements since they are electrochemically active. To preferentially detect the analyte of interest, the operating potential could simply be tuned. This was carried out while adding physiological levels of glucose (5 mM) and interferences (0.2 mM). For both WS₂ and WSe₂, low potentials of 0.10 V and 0.05 V successfully eliminate contributions from the aforementioned compounds. The operating potential is ultimately set at 0.10 V as the intensity of the desired signal is not compromised (Figure 5.9).

Figure 5.9. Chronoamperometry data of both WX_2 -biosensors, (A) WS_2 and (B) WSe_2 , for interference studies. Aside from glucose (5 mM), interferences (0.2 mM) referring to L-ascorbic acid (AA), dopamine hydrochloride (DA) and uric acid (UA), were added sequentially every 100 seconds at varying potentials. Conditions: electrolyte, FcMeOH (2 mM) in pH 7.2 PBS.

After determining the optimum working potential where the interferences species do not affect the biosensors' response, the calibration curves were performed for both biosensor WX_2 materials. WS_2 based biosensor detects glucose in three linear ranges (Figure 5.10A, B), 176- 766 μM ($r = 0.997$), 1.3- 7.7 mM ($r = 0.996$) and 7.7- 22.3 mM ($r = 0.995$) while WSe_2 based biosensor in two (Figure 5.10C, D), 77-274 μM ($r = 0.999$) and 0.77- 22.3 mM ($r = 0.998$). Meanwhile, limit of detection (LOD) as well as limit of quantification (LOQ) are computed following the IUPAC approach.⁴⁵ Representing the lowest concentration of analyte

that can be reliably measured by an analytical procedure, LOD and LOQ were respectively computed to be 82.6 and 275 μM for WS_2 and 52.0 and 173 μM for WSe_2 .

Figure 5.10. Chronoamperometry data and calibration plots for (A, B) WS_2 and (C, D) WSe_2 based electrochemical glucose biosensors. Conditions: electrolyte, FcMeOH (2 mM) in pH 7.2 PBS; potential, 0.1 V.

In terms of analytical aspects, the WSe_2 -based biosensor performed better. Two linear ranges were attained, the second one covering a wide spectrum of concentrations. Furthermore, the LOD and LOQ are lower compared to WS_2 . This is justified by the HET rate where WSe_2 gave rise to narrow ΔE_p implying better capability for electrochemical sensing of glucose. Another notable observation is the other peak corresponding to W (VI) in XPS data. This is contributed by the oxidised species WO_3 that may form during sample preparation and

transfer.⁴⁶ The oxidised peak is significantly prominent in WS₂ than WSe₂, in agreement with another report,⁴⁷ influencing their electrochemical performances.

Finally, the feasibility of the developed WSe₂-based electrochemical glucose biosensor was tested with real sample. Human serum from Sigma Aldrich is used as received. Already containing 3.33-7.78 mM glucose,⁴⁸ the sample is introduced into the electrolyte solution, then generating a response. The concentrations are extrapolated from the calibration graph and obtained to be 2.36 mM $\pm$ 0.78 for WSe₂ (n= 3). The values are close to the lower value of the range stipulated.

Table 5.1. Comparison of TMD-based electrochemical glucose biosensors.

Electrode Platform	Transduction Method	Generation of Biosensor	Linear Range	Detection Limit (μ M)	Ref.
GCE/WS ₂ /GOx/GTA	Chronoamperometry	2 nd Gen	180-770 μ M 1.3-7.6 mM 7.6-22.3 mM	82.6	This Work
GCE/WSe ₂ /GOx/GTA	Chronoamperometry	2 nd Gen	77-274 μ M 0.77-22.3 mM	52.0	
GCE/APTES/Chitosan/rMoS ₂ /GOx	Voltammetry	1 st Gen	3-20 mM	-	49
GCE/AuNPs@MoS ₂ -GOx/Nafion	Voltammetry	1 st Gen	10-300 μ M	2.8	50
Au/AuNPs-MoS ₂ /GOx	Chronoamperometry	2 nd Gen	0.25-13.2 mM	0.042	51

Other TMD-based electrochemical glucose biosensors in literature developed based on MoS₂ were either pre-treated or decorated with Au nanoparticles (Table 5.1). Some of the linear ranges either fall much lower than the physiological levels or in the upper limits. In our case, 2nd generation glucose biosensors are reported based solely on 1T phase WS₂ and WSe₂. This conveys the real sensing capabilities of group 6 TMDs as well as shifts the limelight away from MoS₂. The developed biosensors have linear ranges for different orders of concentration and relatively low LOD, offering flexibility in future developments of electrochemical biosensors for biomedical and environmental applications.

5.3 Conclusion

This chapter puts forth the superior electrochemical properties of tungsten dichalcogenides (WS₂, WSe₂) over their molybdenum counterparts (MoS₂, MoSe₂) in the context of a 2nd generation electrochemical glucose biosensor. These four group 6 TMDs are prepared *via t*-BuLi intercalation and exfoliation prior to a comparison of their electrochemical performance, particularly HET capabilities. With narrower separation between anodic and cathodic peaks in redox probes, both WS₂ and WSe₂ demonstrate faster HET rate. Characterisation data reveals the prominence of metallic 1T phase, a polymorph affirmed to improve electrocatalytic properties. The outstanding electrochemical performance then motivated the incorporation of 1T-phase WX₂ into a 2nd generation glucose biosensor. Analytical parameters were commendably achieved without any pretreatment or functionalisation of noble metals, in contrast to the TMDs-based electrochemical glucose biosensor in literature.

5.4 Methods and Materials

Chemicals. Tungsten sulfide (99.8%), molybdenum (IV) sulfide (99.9%), tungsten selenide (99.8%), and molybdenum (IV) selenide (99.9%) were procured from Alfa Aesar,

Germany. Hexane and tert-butyllithium (*t*-BuLi) in pentane (1.7 M) were respectively procured from Lach-ner and Sigma-Aldrich, Czech Republic. Phosphate buffered saline (pH 7.2) was prepared with potassium chloride (max 0.0001% Al), potassium dihydrogen phosphate and sodium hydrogen phosphate; these three chemicals were acquired from Sigma-Aldrich, Singapore. Potassium hexacyanoferrate (II) trihydrate ($\geq 98.5\%$), potassium hexacyanoferrate (III) ($\sim 99\%$), glucose oxidase from *Aspergillus Niger* (EC 1.1.3.4, type X-S), glutaraldehyde solution (50% in water), D-(+)-glucose ($\geq 99.5\%$), L-ascorbic acid (BioXtra, $\geq 99.0\%$, crystalline), uric acid ($\geq 99\%$, crystalline) and dopamine hydrochloride were also acquired from Sigma-Aldrich, Singapore. Ferrocenemethanol ($>95\%$) was acquired from Tokyo Chemical Industry.

Characterisation of Materials. Field emission JEOL 7600F was used to perform scanning electron microscopy and energy dispersive X-ray spectroscopy, operated at acceleration voltages of 5 and 15 kV. JEOL JEM-2200FS was operated at 200 keV accelerating voltage to obtain high resolution transmission electron microscopy (HR-TEM) data. The sample preparation involved suspension of materials in water (1 mg mL^{-1}), which was drop casted onto a TEM grid (Cu; 200 mesh; Formvar/carbon) and subsequently dried at 60°C for 12 h. X-ray photoelectron spectroscopy (XPS) measurements relied on Phoibos 100 spectrometer (SPECS, Germany), coupled with a monochromatic Mg X-ray radiation source. XPS data was consequently processed with Casa XPS, where the relative sensitivity factor (RSF), area constraint and position constraint were set to obtain a good fitting. The sample preparation for the aforementioned characterisation techniques involved grinding TMDs in solid form using a mortar then placed as a uniform layer onto carbon conductive tape. X-ray diffraction (XRD) was accomplished via Bruker D8 Discoverer and the corresponding Cu $K\alpha$ radiation source ($\lambda = 0.15418 \text{ nm}$). Further processing and analysis were performed using EVA software.

Exfoliation of Bulk Materials. The bulk materials (WS₂, WSe₂, MoS₂, MoSe₂) were exfoliated in close accordance to reported protocols.^{51,52} Each TMD powder was first added to 20 mL *t*-BuLi, thus obtaining four different suspensions. They were then stirred under argon atmosphere at 25 °C for 72 h so as to allow the intercalation of Li. The desired materials were collected via suction filtration under argon atmosphere, prior to repeated washes using hexane and dried over sodium. 100 mL of water was subsequently added and centrifugation (18 000 g) was performed repeatedly until conductivity of less than 20 µS is achieved. Lastly, they were dried for 48 h at 25 °C in a vacuum oven, yielding *t*-BuLi exfoliated TMDs.

Biosensor Preparation. The four *t*-BuLi exfoliated TMDs were suspended in ultrapure water (5 mg mL⁻¹), before they are ultrasonicated for three hours to ensure a homogenous and well-dispersed suspension. For subsequent usage, the suspension would be ultrasonicated for five minutes. The surfaces of working electrodes, referring to glassy carbon, were polished using a slurry of alumina (0.05 µm) on a polishing pad. They were rinsed using ultrapure water before blown dry with N₂ gas. The modification of electrodes firstly involved drop casting 3 µL of *t*-BuLi exfoliated TMD suspension then left to dry in ambient conditions. Once dried, 5 µL glucose oxidase in ultrapure water (0.1 g mL⁻¹, GOx) was drop casted and similarly, left to dry in ambient conditions. 5 µL of glutaraldehyde in ultrapure water (0.25%, GTA) was drop casted last and dried at 35 °C in oven for 30 minutes. As such, a TMD/GOx/GTA modified electrode was prepared and would be stored at 4 °C in the refrigerator when not in use.

Electrochemical Measurements. Electrochemical techniques, including chronoamperometric and voltammetric, were executed using Metrohm µAutolab III through NOVA software version 1.10. A three electrode system was employed, consisting of Ag/AgCl reference electrode, platinum counter electrode and glassy carbon working electrode that was either bare or modified. Heterogenous electron transfer (HET) studies were based on using cyclic voltammetry (CV) at a 100 mV s⁻¹ scan rate, between the potentials of -0.3 V and 0.7 V.

Redox probe potassium hexacyanoferrate (5 mM) was prepared in supporting electrolyte potassium chloride (0.1 M). Other CV measurements, also at 100 mV s^{-1} scan rate, were carried out in ferrocenemethanol (FcMeOH, 2 mM) in phosphate buffered saline solution (PBS, pH 7.2). Specific concentrations of glucose were injected into the electrolyte and stirred for two minutes before each measurement, performed between -0.4 V and 0.6 V. Meanwhile, chronoamperometry ($\Delta t > 1 \text{ ms}$) was performed at a constant potential of 0.1 V. Specific concentrations of glucose were injected into the solution every 100 seconds, where stirring was continuous throughout the measurement. The acquired data was subjected to smoothing and baseline correction for further analysis.

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Part I

Chapter 6

Niobium-Doped Group 4 Titanium Disulfide (TiS₂)

Part of the writing presented in this chapter was published in the following journal article:

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The thesis author was the primary investigator and first author of the above paper.

6.1 Introduction

An attractive aspect of TMDs is elemental composition, apart from dimensionality. There are about 40 layered compounds in the library of TMDs¹, of varying transition metal or chalcogen component, showcasing diverse polymorphs, properties and versatile chemistry.^{2,3} Doping is a feasible strategy to expand the collection of compounds, improving old properties or accessing new ones.^{4,5}

Titanium disulfide (TiS₂) is a group 4 TMD which debuted in the field of intercalation chemistry and batteries. Amongst the layered TMDs, it is deemed as a suitable cathode to pair with lithium anode due to high electronic conductivity, rapid lithium self-diffusion, stability, lightweight and low cost.^{6,7} The material has also been promising for electrochemical^{8,9} and thermoelectric^{10,11} devices. Similar to its group 6 counterparts, MoS₂ and WS₂, structures and properties of TiS₂ can be tailored by doping. The introduction of foreign atoms or compositional variance would enhance its practicability in desired applications. A variety of dopants has been incorporated into TiS₂, especially for thermoelectric applications to reduce thermal conductivity. They include alkali earth metals^{12,13} (Mg), transition metals¹⁴ (V, Nb, Ta^{15,16}, Cr¹⁷, Mn, Fe, Co¹⁸, Ni¹⁹, Cu, Zn, Cd²⁰) and even metalloids²¹ (Ge).

This chapter embarks on the exploration of TiS₂ to unearth its electrochemical properties together with sensing capabilities. This is imperative because to date, there have been no electrochemical biosensors reported based on TiS₂ nor group 4 TMDs. Only group 6 TMDs are extensively studied for that matter, particularly MoS₂, MoSe₂, WS₂ and WSe₂ whose properties are modified by phase engineering as described in the previous chapter. Herein, doping is employed to enhance conductivity whose success hinges on the nature and concentration of dopant. TiS₂, being the parent compound, is doped with varying levels of Nb via controlled heating of elements in stoichiometric ratios. Nb is one of the most promising

candidates as substitutional dopant since it is located in the adjacent group of Ti. The synthesised compounds are referred as $\text{Ti}_{1-x}\text{Nb}_x\text{S}_2$ where $x = 0.05, 0.1, 0.2$. Comparisons between group 4 Ti and group 5 Nb in the aspects of size, polymorph and valency would be drawn to elucidate the outcomes of conducted characterisation techniques. Fundamental electrochemical properties, particularly heterogeneous electron transfer and charge-transfer resistance, would be scrutinised together with their viability as a 2nd generation electrochemical biosensor for glucose detection (Scheme 6.1).

Scheme 6.1. Synthesis of layered 1T- TiS_2 together with TiS_3 nanobelts when doped. These materials are drop casted onto bare glassy carbon electrode for 2nd generation biosensor to detect glucose via enzymatic and mediator reactions.

6.2 Results & Discussion

The focus herein is directed to the effects of niobium (Nb) dopants on the structure and electrochemical sensing performance of titanium disulfide (TiS_2). Varying dopant concentrations are attained, as $\text{Ti}_{1-x}\text{Nb}_x\text{S}_2$ ($x = 0, 0.05, 0.1, 0.2$) are synthesised by controlled heating of a sealed evacuated quartz glass ampoule, containing elements in stoichiometric ratios. Characterisation data obtained from scanning electron microscopy (SEM), X-ray diffraction (XRD), Raman spectroscopy as well as X-ray photoelectron spectroscopy (XPS) would be presented and discussed herein. Heterogeneous electron transfer (HET), a key electrochemical parameter, would be compared across all four compounds followed by their capabilities to detect glucose in a 2nd generation electrochemical biosensor system.

Figure 6.1. Morphologies of pure TiS_2 together with its compounds of increasing dopant concentrations, captured by SEM.

Figure 6.1 presents morphologies of the synthesised compounds, captured at the same magnification using SEM. Pure TiS₂ exists as polygonal and thick multi-layered stacks. When doped with Nb, the structures seem to rupture and the layers become more obvious with increasing dopant concentration. The expansion of layers suggests Nb dopants, of larger ionic radii (0.68 Å), directly substituting Ti⁴⁺ (0.605 Å) in the lattice or intercalating amongst TiS₂ layers.²² Additionally, a striking feature is noticed for Ti_{0.8}Nb_{0.2}S₂ where narrow and seemingly electron-transparent structures populate. These are described in literature as nanobelts^{23,24} or belt-like nanoribbons²⁵.

While different morphologies are observed in SEM, Raman spectra demonstrate the same polymorphic structures for both pure and Nb-doped TiS₂ (Figure 6.2A). The peaks at 235 and 332 cm⁻¹ are assigned to E_g in-plane and A_{1g} out-of-plane vibrational modes accordingly. These two modes correspond to the 1T polymorph, also described by the D_{3d} (*P* $\bar{3}$ m1) space group.²⁶ It has been anticipated that the E_g mode will be of lower intensity than A_{1g}.²⁷ Moreover, there is a shoulder peak adjacent to A_{1g}, at approximately 370 cm⁻¹, characteristic of TiS₂.^{28,29} There are a few postulations to justify the presence of the shoulder peak, including a resonance effect that couple IR and Raman active modes or defects which lead to an excess of interlayer metal atoms.^{27,30} Overall, there are negligible changes in the Raman spectra with regards to peak positions and intensities. It can therefore be implied that the 1T polymorph is retained after doping. This is consistent with data in literature that studied even wider dopant concentrations.³¹⁻³³

The acquired structural information from SEM and Raman spectroscopy is further supported by XRD (Figure 6.2B). Consistent across our synthesised compounds, the first three, also dominant, peaks represent (001), (011) and (012) planes. They are indicative of 1T-TiS₂ (ICSD No. 52195),⁸ affirming the polymorph earlier deduced in Raman spectra. Meanwhile, the (001) peak stands out from the other two peaks with increasing dopant concentration. This

implies a preferential orientation along the c axis which substantiates the layered structures observed in SEM. The primary cause could be the formation of TiS_3 nanobelts upon doping, since they tend to grow in the (001) direction.^{24,34} Agreeably, TiS_3 peaks are identified in the XRD pattern of $\text{Ti}_{0.8}\text{Nb}_{0.2}\text{S}_2$ and marked red.

Figure 6.2. (A) Raman spectra and (B) X-ray diffraction pattern of TiS_2 , and its doped counterparts $\text{Ti}_{1-x}\text{Nb}_x\text{S}_2$ where $x = 0.05, 0.1$ and 0.2 .

XPS is useful to elucidate the consequences of doping. Zooming into the high-resolution spectrum of Nb 3d region (Figure 6.3A), the absence of dopants is ascertained for pure TiS₂. When Nb is introduced, deconvolution of the appeared peak reveals oxidation states of +2 and +5. Each state comes in a doublet where Nb (II) 3d 5/2 and 3/2 occur at 203.9 and 206.7 eV while Nb (V) 3d 5/2 and 3/2 at 206.7 and 209.4 eV. Although Nb (V) coincides with that of Nb₂O₅,³⁵ both of the states are prevalent for NbS₂.^{36,37} This suggests a direct substitution of Nb atoms in TiS₂. If so, the coordination or polymorph has to be considered since TiS₂ and NbS₂ typically exist in 1T and 2H respectively. Nonetheless, it is possible that NbS₂ follows the polymorph of the host compound based on the obtained binding energies being close to values reported for 1T-NbS₂.³⁸ The retention of 1T polymorph, regardless of dopant concentration studied, is in line with the previously discussed characterisation data. This is especially unsurprising in mixed TMDs since one of them, TiS₂ in this case, prefers the 1T phase. A plausible explanation is the number of electrons involved in the mixed system that stabilises the d electron count as well as the respective phase.³⁹ Additionally, a third pair of doublet is detected in HR-XPS spectra of Ti_{0.9}Nb_{0.1}S₂ and Ti_{0.8}Nb_{0.2}S₂ with higher dopant concentrations (x = 0.1, 0.2). They are assigned to Nb⁴⁺ species, where 3d5/2 and 3d3/2 possess binding energies of 205.2 and 207.9 eV respectively. A plausible explanation is Nb matching to the exclusive valency of Ti⁴⁺ in TiS₂ since valency is to be taken into account in substitutional doping. Isomorphic or isovalent substitution is desirable to preserve structural integrity and crystallinity.

The attention is subsequently turned to Ti 2p and S 2p regions to investigate the chemical states and more importantly, validate the relationship between Nb dopants with formation of TiS₃ nanobelts. Figure 6.3B depicts Ti in its most stable +4 oxidation state, either as TiS₂, TiS₃ nanobelts or oxidised form TiO₂. The TiS₃ peaks can only be seen when doped,

Figure 6.3. Deconvoluted XPS spectra of (A) Nb 3d region showcasing the absence of Nb in pure TiS_2 and presence of Nb in doped compounds $\text{Ti}_{1-x}\text{Nb}_x\text{S}_2$ where $x = 0.05, 0.1$ and 0.2 , and (B) Ti 2p region with stable Ti^{4+} oxidation state.

whose 2p_{3/2} and 2p_{1/2} peaks are located at 456.4. They are distinguishable from TiS₂ and TiO₂ whose 2p_{3/2} peaks are at 455.2 and 458.4 eV accordingly.

Likewise, the S 2p region reiterates the formation of TiS₃ nanobelts upon doping (Figure 6.4). TiS₂ constitutes only S²⁻ moieties whereas there are one S²⁻ and two S₂²⁻ for TiS₃.^{24,40} As a result, there is an extra pair of doublet correlating to S₂²⁻ of TiS₃ upon doping. The S₂²⁻ 2p_{3/2} and 2p_{1/2} peaks at 161.1 and 162.3 eV intensifies with increasing dopant concentration. Because they are distinctly observed for Ti_{0.9}Nb_{0.1}S₂ and Ti_{0.8}Nb_{0.2}S₂, TiS₃ nanobelts are especially significant at higher dopant concentrations (x = 0.1, 0.2).

Figure 6.4. Deconvoluted XPS spectra of S 2p region where S₂²⁻ moiety is (A) absent in pure TiS₂, but present in Ti_{1-x}Nb_xS₂ where x = (B) 0.05, (C) 0.1 and (D) 0.2 implying the formation of TiS₃ nanobelts upon doping.

The various characterisation techniques have put forth that doping TiS_2 with Nb atoms would result in TiS_3 nanobelts. The structural implications on the electrochemical properties of Nb-doped compounds would next be of interest. Suspensions of TiS_2 and the Nb-doped compounds are individually drop casted onto bare glassy carbon (GC) electrodes while surface-sensitive ferro/ferricyanide redox probe is employed.⁴¹ Heterogeneous electron transfer (HET), impedance, electron transfer mechanism as well as electrochemically active area would be established. A material is deemed suitable for electrochemical (bio)sensors if it promotes efficient electron transfer on the electrode surface, measured by small peak-to-peak separation (ΔE_p) in cyclic voltammograms.⁴² ΔE_p is extracted from the difference between oxidation and reduction peak potentials (Figure 6.5A). It is the smallest for $\text{Ti}_{0.9}\text{Nb}_{0.1}\text{S}_2$, followed by $\text{Ti}_{0.95}\text{Nb}_{0.05}\text{S}_2$, $\text{Ti}_{0.8}\text{Nb}_{0.2}\text{S}_2$ then TiS_2 with the largest separation. All the Nb-doped compounds surpass pure TiS_2 , favouring the effects of doping. Superior electrochemical performances are therefore expected of the doped compounds. Another observation is the appearance of anodic peaks at ~ 0.4 V specifically in the doped compounds. They grow intensely with increasing dopant concentration, unsurprisingly so as the peaks correspond to Nb oxidation from +4 to +5.³⁶ The oxidation is deemed as irreversible since there are no reduction peaks in response and would disappear in subsequent scans.

In the meantime, electrochemical impedance spectroscopy (EIS) was performed to acquire fundamental information regarding the materials. A graph of the imaginary part of the impedance (Z_i) versus the real part (Z_r), also termed as Nyquist plot, is displayed in Figure 6.5B. The data is fitted using the Randles equivalent circuit (inset) to generate critical values, namely charge-transfer resistance (R_{ct}). R_{ct} is inversely proportional to electron transfer, henceforth smaller values are favoured. The materials are ranked in ascending R_{ct} , championed by $\text{Ti}_{0.95}\text{Nb}_{0.05}\text{S}_2$ (90 Ω) and trailing behind are TiS_2 (686 Ω), $\text{Ti}_{0.8}\text{Nb}_{0.2}\text{S}_2$ (800 Ω) then $\text{Ti}_{0.9}\text{Nb}_{0.1}\text{S}_2$ (1040 Ω).

Figure 6.5. (A) Cyclic voltammograms at 50 mV s^{-1} scan rate, (B) Nyquist plots of GC electrode modified with $\text{Ti}_{1-x}\text{Nb}_x\text{S}_2$ ($x = 0, 0.05, 0.1, 0.2$), for HET and impedance studies respectively. (C) Increasing peak currents resulting from increasing scan rates, represented by $\text{Ti}_{0.95}\text{Nb}_{0.05}\text{S}_2$ modified GCE and (D) current-scan rate plot of all materials studied. Conditions: $10 \text{ mM } [\text{Fe}(\text{CN})_6]^{4-/3-}$ in 0.1 M KCl .

Moving on, it is of interest to report the electron transfer mechanism of the $\text{Ti}_x\text{Nb}_{1-x}\text{S}_2$ modified electrodes. Ferro/ferricyanide redox probe is subjected to cyclic voltammetry and the anodic peak current is measured at different scan rates (Figure 6.5C). There exists a linear relationship with the square root of scan rate, indicating a diffusion-controlled process (Figure 6.5D). The redox species is freely diffusing instead of adsorbing onto the electrode surface. Simultaneously, the steepness of the slopes differ across compounds which allows the determination of electrode area. By relying on the Randles-Sevcik equation, known values of

diffusion coefficient ($D = 7.26 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$) and concentration ($C = 10 \times 10^{-6} \text{ mol cm}^{-3}$) of the redox probe are substituted. The electrochemically active areas are calculated to be 0.024, 0.041, 0.023 and 0.022 cm^2 for TiS_2 , $\text{Ti}_{0.95}\text{Nb}_{0.05}\text{S}_2$, $\text{Ti}_{0.9}\text{Nb}_{0.1}\text{S}_2$ and $\text{Ti}_{0.8}\text{Nb}_{0.2}\text{S}_2$ respectively. It is the largest for $\text{Ti}_{0.95}\text{Nb}_{0.05}\text{S}_2$.

Combining efficient electron transfer rates, low resistance and large electrochemically active area, doping TiS_2 with a specific concentration of Nb dopants has successfully enhanced electrochemical properties. This in agreement with another study which found an increase in electrical conductivity of Nb-doped TiS_2 .¹⁵ Putting forth that Nb is more electron rich, d^1 in Nb^{4+} compared to d^0 in Ti^{4+} , there would be an increase in electron concentration. Since the principle valence band is full in TiS_2 , additional electrons from Nb would partially fill the conduction band. Electrical conductivity consequently increases therefore superior electrochemical sensing performances are anticipated for $\text{Ti}_{0.95}\text{Nb}_{0.05}\text{S}_2$.

Pure and Nb-doped TiS_2 are put to the test where they would be coupled with glucose oxidase enzyme (GOx) before cross-linked by glutaraldehyde (GTA). GTA is selected due to its high reactivity and desired stability, apart from being a simple and gentle cross-linking agent.^{43,44} The efficiency of GTA is demonstrated by several enzymatic-based biosensor previously reported by our group⁴⁵⁻⁴⁸ and others too⁴⁹⁻⁵¹. Drop-casted component-by-component onto bare GC electrodes, the prepared biosensing electrodes would be placed in an electrochemical system to detect glucose. The series of chemical events are deconstructed in the following equations. While processing glucose, the flavin redox center (FAD) of GOx is reduced. Regeneration of the oxidised form is necessary and actualised by ferrocenemethanol (FcMeOH). FcMeOH plays the role of a mediator, whose oxidation at the electrode surface is quantifiable and proportional to glucose levels.⁵² The involvement of the mediator renders the electrochemical biosensor as 2nd generation.

Pure and Nb-doped TiS_2 were incorporated on the electrode surface as an attempt to enhance the signal transduction for glucose detection. Figure 6.6A shows the cyclic voltammograms of FcMeOH upon addition of 1 mM glucose for the respective $\text{Ti}_{1-x}\text{Nb}_x\text{S}_2/\text{GOx}/\text{GTA}$ biosensing electrodes. Instead of the typical pair of reversible redox peaks, the peaks are asymmetric where the anodic peak current is of significantly higher intensity than the cathodic. This implies successful detection of glucose since oxidation of FcMeOH has occurred, a product of enzyme regeneration following the mechanism. Among the materials, $\text{Ti}_{0.95}\text{Nb}_{0.05}\text{S}_2$ generates the largest oxidation peak. Its high sensitivity is evident in chronoamperometry experiments where distinctly large current response is achieved (Figure 6.6B). In contrast, $\text{Ti}_{0.9}\text{Nb}_{0.1}\text{S}_2$ and $\text{Ti}_{0.8}\text{Nb}_{0.2}\text{S}_2$ produce comparable responses to pure TiS_2 . A high dopant concentration of Nb ($x = 0.1, 0.2$) impedes the electrochemical performance due to the formation of TiS_3 nanobelts based on previously discussed characterisation data. Doping would therefore only improve biosensing capabilities at specific dopant concentrations. As the best performing material, subsequent experiments are modelled with $\text{Ti}_{0.95}\text{Nb}_{0.05}\text{S}_2/\text{GOx}/\text{GTA}$ electrodes.

The current response grew with increasing drop-cast concentration (Figure 6.6C). Loading more $\text{Ti}_{0.95}\text{Nb}_{0.05}\text{S}_2$ on the electrode surface emphasises its role in enhancing signal transduction. Meanwhile, the role of other components are investigated by manipulating configurations of the biosensing electrode (Figure 6.6D). No current response is observed for bare GC and $\text{Ti}_{0.95}\text{Nb}_{0.05}\text{S}_2$ electrode, until integrated with GOx. The enzyme is imperative in recognising glucose and triggering the detection mechanism. The current response dramatically increases when GTA is added, an effective cross-linker to stabilise the modified biosensor by keeping the layers intact for reliable glucose measurements.

Figure 6.6. Current responses of biosensing electrodes ($\text{Ti}_{1-x}\text{Nb}_x\text{S}_2/\text{GOx}/\text{GTA}$) in cyclic voltammograms at 100 mV s^{-1} scan rate (A), and chronoamperometry data (B) upon addition of 1 mM and 0.5 mM glucose respectively. Chronoamperometry data of $\text{Ti}_{0.95}\text{Nb}_{0.05}\text{S}_2/\text{GOx}/\text{GTA}$ biosensing electrode when varying drop-cast concentrations (C) and configurations (D). Conditions: 2 mM FcMeOH in PBS pH 7.20 electrolyte.

The aforementioned experiments were executed based on the potential of 0.1 V. Nevertheless, adjustment of the operating potential is necessary if selectivity is not fulfilled. Signals from electrochemically active interferences should be minimised, namely ascorbic acid (AA), dopamine (DA) and uric acid (UA). They are present in concentrations 30 times lower than glucose in blood⁵², hence 0.2 mM were added in relation to 5 mM glucose. In Figure 6.7A, increasing operating potentials generates higher current responses for glucose as well as interferences, unfortunately. Figure 6.7B then compares the responses of the interferences as a

percentage of glucose for the different potentials studied. At 0.1 V, the signals from interferences fall below 15% of glucose unlike 0.15 and 0.2 V. Although a higher potential is desired for a more sensitive biosensor, selectivity is another critical criterion that should not be compromised. Operating potential of 0.1 V is maintained since both sensitivity and selectivity are satisfactory.

Figure 6.7. Chronoamperometry data upon successive additions of 5 mM glucose and 0.2 mM interferences at different operating potentials (A), followed by comparison of current signals from interferences as a percentage of glucose (B). Chronoamperometry data when introducing 50, 500 and 5000 μM glucose in the solution, each concentration added 5 times (C), along with calibration graph of current against glucose concentration with three linear ranges, smallest range is displayed as inset (D). Conditions: $\text{Ti}_{0.95}\text{Nb}_{0.05}\text{S}_2/\text{GOx}/\text{GTA}$ biosensing electrode, 2 mM FcMeOH in PBS pH 7.20 electrolyte.

With all the critical parameters optimised for the $\text{Ti}_{0.95}\text{Nb}_{0.05}\text{S}_2$ -based biosensor, its analytical performance is evaluated. Four different glucose concentrations were prepared (5, 50, 500 and 5000 μM) and each added five times into the measured solution sequentially. No current response is acquired during the addition of 5 μM , but a stepwise increase in current is observed with higher concentrations thereafter (Figure 6.7C). To ensure the repeatability of the developed biosensor, triplicates of the experiment yield a relative standard deviation of 11.7%. A calibration graph of current against glucose concentration was then plotted (Figure 6.7D). Three linear ranges are obtained, 74.6 to 272.9 μM ($r = 0.995$), 0.767 μM to 12.6 mM ($r = 0.995$) and 17.5 to 27.3 mM ($r = 0.990$). A sensitivity of $17.9 \mu\text{A mM}^{-1} \text{cm}^{-2}$ is determined based on the electrochemically active area. The limit of detection and quantification are respectively computed to be 25.7 μM and 85.7 μM . The stability of the biosensor is also emphasised as the measurement was performed over a long duration of 4500 seconds (75 mins) yet glucose was quantified reliably and precisely with low standard deviation.

The developed $\text{Ti}_{0.95}\text{Nb}_{0.05}\text{S}_2$ -based biosensor was put to the test to detect glucose in real sample. Instead of adding prepared concentration of glucose into the solution, human serum procured from Sigma-Aldrich was directly injected. A current response is attained and $2.68 \pm 0.55 \text{ mM}$ ($n=3$) of glucose was quantified after extrapolation from calibration graph and considering the dilution factor. The detected concentration falls short from the specified range of 3.33 – 7.78 mM. Stability of the developed biosensor was also scrutinised over a period of five days. The current responses upon addition of 0.5 mM glucose were expressed as percentage recovery with respect to day 1. The percentage dropped to 86% in day 3 then relatively maintained to 83% in day 5 (Figure 6.8). Finally, other glucose biosensors based on TMDs and other layered materials are compared. Their linear ranges, sensitivities and limit of detections are compiled in Table 6.1. Examining the parameters of the biosensors, $\text{Ti}_{0.95}\text{Nb}_{0.05}\text{S}_2$ -based exhibit wide linear ranges from μM to mM and highest sensitivity.

Figure 6.8. Current response of 0.5 mM glucose as percentage recovery over day 1, 3 and 5. Conditions: $\text{Ti}_{0.95}\text{Nb}_{0.05}\text{S}_2/\text{GOx}/\text{GTA}$ biosensing electrode, 2 mM FcMeOH in PBS pH 7.20 electrolyte.

Table 6.1. Representative glucose biosensors based on layered materials in literature: comparison of linear range, sensitivity and limit of detection (LOD).

Material	Biosensor Architecture (Generation)	Linear Range	Sensitivity	LOD (μM)	Ref
$\text{Ti}_{0.95}\text{Nb}_{0.05}\text{S}_2$	GCE/ $\text{Ti}_{0.95}\text{Nb}_{0.05}\text{S}_2$ / GOx/GTA (2 nd Gen)	74.6 to 272.9 μM 0.767 to 12.6 mM 17.5 to 27.3 mM	17.9 $\mu\text{A mM}^{-1}\text{cm}^{-2}$	25.7	This work
MoS_2	AuE/AuNP- MoS_2 /GOx (2 nd Gen)	0.25 to 13.2 mM	13.8 $\mu\text{A }\mu\text{M}^{-1}\text{cm}^{-2}$	0.042	53
1T- WS_2	GCE/1T- WS_2 / GOx/ GTA (2 nd Gen)	176 to 766 μM 1.3 to 22.3 mM	-----	82.6	54
$\text{Ti}_3\text{C}_2\text{T}_x$	GCE/Au- $\text{Ti}_3\text{C}_2\text{T}_x$ / GOx/Nafion (1 st Gen)	0.1 to 18 mM	4.2 $\mu\text{A mM}^{-1}\text{cm}^{-2}$	5.9	55
$\text{Ti}_3\text{C}_2\text{T}_x$	GCE/ $\text{Ti}_3\text{C}_2\text{T}_x$ - Graphene/ GOx/ Nafion (1 st Gen)	0.2 to 5.5 mM	12.10 $\mu\text{A mM}^{-1}$	100	56

6.3 Conclusion

TiS₂ has been successfully doped with Nb, Ti_{1-x}Nb_xS₂, and this study aimed to address the doping outcomes influenced by nature as well as concentration of dopants ($x = 0.05, 0.1, 0.2$). Characterisation data from various techniques validate the synthesis of TiS₂ and bring to light the changes in its structure upon doping. Aspects of size, polymorph and valency are included in the discussion. The 1T polymorph is retained across all dopant concentrations, however TiS₃ nanobelts are formed especially at higher concentration of $x = 0.2$. With the structural effects in mind, fundamental electrochemical properties of the materials were compared. Ti_{0.95}Nb_{0.05}S₂ demonstrated largest electrochemically active area, superior conductivity and least resistance to electron transfer based on HET and impedance studies. When applied in glucose detection, Ti_{0.95}Nb_{0.05}S₂ generated the highest response. A sensitive and selective 2nd generation electrochemical biosensor is developed based on Ti_{0.95}Nb_{0.05}S₂/GOx/GTA. The linear ranges are 74.6 to 272.9 μM ($r = 0.995$), 0.767 μM to 12.6 mM ($r = 0.995$) and 17.5 to 27.3 mM ($r = 0.990$), while 25.7 μM and 85.7 μM are achieved as the limit of detection and quantification respectively. Electrochemical properties and sensing capabilities have been enhanced by doping TiS₂ with a specific concentration of Nb. Higher concentrations would result in structural implications of TiS₃ nanobelts which may impede performance.

6.4 Methods and Materials

Chemicals. Sulfur (99.999%), titanium (99%) and niobium powder (99.8%) were procured from STREM Chemicals. Sodium hydrogen phosphate (98+%), potassium dihydrogen phosphate (98+%) and potassium chloride (99%) were procured from Alfa Aesar. Potassium hexacyanoferrate (II) trihydrate ($\geq 98.5\%$), potassium hexacyanoferrate (III) ($\sim 99\%$), glucose oxidase from *Aspergillus niger* (EC 1.1.3.4, type X-S), glutaraldehyde

solution (50% in water), D-(+)-glucose ($\geq 99.5\%$), L-ascorbic acid (BioXtra, $\geq 99.0\%$, crystalline), dopamine hydrochloride and uric acid ($\geq 99\%$, crystalline) were procured from Sigma-Aldrich. Ferrocenemethanol ($>95\%$) was acquired from Tokyo Chemical Industry.

Characterisation of Materials. Sample preparation for scanning electron microscopy (SEM) and X-ray photoelectron spectroscopy (XPS) entails a thin, uniform layer of the solid compound deposited onto a carbon-conductive tape. Field emission SEM (Tescan Lyra dual beam microscope) was operated to capture morphologies of the materials. XPS measurements relied on Phoibos 100 spectrometer (SPECS, Germany), coupled with a monochromatic Mg X-ray radiation source. The data was consequently processed with Casa XPS, where the relative sensitivity factor (RSF), area constraint and position constraint were set to obtain a good fitting. Raman spectroscopy was performed using Renishaw inVia Raman microscope in backscattering mode with CCD detector, a 532 nm laser as well as 50x magnification objective lens. The instrument was calibrated according to the peak of silicon at 520 cm^{-1} . X-ray diffraction (XRD) was accomplished via Bruker D8 Discoverer and the corresponding Cu $K\alpha$ radiation source ($\lambda = 0.15418\text{ nm}$). Further processing and analysis were performed using EVA software.

Preparation of Nb-Doped TiS₂. Titanium, sulfur and niobium were weighed in stoichiometric amounts, summing up to 10 g. They were placed in a quartz glass ampoule (30 x 130 mm; 1.5-2 mm wall thickness) which was evacuated to 5×10^{-5} mbar and melt-sealed by oxygen-hydrogen welding torch. Thereafter, it was heated at 425 °C and 500 °C sequentially in a muffle furnace over 24 hours for each temperature. Upon the natural cooling of the ampoule to room temperature, it was again subjected to heating. The conditions were 425 °C, 800 °C and 850 °C for 24, 48 and 12 hours respectively. Finally, the furnace cooled down naturally to ambient temperature. A ramping rate of 5 °C per minute was used throughout the synthesis.

Preparation of Biosensing Electrodes. TiS_2 doped with varying amounts of Nb were suspended in ultrapure water, in concentrations of 5.0 and 7.5 mg mL^{-1} before and after optimisation respectively. They were ultrasonicated for three hours to attain a homogenous and well-dispersed suspension. The surfaces of working electrodes, referring to glassy carbon, were polished using a slurry of alumina (0.5 and 0.05 μm) on a polishing pad. They were rinsed using ultrapure water before blown dry with N_2 gas. The modification of electrodes firstly involved drop casting 3 μL of suspension, then left to dry in ambient conditions. Once dried, 5 μL glucose oxidase in ultrapure water (0.1 g mL^{-1} , GOx) was drop casted and similarly, left to dry in ambient conditions. 5 μL of glutaraldehyde in ultrapure water (1% v/v GTA) was drop casted last and dried at 35 $^\circ\text{C}$ in oven for 30 minutes. The configuration of the biosensing electrode is referred as $\text{Ti}_{1-x}\text{Nb}_x\text{S}_2/\text{GOx}/\text{GTA}$ and stored overnight at 4 $^\circ\text{C}$ in the refrigerator.

Electrochemical Measurements. Electrochemical techniques, including chronoamperometric and voltammetric, were executed using Metrohm Autolab PGSTAT204 through NOVA software version 1.10. A three electrode system was employed, consisting of Ag/AgCl reference electrode, platinum counter electrode and glassy carbon working electrode that was either bare or modified. Heterogenous electron transfer (HET) studies were based on using cyclic voltammetry (CV) at a 100 mV s^{-1} scan rate, between the potentials of -1.0 V and 1.0 V. The redox probe, potassium hexacyanoferrate (10 mM) was prepared in supporting electrolyte potassium chloride (0.1 M). The detection of glucose was performed using $\text{Ti}_{1-x}\text{Nb}_x\text{S}_2/\text{GOx}/\text{GTA}$ biosensing electrodes. Phosphate buffered saline (PBS) electrolyte was prepared by adding 0.356 g of potassium dihydrogen phosphate, 0.746 g of sodium hydrogen phosphate and 0.746 g of potassium chloride in 100 mL ultrapure water. The pH was adjusted to 7.20 by the addition of 1 M potassium hydroxide. Ferrocenemethanol (FcMeOH), mediator in the detection system, was prepared in concentration of 2 mM in PBS. In the chronoamperometric mode, the operating potential was set at 0.1 V. The current was allowed

to stabilise for about 1000 s before introducing glucose into the solution. Baseline correction was performed to facilitate analysis and comparison.

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Part I

Chapter 7

Electrochemical Pretreatment of Group 10 Transition Metal
Dichalcogenides (PtS₂, PtSe₂, PtTe₂) for Non-Enzymatic Sensing

Part of the writing presented in this chapter was published in the following journal article:

N. Rohaizad, C. C. Mayorga-Martinez, Z. Sofer, R. D. Webster, M. Pumera*. Layered Platinum Dichalcogenides (PtS₂, PtSe₂, PtTe₂) for Non-Enzymatic Electrochemical Sensor. *Appl. Mater. Today* **2020**, 19, 100606.

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The thesis author was the primary investigator and first author of the above paper.

7.1 Introduction

Platinum (Pt), a group 10 element also known as a noble metal, is renowned for its catalytic and electrocatalytic properties, as well as resistance to corrosion.¹ Granted such desirable properties, there lies a plethora of applications leveraging on platinum namely catalytic converters, industrial catalysts, anti-cancer drugs, data storage, even jewellery. The noble metal is also involved in the development of fuel cells and sensors.²⁻⁴ However, its rare availability and cost is a hindrance towards feasibility and commercialisation. There have consequently been various efforts by the scientific community to utilise fractional amounts yet surpassing the performance of pure platinum. Strategies include modification of their composition⁵, support^{6,7}, size⁸ and structure⁹.

Platinum when bonded with chalcogens fall under a class of layered materials known as transition metal dichalcogenides (TMDs), yielding a chemical formula of PtX_2 ($\text{X} = \text{S}, \text{Se}, \text{Te}$).^{10,11} Being layered, these materials offer large surface area and possibilities of exfoliation to tune properties according to thickness. A classic example is the transition from indirect to direct band gap semi-conductor upon thinning the extensively studied group 6 TMDs (MoS_2 , WS_2).^{12,13} Meanwhile, group 10 TMDs like PtX_2 deserve attention too as they showcase even dramatic layer dependence.¹⁴ The difference lies in their bonding involving large number of d electrons and close proximity between energy levels of Pt 5d and chalcogen's p orbitals. A greater extent of hybridisation and mixing occur between the orbitals, diminishing the M-X ionic character while giving rise to covalent bonding.¹⁵ Concurrently, there is substantial hybridisation between p_z orbitals of interlayer chalcogen atoms resulting in a covalent-like bonding instead of the typical van der Waals'.¹⁶ A chalcogen dependence is developed as the interlayer interaction grows stronger on going down the group. Different bonding translates to different electronic properties where PtS_2 is semi-conducting, PtSe_2 is semi-metallic while PtTe_2 is metallic.^{15,17}

Layered materials have been pursued as sensors amongst many applications, and PtX₂ is no exception. They have been developed for gas and pressure sensing based on PtSe₂,¹⁸⁻²⁰ attributed to its high stability and mobility together with wide tuneable bandgap.^{21,22} It would therefore be of interest to investigate PtX₂ (X = S, Se, Te) on electrochemical sensing capabilities, exploring their potential applications while observing chalcogen dependence. Moreover, PtX₂ possess significantly lower toxicity than the esteemed oxygen reduction reaction electrocatalyst Pt/C, suggesting its biocompatibility for sensing.²³ Hydrogen peroxide (H₂O₂) is a biomarker under the category of reactive oxygen species (ROS), encompassing both oxygen radicals as well as certain non-radicals which are oxidizing agents and/or are easily converted into radicals.²⁴ It biologically functions as important messengers for cellular signalling processes and physiological pathways.²⁵ An overproduction of ROS, termed as “oxidative stress”, would implicate living activities resulting in cardiovascular as well as neurodegenerative diseases like Alzheimer’s.²⁶ The detection of such a valuable biomarker is therefore imperative.

Combining the elemental superiority of platinum and advantageous layered structures when bonded to chalcogens, it would herein be of interest to delve into the electrochemical properties together with sensing performances of these unique noble TMDs. Specific to this study, there are three materials which will be prepared and characterised: platinum of varying chalcogen composition (PtS₂, PtSe₂ and PtTe₂) (Scheme 7.1). Preliminary studies will be carried out to identify the best performing platinum dichalcogenide in the context of electrochemical sensors for H₂O₂, prior to its consequent development and optimisation. Electrochemical pretreatment will also be incorporated as a strategy to improve the material’s capability. Lastly, analytical parameters including selectivity, sensitivity as well as stability will be established.

Scheme 7.1. Schematic illustration of (A) synthesised layered Pt dichalcogenides which are (B) drop casted onto a bare glassy carbon electrode and subjected to electrochemical pretreatment, prior to (C) the detection of H_2O_2 .

7.2 Results & Discussion

With the primary aim to study the electrochemical sensing capabilities of group 10 TMDs, particularly noble metal Pt with varying chalcogen component (PtS_2 , PtSe_2 , and PtTe_2), these materials of interest were firstly synthesised by heating their respective elements in stoichiometric amounts. Characterisation was performed such as transmission electron microscopy (TEM), X-ray diffraction (XRD) as well as X-ray photoelectron spectroscopy (XPS) to attain structural and chemical information. These characterisation data, together with fundamental electrochemical studies, would be useful in elucidating the materials' sensing performance. A preliminary comparison of the materials in detecting H_2O_2 is herein presented (Figure 7.1), upon drop casting them on surfaces of the glassy carbon working electrodes. It is to be put forth that PtTe_2 demonstrates the most intense and sensitive response towards the

analyte. Distinct peaks arising from the reduction of H_2O_2 are observed, therefore subsequent development and optimisation of a sensor would be based on PtTe_2 .

Figure 7.1. Cyclic voltammograms of PtS_2 , PtSe_2 , PtTe_2 modified electrodes before (dashed lines) and after (solid lines) addition of 3.5 mM H_2O_2 . Conditions: electrolyte PBS pH 7.4, scan rate 50 mV s^{-1} .

A combination of characterisation techniques reveals the layered structures of the synthesised materials. TEM images (Figure 7.2A) show flakes of numerous layers with particle size up to $1 \mu\text{m}$. Their selected area electron diffraction (SAED) insets and high resolution TEM (HR-TEM) micrographs presented in Figure 7.2B demonstrate hexagonal in-plane structure corresponding to $P3m1$ space group. Scanning electron microscopy (SEM) affirms the layered morphology (Figure 7.2C) while energy dispersive X-ray spectroscopy (EDS) validates the elemental composition of Pt (Figure 7.2D) and respective chalcogen (Figure 7.2E). The theoretical chalcogen-metal ratio is 2 whereas the computed values are 1.77, 2.03, and 2.14 for PtS_2 , PtSe_2 and PtTe_2 respectively. Additionally, atomic force microscopy (AFM) in tapping mode was executed to acquire thickness distribution diagrams. Height images of the materials together with their profiles ranging from 150 to 400 nm reiterate the bulk layered morphology (Figure 7.3).

Figure 7.2. Characterization data of PtS₂, PtSe₂ and PtTe₂. (A) TEM with SAED insets, (B) HR-TEM, (C) SEM, (D) EDS of Pt, and (E) EDS of chalcogens with their respective elemental atomic percentages.

Figure 7.3. AFM images together with cross-sectional height profile of the synthesised Pt dichalcogenides. Sample scan sizes are 6.7 μm for PtS₂, 4.9 μm for PtSe₂ and PtTe₂.

Particularly for PtS₂, the significant decrease in chalcogen-metal ratio can be explained by XRD measurements as 2-4% of PtS is detected (Figure 7.4). Apart from the extra peaks corresponding to PtS, the peaks of PtS₂ resemble are in line with literature (ICSD No. 41375). On the other hand, XRD disclose high preferential orientation and purity for both PtSe₂ and

PtTe₂. They exist as few layers in a well-ordered stacking structure based on their conspicuous (00l) diffraction peaks. A plausible explanation is the stronger interlayer covalent bonding compared to PtS₂. Further structural information can be obtained with Raman spectroscopy. Two characteristic peaks are observed in each Raman spectra (Figure 7.5), which are assigned to E_g and A_{1g} modes, corresponding to CdI₂ crystal structure and D_{3d} symmetry of the materials.²⁷ The E_g mode of lower Raman shift describes an in-plane vibration of chalcogen atoms moving opposite from each other while the A_{1g} mode of higher Raman shift refers to out-of-plane vibration of chalcogen atoms moving away from each other. The pairs of modes appear at 306 and 339 cm⁻¹, 174 and 205 cm⁻¹, 109 and 154 cm⁻¹ for PtS₂, PtSe₂ and PtTe₂ respectively, which are in close agreement with literature.²⁸⁻³²

Figure 7.4. X-ray diffraction patterns of PtS₂, PtSe₂, PtTe₂. Presence of PtS (2-4%) is detected in PtS₂.

Figure 7.5. Raman spectra together with E_g and A_{1g} peak assignments of (A) PtS₂, (B) PtSe₂, and (C) PtTe₂. $\Lambda_{\text{exc}} = 514.5 \text{ nm}$.

XPS is carried out on the platinum dichalcogenides to probe more information regarding their oxidation states, in addition to elemental composition. Deconvolution of the XPS spectra reveals three oxidation states of the Pt metal component across all materials (Figure 7.6A-C). They are Pt (0), Pt (II) as well as Pt (IV), and their binding energies are tabulated in Table 7.1. It is to be pointed out that PtTe₂ possess significantly higher proportion of Pt (IV) chemical state, which may influence its electrochemical sensing capabilities. Nonetheless, Pt (II) is the most prevalent as it amounts to 66.9%, 60.1% and 54.9% for PtS₂, PtSe₂ and PtTe₂ respectively. The oxidation state of Pt metal is anticipated to deviate from +4 to 0 or +2 while the chalcogen will be 0, instead of -2. Especially for PtTe₂, this is explained

by a weak covalent Te-Te bond and a stronger covalent bond between Pt and Te.³³ Te (0) peak is consequently dominant among other oxidation states (Figure 7.6F).

Figure 7.6. XPS spectra of (A-C) Pt metal component and (D-F) varying chalcogen of PtS₂, PtSe₂ and PtTe₂, in order.

Table 7.1. Specific binding energies of Pt 4f 7/2 and Pt 4f 5/2 in various oxidation states for the three Pt dichalcogenides, together with values obtained from deconvoluted XPS spectra.

	PtS ₂	PtSe ₂	PtTe ₂
Pt (0)	72.36, 75.69 eV (19.0%)	71.99, 75.32 eV (27.2%)	71.74, 75.07 eV (4.6%)
Pt (II)	73.72, 77.05 eV (66.9%)	73.10, 76.43 eV (60.1%)	72.79, 76.12 eV (54.9%)
Pt (IV)	74.34, 77.67 eV (14.1%)	73.65, 76.98 eV (12.7%)	73.01, 76.34 eV (40.6%)

Upon knowing the morphologies as well as structural characteristics of the different Pt dichalcogenides, the pursuit to develop a non-enzymatic sensor for H₂O₂ detection continues. Figure 7.1 earlier not only convey the sensing capabilities of Pt dichalcogenides, but also provide a glimpse of their inherent electrochemistry. Since PtTe₂ is the superior material amongst its counterparts, its inherent electrochemistry is then studied by performing 10 consecutive scans of cyclic voltammograms. In the first scan, a reduction peak occurred at -0.4 V followed by an oxidation peak at -0.15 V (Figure 7.7A). After each cycle, these peaks diminish and signal flattens, implying the stable state of the material. This suggests that pretreatment is necessary prior to the detection of H₂O₂. The same reduction peak is previously observed when investigating the ORR capabilities of these materials.²⁵ Notably, it would diminish in N₂-saturated conditions. Experiments are subsequently conducted in N₂-saturated conditions where signal-to-noise ratio is improved (Figure 7.8).

Figure 7.7. Electrochemical response of PtTe₂-modified electrode (A) in PBS electrolyte for 10 scans, scan rate 100 mV s⁻¹, (B) upon addition of 0.1 mM H₂O₂ followed by 0.5 mM interferences at varying operating potential, and (C) addition of 0.1 mM H₂O₂ every 100 s after pretreatment at various potentials for 500 s. Conditions: N₂-saturated electrolyte PBS pH 7.4.

Developing the PtTe₂-based sensor for H₂O₂ detection involves optimisation of several parameters to meet analytical requirements. There are two potentials that have to be set, pretreatment as well as operating potential. As the name already suggests, the former refers to the potential applied to change the surface properties of the modified electrodes before detecting H₂O₂, while the latter would then be applied for detection. Looking into the operating potential first, selectivity studies was carried out. Various interferences in 0.5 mM were introduced into the solution every 100 seconds, starting with ascorbic acid (AA), glucose (Glu) followed by uric acid (UA). Selectivity for H₂O₂ is optimised by tuning the potential (Figure

7.7B). Eventually, -0.25 V was chosen for the operating potential of the system due to negligible response from undesired analytes.

Moving on to pretreatment studies, activation of electrodes would increase the reactivity of electrodes for the detection of a specific analyte.³⁴ Electrochemical reduction offers a promising strategy for platinum dichalcogenides since faster heterogeneous electron transfer rates and improved hydrogen evolution as well as oxygen reduction reactions have been reported.³⁵ Applying the same potential as the operating potential for 500 s grant a stable signal as well as an improved signal-to-noise ratio (Figure 7.7C).

Figure 7.8. Chronoamperometry data of PtTe₂-based electrode, electrochemically pre-treated at -0.25 V for 500 s, upon addition of 0.1 mM H₂O₂ every 100 s in ambient and N₂-saturated atmosphere. Conditions: PBS electrolyte pH 7.2, operating potential -0.25 V.

The last optimised parameter is the concentration of the material drop casted onto the electrode (Figure 7.9A). The current increases with increasing concentration, reinstating the role of PtTe₂ in enhancing the electrochemical performance of the system. After optimisation, the three different materials were compared again. PtTe₂ performed the best in enhancing the electrochemical signal, followed by PtSe₂ then PtS₂ (Figure 7.9B). A calibration graph is embedded as an inset to illustrate the materials' sensitivities towards H₂O₂. The highest sensitivity of $10.9 \mu\text{A mM}^{-1}$ is achieved by PtTe₂, surpassing 6.28 and $1.04 \mu\text{A mM}^{-1}$ by PtSe₂

and PtS₂ respectively. Their electrochemical sensing capabilities are influenced by conductivity, granted that PtS₂ is semi-conducting, PtSe₂ is semi-metallic while PtTe₂ is metallic.

Figure 7.9. Chronoamperometric data of (A) PtTe₂-modified electrode of varying drop cast concentrations and (B) PtX₂ of varying chalcogen component, upon successive injections of 0.1 mM H₂O₂. Conditions: cathodic pretreatment at -0.25 V for 500 s, N₂-saturated electrolyte PBS pH 7.4, operating potential -0.25 V, addition of 0.1 mM H₂O₂ every 100 s. (C) Cyclic voltammograms of scan rate 100 mV s⁻¹ and (D) Nyquist plots with Randle's equivalent circuit as inset, both employing 2 mM [Fe(CN)₆]^{4-/3-} redox probe in 0.1 M KCl to establish fundamental electrochemical properties of PtX₂.

To further probe electrochemical properties of the materials, heterogenous electron transfer (HET) and impedance experiments were carried out in 2 mM [Fe(CN)₆]^{4-/3-}. The

ferro/ferricyanide redox probe is established to be sensitive towards the surface of electrode materials.³³ Fast HET rates are reflected by small peak-to-peak separation (ΔE_p) between oxidation and reduction peak potentials in cyclic voltammograms. ΔE_p for PtTe₂ (68 mV) is considerably smaller than its counterparts, PtSe₂ (186 mV) and PtS₂ (413 mV), asserting PtTe₂ as a commendable electrocatalyst (Figure 7.9C). In parallel, Nyquist plots are generated from impedance studies with the equivalent circuit displayed as inset (Figure 7.9D). The equivalent circuit is modelled using Randle's approach modified with Warburg impedance (Z_w) to determine critical values. The semi-circle decreases drastically on going down the chalcogen group. This translates to decreasing charge transfer resistance (R_{ct}) where less opposition force is experienced to electron movement. The R_{ct} are extracted to be 19 726 Ω , 4728 Ω and 956 Ω for PtS₂, PtSe₂ to PtTe₂. PtTe₂ overall demonstrates the most favourable electrochemical properties, giving rise to superior electrochemical sensing capabilities.

The analytical parameters of the developed PtTe₂-based sensor are subsequently assessed to validate its sensitivity, repeatability, practicability and stability. Varying concentrations of H₂O₂ were injected into the solution, starting from 0.2, 2, 20 and 200 μ M (Figure 7.10A). There is no quantifiable response for 0.2 μ M, nonetheless electrochemical response is generated from 2 μ M onwards. A calibration graph is then plotted, establishing two linear relationships between current and concentration (Figure 7.10B). The two linear ranges are 3 to 300 μ M ($r = 1.00$) and 500 to 1100 μ M ($r = 0.98$), while the limit of detection and quantification are computed³⁶ to be 1.2 and 4.0 μ M respectively. The computed average relative standard deviation percentage (% RSD) across concentrations is 10.9%. Next, the practicability of the developed sensor is put to the test using a real sample. Human serum is diluted in the PBS electrolyte in the ratio of 1:1. The response is not compromised upon addition of H₂O₂ (Figure 7.10C). Stability is also investigated and the response dropped below 80% after 6 days (Figure 7.10D).

Figure 7.10. Analytical parameters of the developed PtTe₂-based sensor for the detection of H₂O₂. (A & B) Chronoamperometric data, together with calibration plot to demonstrate linear sensitivity and linear ranges. (C) Chronoamperometric data comparing real sample of serum diluted in PBS 1:1 ratio. (D) Current response after several days for stability test.

It is of interest to assess the performance of the developed PtTe₂-based sensor relative to those in literature. There are a multitude of materials but the ones shortlisted in Table 7.2 are more relevant to PtTe₂. Other layered materials such as group 6 TMDs (MoS₂, WS₂) and black phosphorus sensors have been included. Only pure materials are considered, ruling out composites with an exception of MoS₂-Pt. It is a common strategy to incorporate noble metal nanoparticles, especially catalytic Pt, with MoS of large surface area. Pt-based structures are compared since shape is an important factor.³⁷ The analytical performance of the developed sensors are comparable in terms of linear range and LOD. The advantages of PtTe₂ of large

surface area, good conductivity and electrochemical properties are to be recognised. In addition, Pt chalcogenides are explored to reduce Pt loading, utilising fractional amount of Pt yet yielding comparable or improved performance. Hence, it would be recommended to state the Pt loading to facilitate future comparison.³⁸ The value is generated by dividing mass by geometrical area, which is 0.106 mg cm⁻² for the PtTe₂-based sensor.

Table 7.2. Table of comparison of non-enzymatic sensors for H₂O₂ detection.

Electrode	Linear Range	LOD	Ref.
GCE/ PtTe ₂	3 to 300 μ M 500 to 1100 μ M	1.2 μ M	Our work
GCE/ MoS ₂ -Pt	4 μ M to 48.5 mM	1 μ M	39
C/ 1T-WS ₂ (Lab-on-a-chip)	20 nm to 200 μ M 100 μ M to 2 mM	2.0 nM	40
GCE/ Black Phosphorus	0.1 to 50 μ M	0.1 μ M	41
Mesoporous Pt microelectrode	20 μ M to 40 mM	4.5 μ M	42
GCE/ Pt Nanowire	5 μ M to 3 mM	9.6 μ M	43
PtE/ Pt Nanoflower	10 to 400 μ M	0.32 μ M	44

7.3 Conclusion

Three platinum compounds with varying chalcogen compounds were synthesised, PtS₂, PtSe₂ and PtTe₂. Extensive characterisation including TEM, EDS as well as XPS ascertained their layered structures and chemical states. With regards to H₂O₂ detection, PtTe₂ demonstrates superior performance, followed by PtSe₂ and PtS₂ the worst. This is attributed to their conductivity of metallic, semi-metallic and semi-conducting respectively. HET and

impedance studies substantiate the electrochemical sensing capabilities, with PtTe₂ demonstrating favourable electrochemical properties. Further developed and optimised as a sensor, analytical parameters were established. It is selective to H₂O₂, unaffected by other interfering compounds like ascorbic acid, uric acid and glucose. It is additionally sensitive (10.9 $\mu\text{A mM}^{-1}$), with limits of detection and quantification of 1.2 and 4.0 μM respectively. Moreover, there are two wide linear ranges of 3 to 300 μM and 500 to 1100 μM . The stability as well as feasibility in real sample of human serum was assessed too.

7.4 Methods and Materials

Chemicals. Tellurium (99.999%) and selenium (99.999%) were acquired from Chempur, Germany while sulfur (99.999%) was acquired from STREM Chemicals, Germany. Ammonium hexachloroplatinate (IV) was acquired from Safina, Czech Republic. Hydrogen peroxide (35% w/w aqueous solution) was acquired from Alfa Aesar, Singapore. Phosphate buffered saline tablet, D-(+)-glucose ($\geq 99.5\%$), L-ascorbic acid (BioXtra, $\geq 99.0\%$, crystalline) and uric acid ($\geq 99\%$, crystalline) were acquired from Sigma-Aldrich, Singapore.

Characterisation of Materials. Raman spectroscopy was performed using Horiba LabRam HR, a confocal Raman microscope, in backscattering geometry with CCD detector, 514.5 nm argon-ion excitation laser as well as 100x magnification objective lens. The sample preparation involved drop casting the suspension of materials onto silicon wafer and subsequently dried, while the instrument was calibrated according to silicon peak at 520 cm^{-1} . X-ray photoelectron spectroscopy (XPS) measurements relied on Phoibos 100 spectrometer (SPECS, Germany), coupled with a monochromatic Mg X-ray radiation source. The samples were prepared by grinding solid forms of TMDs in a mortar, thereafter placed as a uniform thin layer onto a carbon conductive tape. XPS data was consequently processed and deconvoluted with Casa XPS, where the relative sensitivity factor, area constraint, and position constraint

were set to obtain a good fitting. For the quantification of oxidation states for the materials, the area and relative sensitivity factor were considered. JEOL JEM-2200FS was operated at 200 keV accelerating voltage to obtain high resolution transmission electron microscopy (HR-TEM) data. The sample preparation involved suspension of materials in water (1 mg mL^{-1}), which was drop casted onto a TEM grid (Cu; 200 mesh; Formvar/carbon) and subsequently dried at 60°C for 12 h. X-ray diffraction (XRD) was accomplished via Bruker D8 Discoverer and the corresponding Cu $K\alpha$ radiation source ($\lambda = 0.15418 \text{ nm}$). Further processing and analysis were performed using EVA software.

Synthesis of Platinum Dichalcogenides. Platinum was firstly obtained from ammonium hexachloroplatinate (IV) that was reduced by hydrogen at 500°C for 1 h. The three materials of interest (PtS_2 , PtSe_2 and PtTe_2) were synthesised based on their respective elements in stoichiometric amounts. They were placed in a quartz glass ampoule (3 mm wall thickness, 18 mm outside diameter, 18 mm long) which was evacuated to $1 \times 10^{-3} \text{ mbar}$ and melt-sealed by oxygen-hydrogen welding torch. For PtS_2 , the ampoule was heated at 800°C for 90 days, where both heating and subsequent cooling rate were 5°C min^{-1} . Unlike its chalcogen counterparts, a shorter duration would yield a mixture of monosulfides (PtS) and disulfides (PtS_2). Henceforth, a longer time is necessary to optimise the synthesis of disulfides. PtSe_2 , on the other hand, was synthesised by heating the ampoule at 800°C for 48 h and thereafter at 1270°C for 1 h. Meanwhile, the ampoule with PtTe_2 was heated at 1000°C for 48 h followed by 1150°C for 1 h. The heating and subsequent cooling rate were $10^\circ\text{C min}^{-1}$ and 1°C min^{-1} respectively for both PtSe_2 and PtTe_2 .

Preparation of Electrodes. Suspensions of platinum dichalcogenides were prepared by dispersing the respective materials in ultrapure water (7.5 mg mL^{-1}). They were ultrasonicated for three hours to ensure exfoliated, homogenous and well-dispersed suspensions. For subsequent usage, the suspension would be ultrasonicated for five minutes.

The surfaces of working electrodes, referring to glassy carbon, were polished using a slurry of alumina (5.0 and 0.05 μm) on a polishing pad. They were rinsed using ultrapure water before blown dry with N_2 gas. The modification of electrodes firstly involved drop casting 4 μL of platinum dichalcogenides suspension then left to dry in ambient conditions.

Electrochemical Measurements. Electrochemical techniques, including chronoamperometric and voltammetric, were executed using Metrohm $\mu\text{Autolab III}$ through NOVA software version 1.10. A three electrode system was employed, consisting of Ag/AgCl reference electrode, platinum counter electrode and glassy carbon working electrode that was either bare or modified. Phosphate buffered solution (PBS) was purged with N_2 gas for 15 minutes prior to its use as the electrolyte, unless otherwise stated. PBS (pH 7.4, at 25°C) was prepared by dissolving the tablet in 200 mL deionised water to yield 0.01 M phosphate buffer, 0.137 M sodium chloride and 0.0027 M potassium chloride. Cyclic voltammetry was performed at a 50 mV s^{-1} scan rate, from 0 V to -0.7 V. Specific concentrations of H_2O_2 were injected into the PBS electrolyte and stirred for two minutes before each measurement. Additionally, the modified electrodes were subjected to electrochemical pretreatment prior to chronoamperometric measurements. Electrochemical pretreatment was performed using chronoamperometry ($\Delta t > 1 \text{ ms}$) at a fixed potential of -0.25 V for 500 seconds before measurements at the same potential. Specific concentrations of H_2O_2 were injected into the solution every 100 seconds, where stirring was continuous throughout the measurement. The acquired data was subjected to baseline correction for further analysis.

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PART II

THREE-DIMENSIONAL PRINTING

Part II

Chapter 8

Background of Three-Dimensional (3D) Printing

8.1 Introduction to 3D Printing

Three-dimensional (3D) printing is defined as “the fabrication of objects through the deposition of a material using a print head, nozzle, or another printer technology”.¹ At a glance, the printing process requires modelling of the object using computer-aided design (CAD) software, 3D scanner or photogrammetry.² The virtual representation would then be translated into a series of coordinates and horizontal planes before the printer produces the real object layer by layer.³ 3D printing is synonymous with additive manufacturing which refers to the process of joining materials typically in consecutive layers to fabricate parts following the 3D model data. Not only are the descriptions fitting of each other, but both technologies also share similar manufacturing principles which contrast to subtractive manufacturing. The terminologies are used interchangeably, nonetheless, 3D printing is presently associated with machines that are modest in price and overall capability.

3D printing conveys an enthusiastic vision of production which is accessible, customisable, and rapid. It is portrayed that users, regardless of location, can simply create or modify any designs conveniently downloaded from the internet. Even complex designs can materialise into products, rapidly and at low cost, using a portable and robust device.⁴ Such vision has transcended the historical expectations of prototype technology, initially set out to overcome the lengthy and counterproductive fabrication process. Building a prototype consumed 1-2 months, further burdened by the numerous attempts to refine the design.³ 3D printing remarkably accelerates the process as well as extends beyond prototyping to accomplish the actual product.

Apart from the apparent improvement in the fabrication process, there are implied time and cost benefits of 3D printing. The on-demand and decentralised printing capability promote self-sufficiency as there is less reliance on external procurement, especially meritorious when

parts are expensive or considered obsolete. Shortening the supply chain by reducing logistics and transport would bring about favourable economic and environmental consequences.⁵ 3D printing also offers design flexibility and materials efficiency, owing to its additive manufacturing principle. The object is fabricated layer by layer, thereby enabling mass customisation and minimal material wastage. Furthermore, leftover materials can often be reused with little processing.⁶ This is a stark contrast to conventional manufacturing techniques which remove large amounts of materials to fabricate a product as well as require auxiliary cutting tools to obtain defined and complex structures.⁷

Spurred by the advantages and potential of 3D printing, together with the patent expiry of in-demand printing techniques like stereolithography and fused deposition modelling, research has been dynamic to expand the technology's possibilities. A prominent research direction is to broaden the diversity of printable materials.⁸ Polymers, metals, ceramics, and concrete are materials successfully printed, yet efforts are still ongoing to enrich their properties and performances.⁹ In addition, unique materials ranging from biological elements^{10,11} to green materials¹², nanomaterials¹³ and even food¹⁴ are explored, either by integrating them into established materials or innovating printing systems. This would greatly facilitate access to a multitude of applications, including healthcare.^{5,9} There are already commercial products such as customised dental crowns, bridges and clear aligners, as well as hearing aids that profit from the efficiency of 3D printing.^{6,15} The progress in electrochemical biosensors to detect biomarkers, on the other hand, would be discussed in the succeeding subsection.

8.2 3D Printing for Electrochemical Biosensors

The advantages of 3D printing are alluring to electrochemical biosensors since the direct design-to-object workflow advances customisation as well as the manufacture of devices and components.¹⁶ Prototypes can be efficiently modified and optimised before finalising a product for the intended application. Adding on to the ease of manufacturing, the 3D printing process is fully automated where a consistent fabrication quality is expected and thus, promote the reproducibility of biosensors. These advantages, coupled with the on-demand and decentralised printing capability, are especially attractive for point-of-care testing.¹⁷ Field laboratories can be equipped with cost-efficient 3D printers to produce necessary components for remote testing.¹⁸ This sub-section herein presents a literature review on the integration of 3D printing into electrochemical biosensors. There would be a systematic elaboration on microfluidic devices and electrodes which the technology has successfully printed. Advantages, prominently design flexibility and various printable materials, along with challenges would be highlighted.

3D printing holds promises to enhance the overall efficiency and accessibility of microfluidic devices which are reputed for their low cost and rapid biosensing capabilities. Such devices reproduce laboratory-scale processes using small volumes of fluid ranging from microlitre to as low as attolitre.¹⁹ Processing and analysis events have been integrated within a miniaturised system that is portable as well as consumes significantly less reagent and sample volumes. These devices are mostly based on thermoplastic and polydimethoxysilane (PDMS), fabricated by the traditional techniques of injection moulding and soft lithography. However, they require a large-scale purpose-built facility and are too manually intensive as well as costly to be viable in academic laboratories or limited resourced areas.^{7,20} In turn, paper-based microfluidic devices are adopted because of simplicity, low cost and the material's ubiquitous abundance.²¹ The fluid moves according to the capillary effect, simultaneously insinuating

limited control and manipulation over it. 3D printing, therefore, demonstrates the potential to overcome the mentioned drawbacks by offering ease of production and design freedom while upholding cost-effectiveness. This promotes the development of microfluidics in academic and field laboratories, even possibly transitioning to industries for mass production, granted the compatibility with a handful of 3D printing's large material pool.¹⁷

A microfluidic device for influenza virus detection has been produced by fused deposition modelling (FDM), one of the techniques of 3D printing.²² Thermoplastic filament, polylactic acid (PLA) in this case, was heated to a semi-molten state prior to its extrusion and deposition onto a stage in consecutive layers. The material fused together during printing and solidified to accurately fabricate the designed microfluidic device. Reagents, starting with streptavidin-modified paramagnetic beads, were introduced at the device's inlet and channelled to a reaction chamber. There, the beads were anchored by a magnetic pad to further build the biosensing structure, instead of continuing the flow and leaving the device by an outlet. With washing steps in between, biotinylated glycan and hemagglutinin labelled with CdS quantum dots were sequentially added. Glycan, immobilised onto the magnetic beads via streptavidin-biotin interaction, was the biorecognition element to capture hemagglutinin which is an influenza A surface antigen. Upon successful binding, the modified magnetic beads were released by retracting the magnetic pad and then deposited onto commercial electrodes that were fitted before the device's outlet for voltammetric analysis. Cadmium of CdS quantum dots subsequently underwent electrochemical reduction to quantify hemagglutinin. This work emphasises the efficiency of the overall process, from the production of the device by 3D printing to the detection application. Printers based on FDM technology are affordable and simple to operate, while the printing material PLA is biodegradable and non-toxic.

Another development of a microfluidic device showcased the design flexibility of 3D printing, as a bespoke on-body biosensor was assembled to continuously monitor dynamic

changes of glucose and lactate for sports applications.²³ Since 3D printing facilitates numerous design modifications, different channel sizes were fabricated to optimise the performance of the microfluidic device. The smallest among the tested dimensions attained the fastest linear rate and response time, governed by the cross-sectional area and broadening of concentration change. In addition, the microfluidic device was designed to integrate with commercially available components. FDA-approved microdialysis probes were inserted subcutaneously on test cyclists while their outlets were connected to the printed microfluidic device for further analysis.

The same microfluidic device was accompanied by needle-type biosensing electrodes. Keen on modular biosensors to monitor relevant biomarkers, there were two working electrodes modified with glucose oxidase and lactate oxidase to respectively analyse glucose and lactate. The challenge lied in securing these electrodes in the microfluidic channel. Attempts with commercial fittings were futile as they were too large to tightly seal the microfluidic chip, resulting in leakages. Henceforth, 3D printing was implored to fabricate customised electrode holders. The technique of polyjet printing was relied on where liquid photopolymer was deposited then cured by a UV light source, in a layer-by-layer fashion. A remarkable feature of this technique was the simultaneous printing of different materials properties. Both hard and soft plastics were printed for the electrode holder since the rubber-like, compressible nature of the latter would prevent leakages by ensuring a good seal with the microfluidic device. The electrode holder was also designed to guide the placement of electrodes and fixate them in the microfluidic channels, preventing their repositioning when users move. Minimal noise was generated for improved sensitivity, on top of the commendable reproducibility achieved. Constituting the biosensing electrodes together with the holder, microfluidic device as well as commercial microdialysis probes, the entire setup was compactly

designed and attached securely on the lower backs of test cyclists. This articulates the potential contribution of 3D printing in wearable biosensing technologies.

Meanwhile, 3D printing of electrodes is integral to the complete manufacturing of electrochemical biosensors. Because they are key components for molecular recognition and transduction, the fabrication of electrodes would greatly appeal to point-of-care applications. An obvious prerequisite is good conductivity of printable materials where metal powders, silver inks and carbon filaments have been explored. These materials would be discussed, together with any necessary post-fabrication steps to enhance their conductivity. One coveted advantage of 3D printing is the freedom to fabricate electrodes with unique designs and material compositions. Electrodes in the form of mesh²⁴, ribbon, and gauzed basket²⁵ have been actualised, while a comparison of their performances for electrochemical oxygen evolution reaction (OER) is made.²⁶ This reiterates the capability of 3D printing to fabricate complex designs as well as tailor pivotal aspects, including geometry, roughness and porosity.

A helical-shaped stainless steel electrode, for DNA detection, was fabricated based on the 3D printing technique of selective laser melting (SLM).²⁷ The helical design exemplified an irregular structure that may be complex and time-consuming if fabricated by subtractive manufacturing methods but is relatively straightforward and easy to print with high precision via 3D printing.²⁸ In the SLM technique, stainless steel powder was deposited according to the horizontal cross-sections of the intended design before being subjected to a high intensity laser beam to fuse the particles. The process was repeated in successive layers, adding up to the helical-shaped electrodes. Despite the fabricated electrodes exhibiting normal metal conductivity when tested with a multimeter, they suffered from high charge transfer resistance and slow electron transfer. Electroplating the stainless steel electrodes with gold offered an attractive solution to improve the electrochemical sensing performance^{29,30} as well as facilitate the immobilisation with thiol-functionalised probe DNA through thiol-gold covalent bonds.

The resulting biosensor selectively hybridised with complementary DNA target to form the double helix structure, where electroactive methylene blue label intercalated. As such, quantification was derived from the reduction intensity of the label.

The design feature of conical microarrays has been introduced on a planar electrode surface to increase surface roughness as well as active surface area.³¹ Relevant to biosensors, a microelectrode array was developed to detect glucose with satisfactory analytical performance.³² Their superiority over conventional single microelectrodes arose from enhanced mass transfer and decreased solution resistance. An array of conductive silver electrodes was fabricated by the 3D printing technique of direct write. It is an extrusion-based technique, also referred to as direct-ink writing or robocasting. The printable precursor is a highly viscous ink that hardens upon deposition on substrates. In this work, an ultrasonic atomizer was engaged to dispense dense mists of Ag NPs onto a hydrophilic glass slide. Photocurable polymer traces were afterwards printed perpendicular to the linear traces of Ag NPs and instantaneously cured by UV source to create the grid or array of microelectrodes. Printing parameters were optimised, particularly the width of polymer traces that were equivalent to the spacing between silver microelectrodes, for a maximum signal to noise ratio. A lower dimensional limit was established to avoid diffusion overlap with neighbouring microelectrodes. For further development into a biosensor, the conductive array was systematically modified with glucose oxidase, Nafion and glutaraldehyde via drop casting to enable the detection of glucose.

3D printed electrodes are compatible with wearables technology when fabricated onto flexible substrates. Again using the direct write technique, Ag NP ink was deposited onto polyethylene terephthalate (PET) substrate to produce a biosensor patch.³³ The commendable mechanical properties of both the viscous ink as well as flexible substrate offered good conformability of the patch with the human body. Electrodes based on the deposited Ag NP

ink were freestanding yet conductivity was further enhanced by a thermal post-fabrication treatment. Annealing conditions were optimised at 70°C for an hour, lower than the glass transition temperature of PET (110 °C) to retain the mechanical integrity of the substrate. Upon acquiring low resistivity, the electrodes were modified by drop casting lactate oxidase enzyme or chloridized with bleach to ascertain the working and reference electrodes respectively. The developed biosensor patch was coupled with a potentiostat, also 3D printed, to detect lactate.

A laudable advancement is the direct 3D printing of biorecognition elements, instead of immobilising them onto electrodes after fabrication. An enzyme ink has been prepared for the 3D printing technique of direct write, by combining a solution of glucose oxidase with a formulated sol-gel, adding cellulose next, and finally cooled.³⁴ Desirable printing behaviour, during extrusion and after deposition, were observed for the enzyme ink based on rheological tests to affirm its printability. The enzyme was printed on bare carbon electrodes, which are firstly fabricated by depositing and drying Prussian blue carbon conductive ink on temporary tattoo paper. Multiple materials, referring to the enzyme as well as Prussian blue carbon, were precisely integrated to establish the biosensing electrode. Prussian blue served as a mediator to efficiently transfer electrons between the glucose oxidase enzyme and carbon electrode. Another striking aspect of the study was the comparison between 3D printing and conventional screen printing. The latter method fabricated electrodes by laser cutting stencils of the same electrode design then casting the same carbon ink. Nonetheless, 3D printed electrodes flaunted a more uniform and homogenous distribution of materials, in addition to the distinct edges and minimal defects. The superior printing quality was attributed to the higher deposition resolution of the method as well as ink homogeneity, leading to better analytical performance. Wider linear range and higher sensitivity for glucose detection were achieved for the 3D printed biosensors. At the same time, there was a significant reduction of material waste as the 3D printing method almost halved the material volume involved in screen printing. This study

consolidates the advantages of 3D printing, namely the possibility of directly printing biological elements onto flexible substrates as well as superiority over conventional methods.

Carbon-based materials are prominent in the fabrication of electrodes, particularly incorporated in thermoplastic filaments for printing by FDM. Variants of carbon include carbon black, graphene and nanographite, all of which confer conductivity. Unlike SLM or direct write techniques that fabricate the aforementioned metallic electrodes, FDM is a more affordable printer and has simpler printing mechanics. Additionally, different materials can be printed simultaneously with multiple nozzles. Carbon-PLA and PLA filaments have been extruded to fabricate three electrodes (working, counter, reference) and the holder respectively.³⁵ Only the working electrode is modified, immersed in a solution of glucose oxidase enzyme and Nafion, to prepare a glucose biosensor. The established linear concentration range in detecting glucose is 2 to 28 mM, although a lower detection range of 0.1 to 1 mM was attained for both silver and Prussian blue carbon electrodes fabricated by direct write printing technique.^{32,34} This was due to the low amounts of conductive carbon within the printable filament, about 10% or less, which poses a considerable challenge for the FDM technique.^{36,37} A higher percentage of carbon would compromise on the printability, since aggregation of the material may occur and block the nozzle. In contrast, a lower percentage of thermoplastic led to brittle filaments and irreproducible fabrication. Efforts to optimise conductivity as well as printability results in 28% of carbon³⁷, but still far from the suggested percentage of 50% in order to possess satisfactory conductivity for electrochemical applications.³⁸

Post-fabrication modifications are therefore called upon to maximise carbon content printed with thermoplastic filaments, aimed to increase conductivity and electrochemical sensing performance. A nature-inspired activation process, outstanding and relevant to biosensors, has been ventured to modify graphene/PLA electrodes.³⁹ Delving into the biodegradability of PLA, it underwent abiotic hydrolysis that can be accelerated by certain

enzymes such as proteinase K. The graphene/PLA electrode was consequently incubated with proteinase K where selective digestion of PLA occurred, leading to the exposure of conductive graphene sheets that were previously concealed. It was imperative to optimise the duration of digestion, to efficiently minimise PLA content yet maintaining mechanical properties of the electrodes which would disintegrate otherwise. In the development of a biosensor, proteinase K was washed off and the modified electrode was incubated with another enzyme, alkaline phosphatase, to convert electro-inactive 1-naphthyl phosphate substrate into electroactive 1-naphthol for quantification. The successful activation, apparent from characterisation and electrochemical experiments, has overcome the main hurdle of FDM technique in fabricating conductive electrodes.

All in all, the combination of 3D printing and electrochemical biosensors has seen the fabrication of microfluidic devices, physical housing and support structures, as well as electrodes. Freedom of design, offered by 3D printing, liberates the development of microfluidic devices with desired flow and optimised channel dimensions. The wide range of printable materials and printing techniques are leveraged in the fabrication of electrodes, including stainless steel powder in SLM, silver inks in direct write and carbon-based filaments in FDM. Nonetheless, there is still much space to explore while following the more established footsteps of optical biosensors and other electrochemical devices.^{2,40} A fully 3D printed electrochemical biosensor, the device together with electrodes, would significantly advance point-of-care and wearable technologies.

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Part II

Chapter 9

3D Printed Ag/AgCl Pseudo-Reference Electrodes

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The thesis author was the primary investigator and first author of the above paper.

9.1 Introduction

Three-dimensional (3D) printing is analogous to a handful of terminologies, namely additive manufacturing and rapid prototyping. Nonetheless, it offers the advantages of customisation, reproducibility, and rapid production. There are various reviews and articles addressing the different aspects of the technology- materials¹ (including biological^{2,3} and green materials^{4,5}), printing techniques⁶, as well as applications. Further elaborating on applications, there is a diverse spectrum ranging from construction⁷, energy^{8,9} to chemical sciences and biomedical^{10,11} together with (bio) sensors^{12,13}.

Along with the advancements in 3D printing, there has been a significant interest for on-demand, customized fabrication of electrochemical devices.^{14,15} An essential component is the reference electrode (RE), which measures and controls the potential of the working electrode. It is therefore imperative for the RE to have a constant and stable potential, despite the changes occurring in the electrolyte solution. Amongst the types of REs, Ag/AgCl is the most frequently used attributed to its simple design, easy fabrication, high stability and non-toxicity. The current trend towards on-demand, customizable remote fabrication of the complete setup of electrochemical flow cells¹⁶, sensors and energy devices^{17,18} has meant the need for 3D printing of not just the main working electrode^{19,20}, but also of the RE.²¹

The preparation of Ag/AgCl pseudo-reference electrodes (p-RE) is herein described, relying on the 3D printing technique of FDM. The electrodes are firstly printed using graphene/PLA filament which is extremely cost-effective and available in any place. Moreover, it is conductive enough, allowing silver electrodeposition for further modification. Bleaching is subsequently required to attain a layer of Ag/AgCl on the 3D printed graphene/PLA electrode. The fabrication process is optimised, followed by their characterisation using scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS). Lastly,

the fabricated electrodes would be put to the test by studying their performance in heterogeneous electron transfer (HET) reactions using two different redox systems.

9.2 Results & Discussion

The fabrication of the 3D printed Ag/AgCl pseudo-reference electrode (p-RE) commenced with its design of a long enough rod for convenient insertion into the electrochemical cell and easy attachment to the cables connected to the potentiostat. At the same time, a circular disc at the end of the rod provides a surface for electrochemical reactions to take place. The design was then materialised as it was 3D printed using a conductive graphene/polylactic acid (PLA) filament (Scheme 9.1). Prior to its intended function as p-RE, further modification was necessary. This included electrodeposition in a silver plating solution before immersing it into bleach. The bleach, consisting of OCl^- as well as Cl^- , leads to the formation of AgCl on the surface of the electrode, giving rise to 3D printed Ag/AgCl electrodes.²²

Scheme 9.1. Schematic representation illustrating the 3D printing process of graphene/PLA electrode, followed by silver electrodeposition then bleaching to obtain Ag/AgCl pseudo-reference electrode. The WE in the schematic diagram is later used as the RE.

A potential of -0.9 V was preliminarily applied for 600 seconds to electrodeposit silver onto the electrode followed by bleaching for 30 seconds. The modification process was validated based on open circuit potential (OCP) measurements against the commercial Ag/AgCl reference electrode in 3.5 M KCl supporting electrolyte. The values represent the potential difference between the fabricated and commercial electrodes, where 17.2 ± 0.2 mV was initially obtained. Nonetheless, motivated by the fabrication feasibility of 3D printed pseudo-reference electrodes, the parameters were subsequently optimised to improve the OCP values close to the commercial ones. Figure 9.1 conveys the effect of electrodeposition duration and potential, as well as bleach duration on the OCP values. The best result was achieved when electrodeposition occurs at -0.9 V for 800 seconds together with bleach duration of 90 seconds. The OCP value of 14.1 ± 0.3 mV is satisfactory, especially stable and reproducible, compared to the wide range of OCP values of the 3D printed graphene/PLA electrode before modification (70.4 to 406.3 mV).

Figure 9.1. Optimisation of modification parameters- (A) varying duration of electrodeposition with fixed potential of -0.9 V and 30 seconds in bleach, (B) varying electrodeposition potential with fixed duration of 800 seconds and 30 seconds in bleach, and (C) varying bleach duration with fixed electrodeposition potential of -0.9 V for 800 seconds.

Scanning electron microscopy (SEM) was carried out to capture the drastic morphology changes of the electrode throughout the fabrication process. As shown in Figure 9.2A, nanowires of random orientation are observed for the initial 3D printed graphene/PLA

electrode. This is in agreement with another report, which comprehensively characterised both the filament and 3D printed product, as they described the surface to be less uniform with the appearance of cracks and ridges.²³ The nanowires observed are assigned to the PLA structure/binder. Upon the electrodeposition of silver, the electrode is then covered with flakes although noticeable pores are present (Figure 9.2B). These pores imply an incomplete electrodeposition. Lastly, particles of AgCl are observed in Figure 9.2C after immersing the electrode in bleach solution.

Figure 9.2. SEM images of (A) 3D printed graphene/PLA electrode, (B) 3D printed Ag electrode, followed by (C) 3D printed Ag/AgCl p-RE. Scale bar represents 1 μm .

Energy dispersive X-ray spectroscopy (EDS) was performed to examine the elemental composition of the electrode after each modification step. In Figure 9.3, the elemental maps highlight the dominant element through colour intensity and numerical atomic percentage. They correlate to the modification process as the dominant element is C due to graphene/PLA (Figure 9.3A), Ag after electrodeposition (Figure 9.3B), followed by both Ag and Cl since AgCl is formed during bleaching. The ratio of Ag (47.6%) and Cl (27.8%) is calculated to be 1.7:1, which suggest the composition of the redox couple Ag/AgCl in both forms.

Figure 9.3. EDS spectra together with individual elemental composition of (A) initial 3D printed graphene/PLA electrode, (B) 3D printed Ag electrode and (B) 3D printed Ag/AgCl p-RE.

Subsequently, the performance of our 3D printed Ag/AgCl p-RE is tested in electrochemical measurements. Cyclic voltammetry is popular amongst the many electrochemical techniques due to its usefulness in studying reduction and oxidation processes of molecular species as well as electron transfer-initiated chemical reactions. Two redox systems of different surface sensitivity were employed, $[\text{Fe}(\text{CN})_6]^{3-/4-}$ and $[\text{Ru}(\text{NH}_3)_6]^{2+/3+}$, to compare the heterogeneous electron transfer (HET) and stability properties between 3D printed pseudo-reference and commercial reference electrodes. Measurements were carried out in triplicate, so average values and voltammograms are reported herein.

Figure 9.4 depicts voltammograms in the respective redox systems where the first scan for both reference electrodes has been excluded as a result of its inconsistency and irregularity with subsequent scans. Analysing their 2nd scans, both commercial and fabricated electrodes

give the same shape and intensity but different peak potentials. A shift to the left is observed in the latter's voltammogram. For the $[\text{Fe}(\text{CN})_6]^{3-/4-}$ redox system, the anodic (oxidative) and cathodic (reductive) peak shifted by 85 mV and 34 mV respectively, whereas there was a consistent shift of 60 mV for both oxidative and reductive peaks of $[\text{Ru}(\text{NH}_3)_6]^{2+/3+}$ redox system. Additional information regarding peak-to-peak separation (ΔE_p) is attained by subtracting cathodic peak potential (E_{pc}) from the anodic peak potential (E_{pa}). All the values are compiled in Table 9.1.

Figure 9.4. Cyclic voltammograms of 10 mM (a) $[\text{Fe}(\text{CN})_6]^{3-/4-}$ and (b) $[\text{Ru}(\text{NH}_3)_6]^{2+/3+}$ when using commercial Ag/AgCl RE and 3D printed Ag/AgCl electrode p-RE, with numerous scans. Conditions: phosphate buffered saline solution (pH 7.2) and scan rate, 100 mV s^{-1} .

Another crucial requirement of a RE is stability. This is investigated by numerous cycling of the electrode. Up to 50 cycles was tested for the $[\text{Ru}(\text{NH}_3)_6]^{2+/3+}$ system, which takes about 30 minutes. Even so, there is not much deviation from the commercial electrodes implying the stability in a continuous measurement. Overall, our fabricated p-RE electrode behaves similarly to the commercial RE based on shifts in potential and current.

3D printing has made possible rapid and customisable production of p-RE, adding to the advantages of the electrochemical component. The advantages include simplicity since they can be immersed directly into the electrolyte, small ohmic resistance effect as well as absence

of liquid junction potential.²⁴ At the same time, the disadvantages of p-RE should also be raised especially the lack of thermodynamic equilibrium. It should therefore be emphasised that a calibration by a reference redox system is performed to establish their potential.

Table 9.1. Comparison between commercial and fabricated reference electrodes of values extracted from cyclic voltammograms, where redox systems $[\text{Fe}(\text{CN})_6]^{3-/4-}$ and $[\text{Ru}(\text{NH}_3)_6]^{2+/3+}$ are employed.

$[\text{Fe}(\text{CN})_6]^{3-/4-}$	E_{pa} [V]	I_{pa} [μA]	E_{pc} [V]	I_{pc} [μA]	ΔE_{p} [V]
Commercial Electrode	0.406	73.3	0.032	-70.4	0.363
Fabricated Electrode	0.321	81.6	-0.002	-77.7	0.300
$[\text{Ru}(\text{NH}_3)_6]^{2+/3+}$	E_{pa} [V]	I_{pa} [μA]	E_{pc} [V]	I_{pc} [μA]	ΔE_{p} [V]
Commercial Electrode	-0.067	123.0	-0.269	-121.6	0.203
Fabricated Electrode	-0.127	122.7	-0.328	-119.7	0.201

9.3 Conclusion

A straightforward, fast and relatively simple method is herein proposed to prepare an Ag/AgCl pseudo-reference electrode which was based on 3D printing technology. The fabrication process involves electrodeposition of silver followed by bleaching to form AgCl on the electrode's surface. Optimisation on the electrodeposition and bleaching parameters were

studied based on open circuit potential measurements against commercial reference electrodes. Thereafter, their surface morphologies and elemental composition were captured using scanning electron microscopy and energy dispersive X-ray spectroscopy. The electrodes were put to the test, through cyclic voltammetry in two redox systems. They exhibit similar voltammogram shape and intensity, with a shift to the more negative peak potential. The ability to fabricate reproducible and stable 3D printed reference electrodes will allow full integration and printable electrochemical systems.

9.4 Methods and Materials

Chemicals. Conductive graphene/polylactic acid filament, silver plating solution and concentrated liquid chlorine bleach were purchased from Black Magic 3D, Technic Inc. and GB Chemicals respectively. Potassium chloride (for molecular biology, $\geq 99.0\%$), potassium hexacyanoferrate (II) trihydrate ($\geq 98.5\%$), potassium hexacyanoferrate (III) ($\sim 99\%$), hexaammineruthenium (III) chloride (98%) and phosphate buffered saline tablet were procured from Sigma Aldrich.

Instrumentation. Electrochemical experiments, both electrodeposition and heterogenous electron transfer, were performed using Metrohm Autolab PGSTAT204 through NOVA software version 1.10. Field emission JEOL 7600F was used to perform scanning electron microscopy and energy dispersive X-ray spectroscopy, operated at acceleration voltages of 5 and 15 kV. Specimens were prepared by placing the 3D printed electrodes of interest on an aluminium stub using carbon tape.

Fabrication of 3D Printed Ag/AgCl Pseudo-Reference Electrodes. The electrodes were virtually modelled with Autodesk Fusion 360 CAD software. An open source Slic3r software was used to convert the design to .STL and subsequently .GCODE files. Meanwhile, the bed and brass nozzle of Prusa i3 MK3 printer were respectively set at temperatures of 60°C

and 205 °C to fabricate the electrodes. Other printing parameters were based on the PLA configuration from Prusa Slic3r. Upon the fabrication of the electrodes, a three electrode system was employed for electrodeposition of silver. They consist of 3D printed graphene/PLA electrode, platinum and Ag/AgCl serving as the working electrode, counter electrode and commercial reference electrode respectively. These electrodes were placed in silver plating solution and a constant potential of -0.9 V was applied for 800 seconds. The silver electrodeposited electrodes were referred as 3D printed Ag electrodes. They were subsequently dipped into concentrated liquid chlorine bleach for 90 seconds, giving rise to 3D printed Ag/AgCl electrodes. To rinse, the electrodes were dipped into ultrapure water and blown dry with compressed nitrogen.

Electrochemical Measurements. Open circuit potentials of the 3D printed electrodes were determined with respect to commercial Ag/AgCl reference electrodes, in a supporting electrolyte of KCl (3.5 mM). The relative potentials were measured using a Fluke 114 Electrical Multimeter that was allowed to stabilise over a period of 120 seconds before recording. Heterogeneous electron transfer studies were carried out with a three-electrode system: a glassy carbon working electrode, a platinum counter electrode, together with either a commercial Ag/AgCl reference electrode or a 3D printed Ag/AgCl pseudo-reference electrode. Cyclic voltammetry was swept at 100 mV s⁻¹ scan rate, between the potentials of -1.0 and 1.0 V. Two redox systems were employed; [Fe(CN)₆]^{3-/4-} and [Ru(NH₃)₆]^{2+/3+}, both concentrations of 10 mM in phosphate buffered saline solution. The former was obtained by mixing potassium hexacyanoferrate (II) trihydrate and potassium hexacyanoferrate (III) while hexaammineruthenium (III) chloride was used for the latter.

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Chapter 10

Conclusion & Future Outlook

10.1 Conclusion

This thesis delves into two paramount technologies, 2D materials and 3D printing, aimed to advance the development of electrochemical biosensors. 2D materials are sought to push the limits of detection, enabling ultrasensitive responses for low concentrations of analytes within short response times. Meanwhile, 3D printing offers a rapid, affordable as well as customised fabrication of electrochemical devices and components. These technologies are in line with the research trends of electrochemical biosensors, towards point-of-care devices and wearables.

A material is deemed attractive for electrochemical biosensors if these three key attributes are addressed: electrical conductivity, specific surface area and electrochemical activity. 2D nanomaterials, discussed in **Part I** of the thesis, are regarded highly as superior electrode materials due to their intriguing size and dimensionality. Demonstrating enhanced mass transport as well as signal-to-noise ratio, they have been steadfastly studied and developed for electrochemical biosensors. Graphene, together with its derivatives, overshadow its analogues of other elemental compositions amongst the wide spectrum of 2D nanomaterials. Nonetheless, TMDs is a rising class with MoS₂ being the most intensively studied. It is to be pointed out that MoS₂ is not a representative material for this particular class of 2D nanomaterials. As such, this thesis investigates TMDs of other elemental compositions, namely group 4 TMDs (TiS₂), group 6 TMDs (MoSe₂, WS₂, WSe₂), along with group 10 TMDs (PtS₂, PtSe₂, PtTe₂). A concrete understanding of these materials, especially their limitations, would pave the way for innovative strategies. Phase engineering, doping, as well as electrochemical pretreatment are explored towards optimising the materials in developing successful electrochemical biosensors. There is no incorporation of any metal nanoparticles, in contrast to those in literature, to demonstrate the real potential of 2D materials.

In **Chapter 5**, group 6 TMDs (MoS_2 , MoSe_2 , WS_2 , and WSe_2) are chemically exfoliated via *t*-BuLi intercalation. The phase engineering via lithium intercalation and exfoliation transforms the natural semiconducting 2H phase to metallic 1T, resulting in a satisfactory conductive material with increased electrocatalytic sites. Their HET capabilities are subsequently studied where strikingly, tungsten dichalcogenides (WS_2 and WSe_2) exhibit superior HET properties compared to that of their molybdenum counterparts (MoS_2 and MoSe_2). When incorporated into second generation glucose biosensors, WS_2 and WSe_2 generated higher electrochemical responses than that of MoS_2 and MoSe_2 , following the same trend as expected. The commendable performance by WX_2 is attributed to the dominance of 1T phase, revealed by characterization data. The developed and optimized 1T WX_2 -based biosensor achieved analytical requirements of selectivity, wide linear ranges, as well as low limits of detection and quantification. In the completed project, it is showcased the superior electrocatalytic capabilities of WS_2 and WSe_2 , consequently shifting the limelight away from MoS_2 . This would spur more studies on other layered TMDs composing of transition metals from other groups.

Chapter 6 is centred on TiS_2 , a group 4 TMD which possess high electronic conductivity and light weight amongst other attractive features. In consideration for electrochemical and thermoelectrical applications, doping is a promising approach to enhance its practicability. The introduction of foreign atoms or compositional variance may improve existing properties or grant access to new ones. Identifying that Ti and Nb are a group apart in the periodic table, their sizes, valencies and polymorphs have been discussed to understand the doping effects. More importantly, Nb is more electron rich and would partially fill the originally empty conduction band of TiS_2 . The increase in electrical conductivity substantially improves defining electrochemical properties for biosensing, namely fast HET, low resistance and large electroactive surface area. However, too high a dopant concentration results in the

formation of TiS_3 nanobelts that impede electrochemical performance. Subsequently developed into a 2nd generation glucose biosensor, group 4 TiS_2 optimally doped with Nb demonstrates competitive advantages such as wider linear range and higher sensitivity than group 6 TMDs.

Noble metal platinum, a group 10 element, is highly prestigious for its catalytic and electrocatalytic properties. Their compounds with chalcogens (S, Se, Te) are layered which may give an edge in terms of structure, in addition to composition. The bonding of platinum dichalcogenides (PtX_2) deviates from their extensively studied group 6 counterpart, MoS_2 . Pt-X is covalent and a strong interlayer interaction exists between the chalcogenides, ensuing a dramatic layer and chalcogen dependence in their electronic properties. **Chapter 7** studies PtS_2 , PtSe_2 and PtTe_2 where extensive characterisation has been carried out to elucidate structural and chemical properties of the materials. Their electrochemical capabilities in enhancing signal transduction upon detection of H_2O_2 are compared where PtTe_2 performs outstandingly, granted its metallic conductivity. Furthermore, electrochemical pretreatment is employed to improve sensitivity.

Moving on to **Part II** of the thesis, advantages of 3D printing for electrochemical biosensors are highlighted. Devices, along with electrochemical components, are produced on-demand with commendable degree of customisation, reproducibility, and speed. Additionally, printing on flexible substrates realises the possibility of wearables. Fabrication of stable 3D-printed reference electrodes represents a key step in the production of fully 3D-printable electrochemical systems. **Chapter 9** describes the fabrication of an Ag/AgCl pseudo-reference electrode by 3D printing, the technique of FDM specifically. The printer, affordable and simple to operate, extrudes conductive graphene-PLA filament according to the intended electrode design. The fabrication process continues with electrodepositioning silver followed by bleaching

to form AgCl on the surface of the electrode. It is overall a straightforward, fast and simple method, by virtue of 3D printing.

10.2 Future Outlook

Among 2D materials beyond graphene, TMDs are the most prominent across biosensors regardless of biorecognition elements or transduction modes. Electrochemical biosensors based on various elemental compositions of TMDs, as presented in this thesis, fulfil the fundamental requirements of sensitivity and selectivity. There are also other properties relevant to biosensors that are investigated, including stability as well as feasibility of detection for real samples. Nonetheless, MXenes are rising in popularity for electrochemical biosensors due to their commendable conductivity and hydrophilicity. Monoelemental Xenes such as phosphorene, on the other hand, are less visible in the biosensing landscape due to their main drawback of chemical stability. Since 2D materials of different elemental composition exhibit diverse properties, one enticing strategy is to precisely select and sequence the materials layer by layer. These designer compounds are known as van der Waals' heterostructures which put together desired properties best for an application.¹ Furthermore, neighbouring crystals can induce structural changes and charge redistribution, leading to improved or novel performances.

Research on van der Waals' heterostructures is gathering momentum with the development of preparation methods² and sophisticated devices³. Specific to biosensors, monolayer graphene has been manually stacked on top of monolayer MoS₂.⁴ The latter is responsible for the change in photoluminescence intensity corresponding to the label-free detection of target DNA. Concurrently, graphene protects MoS₂ from moisture and oxygen in the air as well as provide a biocompatible interface layer to host DNA. Strikingly when stacked,

electron concentration of the initially n-doped MoS₂ reduces as electrons are transferred to graphene. The effect is laudable as high sensitivity of the biosensor is achieved.

In parallel, developed electrochemical biosensors should evolve from proof-of concept to actualization. Rigorous criteria such as stability for a specified duration and comparison with current technology would be instrumental, in addition to sensitivity and selectivity.⁵ With the advent of 3D printing, there are limitless opportunities to fabricate electrochemical devices and their components. Great design flexibility enables integration with commercial components, as well as efficient tailoring of dimensions for optimised performance. A significant hurdle is the fabrication of conductive biosensing electrodes, to successfully print biorecognition elements and conductive materials. If overcome, moreover with the incorporation of 2D materials, it would indeed be a breakthrough for electrochemical biosensors.

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