Layered materials : electrochemical studies for sensing, energy applications and toxicity studies

Chia, Hui Ling

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**LAYERED MATERIALS: ELECTROCHEMICAL STUDIES
FOR SENSING, ENERGY APPLICATIONS AND TOXICITY
STUDIES**

CHIA HUI LING

Interdisciplinary Graduate School

NTU Institute for Health Technologies (HealthTech NTU)

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**Interdisciplinary Graduate School
NTU Institute for Health Technologies (HealthTech NTU)**

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

2021

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.

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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.

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Richard D. WEBSTER

Authorship Attribution Statement

This thesis contains material from 5 papers published in the following peer-reviewed journal(s) where I was the first and/or corresponding author.

Chapter 3 is published as Chia, H. L.; Mayorga-Martinez, C. C.; Antonatos, N.; Sofer, Z.; Gonzalez-Julian, J. J.; Webster, R. D.; Pumera, M., MXene Titanium Carbide-based Biosensor: Strong Dependence of Exfoliation Method on Performance. *Anal. Chem.* **2020**, 92, 2452-2459. DOI: [10.1021/acs.analchem.9b03634](https://doi.org/10.1021/acs.analchem.9b03634)

The contributions of the co-authors are as follows:

- Prof. Pumera supervised the research and helped to revise the manuscript drafts.
- Dr. Mayorga-Martinez designed the study, provided suggestions to improve the project and revised the manuscript.
- Dr. Antonatos assisted in the collection of TEM, EDX, AFM, XRD, Raman and XPS data.
- Prof. Sofer synthesised the MXenes materials through exfoliation and delamination processes and aided in the revision of the manuscript drafts.
- Prof. Gonzalez-Julian synthesised the Ti_3AlC_2 compound and helped to revise the manuscript drafts.
- Prof. Webster contributed to the scientific discussions of the project and aided in the revision of the manuscript.
- I performed all the electrochemical measurements in the study, data analysis and prepared the manuscript.

Chapter 5 is published as Chia, H. L.; Mayorga-Martinez, C. C.; Gusmão, R.; Novotny, F.; Webster, R. D.; Pumera, M., A highly sensitive enzyme-less glucose sensor based on pnictogens and silver shell–gold core nanorod composites. *Chem. Commun.* **2020**, 56, 7909-7912. DOI: [10.1039/D0CC02770G](https://doi.org/10.1039/D0CC02770G)

The contributions of the co-authors are as follows:

- Prof. Pumera supervised the research and helped to revise the manuscript drafts.

- Dr. Mayorga-Martinez designed the study, provided suggestions to improve the project and revised the manuscript.
- Dr. Gusmão synthesised the pnictogen nanosheets and revised the manuscript.
- Dr. Novotny synthesised the Au@Ag core-shell nanorods, assisted with SEM characterisation and helped to revise the manuscript drafts.
- Prof. Webster contributed to the scientific discussions of the project and aided in the revision of the manuscript.
- I characterised the materials via XPS and zeta potential analysis, performed all the electrochemical measurements in the study, data analysis and prepared the manuscript.

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- Dr. Mayorga-Martinez designed the study, provided suggestions to improve the project and revised the manuscript.
- Dr. Lazar assisted with the density functional theory studies and revised the manuscript.
- Prof. Sofer synthesised the vanadium-doped molybdenum dichalcogenides, performed Raman spectroscopy and aided in the revision of the manuscript drafts.
- Prof. Webster contributed to the scientific discussions of the project and aided in the revision of the manuscript.
- I characterised the materials through SEM and EDX, performed all the electrochemical measurements in the study, analysed the data obtained and prepared the manuscript.

Chapter 7 is published as Chia, H. L.; Latiff, N. M.; Sofer, Z.; Pumera, M., Cytotoxicity of Group 5 Transition Metal Ditellurides (MTe₂; M=V, Nb, Ta). *Chem. Eur. J.* **2018**, 24, 206-211. DOI: [10.1002/chem.201704316](https://doi.org/10.1002/chem.201704316)

The contributions of the co-authors are as follows:

- Prof. Pumera provided the project direction, supervised the research and helped to revise the manuscript drafts.

- Dr. Latiff provided suggestions to improve the project and revised the manuscript.
- Prof. Sofer synthesised the Group 5 transition metal ditellurides, characterised the as-synthesised materials via XRD and DLS, and aided in the revision of the manuscript.
- I performed the materials characterisation via SEM, STEM and EDX, cytotoxicity studies, data analysis and prepared the manuscript.

Chapter 8 is published as: Chia, H. L.; Latiff, N. M.; Gusmão, R.; Sofer, Z.; Pumera, M., Cytotoxicity of Shear Exfoliated Pnictogen (As, Sb, Bi) Nanosheets. *Chem. Eur. J.* **2019**, *25*, 2242-2249. DOI: [10.1002/chem.201804336](https://doi.org/10.1002/chem.201804336)

The contributions of the co-authors are as follows:

- Prof. Pumera provided the project direction, supervised the research and helped to revise the manuscript drafts.
- Dr. Latiff provided suggestions to improve the project and revised the manuscript.
- Dr. Gusmão synthesised the pnictogen nanosheets, performed AFM measurements and revised the manuscript.
- Prof. Sofer characterised the as-synthesised materials via TEM, EDX and DLS, and aided in the revision of the manuscript.
- I performed the materials characterisation via SEM and zeta potential analysis, cytotoxicity studies, data analysis and prepared the manuscript.

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Summary

As society has revolutionised, constant advancements in sensor and energy technologies are required. With the increasing complexity of samples and demand for more analysis to be made, it is of utmost importance to constantly improve the analytical capabilities of sensing platforms and develop sensors that are highly specific, have high sensitivity, are low in cost, easy to use, rapid and reliable. Furthermore, the current state-of-the-art electrocatalysts often involve precious metals, which are scarce, costly and suffer from poor stability, hampering their widespread application. Meanwhile, the ground-breaking discovery of graphene from graphite and the subsequent revelation of graphene's extraordinary properties, has paved the way for the rediscovery of a plethora of layered materials. Layered materials possess a myriad of fascinating properties and hold great promise for a variety of applications. As such this thesis explored the electrochemistry of layered materials and exploited layered materials for the development of electrochemical sensing platforms and electrocatalysts. Herein, the dissertation investigated the viability of using MXenes and Group 14 graphene analogues for the development of highly sensitive second-generation electrochemical glucose biosensors. A highly sensitive glucose sensor with an ultralow limit of detection was also fabricated upon core-shell gold@silver nanorods decorated pnictogen nanosheets. Additionally, vanadium-doped molybdenum dichalcogenides were explored for the hydrogen evolution reaction, the oxygen evolution reaction and oxygen reduction reaction and the effects of varying the stoichiometry of vanadium dopants in bulk molybdenum dichalcogenides on their electrocatalytic activities were also investigated. Apart from studying the electrochemical properties and applications of layered materials, it is also perceived that there are growing concerns over the potential of these layered materials in causing new hazards and risks. As such, the thesis explored the cytotoxicity profiles of various emerging classes of layered materials, namely Group 5 transition metal ditellurides and pnictogen nanosheets. The findings

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List of Abbreviations

ΔE_p	Peak-To-Peak Separation
ΔG_H	Differential Free Energy of Hydrogen Adsorption
2D	Two-Dimensional
A549	Human Lung Adenocarcinoma Epithelial Cell Line
AFM	Atomic Force Microscopy
BEAS-2B	Human Lung Bronchial Epithelial Cell Line
BP	Black Phosphorus
CV	Cyclic Voltammetry
DET	Direct Electron Transfer
DLS	Dynamic Light Scattering
DMF	<i>N,N</i> -Dimethylformamide
DMSO	Dimethyl Sulfoxide
DPV	Differential Pulse Voltammetry
EDX	Energy Dispersive X-ray Spectroscopy
FAD	Flavin Adenine Dinucleotide
FcMeOH	Ferrocenemethanol
GC	Glassy Carbon
GOx	Glucose Oxidase
GTA	Glutaraldehyde
HER	Hydrogen Evolution Reaction
HET	Heterogeneous Electron Transfer
LOD	Limit of Detection
LOQ	Limit of Quantification
LSV	Linear Sweep Voltammetry

MTT	Methyl Thiazolyldiphenyl-Tetrazolium Bromide
NRs	Nanorods
OER	Oxygen Evolution Reaction
ORR	Oxygen Reduction Reaction
PBS	Phosphate Buffer Saline
RHE	Reversible Hydrogen Electrode
SAED	Selected Area Electron Diffraction
SEM	Scanning Electron Microscopy
STEM	Scanning Transmission Electron Microscopy
TEM	Transmission Electron Microscopy
TMDs	Transition Metal Dichalcogenides
XPS	X-ray Photoelectron Spectroscopy
XRD	X-ray Diffraction
WST-8	Water-Soluble Tetrazolium Salt

List of Publications

1. **Chia, H. L.**; Mayorga-Martinez, C. C.; Sofer, Z.; Lazar, P.; Webster, R. D.; Pumera, M., Vanadium Dopants: A Boon or a Bane for Molybdenum Dichalcogenides-Based Electrocatalysis Applications. *Adv. Funct. Mater.* **2020**, 2009083.
2. **Chia, H. L.**; Mayorga-Martinez, C. C.; Gusmão, R.; Novotny, F.; Webster, R. D.; Pumera, M., A highly sensitive enzyme-less glucose sensor based on pnictogens and silver shell–gold core nanorod composites. *Chem. Commun.* **2020**, 56, 7909-7912.
3. **Chia, H. L.**; Mayorga-Martinez, C. C.; Antonatos, N.; Sofer, Z.; Gonzalez-Julian, J. J.; Webster, R. D.; Pumera, M., MXene Titanium Carbide-based Biosensor: Strong Dependence of Exfoliation Method on Performance. *Anal. Chem.* **2020**, 92, 2452-2459.
4. **Chia, H. L.**; Latiff, N. M.; Gusmão, R.; Sofer, Z.; Pumera, M., Cytotoxicity of Shear Exfoliated Pnictogen (As, Sb, Bi) Nanosheets. *Chem. Eur. J.* **2019**, 25, 2242-2249.
5. **Chia, H. L.**; Latiff, N. M.; Sofer, Z.; Pumera, M., Cytotoxicity of Group 5 Transition Metal Ditellurides (MTe₂; M=V, Nb, Ta). *Chem. Eur. J.* **2018**, 24, 206-211.
6. Kong, L.; Rosli, N. F.; **Chia, H. L.**; Guan, J.; Pumera, M., Self-Propelled Autonomous Mg/Pt Janus Micromotor Interaction with Human Cells. *Bull. Chem. Soc. Jpn.* **2019**, 92 (10), 1754-1758.
7. Dong, Q.; Mohamad Latiff, N.; Mazánek, V.; Rosli, N. F.; **Chia, H. L.**; Sofer, Z.; Pumera, M., Triazine- and Heptazine-Based Carbon Nitrides: Toxicity. *ACS Appl. Nano Mater.* **2018**, 1 (9), 4442-4449.

Thesis Organisation

This thesis is divided into nine (9) chapters. In Chapter 1, readers are presented with an outline of the objectives and motivations of this thesis. In the following chapter, readers are provided with a general introduction to layered materials, sensors, the global energy crisis and cytotoxicity assessment techniques. An in-depth review of previous studies regarding the use of layered materials in electrochemical-based sensors, biosensors and energy applications and the cytotoxicity effects of layered materials would also be detailed in Chapter 2. Following the comprehensive introduction and literature review, a compilation of research towards the objectives outlined in Chapter 1 will be presented in Chapters 3-8.

Chapters 3 and 4 demonstrate the use of uprising classes of layered materials, namely transition metal carbides (MXenes) and Group 14 graphane analogues (germanane, methyl germanane and siloxene) as transducer platforms in enzymatic-based biosensors. Chapter 5 touches on the application of pnictogens-based composites for electrochemical sensing while Chapter 6 discusses the electrocatalytic performances of heteroatom doped molybdenum dichalcogenides for energy applications. Chapters 7 and 8 evaluate the cytotoxicity of emerging classes of layered materials such as Group 5 transition metal ditellurides and pnictogen nanosheets.

A summary, as well as the future outlook of the works presented in this dissertation, are outlined in Chapter 9.

Chapter 1

Objectives of Thesis

The ground-breaking discovery of graphene from graphite by Novoselov and co-workers and the subsequent revelation of graphene's extraordinary properties, has generated a great amount of excitement amongst researchers, paving way for the rediscovery of numerous two-dimensional (2D) layered materials.^{1,2} It has been reported that layered materials represent a disparate and widely unexplored source of 2D systems that possess features which are important for applications in the field of catalysis, sensors and energy storage, for instance, fascinating electronic properties and high surface areas.^{3,4}

In the 21st century, sensors have found their way into a myriad of applications in our daily life and industrial settings. Such applications include measuring glucose, cholesterol levels and detecting emerging infectious diseases for biomedical purposes, smart homes, environmental monitoring, industrial safety and food analysis.^{5,6} As society revolutionised, constant advancements in sensor technologies are required to meet the ever-increasing number of analyses to be made. Therefore, it is of utmost importance to constantly improve the analytical capabilities of sensing platforms and develop sensors that are highly specific, sensitive, low in cost, easy to use, rapid and reliable.⁷⁻⁹

The use of energy is central to human activities, ranging from preparation of food, powering of vehicles and appliances, warming of houses, production of goods to the monitoring of personal health with sensor technologies. For instance, for individuals diagnosed with diabetes to regularly monitor their blood glucose level, electrical energy is required to power and operate the sensor and prior to that, energy is also used in industrial settings to produce the devices, and to power vehicles to transport the sensors to malls or households. The continual usage of fossil fuels to produce energy has raised alarming concerns with regards to energy shortages and climate changes, making the search for clean and renewable energy resources a pressing

priority for the sustainable development of societies. Fundamental to this is the development of advanced energy conversion technologies, which require the use of highly efficient, low cost and sustainable electrocatalysts.¹⁰⁻¹³ Although conventional precious metal-based catalysts, for instance, iridium, platinum and ruthenium are able to efficiently catalyse the conversion of chemical energy to electrical energy, they are usually very costly, scarce and suffer from poor stability, hence impeding their widespread application. The limitations of the state-of-the-art catalysts have propelled tremendous efforts in search of alternative catalysts.^{10,14}

Rapid advancements in science and technology have resulted in a huge growth in the number of newly engineered sophisticated materials. Layered materials proffer numerous advantageous properties and showcase unlimited potentials for a myriad of applications. Despite its bright prospects, the scientific community has raised much concern about the potential of these novel layered materials in causing new hazards and risks.¹⁵⁻¹⁷ Hence, it is imperative to consider the possible risks posed prior to the inclusion of such materials into products that might eventually enter the marketplace.

As such, the thesis entitled “Layered Materials: Electrochemical Studies for Sensing, Energy Applications and Toxicity Studies” aims to explore the promising properties of emerging classes of layered materials, exploit them for sensors and energy applications and investigate their toxicity effects. The dissertation is separated into two parts where Part I details the electrochemical studies of emerging classes of layered materials for sensing and energy applications while Part II touches on the safety aspects of layered materials.

Part I: Layered Materials for Electrochemical Sensing and Energy Applications

In view of their massive specific surface areas, remarkable electrical and thermal conductivity, abundance of catalytic sites, ease of functionalisation and tuneable electronic structures, layered materials hold promising potentials for the development of highly sensitive sensing platforms as well as electrocatalysts for the generation of alternative energy sources.¹⁸⁻²⁰ However, much of the research has been predominantly focused on using carbon-based materials and Group 6 transition metal dichalcogenides such as MoS₂ and WS₂. There is a need to quest for alternative materials which could potentially outperform and improve research in these directions to improve current technologies. Part I focuses on harnessing the fascinating properties of emerging classes of layered materials to improve the analytical performances of electrochemical sensing platforms and electrocatalysts. Chapters 3 and 4 investigated the feasibility of using MXenes and Group 14 graphane analogues as transducer platforms for the development of second-generation glucose biosensors. Although layered materials behold many beneficial attributes for the development of sensors and electrocatalysts, the properties as well as the electronic structure of layered materials could be fine-tuned through doping techniques or decorating with metal nanostructures to further enhance the performance of sensors and electrocatalysts. Chapter 5 aims to study the synergistic effects of decorating pnictogen nanosheets with precious metal-based nanostructures and the as-synthesised pnictogen nanosheets-gold@silver nanorods (Au@Ag NRs) composites were applied towards the sensitive detection of glucose. Chapter 6 explores the effects of doping various amounts of vanadium on molybdenum dichalcogenides (MoX₂) on their electrocatalytic activities towards the hydrogen evolution reaction (HER), oxygen evolution reaction (OER) and oxygen reduction reaction (ORR).

Part II: Cytotoxicity of Emerging Layered Materials

Part II discusses the safety aspects of various uprising classes of layered materials, in particular Group 5 transition metal ditellurides and pnictogen (arsenic, antimony, and bismuth) nanosheets. The unique and advantageous properties proffered by Group 5 transition metal ditellurides and pnictogen nanosheets have spurred intensive research efforts and led to the discovery of their numerous and diversified applications. Some of these applications include electrocatalysts, field-effect transistors (FETs), flexible 2D electronics, photovoltaics, lubricant additives, sensors and supercapacitors.²¹⁻²⁷ In recent years, a surge in the number of patents filed by multinational companies regarding new technologies that make use of these materials is observed. Companies such as Samsung Electronics,^{28,29} Huawei Technologies,³⁰ and Medibotics³¹ have filed for patents using these materials to fabricate electronic devices and develop sensor platforms. Although intensive research efforts have been put in place to study the synthesis, properties, and applications of these materials, little effort has been devoted to determining the toxicological behaviour of these nanosheets. In view of the probable implementation of these materials in industrial settings and our daily life, it is important for this literature gap to be explored to notify users of the possible health hazards. As such, investigations of the cytotoxicity effects of Group 5 transition metal ditellurides and pnictogen nanosheets are presented in Chapters 7 and 8.

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Chapter 2

Literature Review

2.1 Introduction to Layered Materials

2.2 Introduction to Sensors and Biosensors

2.3 Global Energy Crisis and Energy-Related Processes

2.4 Layered Materials for Electrochemical Sensing and Energy

Applications

2.5 Toxicity Studies of Layered Materials

2.1 Introduction to Layered Materials

The ground-breaking discovery of graphene from graphite by Novoselov and co-workers and the subsequent revelation of graphene's extraordinary properties, has paved the way for the rediscovery of numerous two-dimensional (2D) layered materials.^{1,2} Some of these materials include transition metal dichalcogenides (TMDs), pnictogen nanosheets, transition metal carbides or nitrides (MXenes), Group 14 graphane analogues (germanane and siloxene), graphitic carbon nitride, and hexagonal boron nitride.³⁻¹² Layered materials generally refer to solids that possess strong in-plane chemical bonds within each layer and weak out-of-plane van der Waals forces of attraction between adjacent layers. Hence, layered materials can undergo parallel exfoliation or normal expansion with regard to the in-plane direction. It has been reported that layered materials represent a disparate and widely unexplored source of 2D systems that possess features which are important for applications in the field of catalysis, sensors and energy storage, for instance, fascinating electronic properties and high surface areas.^{3,13} Herein, the dissertation will focus on four groups of layered materials, namely TMDs, MXenes, pnictogen nanosheets and Group 14 graphane analogues.

2.1.1 TMDs

Amongst the vast range of layered compounds reported, TMDs have been extensively researched on and are regarded to be amongst the most commonly explored layered compounds owing to their unique and advantageous electrochemical properties. Various combinations of transition metal and chalcogens result in about 60 different kinds of TMDs that possess diverse inherent electronic properties ranging from semi-metallic to superconductors. By and large, Group 4–7 transition metals yield compounds that possess mainly layered structures whilst a number of Group 8–10 transition metals generate crystal compounds that are non-layered (Figure 2.1).¹⁴⁻¹⁶ Various synthetic routes have been reported to generate TMDs nanosheets,

some of the more commonly used methods include liquid exfoliation with alkali metal intercalants, sonication-assisted exfoliation, sulfurization or selenization of thin metal films and vapour phase reaction using transition metal oxides or halides with chalcogen precursors.¹⁷⁻²²

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 2.1. Transition metal and chalcogen elements which are highlighted mainly exist as layered compounds while partially highlighted elements in the periodic table indicate that only certain TMDs form layered structures. Reproduced from reference 14.

Layered TMDs possess lamellar structures akin to graphite, where interlayer bonds are of strong covalent nature while weaker van der Waals forces of attractions are present amidst adjacent layers. Within every layer of TMD, a hexagonally arranged layer of transition metal (M) is flanked by two chalcogen (X) layers, hence giving rise to the MX_2 stoichiometry. The formal oxidation state of M and X have been reported to be +4 and -2 respectively. Bulk TMDs are found to exist in different polymorphs (Figure 2.2), 1T trigonal phase, 2H hexagonal phase and 3R rhombohedral phase while monolayer TMDs only display 1T trigonal (metallic) and 2H hexagonal (semiconducting) phases. The electronic structure of layered TMDs is heavily influenced by the number of layers whereby the band gap transits from indirect in bulk phase to direct in single-layered 1T phase TMDs, enhancing photoluminescence properties.^{2,14,23-27} Layered TMDs also possess fascinating lubricating properties and low shear stress as a result of the weak van der Waals attractions found amidst adjacent layers.^{28,29} The numerous beneficial

properties of TMDs such as tuneable band gaps, high electron mobility and Young's modulus, excellent tribological properties and large surface area allow TMDs to be utilised in an array of applications, for instance, field-effect transistors, electrocatalysts, lubricant additives and sensors.^{14,26,28,30-35}

Figure 2.2. An illustration showcasing the metal coordination and stacking sequences of the various polymorphs of TMDs. Reproduced from reference 25.

2.1.2 MXenes

MXenes, also known as transition metal carbides or nitrides, is a new and uprising class of layered materials that is taking material scientists by storm. MXenes are often generated via selective etching of ‘A’ layers from their layered, ternary parent compounds, MAX phases (Figure 2.3). MAX phases generally possess a chemical formula of $M_{n+1}AX_n$ ($n = 1-3$), whereby M corresponds to early *d*-transition metals, A denotes elements from either Group 13 or 14, and X corresponds to carbon or nitrogen.^{4,5,36-39} M layers are densely packed with X atoms lining amidst the octahedral sites and the $M_{n+1}X_n$ layers are interposed between layers of A atoms. The M-X bonds have been reported to be remarkably strong and possess a mixture of covalent, and ionic characteristics, whereas M-A bonds are of a weaker nature and possess

metallic characteristics. As a result of the weaker M-A bonds, 'A' layers can be selectively removed from MAX phases via heat treatment under vacuum conditions or in molten salts or via chemical etching with hydrofluoric acid treatment. The selective etching of 'A' layers yields $M_{n+1}X_nT_x$ layers that possess hexagonal crystal lattice structures and surface terminating moieties (T_x) such as oxygen, hydroxyl or fluorine groups.⁴⁰⁻⁴⁴ The etched products could be further subjected to delamination with intercalants like dimethyl sulfoxide (DMSO), tetrabutylammonium hydroxide (TBAOH) and isopropyl alcohol to generate mono- or few-layered MXenes. Akin to many layered materials, single- or few-layered MXenes could also be synthesised via bottom-up techniques, for instance, chemical vapour deposition and plasma-enhanced pulsed laser deposition.⁴⁵⁻⁴⁷ To date, more than 60 distinct types of pure MAX phases and 20 types of MXenes have been discovered.³⁶ MXenes possess several beneficial properties such as its metallic-like conductivity, hydrophilicity inherited from the surface terminating groups, high Young's modulus, outstanding optical transparency (~91.2%) and intrinsic magnetic properties, allowing it to find applications in the field of sensing, supercapacitors, electrocatalysts and electromagnetic interface shields.^{40-42,46,48-52}

Figure 2.3. An illustration depicting the selective etching of 'A' layers from MAX phases to generate $M_{n+1}X_nT_x$ layers. Reproduced from reference 37.

2.1.3 Pnictogen Nanosheets

The rediscovery of black phosphorus (BP) has sparked researchers to be in search of other pnictogens-based mono elemental nanomaterials. Pnictogens are elements from Group 15 of the periodic table, consisting of gaseous nitrogen (N) and crystalline phosphorus (P), arsenic (As), antimony (Sb) and bismuth (Bi), which display increasing metallic characteristics down the Group.^{8,53} Numerous techniques have been detailed for the synthesis of pnictogen nanosheets, including shear exfoliation, mechanical cleavage using the classical scotch-tape method, electrochemical exfoliation and van der Waals epitaxy techniques.^{6,8,53,54} Thermodynamically stable allotropic form of BP crystallises in an orthorhombic structure, while layered heavy pnictogens (As, Sb and Bi) manifest as rhombohedral crystal structures (Figure 2.4). Weak van der Waals interactions are found amidst adjacent layers of phosphorene whereas interlayer bonds between individual layers of heavy pnictogens possess covalent characteristics.^{8,53,55-57} Pnictogen nanomaterials reportedly possess a myriad of fascinating properties such as a broad range of tuneable band gap (0.36 to 2.62eV), high specific surface area due to their unique wrinkled and buckled structures, high carrier mobility of several thousand $\text{cm}^2 \text{V}^{-1} \text{s}^{-1}$ and intrinsic anisotropic properties. Pnictogen nanosheets have been applied to many domains ranging from field-effect transistors (FET), sensors, catalysts to photodynamic therapeutic agents.^{8,58-64}

Figure 2.4. Crystal structures of (a) orthorhombic black phosphorus and (b) rhombohedral grey arsenic, antimony and bismuth. Reproduced from reference 55 and 56.

2.1.4 Group 14 Graphane Analogues

Down Group 14, analogues of graphene, namely silicene and germanene have been explored extensively over the last few years. Akin to graphene, silicon and germanium atoms are arranged in a honeycomb geometry in both silicene and germanene. However, the larger ionic radius of these elements promotes sp^3 hybridisation, causing bonds to exhibit a mixture of sp^2 and sp^3 hybridisation. As a result, these materials possess a low-buckled structure as opposed to graphene's planar structure (Figure 2.5a).⁶⁵⁻⁶⁸ Despite these materials possessing several fascinating properties that are similar to graphene, these materials possess zero bandgaps, have poor thermodynamic stability and are prone to oxidation, limiting their applications. As such, silicene and germanene have been subjected to numerous functionalisations to modify their physical, chemical and electronic properties, thus improving their stability and widening their bandgaps. Some of these modified analogues include germanane (Ge_6H_6), methyl germanane ($\text{Ge}_6(\text{CH}_3)_6$) and siloxene ($\text{Si}_6\text{H}_3(\text{OH})_3$).^{66,68-72}

Germanane, methyl germanane and siloxene can be easily synthesised via topotactical deintercalation of the layered Zintl phases (CaGe_2 and CaSi_2) with either aqueous acid or methyl halide. Germanane and methyl germanane possess buckled honeycomb structures that

are analogous to sp^3 hybridised graphane, where every germanium atom is covalently functionalised with either -H or -CH₃, above and below the layer (Figure 2.5b and 2.5c).^{10,11,70,73-76} Hydrogenation of germanene leads to band gap opening, causing germanane to possess a direct band gap of approximately 1.6 eV. Furthermore, germanane possesses high electron mobility up to 18 000 cm² V⁻¹ s⁻¹ and is highly resistant to oxidation.^{11,70,77-79} Replacing the hydrogen terminations in Ge₆H₆ with methyl functional groups increases the band gap to approximately 1.7 eV and improves the thermal stability significantly, where Ge₆(CH₃)₆ is thermally stable up till 250 °C as opposed to 75 °C for Ge₆H₆. Methyl germanane also exhibits remarkable photocatalytic activities, photoluminescence properties and band edge fluorescence.^{10,80,81} Meanwhile, silicon layers within siloxene are terminated by hydroxyl groups on one side and hydrogen moieties on the other (Figure 2.5d). Siloxene has been reported as a direct bandgap semiconductor that possesses high specific capacitance (2.18 mF cm⁻²) and energy density (9.82 mJ cm⁻²) and exhibits strong photoluminescence.^{9,70,82-84} As a result of their fascinating properties, these emerging Group 14 graphane analogues have found their way into a myriad of applications including supercapacitors, photocatalysts, anodes in Li-ion batteries and sensors.^{9,70,79,81,85-88}

Figure 2.5. (a) Top and side view of silicene and germanene. Reproduced from reference 67. Crystal structures of (b) germanane and (c) methyl germanane, reproduced from reference 74. (d) Schematic illustration of siloxene. Reproduced from reference 82.

2.2 Introduction to Chemical Sensors and Biosensors

In the 21st century, sensors have found their way into a myriad of applications in our daily life, for instance, measuring glucose, cholesterol levels and detecting emerging infectious disease for biomedical purposes, automobiles, smart homes, environmental monitoring and food analysis.^{89,90} Chemical sensors are commonly deemed as instruments that transform chemical information such as concentrations of analytes into measurable signals and have been proven to be effective for the differentiation of analytes.⁹¹⁻⁹⁵ Meanwhile, biosensors, a sub class of chemical sensors, have been defined by IUPAC as independent integrated devices that are competent in providing precise quantitative or semi-quantitative analytical data through the use of biological recognition elements such as enzymes, nucleic acids, proteins and antibodies, which are in direct spatial contact with the transducer components.^{96,97} A chemical sensor typically comprises of two components: (i) a recognition element, also known as the receptor and (ii) a transducer that transforms chemical or biological signals into recordable and analytically useful output signals (Figure 2.6).^{91,94,95}

Figure 2.6. An allegorical representation of a chemical sensor. Reproduced from reference 95.

2.2.1. Transducer and Transduction Methods

Chemical sensors and biosensors can be categorised based upon the type of transducers. Transducers can be divided into many classes such as electrochemical, optical, thermometric, piezoelectric and magnetic transducers.^{95,98-100}

Sensors that comprise electrochemical transducers function by reacting with the target molecules and generating a measurable electrical signal that is typically correlated with the analyte's concentration. Electrochemical-based sensors can be further categorised according to their mode of operations, namely potentiometric, amperometric, and conductimetric which relies on the measurements of potential, current and conductivity respectively.^{90,95,98,101}

Optically transduced sensors function by transforming changes in optical phenomena, that arises as a consequence of analyte interactions with the receptor, into analytically useful and readable signals. Optical sensors can be categorised based upon their radiation source and optical phenomenon, namely colourimetric, fluorometric, luminescence, surface plasmon resonance and surface-enhanced Raman sensors.^{98,102-105}

Thermally transduced sensors functioned by measuring changes in thermal effects that arise as a result of the analyte recognition process or specific chemical reactions.^{95,98} Piezoelectric transducer in sensors works by transforming external stresses such as mass changes at the surface of sensing platforms, which arises as a result of analyte interactions with the receptor, into electrical signals.¹⁰⁶⁻¹⁰⁸ Meanwhile magnetic transducers within sensors function by detecting changes in the paramagnetic properties and magnetic fields and converting them into useful electronic signals.^{98,109}

2.2.2. Electrochemical Techniques in Sensing Applications

Electrochemical transducers have been thoroughly examined for the development of chemical sensors and biosensors due to their ease of use, portability, minimal power requirements, specificity, and low cost. Electrochemical sensors can be categorised according to their mode of operations, namely amperometric, conductometric or potentiometric.^{90,94,101,110}

2.2.2.1 Amperometric Sensors

Amperometric sensors measure the current responses resulting from redox reactions of electroactive species at the surface of the electrodes. These electrochemical reactions are often driven by an applied electrical potential. Amperometric sensors could be further classified based upon their mode of operations, amperometry and voltammetry. Amperometry refers to the measurement of current response at fixed potentials while voltammetry refers to the measurement of current across a range of potentials.^{90,111,112}

In amperometry, constant oxidising or reducing potentials are supplied to the working electrodes and the resulting steady-state currents are recorded as functions of time. The current response obtained correlates proportionally with the concentration of analyte in the sample.

Amperometry offers sensors with selectivity as the applied reduction or oxidation potential is usually specific to that of the analyte species undergoing redox reactions. Amperometry has also been reported to be a sensitive technique that does not require labelling of analytes. Additionally, amperometry techniques produce negligible charging currents which greatly reduce the interferences from background signals, enhancing the signal obtain and improve the detection limits. As such, amperometry has been highly sought after for the development of chemical sensors and biosensors.¹¹¹⁻¹¹⁵

Voltammetry involves a range of applied potentials where the potential is swept from one pre-set value to the next and the current response is obtained as a function of applied potentials. The current response obtained in voltammetry is proportional to the concentration of the analyte. The potential at which the redox peak is obtained is characteristics of the analyte, thus it is possible to simultaneously detect various analytes with distinct redox signature potentials in a single voltammetric measurement. Voltammetry is highly versatile and exists in many forms including cyclic voltammetry, linear sweep voltammetry, polarography, stripping voltammetry and differential pulse voltammetry.^{111-113,116}

2.2.2.2 Conductometric Sensors

Conductometric sensors measure changes in the electrical conductivity of an analyte or medium. Although conductometric sensors possess several attractive advantages such as low cost, the possibility for direct real-time monitoring and ease of use, they are often reported to be non-selective. Modification of electrode surfaces with recognition elements such as enzymes are required to render conductometric sensors with selectivity. Furthermore, conductometric sensors are susceptible to interferences that arise as a result of the non-reacting ions existing in the buffer solution. Careful considerations of the buffer used are required to

reduce the ratio between the noise and signal level to improve the sensitivity of the sensing platform.^{111,112,116,117}

2.2.2.3 Potentiometric Sensors

Potentiometric sensors measure the charge potentials accumulated at the working electrode with reference to a silver-silver chloride or calomel reference electrode, with negligible current flowing between the two electrodes. Potentiometry is governed by the Nernst equation where the potential is correlated to the logarithm of the analyte concentration and provides information about the ion activities in the analyte. Examples of commonly used potentiometric sensors are ion-selective electrode and glass pH sensor.^{94,112,116,118}

2.3 Global Energy Crisis and Energy-Related Processes

Fossil fuels have proffered mankind with astounding opportunities throughout the 20th and early part of the 21st century, spurring the growth and development of many societies. However, the energy bonanza comes at a price, where the continual usage of fossil fuels led to detrimental environmental issues.¹¹⁹ The ever-increasing concerns with regard to dwindling supplies of fossil fuels as well as climate change have prompted the search for green and sustainable energy resources, for instance, solar, geothermal and wind energies. As such, it is fundamental to develop advanced energy conversion and storage technologies to fully optimise the utility of such intermittent renewable energy sources. Examples of these technologies include electrocatalysis processes, supercapacitors and metal-air batteries, which heavily relies on electrochemistry principles to transform chemical energy into electrical energy.¹²⁰⁻¹²² Some of these important energy-related electrochemical conversion reactions include hydrogen evolution reaction (HER), oxygen evolution reaction (OER) and oxygen reduction reaction (ORR). The current state-of-the-art of electrocatalysts involve precious metals namely iridium

(Ir), platinum (Pt) and ruthenium (Ru), which are scarce, costly and suffer from poor stability, hence hampering their widespread application. As such, tremendous efforts have been committed towards the quest for alternative catalysts that are highly abundant, low in cost and highly catalytic.^{121,123-125}

2.3.1 Hydrogen Evolution Reaction

With the turn of the 21st century, dwindling amount of fossil fuels and increasing concerns regarding climate changes have caused government worldwide to come up with road maps to decarbonize their economy, transit into hydrogen economies and mitigate the detrimental effects of global warming.¹²⁶ The term hydrogen economy was first coined by John Bockris in the 1970s and refers to economies that mainly rely upon hydrogen as the commercial fuel.¹²⁶⁻¹²⁸ Hydrogen has come forth as an outstanding energy carrier contender and has been reported to be highly abundant, possesses high gravimetric energy density, non-toxic and clean as it only produces water upon combustion.^{128,129} Even though hydrogen has been reported to be an earth abundant element, it does not manifest as hydrogen (H₂) fuel. One common method to produce hydrogen fuel is through water splitting reactions. HER, the cathodic process during water splitting, can occur in both acidic and alkaline medium and involves a two-electron transfer process, which can be expressed as the following equations:¹³⁰⁻¹³³

HER is an energy intensive reaction, where a large amount of energy input is required to overcome the overpotential resulted by the activation energy barrier. As such electrocatalysts that can reduce HER overpotentials are highly sought after. HER in acidic medium typically

involves a two-step process which occurs either through the Volmer–Heyrovsky or the Volmer–Tafel mechanism. The two pathways are initiated by the Volmer reaction where the proton (H^+) associates with an electron and adsorbs onto the surface of the electrocatalyst forming H_{ads} . Thereafter, hydrogen can be generated via the Heyrovsky or Tafel reaction. In Heyrovsky reaction, the adsorbed hydrogen atom interacts with a H^+ and an electron from the catalyst to yield H_2 fuel while Tafel reaction involves the combination of two adsorbed hydrogen. Chemical equations with regard to Volmer, Heyrovsky and Tafel reactions in acidic media are as follows:^{120,131,133,134}

Likewise, HER also undergoes either the Volmer–Heyrovsky or Volmer–Tafel reaction pathway in alkaline media. However, in alkaline medium, additional dissociation of water molecules takes place during the Volmer reaction step to generate protons needed for both the Volmer and Heyrovsky reaction steps. Breaking the strong covalent H–OH bond may introduce additional energy barriers making HER even more kinetically challenging. As such, acidic electrolytes are highly preferred for HER. The reaction processes involved in HER under alkaline conditions are as follows:^{120,131,133-136}

In accordance with the Sabatier principle, HER kinetic is highly dependent on the interactions between hydrogen species and the electrocatalysts. Optimal HER electrocatalysis has been reported to occur when the differential Gibbs free energy of the adsorbed hydrogen atom (ΔG_H) approaches thermoneutral value, $\Delta G_H \approx 0$ eV. This means that the interactions between the hydrogen species and the electrocatalysts should be of intermediary strength, whereby interactions that are too strong would limit the desorption reaction during the Heyrovsky and Tafel processes and poison the electrocatalysts while interactions that are too weak would hinder the Volmer reaction.^{132,134,137} The Sabatier principle is exemplified by the volcano plot obtained through density functional theory (DFT) studies done by Nørskov and colleagues.¹³⁸ The volcano plot (Figure 2.7) correlates the apparent HER exchange current density (i_0) with ΔG_H and is a useful tool to predict the activity trends of electrocatalysts.

Figure 2.7. Volcano plot correlating the exchange current density and ΔG_H of noble and transition metals. Reproduced from reference 138.

Platinum, situated at the apex of the volcano plot, has been reported to possess superior HER activities. However, platinum is scarce and costly, limiting its widespread application. This has spurred the scientific community in quest of other alternative electrocatalysts that could effectively lower the overpotential for HER.¹³⁸⁻¹⁴⁰ Some of these alternatives include TMDs,^{140,141} MXenes⁴⁹

and metal phosphorus trichalcogenides.^{142,143} Notably, TMDs have emerged as outstanding alternatives as a result of their natural abundance, affordable cost and remarkable HER catalytic activities.^{14,140,144}

2.3.2 Oxygen Evolution Reaction

OER, the complementary anodic reaction of water splitting, is another crucial energy-related reaction required for the development of technologies for renewable and alternative energy sources, namely water electrolyzers, metal-air batteries and fuel cells.¹⁴⁵ Unlike HER, OER typically entails a four-electron transfer process and requires higher energy to overcome the larger kinetic barrier. OER can occur in both acidic and alkaline medium as follows:¹⁴⁶⁻¹⁴⁸

OER involves the transfer of four electrons and often engages multiple step reactions, making it a kinetically challenging process, thus it is imperative to explore catalyst with low overpotential and excellent catalytic performance to overcome the large energy barrier. To date, numerous reaction pathways have been proposed for heterogeneous electrocatalysis of OER, for instance, electrochemical oxide path, Bockris path, Krasil'shchikov path, oxide path, Yeager's path and Wade and Hackerman's path. However, none of these suggested OER mechanisms have been fully validated experimentally.^{145,149,150}

The OER kinetic is highly influenced by the interactions between oxygen species (O_{ads} , OH_{ads} and OOH_{ads}) and the electrocatalysts.^{148,149} Studies by Koper,¹⁵¹ revealed that the binding energies of OH_{ads} and OOH_{ads} are correlated with a fixed energy value of circa 3.2 eV at $U = 0$

V. As such, Man et al.,¹⁵² assumed that the variation in OER overpotential from an adsorbed oxygen species to the next is determined by the adsorption energy of O_{ads} and established $\Delta G_{O_{ads}} - \Delta G_{OH_{ads}}$ as the descriptor for OER activity (Figure 2. 8). Following the Sabatier principle, OER would be limited by the formation of OOH_{ads} species if the interactions between the electrocatalysts and oxygen are too strong, while interactions that are too weak cause OER to be limited by the oxidation of OH_{ads} species.

Figure 2.8. Volcano plot correlating the overpotential of OER and $\Delta G_{O_{ads}} - \Delta G_{OH_{ads}}$ of noble and transition metal oxides. Reproduced from reference 152.

Sitting near the peak of the volcano plot, iridium oxide and ruthenium oxide possess superior electrochemical properties and have been the benchmark electrocatalysts for OER. These noble metal oxides are not only costly and scarce but are also unstable in aqueous media and easily oxidise under high anodic potentials, impeding their widespread application.^{145,149} As such, it is imperative to scout for alternative electrocatalysts that are highly abundant, stable under high anodic potentials and highly electrocatalytic towards OER.

2.3.3 Oxygen Reduction Reaction

ORR, an imperative cathodic half-reaction of oxygen electrode reactions, is widely applied in electrochemical energy conversion and storage devices including metal-air batteries and fuel

cells. Unlike HER and OER, ORR can take place in three different medium, namely acidic, alkaline and nonaqueous aprotic solvents.^{139,147,153,154}

In an acidic medium, ORR can occur via two different pathways, a 2-electron transfer process or a direct 4-electron process to yield water. The selectivity toward the 2-electron or 4-electron transfer pathways is influenced by the adsorption energies of the intermediary oxygenated species and the reaction barriers on the surface of the electrocatalyst. The direct 4-electron transfer process is preferred over the 2-electron transfer pathway as it delivers a much higher current efficiency. Furthermore, the 2-electron transfer pathway yields hydrogen peroxide as an intermediate in acidic media, which is highly undesirable in fuel cell applications.^{120,153-155} The 2-electron or 4-electron transfer processes occurring on the surface of electrocatalyst in acidic media can be described by the following equations:^{153,154}

Likewise, ORR can also occur via the 2-electron or 4-electron transfer process in an alkaline medium. Instead of forming water as the product, ORR in alkaline medium yields hydroxide ions as the end product and superoxide ion is formed as the intermediate species in the two-step 2-electron transfer pathway. ORR is preferentially conducted in alkaline medium over acidic media as it confers electrocatalyst with a less corrosive environment and faster ORR kinetics. The reaction pathways in alkaline media are as follows:^{139,153,154}

ORR has also been reported to occur in non-aqueous aprotic solution via a 1-electron transfer pathway to generate superoxide:¹⁵⁴

ORR is a highly complex reaction that involves numerous steps and intermediate species, depending on the nature of the electrodes, electrocatalysts and electrolytes used. To date, the mechanistic aspect of ORR is still poorly understood, despite extensive efforts being devoted to elucidating it. Even though the mechanism of ORR is poorly understood, several studies have come to the consensus that the high energy input required for the adsorption of oxygen molecules onto the working electrode, activation of oxygen-oxygen bond and dissociation of the strong double bond renders ORR with sluggish kinetics.^{139,153,156,157} Through DFT studies, Nørskov et al.¹⁵⁸ were able to establish a relation between the electrocatalytic activities of noble and transition metals towards ORR and their oxygen binding energies of oxygen (ΔE_{O}) (Figure 2.9). They also reported that too strong of an interaction amidst the metal catalyst and oxygen molecules would hinder the proton transfer step to O_{ads} and OH_{ads} while interactions that are too weak hinder the oxygen dissociation process.

Figure 2.9. Volcano plot correlating the electrocatalytic activities and the binding energy of oxygen (ΔE_O) on the surface of noble and transition metals. Reproduced from reference 158.

Although the current state-of-the-art ORR electrocatalyst, platinum, is able to catalyse ORR rather efficiently, its low abundance and high cost hinder its viability in industrial applications. As such, the search for alternative electrocatalyst has been highly endeavoured. In recent years, layered materials have been extensively explored as alternative ORR electrocatalysts due to their massive specific area, fascinating electronics properties and high surface density of active catalytic sites.^{120,139,155,159}

2.4 Layered Materials in Electrochemical Sensing and Energy Applications

Layered materials are highly favoured for electrochemical energy and sensing applications due to their vast range of fascinating properties. Layered materials possess large specific surface areas thus increasing the number of electrochemically active sites, enhancing electrocatalytic reaction kinetic in electrochemical energy applications. Furthermore, the large specific surface areas proffered by layered materials allow more recognition elements and substrates to be adsorbed, hence improving the sensitivity of electrochemical sensors. The electronics properties of layered materials could also be easily tuned through altering the number of layers, doping or functionalising to improve electrochemical sensing and catalysis

performances.^{3,13,160,161} Herein, a review of the inherent electrochemistry, electrochemical sensing and energy applications of TMDs, MXenes, pnictogen nanosheets and Group 14 graphane analogues would be provided in the preceding sections.

2.4.1 Transition Metal Dichalcogenides

As mentioned above in Section 2.1.1, various combinations of transition metal and chalcogens result in a vast range of TMDs that possess diverse inherent properties. As a result of these distinct and fascinating properties, TMDs have found their way into a myriad of applications.

2.4.1.1 Electrochemical Properties of Transition Metal Dichalcogenides

Prior to exploring the electrochemical catalytic applications of TMDs, it is fundamental to study their electrochemical properties, for instance, inherent electrochemistry and heterogeneous electron transfer (HET) rate to determine their suitability. Herein, inherent electrochemistry outlines the intrinsic redox behaviours of layered materials employed as electrode materials and influences the operational potential window. HET rate is another analytical parameter of paramount importance for assessing the suitability of layered materials for electrochemical applications. Inherently fast HET rate efficiently reduces the overpotentials necessary for electrochemical reactions to occur and is thus considered an alluring characteristic of prospective electrode materials.^{25,30,147,162,163}

Bonde et al. were amongst one of the pioneering groups who studied the innate electrochemical properties of MoS₂ and WS₂ and demonstrated the adverse implications of the intrinsic oxidation of MoS₂ and WS₂ towards HER.¹⁶⁴ Cyclic voltammetry studies were conducted in acidic media mimicking the experimental conditions of HER experiments and the cyclic voltammograms are portrayed in Figure 2.10. The cyclic voltammogram of MoS₂ in Figure

2.10 illustrates the presence of a broad and irreversible oxidation peak at +0.98 V *vs.* NHE and a significant loss in HER activity in subsequent scans. Closer scrutinisation reveals that the broad anodic peak corresponds to the oxidation of the edge (+0.7 V *vs.* NHE) and basal (+0.98 V *vs.* NHE) planes of MoS₂. XPS analysis was also conducted, where peaks corresponding to MoO₃ were observed upon subjecting MoS₂ to an oxidation treatment. Similar observations were also noted for WS₂ (Figure 2.10b), where the irreversible oxidation of the material resulted in a loss in HER activities.

Figure 2.10. Cyclic voltammograms of (a) MoS₂ and (b) WS₂ acquired through cyclic voltammetry studies conducted in N₂ purged H₂SO₄. Reproduced from reference 164.

After the study by Bonde et al., reports investigating the effects of exfoliation on the electrochemical properties of TMDs have also surfaced. Study conducted by Ambrosi et al.¹⁶⁵ reported an increase in inherent redox current with efficient exfoliation of MoS₂. Exfoliation of MoS₂ with n-butyllithium and tert-butyllithium result in thinner MoS₂ flakes that possess larger surface areas as compared to MoS₂ exfoliated with methylithium. As such, the redox peaks of n-butyllithium and tert-butyllithium exfoliated MoS₂ are much higher in intensities as compared to the bulk or methylithium exfoliated MoS₂. A parallel study was also carried out by Eng et al.,¹⁶³ where he investigated the effects of exfoliation on MoSe₂, WS₂ and WSe₂ (Figure 2.11d-m). The authors noted a positive shift in potentials upon exfoliating the Group 6

TMDs and ascribed the phenomena to the increased exposure of basal planes. The study also highlighted that different combinations of transition metal and chalcogens result in significantly different electrochemical properties. For instance, tungsten dichalcogenides reportedly possess broader recurrent oxidation peaks compared to their molybdenum counterparts, suggesting that different oxidation routes might have occurred. The authors also compared the oxidation peaks with MoS₂ and found that the Group 6 transition metal diselenides have lower oxidation potentials as compared to the Group 6 transition metal disulfides and the oxidation peaks potentials are rank as follows, WSe₂ < MoSe₂ < WS₂ < MoS₂, with MoS₂ possessing the largest oxidation overpotential.

Figure 2.11. Cyclic voltammograms of (a) bulk MoS₂ and (b-d) methyllithium, n-butyllithium and tert-butyllithium exfoliated MoS₂ in 0.1 M KCl. Reproduced from reference 165. Cyclic voltammograms detailing the innate electrochemistry of (e-g) bulk, (h-j) methyllithium

exfoliated and (k-m) n-butyllithium exfoliated MoSe₂, WS₂ and WSe₂. Reproduced from reference 163.

In very recent years, effects of dopants on TMDs have also been looked into, where Chua and colleagues demonstrated that tantalum dopant causes MoS₂ to oxidise much more readily as compared to niobium dopant.¹⁶⁶ In another instance, Chia et al. doped WSe₂ with vanadium, niobium and tantalum dopants and reported that dopants cause a drastic change in the electrochemical behaviour of WSe₂, where doped WSe₂ display anodic peaks with smaller peak currents in subsequent scans as opposed to the complete disappearance of the anodic peak in the subsequent scan of undoped WSe₂.¹⁶⁷ Recently, electrochemical studies investigating the inherent electrochemistry of other groups of TMDs (apart from Group 6 TMDs) have also been reported. For instance, the inherent electrochemistry of Group 4 and Group 5 TMDs were studied by Toh and Chia et al. respectively.^{30,168} Chia and co-workers also reported the innate electrochemistry behaviours of platinum dichalcogenides.¹⁶⁹

Apart from studying the inherent electrochemical behaviours, HET rates of TMDs is another important parameter to be explored when considering the potential electrochemical applications of TMDs. Similar to graphene, layered TMDs possess both basal and edge planes and display distinct electron transfer properties at the two planes. The electron transfer kinetics of edge and basal planes of MoS₂ were first probed by Ahmed and Gerischer using a series of redox probes, namely [Fe(CN)₆]^{3-/4-}, Fe^{3+/2+} and Cu^{2+/+}.¹⁷⁰ The study reported that the edge plane of MoS₂ displays superior electron transfer properties as opposed to the basal plane and ascribed the phenomenon to greater overlapping between the orbitals of molybdenum atoms (d_{xy} and $d_{x^2-y^2}$) and the orbitals of redox probes. Tan et al. further affirmed the superior electron transfer properties of edge plane MoS₂ where edge plane MoS₂ was calculated to

possess a HET rate constant (k_{obs}^0) of $9.3 \times 10^{-4} \text{ cm s}^{-1}$ in $[\text{Ru}(\text{NH}_3)_6]^{3+/2+}$ redox probes while the k_{obs}^0 of basal plane MoS_2 was computed to be $1.1 \times 10^{-3} \text{ cm s}^{-1}$.¹⁷¹

Electron transfer properties of TMDs could be altered via exfoliation or the introduction of dopants. Mixed results have been observed for exfoliated TMDs where the exfoliated products can either enhance or impede the HET process. Studies by Tan and Eng et al. showed that exfoliating MoSe_2 and WS_2 generally reduces the ΔE_p and enhances the HET kinetics in $[\text{Fe}(\text{CN})_6]^{3-/4-}$ redox probes.^{163,172} Meanwhile, depending on the type of intercalants used, exfoliated MoS_2 and WSe_2 show mixed results where both improvement and worsening of HET rates were observed.

Likewise, the introduction of dopants could also improve or worsen HET rates. In a study by Chua and co-workers,¹⁶⁶ they demonstrated that doping MoS_2 with niobium and tantalum result in an increase in ΔE_p in $[\text{Fe}(\text{CN})_6]^{3-/4-}$ redox probes and worsen the HET rates. However, when the same dopants were introduced to WS_2 , the ΔE_p in $[\text{Fe}(\text{CN})_6]^{3-/4-}$ redox probe decrease significantly, suggesting an enhancement in HET kinetics. In another instance, Urbanová and colleagues established that titanium and vanadium dopants can improve the HET rates of MoS_2 and WS_2 in $[\text{Fe}(\text{CN})_6]^{3-/4-}$ while doping TMDs with manganese and iron result in sluggish HET kinetics.¹⁷³ Apart from varying the type of dopants, the amount of dopants introduced could also affect the HET rates of TMDs. This effect is especially evident in the study by Rahmanian et al.¹⁷⁴ Although they demonstrated improvements in the HET rates upon incorporating varying amounts (5, 10 and 20% with respect to Ti) of niobium dopant into TiS_2 , TiS_2 doped with 5% of niobium shows the greatest enhancement in HET kinetics, suggesting that controlled introduction of dopant is required to achieve optimal HET rates in TMDs.

2.4.1.2 Energy Applications of Transition Metal Dichalcogenides

With an increasing number of governments attempting to transit into hydrogen-based economies, there is a pressing need to produce hydrogen energy carrier efficiently and economically. The current state-of-the-art platinum is scarce and costly, limiting their economic feasibility, thus prompting the scientific community to search for other alternative electrocatalysts. Notably, TMDs have emerged as outstanding alternatives owing to their natural abundancy, low cost and remarkable HER catalytic activities. To date, vast amounts of efforts have been committed to studying the HER performances of different TMDs, the factors influencing HER performances of TMDs as well as strategies to enhance the HER activities of TMDs.

It is prudent to determine the active sites of TMDs for HER, to have a better understanding of the differing electrocatalytic activities exhibited by TMDs and provide insights for the development of strategies to improve HER activities. A study pioneered by Hinnemann and co-workers,¹⁷⁵ revealed the edges of MoS₂ as the electrocatalytic active sites catalysing HER while basal planes are deemed to be catalytically inactive. Through DFT studies, they discovered that the ΔG_H of MoS₂ edges is relatively similar to those of platinum and nitrogenase models, close to the thermoneutral value (Figure 2.12a). This was exemplified by a study performed by Tan et al.,¹⁷¹ where they demonstrated that the edge planes of MoS₂ crystals are highly catalytic towards HER while the basal planes fare worse when compared with bare glassy carbon electrode and produce negligible HER activity (Figure 2.12b). In another study, Tsai and colleagues also reported the edge planes of MoSe₂ and WSe₂ as the electrocatalytic active sites for HER.¹⁷⁶

Figure 2.12. (a) Calculated free energy diagram for HER using DFT studies. Reproduced from Reference 175. (b) LSV illustrating the HER performances of edge and basal-planes of MoS₂, where the HER study was performed in 0.5 M sulfuric acid at a scan rate of 2 mV s⁻¹. Reproduced from Reference 171.

Inspired by the excellent HER performances of Group 6 TMDs, researchers have also ventured out to examine the HER electrocatalytic activities of other TMDs in recent years. The electrocatalytic performances of Group 4 TMDs towards HER were first explored by Toh et al.¹⁶⁸ LSV was employed to study the HER electrocatalytic performances of Group 4 TMDs and revealed HfS₂ to possess the smallest overpotential at -10 mA cm⁻², suggesting that HfS₂ exhibits the finest electrocatalytic activity amongst the Group 4 TMDs. The study also reported a strong metal dependence amongst the Group 4 transition metal disulfides where a significant decrease in overpotential at -10 mA cm⁻² was observed down the group (Figure 2.13a), while the overpotential remains relative unchanged down Group 4 transition metal diselenides and ditellurides (Figure 2.13c and e). A study by Chia and co-workers revealed that Group 5 TMDs generally exhibit poorer activities compared to Group 6 TMDs.³⁰ Amongst the Group 5 TMDs, only VTe₂ stands out to be an excellent electrocatalyst for HER. Close scrutinisation reveals that Group 5 transition metal ditellurides exhibit a strong metal dependence where the overpotentials increase significantly down Group 5 transition metal ditellurides (VTe₂ < NbTe₂ < TaTe₂). Meanwhile, negligible changes in overpotentials were observed down Group 5

transition metal disulfides and diselenides, suggesting the absence of metal dependency (Figure 2.13).

Figure 2.13. LSV curves of (a,c,e) Group 4 and (b,d,f) Group 5 TMDs. LSV sweeps were performed in 0.5 M H₂SO₄ at a scan rate of 2 mV s⁻¹. Bar charts depicting the average overpotential of (g) Group 4 and (h) Group 5 TMDs at a current density of -10 mA cm⁻². Data pertaining to Group 4 TMDs were reproduced from reference 168, while data corresponding to Group 5 TMDs was reproduced from reference 30.

In the hope of improving the electrocatalytic performances of TMDs, diverse strategies have been employed to modulate the electrochemical properties of TMDs. Chemical exfoliation has

been extensively explored to facilitate phase transitions from semiconducting 2H to metallic 1T polytypes in TMDs. 2H and 1T phases have been reported to exhibit significantly different HER electrocatalytic performances where 1T phase TMDs reportedly possess superior HER electrocatalytic activities as a result of their innately low charge transfer resistance.¹⁷⁷ This was exemplified by the studies conducted by Tan and Ambrosi et al.^{172,178} Both studies showed that Group 6 TMDs (MoS₂, MoSe₂, WS₂, WSe₂) exfoliated using phenyllithium, sodium naphthalenide sodium anthracenide and *tert*-butyllithium intercalants possess significantly lower overpotentials at -10 mA cm^{-2} as opposed to their bulk form. The improvement in HER activities was attributed to the significant increase in metallic 1T phases in TMDs which were observed in their XPS analysis.

Another commonly used method to alter the electrochemical properties of TMDs is through the introduction of dopants. Several studies have reported that when TMDs are doped with metallic heteroatoms, their ΔG_H gets closer to the thermoneutral value and the proton transfer kinetics get accelerated, which are beneficial for hydrogen evolution reaction.^{179,180} This is illustrated in the study conducted by Chia and colleagues,¹⁶⁷ where a decrease in overpotentials at -10 mA cm^{-2} were observed for the Group 5 transition metal-doped WSe₂ (Figure 2.14a and e). In another study, a continual decrease in overpotential was observed with an increasing amount of dopant niobium dopant in TiS₂ (Figure 2.14b and f).¹⁷⁴ Despite numerous studies showing improvements in the HER electrocatalytic activities upon the introduction of dopants, there are also studies reporting the detrimental effects of dopants on the electrocatalytic performance of TMDs. The study conducted by Chua and colleagues demonstrated a stark increase in HER overpotentials at -10 mA cm^{-2} when MoS₂ and WS₂ were doped using niobium and tantalum (Figure 2.14c and g).¹⁶⁶ Urbanová et al. also reported a decrease in the HER electrocatalytic performances of MoS₂ that were doped with titanium, manganese,

vanadium and niobium (Figure 2.14d and h).¹⁷³ The group also conducted DFT studies and reported that the ΔG_H of doped MoS₂ compounds deviates further away from thermoneutral values, hence resulting in declines in HER electrocatalytic performances.

Figure 2.14. (a-d) LSV curves and (e-f) their corresponding bar charts depicting the average overpotential of various doped TMDs at -10 mA cm^{-2} current density. Reproduced from reference 166 (Figure c and g), 167 (Figure a and e), 173 (Figure d and h) and 174 (Figure b and f).

Apart from being outstanding alternative electrocatalysts for HER, TMDs have also shown promising results for ORR electrocatalysis. Earlier works on TMDs based ORR electrocatalysis focused on TMDs based upon first row transition metals. For instance, pioneering research directed by Susac et al. demonstrated that electrodes modified with FeS₂ displayed larger current signals upon saturating the electrolyte with oxygen gas, suggesting that there is an increase in ORR activities (Figure 2.15a).¹⁸¹ Even though FeS₂ was shown to exhibit ORR electrocatalytic activities, the current achieved was three orders of magnitude lower than the benchmark platinum electrocatalyst, suggesting that the electrocatalytic performance of FeS₂ is much more inferior than that of platinum. In another study, Zhu and co-workers showed that larger cathodic currents were achieved by CoS₂ and NiS₂ in oxygen saturated HClO₄

compared to oxygen free HClO_4 , suggesting that both TMDs possess ORR electrocatalytic activities (Figure 2.15b).¹⁸² They also reported CoS_2 to be more stable than NiS_2 where negligible changes were observed in the XPS and Auger electron spectroscopy of CoS_2 after subjecting it to numerous hours of oxygen reduction conditions. The same group also demonstrated CoSe_2 to possess electrocatalytic activity towards ORR in another study and reported that electrochemical treatment could passivate the surface of CoSe_2 , enhancing its corrosion resistance, thus allowing CoSe_2 to remain stable for longer hours under oxygen reduction conditions.¹⁸³

Figure 2.15. (a) LSV plots of FeS_2 conducted in oxygen free and oxygen saturated HClO_4 electrolyte (0.1 M). Reproduced from reference 181. (b) LSV curves of CoS_2 and $(\text{Co,Ni})\text{S}_2$ (curve a) and NiS_2 (curve b) performed in oxygen free- HClO_4 electrolyte (0.1 M) and LSV plots of NiS_2 (curve c), CoS_2 (curve d) and $(\text{Co,Ni})\text{S}_2$ (curve e) conducted in oxygen saturated HClO_4 electrolyte (0.1 M). Reproduced from reference 182.

Although many TMDs have been reported to possess electrocatalytic activities towards ORR, their performances were inferior compared to platinum catalyst. As such, numerous strategies have been devised to alter the electrocatalytic performances of TMDs towards ORR. The introduction of heteroatom dopants into TMDs have been widely studied in the hope of improving the electrocatalytic performances of TMDs, however, mixed results have been

attained by numerous groups. A study by Chua et al. revealed a worsening in ORR performance upon the introduction of niobium and tantalum into MoS₂, while the introduction of the above-mentioned dopants induced negligible changes in the ORR electrocatalytic performances of WS₂ (Figure 2.16a-c).¹⁶⁶ In another study, Urbanová and colleagues demonstrated that manganese and iron dopants could improve the ORR catalytic activities of MoS₂ and WS₂, while titanium and vanadium dopants resulted in a slight suppression of the ORR catalytic activities of MoS₂ and WS₂ (Figure 2.16d-g).¹⁷³ Meanwhile, doping Group 6 TMDs with non-metal dopants such as nitrogen and phosphorus have also been reported to induce enhancement in ORR performance.^{184,185}

Figure 2.16. ORR LSV plots (a and b) and (c) bar chart showing the ORR onset potential of niobium- and tantalum-doped MoS₂ and WS₂. Reproduced from reference 166. ORR LSV curves (d and f) and histogram plots (e and g) depicting the ORR onset potential of titanium-, vanadium-, manganese- and iron-doped MoS₂ and WS₂. Reproduced from reference 173.

In comparison with HER and ORR, far fewer studies have been carried out to evaluate the feasibility of using TMDs as alternative OER electrocatalysts. One of the first studies to investigate the electrocatalytic activity of TMDs towards OER was conducted by Liu et al.,¹⁸⁶ where they demonstrated CoSe₂ as a viable OER catalyst. They reported that atomically thick CoSe₂ nanosheets are more favourable towards water molecule adsorption and can facilitate mass transport more efficiently, hence allowing single atom thick CoSe₂ nanosheets to possess superior OER activities as opposed to bulk CoSe₂. They also showed that the atomically thick CoSe₂ nanosheets are highly stable under OER conditions, where negligible changes in catalytic activity were observed upon 1000 OER cycles. In another study, Wu and colleagues reported the use of MoS₂ and TaS₂ nanosheets for OER applications and revealed that 1T phase TMDs exhibit greater OER electrocatalytic activities as compared to their 2H phase counterparts.¹⁸⁷

2.4.2 MXenes

A vast range of MXenes that possess distinct properties can be generated by altering the elemental compositions or surface functional moieties. The metallic-like conductivity, large surface areas, hydrophilicity and excellent biocompatibility of MXenes, have rendered them excellent candidates for sensing applications.^{40,188,189} In the ensuing sections, an introduction to the electrochemical properties and electrochemical sensing applications of MXenes would be provided.

2.4.2.1 Electrochemistry of MXenes

Prior to exploring the electrochemical sensing applications of MXenes, it is cardinal to study their inherent electrochemistry and HET properties to determine their operational potential

window and their suitability for various sensing applications. Lorencova et al. pioneered the study on the innate electrochemistry of $\text{Ti}_3\text{C}_2\text{T}_x$ and revealed a narrow anodic potential window for $\text{Ti}_3\text{C}_2\text{T}_x$ electrochemical applications.¹⁹⁰ Cyclic voltammetry studies revealed the occurrence of an anodic peak that starts at +0.2 V and reaches a maximum at +0.43 V (Figure 2.17a). The peak was also reported to diminish significantly in the subsequent scans suggesting the occurrence of an irreversible oxidation process. As such, $\text{Ti}_3\text{C}_2\text{T}_x$ could only be employed for electrochemical applications involving potential windows that ranges from the cathodic region up to +0.2 V of the anodic region. In another study, Rasheed et al. explored the inherent electrochemical behaviours of Nb_2CT_x and $\text{Nb}_4\text{C}_3\text{T}_x$ and found them to be vastly different from those of $\text{Ti}_3\text{C}_2\text{T}_x$.¹⁹¹ Cyclic voltammograms of both niobium based MXenes revealed the occurrence of irreversible oxidative processes, where the anodic peaks that start at +0.5 V diminish rapidly in intensities in subsequent cycles (Figure 2.17b). This suggests that Nb_2CT_x and $\text{Nb}_4\text{C}_3\text{T}_x$ can be used for electrochemical applications that have operational potential windows up to +0.5 V. The study also revealed that $\text{Nb}_4\text{C}_3\text{T}_x$ have a much larger oxidative peak compared to Nb_2CT_x and attributed the phenomena to the superior electron transfer kinetics of $\text{Nb}_4\text{C}_3\text{T}_x$.

Figure 2.17. (a) Cyclic voltammogram depicting the inherent electrochemical behaviour of $\text{Ti}_3\text{C}_2\text{T}_x$ in phosphate buffer. Reproduced from reference 190. (b) Cyclic voltammogram illustrating the inherent electrochemistry of Nb_2CT_x and $\text{Nb}_4\text{C}_3\text{T}_x$ through 5 consecutive scans in 0.1 M phosphate buffer. Reproduced from reference 191.

Recent studies have demonstrated that the inherent electrochemical behaviour of MXenes could be modulated by functionalising MXenes and tuning the number of layers within MXenes. In a separate study, Lorencova et al. showed that functionalising $\text{Ti}_3\text{C}_2\text{T}_x$ with platinum nanoparticles could greatly enhance its electrochemical stability.¹⁹² Cyclic voltammetry study of the functionalised $\text{Ti}_3\text{C}_2\text{T}_x$ revealed the absence of +0.43 V peak associated with the oxidation of titanium in $\text{Ti}_3\text{C}_2\text{T}_x$ and the functionalised $\text{Ti}_3\text{C}_2\text{T}_x$ depicted a much smaller decrease in current signal at +0.43 V as compared to pristine $\text{Ti}_3\text{C}_2\text{T}_x$ after 36 scans (Figure 2.18a). Meanwhile, Nayak et al. detailed different electrochemical behaviours between few- and multi-layered $\text{Ti}_3\text{C}_2\text{T}_x$, suggesting that the thickness of the materials could influence the electrochemical behaviours of MXenes.¹⁹³ Anodic scans of few-layered $\text{Ti}_3\text{C}_2\text{T}_x$ revealed the existence of a distinct oxidation peak at +0.7 V as well as a small overlapping shoulder peak at +0.37 V, whilst anodic scans of multi-layered $\text{Ti}_3\text{C}_2\text{T}_x$ revealed the appearance of an oxidation peak of lower intensity at +0.34 V. Subsequent scans of both few- and multi-layered $\text{Ti}_3\text{C}_2\text{T}_x$ depict an absence of the anodic peak, suggesting the occurrence of an irreversible oxidation process.

Figure 2.18. (a) Cyclic voltammogram illustrating the 1st and 36th scans of $\text{Ti}_3\text{C}_2\text{T}_x$ (black curve) and $\text{Ti}_3\text{C}_2\text{T}_x$ functionalised with platinum nanoparticles (red curve) in 0.1 M phosphate buffer. Reproduced from reference 192. Cyclic voltammograms portraying the intrinsic

electrochemistry of few-layered $\text{Ti}_3\text{C}_2\text{T}_x$ (b) and multi-layered $\text{Ti}_3\text{C}_2\text{T}_x$ (c) in 0.1 M phosphate buffer. Reproduced from reference 193.

Apart from studying the inherent electrochemical behaviours, it is also cardinal to investigate its electron transfer properties. The study by Shahzad et al. reported that their as-synthesised ultrathin $\text{Ti}_3\text{C}_2\text{T}_x$ (3 nm thick) exhibited a much smaller ΔE_p and larger peak current as compared to reduced graphene oxide in ferro/ferricyanide redox probes, suggesting that $\text{Ti}_3\text{C}_2\text{T}_x$ possesses excellent electron transfer properties.¹⁹⁴ Meanwhile, the study by Nayak and co-workers revealed that apart from altering the inherent electrochemistry of MXenes, tuning the thickness of materials also modulates their HET properties.¹⁹⁴ They detailed that few-layered $\text{Ti}_3\text{C}_2\text{T}_x$ exhibits superior electron transfer properties over multi-layered $\text{Ti}_3\text{C}_2\text{T}_x$ in ferro/ferricyanide redox probes, where few-layered $\text{Ti}_3\text{C}_2\text{T}_x$ records a much smaller ΔE_p and possesses a HET rate constant that is an order of magnitude faster.

2.4.2.2 Electrochemical Sensing Applications of MXenes

Till date, most MXenes-based electrochemical sensing studies have been focused on $\text{Ti}_3\text{C}_2\text{T}_x$, with only one study recounting the use of $\text{Nb}_4\text{C}_3\text{T}_x$ for the fabrication of electrochemical sensor. A summary of the latest literature examples of MXenes-based electrochemical (bio)sensor is displayed in Table 2.1.

Table 2.1. Compilation of electrochemical (bio)sensors based upon MXenes, pnictogen nanosheets and Group 14 graphane analogues.

Layered Material Class	Analytical System	Target Analyte	Limit of Detection	Linear Range	Ref.
MXenes	GC/Ti ₃ C ₂ T _x	Dopamine	3 nM	15 nM – 10 μ M	194
	GC/Nb ₄ C ₃ T _x	Dopamine	2.3 nM	50 nM – 1 μ M 1 μ M – 10 μ M	191
	GC/Ti ₃ C ₂ /Hb/Nafion	H ₂ O ₂	0.02 μ M	0.1 – 260 μ M	195
	GC/Ti ₃ C ₂ /Hb/Nafion	NO ₂ ⁻	0.12 μ M	0.5 – 11800 μ M	196
	GC/Tyr-MXene-Chi	Phenol	12 nM	0.05 – 15.5 μ M	197
	GC/Ti ₃ C ₂ T _x /PtNP	H ₂ O ₂	448 nM	–	192
	GC/Au/Ti ₃ C ₂ T _x /Nafion/GOx	Glucose	5.9 μ M	0.1 – 18 mM	198
Pnictogen Nanosheets	GC/TiO ₂ -Ti ₃ C ₂ /Hb/Nafion	H ₂ O ₂	14 nM	0.1 – 380 μ M	199
	GC/FL-BP	H ₂ O ₂	0.1 μ M	0.1 – 50 μ M	203
	GC/BP-Nf(IP)	Clenbuterol	3.7 nM	0.06 – 24 μ M	204
	GC/BP	Ochratoxin	0.18 μ g mL ⁻¹	0.3 – 10 μ g mL ⁻¹	205
	GC/Phosphorene/HRP/Glu	H ₂ O ₂	0.14 μ M	5 – 275 μ M	206
Group 14 Graphane Analogues	GC/Antimonene/Tyr/Glu	Phenol	255 nM	500 – 2500 nM 7.5 – 27.5 μ M	59
	GC/Siloxene	Dopamine	0.327 μ M	10 – 190 μ M 200 – 1100 μ M	211
	GC/Siloxene	Dopamine	19.1 μ M	30 – 100 μ M	70

In a recent study, Shahzad and co-workers were able to yield ultrathin Ti₃C₂T_x flakes that are approximately 3 nm thick using a lithium fluoride/hydrochloric acid etching solution and a subsequent ultrasonication delamination process.¹⁹⁴ The ultrathin Ti₃C₂T_x was then employed for the sensitive detection of dopamine. The authors revealed the sensing platform to be highly selective and sensitive with a low limit of detection (LOD) of 3 nM. The sensing platform also possesses a wide linear detection range from 0.015 – 10 μ M. The outstanding performance of the sensing device was ascribed to the small charge transfer resistance, remarkable inherent electrical conductivity, and massive surface area of Ti₃C₂T_x. Additionally, the authors explored

the viability of using the sensing platform for real sample analysis and reported excellent dopamine recoveries between 95 to 98.5% in urine and serum samples. The proposed dopamine sensor also yields a low relative standard deviation of ~3% during real sample analysis, illustrating that it is highly reproducible. In another study, Rasheed et al. fabricated a dopamine sensor based upon pristine Nb₄C₃T_x.¹⁹¹ Differential pulse voltammetry studies revealed an increase in peak current with increasing concentrations of dopamine and the sensing platform was reported to detect dopamine linearly over two ranges, 50 nM to 1 μ M and 1 μ M to 10 μ M. The Nb₄C₃T_x based sensor also possesses a relatively low LOD of 23 nM.

Apart from non-enzymatic detection of small molecules, MXenes have also been employed for the construction of biosensors. Wang and co-workers synthesised a multi-layered organ-like Ti₃C₂ by etching its corresponding MAX phase, Ti₃AlC₂, with 40% hydrofluoric acid.¹⁹⁵ The as-synthesised Ti₃C₂ was subsequently used to immobilise haemoglobin for the development of a mediator-free hydrogen peroxide biosensor. Using chronoamperometry, the authors reported that the proposed biosensor has a quick response time (< 3 s), possesses a low LOD of 0.02 μ M and is able to detect hydrogen peroxide linearly over a vast range from 0.1 to 260 μ M. In the following year, the same group applied the above as-synthesised biosensor for the sensitive detection of nitrite. The sensing platform was reported to detect nitrite over an extensive linear range of 0.5 μ M – 11.8 mM and possesses an ultralow detection limit of 0.12 μ M.¹⁹⁶ In both studies, the fabricated biosensors were determined to be highly stable where more than 90% of the initial responses were retained after numerous weeks of storage. Ti₃C₂ was also employed for the construction of a phenol biosensor, where Ti₃C₂ was first mixed with tyrosinase followed by chitosan and 5 μ L of the resulting composite was deposited onto an electrode to yield the biosensor. The constructed biosensor possesses an ultralow detection limit of 12 nmol L⁻¹, high sensitivity of 414.4 mA M⁻¹ and detects phenol linearly

over a broad range of 50 nmol L^{-1} to $15.5 \text{ } \mu\text{mol L}^{-1}$. To determine the feasibility of using the biosensing platform for real sample analysis, tap water was spiked with varying amount of phenol and the biosensing platform recorded good recovery percentages from 86.8 to 106% with a low relative standard deviation of 1.3%.¹⁹⁷

In the last decade, numerous studies have reported that functionalising MXenes with nanostructures could greatly enhance their sensing performances. For instance, the hydrogen peroxide electrochemical sensor proposed by Lorencova et al. revealed that the decoration of $\text{Ti}_3\text{C}_2\text{T}_x$ with platinum nanoparticles led to a positive shift in the reduction potential of hydrogen peroxide, suggesting that the composite can reduce hydrogen peroxide much more effectively.¹⁹² The study also reported that the composite shows improvements in terms of electrochemical stability and limit of detection (approximately 2000 times lower) compared to pristine $\text{Ti}_3\text{C}_2\text{T}_x$. In another instance, Rakhi and colleagues demonstrated that glucose biosensor constructed with gold nanoparticles functionalised $\text{Ti}_3\text{C}_2\text{T}_x$ yields much better analytical performances with regard to response times, LOD and linear detection range.¹⁹⁸ Through chronoamperometric studies, the authors noted that a much shorter time (10 s) is required by the functionalised $\text{Ti}_3\text{C}_2\text{T}_x$ -based biosensor to achieve a stable current as compared to the biosensor based upon pristine $\text{Ti}_3\text{C}_2\text{T}_x$ (20 s). The glucose biosensing platform based upon the functionalised $\text{Ti}_3\text{C}_2\text{T}_x$ exhibits a much wider linear detection range (0.1–18 mM) and lower LOD ($5.9 \text{ } \mu\text{M}$) as opposed to the pristine $\text{Ti}_3\text{C}_2\text{T}_x$ based biosensor (0.5–6 mM and $100 \text{ } \mu\text{M}$ respectively). Wang et al. demonstrated that decorating Ti_3C_2 with TiO_2 nanoparticles can enhance the sensitivity of haemoglobin-based biosensor and improves its stability.¹⁹⁹ The biosensor developed using TiO_2 functionalised Ti_3C_2 displays a lower LOD of 14 nM and possesses a wider linear detection range for hydrogen peroxide (0.1–380 μM) as opposed to the biosensor based upon pristine Ti_3C_2 which possesses a detection limit of $0.02 \text{ } \mu\text{M}$ and a

linear detection range of 0.1–260 μM . The biosensor fabricated using TiO_2 functionalised Ti_3C_2 was reported to retain 94.6% of the original response following two months of storage while pristine Ti_3C_2 -based biosensor only managed to retain 93% of the initial response after 21 days of storage. This suggests that functionalising Ti_3C_2 with TiO_2 helps to enhance its biocompatibility and provides a more conducive microenvironment for haemoglobin to retain its bioactivity, thus improving the biosensor's stability.

2.4.3 Pnictogens

The recent experimental achievements of pnictogen nanosheets along with the discovery of their numerous fascinating properties have resulted in an explosive growth of interest in these materials. The large surface area, high carrier mobilities and the broad range of band gaps make pnictogen nanosheets ideal candidates for sensing applications.²⁰⁰ For this thesis, the inherent electrochemical behaviours and electrochemical sensing applications of pnictogen nanosheets would be briefly discussed in the ensuing sections.

2.4.3.1 Electrochemistry of Pnictogen Nanosheets

As a recent addition to the family of layered materials, the innate electrochemistry of pnictogen nanosheets is still poorly understood. One of the first studies that detailed the intrinsic redox behaviours of pnictogens was conducted by Wang and co-workers.²⁰¹ They observed an anodic oxidation peak at +0.6 V for black phosphorus and attributed it to the oxidation of P^0 to P^{5+} (Figure 2.19a). Likewise to many layered materials, the edge and basal planes of black phosphorus exhibit distinct inherent electrochemistry. Sofer and colleagues revealed that the edge planes of black phosphorus possess a much narrower anodic electrochemical potential window compared to the basal planes of black phosphorus where the edges of black phosphorus exhibited a significant oxidation peak that starts at +0.5 V.²⁰² Furthermore, the group also noted

weak reduction peaks (0.0 and -0.2 V) for basal plane black phosphorus and attributed them to the reduction of P^{5+} to P^{3+} and P^{1+} respectively (Figure 2.19b). A study carried out by Gusmão et al. revealed that through shear exfoliation, the inherent electrochemistry of layered arsenic, antimony and bismuth could be altered drastically (Figure 2.19c-e).⁸ The authors revealed bulk arsenic, antimony and bismuth to be electrochemically inert while the exfoliated forms are deemed more unstable. The anodic oxidation peaks of exfoliated pnictogen nanosheets noted a positive shift down Group 15, from $+0.10$ V for arsenic nanosheets to $+0.19$ V for bismuth nanosheets. Interestingly, the authors also noted an absence of reduction peak for antimony nanosheets and that bismuth nanosheets displayed additional redox peaks at $+0.19$ and -0.78 V after the third scan.

Figure 2.19. (a) Cyclic voltammogram illustrating the inherent electrochemistry of layered black phosphorus, reproduced from reference 201. (b) Cyclic voltammogram depicting the innate electrochemistry of edge and basal plane black phosphorus. Reproduced from reference 202. Cyclic voltammograms detailing the intrinsic electrochemistry of bulk and shear exfoliated (c) arsenic, (d) antimony and (e) bismuth. Reproduced from reference 8.

HET studies conducted using the common ferro/ferricyanide redox probes revealed superior electron transfer properties along the edges of black phosphorus in contrast to the basal plane where clear, discernible redox peaks were noted for the edge plane of black phosphorus. Meanwhile, an absence of redox peaks was noted for the basal plane of black phosphorus, suggesting that HET barely occurred on the surface of the basal planes.²⁰² Results from the study by Gusmão et al. demonstrated an enhancement in the HET rates of heavy pnictogens upon shear exfoliation.⁸ Additionally, the authors noted larger peak-to-peak separations for both bulk and exfoliated arsenic, suggesting that arsenic exhibits slower HET compared to antimony and bismuth.

2.4.3.2 Electrochemical Sensing Applications of Pnictogen Nanosheets

As of writing this thesis, most of the works based on pnictogen-based electrochemical sensors are conducted using black phosphorus (or phosphorene), reports involving heavy pnictogens are tremendously rare (Table 2.1). Yan et al. managed to synthesise few-layered black phosphorus through a supercritical carbon dioxide assisted technique and developed a non-enzymatic hydrogen peroxide sensor using the as-synthesised black phosphorus nanosheets.²⁰³ AFM study revealed that the as-synthesised black phosphorus nanosheets contain around three to five layers of phosphorene. The fabricated sensor displays a low LOD of 1×10^{-7} M for hydrogen peroxide detection and has a linear detection range up to 5×10^{-5} M. In another instance, an electrochemical clenbuterol sensor was constructed using a nanocomposite synthesised from black phosphorene and isopropanol treated nafion. Through differential pulse voltammetry study, the sensor reportedly detects clenbuterol over an extensive linear detection range of 0.06–24 μ M and exhibits a low LOD of 3.7 nM. The sensor also demonstrated high selectivity against a wide variety of amino acids, carbohydrates and biogenic amines and

excellent recovery of clenbuterol of 100.33% from bovine serum for real sample analysis.²⁰⁴

In another instance, an ochratoxin sensor was developed using Ag^+ functionalised black phosphorus nanosheets. Using differential pulse voltammetry study, the sensor displayed a linear increase in current with sequential additions of ochratoxin from 0.3–10 $\mu\text{g/mL}$ and a low LOD of 0.18 $\mu\text{g/mL}$. Analysis using beer and grape juice also revealed excellent recovery capabilities of the sensing platform, suggesting that the proposed sensing platform is highly viable for real sample analysis.²⁰⁵

Pnictogen nanosheets have also been utilised for the creation of electrochemical enzymatic-based biosensors. For instance, phosphorene was employed by Mayorga-Martinez and colleagues for the development of a binary oxidoreductase enzymatic system.²⁰⁶ Using glucose oxidase and horseradish peroxidase as the biorecognition elements, the sensors function by detecting the redox process undergone by the ferrocene methanol mediator and the signals yielded are proportional to the concentrations of the target analytes. Interestingly, the authors noted an enhancement in enzymatic activity for the reductive peroxidase-based biosensor only and attributed the phenomena to the inherent electrochemical behaviours of phosphorene. Glucose oxidase biosensing systems function in the anodic potential windows, under such oxidative conditions, phosphorene is easily oxidised and hence a decline in enzymatic activity was observed. In another study, the same group employed shear exfoliated phosphorene, arsenene, antimonene and bismuthene for the development of a tyrosinase-based phenol biosensor. The authors reported that arsenene fared worst among the four materials tested and attributed the poor performance to the thiophilic nature of arsenene which binds strongly to the sulphur atoms in the enzyme, disrupting the enzyme's structure. Meanwhile, the biosensing system based upon antimonene displayed the best performance. TEM studies revealed antimonene to be highly exfoliated and XPS analysis revealed the lowest oxidised content,

hence antimonene was able to greatly enhance the HET process. The antimonene based phenol biosensor displayed an ultralow LOD of 255 nM, ten times lower than the recommended limit by the United States Environmental Protection Agency.^{59,207}

2.4.4 Group 14 Graphane Analogues

Functionalisation of germanene and silicene yield Group 14 graphane analogues (such as germanane, methyl germanane and siloxane) that possess direct bandgaps and enhanced stability. Furthermore, these emerging Group 14 graphane analogues possess numerous fascinating properties such as high electron mobilities, photoluminescence properties, high resistance to oxidation and high specific capacitance, allowing them to be applied to a myriad of fields. Some of these applications include supercapacitors, photocatalysts, anodes in Li-ion batteries and sensors.^{9,70,78,80,85-88} In the ensuing sections, the thesis would briefly review the innate electrochemical properties of the above-mentioned Group 14 graphane analogues and the sensing applications of these materials.

2.4.4.1 Electrochemistry of Group 14 Graphane Analogues

Despite germanane, methyl germanane and siloxene bespeaking great promises for a myriad of applications, little studies were conducted to probe the inherent electrochemical properties of these materials. Through cyclic voltammetry, Rosli and colleagues revealed a prominent irreversible oxidative peak at +0.7 V for methylgermanane while nominal redox peaks were observed for germanane and siloxene.⁷⁰ The peak at +0.7 V was ascribed to the oxidation of Ge^+ to Ge^{2+} or Ge^{3+} species. The authors suggested that the presence of bulky methyl groups prevent germanene from being fully functionalised, causing small amounts of germanium atom to be exposed on the material's surface, causing oxidative peaks to be observed in methyl

germanane. The authors also proposed that siloxene and germanane can be applied towards a wider variety of electrochemical applications due to their larger operational potential window.

Apart from studying the inherent redox behaviours of germanane, methyl germanane and siloxene, Rosli et al. also investigated the HET properties of the above-mentioned materials using ferro/ferricyanide redox probes.⁷⁰ The authors revealed that all three materials exhibit smaller peak-to-peak separations as compared to bare glassy carbon electrode, suggesting that working electrodes modified with these materials would exhibit enhanced HET rates. The authors also calculated the HET rate constant using the Nicholson approach and revealed that germanane, methyl germanane and siloxene possess HET rate constants that are 17, 21 and 5 times greater than that of glassy carbon electrode. This signifies that methyl germanane exhibits the fastest HET rate accompanied by germanane then siloxene.

2.4.4.2 Electrochemical Sensing Applications of Group 14 Graphane Analogues

As an uprising class of layered materials, few studies have experimentally investigated the applications of these materials towards sensing platforms (Table 2.1), many of the sensing applications of Group 14 graphane analogues were studied through DFT studies. Computational studies conducted by Chandiramouli's group have predicted germanane to be an excellent surface for the detection of liver cirrhosis biomarkers, nucleobases, alcohol and aldehyde molecules.²⁰⁸⁻²¹⁰ Ramachandran et al. was amongst one of the first groups to experimentally determine the feasibility of using siloxene for the construction of electrochemical sensors.²¹¹ The group reported that the siloxene-based dopamine sensor exhibited a low LOD of 0.327 μM and a broad linear detection range spanning from 10 to 1100 μM . The fabricated sensor also exhibited outstanding selectivity against a wide range of endogenous interfering species including glucose, hydrogen peroxide, uric acid, L-cysteine and

adenosine triphosphate. Furthermore, the sensing platform demonstrated high stability where the response attained after two weeks of storage was determined to be 96.3% of the initial signal. In another study, Rosli and co-workers demonstrated that germanane-based materials are more selective and sensitive towards dopamine detection.⁷⁰ Through cyclic voltammetry, germanane-based materials were able to yield separate oxidative peaks to distinguish dopamine from a solution mixture containing dopamine, ascorbic and uric acid. Close scrutinisation of the cyclic voltammograms revealed that the peak current achieved by germanane is much higher than methyl germanane, suggesting that germanane is much more sensitive towards dopamine detection. The group subsequently studied the analytical parameters of the dopamine sensor based upon germanane and revealed that the sensor possesses a low LOD of 19.1 μM and a linear detection range from 30 to 100 μM .

2.5 Toxicity Studies of Layered Materials

With the rapid advancement in science and technology, there has been a huge growth in the number of newly engineered sophisticated materials. The scientific community has raised much concerns about the potential of these novel materials in causing new hazards and risks, therefore careful consideration of the possible novel risks posed is imperative before the inclusion of such materials into products that might eventually enter the market place. Previous studies have suggested that certain physiochemical properties of materials, for instance, size, chemical compositions, surface charge, surface area and shape play important roles in biological interactions and responses, which could lead to adverse side effects.²¹²⁻²¹⁷

To have a better comprehension of the novel material's deleterious effects, procedures and interpretation of the data have to be carried out correctly and assumptions have to be taken into consideration. There are two main methods to study the toxicity of materials, namely through

in vitro and *in vivo* toxicological studies. *In vitro* techniques are preferred by many to conduct preliminary toxicity studies, as it eliminates the ethical and administrative challenges that one must deal with when conducting *in vivo* studies. During the investigation of material's toxicity via *in vitro* toxicological studies, mammalian cells are used to determine cell viability or changes in activity in the designated biological pathway against the test materials. There is a wide range of well-established and commercially available cell viability assays such as tetrazolium reduction and protease activity assays which measure certain aspects of metabolism or enzymatic activities. One of the more commonly used methods is the tetrazolium reduction assays where compounds such as MTT (methylthiazolyldiphenyl-tetrazolium bromide) or WST (water-soluble tetrazolium salt) are employed. In the presence of metabolically active cells, cellular dehydrogenases would catalyse the reduction of the tetrazolium compounds to yield purple or orange formazan dyes for MTT and WST-8 assays respectively.^{214,218-222}

2.5.1 Toxicity Profiles of Transition Metal Dichalcogenides

The fascinating properties proffered by TMDs, have caused them to become outstanding contenders for a vast range of applications. Prior to the wide scale usage of TMDs, it is of utmost importance to investigate the inherent toxicities of TMDs to minimise the risk of causing adverse effects to manufacturers and end users.

Chemical compositions undertake a vital role in influencing the toxicological effects of layered materials, different combinations of transition metals and chalcogens can give rise to TMDs that exhibit drastically different toxicological profiles. This was exemplified by the study conducted by Teo et al. where cytotoxicity studies conducted on adenocarcinoma human lung epithelial cells (A549 cells) using WST-8 and MTT assays revealed WSe₂ to be significantly more toxic than MoS₂ and WS₂.²²³ A dose-dependent effect was observed for WSe₂ where

increasing amounts of WSe₂ lead to declines in cell viabilities and only 31.8 % of the cells remained viable at the maximum tested concentration of 400 µg mL⁻¹. Meanwhile, MoS₂ and WS₂ displayed high cell viability over 60 and 80% respectively at high TMDs concentrations of 400 µg mL⁻¹. In another study, Latiff and co-workers explored the innate cytotoxicity behaviours of vanadium dichalcogenides towards A549 cells and revealed dose-dependent toxicity trends for VS₂, VSe₂ and VTe₂.²²⁴ The authors revealed VTe₂ to be highly toxic, while VS₂ and VSe₂ exhibited moderate toxicity and the innate toxicity of vanadium dichalcogenides are ranked as follows: VS₂ < VSe₂ < VTe₂. The authors also compared the cell viabilities studies of vanadium dichalcogenides with the study conducted by Teo et al. and concluded that vanadium dichalcogenides are typically more cytotoxic as opposed to Group 6 TMDs. The study by Rosli et al. also revealed dose-dependent toxicity trends induced by PtSe₂ and PtTe₂ towards A549 cells and human lung epithelial cell line (BEAS-2B cells).²²⁵ Cytotoxicity studies conducted using WST-8 revealed PtS₂ to be the least toxic where it displayed nominal toxicity across a vast range of PtS₂ concentrations (0 to 200 µg mL⁻¹). The cytotoxicity profiles of the platinum dichalcogenides were ranked as such: PtS₂ < PtSe₂ < PtTe₂. Close scrutinization of these study reveals an increase in toxicity down the chalcogen group for TMDs with the same transition metal element.

Apart from chemical composition, size effects and surface functionalisation have also been reported to influence the cytotoxicity of TMDs. Chng et al. demonstrated that exfoliating MoS₂ with different organic intercalants results in MoS₂ nanosheets with different thickness and these resultant MoS₂ nanosheets exhibit different cytotoxicity trends.²²⁶ The authors reported that MoS₂ exfoliated with *n*-butyllithium and *tert*-butyllithium results in thinner MoS₂ nanosheets as compared to methyllithium exfoliated MoS₂ and these materials exhibited higher degrees of toxicity. The extent of cytotoxicity exhibited by the different MoS₂ was rank as follows, bulk

MoS₂ being the least toxic, followed by methyllithium MoS₂, *n*-butyllithium MoS₂ and *tert*-butyllithium MoS₂. The authors proposed that well exfoliated MoS₂ might contain a larger number of edge sites, which could interact with the cell membrane causing membrane stress and hence causing greater degrees of cytotoxicity. Meanwhile, both Yin and Rosli et al. demonstrated that modification of MoS₂ with chitosan and thiobarbituric acid can greatly reduce their toxicities.^{227,228}

2.5.2 Toxicity Profiles of Pnictogens

As one of the newer additions to the layered materials family, the toxicological profiles of layered pnictogens are poorly understood. Most of the toxicity studies conducted were based upon black phosphorus and phosphorene. Toxicity studies conducted by Latiff et al. revealed that black phosphorus exhibits a dose-dependent toxicity trend and significantly reduces cell viabilities to 48 % at 50 µg mL⁻¹ of test materials.²²⁹ By comparing the data from cytotoxicity studies conducted on other layered materials, the authors suggest that the extent of cytotoxicity exhibited by black phosphorus is in between that of graphene oxides and exfoliated TMDs. The same group also demonstrated that different allotropic forms of phosphorus nanosheets could induce different extent of toxicity. The authors revealed black phosphorus to be much more toxic than red and violet phosphorus and result in higher production of reactive oxygen species.²³⁰ In a separate study, Zhang and co-workers reported that black phosphorus nanosheets that are greater in lateral dimension and thickness exhibit higher degrees of cytotoxicity.²³¹ The authors reported that larger and thicker flakes of black phosphorus cause greater disruption to cell membrane integrity while dihydrodichlorofluorescein diacetate assay reveals a comparable amount of reactive oxygen species produced across black phosphorus of all sizes.

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Part I

Chapter 3

Titanium Carbide-based Glucose Biosensor: Strong
Dependence of Exfoliation Method on Performance

3.1 Introduction

3.2 Results and Discussion

3.3 Conclusion

3.4 Experimental Section

3.5 Supporting Information

The results and discussion in this chapter were published in the following journal article:

MXene Titanium Carbide-based Biosensor: Strong Dependence of Exfoliation Method on Performance

H. L. Chia, C. C. Mayorga-Martinez, N. Antonatos, Z. Sofer, J. J. Gonzalez-Julian, R. D. Webster, M. Pumera, *Anal. Chem.* **2020**, 92, 2452-2459.

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3.1 Introduction

Over the last few years, the family of 2D materials has been extended by a novel and rapidly growing collection of early transition metal carbides known as MXenes.^{1,2} MXenes are generated via the selective etching of ‘A’ layers from their layered, ternary parent compounds, MAX phases. MAX phases possess a generic chemical formula of $M_{n+1}AX_n$, whereby M corresponds to early d-transition metals, A being elements from either Group 13 or 14, and X corresponds to carbon or nitrogen.³⁻⁷ M layers are closely arranged with X atoms lining amidst the octahedral sites and the $M_{n+1}X_n$ layers are sandwiched between layers comprising of A atoms. The M-X bonds are found to be remarkably strong, possessing a mixture of covalent, and ionic characteristics, while M-A bonds are of a weaker nature and possess metallic characteristics.^{1,8} As a consequence of weaker M-A bonds, it is viable to selectively remove ‘A’ layers from MAX phases via heat treatment under vacuum conditions or in molten salts or via chemical etching with hydrofluoric acid treatment. The selective etching of ‘A’ layers yields $M_{n+1}X_nT_x$ layers (MXenes), whereby T_x are surface terminating moieties such as oxygen, hydroxyl or fluorine groups.⁹⁻¹³ To date, more than 60 distinct types of pure MAX phases and 20 types of MXenes have been discovered. MXenes uniquely integrate the metallic-like conductivity inherited from their parent MAX phases and the hydrophilic nature of their surface terminating moieties. Due to the unique mechanical, chemical, physical and electrical properties presented by MXenes, a vast amount of research has been conducted on MXenes to explore their utilities. MXenes show promising applications in numerous fields, such as transparent conductive films, supercapacitors, catalysts, electromagnetic interface shield, Li-ion batteries and sensors.^{1,10,14-21}

Diabetes mellitus (DM) is listed among one of the most critical health challenges faced globally and is deemed as one of the foremost reasons causing mortality and disability throughout the

globe.²²⁻²⁴ The prevalence of DM is rapidly on the rise, whereby the number of people with DM was projected to rise from approximately 347 million people in 2013 to 552 million by 2030.²⁵ DM is a metabolic disorder that arises as a result of insulin inadequacy and hyperglycemia, and is indicated by blood glucose concentrations larger than the conventional range of approximately 3.9–6.2 mM on an empty stomach or 3.9–7.8 mM following food consumption.^{23,26} The quantitative monitoring of glucose concentration in blood is cardinal for the diagnosis and management of DM, which can significantly reduce micro and macrovascular complications such as nephropathy, cardiovascular diseases and retinopathy.^{24,27,28} As a consequence, enormous effort has been devoted to developing glucose-measuring devices. Over the past decades, biosensor technology has seen numerous breakthroughs and plays a crucial role as a powerful analytical tool in the medical field. Glucose biosensors have been dominating the biosensor market and account for approximately 85% of the market share. Today, a vast majority of the commercially available glucose biosensors belong to the electrochemical class, because of their numerous advantages such as high sensitivity, ability to give a fast response, low cost of operation and easy maintenance.^{22,24,26,29,30}

Direct electron transfer (DET) amidst enzymes and electrodes is cardinal for the construction of electrochemical biosensors. However, electroactive centres are often deeply embedded within the protein core of enzymes, making it challenging for proteins to accomplish DET processes on the bare electrodes. To better facilitate the electrical contact amidst redox centres of glucose oxidase (GOx) and electrode surfaces and accelerate the transfer of electrons, various novel nanomaterials have been utilised for GOx immobilisation.^{26,31-34} MXenes possess numerous distinct properties, for instance, large specific surface area and outstanding electrical conductivity, therefore MXenes could possibly be introduced as an efficient pathway to facilitate DET. Herein, the thesis aspires to investigate the potential of incorporating MXenes

towards the development of 2nd generation electrochemical glucose biosensors. The heterogeneous electron transfer (HET) capabilities of bulk Ti_3AlC_2 and Ti_3C_2 synthesised via HF etching ($\text{Ti}_3\text{C}_2\text{-HF}$) and subsequent delamination by tetrabutylammonium hydroxide, TBAOH ($\text{Ti}_3\text{C}_2\text{-HF/TBA}$) were investigated and the superior material was then utilised to develop a glucose biosensor. Fabrication of the glucose biosensor was accomplished by pipetting MXene materials onto the surface of the glassy carbon (GC) electrode, then glucose oxidase (GOx) and lastly cross-linking with glutaraldehyde (GTA) to yield the biosensor. The as-synthesised MXene-based biosensor allows selective, sensitive and fast amperometric detection of glucose, as portrayed in Figure 3.1.

Figure 3.1. Schematic representation depicting the (a) exfoliation of Ti_3AlC_2 to generate $\text{Ti}_3\text{C}_2\text{-HF}$ via etching with HF and (b) delamination of $\text{Ti}_3\text{C}_2\text{-HF}$ with TBAOH to yield $\text{Ti}_3\text{C}_2\text{-HF/TBA}$. Construction of the proposed glucose biosensing system by (c) modifying the surface of GC electrode with $\text{Ti}_3\text{C}_2\text{-HF/TBA}$, (d) followed by depositing glucose oxidase (GOx), and (e) subsequently cross-linking glutaraldehyde (GTA) with GOx; (f) glucose detection mechanism of the constructed biosensing system.

3.2 Results and Discussion

Prior to the synthesis of the desired Ti_3C_2 , Ti_3AlC_2 MAX phase was first synthesised by a molten salt process with Ti, Al and graphite in a stoichiometric molar ratio of 3:1:2 in KBr melt. The as-prepared Ti_3AlC_2 was then utilised to prepare MXenes via two routes; (1) etching with 40% hydrofluoric acid (HF) (2) etching with 40% HF followed by delamination with 40% TBAOH, termed $\text{Ti}_3\text{C}_2\text{-HF}$ and $\text{Ti}_3\text{C}_2\text{-HF/TBA}$ respectively. The physical and chemical properties of the as-prepared MXene before etching, after etching and after delamination were examined via transmission electron microscopy (TEM), energy X-ray dispersive spectroscopy (EDX), atomic force microscopy (AFM), X-ray diffraction spectroscopy (XRD), Raman and X-ray photoelectron spectroscopy (XPS).

TEM was first utilised to investigate the morphological differences induced by the exfoliation and delamination processes, and the data are illustrated in Figure 3.2. Through TEM data in Figure 3.2a, it is inferred that the MAX phase displays structural features typical of bulk materials and manifest as micron-sized flakes, while SAED and HRTEM studies revealed the hexagonal symmetry possessed by the material. TEM images of the exfoliated $\text{Ti}_3\text{C}_2\text{-HF}$ in Figure 3.2b show an expanded structure due to the etching of aluminium while SAED and HRTEM images show that the hexagonal symmetry of the materials is still retained despite undergoing the etching process with hydrofluoric acid. Meanwhile, the delamination of $\text{Ti}_3\text{C}_2\text{-HF}$ with TBAOH led to the formation of single- and few-layered MXene ($\text{Ti}_3\text{C}_2\text{-HF/TBA}$) while retaining the hexagonal symmetry of the structure.

Figure 3.2. TEM images with corresponding SAED images and high-resolution TEM images of (a) bulk Ti_3AlC_2 , (b) exfoliated $\text{Ti}_3\text{C}_2\text{-HF}$ and (c) delaminated $\text{Ti}_3\text{C}_2\text{-HF/TBA}$.

Next, the study moved on to ascertain the types of elements present in the materials and their distribution via the use of EDX. Through the EDX elemental maps in Figure 3.3, it was observed that Ti, C and Al corresponding to the composition of the starting MAX phase are homogeneously distributed throughout the material. Subsequent exfoliation and delamination of the bulk MAX phase led to the removal of aluminium as depicted in Figure 3.3b and 3.3c. The selective etching of reactive Al layers with hydrofluoric acid also introduces surface terminating functional group containing oxygen and fluorine into the MXenes.

Figure 3.3. TEM images with corresponding EDX elemental distribution maps of (a) bulk Ti_3AlC_2 , (b) exfoliated $\text{Ti}_3\text{C}_2\text{-HF}$ and (c) delaminated $\text{Ti}_3\text{C}_2\text{-HF/TBA}$. Scale bars denote 1 μm for Ti_3AlC_2 and $\text{Ti}_3\text{C}_2\text{-HF/TBA}$ and 500 nm for $\text{Ti}_3\text{C}_2\text{-HF}$.

AFM measurements were conducted to study the thickness of the Ti_3AlC_2 MAX phase, $\text{Ti}_3\text{C}_2\text{-HF}$ and $\text{Ti}_3\text{C}_2\text{-HF/TBA}$. AFM data (refer to Supporting Information Figure 3.S1) suggest that the thickness of Ti_3AlC_2 ranges from 1 to 2 μm , confirming its bulk state. Meanwhile, $\text{Ti}_3\text{C}_2\text{-HF}$ and $\text{Ti}_3\text{C}_2\text{-HF/TBA}$ were measured to possess an average thickness range of ca. 5-6 nm and 1-3 nm respectively, suggesting the successful etching and subsequent delamination of the Ti_3AlC_2 MAX phase.

In addition, XRD and Raman spectroscopy were also performed to determine the extent of exfoliation and are illustrated in Figure 3.S2 and 3.S3 of the Supporting Information respectively. As depicted in Figure 3.S2a, the XRD pattern obtained for bulk Ti_3AlC_2 correlates well with the data recorded in literature,³⁵ with peaks at 2θ values of 9.5° , 19.1° , 34° , 36.8° , 38.9° , 41.7° , 44.9° , 48.4° , 52.3° , 56.4° and 60.1° , which have been assigned to the (002), (004), (101), (103), (104), (105), (106), (107), (108) and (110) planes respectively. Upon treatment with 40% HF, there is a broadening of peaks and a decrease in peak intensities (refer to

Supporting Information Figure 3.S2b), which arises due to a decrease in the crystallinity and structural order of the material. Furthermore, the absence of non-basal peaks especially (104) peak at 38.9° , indicates the successful etching of aluminium atoms from the bulk Ti_3AlC_2 . In addition, a shift of the (002) and (004) peaks towards lower angles were also observed. The phenomenon arises due to an increase in d-spacing originating from the structural expansion during etching and removal of the Al atoms and substitution of Al atoms with functional groups containing oxygen or fluoride.³⁶

The subsequent treatment of $\text{Ti}_3\text{C}_2\text{-HF}$ with TBAOH led to delamination which resulted in a shift of the characteristic (002) peak from 9.2° to 6.2° 2θ and the peak at 27.6° to a higher 2θ angle of 30.7° , indicating the introduction of TBAOH atoms in the lattice of Ti_3C_2 and potential substitution of the functional groups mentioned above (Figure 3.S2c). The significant shift of the (002) peak also indicates a much larger d-spacing.

The Raman spectra of Ti_3AlC_2 depicted in Figure 3.S3a, revealed its characteristic vibrational modes at 154, 262, 409 and 603 cm^{-1} , which coincides well with literature. The exfoliation of the bulk Ti_3AlC_2 with HF results in an intense peak at 144 cm^{-1} along with three smaller peaks at 403, 513 and 628 cm^{-1} . These peaks are characteristic vibrational modes for anatase TiO_2 formed by MXene partial oxidation (Figure 3.S3b). The treatment with HF causes a substitution of Al atoms by OH^- groups based on the following reactions:^{35,37}

Meanwhile the treatment of $\text{Ti}_3\text{C}_2\text{-HF}$ with TBAOH for an hour, causes the peak at 144 cm^{-1} to decrease drastically and the three peaks between 400 and 700 cm^{-1} almost vanish (Figure 3.S3c). These results suggest that the delamination procedure causes anatase nanocrystals to be removed from exfoliated MXene surface.

Furthermore, XPS was executed to assess the surface compositions of the bulk and exfoliated Ti_3AlC_2 . Wide survey XPS spectra (refer to Supporting Information, Figure 3.S4) affirmed the presence of the anticipated elements originating from the MAX compound (Ti, Al, C), while the presence of additional fluorine in $\text{Ti}_3\text{C}_2\text{-HF}$ and $\text{Ti}_3\text{C}_2\text{-HF/TBA}$ arises as a result of the exfoliation of the MAX phase with HF. Meanwhile, the atomic percentages of the elements present in the bulk Ti_3AlC_2 , exfoliated and delaminated MXenes determined by XPS analysis are outlined in Table 3.S1.

Analysis of the high-resolution XPS spectra (Figure 3.4) revealed the presence of a well-defined Ti 2p signal which is split into two binding energies of Ti $2p_{3/2}$ and Ti $2p_{1/2}$. Deconvolution of the Ti 2p peaks revealed that the dominant Ti peak for Ti_3AlC_2 etched with HF belongs to titanium oxide while a significant decrease in the percentage of titanium oxide and an increase in the titanium carbide percentage were observed for the delaminated product (Table 3.S2). This is a further indication of the removal of anatase nanocrystals due to the delamination procedure. A comparison of Al 2s high-resolution XPS spectra is also presented (see Supporting Information, Figure 3.S5) to demonstrate the successful removal of Al atoms via the exfoliation and subsequent delamination of the material.

Figure 3.4. High-resolution XPS spectra of (a) bulk Ti_3AlC_2 , (b) exfoliated $\text{Ti}_3\text{C}_2\text{-HF}$ and (c) delaminated $\text{Ti}_3\text{C}_2\text{-HF/TBA}$.

Prior to the fabrication of the glucose biosensor, it is of utmost importance to assess the electrochemical performances of Ti_3AlC_2 and MXenes ($\text{Ti}_3\text{C}_2\text{-HF}$ and $\text{Ti}_3\text{C}_2\text{-HF/TBA}$) to evaluate any disparities in sensitivity and response towards glucose that might arise as a result of their distinct intrinsic properties. The HET rate is considered an important electrocatalytic

parameter that determines the suitability of materials for sensing applications. HET rate is commonly associated with the peak-to-peak separation (ΔE_p), whereby small potential differences between the anodic and cathodic peaks generally equate to fast HET rates. Typically, materials with fast intrinsic HET rate are preferred to be used as electrode materials as they efficiently lessen the overpotentials needed for electrochemical reactions to occur.³⁸ As such, cyclic voltammetry was performed with a widely used ferro/ferricyanide redox probe to determine any differences in the heterogeneous electron transfer (HET) capabilities of the materials. As depicted in Figure 3.5a, all materials have smaller ΔE_p as compared to GC and the ΔE_p is ranked as follows: $\text{Ti}_3\text{C}_2\text{-HF/TBA} < \text{Ti}_3\text{AlC}_2 < \text{Ti}_3\text{C}_2\text{-HF}$. The data suggest that all three materials have HET rates that are faster than GC and $\text{Ti}_3\text{C}_2\text{-HF/TBA}$, with the narrowest ΔE_p , would have the fastest HET rate and would thus present superior electrocatalytic performance for sensing devices.

Figure 3.5. (a) Cyclic voltammograms of Ti_3AlC_2 , $\text{Ti}_3\text{C}_2\text{-HF}$ and $\text{Ti}_3\text{C}_2\text{-HF/TBA}$ versus bare GC electrode and the inset illustrates the peak separations for the probe. Measurements were performed in 0.1 M KCl supporting electrolyte supplemented with 5 mM $[\text{Fe}(\text{CN})_6]^{4-/3-}$ at 50

mV s⁻¹ scan rate. (b) Chronoamperometry data of the second-generation electrochemical glucose biosensors predicated on Ti₃AlC₂, Ti₃C₂-HF and Ti₃C₂-HF/TBA where 50 µM of glucose was added every 200 s for 1000 s followed by 500 µM addition at 200 s intervals for another 1000 s. Chronoamperometry measurements were conducted at 0.15 V in PBS (pH 7.2) supplemented with 2 mM of FcMeOH. (c) Chronoamperometry data of the different electrode configurations of the proposed second-generation electrochemical glucose biosensor, where 50 µM of glucose was added every 200 s for 1000 s followed by 500 µM addition at 200 s intervals for another 1000 s. Chronoamperometry measurements were conducted at 0.15 V in PBS (pH 7.2) supplemented with 2 mM of FcMeOH. (d) Chronoamperometry data obtained from the interference studies of Ti₃C₂-HF/TBA biosensors, whereby successive addition of 5 mM glucose, 0.2 mM L-ascorbic acid, 0.2 mM dopamine hydrochloride, 0.2 mM uric acid and 5 mM glucose occurs at every 300 s. The measurement was conducted in pH 7.2 PBS supplemented with 2 mM FcMeOH at 0.15 V potential.

Upon determining the HET performances, second-generation titanium carbide-based glucose biosensors are being fabricated and their response behaviours towards glucose detection are being assessed. In second-generation glucose biosensors, oxygen is replaced with non-physiological mediators, which allows an enhancement in the transfer of electrons to the electrode surface as FcMeOH is sufficiently minute to access the thoroughly embedded flavin adenine dinucleotide (FAD) redox centre. The glucose detection mechanism of a typical second-generation biosensor is initiated via an electron transfer from glucose to the catalytic FAD core of GOx (eq 1). Subsequently, FAD-GOx is regenerated via the intervention of ferrocenium ion (Fc⁺MeOH) as the mediator (eq 2), to generate FcMeOH, and lastly, an oxidation signal corresponding to the glucose concentration is generated when FcMeOH oxidises at the electrode to regenerate the mediator Fc⁺MeOH (eq 3).^{24,26,39,40}

Upon determining the inherent HET capabilities of the materials, we moved on to explore the feasibility of the suggested biosensing system to detect glucose and examine the performances of Ti_3AlC_2 and MXenes ($\text{Ti}_3\text{C}_2\text{-HF}$ and $\text{Ti}_3\text{C}_2\text{-HF/TBA}$) towards glucose detection. To evaluate the response and determine differences in the sensitivity of the glucose biosensing system fabricated using Ti_3AlC_2 and MXenes, chronoamperometric measurements were carried out with sequential addition of glucose. From Figure 3.5b, the glucose detection responses amongst the materials compared are ostensibly distinct, whereby $\text{Ti}_3\text{C}_2\text{-HF/TBA}$ yielded the largest response, accompanied by Ti_3AlC_2 then $\text{Ti}_3\text{C}_2\text{-HF}$. The chronoamperometry data trend is in good agreement with the HET study whereby $\text{Ti}_3\text{C}_2\text{-HF/TBA}$ indeed yields the finest electrocatalytic performance for glucose sensing among the three materials compared. One plausible reason that could account for the superior performance of $\text{Ti}_3\text{C}_2\text{-HF/TBA}$, could be ascribed to the thinness and huge surface area of the materials. Delamination of $\text{Ti}_3\text{C}_2\text{-HF}$ with TBAOH led to the formation of single- and few-layered $\text{Ti}_3\text{C}_2\text{-HF/TBA}$, which decreases the distance between the enzyme and the electrode as compared to the bulk and exfoliated counterparts, thus allowing efficient electron transfer amidst the working electrode and enzyme. Furthermore, the larger surface area to volume ratio provides more active surface area for protein binding and entrapment of substrates, thus permitting the access of a large quantity and homogeneous distribution of enzymes to be immobilised on the nanosheet.⁸ Additionally, MXenes inherit the metallic nature of their parent MAX phases which endows them with excellent conductivity. The delamination process causes the removal of surface terminating

groups on the MXene which further enhances the electrical conductivity of the MXene material and thus better facilitating the electron transfer process amidst the electrode and the enzyme.^{8,11,41} Motivated by the excellent electrochemical properties of $\text{Ti}_3\text{C}_2\text{-HF/TBA}$, the material was selected to develop a second-generation glucose biosensor for subsequent studies.

In order to yield a comprehensive understanding and verify the roles of each respective component, individual biosensor component was examined via chronoamperometry measurements through sequential introduction of glucose to the different modified layers. Chronoamperometric responses were recorded and depicted in Figure 3.5c. The significance of enzyme GOx as the biorecognition element of glucose is evident, as it triggers the detection mechanism and generates distinct responses after injection of glucose (green curve) and along with $\text{Ti}_3\text{C}_2\text{-HF/TBA}$, GOx signal increases even more significantly (blue curve) when glucose was introduced. Very often, the sensing capability of amperometric glucose biosensors is limited by the electron transfer process amidst GOx active sites and the electrode surfaces. Due to the thick layer of protein enveloping the FAD redox center, an inherent barrier towards direct electron transfer is being introduced and hence GOx is unable to transfer electrons directly to traditional GC electrodes. As such, several innovative measures including the use of nanomaterials with extraordinary electrical property and large specific surface area have been looked into to construct and enhance the electrical contact amidst the FAD core of GOx and electrode surfaces.^{23,37} MXenes have been reported to have excellent conductivity and its huge surface area significantly increases the active surface area for proteins to bind onto and substrate entrapment.³³ As such, $\text{Ti}_3\text{C}_2\text{-HF/TBA}$ could have possibly aid in ameliorating the sensitivity and response signal of the glucose sensing platform, hence enhancing the signal. The performance of the biosensor is further enhanced upon the addition of glutaraldehyde (red curve) as glutaraldehyde aids in the cross-linking of matrixes, helping GOx to maintain its

structural rigidity, entrapping and stabilising the $\text{Ti}_3\text{C}_2\text{-HF/TBA}$ and GOx layers on the GC electrode.

Having verified the feasibility of the enzymatic glucose biosensor platform, the study moved on to examine if commonly found interferences would cause any probable signal induction on the proposed system to determine its selectivity. Chronoamperometric analysis was carried out with the following interferences; 0.2 mM of L-ascorbic, dopamine hydrochloride and uric acid. These compounds were selected as they are commonly present in blood and are electrochemically active.^{29,41-43} As depicted in Figure 3.5d, the addition of interfering compounds did not give rise to significant current changes, while well-defined responses were recorded with additions of glucose, validating the use of the low working potential. This exemplary selectivity can be attributed to the fact that the biosensing system likely reacts preferentially with glucose rather than other compounds. Utilising the information obtained, we next set forth to perform subsequent chronoamperometry measurements at 0.15 V.

Upon optimising the parameters, the analytical response of the biosensing system was then evaluated via chronoamperometry, whereby distinct concentrations of glucose were sequentially introduced and continually stirred into PBS (pH 7.2) supplemented with 2 mM of FcMeOH. As depicted in Figure 3.6a, increasing the concentration of glucose causes an increase in oxidation current. Meanwhile, the linear plot for glucose concentrations between 50 to 27,750 μM is displayed in Figure 3.6b, where it shows that the $\text{Ti}_3\text{C}_2\text{-HF/TBA}$ -based biosensing system detects glucose over two linear ranges, 50–250 μM ($r = 0.997$) and 750–27,750 μM ($r = 0.997$). Utilising the approach defined by IUPAC,⁴⁴⁻⁴⁶ the limit of detection (LOD) was computed by multiplying the ratio between the standard deviation of the chronoamperometric signal obtained from the lowest concentration of glucose and the gradient

attained from the calibration plot by a factor of 3, while the limit of quantification (LOQ) is acquired by multiplying with a factor of 10. The biosensor possesses a low LOD and LOQ of 23.0 and 76.8 μM respectively, demonstrating its high sensitivity towards glucose detection.

Figure 3.6. Chronoamperometry curves (a) and calibration data (b) of $\text{Ti}_3\text{C}_2\text{-HF/TBA}$ -based electrochemical glucose biosensor. Data were attained using 2 mM of FcMeOH in pH 7.2 PBS (electrolyte) at a constant potential of 0.15 V.

Lastly, the proposed MXene-based glucose biosensor was examined with real samples to determine its feasibility for biomedical and food applications. Human serum containing 3.33–7.78 mM of glucose was acquired from Sigma-Aldrich,⁴⁷ introduced to the electrolyte solution and the response generated was recorded. The glucose concentration of the serum was inferred from the calibration plot and calculated to be 1.83 ± 0.27 mM ($n = 3$). Recovery experiments were also performed to illustrate the practicality of using the fabricated biosensors with real samples for food analysis. A recovery percentage of 95.2 % was attained for glucose concentration of 500 μM in a sugar-free drink bought from a local grocer. These results attained imply that the fabricated glucose biosensing system could possibly be applied for real samples testing.

To yield a more comprehensive understanding of the proposed glucose biosensor performance, the analytical parameters of various 2D materials-based biosensors are compared and summarised in Table 1. Gold nanoparticles have demonstrated unique abilities to provide enzymes with suitable microenvironments to retain their biological activity during immobilisation processes and further promote the transfer of electrons amidst the redox center of the immobilised biocatalyst and the electrode substrates.⁴⁸⁻⁵⁰ As such pre-treatments of layered materials with Au nanoparticles were commonly reported for 2D materials-based electrochemical glucose biosensors (Table 3.1), in the hope of improving the sensing performance of the biosensors, yielding lower detection limits. Though many of these biosensors yielded high sensitivity, some of their linear ranges fall out of the levels commonly reported in physiological fluids. In this work, we were able to present a second-generation glucose biosensor based simply upon pristine MXene ($\text{Ti}_3\text{C}_2\text{-HF/TBA}$), with performances being on par with or even better than several of the best performing sensors published, without needing to pre-treat MXene with Au nanoparticles. The delaminated MXene possesses extraordinary electrochemical properties which greatly promotes the electron transfer process amidst GOx and the working electrode, endowing the biosensor with excellent sensing performance and high sensitivity, outperforming biosensor based simply upon transition metal dichalcogenides. Our proposed biosensing system offers linear ranges spanning over different magnitudes of glucose concentration and low LOD, proffering flexibility for the creation of future electrochemical biosensors with high sensitivity for an array of applications, spanning from biomedical to food analysis.

Table 3.1. Comparison of the analytical performances of GC/Ti₃C₂-HF/TBA/GO_x/GTA glucose biosensor with other electrochemical glucose biosensors predicated on layered materials.

Electrode	Generation of Sensor	Linear Range (μM)	Detection Limit (μM)	Ref.
GC/Ti ₃ C ₂ -HF/TBA/GO _x /GTA	Second	50–250 750–27,750	23.7	This work
GC/MXene/Au/Nafion/GO _x	First	100-18,000	5.9	51
GC/WS ₂ /GO _x /GTA	Second	180–770 1300–7,600 7,600–22,300	82.6	29
GC/WSe ₂ /GO _x /GTA	Second	77–274 770 – 22,300	52.0	
GC/Graphene/AuNPs/GO _x	First	20 – 2,360	4.10	52
GC/MoS ₂ /AuNPs/GO _x	Second	250 – 13,200	0.042	53

3.3 Conclusion

Inspired by the huge specific surface area and outstanding electrical properties of MXenes, the thesis sets forth to investigate the feasibility of incorporating MXene into a second-generation electrochemical glucose biosensor. Herein, the study investigated the inherent HET capabilities of the MAX and MXene materials and found that Ti₃C₂-HF/TBA (produced from the etching of MAX phase with HF followed by subsequent delamination with TBAOH) gives rise to the

smallest ΔE_p , has the fastest HET rate and would present superior electrocatalytic performance for sensing devices. Motivated by the excellent electrochemical properties of $\text{Ti}_3\text{C}_2\text{-HF/TBA}$, the material was selected to develop a second-generation glucose biosensor. Chronoamperometric studies show that the proposed biosensing system exhibits high selectivity and outstanding electrocatalytic activity towards the detection of glucose, spanning over a wide linear range and possesses a low limit of detection of $23.0\ \mu\text{M}$. The findings reported in this study conceptually proves the probable applications of pristine MXenes for future developments of highly selective and sensitive electrochemical biosensors for biomedical and food sampling applications.

3.4 Experimental Section

3.4.1 Synthesis of Ti_3AlC_2

The MAX phase, Ti_3AlC_2 , was synthesised by mixing Ti, Al and graphite powders (Alfa Aesar) in a stoichiometric molar ratio of 3:1:2 with KBr in a weight ratio of 1:1. The 24 hours mixing process was carried out in a multidirectional mixer (Turbula, WAB, Switzerland), using ethanol as liquid media and 5 mm diameter zirconia milling balls. A steel die was then used to uniaxially pressed the dried powder mixture at a pressure of 200 MPa. The pressed powder was then subjected to further encapsulation with KBr and placed in an alumina crucible lined with KBr salt bed. The resulting mixture was heated at $1300\ ^\circ\text{C}$ for 60 minutes with a heating rate of $5\ ^\circ\text{C}/\text{min}$. Upon cooling, Ti_3AlC_2 was obtained by dissolving the reaction mixture in water and subsequent washing with hot water to eliminate the residual KBr salt content. Lastly, to prevent agglomeration, the Ti_3AlC_2 powder was rinsed in ethanol, dried overnight at $70\ ^\circ\text{C}$ in an oven and sieved through $63\ \mu\text{m}$ sieve.

3.4.2 Synthesis of MXenes (Ti₃C₂-HF and Ti₃C₂-HF/TBA)

Ti₃C₂-HF: 2g of Ti₃AlC₂ was immersed in 100 mL 40% HF solution (Sigma-Aldrich) and stirred continuously under ambient conditions for 7 days. After which, the samples were separated via repeated rounds of centrifugation and redispersion in water and subsequently via suction filtration. Thereafter, the etched sample was dried for a day in a vacuum oven set at 50 °C.

Ti₃C₂-HF/TBA: 1g of Ti₃C₂-HF was stirred continually with 50 mL of 40% TBAOH (Sigma-Aldrich) under ambient conditions for an hour. The sample was then ultrasonicated for 0.5 hours (12 L bath, 20 °C, 400 W), separated via suction filtration, accompanied by washing with deionised water. After which, the delaminated material was dried for a day in a vacuum oven set at 50 °C.

3.4.3 Characterisation of Ti₃AlC₂ and MXene Materials

EFTEM JEOL 2200 FS microscope was employed to acquire TEM images. Desired sample suspensions of 1 mg mL⁻¹ were drop cast onto TEM grids to yield the TEM samples. TEM-EDX spectra were attained via the use of Oxford Instruments' SDD detector X-MaxN 80 T S.

Raman spectra were obtained in backscattering geometry mode, utilising an inVia Raman microscope (Renishaw, England) coupled with a DPSS laser of 532 nm wavelength, a 50x objective lens and a charge-coupled device (CCD) detector. Preceding sample measurements, calibration procedures were accomplished using silicon references at 520 cm⁻¹ to attain peak position resolutions of less than 1 cm⁻¹.

XPS measurements were accomplished using a Phoibos 100 spectrometer equipped with a monochromatic magnesium X-ray irradiation source (SPECS, Germany). Both wide-scan and high-resolution spectra were collected for individual samples.

XRD measurements were performed via an X-ray powder diffractometer (Bruker D8 Discoverer) furnished with a CuK α radiation X-ray source ($\lambda = 0.15418$ nm, U = 40 kV, I = 40 mA) under Bragg–Brentano parafocusing geometry mode. The diffraction data were acquired throughout the angular range of 5° to 80° of 2 θ with 0.019° (2 θ) step sizes. The EVA software package was employed for XRD data analysis.

AFM measurements were executed via tapping mode with a Ntegra Spectra from NT-MDT. Cantilevers employed for all data acquisitions were fitted with a silicon tip that has a curvature radius below 10 nm and a strain constant of 1.5 kN.m⁻¹. Desired material suspensions (1 mg/mL) were drop cast onto mica substrates that were freshly cleaved to generate AFM samples. AFM measurements were performed at a scan rate of 1 Hz and scan line of 512 under ambient conditions and data were evaluated via the Gwyddion software.

3.4.4 Biosensor Preparation

Preceding each usage, the surfaces of GC electrodes were renewed via polishing using a series of alumina particle slurry (9 μ m, 5 μ m, 1 μ m, 0.3 μ m sequentially) on a polishing pad. After which, the electrodes were rinsed with ultrapure water then dried via dry polishing on a polishing pad.

Ti₃AlC₂ or MXenes were added to dimethylformamide to yield separate 5 mg mL⁻¹ suspensions and preceding each electrochemical measurement the suspension was ultrasonicated for 1 min

to make certain that the material of interest is homogeneous. 0.75 μL of material suspension was then pipetted on the electrode surface and dried under ambient conditions. Subsequently, 0.1 mg of GOx in ultrapure water (20 mg mL^{-1}) was pipetted onto the material modified electrode and left to dry under ambient conditions. Lastly, 1% v/v glutaraldehyde solution (5 μL) was pipetted onto the electrode surface to cross-link the GOx enzyme and dried for an hour in an oven set at 35 $^{\circ}\text{C}$. The final modified electrodes were denoted as GC/Ti₃AlC₂/GOx/GTA, GC/Ti₃C₂-HF/GOx/GTA and GC/Ti₃C₂-HF/TBA/GOx/GTA and were kept at 4 $^{\circ}\text{C}$ when not in use.

3.4.5 Electrochemical Measurements

Voltammetric and chronoamperometric measurements were conducted via an Eco Chemie $\mu\text{Autolab}$ type III electrochemical analyser linked to a laptop and the parameters were set up via the NOVA version 1.10 software. All measurements in the study were performed under ambient conditions via the use of a three-electrode configuration in a 10 mL electrochemical glass cell comprising of a modified working electrode, platinum auxiliary electrode, and Ag/AgCl reference electrode.

Heterogeneous electron transfer (HET) measurements were performed using cyclic voltammetry (CV) with potentials ranging from -0.2 to 0.5 V, a scan rate of 50 mV s^{-1} and 0.1 M KCl as supporting electrolyte for ferro/ferricyanide redox probe (5 mM). Chronoamperometry measurements ($\Delta t > 1$ ms) were carried out at a fixed potential of 0.15 V, where distinct concentrations of glucose were sequentially introduced into the ferrocenemethanol solution (FcMeOH, 2 mM) at every 200 s intervals and the solution was continually stirred across the measurements. The data attained were smoothed and subsequently administered with baseline correction for further analysis.

3.5 Supporting Information

Figure 3.S1. AFM images (left) and representative height profiles (right) of (a) bulk Ti_3AlC_2 , (b) exfoliated $\text{Ti}_3\text{C}_2\text{-HF}$ and (c) delaminated $\text{Ti}_3\text{C}_2\text{-HF/TBA}$.

Figure 3.S2. XRD patterns of (a) bulk Ti_3AlC_2 , (b) exfoliated $\text{Ti}_3\text{C}_2\text{-HF}$ and (c) delaminated $\text{Ti}_3\text{C}_2\text{-HF/TBA}$.

Figure 3.S3. Raman spectra of (a) bulk Ti_3AlC_2 , (b) exfoliated $\text{Ti}_3\text{C}_2\text{-HF}$ and (c) delaminated $\text{Ti}_3\text{C}_2\text{-HF/TBA}$.

Figure 3.S4. Wide survey XPS spectra of (a) bulk Ti_3AlC_2 , (b) exfoliated Ti_3C_2 -HF and (c) delaminated Ti_3C_2 -HF/TBA.

Table 3.S1. Atomic percentages of elements in bulk Ti_3AlC_2 , exfoliated and delaminated Ti_3C_2 determined by XPS analysis.

Compound	C	O	F	Ti	Al
Ti_3AlC_2	43.50	31.04	N/A	9.19	16.27
$\text{Ti}_3\text{C}_2\text{-HF}$	36.94	9.00	3.75	42.49	7.81
$\text{Ti}_3\text{C}_2\text{-HF/TBA}$	41.30	8.61	1.06	47.19	1.83

Table 3.S2. Atomic percentages of deconvoluted titanium peaks from high-resolution Ti 2p XPS spectra of bulk Ti_3AlC_2 , etched $\text{Ti}_3\text{C}_2\text{-HF}$ and delaminated $\text{Ti}_3\text{C}_2\text{-HF/TBA}$.

	Ti (0)	Ti-O	Ti-C
Ti_3AlC_2	18.81	36.41	44.78
$\text{Ti}_3\text{C}_2\text{ - HF}$	45.58	30.02	24.40
$\text{Ti}_3\text{C}_2\text{ - HF/TBA}$	42.55	2.69	54.75

Figure 3.S5. Comparison of high-resolution Al 2p XPS spectrum between (a) bulk Ti_3AlC_2 and (b) delaminated $\text{Ti}_3\text{C}_2\text{-HF/TBA}$.

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Part I

Chapter 4

Functionalised Germernene and Silicene

Enzymatic Systems

4.1 Introduction

4.2 Results and Discussion

4.3 Conclusion

4.4 Experimental Section

4.5 Supporting Information

4.1 Introduction

The discovery of the wonder material, graphene, has sparked immense zeal amongst the scientific community, paving way for the discovery of various layered materials, in particular mono elemental layered materials. Down Group 14, graphene analogues of silicene and germanene have been studied extensively in very recent years.¹⁻⁴ However, these materials are plagued with a multitude of disadvantageous properties such as zero band gap, poor thermodynamic stability and are prone to oxidation, limiting their applications. As such, numerous functionalisations have been performed on silicene and germanene to modify their physical, chemical and electronic properties, thus improving their stability and widening their band gaps. Some of these modified analogues include germanane (Ge_6H_6), methyl germanane ($\text{Ge}_6(\text{CH}_3)_6$) and siloxene ($\text{Si}_6\text{H}_3(\text{OH})_3$).^{2,4-9}

Germanane and methyl germanane can be easily synthesised via topotactical deintercalation of the layered Zintl phase calcium germanide (CaGe_2) with aqueous acid or methyl halide respectively. Germanane and methyl germanane possess buckled honeycomb structures that are analogous to sp^3 hybridised graphane where every germanium atom is covalently functionalised with either $-\text{H}$ or $-\text{CH}_3$, above and below the layer.¹⁰⁻¹³ Hydrogenation of germanene leads to band gap opening, causing germanane to possess a direct band gap of approximately 1.6 eV. Furthermore, germanane possesses high electron mobility of circa $18\,000\text{ cm}^2\text{ V}^{-1}\text{ s}^{-1}$ and is highly resistant to oxidation.^{7,11,14-16} Replacing the hydrogen terminations in Ge_6H_6 with methyl functional groups increases the direct band gap to approximately 1.7 eV and improves the thermal stability significantly where $\text{Ge}_6(\text{CH}_3)_6$ is thermally stable up till $250\text{ }^\circ\text{C}$ as opposed to $75\text{ }^\circ\text{C}$ for Ge_6H_6 . Methyl germanane also exhibits remarkable photocatalytic activities, photoluminescence properties and band edge fluorescence.^{12,17,18} Siloxene can be synthesised via deintercalation of calcium ions from the

layered Zintl phase calcium silicide (CaSi_2) in the presence of aqueous acid, generating silicon layers that are terminated by hydroxyl groups on one side of the plane and hydrogen atoms on the other. Siloxene is a direct band gap semiconductor that possesses high specific capacitance (2.18 mF cm^{-2}) and energy density (9.82 mJ cm^{-2}) and exhibits strong photoluminescence.^{7,19-21} As a result of their fascinating properties, these emerging Group 14 graphane analogues have been applied to a myriad of fields including supercapacitors, photocatalysts, anodes in Li-ion batteries, field-effect transistors and sensors.^{7,16,18,20,22-25}

Detection of glucose is of paramount importance in many domains, such as clinical diagnostics, food industries and medicinal industries.^{26,27} One common and important example of glucose detection is the regular monitoring of blood glucose levels for patients diagnosed with diabetes mellitus (DM). DM arises as a consequence of insulin deficiency and hyperglycemia and is characterised by blood sugar level being greater or lower than the norm of 4.4-6.6 mM. DM is becoming more prevalent and has been deemed as one of the world's major health issues that causes fatality and disability.^{26,28-30} Therefore, it is of utmost clinical significance to quantitatively monitor glucose for the diagnosis and management of DM, which can notably diminish the risk of DM-induced diseases such as stroke, retinopathy, heart disease and nephropathy.^{26,28,31} Consequently, tremendous efforts have been dedicated to fabricating glucose-measuring devices. Biosensor technologies have seen multiple breakthroughs over the years and assume important roles, providing powerful analytical techniques in the medical field, with glucose sensors accounting for roughly 85% of the market. Electrochemical glucose biosensor dominates the market of commercially available glucose biosensors as a result of its countless benefits, such as high sensitivity, low operating costs, ease of use and maintenance.^{26,28-30,32,33}

Glucose oxidase (GOx) has been commonly utilised for the fabrication of glucose biosensor due to its commercial availability, high selectivity for glucose, low cost, high bioactivity and stability.²⁹ However, the electroactive redox centres of GOx are often reported to be deep-seated within the protein cores, physiologically hampering the electron transfer process amidst the flavin adenine dinucleotide (FAD) electroactive centre and surfaces of conventional electrodes. As such, numerous techniques such as the use of mediators and incorporation of nanomaterials have been devised to enhance the electrical contact amidst the FAD electroactive centres of GOx and electrode surfaces and facilitate the direct electron transfer (DET) process.^{26,28,29,34} Layered materials, in particular, hold promising potentials for the fabrication of electrochemical glucose biosensors as they present diverse beneficial physical, chemical, electronic, and optical attributes such as large specific surface areas, excellent electrical conductivity, abundance of catalytic sites, ease of functionalisation and tuneable electronic structures.³⁵⁻³⁸ Although it has been reported that Group 14 graphane analogues are suitable candidates for the construction of biosensors, they are mainly based upon computational studies, not much experimental studies have been conducted.^{39,40} Herein, this chapter aims to experimentally investigate the feasibility of using germanane- and siloxene-based materials towards the construction of second-generation electrochemical glucose biosensors. The heterogeneous electron transfer (HET) kinetics of the as-synthesised germanane, methyl germanane and siloxene were first examined and the most outstanding material was then employed to construct the electrochemical glucose biosensor. The glucose biosensor was constructed by depositing the Group 14 graphane analogues onto the glassy carbon (GC) electrodes, accompanied by GOx and lastly glutaraldehyde (GTA). The fabricated glucose biosensor was also utilised to distinguish glucose in human serum, demonstrating the viability of the sensing platform for real sample analysis.

4.2 Results and Discussion

Germanane, methyl germanane and siloxene were synthesised by deintercalation of their Zintl phase precursors with either hydrochloric acid or methyl iodide and subjected to an array of characterisation techniques including scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDX) and X-ray photoelectron spectroscopy (XPS). These Group 14 graphane analogues were also previously characterised by the group via atomic force microscopy, X-ray powder diffraction, high-resolution transmission electron microscopy and Fourier transformed infrared spectroscopy.⁷

SEM images in Figure 4.1 reveal micron-sized structures with closely stacked layers, suggesting that all Group 14 graphane analogues exist in the bulk form. Thereafter, EDX was employed to evaluate the elemental composition of the materials as well as their distribution. The elemental mappings illustrated in Figure 4.S1 indicates the homogeneous distribution of germanium throughout germanane, germanium and carbon in methyl germanane and silicon and oxygen for siloxene. EDX analysis of germanane also indicates the presence of carbon, which could be attributed to the substrate (carbon tape) that the materials were deposited on and the presence of atmospheric adventitious carbon.

Figure 4.1. SEM images of (a) germanane, (b) methyl germanane and (c) siloxene.

XPS was also implemented to determine the surface composition of the Group 14 graphane analogues, germanane, methyl germanane and siloxene. The existence of the anticipated elements Ge, C, Si and O corresponding to the respective germanane, methyl germanane and siloxene compounds were verified through XPS survey analysis (refer to Supporting Figure 4.S2). The presence of residual chlorine that originates from the hydrochloric acid used to deintercalate the Zintl phases was also observed in germanane while the additional iodine peak in the survey XPS spectrum of methyl germanane originates from methyl iodide used during the synthesis process. Deconvolution analysis of the high-resolution XPS (HR-XPS) of germanium in Figure 4.2a (germanane) and 4.2c (methyl germanane), reveal peaks at 30.1 and 31.1 eV which corresponds to Ge-H and Ge-C,^{7,41} suggesting the successful functionalisation of germanene with hydrogenated and methyl groups respectively. A greater shift in binding energy was observed for Ge-C as opposed to Ge-H as a result of the higher electronegativity of carbon.^{7,42} HR-XPS of carbon from germanium in Figure 4.2b reveals the presence of adventitious carbon only while the HR-XPS of carbon from methyl germanium in Figure 4.2d reveals an additional peak at ~281.7 eV, affirming the presence of Ge-C bond. HR-XPS of silicon (Figure 4.2e) from siloxene illustrates two peaks at 100.3 and 102.8 eV, corresponding to Si-H and Si-O while the HR-XPS of oxygen (Figure 4.2f) reveals a Si-O peak at 532.4 eV, affirming the successful functionalisation of siloxene.^{7,20,43}

Figure 4.2. HR-XPS spectra of (a) germanium, (b) carbon from germanane, (c) germanium, (d) carbon from methyl germanane, (e) silicon and (f) oxygen from siloxene.

Thereafter, cyclic voltammetry was employed to probe the innate HET properties of germanane, methyl germanane and siloxene. HET rate, a critical and useful electrocatalytic parameter, is often used to assess the suitability of the material of interest for electrochemical

applications, in particular for the construction of electrochemical sensors. Typically, a narrow peak-to-peak separation (ΔE_p) would suggest a fast HET rate and materials that exhibit intrinsically fast HET rates are favoured as they can reduce the overpotential needed for an electrochemical reaction to occur.⁴⁴⁻⁴⁶ To probe the HET properties of the Group 14 graphane analogues, cyclic voltammetry was carried out using the commonly used ferro/ferricyanide redox probe ($[\text{Fe}(\text{CN})_6]^{4-/3-}$). Figure 4.3a reveals that germanane and methyl germanane exhibit smaller ΔE_p in contrast to bare GC while siloxene possesses a wider ΔE_p compared to bare GC, and the ΔE_p of the materials is ranked as follows: $\text{Ge}_6\text{H}_6 < \text{Ge}_6\text{Me}_6 < \text{Bare GC} < \text{Si}_6\text{H}_3(\text{OH})_3$. This suggests that germanane and methyl germanane exhibit faster HET rates as compared to bare GC and would facilitate electron transfer while modifications of electrode with siloxene would hinder electron transfer at the surface of the working electrodes. The larger ΔE_p exhibited by siloxene could be attributed to the presence of hydroxyl moieties, which might electrostatically repel the negatively charged redox probe and hinder the electron transfer reaction.⁴⁷ Through the cyclic voltammetry study, germanane reveals the narrowest ΔE_p , suggesting that it possesses the fastest HET rate and would exhibit the finest electrocatalytic performance for sensing devices.

Figure 4.3. (a) Cyclic voltammograms of 5 mM ferro/ferricyanide redox probes ($[\text{Fe}(\text{CN})_6]^{4-/3-}$) in 0.1 M of KCl supporting electrolyte using germanane, methyl germanane and siloxene modified working electrodes. Cyclic voltammetry was performed at a scan rate of 50 mV s^{-1} .

(b) Chronoamperometry plots of the glucose oxidase-based electrochemical glucose biosensor constructed using germanane, methyl germanane and siloxene where 500 μM of glucose was successively introduced every 200 s. (c) Chronoamperometry curves of the various electrode configurations of the suggested electrochemical glucose biosensor with successive additions of 500 μM of glucose at 200 s intervals. Chronoamperometry studies were performed using PBS (pH 7.2) complemented with 2 mM of FcMeOH at 0.15 V.

After evaluating the HET performances, second-generation Group 14 graphane analogues-based glucose biosensors were constructed and chronoamperometry was utilised to yield a better understanding of the analytical responses of the differently configured biosensors towards glucose detection. In second-generation glucose biosensors, oxygen is superseded by synthetic mediators to reduce the strong dependence on ambient oxygen substrates. Using mediators instead of oxygen also helps to lower the high potentials required to reduce oxygen or oxidise hydrogen peroxide, thereby diminishing the interference effects induced by electroactive species, namely ascorbic and uric acid.^{28,29} Mediators are redox active compounds that are typically small and soluble and are competent of undergoing fast and reversible redox reactions, facilitating electron transfers amidst the redox centres of enzymes and surfaces of the working electrodes. Examples of non-physiological mediators include ferrocene analogues, ferrocyanide, tetrathiafulvalene, methylene blue and quinones.^{26,29} The glucose detection mechanism of a second-generation amperometric glucose biosensor typically involves three processes where electrons are first transferred from glucose to the FAD electroactive centres of GOx (Equation 1). Thereafter, electrons are transferred from the reduced FAD centres to the mediator, ferrocene methanol in this study (Equation 2) and lastly, the oxidised mediator is regenerated via the transfer of electrons from the synthetic mediator to the electrode's surface

(Equation 3), generating current signals that are proportional to glucose concentration.^{26,28,34,48,49}

Chronoamperometry was performed with successive additions of glucose to assess the analytical performances of the proposed germanane-, methyl germanane- and siloxene-based biosensing platforms towards the detection of glucose and the amperometric curves are illustrated in Figure 4.3b. The glucose sensing responses amidst the compared materials are apparently disparate, with germanane recording the greatest current response followed by methyl germanane then siloxene. The data obtained from the chronoamperometry studies corroborates well with the HET study where germanane gave rise to the finest electrocatalytic performance while siloxene exhibits the poorest electrocatalytic capabilities towards glucose detection. One conceivable reason for the excellent sensing capabilities of germanane could be due to the high carrier mobility possessed by germanane ($\sim 18\,000\text{ cm}^2\text{ V}^{-1}\text{ s}^{-1}$),¹¹ thus greatly enhancing the DET process amidst the redox centre of GOx and the working electrode. Furthermore, previous study has demonstrated that the germanane materials manifest as micron size flakes with greater lateral dimension and are thinner as compared to methyl germanane and siloxene,⁷ suggesting that germanane would possess a larger surface area to volume ratio. This would in turn provide a larger surface area for binding of enzymes and trapping of substrates, thereby allowing larger amounts of enzymes to be homogeneously immobilised on the germanane. Additionally, germanane possessing a

smaller thickness as opposed to methyl germanane and siloxene would suggest a shorter distance amidst the electroactive centre of GOx and the surface of the working, allowing DET to take place more efficiently amidst the electrode and enzyme.

To generate a thorough comprehension of the roles played by the individual components and the synergistic effects of combining distinct components in the biosensing system, chronoamperometry measurements were performed with successive additions of glucose using differently configured biosensing electrodes. From Figure 4.3c, it can be observed that the introduction of glucose to the germanane and GTA modified electrode (orange curve) results in nominal increment in current response, thus verifying the absence of the enzyme's catalytic activity. Modification of electrodes with GOx enzyme results in distinct increments in signal currents upon the introduction of glucose, authenticating the importance of GOx in the biosensing system as GOx is required to induce the glucose detection mechanism. Meanwhile, the introduction of germanane to the biosensing platform elicits significant increment in current responses with successive additions of glucose. The electroactive redox centres GOx are often reported to be deep-seated within the protein cores, physiologically hampering the electron transfer process amidst the FAD electroactive centres and surfaces of conventional electrodes. As such, numerous classes of emerging layered materials with remarkable electrical properties and high specific surface area have been employed for GOx immobilisation, in the hope of improving the electrical contact amongst the FAD electroactive centres of GOx and electrode surfaces, thus facilitating the DET process. Germanane has been accounted to possess high carrier mobility, which might greatly enhance the electron transfer process. Furthermore, the large surface area of germanane could have possibly provided a larger surface area for binding of enzymes and trapping of substrates, thereby allowing larger amounts of enzymes to be homogeneously immobilised, enhancing the sensitivity of the glucose sensing platform. The

biosensing platforms were also modified with GTA which functions as a cross-linking matrix, helping GOx to preserve its structural rigidity and to entrap and stabilise the germanane and GOx layers on the surface of the working electrode.^{32,33,50} The importance of GTA is evident in this study, where germanane and GOx were observed to detach from the working electrode during chronoamperometric measurements in the absence of GTA, resulting in a much smaller current response (grey curve) as compared to the biosensing system modified with GTA (purple curve).

Having validated the viability of the electrochemical glucose biosensing platform, the study moved on to investigate the analytical parameters of the fabricated biosensor via chronoamperometric studies with successive introduction of glucose into PBS (pH 7.2) supplemented with 2mM of FcMeOH at 0.15 V under continuous stirring conditions. The chronoamperometric data in Figure 4.4a reveals an increase in current response with increasing concentrations of glucose while Figure 4.4b illustrates the calibration curve of the current response attained against 50 to 27,750 μM of glucose for the fabricated biosensing system. Through Figure 4.4b, it can be observed that the germanane-based biosensing platform detects glucose over three linear ranges, 50-1750 μM ($r^2=0.991$), 1750-12750 μM ($r^2=0.993$) and 12750-27750 μM ($r^2=0.992$). The limit of detection (LOD) and limit of quantification (LOQ) were then computed using the method outline by IUPAC,^{51,52} and were determined to be 6.3 and 26.5 μM respectively. The low LOD of the biosensing system suggests that it is highly sensitive towards the detection of glucose.

Figure 4.4. (a) Chronoamperometric responses achieved by the germanane-based electrochemical glucose biosensor upon successive introduction of 50, 500 and 5000 μM of glucose into PBS electrolyte (pH 7.2) supplemented with FcMeOH (2 mM). Five successive additions of each glucose concentrations were introduced into the continually stirred PBS electrolyte and chronoamperometry was performed at 0.15 V. (b) Calibration curve illustrating the current response attained by the biosensor vs concentration of glucose.

Inspired by the excellent sensing capabilities of the germanane-based glucose biosensing platform, we proceeded to probe the viability of using the constructed biosensor for glucose detection in real samples. Chronoamperometry study was employed to investigate the interference effects of electrochemically active compounds that are commonly found in blood, namely L-ascorbic acid (AA), dopamine hydrochloride (DA) and uric acid (UA). The chronoamperometric curve (Figure 4.5) reveals the current response of the proposed biosensing platform with sequential introduction of physiological amounts of glucose (5 mM), the above-mentioned interferences (0.2 mM) and glucose (5 mM).^{32,53} It can be observed from Figure 4.5 that the introduction of the above-mentioned endogenous interference compounds result in insignificant changes in the oxidation current, while addition of glucose analyte elicits significant increase in oxidation current. This suggests that the biosensing system is highly

selective towards glucose where it reacts preferentially with glucose instead of other compounds.

Figure 4.5. Chronoamperometric curve illustrating the effects of interference analytes on germanane-based glucose biosensing platform. 5 mM glucose, 0.2 mM L-ascorbic acid, 0.2 mM dopamine hydrochloride, 0.2 mM uric acid and 5 mM glucose were successively injected into the PBS electrolyte (pH 7.2) supplemented with 2 mM FcMeOH and chronoamperometric measurements were performed at 0.15 V potential.

Lastly, this chapter investigated the practical applicability of the germanane-based biosensor towards glucose detection in real samples, using human serum procured from Sigma-Aldrich. Utilising the current response obtained, glucose concentration was determined to be 2.93 ± 0.82 mM ($n = 3$) from the calibration curve, falling slightly short of the manufacturer's specified range of 3.33–7.78 mM.⁵⁴

Moreover, a comparison of the analytical performances with previous works reported using other 2D materials-based biosensors (Table 4.1) reveals the superior analytical capability of the proposed GC/Ge₆H₆/GOx/GTA biosensor, where it displays one of the finest analytical

ranges and detection limits. The proposed biosensor outperforms works based upon pristine MXenes and transition metal dichalcogenides (namely WS₂ and WSe₂),^{32,55} where it possesses a much lower limit of detection. Layered materials have been commonly decorated with gold nanoparticles to augment the sensing capabilities of biosensors as gold nanoparticles have been reported to aid enzymes in retaining their biological activities during immobilisation processes and further facilitate the electron transfer process amidst the redox centre of the immobilised biorecognition element and the surface of electrodes.⁵⁶⁻⁵⁸ Herein, we were able to construct the biosensor with pristine germanane that possesses LOD that is comparable with works based upon gold-functionalised graphene and MXene,^{59,60} without needing to decorate with the scarce and costly noble metal, gold. Furthermore, the proposed biosensor possesses a much wider linear detection range as compared to many of the above-reported works. The wide linear detection range and low LOD of our proposed biosensing platform, showcase the potential of applying these Group 14 graphane analogues for prospective developments of highly selective and sensitive biosensors.

Table 4.1. Comparison of the analytical performances of glucose biosensors based upon various 2D materials.

Electrode	Linear Range (μM)	Limit of Detection (μM)	Ref.
GC/Ge ₆ H ₆ /TBA/GOx/GTA	50–1,750 1,750–12,750 12,750–27,750	6.3	This Work
GC/Ti ₃ C ₂ -HF/TBA/GOx/GTA	50–250 750–27,750	23.7	55

GC/WS ₂ /GO _x /GTA	180–770	82.6	32
	1,300–7,600		
	7,600–22,300		
GC/WSe ₂ /GO _x /GTA	77–274	52.0	
	770–22,300		
GC/Graphene/AuNPs/GO _x	20–2,360	4.10	59
GC/MXene/Au/Nafion/GO _x	100–18,000	5.9	60

4.3 Conclusion

Impelled by the remarkable electrochemical properties of Group 14 graphane analogues, as well as the computational achievements of using these analogues for sensing applications, this chapter sets forth to experimentally explore the feasibility of using germanane- and siloxene-based materials towards the construction of second-generation electrochemical glucose biosensor. Herein, the inherent HET properties of the germanane- and siloxene-based materials were first probed and germanane was determined to possess the smallest ΔE_p . The small ΔE_p suggests that germanane possesses innately fast HET kinetics and could possibly proffer superior electrocatalytic performance. Encouraged by the excellent electrochemical performance exhibited by germanane, germanane was chosen for the fabrication of a second-generation electrochemical glucose biosensor. The fabricated biosensing platform delivered excellent analytical performances, displaying good linearity over numerous magnitudes of glucose concentrations with a low detection limit of 6.3 μM . Chronoamperometry study also demonstrated the viability of the as-synthesised biosensor for glucose detection in human serum. The findings reported herein showcase the potential of applying these Group 14 graphane analogues for prospective developments of highly selective and sensitive biosensors.

It is believed that the proposed 2D germanane and siloxane enzymatic systems can be used not only for versatile biosensors applications but in general as biorecognition platforms.

4.4 Experimental Section

4.4.1 Synthesis of Germanane, Methyl germanane and Siloxene

Germanane (Ge_6H_6) was prepared using the protocol outlined by Bianco et al.,¹¹ where 1.0 g of CaGe_2 was introduced to 100 mL of 35% aqueous hydrochloric acid (HCl) and stirred continuously for 7 days at -35°C . Thereafter, a grey solid was collected via filtration and rinsed twice with 25 mL of HCl, then 5 times with 100 mL of ultrapure water and lastly twice with 50 mL of acetone.

Methyl germanane (Ge_6Me_6) was produced by placing 0.4 g of CaGe_2 and 11.4 g of methyl iodide into a sintered glass funnel with a S4 pore size and stirred continuously for 7 days. The funnel has its bottom part filled with water and the funnel was sealed with parafilm during the stirring process. Filtration technique was employed to collect the as-synthesised compound and the solid was washed twice with 100 mL of acetone, 5×100 mL of water and subsequently 3×50 mL of acetone.

Siloxene ($\text{Si}_6\text{H}_3(\text{OH})_3$) was synthesised by stirring 0.5 g of CaSi_2 in 50 mL of 35% HCl for a day under ambient conditions. The greenish-yellow compound was then collected via filtration and rinsed similarly as Ge_6H_6 .

The as-synthesised materials were dried under vacuum and stored away from light in an argon atmosphere.

4.4.2 Characterisation of Materials

Both SEM and EDX were accomplished using a Jeol 7600F SEM instrument acquired from Joel, Japan. SEM was accomplished using an acceleration voltage of 3 kV while EDX was executed at 15 kV. Survey and high-resolution XPS spectra of individual samples were acquired using a monochromatic magnesium X-ray radiation source and Phoibos 100 spectrometer from SPECS, Germany. EDX and XPS samples of germanane and siloxene were prepared on carbon tape while methyl germanane sample was prepared on a silicon wafer.

4.4.3 Preparation of Glucose Biosensor and Electrochemical Measurements

Preceding each measurement, GC electrodes would be sequentially polished on a polishing pad with 5 and 0.05 μm alumina particle slurry to renew its surface. Thereafter, the electrodes were rinsed carefully with ultrapure water and blown dry with a stream of nitrogen. Germanane, methyl germanane and siloxene were suspended in dimethylformamide to generate separate 5 mg mL^{-1} suspensions and ultrasonicated for 2 hours to generate well dispersed, homogeneous solutions. Prior to the surface modification of the GC electrode, the material of interest was ultrasonicated for 10 min to ensure its homogeneity. Thereafter, 0.75 μL of the desired material suspension was carefully pipetted on the electrode surface and left to dry under ambient conditions. 5 μL of glucose oxidase (GOx, 20 mg mL^{-1} in water) was then aliquoted onto the electrode and left to dry at room temperature. Last of all, 5 μL of glutaraldehyde solution (1% v/v) was deposited onto the surface of the electrode to induce cross-linking with the GOx enzyme and dried in an oven for an hour at 35 $^{\circ}\text{C}$. The fully modified electrodes are named GC/Ge₆H₆/GOx/GTA, GC/Ge₆Me₆/GOx/GTA and GC/Si₆H₃(OH)₃/GOx/GTA and these electrodes were stored at 4 $^{\circ}\text{C}$ when not in use.

Electrochemical measurements were performed via the use of an Eco Chemie μ Autolab type III electrochemical analyser linked to a desktop and the NOVA version 1.10 software was employed to set the analytical parameters. All electrochemical measurements within this study were carried out under atmospheric conditions, using a 10 mL electrochemical glass cell and the conventional three-electrode system (modified glassy carbon working electrode, platinum auxiliary electrode, and Ag/AgCl reference electrode).

Heterogeneous electron transfer (HET) studies were executed via cyclic voltammetry (CV) in 5 mM ferro/ferricyanide redox probe and 0.1 M KCl (supporting electrolyte), at a scan rate of 50 mV s⁻¹ with potentials starting from -0.2 to 0.5 V. To study the glucose sensing responses of the biosensors, chronoamperometry studies ($\Delta t > 1$ ms) were conducted at a constant potential of 0.15 V in 2 mM ferrocenemethanol (FcMeOH) and stirred continuously throughout the measurements. The chronoamperometric curves obtained were smoothed and subjected to baseline corrections prior to further analysis.

4.5 Supporting Information

Figure 4.S1. SEM-EDX images of (a) germanane, (b) methyl germanane and (c) siloxene.

Figure 4.S2. Wide survey XPS spectra of germanane, methyl germanane and siloxene.

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Part I

Chapter 5

Highly Sensitive Enzyme-less Glucose Sensor
Based on Pnictogens and Silver Shell-Gold Core
Nanorods Composite

5.1 Introduction

5.2 Results and Discussion

5.3 Conclusion

5.4 Experimental Section

5.5 Supporting Information

The results and discussion in this chapter were published in the following journal article:

A highly sensitive enzyme-less glucose sensor based on pnictogens and silver shell–gold core nanorod composites

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5.1 Introduction

Recently the rediscovery of black phosphorus, along with its extraordinary electronic and optical properties, has led to a surge in research interest in other pnictogens-based elemental 2D nanomaterials. Pnictogens are elements from Group 15 of the periodic table, which consist of the following elements: Nitrogen (N), Phosphorus (P), Arsenic (As), Antimony (Sb) and Bismuth (Bi). Heavy pnictogens (As, Sb and Bi) possess rhombohedral crystalline layered structures and have dative electron lone pairs situated on the surface atoms, endowing pnictogen-based 2D materials with high carrier mobilities and good adsorption capabilities with unsaturated organic molecules.¹⁻⁴ These rhombohedral-structured pnictogen layered materials are also brittle and possess high anisotropy, allowing them to undergo exfoliation easily to give rise to arsenene, antimonene, and bismuthene nanosheets. Additionally, pnictogen monolayers possess semiconductor characteristics and an extensive range of band gaps spanning from 0.36 to 2.62 eV. The exemplary electronic, optical and physical properties exhibited by pnictogen nanosheets have impelled much research vigour and opened new horizons for pnictogen nanosheets applications. Pnictogen nanosheets have been applied to an extensive field ranging from optoelectronics, energy storage, catalysis, biosensing and biomedical fields.³⁻⁹

In the past decade, nanomaterials that possess unique and extraordinary chemical and physical properties have been widely utilised to develop chemosensing and biosensing devices. Amongst the vast range of nanomaterials, metal-based nanomaterials have been commonly applied to sensing systems in the hope of enhancing catalytic reactions, through increasing surface area and promoting electron transfers.¹⁰⁻¹⁴ Precious metal-based nanostructures, in particular, silver and gold nanostructures have been thoroughly studied for sensing applications due to their numerous advantages, such as biocompatibility, excellent conductivity,

extraordinary catalytic activities and surface chemical properties. In addition, these precious metal-based nanostructures also exhibit fascinating activity towards the oxidation of glucose, making them ideal candidates for the design of non-enzymatic electrochemical glucose sensors.^{13,15-20} Considering the distinct and beneficial properties of both pnictogen nanosheets and precious metal-based nanostructures, this study aspires to synthesise novel pnictogen nanosheets-gold@silver nanorods (Au@Ag NRs) composites that could possibly synergistically combine these outstanding properties. Herein, the composites were synthesised simply via continual mixing of pnictogen nanosheets and gold@silver nanorods overnight and the as-synthesised composites were then applied towards the sensitive detection of glucose, as illustrated in Figure 5.1.

Figure 5.1. Schematic illustration picturing the (a) exfoliation of bulk pnictogens to shear exfoliated pnictogen nanosheets (pnictogens_{SE}) using a kitchen blender, (b) synthesis of pnictogens_{SE}-Au@AgNRs composites, (c) modification of glassy carbon electrode with pnictogens_{SE}-Au@AgNRs composites and (d) electrochemical sensing of glucose.

5.2 Results and Discussion

To synthesise the pnictogen_{SE}-Au@AgNRs composites, silver coated gold nanorods were first prepared using a seeded-growth method in the presence of silver nitrate solution while the pnictogen nanosheets were shear exfoliated using kitchen blenders. The as-synthesised pnictogen nanosheets had previously been thoroughly characterised in Rui et al.,⁸ and our previous reports via scanning electron microscopy (SEM), transmission electron microscopy (TEM), energy dispersive X-ray (EDX) spectroscopy, atomic force microscopy (AFM), Raman etc.⁶ Meanwhile, detailed characterisation of the Au@AgNRs has been reported previously by Novotný et al.²¹ SEM and EDX characterisation data of the Au@AgNRs are depicted in Figure 5.2a. SEM images reveal the formation of rectangular uniform Au@AgNRs that have a length of approximately 90 nm, while EDX affirms the presence and homogeneous dispersion of silver and gold throughout the nanorods. In addition, the gold elemental map displays structures that are slimmer than the rod structures in the silver elemental map, signifying the successful synthesis of a silver shell around the gold core nanorods (Au@AgNRs). After which, desired volume of pnictogen nanosheets samples were added to the Au@AgNRs, shaken and incubated overnight to yield the various pnictogen_{SE}-Au@AgNRs composites. The as-synthesised composites were then subjected to characterisation via SEM, EDX, XPS and zeta potential analysis.

SEM was utilised to determine the morphological structures of the pnictogen_{SE}-Au@AgNRs composites and the distribution of the Au@AgNRs on the pnictogen nanosheets while EDX was conducted to evaluate the elemental constitutions and distributions in the pnictogen_{SE}-Au@AgNRs composites. SEM and the elemental mappings of As_{SE}-Au@AgNRs composite are illustrated in Figure 5.1b. Through SEM, small rod-like structures, Au@AgNRs, were observed to be homogeneously distributed on the arsenic nanosheets indicating the successful

synthesis of $\text{As}_{\text{SE}}\text{-Au@AgNRs}$ composites. In addition, the elemental mappings reveal rod-like structures made up of silver and gold being homogeneously distributed throughout the arsenic nanosheets, thus affirming the successful synthesis of the $\text{As}_{\text{SE}}\text{-Au@AgNRs}$ composites.

Figure 5.2. (a) SEM (left panel) and SEM-EDX of Au@AgNRs (core-shell gold-silver nanorods). (b) SEM image and SEM-EDX of the $\text{As}_{\text{SE}}\text{-Au@AgNRs}$ composite.

The SEM and EDX data presented in Figure 5.S1 and 5.S2 (refer to Supporting Information) correspond to $\text{Sb}_{\text{SE}}\text{-Au@AgNRs}$ and $\text{Bi}_{\text{SE}}\text{-Au@AgNRs}$ composites respectively. Through SEM, it is interesting to note that Au@AgNRs were not found on the antimony nanosheets, meanwhile, Au@AgNRs, were observed to be homogeneously distributed on the bismuth nanosheets indicating the successful synthesis of $\text{Bi}_{\text{SE}}\text{-Au@AgNRs}$ composites. One reason that could possibly account for the absence of Au@AgNRs on antimony nanosheets is that gold-antimony complexes undergoes oxidation readily in the presence of oxygen and decomposes in solution.^{22,23}

EDX data of the $\text{Sb}_{\text{SE}}\text{-Au@AgNRs}$ composite corroborates well with the SEM data, whereby both Ag and Au elements are absent from the materials, suggesting the absence of the nanorods on the antimony nanosheets. However, it is worth noting from Figure 5.S2, that despite the

presence of rod-like clusters of silver element, gold was absent from the Bi_{SE}-Au@AgNRs composite.

XPS studies were also conducted to compare the surface compositions of Au@AgNRs and As_{SE}-Au@AgNRs composite. Wide survey XPS spectrum of Au@AgNRs (see Supporting Information, Figure 5.S3) verified the presence of silver element which makes up the shell of the nanorods, carbon, nitrogen and chlorine, which originates from the cetyltrimethylammonium chloride (CTAC) solution that the nanorods were dispersed in. It is worth noting that there is an absence of Au-signal in the XPS spectrum, suggesting the formation of closed silver shells with an insignificant amount of residual bare Au cores. On the other hand, an additional peak corresponding to arsenic was also observed in the wide survey XPS spectra of As_{SE}-Au@AgNRs composite in Figure 5.3a, suggesting the successful synthesis of the composite. High-resolution XPS (HR-XPS) was also conducted to further investigate the elements' chemical states in the As_{SE}-Au@AgNRs composite and the spectra of silver and arsenic are displayed in Figure 5.3b and 5.3c respectively. The Ag spectrum illustrated in Figure 5.3b displays two peaks at 367.8 and 373.3 eV denoting the Ag 3d_{5/2} and Ag 3d_{3/2} peaks of elemental silver (Ag(0)) respectively. Meanwhile in Figure 5.3c, the peaks at 41.6 and 42.3 eV accord with the As 3d_{5/2} and As 3d_{3/2} state of elemental arsenic (As(0)) respectively while the peaks at 45.0 and 45.7 eV accord with As 3d_{5/2} and As 3d_{3/2} of arsenic oxide (As₂O₃). Although the results obtained from XPS analysis suggest the successful synthesis of the As_{SE}-Au@AgNRs composite, they do not give insights into the interactions between arsenic nanosheets and Au@AgNRs.

Figure 5.3. (a) Wide survey XPS spectra of AsSE-Au@AgNRs composite. HR-XPS spectra of (b) silver, Ag and (c) arsenic, As.

To further probe the interactions between the arsenic nanosheets and the Au@AgNRs, zeta potentials of the materials were investigated. The zeta potentials of the arsenic nanosheets and the Au@AgNRs were determined to be -28.9 mV and 20.6 mV respectively, suggesting that the Au@AgNRs could have possibly adsorbed on the arsenic nanosheets through electrostatic attractions to yield the AsSE-Au@AgNRs composite.

After characterising the materials, the study then moved on to investigate the probability of incorporating the pinctogen_{SE}-Au@AgNRs composite to develop enzyme-free glucose sensors. Typically, the non-enzymatic glucose detection mechanism of metals (M) starts with the formation of hydroxide moieties on the electrode's surface to form a M-OH premonolayer. Glucose then gets adsorbed onto the active sites of the electrodes through a nucleophilic attack by the OH adsorbed on M on the C1 carbon on glucose and a C1 dehydrogenation. Glucose

subsequently undergoes an electro-oxidation process to produce glucono- δ -lactone which then further undergoes oxidation into gluconic acid. In addition, the catalytic effect of M is more pronounced at high pH as an increase in OH⁻ concentration greatly enhances the formation of M-OH.^{12,24-27} To assess the electrochemical response of the glucose sensing platform developed from the pinctogen_{SE}-Au@AgNRs composites, cyclic voltammetry was carried out with 50 μ M of glucose in 50 mM sodium hydroxide (NaOH) and the data obtained are illustrated in Figure 5.4 (As_{SE}-Au@AgNRs) and Figure 5.S4 (Sb_{SE}-Au@AgNRs and Bi_{SE}-Au@AgNRs).

From Figure 5.4a, it was observed that pure As_{SE} does not give rise to any signal in the presence of glucose. However, when shear exfoliated arsenic nanosheets were shaken overnight with the Au@AgNRs to produce the As_{SE}-Au@AgNRs composites, sharp distinguishable signals were produced in the presence of glucose for all composites made up of different ratios of As_{SE} and Au@AgNRs. Even though there is a decrease in sensitivity for the composite materials as compared to the Au@AgNRs (see Supporting Information, Figure 5.S4a), the anodic peak shifted to a significantly lower potential for all three composites as compared to pure Au@AgNRs, suggesting that the presence of As_{SE} nanosheets enhances the oxidative catalysis of glucose. This could possibly be ascribed to the high carrier mobility of the arsenic nanosheets,⁴ hence allowing better electron transfer kinetics. When comparing Figure 5.4a and Fig 5.S4, it is worth noting that amongst the three composites synthesised, only As_{SE}-Au@AgNRs composites produced distinguishable signals in the cyclic voltammograms in the presence of glucose. A possible reason that could account for this lack of response could be due to the absence of Au element in the Sb_{SE}-Au@AgNRs and Bi_{SE}-Au@AgNRs composites as observed in the EDX data, as gold has been proven to possess fascinating activity towards the oxidation of glucose.¹⁵

The $\text{As}_{\text{SE}}\text{-Au@AgNRs}$ composite (with the ratio of 25% As_{SE} : 75% Au@Ag NRs) gave rise to a signal at the lowest potential, suggesting that it has excellent electrocatalytic ability towards the oxidation of glucose and was chosen to develop a non-enzymatic glucose sensor. Voltammetric quantifications of glucose were then performed via differential pulse voltammetry techniques, whereby increasing concentration of glucose was introduced to 50 mM NaOH. As depicted in Figure 5.4b, increments of glucose concentrations result in an increase in oxidation current signal. A linear calibration graph of current density against glucose concentration was computed and displayed in Figure 5.4c, where it shows that the sensing system distinguishes glucose linearly ranging from 50 pM - 555.550 nM ($r=0.915$). The proposed glucose sensor also displays a rather satisfactory reproducibility with a relative standard deviation below 15%. To have a better understanding of the glucose sensing system's analytical performance, the limit of detection (LOD) and limit of quantification (LOQ) was determined via the approach defined by IUPAC.^{28,29} The glucose sensing platform was found to possess low LOD and LOQ of 3.5 and 11.6 pM respectively, demonstrating that the sensing platform is highly sensitive towards glucose detection.

Figure 5.4. (a) Cyclic voltammograms of 50 μM glucose in 50 mM sodium hydroxide (NaOH) with shear exfoliated arsenic nanosheets (As_{SE}) and $\text{As}_{\text{SE}}\text{-Au@AgNRs}$ composites. Differential

pulse voltammetry data (b) and calibration plot (c) for 25% AsSE-Au@AgNRs-based electrochemical glucose sensor, measurements were conducted in 50 mM NaOH.

To generate an in-depth knowledge of the proposed sensor's glucose sensing performance, a comparison of the analytical parameters of sensors constructed using metal-decorated layered materials are outlined in Table 5.1. Herein, we were able to develop a non-enzymatic glucose sensor based upon arsenic nanosheets and Au@AgNRs that allows glucose to be detected at a much higher sensitivity as compared to other layered materials that were decorated with metal-based nanomaterials. Pnictogen nanosheets possess high carrier mobilities up to several thousand $\text{cm}^2 \text{V}^{-1} \text{s}^{-1}$,^{30,31} hence they might help to promote fast electron transfer kinetics for the oxidation of glucose. Furthermore, pnictogens have dative electron lone pairs situated on the surface atoms, good adsorption capabilities and are known to interact with biomolecules such as glucose at high pH,^{2,32} thus it could also help to facilitate the adsorption process bringing glucose molecules into close proximity with the electrocatalytic Au@AgNRs. The high carrier mobility of arsenene and the excellent catalytic activity of gold nanomaterials towards oxidation of glucose synergistically combine to allow efficient electron transport and enhances the catalysis of glucose oxidation, thus endowing the sensor with extraordinary sensing performance and high sensitivity of ultralow LOD. Additionally, studies have demonstrated that the interactions of noble metal (Ag or Au) nanomaterials with pristine graphene are weak, the noble metal nanomaterials or graphene have to be treated or functionalised in order to effectively load the nanomaterials.³³⁻³⁶ Meanwhile pnictogens, in particular arsenic, is known to have good affinities with gold and silver,³⁷⁻³⁹ hence we were able to bypass the functionalisation step, generating a composite that could be easily synthesised without modifying the pnictogen nanosheets or Au@AgNRs. Additionally, pnictogens have better hydrophilicity as compared to graphene, preventing metallic alloy

nanoparticles from aggregating, and allowing the composite to be dispersed in water thus reducing the use of harmful organic solvents.⁴⁰⁻⁴²

Table 5.1. Comparison of the analytical performances of GC/As_{SE}-Au@AgNRs glucose sensor with various electrochemical glucose sensors based upon layered materials decorated with metals.

Electrode	Linear Range (μM)	LOD (μM)	Ref.
GC/As _{SE} -Au@AgNRs	0.05 nM - 555.55 nM	3.5 pM	This work
GC/Colloidal AgNPs-MoS ₂	0.1 - 1000	0.003	43
GC/NiNPs-MoS ₂	0 - 4000	0.31	44
GC/PTNFs/Graphene Oxide	2 - 10300 10.3 mM - 20.3 mM	2	45
GC/Au@PtNPs-MoS ₂	10 - 3000	1.08	46
GC/AuNPs-Graphene	100 - 2000 2 mM - 16 mM	25	47

5.3 Conclusion

Inspired by the remarkable electronic, physical and catalytic properties exhibited by pnictogen nanosheets and gold nanomaterials, this chapter sets forth to investigate the feasibility of applying pnictogen/gold nanomaterial composites as novel electrode materials towards glucose sensing. Herein, the chapter reported the synthesis of various novel pnictogen_{SE}-Au@AgNRs composites by simply adding and incubating the desired volume of pnictogen nanosheets samples with the as-synthesised Au@AgNRs. The results obtained suggest that As_{SE}-based composites display excellent electrocatalytic activity towards glucose detection. In addition, differential pulse voltammetry studies demonstrated the high sensitivity of the proposed

sensing system towards the detection of glucose, with linear ranges spanning multiple magnitudes and an ultralow LOD of 3.5 pM for glucose. The discoveries accounted in this study conceptually prove and open up new horizons for the probable incorporation of As_{SE}-based composites for future development of electrochemical sensors for biomedical, pharmaceutical and food industries.

5.4 Experimental Section

5.4.1 Synthesis of gold@silver nanorods (Au@AgNRs)

Synthesis of the silver coated gold nanorods were performed as reported by Novotný et al.,²¹ using a two-stage method, a modification of previous work.⁴⁸ In brief, the core gold nanorods (AuNRs) were first fabricated via the well-known seeded-growth technique in the presence of silver nitrate and cetyltrimethylammonium bromide.⁴⁹ To clean the as-synthesised AuNRs from the synthesis by-products, the resultant colloid was then subjected to centrifugation and the AuNRs pellets were collected and re-dispersed into cetyltrimethylammonium chloride (CTAC) solution (10×10^{-3} M).

Subsequently, silver shell was grown using silver nitrate on the synthesised AuNRs using elevated pH (sodium hydroxide), ascorbic acid and elevated temperature (60 °C) in the presence of CTAC. After 2 hours of the start of shell overgrowth, the mixture was cooled to ambient temperature and silver coated gold nanorods were purified via centrifugation followed by redispersion into CTAC solution (10×10^{-3} M).

5.4.2 Synthesis of Pnictogen Nanosheets

Synthesis of shear exfoliated pnictogen nanosheets was performed as reported previously by Gusmão et al.⁸ Briefly, bulk pnictogen materials procured from Alfa Aesar (Germany) were

first subjected to sonication in aqueous sodium cholate (SC) surfactant in an ice bath to remove any inorganic impurities and oxides. The resultant mixtures were then centrifuged, and the pellets containing the pnictogens were dried then subjected to shear exfoliation in SC for 2 hours via the use of common kitchen blenders. Upon the 2 hours exfoliation process, the resultant suspensions were subjected to centrifugation and the top 75% of the suspensions were recovered to yield As_{SE} (3.9%), Sb_{SE} (8.2%) and Bi_{SE} (8.8%) from the respective pnictogen starting materials.

5.4.3 Synthesis of pnictogen_{SE}-Au@AgNRs composites

Desired volume (50, 100 or 150 μ L) of 5 mg/mL of pnictogen samples were added to the gold@silver nanorods (Au@AgNRs) to yield a mixture of 200 μ L solution, incubated overnight at 25 °C and gently agitated at 400 rpm using a TS-100 Thermo-Shaker from Biosan. Subsequently, the supernatant containing free Au@AgNRs was removed and the remaining pnictogen_{SE}-Au@AgNRs pellet was resuspended in 100 μ L of ultrapure water.

5.4.4 Characterisation of pnictogen_{SE}-Au@AgNRs composites

Scanning electron microscopy was performed using the Tescan Maia 3 electron microscope. 3 μ L of the desired sample suspensions were drop cast onto individual 200 mesh TEM grids during the preparation of the SEM samples. EDX spectra were acquired via an energy dispersive spectrometer from Oxford Instruments and analysis was performed via the Aztec software. XPS data were acquired via the use of a Phoibos 100 spectrometer (SPECS, Germany) that has been fitted with a monochromatic magnesium X-ray radiation anode. The zeta potential measurements of the arsenic nanosheets and the Au@AgNRs were conducted via the use of the Zetasizer Nano ZS (Malvern Instruments, England) under zeta potential mode at 20°C.

5.4.5 Electrochemical Measurements

Cyclic and differential pulse voltammetry were executed with an Eco Chemie μ Autolab type III electrochemical analyser linked to a laptop and the NOVA version 1.10 software was utilised to set the experimental parameters. In this study, all measurements were conducted under ambient condition using a classic three-electrode configuration, composing of a modified glassy carbon working electrode, an Ag/AgCl reference electrode and a platinum auxiliary electrode, in a 10 mL electrochemical glass cell.

Preceding each measurement, GC electrodes' surfaces were carefully renewed via polishing with a range of alumina particle slurry (5 μm , 1 μm , 0.3 μm successively) on a polishing pad. Thereafter, electrodes were rinsed using ultrapure water and were dried via dry polishing on a polishing pad. 3 μL of pniictogen_{SE}-Au@AgNRs solution was subsequently deposited onto the electrode's surface and dried under ambient conditions. Cyclic voltammetry measurements were carried out under the potential range of -0.2 to 0.5 for glucose detection, with a 50 mV s^{-1} scan rate in 50 mM NaOH. Differential pulse voltammetry measurements were conducted in 50 mM NaOH at an amplitude pulse of 50 mV and a scan rate of 10 mV s^{-1} .

5.5 Supporting Information

Figure 5.S1. SEM and SEM-EDX of Sb_{SE}-Au@AgNRs with the ratio of 25% Sb_{SE} : 75% Au@AgNRs.

Figure 5.S2. SEM and SEM-EDX of Bi_{SE}-Au@AgNRs composite with the ratio of 25% Bi_{SE} : 75% Au@AgNRs.

Figure 5.S3. Wide survey XPS spectra of Au@AgNRs.

Figure 5.S4. Cyclic voltammograms of 50 μ M glucose in 50 mM sodium hydroxide with Au@AgNRs, shear exfoliated pnictogen nanosheets and pnictogen_{SE}-Au@AgNRs composites.

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Part I

Chapter 6

Vanadium-doped Molybdenum Dichalcogenides
for Electrocatalytic Energy Applications

6.1 Introduction

6.2 Results and Discussion

6.3 Conclusion

6.4 Experimental Section

6.5 Supporting Information

The results and discussion in this chapter were published in the following journal article:

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6.1 Introduction

The ever-rising concerns with regard to energy shortages and climate change have made the search for clean and renewable energy sources a pressing priority for the sustainable development of societies as well as alleviating the environmental hazards posed by fossil fuels. As such, a noteworthy amount of research vigour has been dedicated to exploring alternative sources of energy including solar, geothermal and wind energies.¹⁻³ In order to fully maximise the utility of such intermittent renewable energy sources, it is of great importance to develop advanced energy storage and conversion technologies, for instance, electrocatalysis processes, supercapacitors and metal-air batteries. These technologies often rely heavily on electrochemistry principles which aid in converting chemical energy into electrical energy.⁴⁻⁶ Even though conventional precious metal-based catalysts such as platinum (Pt), iridium (Ir) and ruthenium (Ru) are able to catalyse these conversions efficiently, they are usually very costly, scarce and suffer from poor stability, hence impeding their widespread application. Therefore, tremendous efforts have been put in place to search for alternative catalysts that are abundant, low in cost and highly catalytic. Some of these alternatives include carbon nanomaterials, transition metal dichalcogenides (TMDs), transition metal oxides and hydroxides and transition metal carbides.^{1,2,6-9}

The isolation of the wonder material, graphene, from graphite by the Manchester group has created a great deal of fervour among the scientific community, paving way for the rediscovery of a multitude of layered materials.^{10,11} Examples of layered materials include TMDs,¹² transition metal carbides or nitrides (MXenes),¹³ pnictogen nanosheets,¹⁴ and metal phosphorus trichalcogenides.^{15,16} Layered materials possess a vast range of fascinating physical, chemical, electronic, and optical properties, for instance, large specific surface areas, remarkable electrical and thermal conductivities, abundance of catalytic sites, ease of functionalisation and

tuneable electronic structures. Notably, TMDs have spurred much scientific enthusiasm because of their natural abundancy, low cost and remarkable catalytic activities.^{9,12} TMDs is a prominent class of layered materials that constitute of a transition metal (M) with an oxidation state of +4 and a chalcogen (X) of -2 oxidation state. Group 4 – 7 TMDs have been reported to possess layered structures analogous to graphite whereby covalent bonds are found within each layer while van der Waals forces of attractions are found between adjacent MX₂ layers. Within each layer, transition metal sits amidst two chalcogen layers hence giving rise to the MX₂ stoichiometry.^{12,17-19} TMDs possess numerous astounding properties including tuneable band gaps, superconductivity, large specific surface areas and low shear resistance, hence attracting much research zeal, leading to a myriad of promising applications, for example, catalysts, lubricants, energy storage and conversion systems and sensors.²⁰⁻²⁴

Numerous studies have been carried out to modulate the properties of TMDs in order to fully exploit them for various applications. Some of these methods include the application of mechanical strains, doping, varying the number of layers present in the material and alloying.²⁵⁻
²⁷ Atomic doping is one of the most commonly used techniques to alter the structures or properties of TMDs. In general, doping can occur via either direct substitution of atoms or via interstitial sites amidst atoms found within the crystal lattice or through intercalation amidst layers in layered TMDs (Figure 6.1).^{28,29} Doping of TMDs can possibly enhance their electronic properties and enhance the density of active sites, hence improving their catalytic activities. For instance, studies have reported that when TMDs are doped with metallic heteroatoms, their differential free energy of hydrogen adsorption (ΔG_H) gets altered, proton transfer kinetic was accelerated, in which these modulations are beneficial for hydrogen evolution reaction.³⁰⁻³⁵ Doping TMDs with vanadium (V) reportedly improves the electrical properties of Group 6

TMDs as it changes the semi-conducting behaviours to semi-metallic, enhances electrical conductivity and increases carrier concentrations.^{29,36-38}

Figure 6.1. Structure of heteroatom doped TMDs.

Herein, this chapter investigated the effects of doping various amounts of vanadium on molybdenum dichalcogenides (MoX_2) on their electrocatalytic activities towards hydrogen evolution reaction (HER), oxygen evolution reaction (OER) and oxygen reduction reaction (ORR). Doped MoX_2 were synthesised via mixing a stoichiometric amount of elemental powders under high pressure and temperature. The as-synthesised vanadium-doped MoX_2 were then characterised via scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDX) and Raman spectroscopy. Lastly, voltammetry was employed to provide insights on the effects of vanadium doping on the materials' intrinsic electrochemical properties and the implications of varying the amount of vanadium dopants in TMDs towards a myriad of electrochemical applications such as HER, OER and ORR.

6.2 Results and Discussion

Prior to the electrochemical studies of V-doped molybdenum dichalcogenides, these materials were subjected to characterisation techniques, such as SEM, EDX and Raman microscopy, to have a better understanding of their morphological and structural characteristics as well as their chemical compositions.

SEM has been exploited to determine the morphologies of the V-doped molybdenum dichalcogenides. SEM data in Figure 6.2, reveal close stacking of layers, suggesting that all nine materials exist in the bulk state. All V-doped MoS_2 manifest in large, flat polygonal structures while V-doped MoSe_2 and MoTe_2 appear to be irregular in shape, with $\text{Mo}_{0.75}\text{V}_{0.05}\text{Te}_2$ appearing to be stacks of irregular platelet-like structures. Meanwhile, EDX was employed to carry out elemental analysis and chemical characterisation of the V-doped molybdenum dichalcogenides. The elemental mappings from EDX (displayed in Figure 6.S1-6.S3) reveal the homogeneous distribution of molybdenum, vanadium and chalcogens corresponding to the composition of the respective V-doped molybdenum dichalcogenides, suggesting uniform vanadium doping throughout these materials. Additionally, atomic percentage (at%) distributions of the V-doped molybdenum dichalcogenides arrived from EDX are tabulated in Table 6.S1. Although the calculated vanadium to molybdenum (V/Mo) ratio in Table 6.S1 deviates from the expected stoichiometry, a general increase in V/Mo ratio is observed when an increasing amount of vanadium dopant is used during the synthesis process. It was also observed that with increasing amount of vanadium dopants, the X:M ratio increases for the doped MoS_2 and MoTe_2 compounds while a decrease in X:M ratio was noted for the MoSe_2 materials with increasing stoichiometry of dopants.

Figure 6.2. SEM images of (a) $\text{Mo}_{0.95}\text{V}_{0.05}\text{S}_2$, (b) $\text{Mo}_{0.90}\text{V}_{0.10}\text{S}_2$, (c) $\text{Mo}_{0.75}\text{V}_{0.25}\text{S}_2$, (d) $\text{Mo}_{0.95}\text{V}_{0.05}\text{Se}_2$, (e) $\text{Mo}_{0.90}\text{V}_{0.10}\text{Se}_2$, (f) $\text{Mo}_{0.75}\text{V}_{0.25}\text{Se}_2$, (g) $\text{Mo}_{0.95}\text{V}_{0.05}\text{Te}_2$, (h) $\text{Mo}_{0.90}\text{V}_{0.10}\text{Te}_2$ and (i) $\text{Mo}_{0.75}\text{V}_{0.25}\text{Te}_2$, with scale bars corresponding to 1 μm .

To yield a more comprehensive understanding of the structures of the V-doped molybdenum dichalcogenides, Raman spectroscopy was conducted, and data obtained are depicted in Figure 6.3. In Figure 6.3a, distinct peaks at ~ 381 and ~ 407 cm^{-1} , corresponding to the in-plane E_{2g}^1 and out-of-plane A_{1g} vibrational modes of MoS_2 were observed. Broad separation (~ 26 cm^{-1}) between these two peaks further affirms that the V-doped MoS_2 exist in their bulk form. Furthermore, the positions of the E_{2g}^1 and A_{1g} peaks remain relatively unchanged, suggesting that increasing the amount of vanadium dopant would not alter the thickness of the materials. Additionally, it was noted that E_{2g}^1 and A_{1g} peaks for $\text{Mo}_{0.75}\text{V}_{0.25}\text{S}_2$ became broader and weaker, suggesting that the vanadium dopant might have caused some disruption to the MoS_2

crystal structure.^{36,39-41} In Figure 6.3b, distinct peaks at ~ 284 and ~ 242 cm^{-1} , corresponding to the in-plane E_{2g}^1 and out-of-plane A_{1g} vibrational modes of MoSe_2 were noted, which corroborate well with the literature values for multi-layered MoSe_2 .^{39,42,43} Lastly, peaks at ~ 162 , ~ 118 and ~ 227 cm^{-1} corresponding to A_{1g} , E_{1g} and E_{2g}^1 of MoTe_2 were observed for the V-doped MoTe_2 . Interestingly, peaks at ~ 91 and ~ 137 cm^{-1} , which have been reported to be the E_g and A_{1g} vibrational modes of VTe_2 were also observed, suggesting that there might be some formation of VTe_2 .⁴⁴⁻⁴⁷

Figure 6.3. Raman spectra of (a) $\text{Mo}_{1-y}\text{V}_y\text{S}_2$, (b) $\text{Mo}_{1-y}\text{V}_y\text{Se}_2$ and (c) $\text{Mo}_{1-y}\text{V}_y\text{Te}_2$ compounds.

The inherent electrochemistry, a fundamental parameter to consider when devising electrochemical applications, provides insights into the redox properties of the materials. The innate signals arising from the materials may restrict their operational electrochemical potential window, thus limiting their applications especially in the field of electrochemical sensors.^{20,48,49} Herein, the inherent electrochemistry of the V-doped molybdenum dichalcogenides was assessed by performing cyclic voltammetry in a phosphate buffer solution (PBS) in both anodic and cathodic scan directions.

Data obtained from the anodic scans and cathodic scans of the V-doped molybdenum dichalcogenides are displayed in Figure 6.S4 to 6.S6 (see Supporting Information). From

Figure 6.S4, intense oxidation peaks were observed at around ~ 1.6 V *vs* Ag/AgCl for both anodic and cathodic scans, suggesting that the MoS₂ materials are getting oxidised. Additionally, a peak at ~ -1.4 V *vs* Ag/AgCl was also noted for all V-doped MoS₂, which could be assigned to the reduction of V(V) to V(IV) species.^{20,50} These two peaks were found to slowly diminish in the subsequent scans. It is also worth noting that when comparing the first anodic scan of all V-doped MoS₂, an additional oxidative peak at ~ 0.9 V *vs* Ag/AgCl was generated for Mo_{0.75}V_{0.25}S₂, which could be ascribed to the oxidation peak of V(IV) to V(V) species.⁵¹ Additionally, a prominent oxidative peak at ~ 0.9 V *vs* Ag/AgCl appears only in the second and third anodic scans of Mo_{0.90}V_{0.10}S₂, suggesting that the vanadium species existing in Mo_{0.90}V_{0.10}S₂ are in the V(V) state and that the V(IV) is generated after a reduction process has occurred in the first scan at ~ -1.4 V *vs* Ag/AgCl.

From the cyclic voltammograms of vanadium-doped molybdenum diselenides in Figure 6.S5, it could be observed that large oxidative peaks that appeared in the initial oxidative sweep for both anodic and cathodic scans diminish rapidly, making it hardly discernible in the subsequent scans, implying that an irreversible oxidation process has occurred. The oxidative peak at approximately ~ 1.2 V *vs* Ag/AgCl in the anodic scans of vanadium-doped molybdenum diselenide compounds manifest as broad peaks suggesting that more than one oxidative reaction could have taken place. In the cathodic scan, the broad peak evolves to two peaks at ~ 0.9 V and ~ 1.2 V *vs* Ag/AgCl, indicating that at least two different oxidative processes are taking place on the surfaces of vanadium-doped molybdenum diselenide compounds. Similar to the V-doped MoS₂ compounds, a minute peak at ~ -1.3 V *vs* Ag/AgCl was observed, suggesting the occurrence of a reduction of V(V) to V(IV) species.

All anodic and cathodic scans of V-doped MoTe₂ in Figure 6.S6, reveal oxidative signals at approximately ~ 0.3 V *vs* Ag/AgCl, which accords to the oxidative stripping of tellurium. Additionally, a reductive peak at ~ -0.8 V *vs* Ag/AgCl was also noted in all scans, which could be ascribed to the cathodic stripping of tellurium.^{20,52} Both of these peaks are still fairly discernible in the subsequent scans suggesting that some form of reversible oxidative/reduction process is going on, where the peak at ~ 0.3 V *vs* Ag/AgCl accords to the oxidation of the product from the reductive process at ~ -0.8 V *vs* Ag/AgCl and vice versa. Furthermore, close scrutinization of the cathodic scans of the V-doped MoTe₂ reveals the presence of an additional oxidative peak at ~ 0.6 V *vs* Ag/AgCl for the Mo_{0.95}V_{0.05}Te₂ material, suggesting the formation of a new tellurium species through an oxidation process. Likewise to the V-doped MoS₂ and V-doped MoSe₂ compound, a minuscule peak at ~ -1.3 V *vs* Ag/AgCl was observed, suggesting the occurrence of a reductive process of V(V) to V(IV) species.

Thereafter, the study moved on to ascertain the heterogeneous electron transfer (HET) rates of the vanadium-doped molybdenum dichalcogenides. HET rate is another important analytical parameter that is often used to determine the suitability of materials for electrochemical applications, particularly so for sensors development. Generally, materials with inherently fast HET rate are preferred as they reduce the overpotential necessary for an electrochemical reaction to occur efficiently. Cyclic voltammetry was accomplished via the aid of a conventional ferro/ferricyanide redox probe and the observed HET rate constant, k_{obs}^0 , was then calculated according to the approach reported by Nicholson.⁵³ Through Nicholson's report, one could infer that the peak-to-peak separation (ΔE_p) is correlated with k_{obs}^0 , where wide ΔE_p typically accords to a slow HET rate. The voltammograms derived from HET studies are depicted in Figure 6.4 while the tabulated k_{obs}^0 data is reported in Table 6.S2 (see Supporting Information).

Figure 6.4. Cyclic voltammograms of (a) $\text{Mo}_{1-y}\text{V}_y\text{S}_2$, (c) $\text{Mo}_{1-y}\text{V}_y\text{Se}_2$ and (e) $\text{Mo}_{1-y}\text{V}_y\text{Te}_2$ in $\text{Fe}(\text{CN})_6^{3-/4-}$ redox probe. Peak-to-peak separations of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ on the surfaces of (b) $\text{Mo}_{1-y}\text{V}_y\text{S}_2$, (d) $\text{Mo}_{1-y}\text{V}_y\text{Se}_2$ and (f) $\text{Mo}_{1-y}\text{V}_y\text{Te}_2$. Cyclic voltammetry was performed at a scan rate of 50 mV s^{-1} with $5 \text{ mM Fe}(\text{CN})_6^{3-/4-}$ redox probe in 0.1 M KCl .

Through the cyclic voltammograms, it could be observed that all vanadium-doped molybdenum dichalcogenides have narrower ΔE_p as compared to bare glassy carbon (GC) electrode, suggesting that HET is faster in these compounds in contrast to bare GC. Indeed, the computed k_{obs}^0 values (see Supporting Information Table 6.S2) reveal GC to possess the slowest HET rate at $1.51 \times 10^{-4} \text{ cm s}^{-1}$. Another trend ascertained from the HET studies is that the vanadium-doped MoTe_2 materials, generally have wider peak-to-peak separations as compared to their MoS_2 and MoSe_2 counterparts. Vanadium-doped MoTe_2 materials display electron transfer rates that are rather slow, with k_{obs}^0 calculated to be within the $\sim 10^{-4} \text{ cm s}^{-1}$

range, approximately one order of magnitude slower than V-doped MoS₂ and MoSe₂ materials. One plausible reason that could account for this slow HET rate is that tellurium is more liable towards surface oxidation as opposed to the other chalcogens, tellurium oxides existing on the surface of these materials might have electrostatically repelled the negatively charged ferro/ferri redox probe, hence hindering the electron transfer reaction.²⁰ Lastly, it could also be perceived that increasing the amount of vanadium dopants causes a decrease in the ΔE_p of the doped MoS₂ and MoTe₂ materials, resulting in faster electron transfer rates. Meanwhile, the opposite trend is observed for the V-doped MoSe₂ compounds, where increasing the amount of vanadium dopant results in wider peak-to-peak separations and slower HET rates.

Having shed some light on the effects of varying the amount of vanadium dopants on the physical, chemical and inherent electrochemistry of the molybdenum dichalcogenides, this study moved on to investigate the effects of varying dopant amounts on electrochemical applications, in particular HER, OER and ORR.

HER, the cathodic half reaction in water splitting, is a reaction of paramount importance in the pursuit of obtaining alternative fuels, particularly hydrogen. The current state-of-the-art HER catalysts often involve precious metals such as platinum which are scarce and costly, spurring the scientific community to search for other alternative electrocatalysts that could effectively lower the overpotential for HER.^{3,54} Herein, linear sweep voltammetry was employed to probe the effects of varying dopant amounts on HER and the polarisation curves are presented in Figure 6.5. A comparison of the overpotential in Figure 6.5g reveals that all the doped molybdenum dichalcogenides possess significantly lower HER overpotentials as compared to bare GC. Unsurprisingly, the electrode modified with the commercially available platinum on graphitized carbon (Pt/C) resulted in the lowest overpotential of ~0.1 V (vs reversible hydrogen

electrode, RHE) at -10 mA/cm^2 . This implies that these doped materials possess superior electrocatalytic activities compared to bare GC but exhibit poorer electrocatalytic performances when compared with Pt/C. Amongst the vanadium-doped molybdenum dichalcogenides, $\text{Mo}_{0.95}\text{V}_{0.05}\text{Te}_2$ displays the lowest overpotential. Further scrutinization of the overpotentials reveals that increasing the amount of vanadium dopant decreases the overpotential required to reach -10 mA/cm^2 for the doped MoS_2 compounds, suggesting that there is an improvement in electrocatalytic behaviour with increasing vanadium dopants. Meanwhile, no clear correlations could be drawn between the amount of vanadium dopant and the overpotentials for MoSe_2 materials. Lastly, an increase in the overpotential is observed with increasing stoichiometry of dopants for the V-doped MoTe_2 compounds, suggesting that excessive amounts of dopants could partially suppress the electrocatalytic capabilities of MoTe_2 compounds.

Figure 6.5. HER polarisation curves and the corresponding Tafel plots for Mo_{1-y}V_yS₂ (a, b), Mo_{1-y}V_ySe₂ (c, d) and Mo_{1-y}V_yTe₂ (e, f). Consolidated data of the average overpotential at -10 mA cm⁻² current density (g) and average Tafel slopes (h) in bar charts. HER measurements were conducted in 0.5 M sulfuric acid and at a scan rate of 2 mV s⁻¹.

To rationalise the results and elucidate the intrinsic effects of doping on HER activities, density functional theory (DFT) calculations of hydrogen bonding onto edges of V-doped MoS₂ and MoTe₂ were performed. The activity for HER was assessed by ΔG_H . Although hydrogen atoms were bound directly to the chalcogen (S/Te) atoms instead of metallic dopants covering the

edge, vanadium doping was observed to have a pronounced effect on hydrogen binding (Figure 6.6). Binding of hydrogen was systematically stronger at the chalcogens bonded to vanadium compared to the molybdenum counterparts. Effect of vanadium doping was apparent for the fully substituted edge of $\text{Mo}_{0.76}\text{V}_{0.24}\text{S}_2$, where thermoneutral ΔG_{H} was reached at high H coverages ($>50\%$ H), thereby explaining the enhanced HER performance of $\text{Mo}_{0.75}\text{V}_{0.25}\text{S}_2$ obtained experimentally, see Figure 6.5g. Overall, stronger and more variable bonding of H at the edge of V-doped MoS_2 could lead to an improvement in HER performance for V-doped MoS_2 . On the other hand, vanadium doping had a negligible effect on hydrogen binding at MoTe_2 interfaces, where hydrogen binding was not strengthened and negligible changes in ΔG_{H} were noted with increasing dopant concentrations. It should be noted that ΔG_{H} only assesses the thermodynamic part of HER, while the kinetic part, determined by reaction barriers, could be affected by doping differently. The process of doping could also alter the HER efficiency indirectly by changing the surface/edge properties of samples.

Figure 6.6. The differential Gibbs free energies of H adsorption (ΔG_{H}) as a function of the H coverage at the edge. The V atoms substituting 25%, 50%, and 100% edge Mo atoms

correspond to $\text{Mo}_{0.94}\text{V}_{0.06}\text{S}_2$, $\text{Mo}_{0.88}\text{V}_{0.12}\text{S}_2$, and $\text{Mo}_{0.76}\text{V}_{0.24}\text{S}_2$ composition, respectively. The values for pristine MoTe_2 were taken from our previous study in reference 55.

Aside from comparing the HER overpotentials, Tafel slopes were also evaluated to determine the electrocatalytic capabilities of the materials for HER and to gain insights into the HER mechanism undergone by the various doped compounds. Typically, low Tafel values are preferred for HER electrocatalyst, as they would be able to efficiently increase the current density with small voltage inputs. The reactions involved in the hydrogen evolution mechanism are well-reported and are as follows:^{56,57}

Similar to the overpotential studies, all vanadium-doped molybdenum dichalcogenides have smaller Tafel slopes compared to bare GC (158 mV dec^{-1}) but higher Tafel values than Pt/C (22 mV dec^{-1}). All Tafel values of the vanadium-doped molybdenum dichalcogenides except for $\text{Mo}_{0.90}\text{V}_{0.10}\text{Se}_2$ fall between 40 mV dec^{-1} and 120 mV dec^{-1} , suggesting that these materials undergo a Volmer–Heyrovsky mechanism. Meanwhile, $\text{Mo}_{0.90}\text{V}_{0.10}\text{Se}_2$ has a high Tafel slope of 137 mV dec^{-1} implying that it has poor electrocatalytic performance and HER is predominantly influenced by the Volmer adsorption reaction. Likewise to the overpotential studies, increasing vanadium dopants results in smaller Tafel values in MoS_2 compounds, implying an improvement in electrocatalytic behaviour. However, no clear correlations could be drawn between the amount of vanadium dopants and the Tafel slopes for MoSe_2 and MoTe_2 materials.

OER, the anodic half reaction of water splitting reaction is yet another cardinal reaction required for the development of technologies for renewable and alternative energy sources, such as metal-air batteries and the production of solar fuels. OER involves the transfer of four electrons and often engages multiple step reactions making it a kinetically challenging process, hence it is critical to search for catalyst with low overpotential and excellent catalytic performance to overcome the large energy barrier.^{58,59} OER studies were carried out in 1.0 M KOH using linear sweep voltammetry and the OER polarisation curves are displayed in Figure 6.S7 (see Supporting Information). It is evident that all vanadium-doped molybdenum dichalcogenides possess high overpotentials (~ 2.45 V *vs* RHE) that are similar to bare GC suggesting that these materials exhibit nominal electrocatalysis towards OER. It is also noted that increasing the vanadium dopant concentrations have minimal effects on the electrocatalytic properties of these vanadium-doped molybdenum dichalcogenides for OER.

Lastly, the study examined the effects of varying amount of vanadium dopants in molybdenum dichalcogenides on the electrocatalytic performances for ORR, a cardinal process in fuel cell technologies.^{60,61} Linear sweep voltammograms for ORR and a comparison of the overpotentials required to reach 10% of the maximum current density are illustrated in Figure 6.7. Unsurprisingly, Pt/C possesses the lowest onset potential at approximately -0.08 V (*vs* Ag/AgCl), while the doped materials have onset potentials that are much higher compared to Pt/C. This implies that the electrocatalytic behaviours of these materials are far below par compared to the current state-of-the-art platinum catalyst. Across all the doped molybdenum dichalcogenides, it is observed that the doped MoSe₂ compounds generally have lower overpotentials compared to the doped MoS₂ and MoTe₂ counterparts. It is also noted that the ORR potentials of vanadium-doped MoSe₂ shifted negatively with increasing amount of vanadium dopants, suggesting that excessive amount of dopants could partially suppress the

electrocatalytic behaviour of MoSe₂ compounds. Meanwhile, further inspection reveals that even though slight reductions in overpotentials were observed with increasing dopant concentrations, vanadium-doped MoTe₂ materials exhibit inferior catalytic properties with higher negative overpotentials in comparison with bare GC (~-0.32 V vs Ag/AgCl). Likewise to vanadium-doped MoTe₂, increasing the amount of vanadium dopants enhances the ORR performances of MoS₂

Figure 6.7. Linear sweep voltammograms of ORR for (a) Mo_{1-y}V_yS₂, (b) Mo_{1-y}V_ySe₂, (c) Mo_{1-y}V_yTe₂ and a bar chart of the average onset potentials required to achieve 10% of the maximum current density (d). ORR studies were executed in 0.1 M potassium hydroxide at a scan rate of 5 mV s⁻¹.

6.3 Conclusion

Herein, the chapter examined the effects of varying the stoichiometry of vanadium dopants in bulk molybdenum dichalcogenides on HER, ORR and OER electrocatalytic performances. Extensive characterisation reveals that varying the amount of dopants in the bulk molybdenum dichalcogenides does indeed influence their physical, chemical and inherent electrochemistry

properties. With regard to the electrocatalytic performances, mixed results were obtained for different molybdenum dichalcogenides and different reactions. Increasing the ratio of vanadium dopants enhances both HER and ORR performances of V-doped MoS₂ compounds, while no clear correlations between the stoichiometry of dopants and electrocatalytic HER performance were observed for the doped MoSe₂ compounds. Additionally, increasing the amount of vanadium dopants partially suppresses the electrocatalytic ORR performance of doped MoSe₂ compounds. Meanwhile, a different trend is observed for V-doped MoTe₂, where increasing the stoichiometry of vanadium dopants generally worsens the HER capabilities and improves ORR capabilities. DFT studies revealed that increasing the vanadium dopant concentrations in MoS₂ results in stronger and more variable bonding of H at the edge of V-doped MoS₂ and a more thermoneutral ΔG_H , thus yielding enhanced HER catalytic performances. Lastly, OER studies revealed that varying the concentration of vanadium dopants yield no effect on the electrocatalytic behaviour across all doped compounds. There are many factors involved in the mechanisms of these complex reactions, there is no magic bullet that could enhance the electrocatalytic performance of materials for all HER, ORR and OER applications. Increasing the amount of vanadium dopants can perhaps enhance the catalytic capability of a certain material for a specific reaction, but the same results might not be observed for another material or another energy-related reaction.

6.4 Experimental Section

6.4.1 Synthesis of Vanadium-doped Molybdenum Dichalcogenides

Doped molybdenum dichalcogenides were synthesised from their elements in powdered form. A stoichiometric amount of elemental powder corresponding to 10g of doped molybdenum dichalcogenides, Mo_(1-y)V_yX₂ (where y = 0.05, 0.10, 0.25; and X = S, Se and Te) were mixed

and the mixtures were sealed in quartz glass ampoules under high vacuum. The ampoules were then heated at 850 °C for 7 days, with a heating and cooling rate of 5 °C/min.

6.4.2 Characterisation of Vanadium-doped Molybdenum Dichalcogenides

To obtain morphological information and elemental mappings of V-doped molybdenum dichalcogenides, SEM and EDX were executed with the aid of a JEOL 7600F field-emission scanning electron microscope, at 5 kV and 15 kV acceleration voltage respectively. Raman spectroscopy was achieved using an inVia Raman microscope from Renishaw (England) under the backscattering geometry mode. The microscope was equipped with a charge-coupled device detector, a 50x objective lens alongside a 532 nm DPSS laser.

6.4.3 Electrochemical Studies of Vanadium-doped Molybdenum Dichalcogenides

Individual V-doped molybdenum dichalcogenides (5.0 mg mL^{-1}) were suspended in *N,N*-dimethylformamide (DMF), and subjected to ultrasonication for 20 mins to yield homogeneous suspensions. The surfaces of the glassy carbon (GC) electrodes were polished prior to each measurement with 9 μm , 5 μm , 1 μm and 0.3 μm alumina slurries sequentially, rinsed with ultrapure water and thoroughly dried by means of dry polishing on a polishing pad. Before pipetting the materials of interest onto the GC, the materials were sonicated for 1 min to maintain the well dispersed suspensions. Thereafter, aliquots of the material suspension (3 μL) were pipetted onto the surface of GC and left to dry under ambient conditions.

A μ Autolab type III electrochemical analyser (Eco Chemie, The Netherlands) was employed to perform cyclic voltammetry (CV) and linear sweep voltammetry (LSV) measurements. All electrochemical measurements were performed using a 10 mL electrochemical cell under ambient conditions with a classical three-electrode setup, where the modified GC functions as

the working electrode, Ag/AgCl as reference electrode and a platinum electrode as the auxiliary electrode, with the exception of electrocatalytic HER, OER and ORR studies which uses GC electrode as the auxiliary electrode instead of platinum. Inherent electrochemistry studies were carried out in phosphate buffer solution (50 mM PBS, pH 7.2) using the cyclic voltammetry mode at a scan rate of 100 mV s^{-1} . Heterogeneous electron transfer (HET) rates of V-doped molybdenum dichalcogenides were investigated using ferro/ferricyanide redox probe (5 mM) in KCl (0.1 M) supporting electrolyte, under CV mode at 50 mV s^{-1} scan rate. Meanwhile, HER, ORR and OER studies were executed using the LSV mode in 0.5 M sulfuric acid, 0.1 M potassium hydroxide (KOH) and 1.0 M KOH respectively. The scan rate used to perform LSV for HER, ORR and OER are as follow; 2 mV s^{-1} , 5 mV s^{-1} and 5 mV s^{-1} .

6.4.4 DFT Calculations

First principle DFT calculations were conducted with the aid of the Vienna ab initio simulation package (VASP).^{62,63} The cut-off energy of the plane-wave basis was calibrated to 250 eV and the exchange-correlation energy was approximated utilising the van der Waals-corrected functional optB86b-vdW.^{64,65} The edge was modelled using a 1D nanostripe cut out of a supercell of a single layer of the 2H phase, where the supercell was four unit cells thick and had an edge length of four unit cells (4x4). Although this model allows one to calculate the various dopant concentrations and hydrogen coverages of the edge, it is limited to a two-fold reconstruction of the edge.⁶⁶ Sampling of the Brillouin zone was conducted at $1 \times 3 \times 1$ k-points in which the three k-points were situated along the y axis at which the nanostripe was repeated systematically. The nanostripe layers were segregated by approximately $10 \text{ \AA}$ of vacuum in both non-periodic directions, with Mo terminated edge being exposed at one side of the nanostripes and S terminated edge on the other. The Mo-edge was found to be energetically favoured over the S-edge under atmospheric conditions.⁶⁷ Undercoordinated Mo atoms along

the edges were stabilised by introducing supplementary sulphur atoms, giving the edge an S coverage of 50 %. HER activities of the edges were examined via computing the ΔG_H .⁶⁸ In accordance with Sabatier principle, ΔG_H of an exemplar catalyst for HER should be as near to zero as possible, thus materials with thermoneutral ΔG_H (i.e. $\Delta G_H \approx 0$ eV) are deemed effective for HER catalysis. The ΔG_H was acquired by calculating the differential adsorption energy of hydrogen ΔE alongside the addition of thermal corrections (the difference in the zero-point energy, entropy, and internal energy amidst the adsorbed hydrogen and free hydrogen). The corrections have been reported to be independent of the specific adsorption site, hence ΔG_H was computed as ΔE plus a thermal correction constant of 0.29 eV under standard conditions.⁶¹ In this study, we focused on the binding of hydrogen onto the metal edge of MoS₂ and MoTe₂, as edges have been reported as the active sites for HER, while the (0001) basal planes were found to be inert.⁶⁹ The vanadium dopants were introduced by substituting a Mo atom along the metal edge with V. The edge atoms were substituted because according to our calculation, dopants prefer to substitute at the edge rather than in the bulk for both MoS₂ and MoTe₂. We substituted 25%, 50%, and 100% edge atoms, which corresponded to Mo_{0.94}V_{0.06}S₂, Mo_{0.88}V_{0.12}S₂, and Mo_{0.76}V_{0.24}S₂ bulk composition of alloy.

6.5 Supporting Information

Figure 6.S1. SEM-EDX images of (a) $\text{Mo}_{0.95}\text{V}_{0.05}\text{S}_2$, (b) $\text{Mo}_{0.90}\text{V}_{0.10}\text{S}_2$ and (c) $\text{Mo}_{0.75}\text{V}_{0.25}\text{S}_2$ with scale bars corresponding to 25 μm .

Figure 6.S2. SEM-EDX images of (a) $\text{Mo}_{0.95}\text{V}_{0.05}\text{Se}_2$, (b) $\text{Mo}_{0.90}\text{V}_{0.10}\text{Se}_2$ and (c) $\text{Mo}_{0.75}\text{V}_{0.25}\text{Se}_2$ with scale bars corresponding to 25 μm .

Figure 6.S3. SEM-EDX images of (a) $\text{Mo}_{0.95}\text{V}_{0.05}\text{Te}_2$, (b) $\text{Mo}_{0.90}\text{V}_{0.10}\text{Te}_2$ and (c) $\text{Mo}_{0.75}\text{V}_{0.25}\text{Te}_2$, with scale bars corresponding to 25 μm .

Table 6.S1. Elemental compositions of vanadium-doped molybdenum dichalcogenides based upon energy dispersive X-ray spectroscopy.

Materials	Mo at. %	V at. %	S at. %	Se at. %	Te at. %	C at. %	O at. %	V/Mo	X/Mo
Mo _{0.95} V _{0.05} S ₂	11.23	0.12	15.08			69.25	4.33	0.0107	1.342
Mo _{0.90} V _{0.10} S ₂	16.00	0.59	20.99			56.97	5.45	0.0369	1.312
Mo _{0.75} V _{0.25} S ₂	18.75	2.13	26.40			45.60	7.11	0.1136	1.408
Mo _{0.95} V _{0.05} Se ₂	8.18	0.09		13.90		72.27	5.57	0.0110	1.699
Mo _{0.90} V _{0.10} Se ₂	13.58	0.28		18.45		58.37	9.32	0.0206	1.359
Mo _{0.75} V _{0.25} Se ₂	9.71	0.83		13.14		62.79	13.53	0.0855	1.353
Mo _{0.95} V _{0.05} Te ₂	13.3	0.61			16.05	62.62	7.42	0.0457	1.207
Mo _{0.90} V _{0.10} Te ₂	6.63	0.43			8.86	77.14	6.95	0.0649	1.336
Mo _{0.75} V _{0.25} Te ₂	16.28	2.95			25.72	46.55	8.51	0.1812	1.580

Figure 6.S4. Cyclic voltammograms depicting the inherent electrochemistry of vanadium-doped molybdenum disulfides during the (a) anodic and (b) cathodic scans. Cyclic voltammetry was conducted using 50 mM PBS (pH 7.2). Cyclic voltammetric scans were initiated at 0.0 V vs Ag/AgCl and the arrows indicate the direction of the initial scan.

Figure 6.S5. Cyclic voltammograms depicting the inherent electrochemical behaviours of vanadium-doped molybdenum diselenides during the (a) anodic and (b) cathodic scans. Cyclic voltammetry was conducted using 50 mM PBS (pH 7.2). Cyclic voltammetric scans were initiated at 0.0 V vs Ag/AgCl and the arrows indicate the direction of the initial scan.

Figure 6.S6. Cyclic voltammograms depicting the innate electrochemistry of vanadium-doped molybdenum ditellurides during the (a) anodic and (b) cathodic scans. Cyclic voltammetry was conducted using 50 mM PBS (pH 7.2). Cyclic voltammetric scans were initiated at 0.0 V vs Ag/AgCl and the arrows indicate the direction of the initial scan.

Table 6.S2. Table of calculated heterogeneous electron transfer (HET) rate constant, k_{obs}^0 , for bare GC and vanadium-doped molybdenum dichalcogenides.

Materials	K_{obs}^0 (cm/s)
Bare GC	1.51E-04
$\text{Mo}_{0.95}\text{V}_{0.05}\text{S}_2$	1.63E-03
$\text{Mo}_{0.90}\text{V}_{0.10}\text{S}_2$	1.95E-03
$\text{Mo}_{0.75}\text{V}_{0.25}\text{S}_2$	3.17E-03
$\text{Mo}_{0.95}\text{V}_{0.05}\text{Se}_2$	4.41E-03
$\text{Mo}_{0.90}\text{V}_{0.10}\text{Se}_2$	4.00E-03
$\text{Mo}_{0.75}\text{V}_{0.25}\text{Se}_2$	3.58E-03
$\text{Mo}_{0.95}\text{V}_{0.05}\text{Te}_2$	3.64E-04
$\text{Mo}_{0.90}\text{V}_{0.10}\text{Te}_2$	6.26E-04
$\text{Mo}_{0.75}\text{V}_{0.25}\text{Te}_2$	5.70E-04

Figure 6.S7. OER polarisation curves for (a) Mo_{1-y}V_yS₂, (b) Mo_{1-y}V_ySe₂ and (c) Mo_{1-y}V_yTe₂.

Bar chart displaying the consolidated data of the average overpotentials at a current density of 10 mA cm⁻² (d). OER measurements were conducted in 1.0 M potassium hydroxide at a scan rate of 5 mV s⁻¹.

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Part 2

Chapter 7

Cytotoxicity of Group 5 Transition Metal

Ditellurides

7.1 Introduction

7.2 Results and Discussion

7.3 Conclusion

7.4 Experimental Section

7.5 Supporting Information

The results and discussion in this chapter were published in the following journal article:

Cytotoxicity of Group 5 Transition Metal Ditellurides (MTe₂; M=V, Nb, Ta)

H. L. Chia, N. M. Latiff, Z. Sofer, M. Pumera, *Chem. Eur. J.* **2018**, *24*, 206-211.

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7.1 Introduction

Ever since the isolation of graphene from graphite by Novoselov et al. in 2004,¹ two-dimensional (2D) nanomaterials have gained intensive academic and industrial research interest due to their extraordinary and unique properties. To date, a vast range of 2D nanomaterials have been reported, such examples include transition metal dichalcogenides (TMDs), graphitic carbon nitride (g-C₃N₄), hexagonal boron nitride (h-BN) and black phosphorus.²⁻⁸ Among 2D nanomaterials, TMDs are deemed as one of the most widely explored classes of layered compounds due to their extraordinary electrochemical properties.⁹⁻

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TMDs have a generic chemical formula of MX₂, where M denotes a transition metal (for instance Ti, V, Nb, Ta, Mo, W and so on) of a +4 oxidation state and X corresponds to a chalcogen (S, Se or Te) with a -2 oxidation state.^{9,13,14} Different permutations of these elements give rise to approximately 60 different TMDs, where two-thirds of these compounds are reported to assume layered structures. Generally, transition metals from Group 4–7 generate compounds that are predominantly layered while some Group 8–10 transition metals give rise to three-dimensional crystal compounds.¹⁴⁻¹⁶ Many TMDs possess a layered structure akin to graphite and within each layer of TMDs, the transition metal is sandwiched amidst two chalcogens, hence resulting in the MX₂ stoichiometry. It has been reported that the bonds within each layer are covalent, whereas the bonds between two adjacent MX₂ layers are typically van der Waals forces of interactions, thus allowing TMDs to be exfoliated down to single layers.^{9,13-15,17-19}

TMDs possess unique and advantageous properties, including large surface area, tuneable band gaps and stability against photocorrosion. They also exhibit low shear resistance as a consequence of the weak van der Waals interactions amidst layers. As such TMDs have attracted much research

attention and led to numerous and diversified applications.^{13,14,20,21} Such applications include electrocatalysts, high performance electrochemical supercapacitors, biosensors, field-effect transistors (FETs), photodetectors, lubricant additives, heterostructure junctions and photovoltaics.^{5,9,13,14,17-32} In the last few years, enormous efforts have been committed to exploring the different applications of Group 5 transition metal ditellurides with VTe₂ being deemed as a promising hydrogen evolution reaction (HER) electrocatalyst,³³ NbTe₂ being reported to be an excellent lubricant additive,²⁰ and TaTe₂ having potential applications as supercapacitors.³⁴ Hitherto potential toxicities presented by Group 5 transition metal ditellurides is poorly understood. There is still much to address to comprehend the cytotoxicity behaviours of TMD materials, in particular bulk Group 5 transition metal ditellurides, which to our knowledge has been undetermined. It is imperative to fill this literature gap to inform users of the possible health implications involved in light of their probable implementation in industries.

As such, we conducted the cytotoxicity study of bulk Group 5 transition metal ditellurides (MTe₂; VTe₂, NbTe₂, TaTe₂) with A549 human lung carcinoma epithelial cells. The cell line was specifically selected as lungs is one of the main potential target organs during the processing of nanomaterials and it is one of the most frequently used cell types in inhalation toxicity studies, allowing easy comparison between our results obtained with other reports.^{10,35-}
³⁹ The remaining cell viability of A549 cells following 24 h of incubation with various MTe₂ concentrations were measured and analysed using water-soluble tetrazolium salt (WST-8) assay. Control experiments were also performed in the absence of cells to determine potential sources of particle-induced interferences amidst MTe₂ and the cell viability assay markers, which could cause false-positive or false-negative results to be produced.³⁹⁻⁴¹

7.2 Results and Discussion

It has been reported that physicochemical properties such as size and chemical compositions could contribute to the nanomaterials' toxicities.⁴²⁻⁴⁵ Hence prior to assessing the cytotoxicity of the Group 5 transition metal ditellurides, characterisation of the materials were necessary and carried out through scanning electron microscopy (SEM), scanning transmission electron microscopy (STEM), energy dispersive X-ray spectroscopy (EDX), X-ray diffraction (XRD) and dynamic light scattering (DLS).

Through the SEM and STEM images in Figure 7.1, it is deduced that all samples (VTe_2 , NbTe_2 and TaTe_2) display structural features typical of bulk materials. VTe_2 and TaTe_2 are predominantly seen to be layered structures while NbTe_2 manifest in irregular shapes. Furthermore, STEM unveils an extensive range of particle sizes spanning from 80 nm to 3 μm in the lateral dimension.

Figure 7.1. SEM (a,c,e) and STEM (b,d,f) images of VTe_2 , NbTe_2 and TaTe_2 respectively. The scale bars represent 1 μm .

Another characterisation technique used was EDX, which provides elemental mappings of the elements present and illustrates the elemental distributions throughout the materials. The SEM-EDX images in Figure 7.2 suggest that the Group 5 transition metals and tellurium are well distributed in the materials and that the main difference present is the transition metal element.

Figure 7.2. SEM-EDX images of bulk VTe_2 , NbTe_2 and TaTe_2 . The scale bars represent 10 μm .

XRD data (refer to Supporting Information, Figure 7.S1) show single phase purity for all ditellurides with a strong preferential orientation along (00l) direction, which originates from the layered character of the materials. It was also determined that all phases are monoclinic structures with a C2/m space group. The particle size distribution measurements conducted via DLS (Figure 7.S2) showed that all three materials exhibit multimodal characteristics, which corroborates with the wide range of sizes determined from STEM. However, the DLS size ranges differ from the STEM measurements, where DLS data show a predominant distribution peak with sizes ranging 500–1000 nm and two smaller fractions with sizes around 100 and 200 nm. This narrower size range from DLS measurements could be attributed to the faster settling of the larger particles.

Upon the successful characterisation of the materials, we proceeded to determine the toxicity effects of these Group 5 transition metal ditellurides towards A549 cells. To evaluate the materials' toxicities, A549 cells were exposed to Group 5 transition metal ditellurides and WST-8 assay was utilised to measure the remaining cell viabilities. WST-8, a mitochondrial activity-based assay, works on the principle that soluble formazan is produced as a result of cellular reduction by dehydrogenase activities in viable cells.^{10,46-48} By normalising the absorbance intensities obtained with a control setup, in which cells were not incubated with test materials, the extent of toxicity exhibited by the Group 5 ditellurides TMDs towards A549 cells could be determined. Data acquired from WST-8 assay is illustrated in Figure 7.3.

Figure 7.3. Cell viability percentages were measured using WST-8 assay after incubating varying concentrations of Group 5 transition metal ditellurides with A549 cells for 24 hours.

As depicted in the data above, a dose-dependent trend for VTe₂ was observed. Furthermore, VTe₂ exhibited significant cytotoxicity towards A549 cells, whereby even at low concentrations of 25 µg/mL more than 50% of the cells were not viable and at the highest concentration of 200 µg/mL less than 10% of the cells remained viable. This result is in line

with previous toxicity studies conducted on exfoliated VTe₂, which demonstrated that VTe₂ was highly toxic.¹⁰ On the other hand, the cell viability percentages for NbTe₂ and TaTe₂ remained relatively high ($\geq 60\%$), even at high material concentrations above 50 $\mu\text{g/mL}$, denoting that these two Group 5 transition metal ditellurides induce low toxicological effects towards the A549 cells. Therefore, in this study, the degree of cytotoxicity displayed by the bulk Group 5 transition metal ditellurides can be deemed as VTe₂ being the most toxic while NbTe₂ and TaTe₂ are of comparable toxicity.

One plausible reason that could account for VTe₂ being more toxic is that VTe₂ might produce more reactive oxygen species (ROS) than NbTe₂ and TaTe₂. Studies have suggested that solid surfaces could directly interact with endogenous biological target molecules or indirectly interact with hydrogen peroxide or oxygen molecules, by transferring electron or H[•] to/from solid surfaces to produce ROS. These ROS may in turn cause oxidative stress to the cells and damage cellular proteins, lipids and nucleic acids.^{49,50} A study by Chia and colleagues demonstrated that the heterogeneous electron transfer (HET) rate of VTe₂ is significantly faster than NbTe₂ and TaTe₂.³³ A faster HET rate indicates that a lower overpotential is required for an electrochemical reaction to occur, hence more oxidation/reduction reactions could have occurred on the surface of VTe₂ to produce more ROS, causing VTe₂ to be significantly more toxic.

From the EDX images in Figure 7.2, the main fundamental difference between the materials is the transition metals present, hence one could deduce that the different transition metal present in the Group 5 transition metal ditellurides is the major influencing factor for the cytotoxicity properties. The trend observed in this study coincides with the trend from the toxicities study of the Group 5 transition metal (IV) compounds, whereby it was observed that vanadium compounds are generally the most toxic among the three elements while niobium and tantalum

compounds are considered to be relatively non-toxic.⁵¹ Toxicity studies on rats have shown that the LD₅₀ values for vanadium compounds range from a few tens to hundreds mg/kg body weight,^{52,53} while the reported LD₅₀ values for niobium and tantalum compounds ranges from several hundreds to thousands mg/kg body weight.⁵⁴⁻⁵⁶

Several studies have reported that nanomaterials could interact with the viability markers in cell viability assays, thus causing false readings to be produced.³⁹⁻⁴¹ As such, control experiments were performed in the absence of cells to determine potential sources of material-induced interferences and assess the suitability of the assays. Therefore, we set out to determine possible forms of material-induced interferences for two commonly used assays that have similar working mechanisms, WST-8 and methyl thiazolyldiphenyl-tetrazoliumbromide (MTT) assays.

One possible interference induced by the nanomaterials on WST-8 is that the nanomaterials have the ability to reduce the active tetrazolium salt to form formazan, thereby generating false-positive results, causing an overestimation in the number of viable cells.⁵⁷⁻⁵⁹ In order to check for any interference, a control experiment was carried out by incubating varying concentrations of test materials with WST-8 assay for an hour in a cell-free condition to check for potential formazan production through materials-induced reductions. Figure 7.4 details the data from the WST-8 control experiments.

Figure 7.4. WST-8 control experiment: Formazan generated upon an hour of WST-8 incubation with varying concentrations of Group 5 transition ditelluride materials.

It can be observed that there was nominal material-induced reduction of WST-8 by the bulk Group 5 transition metal ditellurides, where the amount of formazan produced after an hour of incubation with the test materials were within the $\pm 15\%$ of the quantity attained from the control containing only 10% WST-8. Furthermore, during the cell viability assay, a washing step was introduced prior to the introduction of the assay reagent, to minimise the test material-induced interference, hence it is postulated that the interference induced by Group 5 transition metal ditellurides is insignificant.

Apart from using WST-8 assay, another commonly used MTT assay was also considered for use as a cell viability assay in this study. MTT working principle is similar to WST-8 such that in the presence of viable cells, MTT will be converted to purple coloured formazan crystals with a maximum absorbance near 570 nm. There exist two possible interferences induced by nanomaterials on MTT. Firstly, similar to WST-8, the nanomaterials could reduce the active tetrazolium salt to form formazan on its own, thereby generating a false-positive result.

Secondly, the nanomaterials could interfere through binding with the formazan crystals formed thereby generating a false-negative result causing an underestimation in the number of viable cells.^{41,57-62} To investigate whether the test materials could induce a reduction of the active tetrazolium salt, different concentrations of the Group 5 transition metal ditellurides were exposed with the MTT assay in a cell-free environment. To study the probable binding effect, ascorbic acid was added to different concentrations of MTe₂ to induce the formazan crystals formation from the MTT assay. If MTe₂ materials bind to the formazan crystals, one would expect to see a decrease in the percentage of formazan with increasing amounts of the test materials. The results from the MTT control experiment are displayed in Supporting Information, Figure 7.S3.

It was found that a high amount of interference was observed between VTe₂ with the MTT assay, whereby the amount of formazan produced increases significantly to approximately 280% at the highest concentration tested whereas material-induced reduction of the MTT by NbTe₂ and TaTe₂ is insignificant. It was also revealed that there is minimal binding of the Group 5 transition metal ditellurides with the formazan crystals. Due to the significant amount of tetrazolium salt reduction by VTe₂, it was deemed that the MTT assay would not be a fair assay to compare the toxicity between the Group 5 transition metal ditelluride materials and hence MTT cell viability assay was omitted in this study.

To form a better understanding of Group 5 transition metal ditellurides' relative toxicities, a comparison study can be conducted between toxicological data obtained from this project and data of other TMDs from literature. Through comparison with findings from Group 6 TMDs (namely MoS₂, WS₂, and WSe₂) cytotoxicity study conducted under identical conditions,⁶³ we noted that the Group 5 transition metal ditellurides are generally more toxic than the Group 6

TMDs. However, an exception was observed in WSe₂ where it is more toxic than the Group 5 transition metal ditellurides with only VTe₂ being more toxic than WSe₂ (Table 7.1).

Table 7.1. Normalised cell viability percentages attained using WST-8 assays, upon 24 h exposure with 200 µg/mL of TMDs. Data for Group 6 TMDs are attained from reference 63.

Materials	Cell Viability (%)					
	MoS ₂	WS ₂	WSe ₂	VTe ₂	NbTe ₂	TaTe ₂
WST-8 Assay	80.7	83.6	45.0	7.5	71.6	65.1

Furthermore, we scrutinized the influence of transition metal elements within the same group on the toxicological behaviour of TMD compounds. For instance, when the chalcogen element is kept constant between MoS₂ and WS₂, an increase in cell viabilities was observed down the transition metal group. A similar trend was also observed in our study where the cell viability generally increases down the transition metal group, suggesting that the toxicities of TMDs could generally decrease down the transition metal group. However, more studies have to be conducted for verification.

7.3 Conclusion

With Group 5 transition metal ditellurides gaining an intensive amount of academic and industrial research interest, there is a need to investigate the potential toxicity effects posed in view of their possible implementation in industries. This chapter investigated the cytotoxicity effects of Group 5 transition metal ditellurides towards A549 cells using WST-8 assay. The findings of this study highlighted that VTe₂ is highly toxic and cell viability is affected by the concentrations of VTe₂ while NbTe₂ and TaTe₂ are both mildly toxic with comparable results.

Control experiments of WST-8 and MTT were carried out to assess the interferences posed by the test materials and the suitability of the assays. Results from the WST-8 suggest minimal test materials-induced reduction of WST-8 indicating that there was insignificant interference posed. On the other hand, MTT data shows a high degree of interference with VTe₂, making MTT an unreliable cell viability assay for a fair comparison between the Group 5 transition metal ditellurides and was thus omitted from this study. In our view, these results constitute an excellent initial step towards the understanding of the toxicity effects of the bulk Group 5 transition metal ditellurides. However, more studies have to be conducted to explore the mechanisms behind the biological responses to nanomaterial exposures to have a full understanding of the toxicological effects of Group 5 transition metal ditellurides.

7.4 Experimental Section

7.4.1 Synthesis of Group 5 Transition Metal Ditellurides

Vanadium, niobium, tantalum and tellurium were purchased from Chempur, Germany. Quartz glass ampoules were employed for the synthesis of the Group 5 transition metal ditellurides under high vacuum. For the synthesis of 6g of TMDs, stoichiometric quantities of metal and chalcogen were deposited in an ampoule, and an excess of 1 wt.% of telluride was used throughout all the synthesis procedures. Thereafter, a vacuum and an oxygen/hydrogen welding torch were employed to seal the ampoules to a base pressure below 5×10^{-3} Pa. The ampoules were heated at 500 °C for two days, followed by 800 °C for another two days and lastly 900 °C for a day. The heating and cooling rate were set at 5 °C/min.

7.4.2 Materials Characterisation

The Group 5 transition metal ditellurides were characterised using JEOL 7600F to obtain the SEM, STEM and EDX images. SEM was carried out under SEI mode with a 5.00 kV accelerating voltage (AV) and a working distance (WD) of 4.7 mm while EDX was conducted at 15.00 kV AV and 14.8 mm WD. STEM was performed using the TED mode at 30.00 kV AV and 8.2 mm WD.

XRD was conducted using a Bruker diffractometer (D8 Discoverer) under Bragg–Brentano para-focusing geometry mode. A CuK_α radiation ($\lambda = 0.15418 \text{ nm}$, $U = 40 \text{ kV}$, $I = 40 \text{ mA}$) was employed as the X-ray source and the diffraction patterns were collated between the angular range of 5° and 80° of 2θ with step sizes equating to 0.015° (2θ). Analysis of the XRD data was carried out using the software package EVA.

Dynamic light scattering measurements of the bulk Group 5 transition metal ditelluride samples were conducted with a Zetasizer Nano ZS from Malvern Instruments (England). Measurements were performed at 20°C with glass cuvettes.

7.4.3 Cell Culture

A549, human lung carcinoma epithelial cells purchased from PAA Laboratories was cultured using Dulbecco's modified eagle medium (Life Technologies) and supplemented with 1 % penicillin/streptomycin mixture from Capricorn and 10% fetal bovine serum from Life Technologies. Incubation took place at 37°C under an atmosphere with 5% CO_2 . Similar incubation conditions were adopted for all incubation procedures in this study, unless stated otherwise.

7.4.4 WST-8 Cell Viability Assay

For cell viability assays, 20,000 cells (300 μ L) were seeded into individual wells of the 24-well plate. Ensuing 24 hours of cell seeding, the wells were rinsed with phosphate buffer solution (PBS, Life Technologies) of pH 7.2 and were exposed to various test materials concentrations (300 μ L). For the negative control setup, cells were not introduced with our materials of interest, while cells were supplemented with 10% dimethyl sulfoxide (DMSO, Tedia) in the positive control setup. Following a day of incubation with the test materials, the wells were rinsed twice with PBS and 300 μ L of 10% WST-8 acquired via Dojindo Molecular Technologies was pipetted into the individual wells and subjected to further incubation (1 hour). After incubating for an hour, the solution mixture was subjected to centrifugation and the supernatant was pipetted into a 96-well plate (100 μ L/well) for absorbance measurement at 450 nm.

7.4.5 WST-8 Control Experiment

To evaluate whether the test materials would induce the reduction of tetrazolium salt, various concentrations of test materials were incubated with 10% WST-8 for an hour. After incubating for one hour, the solution was centrifuged and 100 μ L of the supernatant was pipetted into a 96-well plate for spectroscopic absorbance measurement at a wavelength of 450 nm.

7.4.6 MTT Control Experiment

Two control experiments were executed to test for material-induced formation of formazan and determine whether the test materials would bind to the formazan crystals. To probe for any probable material-induced formation of formazan, 1 mg/mL of MTT was introduced to varying concentrations of test materials and incubated for three hours in a 24-well plate. Following the 3-hour incubation, the MTT-test material mixture (300 μ L/well) was discarded, an equal

amount of DMSO was subsequently added and the plate was gently agitated for 10 minutes to dissolve the formazan crystal formed. The dissolved formazan solution was centrifuged, and the supernatant (100 μ L) was pipetted into a fresh 96-well plate for absorbance measurement at 570 nm wavelength.

To investigate if the test materials would bind to the formazan crystals, 4 mM of ascorbic (160 μ L/well) was introduced to 200 μ L of MTT-test material solution following a three-hour incubation period of 1 mg/mL of MTT assay with the test materials. The wells were further subjected to another hour of incubation, then DMSO (720 μ L/well) was introduced, and the wells were gently agitated for 10 minutes to dissolve the insoluble formazan generated. Finally, absorbance readings of the solutions were acquired spectroscopically in a process akin to the MTT control experiment that was used to test for probable test material-induced formation of formazan.

7.5 Supporting Information

Figure 7.S1. Diffractograms of vanadium, niobium and tantalum ditellurides.

Figure 7.S2. Particle size distributions of Group 5 transition metal ditellurides measured by dynamic light scattering.

Figure 7.S3. MTT control experiments: (a) Formazan generated upon 3 hours of MTT incubation with varying concentrations of Group 5 transition metal ditellurides. (b) Extent of binding between formazan crystals generated and distinct concentrations of Group 5 transition metal ditelluride materials.

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Part 2

Chapter 8

Cytotoxicity of Shear Exfoliated Pnictogen Nanosheets

8.1 Introduction

8.2 Results and Discussion

8.3 Conclusion

8.4 Experimental Section

8.5 Supporting Information

The results and discussion in this chapter were published in the following journal article:

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8.1 Introduction

The isolation of graphene by Novoselov et al. has sparked intensive research interest in two-dimensional (2D) nanomaterials amongst the scientific community.¹ 2D nanomaterials have become one of the hottest research fields in recent years with more than 30,000 published academic articles per year.²⁻⁵ To date, a wide array of 2D nanomaterials including hexagonal boron nitride (h-BN), transition metal dichalcogenides (TMDs), graphitic carbon nitride (g-C₃N₄) and elemental 2D nanomaterials (borophene, silicene, phosphorene, arsenene, antimonene etc.) has been reported.⁴⁻¹⁰

Though graphene has awed the scientific community with its fascinating properties, the zero band gap it possesses leads to a drawback in optoelectronics applications.^{3,9,11-13} On the other hand, the widely explored semiconducting TMDs possess electronic band gaps that are smaller than 2 eV, limiting their applications in flexible displays and their low carrier mobilities causes drawbacks in optoelectronic device applications.^{9,14,15} Owing to these reasons, efforts have been devoted to exploring alternative 2D nanomaterials with superior properties. Meanwhile, the experimental achievement of phosphorene, which exhibits fascinating electronic, physical and optical properties has ignited recent interest in other Group 15 elemental 2D nanomaterials.^{2,9,16}

Down Group 15, with increasing atomic weight, the trends of the pnictogens shift from non-metallic to metallic where nitrogen (N) and phosphorus (P) are reported to be non-metals, while arsenic (As) and antimony (Sb) possess metalloid characteristics and bismuth (Bi) is being deemed as a metal. As such, P reportedly possess many similarities with As but possesses much fewer parallels with Bi.^{17,18} Studies have suggested that pnictogens possess several phases, and the heavier pnictogens (As, Sb and Bi) have been reported to crystallise in their most stable

allotropic form, the rhombohedral layered structure.¹⁸⁻²⁰ These rhombohedral structured pnictogens are brittle and can be easily exfoliated to yield nanosheets of arsenene, antimonene and bismuthene.^{18,21,22}

Group 15 elemental nanosheets possess a broad band gap ranging from 0.36 to 2.62 eV, which corresponds to the light emitting wavelength that spans from near-infrared to visible light. Furthermore, these pnictogen nanosheets present high carrier mobilities up to several thousand square centimetres per volt-second, exhibiting high anisotropy.^{9,13,15,16,23,24} These unique and superior properties of pnictogen nanosheets have spurred intensive research efforts and led to the discovery of their numerous and diversified applications. Some potential applications include optical Kerr materials, photonic devices, pnictogens decorated microfibers, high speed transistors, catalysts, spintronic devices and flexible 2D electronics.^{15,23-29} Though intensive efforts have been put in place to study the synthesis, properties and applications of these heavy pnictogen nanosheets, little effort has been devoted to evaluating the toxicological behaviour of these nanosheets. In view of the probable implementation of pnictogen nanosheets in industrial settings, it is important for this literature gap to be explored to notify users of the probable health hazards.

To provide insights to fill the literature gap, cytotoxicity study of pnictogen (arsenic, antimony and bismuth) nanosheets was carried out on adenocarcinoma human lung epithelial A549 cells. The particular cell line was selected as lungs are deemed to be amongst the primary potential target organs when handling nanomaterials.³⁰⁻³⁵ Furthermore, A549 is widely used in inhalation toxicity studies, allowing comparison to be made easily between results obtained with other reports to yield a comprehensive understanding of pnictogen nanosheets relative toxicities among 2D nanomaterials.^{32,36} Water-soluble tetrazolium salt (WST-8) and methyl-

thiazolyldiphenyl-tetrazolium bromide (MTT) assays were employed to study and analyse the remaining cell viabilities of A549 cells upon 24 hours of treatment with varying pnictogen nanosheets concentrations. Apart from studying the cytotoxic effects of pnictogen nanosheets, this chapter also investigated and compared the cytotoxicity with pnictogen (III) salts to generate a deeper understanding of the nanosheets relative toxicities. Lastly, control studies were carried out in cell-free conditions to determine possible materials-induced interferences between pnictogens and their salts with cell viability assays markers, which could result in false results to be generated.³⁷⁻³⁹

8.2 Results and Discussion

Several studies have suggested that physicochemical properties, for instance, chemical compositions, size, aggregation and surface properties could affect nanomaterials toxicities.⁴⁰⁻

⁴⁴ Hence it is cardinal to characterise the nanomaterials prior to the cytotoxicity assessment of pnictogen nanosheets. Characterizations of the nanosheets were accomplished using scanning electron microscopy (SEM), dynamic light scattering (DLS), zeta potential, transmission electron microscopy (TEM), atomic force microscopy (AFM) and energy dispersive x-ray spectroscopy (EDX).

The morphologies and chemical compositions of the shear exfoliated pnictogen nanosheets were observed via SEM, TEM-EDX and HRTEM and are represented by Figures 8.1, 8.2 and Figure 8.S1 respectively. Both SEM and TEM images of the exfoliated pnictogen nanosheets depicted in Figure 8.1 and Figure 8.S1 depict arrays of heterogenous sub-micron nanosheets. Differences in the morphology of nanosheets amidst individual pnictogens were observed and these differences are attributed to the intense transformations in the degree of anisotropy. Due to its high degree of anisotropy, arsenic was able to be shear exfoliated to form few-layered

nanosheets with large sheet sizes and wrinkled structures. Meanwhile, antimony possesses significantly higher interlayer bonds, thus giving rise to nanosheets that are of few-layer thickness and approximately an order of magnitude smaller when compared with arsenic nanosheets. On the other hand, bismuth undergoes a downsizing process dominantly over exfoliation due to its minimal degree of anisotropy and generated heterogeneous individual particles, with sizes varying from tenth to hundreds of nanometers.

Figure 8.1. SEM images of exfoliated As (a), Sb (b) and Bi (c) nanosheets, with scale bars corresponding to 1 μm .

Another factor that influences the toxicity of the materials is the chemical compositions. Hence, EDX was used as a characterisation technique to do an elemental mapping of the present elements and their distribution. Figure 8.2 below indicates the existence of the respective pnictogen in the nanosheets and the presence of oxygen due to surface oxidation. The data in the EDX study corroborates with the XPS results obtained by Gusmão et al.,¹⁸ which shows partial oxidation of the exfoliated pnictogen nanosheets.

Figure 8.2. TEM-EDX images and respective mapping of elements for the shear exfoliated pnictogens: As_{SE}, Sb_{SE} and Bi_{SE}. The scale bars correspond to 1 μm for Sb_{SE} and 500 nm for As_{SE} and Bi_{SE}.

AFM measurements were also carried out to assess the thickness of the shear exfoliated pnictogen nanosheets. AFM data (see Supporting Figure 8.S2) point out that As_{SE} and Sb_{SE} have comparable thicknesses of an average of *ca.* 5.6 nm while Bi_{SE} yielded significantly thicker nanosheets, with an average thickness of 82.5 nm.

DLS measurements were carried out to determine the size ranges of the shear exfoliated pnictogen nanosheets. DLS data (Figure 8.S3) suggest that the pnictogen nanosheets display broad particle size distribution profiles which arises as a consequence of the anisotropic characteristics of the particles' platelet-like structure. As exhibits a broad size distribution peak ranging from 220–615 nm, with a maxima at 343 nm, while Sb displays a relatively

narrow size distribution ranging from 222–341 nm, with a maxima at approximately 260 nm and Bi having a wide size distribution ranging from 43–630 nm, with a maxima at 236 nm. Furthermore, zeta potential studies reveal that all pnictogen nanosheets possess negative charges, where As, Sb and Bi nanosheets possess a charge of -25.5 , -26.3 and -29.7 mV respectively.

After successfully characterising the materials, this project moved on to study the toxicity effects posed by these shear exfoliated pnictogen nanosheets towards A549 cells. To determine the pnictogens' toxicities, A549 cells were introduced with the nanosheets and the remaining cell viabilities were obtained via WST-8 and MTT assays. Both WST-8 and MTT have similar working principles, whereby corresponding orange and purple formazans are produced as a result of the dehydrogenase activities in viable cells. WST-8 and MTT cell viability assay data are illustrated in Figure 8.3.

Figure 8.3. Remaining cell viabilities obtained from WST-8 assay (a) and MTT assay (b) after incubating A549 cells with varying concentrations of shear exfoliated pnictogen nanosheets for 24 hours.

From the data illustrated in Figure 8.3a, WST-8 assay demonstrates the difference in toxicity trends, whereby cytotoxicity of pnictogens decreases down the group. Shear exfoliated arsenic nanosheets exhibited a dose-dependent trend and is deemed to be the most cytotoxic among the three pnictogens used, while antimony and bismuth nanosheets induce much lower cytotoxicity. When exposed to arsenic nanosheets, cells experienced almost 50% of cell death at a low concentration of 12.5 µg/mL and at the highest test concentration of 200 µg/mL only 12.8% of cells remained viable. On the other hand, the cell viability percentages remained

relatively high at 57.2% and 71.5% following 24 hours of treatment with antimony and bismuth nanosheets respectively.

The results obtained from the MTT assay are presented in Figure 8.3b and display a trend similar to that attained via the WST-8 assay in Figure 8.3a. By and large, the MTT data suggest that pnictogen nanosheets induced a dose-dependent cytotoxicity trend towards A549 cells. Shear exfoliated arsenic nanosheets exhibited a substantial amount of cytotoxic effects towards A549 cells, whereby slightly more than 50% of the cells experienced cell death at a low concentration of 12.5 $\mu\text{g/mL}$ and at 200 $\mu\text{g/mL}$ of arsenic nanosheets, only 8.0% of cells remained viable. Antimony and bismuth nanosheets on the other hand appear to be less toxic whereby slightly more than 50% of the cells experienced cell death at 50 $\mu\text{g/mL}$ of antimony nanosheets and 36.0% cell viability at 200 $\mu\text{g/mL}$ of antimony nanosheets treatment while bismuth nanosheets had a remaining cell viability of 59.2% at 200 $\mu\text{g/mL}$ of bismuth nanosheets. The findings drawn from the MTT data were coherent to that of the WST-8 results and further affirmed the toxicity trend of the pnictogens whereby exfoliated arsenic nanosheets exhibited the highest cytotoxicity effect towards A549 cell, followed by antimony nanosheets and bismuth nanosheets being the least toxic.

Apart from carrying out cell viability studies, control experiments were also executed to determine the reliability of using WST-8 and MTT towards the cytotoxicity assessment of pnictogens. Numerous studies have demonstrated that nanomaterials could interact or adsorb to report markers of *in vitro* toxicological assays, hence distorting the outcomes of the assay, causing false results to be generated. Hence, cell-free experiments were conducted to determine any forms of pnictogens-induced interferences.

Previous studies have mentioned that nanomaterials could interfere with WST-8 by reducing the active tetrazolium salt, thereby causing false-positive cell viability reading to be produced and could lead to an overestimation in the number of viable cells.⁴⁴⁻⁴⁶ To determine whether these pnictogen nanosheets could induce any form of interference towards WST-8 assay, various concentrations of shear exfoliated pnictogen nanosheets ranging from 0 to 200 $\mu\text{g/mL}$ were introduced to WST-8 reagent, incubated and the spectroscopic absorbance data obtained are presented in Figure 8.4a.

Figure 8.4. WST-8 and MTT control experiments: Normalised formazan concentration after an hour of incubation of WST-8 (a) and three hours for MTT assay (b) with distinct concentrations of pnictogen nanosheets.

As seen from Figure 8.4a, both arsenic and bismuth nanosheets induced insignificant amount of WST-8 tetrazolium reduction as the amount of formazan generated upon an hour of

treatment with the pnictogen nanosheets are within $\pm 10\%$ range of the concentration measured from the control setup containing 10% WST-8 solution. On the other hand, shear exfoliated antimony nanosheets induced a moderate amount of interference whereby the concentration of formazan generated increased considerably to about 136% at 200 $\mu\text{g/mL}$ of antimony nanosheets. To minimise and eliminate the presence of interference in the cell viability studies, data analysis of the cell viability studies was conducted using the method described by Rosli et al.,⁴⁷ whereby absorbance readings obtained from the control experiments were deducted from the absorbance data attained from the actual cell viability studies which give rise to the corrected data presented in Figure 8.3a.

Likewise, nanomaterials have also been reported to interfere with MTT by undergoing a redox reaction with the active tetrazolium salt or via binding with the insoluble formazan crystals yielded, thereby leading to the number of viable cells being overestimated or underestimated respectively.⁴⁸⁻⁵¹ To investigate whether these pnictogen nanosheets could induce any form of interference towards MTT assay, various concentrations of shear exfoliated pnictogen nanosheets ranging from 0 to 200 $\mu\text{g/mL}$ were incubated with MTT reagent and the spectroscopic absorbance data obtained are presented in Figure 8.4b.

As depicted in Figure 8.4b, arsenic nanosheets induced the least amount of pnictogens-induced MTT interference, whereby the amount of purple formazan generated upon three hours of incubation with the shear exfoliated arsenic nanosheets is within $\pm 10\%$ range of the concentration attained from the control setup containing 5 mg/mL of MTT solution, indicating an infinitesimal degree of interference induced. Shear exfoliated bismuth nanosheets induced a moderate amount of interference whereby the concentration of formazan generated increased significantly to nearly 137% at 200 $\mu\text{g/mL}$ of bismuth nanosheets. Antimony nanosheets are

deemed to induce the highest amount of interference with the MTT assay among the three pnictogen nanosheets tested, by which the amount of formazan generated increased substantially to about 332% at 200 $\mu\text{g/mL}$ of antimony nanosheets. To eliminate the presence of interference and make the MTT assay a fair assay to compare the toxicological effects of shear exfoliated pnictogens towards A549 cells, data analysis of the remaining cell viability study was conducted similarly to that of the WST-8 data analysis. Absorbance readings obtained from the control experiments were subtracted from the absorbance measurements of the actual cell viability studies to give rise to more reliable data presented in Figure 8.3b.

Apart from carrying out toxicity studies of the pnictogen nanosheets, we also carried out toxicity studies with the corresponding pnictogens (III) oxide salts to yield a better comprehension of their relative toxicities. Cell viability data acquired from WST-8 and MTT assays for the corresponding pnictogens (III) oxide salts are presented in Supporting Information Figure 8.S4a and Figure 8.S5a respectively.

From the data depicted in both Figure 8.S4a and 8.S5a, it was observed that the toxicity trends of the pnictogen (III) oxide salts acquired via both WST-8 and MTT assays are similar to that of the cell viability trends for the shear exfoliated pnictogen nanosheets. Both experiments showed that compounds containing arsenic are the most toxic while antimony and bismuth compounds are found to be comparatively less toxic. Based upon the trends attained from cell viability assessments of both the pnictogen nanosheets and pnictogen (III) salts, it is suggested that the pnictogens present in the shear exfoliated nanosheets play a dominant role in inducing toxicity towards A549 cells.

The trends obtained in this study corroborate well with toxicity studies conducted on pnictogen compounds, whereby the toxicity of pnictogens decreases down the group. Arsenicals are deemed as systemic poisons, with LD₅₀ values ranging from 10 – 250 mg/kg, due to their inhibitory effects on cellular respiratory functions which subsequently decreases mitochondrial energy production. On the other hand, acute antimony and bismuth poisoning are considered remarkably rare where the LD₅₀ values of antimonials range from 152 – 3584 mg/kg,^{52,53} while bismuth compounds range from 2.2 – 22.5 g/kg in rodents.^{54,55}

From both WST-8 and MTT data plotted in Figure 8.5, it is deemed that pnictogen nanosheets are generally less toxic than pnictogen (III) salts, with the exception of arsenic nanosheets being more toxic than their salt counterpart at concentrations above 50 µg/mL. Both bismuth and antimonial drugs have seen long histories in therapeutic purposes, these antimony and bismuth nanosheets could be potentially applied in biomedical fields.⁵⁶⁻⁶¹ Antimony and bismuth nanosheets, like other 2D nanomaterials (graphene and black phosphorus) that possess large surface area to volume ratios and excellent photothermal conversion,^{58,62,63} could possibly hold great promises for clinical translations, such as the development of drug carriers or photothermal transducing agents (PTAs).

Figure 8.5. Comparison of remaining cell viability obtained via (a) WST-8 and (b) MTT assays amidst shear exfoliated pnictogen nanosheets and their corresponding pnictogen (III) oxides across varying test concentrations, upon 24 hours of materials treatment with A549 cell.

To form a better understanding of the pnictogen nanosheets toxicities, a comparison study can also be conducted between toxicological data obtained from this project and data of other 2D materials from literatures. Through comparison with findings from graphene oxides (GO) and black phosphorus (BP) cytotoxicity studies conducted under identical conditions,⁶⁴⁻⁶⁶ it was perceived that in general pnictogen nanosheets are less toxic, with the exception of arsenic nanosheets exhibiting higher toxicity. Data from the comparison study is highlighted in Table 8.1.

Table 8.1. Measurement of WST-8 assay normalised cell viability percentages upon 24 h of introduction with 125 µg/mL of graphene oxide synthesised via Hoffmann’s method (GO-HO), 85 µg/mL of reduced graphene oxide (rGO) and 200 µg/mL of pnictogen nanosheets. Data for graphene oxide, reduced graphene oxide and black phosphorus are obtained from reference 64-66.

Materials	GO - HO	rGO	BP	As _{SE}	Sb _{SE}	Bi _{SE}
Cell Viability (%)	35.0	15.0	54.2	12.8	57.2	71.3

Owing to their unique structures and extraordinary properties, 2D nanomaterials, for instance, graphene and black phosphorus, are generating considerable interest in the field of biomedical applications. The last few years have witnessed an increasing growth in research on biomedical applications of graphene oxides and black phosphorus such as biosensing, drug delivery, PTAs and bioimaging.^{62,67-71} Data in Table 8.1 proposes that antimony and bismuth nanosheets are less toxic than GO, rGO and BP and could possibly be translated to better biocompatibility. Taken together the outstanding and stable inherent electrochemical properties of antimony and bismuth nanosheets and probable low toxic adverse effects, antimony and bismuth nanosheets could potentially serve as alternatives to GO and BP in biosensing schemes and drug delivery carriers.¹⁸

8.3 Conclusion

Recent developments in the studies and applications of pnictogen nanosheets have heightened the need for us to explore their toxicological effects posed in light of their potential industrial implementations. In this study, the toxicological effects of pnictogen nanosheets (arsenic, antimony and bismuth nanosheets) towards A549 cells were investigated using WST-8 and MTT cell viability assays. The evidence from the cytotoxicity study suggests that pnictogen

nanosheets toxicity decreases down the group, whereby arsenic nanosheets are considered to be highly toxic while antimony nanosheets are deemed to be moderately toxic and bismuth nanosheets are being contemplated to induce mild cytotoxicity. To form a better understanding of the relative toxicity of pnictogen nanosheets, a comparison study was also conducted with the corresponding pnictogen (III) salts and pnictogen nanosheets are found to be generally less toxic. Both bismuth (III) and antimonial (III) drugs have been used for therapeutic purposes for centuries. The relatively low toxicity of these antimony and bismuth nanosheets could possibly hold great promises for clinical translations for the development of drug carriers or PTAs. In conclusion, the findings of this study represent a noteworthy first step towards enhancing our understanding of the toxicological effects of arsenic, antimony and bismuth nanosheets. However, further experimental investigations are required to gain more insights into the mechanisms underlying the biological responses towards pnictogen nanosheets.

8.4 Experimental Section

8.4.1 Synthesis of Pnictogen Nanosheets

Starting bulk pnictogen materials purchased from Alfa Aesar (Germany), rhombohedral arsenic, antimony and bismuth crystals, were shear exfoliated following the protocol published by Gusmão et al.¹⁵ Firstly, 1 g of each starting material underwent 20 minutes of sonication in an ice bath, with sodium cholate (SC, 5 mg/mL), to remove inorganic impurities and surface oxides. The resulting suspensions were subjected to centrifugation using a Beckman Coulter Allegra 64R centrifuge for an hour at 4 Krpm. The supernatant was discarded, and the remaining material pellet was dried at 60 °C under vacuum.

The pre-treated powders were then shear exfoliated in aqueous surfactant SC for 2 hours interchanging between two models of kitchen blenders: 600 W Tefal Blendforce Maxi and

Bosch immersion 750 W hand blender. To prevent overheating of appliances and potentially boiling the solvent, 2 min on/1 min off cycles were used for the operation of the blenders, ice cooling was used when shear exfoliating with the top hand blender and the glass jug was stored at -4 °C during off cycles.

Upon shear exfoliation, the suspensions were transferred into 50 mL falcon tubes and centrifuged at 1 Krpm for an hour. Afterwards, suspensions were divided into two separate phases: the top 75% of the dispersions, containing exfoliated material (pnictogen_{SE}). The two phases were processed separately, subjected to cycles of aqueous washing and were centrifuged at various rotation speeds. The as-synthesised solids were subsequently dried under vacuum at 60 °C prior to characterisation and cytotoxicity studies. The production yields of the recovered materials from top 75% of the suspensions were: As_{SE} (3.9%), Sb_{SE} (8.2%) and Bi_{SE} (8.8%)

8.4.2 Materials Characterisation

The pnictogen nanosheets were characterised using JEOL 7600F to obtain the SEM images. Pnictogen nanosheets were first sputtered with a thin layer of 10 nm platinum, then SEM was carried out using the gentle beam mode with a 2.00 kB accelerating voltage and a 7.6 mm working distance.

TEM images were attained via EFTEM JEOL 2200 FS microscope. Preparation of samples was carried out by pipetting 3 µL of desired sample suspensions (1 mg/mL) onto the TEM grids. The SDD detector X-MaxN 80 T S from Oxford Instruments, England) was utilised to obtain the TEM-EDX spectra.

The size distributions and surface charges of the pnictogen nanosheets were acquired via the Zetasizer Nano ZS (Malvern Instruments, England) at 20 °C, through dynamic light scattering measurements and zeta potential mode respectively.

AFM data were acquired using a NT-MTD Ntegra Spectra fitted with silicon cantilevers (strain constant of 1.5 kN/m and curvature radius < 10 nm), under a tapping mode. AFM studies were executed under room temperature and pressure at a scan rate of 1 Hz and 256 scan lines.

8.4.3 Cell Culture

Dulbecco's modified eagle medium and 10% fetal bovine serum acquired from Life Technologies were mixed with 1 % penicillin/streptomycin mixture (Capricorn) to yield the cell culture media (CCM). Human lung carcinoma epithelial cells, A549, purchased from PAA Laboratories was cultured in CCM and incubated at 37 °C under a 5% CO₂ atmosphere. All incubation procedures in this study used similar conditions unless stated otherwise.

8.4.4 WST-8 Cell Viability Assay

For WST-8 cell viability studies, 5,000 cells (100 µL) were seeded into each well of the 96-well plate. Upon 24 hours of cell seeding, CCM was pipetted out and replaced with varying concentrations of test materials, across the range from 3.125 to 200 µg/mL (100 µL). In the negative control, test materials were not introduced to the cells while cells were introduced with 10 % dimethyl sulfoxide (DMSO) in the positive control study. After 24 hours of incubation with the test materials, 10 µL of WST-8 (Dojindo Molecular Technologies) was introduced into individual wells and the mixtures were subjected to further incubation (1 hour). After the one hour incubation period, absorbance measurement was carried out at 450 nm.

8.4.5 WST-8 Control Experiment

To determine if the pnictogen nanosheets would induce the reduction of the tetrazolium salt, varying concentrations of test materials (100 μ L) were incubated in a 96-well plate with 10 μ L of WST-8 for an hour. After incubation for an hour, spectroscopic absorbance measurement was performed at 450 nm.

8.4.6 MTT Cell Viability Assay

For this study, 5,000 cells (100 μ L) were seeded into each well of the 96-well plate. CCM was then discarded and superseded with varying concentrations of test materials, ranging from 3.125 to 200 μ g/mL (100 μ L), after 24 hours of cell seeding. In the negative control, nanomaterials were not introduced to the cells while cells were introduced with 10 % dimethyl sulfoxide (DMSO, Tedia) in the positive control study. After 24 hours of incubation with the test materials, 10 μ L of 5 mg/mL MTT (Sigma-Aldrich) was introduced into each well and the plate was further incubated for three hours. The wells were then introduced to 100 μ L of solubilisation solution (40% vol/vol dimethylformamide, 2% vol/vol glacial acetic acid and 16% wt/vol sodium dodecyl sulphate, SDS) and the plate was gently agitated for 10 minutes to dissolve the insoluble purple formazan crystals generated. Spectroscopic absorbance measurement was performed at a wavelength of 570 nm.

8.4.7 MTT Control Experiment

To test for probable pnictogens induced interferences with the MTT assay, various concentrations of the test materials (100 μL) were incubated with 5 mg/mL of MTT (10 μL) in a 96 well plate for 3 hours. Following the 3-hour incubation period, 100 μL of solubilisation solution was supplemented to the MTT-test material mixture and gently agitated for 10 minutes to dissolve the formazan crystals formed. Spectroscopic absorbance measurement was carried out at 570 nm wavelength.

8.5 Supporting Information

Figure 8.S1. TEM and HRTEM images, with the corresponding SAED patterns of the shear exfoliated pnictogens: As_{SE} (A), Sb_{SE} (B) and Sb_{SE} (C). On the left column, the scale bars

accord to 1 μm (A) and 500 nm (B and C). On the right column, the scale bars represent 5 nm. In the inset, the scale bars correspond to 0.05 nm.

Figure 8.S2. AFM images (A, C and E) and representative height profiles (B, D and F) of shear exfoliated pnictogen nanosheets.

Figure 8.S3. DLS measurements of the particle size distributions for pnictogen nanosheets.

Figure 8.S4. (a) Remaining cell viabilities obtained from WST-8 assay following 24 hours of incubation with distinct concentrations of pnictogen (III) salts towards A549 cells. (b) Normalised formazan concentration after an hour of incubation of WST-8 with different test concentrations of pnictogen (III) salts.

Figure 8.S5. (a) Remaining cell viabilities obtained from MTT assay after 24 hours of incubation with distinct concentrations of pnictogen (III) salts towards A549 cells. (b) Normalised formazan concentration upon three hours of introducing MTT with different test concentrations of pnictogen (III) salts.

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Chapter 9

Conclusion and Future Outlook

9.1 Conclusion

9.2 Future Outlook

9.1 Conclusion

Constant advancements in sensor and energy technologies are cardinal to ensure the continual evolution of societies. The increasing complexity of samples and demand for more analysis to be made, have made it imperative to constantly improve the analytical capabilities of sensing platforms and develop sensors that are highly specific, sensitive, low in cost, easy to use, rapid and reliable. Meanwhile, as more governments try to decarbonize their economies, it is fundamental to develop advanced energy storage and conversion technologies to fully optimise the utility of intermittent renewable energy sources. However, the current state-of-the-art electrocatalysts involve precious metals, which are scarce, costly and suffer from poor stability, hampering their widespread application. The rapid advancement in science and technology has resulted in a huge growth in the number of newly engineered layered materials. Layered materials possess numerous fascinating properties such as massive specific surface areas, remarkable electrical and thermal conductivity, abundance of catalytic sites, ease of functionalisation and tuneable electronic structures, making them ideal candidates for the development of sensing platforms as well as electrocatalysts for the generation of alternative energy sources. Although layered materials bespeak bright prospects for sensing and energy applications, the scientific community has raised many concerns about the potential of these novel materials in causing new hazards and risks. As such the thesis is divided into two parts, where Part I details the electrochemical studies of emerging classes of layered materials for sensing and energy applications while Part II touches on the safety aspects of layered materials.

Part I explored the electrochemical aspects of emerging classes of layered materials and exploited them to improve the analytical performance of electrochemical sensing platforms and electrocatalysts. Chapters 3 and 4 investigated the feasibility of using MXenes and Group 14 graphane analogues as transducer platforms for the development of second-generation glucose

biosensors. Both biosensing systems displayed low limits of detection and offered extensive linear detection ranges spanning over different magnitudes of glucose concentration. Both biosensing platforms also exhibited high selectivity and showcased the potential of applying these systems for real sample analysis. Chapter 5 studied the synergistic effects of decorating pnictogen nanosheets with precious metal-based nanostructures (core-shell gold@silver nanorods) towards the sensitive detection of glucose. The study revealed that As_{SE} -based composite displayed excellent electrocatalytic activity towards glucose detection and possessed an ultralow limit of detection. Chapter 6 explored the effects of doping various amounts of vanadium on molybdenum dichalcogenides on their electrocatalytic activities towards the hydrogen evolution reaction, the oxygen evolution reaction and the oxygen reduction reaction. Increasing the amount of vanadium dopants yielded mixed results for different molybdenum dichalcogenides and different energy reactions. These studies provide fundamental insights into the various classes of layered materials, highlight their inherent electrochemical properties, provide proofs-of-concept and showcase the potentials of applying these materials for prospective developments of sensing platforms and electrocatalysts.

In view of the probable implementation of these materials in industrial settings and our daily life in the near future, Part II of the thesis sets forth to conduct preliminary cytotoxicity studies on various emerging classes of layered materials, namely Group 5 transition metal ditellurides and pnictogen nanosheets. Although intensive research efforts have been put in place to study the synthesis, properties, and applications of these materials, little effort has been devoted to determining the toxicological behaviour of the abovementioned materials. Herein this dissertation, the cytotoxicity effects of Group 5 transition metal ditellurides and pnictogen nanosheets have been investigated in Chapters 7 and 8 respectively. In Chapter 7, VTe_2 was revealed to be highly toxic and exhibited a dose-dependent toxicity trend, while $NbTe_2$ and

TaTe₂ were both mildly toxic with comparable results. Chapter 8 demonstrated a decrease in the cytotoxicity effect down the group of heavy pnictogens, where arsenic nanosheets were considered to be highly toxic, antimony nanosheets were deemed to be moderately toxic and bismuth nanosheets were found to induce mild cytotoxicity. The study also revealed that pnictogen nanosheets were generally less toxic than their corresponding pnictogen (III) salts.

9.2 Future Outlook

Although layered materials behold great promises for electrochemical sensing and energy applications, it is necessary to address several challenges prior to the practical realisation of commercial applications of these materials. Firstly, it is essential to develop methods for the large scale and cost-effective synthesis of layered materials. Secondly, one should also take into account the long-term stability of these materials, where processes such as oxidation and corrosion could have detrimental impacts on their electrochemical performance. Robust sensing platforms and electrocatalysts need to be developed for practical applications. Thirdly, the safety aspects of these materials should also be investigated to avoid causing adverse effects to the environment and users.

Layered materials are expected to assume important roles in empowering future technological advancements, and exposure to these materials would be inevitable in the near future. As such it is imperative to determine the safety aspects of these uprising classes of layered materials. Even though preliminary cytotoxicity studies of layered materials have been published increasingly using various cell lines and incubation times, the toxicity mechanisms of layered materials are still poorly understood. There exist huge gaps in knowledge about the nature of interactions between layered materials and physiological systems. Furthermore, many of these preliminary studies were performed over short periods of time, the long-term toxicity effects

of layered materials are poorly understood. It would also be insightful to progressively move towards *in vivo* toxicity studies to glean on the toxico-kinetics of layered materials.

Research on layered materials is still in the early stages. With rapid breakthroughs in technologies, it is envisioned that more classes of layered materials would surface, and layered materials would continue to exert their revolutionary promises in a plethora of applications.