**NANYANG
TECHNOLOGICAL
UNIVERSITY**

**MATERIALS FOR ELECTROCHEMICAL
ENERGY AND SENSING APPLICATIONS**

TOH ROU JUN

2017

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SENSING APPLICATIONS**

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**SCHOOL OF PHYSICAL AND MATHEMATICAL SCIENCES
NANYANG TECHNOLOGICAL UNIVERSITY**

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MATERIALS FOR ELECTROCHEMICAL ENERGY AND SENSING APPLICATIONS

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School of Physical and Mathematical Sciences

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Summary

In our technology-driven society, constant and sustainable growth requires ongoing advancements in the fields of renewable energy and sensing. However, electrocatalysis of energy-related processes is impeded by the expensive state-of-the-art platinum-based catalysts, thus posing a challenge towards establishing a hydrogen economy. In sensing, a sensitive and selective detection platform towards point-of-care diagnostics is highly sought after. Consequently, this thesis, emphasised on the use of electrochemical methods, has identified three groups of functional materials: transition metal oxides (TMOs), graphene and transition metal dichalcogenides (TMDs), which offer advantageous properties for energy and sensing applications. In particular, TMOs and TMDs, being abundant in nature, also exhibit electrocatalytic activity towards the oxygen reduction reaction and hydrogen evolution reaction respectively. Aimed at the design and development of advanced electrocatalysts, the factors that may govern the electrocatalytic properties of TMOs and TMDs are investigated. Moreover, graphene and TMDs, recognised for their unique structural, physical and electronic properties, present opportunities to be explored for sensing applications. Unfortunately, graphene did not demonstrate any advantageous role in the sensing of haemoglobin. On the other hand, TMDs was successfully introduced as an electroactive label for protein detection, and as a platform for hydrogen peroxide determination – further established in lab-on-a-chip devices. The knowledge garnered and demonstrated applications would serve as a useful platform for future advancements in the usage of these materials for electrochemical energy and sensing applications.

List of Publications

Part I: Electrocatalytic Properties of Materials for Energy Applications

1. **Rou Jun Toh**, Alex Yong Sheng Eng, Zdenek Sofer, David Sedmidubsky, Martin Pumera. Ternary Transition Metal Oxide Nanoparticles with Spinel Structure for the Oxygen Reduction Reaction. *ChemElectroChem* **2015**, 2, 982-987.
2. **Rou Jun Toh**, Zdenek Sofer, Martin Pumera. Transition Metal Oxides for the Oxygen Reduction Reaction: Influence of the Oxidation States of the Metal and its Position on the Periodic Table. *ChemPhysChem* **2015**, 16, 3527-3531.
3. **Rou Jun Toh**, Zdenek Sofer, Martin Pumera. Catalytic Properties of Group 4 Transition Metal Dichalcogenides (MX_2 ; M = Ti, Zr, Hf; X = S, Se, Te): Effect of Electrochemical Pretreatment. *J. Mater. Chem. A* **2016**, 4, 18322-18334.
4. **Rou Jun Toh**, Zdenek Sofer, Jan Luxa, Martin Pumera. Ultrapure Molybdenum Disulfide Shows Enhanced Catalysis for Hydrogen Evolution over Impurities-Doped Counterpart. *ChemCatChem* **2017**, 9, 1168-1171.
5. **Rou Jun Toh**, Zdenek Sofer, Jan Luxa, David Sedmidubsky, Martin Pumera. 3R Phase of MoS_2 and WS_2 Outperforms the Corresponding 2H Phase for Hydrogen Evolution. *Chem. Commun.* **2017**, 53, 3054-3057.

Part II: Nanomaterial-based Electrochemical Sensors towards Point-of-Care Diagnostics

6. **Rou Jun Toh**, Weng Kung Peng, Jongyoon Han, Martin Pumera. Haemoglobin Electrochemical Detection on Various Reduced Graphene Surfaces: Well-Defined Glassy Carbon Electrode Outperforms the Graphenoids. *RSC Adv.* **2014**, 4, 8050-8054.
7. **Rou Jun Toh**, Weng Kung Peng, Jongyoon Han, Martin Pumera, M. Direct In Vivo Electrochemical Detection of Haemoglobin in Red Blood Cells. *Sci. Rep.* **2014**, 28, 4:6209.
8. **Rou Jun Toh**, Carmen C. Mayorga-Martinez, Zdenek Sofer, Martin Pumera. MoSe₂ Nanolabels for Electrochemical Immunoassays. *Anal. Chem.* **2016**, 88, 12204-12209.
9. **Rou Jun Toh**, Carmen C. Mayorga-Martinez, Zdenek Sofer, Martin Pumera. 1T-Phase WS₂ Protein-based Biosensor. *Adv. Func. Mater.* **2017**, 1604923.
10. **Rou Jun Toh**, Carmen C. Mayorga-Martinez, Jongyoon Han, Zdenek Sofer, Martin Pumera. Group 6 Layered Transition-Metal Dichalcogenides in Lab-on-a-Chip Devices: 1T-Phase WS₂ for Microfluidics Non-Enzymatic Detection of Hydrogen Peroxide. *Anal. Chem.* **2017**, 89, 4978-4985.

Thesis Organisation

This thesis is organised into **fourteen (14) Chapters**. The reader is presented with the motivations and ideas towards this thesis in **Chapter 1**. Subsequently, **Chapter 2** will provide the reader with the challenges in electrochemical energy and sensing applications, and a general introduction to transition metal oxide, graphene and transition metal dichalcogenides materials. Background information of energy-related processes and electrochemical sensors, and in-depth reviews on earlier published works in relation to the electrochemical properties of the materials of interest will be included in independent subchapters of **Chapter 3**. With succinct information regarding the scientific background of this thesis, the reader will be directed to **Chapters 4-13**, which is made up of a collection of research towards the objectives established in **Chapter 1**.

In detail, **Chapters 4 and 5** will discuss the factors governing the electrocatalytic activity of transition metal oxides for the oxygen reduction reaction. **Chapters 6, 7 and 8** will cover the fundamental investigations of transition metal dichalcogenides for electrocatalysis of the hydrogen evolution reaction. **Chapters 9 and 10** will touch on the application of graphene for sensing while **Chapters 11, 12 and 13** will emphasise on the applications of transition metal dichalcogenides for sensing and as electrode materials on a lab-on-a-chip detection platform.

A summary and perspective of the work presented in this thesis are collated in **Chapter 14**.

The published version of the results in **Chapters 4-13** are shown in the **Appendices**.

List of Abbreviations

°C	Degree Centigrade
at%	Atomic Percentage
CV	Cyclic Voltammetry
DPV	Differential Pulse Voltammetry
EDS	Energy-Dispersive Spectroscopy
GC	Glassy Carbon
Hb	Haemoglobin
HER	Hydrogen Evolution Reaction
IgG	Immunoglobulin G
LOC	Lab-on-a-Chip
LSV	Linear Sweep Voltammetry
min	Minute
mM	Millimolar
nM	Nanomolar
ORR	Oxygen Reduction Reaction
PBS	Phosphate Buffered Saline
RBC	Red Blood Cell
SEM	Scanning Electron Microscopy
<i>t</i> -BuLi	Tert-Butyllithium
TEM	Transmission Electron Microscopy
TMD	Transition Metal Dichalcogenide
TMO	Transition Metal Oxide
wt%	Weight Percentage

Chapter 1

Objectives of the Thesis

Materials themselves do nothing. Yet, without materials, man can do nothing. Modern society is heavily dependent on the discovery and development of advanced materials: lightweight composites for faster vehicles, bio-inspired materials for regenerative medicine. Materials science is an exciting and promising field and can be extended to a broad range of applications.

Constant and sustainable growth of our technology-driven society requires ongoing advancements in the fields of renewable energy and sensing. Focused on the use of electrochemical methods and considering the challenges faced in energy and sensing applications, this thesis titled “Materials for Electrochemical Energy and Sensing Applications” is aimed at exploring the promising properties of transition metal oxides, graphene and transition metal dichalcogenides to address these challenges. Detailed discussion will be provided in **Chapter 2**.

This thesis is arranged into two parts as depicted in **Figure 1.1**. **Part I** covers the investigation of functional materials as electrocatalysts in energy-related processes like the oxygen reduction reaction (ORR) and hydrogen evolution reaction (HER), while **Part II** covers the development of nanomaterial-based electrochemical sensors towards point-of-care diagnostics. Comprehensive background information of the energy-related processes and electrochemical sensors, and a literature review of the materials of interest, directed at rationalising the objectives of this thesis, will be highlighted in **Chapter 3**.

Part I: Electrocatalytic Properties of Materials for Energy Applications

In **Part I**, transition metal oxides (TMOs) and transition metal dichalcogenides (TMDs) were identified as materials of interest. With high availability and low costs, they serve as promising alternatives to expensive, state-of-the-art platinum (Pt) and Pt-based catalysts for energy-related processes. In order to push the field forward, we aim to investigate possible trends and provide a better understanding of factors that may affect the electrocatalytic properties of TMOs and TMDs. The conclusions drawn have profound implications for the choice and development of efficient electrocatalysts for energy applications.

Identified as active electrocatalysts for the oxygen reduction reaction (ORR), TMOs are subjected to extensive comparisons to establish possible correlations between their electrocatalytic performance and the metal oxidation states and their positions on the periodic table. Furthermore, a systematic study revealed the effect of metal composition and substitution on the ORR electrocatalytic performance of TMOs. Results of the trend studies are discussed in **Chapters 4 and 5**.

On the other hand, the properties emerging from the anisotropy of TMDs have been demonstrated to be useful for advanced energy storage and conversion, specifically as electrocatalysts for the hydrogen evolution reaction (HER). At present, group 6 TMDs are the most highly studied and are hence, representative of this class of materials. For this reason, **Chapters 6 and 7** investigate the elemental and phase purity effects on the HER electrocatalytic properties of group 6 TMDs, while the unexplored electrochemical and HER electrocatalytic properties of group 4 TMDs are addressed in **Chapter 8**.

Part II: Nanomaterial-based Electrochemical Sensors towards Point-of-Care Diagnostics

Part II focuses on exploiting the unique properties of nanomaterials such as graphene and transition metal dichalcogenides (TMDs) to improve the performance of sensing systems. The high specific surface area and excellent electrical conductivity of these materials have spurred us to explore opportunities for their application in the field of electrochemical sensing. The findings are of paramount importance to meet the demands and future needs in the diverse fields of bio-diagnostics, security and environmental control.

In **Chapter 9 and 10**, graphene and graphene-related materials are investigated and compared against the well-defined glassy carbon (GC) electrode for the electrochemical detection of haemoglobin (Hb). Transition metal dichalcogenides are explored for immunoglobulin G (IgG) protein and hydrogen peroxide (H_2O_2) determination in **Chapters 11 and 12** respectively.

Towards the development of low cost and portable devices for point-of-care diagnostics, a simple and low-cost fabrication method to integrate electrochemical sensors into lab-on-a-chip (LOC) microfluidic devices is demonstrated in **Chapter 13**. This is accompanied by the integration of TMD materials within the LOC devices to enhance sensor performance for hydrogen peroxide detection.

Figure 1.1 Overview of the challenges and objectives of the thesis project.

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Chapter 2

Introduction

2.1 Challenges for a Sustainable Energy Future

2.2 Challenges in Electrochemical Sensing

2.3 Functional Materials: A Brief History and Introduction

2.3.1 Transition Metal Oxides (TMOs)

2.3.2 Graphene

2.3.3 Transition Metal Dichalcogenides (TMDs)

2.1 Challenges for a Sustainable Energy Future

Fossil fuels have offered opportunities and driven economic growth in many countries in the 20th century. Meanwhile, oil and electricity have become vital components in meeting our everyday needs. However, the increasing demand for energy is exhausting the proven reserves of fossil fuels at an alarming rate. World energy consumption is projected to see a 48% increase from 2012 to 2040.¹ Moreover, reports of health-threatening pollution and greenhouse gases linked to global warming raises environmental concerns of using fossil fuels.²⁻⁴

The exploitation of fossil fuels surfaces an inevitable challenge to diversify our energy supply in order to maintain a healthy global economic growth.^{5,6} Much effort has been poured into realising the transition from relying on fossil fuels to using other renewable sources of energy. A particularly promising technology is the hydrogen fuel cell, which is often compared to batteries.^{7,8} While both devices generate electricity by a chemical reaction, the fuel cell is capable of doing so without losing its charge as long as the hydrogen fuel is supplied.

As illustrated in Figure 2.1, hydrogen fuel is combusted in fuel cells following a simple reaction where hydrogen and oxygen combine to produce electricity, heat and water.

Each fuel cell consists of two electrodes, the anode and cathode, where oxidation and reduction processes take place respectively. These processes at the electrodes produce electricity, as electrons flow from the anode to the cathode. In the context of an alkaline electrolyte fuel cell, hydroxyl ions (OH^-) react with

hydrogen at the anode to release electrons. On the other hand, oxygen reacts with the electrons and water at the cathode to form new OH^- ions. This reaction is known as the oxygen reduction reaction (ORR).

Figure 2.1 Electrode reactions and charge flow for an alkaline electrolyte fuel cell. Reproduced from ref. 7.

The great appeal of hydrogen fuel cells lies in their ability to generate electricity while producing low levels of pollution as hydrogen and oxygen combine to produce water, a harmless by-product. However, the otherwise sluggish ORR kinetics at the cathode has been a major limiting factor for energy conversion efficiency, stability and bulk commercialisation of fuel cells.⁹ Moreover, as tempting as the idea of replacing oil with hydrogen may sound, the production of hydrogen as a fuel poses a challenge. While twice as much of hydrogen is required in a fuel cell compared to that of oxygen, it is unfortunate that only 0.000053% of the atmosphere of Earth is composed of hydrogen.¹⁰ Simply put, molecular hydrogen cannot be “mined” like fossil fuels, but it has to be “manufactured”. One method of producing hydrogen is by steam reforming of methane gas (CH_4) as shown in the following process:¹¹

Steam reforming of methane gas performed in factories accounts for most of the hydrogen we use today. Another method involves the electrochemical splitting of water in an acidic electrolyte where the hydrogen evolution reaction (HER) takes place:¹²

In the past decades, a focus of electrochemistry has been to explore new electrocatalysts to enhance the kinetics of the ORR and HER. Hitherto, platinum (Pt) and Pt-based materials are recognised to be the most active ORR and HER electrocatalysts.¹³⁻¹⁶ Nonetheless, the rarity of Pt in the Earth's crust and its high costs pose as major bottlenecks to large-scale commercial application. Although encouraging results have been reported on alternative electrocatalysts,¹⁷⁻¹⁹ it remains a challenge to develop electrocatalysts for ORR and HER with high activity, high availability and low cost compared to Pt. This is mainly due to a lack of comprehensive understanding of the factors that govern the electrocatalytic properties of these materials. In particular, transition metal oxides (TMOs) have been explored for the ORR²⁰⁻²³ and transition metal dichalcogenides (TMDs) have been investigated for the HER with promising advances.²⁴⁻²⁶ Prompted, the electrocatalytic properties of TMOs and TMDs, and the factors that may affect them will be studied in **Chapters 4, 5, 6, 7 and 8**.

2.2 Challenges in Electrochemical Sensing

The analysis of biomarkers such as proteins, bacteria, DNA and hydrogen peroxide is fundamental for applications in the fields of medicine, security, forensic science and environmental protection. In contrast to optical, mass and thermal analytical methods, electrochemical techniques are particularly attractive due to their remarkable detection capability in the femtomolar (10^{-15} M) range, simple experimental methods, low costs and ease of miniaturisation.^{27,28} To meet the huge market demand and future need for sensors in the diverse fields, the design and construction of new electrochemical sensors is essential. For this purpose, the concepts of sensitivity, selectivity, detection limits and ruggedness remain extremely important characteristics to be considered and improved on. Favourable due to their high surface-to-volume ratio and excellent electrical conductivity,^{29,30} nanomaterials such as graphene and transition metal dichalcogenides (TMDs) will be explored for applications in electrochemical sensing in **Chapters 9, 10, 11 and 12**.

Moreover, the potential to perform reliable and rapid complex analyses for point-of-care diagnostics has stirred much research interests in the development of low-cost, portable devices. During the past decade, microfluidics and lab-on-a-chip (LOC) devices have progressed a great deal.³¹ A classic example of the successful translation of an electrochemical sensor from the bench of laboratories to the shelf of commercial markets is the glucose sensor (Figure 2.2).³²

Figure 2.2 Commercially available glucose sensor.

However, it is still a challenge in the field of electrochemical LOC devices to provide a sensitive and selective detection platform for analytical applications. To address this, the integration of TMD materials within lab-on-a-chip devices microfluidic devices will be investigated in **Chapter 13**.

2.3 Functional Materials: A Brief History and Introduction

In order to address the challenges in electrochemical energy and sensing as discussed earlier, three classes of functional materials were identified: (1) transition metal oxides, (2) graphene and (3) transition metal dichalcogenides. A brief history and introduction to each material of interest will be provided.

2.3.1 Transition Metal Oxides (TMOs)

Transition metal oxides (TMOs) constitute one of the most interesting classes of solids with a long and distinguished history.^{33,34} The first magnets were found from a natural mineral called magnetite (Fe_3O_4) during the Greek era.³⁵ Following the discovery of high-temperature superconductivity in perovskite-like copper oxides in 1986 by Bednorz and Müller,³⁶ renewed interests in TMOs developed. Apart from simple binary TMOs, the formation of mixed TMOs is also possible. Mixed TMOs have a general formula of $\text{A}_x\text{B}_{3-x}\text{O}_4$ where A, B = Co, Ni, Mn, Fe and so on. They can occur as a mixture of two binary TMOs with the same metal cation (eg. Co_3O_4) or as ternary TMOs composed of two different metal cations (eg. MnCo_2O_4).

The nature of transition metal-oxygen bonding can vary between nearly ionic to highly covalent or metallic. Owing to the distinctive nature of the outer d-electrons, a broad spectrum of structures and properties is observed.³⁷ In particular, TMOs exhibit a remarkable range of electronic properties.³⁸ Oxides such as RuO_2 and ReO_3 display metallic properties while others like BaTiO_3 are highly insulating. Interestingly, TMOs, for example V_2O_3 , is able to traverse both regimes with a change in temperature, pressure or composition.

2.3.2 Graphene

Figure 2.3 Graphene: a single layer of sp^2 hybridised carbon atoms in a honeycomb-like structure.

The term “graphene” was first officially recognised by the International Union of Pure and Applied Chemistry (IUPAC) in 1997. Defined as “a single carbon layer of the graphite structure, describing its nature by analogy to a polycyclic aromatic hydrocarbon of quasi infinite size”,³⁹ graphene is prized as one of the strongest, lightest and most conductive materials known to mankind (Figure 2.3). First discussed by Wallace in 1946,⁴⁰ the isolation and proper characterisation of graphene was only achieved in 2004 by Geim and Novoselov through the mechanical exfoliation of graphite using common adhesive tapes (Figure 2.4).⁴¹ This discovery created waves in the scientific community and the subsequent surge in research efforts revealed the impressive electronic, thermal, optical and mechanical properties of graphene.⁴²⁻⁴⁵

Figure 2.4 Graphene films. (A) Photograph of a multilayer graphene flake with thickness ~ 3 nm on top of an oxidised Si wafer. (B) Atomic force microscope (AFM) image of this flake near its edge ($2\ \mu\text{m} \times 2\ \mu\text{m}$). (C) AFM image of single-layer graphene. (D) Scanning electron microscope image of one of the experimental devices. (E) Schematic view of the device in (D). Reproduced from ref. 41.

Graphene possess zero band gap energy, implying that electrons in the material are forced to consistently travel at the same speed without stopping.⁴⁶ As such, graphene can be exploited as an excellent conductor with quick response to an applied voltage. Moreover, being an ultrathin two-dimensional (2D) material, graphene allows light to pass through (Figure 2.5), thus differentiating itself from the typically opaque conductive materials.^{47,48}

Figure 2.5 An assembled graphene/polyethylene terephthalate touch panel showing outstanding flexibility. Reproduced from ref. 48.

2.3.3 Transition Metal Dichalcogenides (TMDs)

Figure 2.6 Transition metal dichalcogenide (TMD) crystals contain one transition metal atom (green) for every two chalcogen atoms (orange). Some 40 such TMDs – mostly those made with the metals highlighted in dark green in the periodic table – can be split into 2D layers that are flexible, transparent and excellent conductors of electricity. Some are also semiconductors. Reproduced from ref. 51.

The recent breakthrough in graphene discovery and its promising properties reignited a great deal of interest in 2D layered nanomaterials, in particular transition metal dichalcogenides (TMDs).^{49,50} TMDs are layered structures similar to that of graphite. They have a general chemical formula MX_2 , and a structure where the transition metal (M) layer like molybdenum (Mo) or hafnium (Hf) is sandwiched between two chalcogen (X) layers, generally sulphur (S), selenium (Se) or tellurium (Te) (Figure 2.6).⁵¹⁻⁵³ TMDs occur naturally in the Earth's crust with molybdenite (MoS_2) being the most abundant among them.⁵⁴ Characterised by weak van der Waals forces between layers and strong in-plane covalent bonding, parallels have been drawn between graphene and TMDs. However, TMDs differ from graphene as they are semi-conductive materials and possess non-zero band

gap energy. While the lack of a band gap in graphene limits its ability to switch on and off as normally required in logic circuits, TMDs do not face the same constraint.

As illustrated in Figure 2.7, a single-layered TMD generally presents either an octahedral or trigonal prismatic coordination phase. In multi-layered TMDs, a large variety of polymorphic structures arise as each individual layer can possess any of the two coordination phases. The three commonly found polymorphs are defined as 1T, 2H and 3R, where the digit is indicative of the number of layers in the crystallographic unit cell, while the latter designates the type of symmetry exhibited; T stands for tetragonal (D_{3d} group), H represents hexagonal (D_{3h} group), and R denotes rhombohedral (C_{3v}^5 group).

Figure 2.7 Metal coordinations and stacking sequences of TMD structural unit cells. Metal coordination can be either octahedral or trigonal prismatic. The octahedral coordination allows stacking sequences which yield a tetragonal symmetry (1T). Dissimilar stacking sequences of trigonal prismatic single layers can give rise to different symmetries; hexagonal symmetry (2H) and rhombohedral symmetry (3R).

The transition-metal coordination by the chalcogen and the stacking sequences of multiple layers of TMD materials can elicit different electronic properties. For instance, bulk semi-conducting trigonal prismatic TMDs possess an indirect band gap but when exfoliated to a monolayer, can be converted to octahedral TMDs and display direct electronic and optical band gaps. Since initial reports in the 1970s, efforts at obtaining single- or few-layered TMDs have since been on-going where exfoliation is mostly elicited by lithium (Li) intercalation of the bulk TMDs.⁵⁵⁻⁵⁸

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Chapter 3

Background and Literature Review

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3.1 Energy-Related Electrochemical Processes

3.1.1 The Oxygen Reduction Reaction (ORR)

The reduction of oxygen to water plays an essential role as the cathodic reaction in energy-converting systems such as the fuel cell and metal-air battery.^{1,2} Three types of electrolytes may be used for the oxygen reduction reaction (ORR): acidic or alkaline aqueous media, and non-aqueous aprotic solvents. Specifically, the alkaline aqueous media confers an advantage by providing a less corrosive environment to the electrocatalysts, accompanied by more rapid ORR kinetics.^{3,4} For this reason, the focus of this thesis and literature review will be directed towards ORR in the alkaline media. In the proceeding discussions, we give an overview of the thermodynamics and kinetics of the ORR, and electrochemical techniques for electrocatalytic ORR in the alkaline media.

3.1.1.1 Thermodynamics and Kinetics of the ORR

As illustrated in Table 3.1, ORR in aqueous media primarily takes place through two pathways where molecular oxygen is reduced via either a 4-electron process or two 2-electron processes. On the other hand, the 1-electron process from O_2 to superoxide (O_2^-) occurs in non-aqueous aprotic solvents.⁵ Depending on the application, each reduction pathway holds unique significance. While the 1-electron process is paramount to the investigation of ORR mechanisms^{6,7} and the 2-electron process is used for industrial production of hydrogen peroxide (H_2O_2),^{8,9} the direct 4-electron process is highly preferred in fuel cell processes.¹⁰

Table 3.1 Electrochemical ORR pathways in different electrolytes and their corresponding thermodynamic electrode potentials^{3,11}

Electrolyte	ORR Pathways		Thermodynamic electrode potential (V versus standard hydrogen electrode)
Acidic aqueous solution	Direct 4 e ⁻ process	$O_2 + 4H^+ + 4e^- \rightarrow H_2O$	1.229
	Two 2 e ⁻ processes	$O_2 + 2H^+ + 2e^- \rightarrow H_2O_2$ $H_2O_2 + 2H^+ + 2e^- \rightarrow 2H_2O$	0.695 1.776
Alkaline aqueous solution	Direct 4 e ⁻ process	$O_2 + H_2O + 4e^- \rightarrow 4OH^-$	0.401
	Two 2 e ⁻ processes	$O_2 + H_2O + 2e^- \rightarrow HO_2^- + OH^-$ $HO_2^- + H_2O + 2e^- \rightarrow 3OH^-$	-0.076 0.878
Non-aqueous aprotic solvents	Two 1 e ⁻ processes	$O_2 + e^- \rightarrow O_2^-$ $O_2^- + e^- \rightarrow O_2^{2-}$	Strongly dependent on solvent used

The mechanism of the electrochemical oxygen reduction reaction (ORR) at the cathode is highly complex and can involve many intermediates and electronic transfer steps, depending on the nature of the electrode material, catalyst and electrolyte used. Despite extensive research over the decades, mechanistic aspects of oxygen reduction remain poorly understood due to the involvement of four electrons and four protons to O₂. Moreover, adsorption of O=O onto the electrode surface, followed by O-O bond activation and the need to overcome the strong double bond dissociation energy of molecular oxygen (497 kJ mol⁻¹) render the ORR a slow kinetic process. Jonsson and co-workers stated that at metallic surfaces, the adsorbed O₂ and OH⁻ are found to be stable intermediates at the thermodynamic electrode potential (1.229 V vs SHE). The authors further

demonstrated the relationship between the electrocatalytic activities of some metals towards the ORR and the binding energies of O_2 (ΔE_O) on the metallic surfaces using a volcano plot (Figure 3.1).¹²

Figure 3.1 Volcano plot of the ORR activity as a function of the O_2 binding energy. Reproduced from ref. 12.

In order to attain high energy efficiencies in energy-converting systems, it is desirable for the ORR to take place at reversible conditions where the electrode potential is the thermodynamic electrode potential (Table 3.1).¹³ Therefore, the use of electrocatalysts is of utmost importance to minimise the overpotential. Hitherto, Pt and Pt-based materials remain the most practical electrocatalysts for oxygen reduction.¹⁴⁻¹⁷ However, Pt is an expensive electrode material and it is highly sensitive to impurities present in the O_2 phase or in solution.¹⁸ Towards this aim, increased attention has been placed on the design and development of alternative ORR catalysts in the past decades. Non-precious metals,¹⁹⁻²¹ transition metals,^{22,23} doped carbon materials,^{24,25} nitrogen-coordinated metal on carbon matrices,^{26,27} and in particular, transition metal oxides (TMOs) have been explored with promising advances.²⁸⁻³¹

3.1.1.2 Electrochemical Techniques for Electrocatalytic Oxygen Reduction

Reactions

Two of the commonly used techniques for evaluating ORR electrocatalytic performance include steady-state polarization and voltammetric techniques.

3.1.1.2.1 Steady-State Polarization

Taking into account the low solubility of O_2 (1.26×10^{-3} M in aqueous solutions), techniques allowing for the forced transport of reactants in solution greatly aids the evaluation of ORR activity.³ For this purpose, convective electrode systems like the rotating-disk electrode (RDE) and rotating ring-disk electrode (RRDE) can be used as powerful tools. The electrochemical and hydrodynamic properties of a RDE system are correlated with the Koutecký-Levich equation.³² While the RDE is able to monitor the oxygen reduction currents only, the RRDE is further capable of establishing the involvement of each reaction pathway, as discussed in Table 3.1, for a particular electrocatalyst according to the Butler-Volmer equation.³³

Polarization is defined as the shift in the electrode surface potential away from its equilibrium value, resulting in an electrochemical reaction. By either recording the stable current response at a held electrode potential, or recording the stable potential response at a held current density, a steady-state polarization curve is obtained.³⁴ From the obtained curve, the relationship between the electrode potential and current density is established. Figure 3.2 shows a typical ORR polarization curve of carbon-supported Pt catalyst (Pt/C) collected from a RDE system.³ In general, evaluation of the polarization curve depends on its application. In the context of a fuel cell, a high current density at lower

overpotential for the ORR or at higher cell voltage for the fuel cell is translated to maximum power density.

Figure 3.2 Typical ORR polarization curve of Pt/C collected from a RDE system with rotating rate of 2000 rpm. The onset potential (E_{onset}), half-wave potential ($E_{1/2}$), overpotential under a specific current density (η_j) and diffusion-limiting current density (j_L) are indicated accordingly. Reproduced from ref. 3.

3.1.1.2.2 Linear Sweep and Cyclic Voltammetry

Linear sweep voltammetry (LSV) and cyclic voltammetry (CV) are among the most useful electrochemical techniques. Current is sampled as the potential is swept at a fixed rate between two values. Figure 3.3 shows the CV profile of various electrocatalysts for the ORR.²² Information about the inherent ORR electrocatalytic activity can be obtained by looking at the onset potential.³⁴ A less negative ORR onset potential is representative of a higher electrocatalytic activity towards the ORR as less energy is required to drive the reaction. Although convection is not involved, LSV and CV can serve as fast and simple techniques to provide qualitative information about the electrocatalysts and electrochemical reactions. For this reason, evaluation of ORR electrocatalytic performance in this thesis will be narrowed to the employment of linear sweep and cyclic voltammetric techniques.

Figure 3.3 Cyclic voltammetry curves of $\text{Co}_3\text{O}_4/\text{graphene oxide (rmGO)}$ hybrid, $\text{Co}_3\text{O}_4/\text{N-doped graphene oxide (N-rmGO)}$ hybrid and Pt/C on glassy carbon electrodes in O₂-saturated (solid line) or Ar-saturated 0.1 M KOH (dash line). Catalyst loading was 0.17 mg cm⁻² for all samples. Reproduced from ref. 22.

3.1.2 The Hydrogen Evolution Reaction (HER)

Hydrogen possesses high energy density as a fuel. When burned in air, 1 mol of hydrogen can produce 286 kJ of energy.³⁵ The promising future of hydrogen economy has placed enormous attention on the hydrogen evolution reaction (HER). In particular, water electrolysis in acidic electrolyte can be employed as a method to produce hydrogen gas.³⁶ The thermodynamics and kinetics of the cathodic HER and the relevant electrochemical techniques will be discussed subsequently.

3.1.2.1 Thermodynamics and Kinetics of the HER

In a water splitting cell, the HER occurs at the cathode while the oxygen evolution reaction (OER) takes place at the anode:

The energy input required to drive the water splitting reaction at standard conditions is given by the Gibbs free energy, $\Delta G = -237.1 \text{ kJ mol}^{-1}$. This corresponds to a thermodynamic voltage requirement of 1.229 V.^{37,38} Focusing on the HER, it can be divided into two steps: adsorption, followed by desorption. Three possible rate-determining steps for the HER have been reported as in Table 3.2.

Table 3.2 Possible rate-determining steps for the HER^{39,40}

Reaction Steps	Possible Rate-Determining Steps	
1. Adsorption	Volmer step	$\text{H}_3\text{O}^+ + \text{e}^- \rightarrow \text{H}_{\text{ads}} + \text{H}_2\text{O}$
2. Desorption	Heyrovsky step	$\text{H}_{\text{ads}} + \text{H}_3\text{O}^+ + \text{e}^- \rightarrow \text{H}_2 + \text{H}_2\text{O}$
	Tafel step	$\text{H}_{\text{ads}} + \text{H}_{\text{ads}} + \text{e}^- \rightarrow \text{H}_2$

The HER may follow the Volmer-Heyrovsky mechanism or the Volmer-Tafel mechanism. In both cases, initiation of the reaction involves adsorption of atomic hydrogen at the electrode surface, H_{ads} . First discussed by Parsons, the rate of the overall reaction is therefore greatly dependent on the free energy of adsorption of hydrogen, ΔG_{H} .⁴¹ Given that hydrogen is too weakly adsorbed to the electrode surface, the adsorption step will limit the rate of reaction. On the other hand, if the hydrogen is too strongly adsorbed to the electrode surface, the subsequent desorption step will limit the overall rate of reaction.

Density functional theory (DFT)-calculations by Stimming and co-workers demonstrated a “volcano” relationship between the ΔG_{H} of pure metals and their HER exchange current density (i_0) as an indicator of electrocatalytic activity (Figure 3.4).⁴² The computational study emphasised that desirable HER electrocatalysts have Gibbs free energy of adsorbed atomic hydrogen (ΔG_{H}) close to zero; hydrogen is adsorbed neither too weakly nor too strongly. In order to maximise the rate of HER, the development and design of HER electrocatalysts is of paramount importance. While the current state-of-the-art Pt catalysts are not commercially viable,⁴³ this has led to interests and studies of alternatives such as transition metal nitrides,⁴⁴ transition metal phosphides,^{45,46} and transition metal dichalcogenides (TMDs).^{47,48} Among these, TMDs stand out with their stellar HER performance.

Figure 3.4 Volcano plot of the exchange current density as a function of the DFT-calculated ΔG_H for pure metals. Reproduced from ref. 42.

3.1.2.2 Electrochemical Techniques for Electrocatalytic Hydrogen Evolution

Reaction

Electrochemical impedance spectroscopy and voltammetric techniques are two commonly used methods for the evaluation of HER electrocatalytic performance.

3.1.2.2.1 Electrochemical Impedance Spectroscopy

Electrochemical impedance spectroscopy (EIS) is a powerful analytical tool. The impedance spectrum is obtained by varying the frequency of perturbation to a system over a wide range. Perturbation of the system is achieved by a small amplitude sinusoidal ac excitation signal.⁴⁹ EIS can be used to provide information about the cell resistivity, charge transport, mass transport, and electrical charge transfer for water electrolyzers.⁵⁰ Figure 3.5 shows an example of EIS data for HER electrocatalysts. In general, a lower Faradaic impedance or charge-transfer impedance marks a faster HER kinetics, and hence enhanced electrocatalytic performance for the HER.^{40,51}

Figure 3.5 Electrochemical impedance spectroscopy data for carbon nitride/N-doped graphene ($C_3N_4@NG$) hybrid and carbon nitride/N-doped graphene (C_3N_4/NG) mixture in H_2SO_4 . Reproduced from ref. 52.

3.1.2.2.2 Linear Sweep Voltammetry

Linear sweep voltammetry (LSV) serves as a versatile technique to probe the HER electrocatalytic activity.⁴⁰ Three commonly adopted HER performance indicators, namely the onset potential, onset potential and Tafel slope can be extracted from this electroanalytical method. From the LSV profile, the relationship of the current density as a function of the applied potential is established (Figure 3.6A). The onset potential is determined at the potential where a steep decrease is observed for the current density corresponding to proton reduction. Overpotentials were taken at a pre-fixed current density, typically at -10 mA cm^{-2} . Moreover, a plot of overpotential against current density provides information about the Tafel slope (Figure 3.6B). The Tafel slope is characterised by the increase in overpotential required to bring about a unit increase in current density. Tafel slope analysis can give an understanding of the hydrogen evolution mechanism on the different electrocatalyst surfaces, where the rate-determining steps may be accounted. With the Volmer adsorption as rate-limiting step, the

Tafel slope is approximately 120 mV dec^{-1} . For the Heyrovsky and Tafel desorption steps, the Tafel slope is determined to be 40 mV dec^{-1} and 30 mV dec^{-1} respectively.⁴⁰ Overall, LSV is a simple and comprehensive method for the evaluation of HER electrocatalytic performance and will be employed in this thesis.

Figure 3.6 (A) Polarization curves obtained and (B) corresponding Tafel plots recorded on glassy carbon electrodes with several catalysts as indicated. Catalyst loading, 0.28 mg cm^{-2} . Reproduced from ref. 40.

3.2 Nanomaterial-based Electrochemical Sensors

Biomarker analysis is critical from the medical, security and environmental viewpoint. A sensor is a device that provides quantitative or semi-quantitative information regarding the presence of a chemical or biological species. Typically, a sensor is composed of two basic components: (1) a chemical recognition interface, also known as the receptor and (2) a transducer, which converts a chemical or biological signal into an analytically useful signal (Figure 3.7).⁵³

Figure 3.7 Scheme of a biosensor. Reproduced from ref. 53.

In most cases, electrochemical detection is used as the transducer due to experimental simplicity, ease of miniaturisation and low cost.^{54,55} The design of each component of an electrochemical sensor strongly influences its characteristics. A desirable electrochemical sensor is one with high sensitivity, selectivity, detection limits and ruggedness. Specifically, nanomaterials have been explored to enhance the performance of electrochemical sensors.^{56, 57} Nanomaterials are described as materials of which at least a single dimension is sized in the 1 – 100 nm scale. In the following sections, we will discuss the roles of nanomaterials in electrochemical sensors and the commonly used electrochemical techniques for sensing applications.

3.2.1 Roles of Nanomaterials in Electrochemical Sensors

Nanomaterials serve two main roles in electrochemical sensors as the: (1) sensing platform and/or (2) electroactive label. An overview of the roles and corresponding characteristics of nanomaterials is illustrated in Figure 3.8.

Figure 3.8 Overview of the roles and corresponding characteristics of nanomaterials in electrochemical sensors.

3.2.1.1 Nanomaterials as Sensing Platforms

The desirable properties of certain nanomaterials such as high surface-to-volume ratio and excellent electrical conductivity allow them to be employed as a modifier in the construction of electrochemical transducing platforms to enhance sensor performance. As a sensing platform, nanomaterials can serve one or more of these functions: (1) as the electrode material, (2) as the supporting substrate employed to elicit the immobilisation of biological recognition molecules and (3) to improve the electroanalytical signal.⁵⁸⁻⁶⁰ One important consideration when choosing nanomaterials as a sensing platform would be the inherent

electrochemical activity of the material. Electroactive groups present on the nanomaterials would limit the working electrochemical potential window and interfere with the electrochemical detection of reduction or oxidation processes of the analyte of interest.⁶¹

3.2.1.2 Nanomaterials as Electroactive Labels

In order to achieve sensitive detection, a signal enhancing element is typically employed to label target biological molecules. A desirable label material is one with small sizes comparable to that of biological molecules, and possesses long-term stability. Nanomaterials serve as excellent label materials due to their small sizes and hence, compatibility for attachment to target biological molecules. When tagged to the target molecule, the electrochemical signal originating from the nanomaterial can be collected and used to quantify the target molecule. Electrochemical sensors based on nanomaterials as electroactive labels offer advantages in terms of cost efficiency in comparison to traditional methods of bioanalysis such as enzyme-linked immunosorbant assay (ELISA) or polymerase chain reaction (PCR) and have been used in a variety of bioanalytical assays.⁶²⁻⁶⁴

Depending on the electrochemical detection mode, the nanomaterials rely on one or more identifiable characteristics to be probed. When the catalytic detection mode is employed, a characteristic of the nanomaterial would be that it displays electrocatalytic activity towards a specific reaction to be probed. For example, gold nanoparticles (Au NPs) are commonly exploited as electroactive labels for their high catalytic activity towards the hydrogen evolution reaction (HER).^{65,66} While the electrocatalytic detection is an indirect detection strategy of the nanomaterial

labels, a direct detection strategy can be achieved through electrochemical stripping after dissolution.^{67,68}

3.2.2 Electrochemical Techniques for Sensing Applications

In general, electrochemical techniques for sensing applications can be organised into three main classifications: current, impedance and potential.

3.2.2.1 Voltammetry/Amperometry

In voltammetry and amperometry, potential applied to the working electrode elicits a current response due to electrochemical reduction or oxidation processes. This current response is measured. Voltammetry refers to when potential is swept over a fixed range. The response in current typically shows as a peak or plateau and its magnitude is proportional to the analyte concentration. Voltammetric methods possess a wide dynamic range and are advantageous for the quantification of low analyte concentrations. They include cyclic voltammetry, differential pulse voltammetry, linear sweep voltammetry, hydrodynamic voltammetry, stripping voltammetry, square-wave voltammetry, AC voltammetry, and polarography.⁶⁹

Amperometry, on the other hand, is distinguished by the lack of a scanning potential. While holding a constant potential at the working electrode, current is sampled as a function of time.⁷⁰ The current obtained can be correlated to the concentration of electroactive species available in the sample. Moreover, as the reduction or oxidation potential used for detection can be set specific to the analyte species, additional selectivity is conferred.⁷¹ Furthermore, a fixed current elicits a negligible charging current which helps to enhance the limit of detection by minimising the background signal. Amperometric methods are especially useful

in the context of flow systems where samples are flowed across the electrode. Due to their simplicity and low limits of detection, amperometry methods are also widely used with catalytic and affinity sensors.^{72,73}

3.2.2.2 Electrochemical Impedance Spectroscopy

Electrochemical impedance spectroscopy (EIS) is a powerful tool used to measure the resistive and capacitive properties of materials.^{49,74} For the purpose of sensing applications, it is frequently used in affinity sensors to monitor binding events at the electrode interface.^{75,76} Binding events produce changes in electron transfer resistance at the interface between the electrode and the solution. The reflected changes in impedance are representative of the amounts of bound target analytes. EIS provides a direct detection strategy for label-free systems. However, it is highly time consuming and irreproducible to regenerate the sensor surface for subsequent measurements in an impedance sensor. Non-specific binding further poses a challenge to the design of affinity sensors based on impedimetric detection.⁷⁷

3.2.2.3 Potentiometry

Potentiometry is characterised by the measurement of potential while negligible current is drawn. Examples of potentiometric sensors include ion-selective electrodes and glass pH electrodes.⁷¹ Two electrodes (working and reference) are involved in a potentiometric sensor. The working electrode is designed to be able to react selectively with the analyte of interest and the potential difference between the working and reference electrodes provides quantitative information about the analyte.

3.2.2.4 Miniaturised electrochemical transducers

There is a growing trend of miniaturisation in the field of analytical chemistry. The construction of small and portable sensors requires the size of the electrochemical transducer or the electrode to be in the micrometer scale or smaller.⁷⁸ Microelectrodes can easily be manufactured on a surface by vapour deposition and screen printing technologies.⁷⁹⁻⁸¹ Miniaturised sensors open up the possibility of field deployability and analysis of low sample volumes at low costs.^{82,83} Moreover, the small sensing surface area of a microelectrode allows it to be introduced into very small spaces without instigating much disturbance or damage. With miniaturised electrochemical transducers, the first measurements of oxygen concentration in biological tissues were realised in the early 1940s,⁸⁴ while measurements performed at the cellular level ensued.^{85,86}

3.3 Materials for Electrochemical Energy and Sensing Applications

3.3.1 Transition Metal Oxides (TMOs)

In contrast to precious metals like platinum, transition metal oxides (TMOs) are relatively abundant in nature. Known for their wide range of electronic, chemical and mechanical properties, massive attention has been placed on exploring TMOs as technologically important materials for applications such as catalysis, pigment in paints and plastics and high temperature superconductors.^{87, 88} Among these applications, the utilization of TMOs as catalysts is perhaps most technologically advanced and economically important. Specifically, the widely reported electrocatalytic activity of individual TMOs towards the oxygen reduction reaction (ORR) stands out and offers the promising possibility as alternatives to precious metals for electrochemical energy applications. Subsequently, we discuss the electrochemical properties of TMOs and their electrochemical energy applications, narrowing our focus on their electrocatalytic activity towards the ORR.

3.3.1.1 Electrochemical Properties of Transition Metal Oxides

Transition metals are known for their ability to accept a wide variety of oxidation states. In fact, the ability of TMOs to switch between different oxidation states of the metal cations has been reported to be a crucial factor in determining their electrocatalytic activity.⁸⁹ Apart from surface characterisation techniques like X-ray photoelectron spectroscopy (XPS) or energy-dispersive X-ray spectroscopy (EDS), cyclic voltammetry is often employed to provide information on the state

and composition of the oxide surface, and to analyse the electrochemical properties of TMOs.⁹⁰ Given that the metal cation can exist in more than one oxidation states, the resulting voltammetric spectra will exhibit distinctive peaks at the corresponding redox potentials, presenting evidence of surface redox transitions. Herein, we review the well-studied electrochemical properties of Co- and Ni-based TMOs.

The electrochemical profile of Co_3O_4 was first characterised by Chartier and co-workers in 1998.⁹¹ Co_3O_4 reported a wide electrochemical potential window from +0.5 V to -0.75 V vs Hg/HgO under alkaline conditions typically employed for ORR electrocatalysis. At potentials negative of -0.75 V vs Hg/HgO, destruction of the oxide lattice occurs. In the positive potential range, an oxidation peak was observed at +0.52 V vs Hg/HgO while a reduction peak corresponded at +0.51 V vs Hg/HgO (Figure 3.9A). As the oxide film surface changes partially to the hydrated CoOOH phase at rest potential,⁹² this distinctive pair of redox peaks was attributed to the redox couple $\text{CoO}_2/\text{CoOOH}$. Independent of the preparation method, similar electrochemical profiles of Co-based oxides were later reported by other groups and the redox reaction was established to be as follows:^{93,94}

In the same work, Chartier and co-workers demonstrated the electrochemical properties of ternary TMO, NiCo_2O_4 , where the peaks obtained were broader (Figure 3.9B).⁹¹ The width of the peak is an indication of the surface heterogeneity,⁹⁰ explaining the difference in voltammetric profiles of Co_3O_4 and NiCo_2O_4 . Moreover, Pumera and co-workers demonstrated the electrochemical properties of NiO .⁹⁵ In alkaline media, NiO spontaneously generates thin films of

Ni(OH)₂ on the surface. The Ni(OH)₂/NiOOH redox couple is evident at +0.37 V vs Ag/AgCl and +0.55 V vs Ag/AgCl as illustrated in Figure 3.9C following the reaction:

Figure 3.9 (A) Cyclic voltammograms of Co₃O₄ film on CdO-modified glass substrate in Ar-saturated 1 M KOH (vs Hg/HgO). Reproduced from ref. 91. (B) Repetitive cyclic voltammograms of NiO nanoparticles in 0.1 M NaOH (vs Ag/AgCl). Reproduced from ref. 95. (C) Cyclic voltammograms of NiCo₂O₄ film on CdO-modified glass substrate in Ar-saturated 1 M KOH (vs Hg/HgO). Reproduced from ref. 91.

3.3.1.2 Transition Metal Oxides for Electrochemical Energy Applications:

Electrocatalysts for the ORR

The search for alternative ORR electrocatalysts to Pt has directed attention to transition metal oxides (TMOs) as promising materials. Among the simple TMOs, Mn-based oxides are especially recognised for their high electrocatalytic activity towards the ORR, in addition to their environmental and economic advantages. Furthermore, strategies such as changing the stoichiometry, integrating conductive nanostructures, doping and introducing vacancies to tune the electronic and hence electrocatalytic properties of TMOs have been explored with much success.

An early report by Vorkapic and co-workers observed an improved ORR performance of carbon-based air electrodes following the addition of a small amount of MnO_2 .⁹⁶ This led the authors to conclude the electrocatalytic activity of MnO_2 towards ORR. Subsequently, similar conclusions were drawn for Mn-based oxides.^{97,98} Moreover, ORR performances of other manganese oxides, Mn_2O_3 , Mn_3O_4 , Mn_5O_8 and MnOOH , were characterised by Ohsaka and co-workers where MnOOH demonstrated the highest electrocatalytic activity among the other Mn-based oxides.⁹⁹ In a follow-up mechanistic study by the same group, the excellent electrocatalytic activity of Mn-based oxides towards the disproportionation reaction of OH^- was revealed.¹⁰⁰ However, in contrast to Pt-based electrocatalysts, manganese oxides are generally less active in terms of overpotentials and their capability to catalyse the direct 4e^- ORR pathway.

Meadowcroft first reported the superior electrocatalytic activity of mixed valence TMO, $\text{La}_{0.8}\text{Sr}_{0.2}\text{CoO}_3$.¹⁰¹ Since then, effort has been invested into studying the influence of TMO stoichiometry for enhanced electrocatalytic activity towards

the ORR. Gautier and co-workers studied the ORR performance of thin-sprayed films of mixed valence spinel TMOs, with the general formula $\text{Mn}_x\text{Co}_{3-x}\text{O}_4$ ($0 \leq x \leq 1$), on Ti under alkaline conditions.¹⁰² As the manganese concentration increased, they observed that the formation of intermediate peroxide ions (OH^-) increased accordingly with MnCo_2O_4 demonstrating the highest ORR performance. In general, cobalt-based spinel oxides have demonstrated advantages of activity, high stability, simple preparation and low cost.¹⁰³ The presence of multiple oxidation states of the metal cations in such systems provides donor-acceptor chemisorption sites for the reversible adsorption of oxygen. More significant is their generally higher electrical conductivity when compared with simple TMOs due to the relatively low activation energy for electron transfer between the metal cations.¹⁰⁴

The integration of conductive nanostructures such as graphene and carbon nanotubes has also been explored as a strategy to tune the electrocatalytic activity of TMOs. While Co_3O_4 nanoparticles and reduced graphene oxide, by themselves, show little activity towards the ORR, Dai and co-workers synthesised a hybrid material consisting of Co_3O_4 nanocrystals grown on reduced graphene oxide and reported its high performance as an ORR electrocatalyst.²² This ORR performance was further enhanced by nitrogen doping of graphene. This N-doped hybrid displayed comparable activity but superior stability to commercially available carbon-supported Pt catalysts (Pt/C) under alkaline conditions. In a related work by the same group, the hybrid material consisting of CoO nanocrystals grown on nitrogen-doped, multiwall carbon nanotubes (CNTs) showed high ORR activity following the direct $4 e^-$ reduction pathway and further outperformed the earlier synthesised graphene hybrid and commercial Pt/C catalysts.¹⁰⁵

3.3.2 Graphene

Figure 3.10 Scheme of top-down synthetic approach of graphene.

Large-scale production of graphene has been made possible by the top-down approach where graphite is oxidised to graphite oxide and further exfoliated and reduced to produce graphene. Graphene materials involved in the top-down approach are termed chemically modified graphene and can be classified into five main categories: graphite oxide, graphene oxide, thermally reduced graphene oxide (TRGO), chemically reduced graphene oxide (CRGO) and electrochemically reduced graphene oxide (ERGO) (Figure 3.10).¹⁰⁶⁻¹⁰⁸ The obtained graphene is mainly defective in nature and greatly differs from pristine graphene – holes and defects are present in the sp^2 carbon networks. This has rendered them unsuitable for applications such as high-power electronics due to interruption in the ballistic transport and hence, scattering of electrons on the defective graphene. Nonetheless, these graphene materials have found usage in less rigorous applications such as paint/ink, coatings, composites, transparent conductive layers, energy storage and sensing applications.¹⁰⁹ In particular, the excellent conductivity, ease for functionalization and large surface-to-volume ratio of graphene allows it

to be incorporated for electrochemical sensing with promising results. A brief introduction to the electrochemical properties of graphene and its applications in electrochemical sensing will be discussed subsequently.

3.3.2.1 Electrochemical Properties of Graphene

For graphene to be considered for sensing applications in electrochemistry, it is vital to first understand several basic electrochemical parameters of the graphene electrodes: electrochemical potential window and electron transfer rate. According to Dong and co-workers, graphene displays a wide electrochemical potential window of 2.5 V in phosphate buffered saline (0.1 M PBS, pH 7.0). This is comparable to that of graphite, and standard electrodes like glassy carbon and boron-doped diamond electrodes.¹¹⁰⁻¹¹²

Electrochemical processes are fundamentally governed by electron transfer. In order to map the electrochemical properties of graphene, it is important to understand the origin of heterogeneous electron transfer (HET) phenomenon, where the exchange of electrons takes place between a chemical species and the solid-state electrode. The structural features of graphene are basically similar to that of its parent graphite material where both consist of basal- and edge-plane sites. Consequently, parallels can easily be drawn between the electrochemical performances of graphene and graphite. For this reason, the defect-free highly ordered pyrolytic graphite (HOPG) is often used as an experimental substrate for investigation of the specific site of heterogeneous electron transfer on graphene.

Compton and co-workers established that the HET from/to a graphene sheet takes place in the order of $\sim 0.01 \text{ cm s}^{-1}$ on the edge-plane sites while that exhibited on the basal-plane sites is close to zero ($\sim 10^{-9} \text{ cm s}^{-1}$).¹¹³ The basal-plane sites of

graphite are electrochemically inert and in the case when an electrochemical response is elicited, it is solely contributed by the edge-plane defects on the basal-plane.¹¹⁴ Further investigations by Loh and co-workers supported the above argument as they demonstrated the low HET rate of the basal plane of epitaxial-graphene. Upon electrochemical anodization to introduce defects on the epitaxial-graphene, a significant enhancement of HET rate was observed.¹¹⁵

Moreover, the presence or introduction of oxygen-containing groups to graphene can affect its electrochemical properties. The influence of oxygen-containing groups remains controversial. While they can confer electrocatalytic properties in some cases,^{116,117} they have also shown to inhibit electrochemical reactions and even adsorb reaction products.¹¹⁸ Conversely, the number of graphene layers has been determined to have insignificant influence over the electrochemistry of graphene. The electrochemical performances of single, few and multi-layer graphene materials displayed negligible differences based on the electrochemical oxidation of ascorbic acid and dopamine.¹¹⁹

3.3.2.2 Graphene for Electrochemical Sensing

Graphene is an ideal candidate for electrochemical sensing applications given its large surface-to-volume ratio, excellent electrical conductivity and ease of functionalization. Moreover, it is worth pointing out that graphene is expected to be advantageous for electrochemical applications over related materials such as graphite or carbon nanotubes with a much greater surface area of $2630 \text{ m}^2 \text{ g}^{-1}$ compared to that of graphite ($\sim 10 \text{ m}^2 \text{ g}^{-1}$) and carbon nanotubes ($1315 \text{ m}^2 \text{ g}^{-1}$).¹²⁰ Graphene has been demonstrated for the electrochemical detection of important biomarkers such as ascorbic acid (AA), dopamine (DA), uric acid (UA), hydrogen

peroxide (H_2O_2), glucose, proteins and DNA bases.⁵⁶ In most cases, voltammetry and amperometry are used as electrochemical detection techniques.

One of the earliest demonstrations of graphene for electrochemical sensing is by Papakonstantinou and co-workers in 2008.¹²¹ Multi-layer graphene was exploited as a sensing platform for the simultaneous detection of AA, DA and UA with well-resolved peaks. Determination of DA was also achieved at high sensitivities with a detection limit of $0.17 \mu\text{M}$. The graphene-based electrode outperformed conventional solid-state electrodes such as glassy carbon, gold, boron-doped diamond, and was comparable to only the edge-plane pyrolytic graphite (EPPG) electrode. The report further highlighted the vital role played by edge-plane sites for the enhanced electron transfer kinetics.

In another work, Dong and co-workers demonstrated the usage of chemically reduced graphene oxide for DNA bases, H_2O_2 and glucose.¹¹⁰ The electrochemical reactivity of free purine and pyrimidine was greatly enhanced at a chemically reduced graphene oxide-modified glassy carbon (CRGO/GC) electrode. Based on this, simultaneous detection of all four DNA bases (G, A, T and C) in both single and double-stranded DNA were readily achieved at the CRGO/GC electrode at physiological pH without requiring the usual pre-hydrolysis step. Further investigations revealed the superior electrocatalytic activity of the CRGO/GC electrode towards H_2O_2 . Consequently, the incorporation of glucose oxidase into the CRGO/GC electrode resulted in a high performance glucose biosensor (linear range of $0.01 - 10 \text{ mM}$, limit of detection of $2 \mu\text{M}$), outperforming that based on graphite and bare glassy carbon.

However, the electrochemical sensing performances of chemically modified graphene can vary significantly from one another. In the context of DNA base detection, Pumera and co-workers highlighted the enhanced sensitivities of graphite oxide, graphene oxide, TRGO and ERGO towards the determination of adenine and cytosine while only TRGO and ERGO were efficient for the detection of guanine and thymine.¹²² Moreover, there have been reports showing that graphene may not be as advantageous as compared to its parent graphite. Banks and co-workers demonstrated that graphene-based carbon paste electrode did not exhibit any advantageous properties for the detection of AA and UA.¹²³

3.3.3 Transition Metal Dichalcogenides (TMDs)

Following the reignited interest in 2D layered nanomaterials, transition metal dichalcogenides (TMDs) have gained recognition rapidly. Possessing a layered structure similar to that of graphite and being earth-abundant, the electronic, chemical and optical properties emerging from the anisotropy of TMD materials have been demonstrated to be useful for supercapacitors, transistors, photovoltaics, electrochemical catalysis and sensing.^{124,125} Considering TMDs for electrochemical energy applications, of particular interest is their high electrocatalytic activity towards the hydrogen evolution reaction (HER). TMDs could eventually be exploited to support efficient and low-cost production of hydrogen without the reliance on precious Pt-based catalysts. Moreover, when exfoliated to single or few layer sheets, TMDs possess high specific surface area, electronic properties ranging from insulating to metallic and versatility in functionalization, making them excellent candidates for electrochemical sensing applications. A brief introduction of the electrochemical properties of TMDs and consequently, their applications in electrochemical energy and sensing will be included in proceeding discussions.

3.3.3.1 Electrochemical Properties of Transition Metal Dichalcogenides

With the proliferation of TMD research, a large volume of research has been dedicated to the development of TMD-based applications. Despite being crucial for driving and sustaining these developments, efforts made to understand the fundamental electrochemical behaviours of transition metal dichalcogenides are not keeping pace.

Figure 3.11 (A) Cyclic voltammograms of the oxidation and subsequent deactivation of MoS₂ under typical hydrogen evolution reaction conditions of 0.5 M H₂SO₄ (N₂-saturated). Scan rate, 2 mV s⁻¹. Reproduced from ref. 126. (B) Cyclic voltammograms of APTES-MoS₂ modified glassy carbon electrode in aqueous 0.5 M NaCl (N₂-saturated). Scan rate, 50 mV s⁻¹. Reproduced from ref. 127.

Applied reduction and oxidation potentials can give rise to redox reactions inherent to the TMDs. As such, when TMDs are used in electrochemical catalysis or sensing as electrode materials, efficacy of the system may be greatly influenced.⁶¹ The electrochemical properties are independent of the analyte in solution, and can differ according to experimental conditions such as pH, the nature of electrolyte and the potential applied. A prominent work by Chorkendorff and co-workers reported the inherent electrochemical oxidation of MoS₂ nanoparticles supported on carbon paper under conditions typically employed for HER electrocatalysis.¹²⁶ An irreversible oxidation was observed, starting at +0.6 V vs NHE and reaching a maximum at +0.98 V vs NHE (Figure 3.11A). This oxidative process resulted in a decrease in HER activity. The authors further correlated the oxidation peaks at +0.7 and +0.98 V vs NHE to the oxidation of the MoS₂ edge and basal planes respectively. The larger oxidation potential required for the basal planes agreed with the greater resistance towards corrosion. Moreover, the electrochemical reduction of MoS₂ has more recently been demonstrated by Zhang and co-

workers.¹²⁷ At -0.71 V vs Ag/AgCl, an irreversible reduction of MoS₂ in aqueous electrolyte was observed (Figure 3.11B). However, re-oxidation of this reduced state is possible in organic electrolytes like acetonitrile and tetrabutylammonium tetrafluoroborate. According to XPS characterisation, the reduction process corresponded to a phase change in the material.

Figure 3.12 (A-D) Cyclic voltammograms of bulk MoS₂ and after exfoliation with methyllithium, n-butyllithium and tert-butyllithium in 0.1 M KCl electrolyte. Scan rate, 0.1 V s⁻¹. Reproduced from ref. 128. (E-G) Cyclic voltammograms of bulk MoSe₂, WS₂ and WSe₂ in 0.1 M KCl electrolyte. Scan rate, 0.1 Vs⁻¹. Reproduced from ref. 129.

The effects of chemical exfoliation on MoS₂ using organolithium reagents were investigated by Pumera and co-workers, where exfoliations with butyllithium (*n*-BuLi and *t*-BuLi) were most effective in contrast to that with methyllithium (MeLi).¹²⁸ The fundamental electrochemical behaviours of MoS₂ upon reduction and oxidation were recorded for the different exfoliation extents. For bulk MoS₂, an oxidation wave was noted at $+1.3$ V vs Ag/AgCl and a broad reduction peak was observed at -1.0 V vs Ag/AgCl (Figure 3.12A). Interestingly, even as negligible variations in peak potentials were observed with the different extents of exfoliation, the peak current intensities were noted to be largest after BuLi exfoliations (Figure

3.12B-D), agreeing well with the higher exfoliation efficiencies and hence, larger specific surface areas. Exploring beyond MoS_2 , Pumera and co-workers established in a separate work that the electrochemical properties of group 6 TMDs (MoS_2 , MoSe_2 , WS_2 , WSe_2) are dependent on their constituent elements (Mo/W and S/Se).¹²⁹ A general increasing trend in oxidation potentials in the order $\text{WSe}_2 < \text{MoSe}_2 < \text{WS}_2 < \text{MoS}_2$ was further observed (Figure 3.12A and Figure 3.12E-G). Efforts to characterise the electrochemical properties of group 5 TMDs were also made by the same group where the electrochemistry of the tellurium chalcogen is prominent in the voltammograms of group 5 tellurides.¹³⁰

Figure 3.13 Potential-pH diagrams illustrating thermodynamic stabilities of a (A) molybdenum-sulphur-oxygen-hydrogen system and (B) tungsten-sulphur-oxygen-hydrogen system. Reproduced from ref. 133.

Understanding the inherent electrochemical properties of TMDs and the products generated at specific potentials is of paramount importance to developing TMD-based applications. Often, they can pose as challenges to TMD applications due to the limited electrochemical potential window. In other cases, they can be

exploited to enhance the electrocatalytic performance of TMDs; Pumera and co-workers demonstrated the activation of group 6 TMDs for HER electrocatalysis by reductive treatment.¹³¹ Considering that such redox behaviours occur as surface processes, X-ray photoelectron spectroscopy (XPS) is a highly suitable and commonly used technique employed to probe compositional changes in the materials.^{126,129,132} In addition, reference can be taken from potential-pH or Pourbaix diagrams to provide preliminary insights on the thermodynamic stability of TMDs at different potentials and pH conditions.¹³³ Under acidic HER conditions (pH ~0.5), it is suggested that TMDs like MoS₂ and WS₂ are fully stable only within a narrow potential window just positive of potentials where hydrogen evolution takes place (Figure 3.13). Nonetheless, it is possible for parallel reactions to take place at the TMDs and their surface oxides and even so, it is likely that these reactions proceed only on a fraction of the total electrode surface. For these reasons, the conclusive determination of reaction mechanisms corresponding to the electrochemical behaviours of TMDs remains an arduous task.

Moreover, the electron transfer rate of TMDs is an important parameter to be investigated. Similar to graphene, layered TMDs possess basal- and basal-plane sites. Ahmed and Gerischer reported a much higher heterogeneous electron transfer (HET) to/from MoS₂ sheets at the edge-plane sites compared to the basal-plane sites.¹³⁴ The authors explained this phenomenon by the greater overlapping of the d_{xy} and d_{x²-y²} orbitals of the conduction band of the Mo metal with the orbitals of the redox probe Fe(CN)₆^{3-/4-}. A separate work by Pumera and co-workers showed the rate of HET to be 4.96 x 10⁻⁵ cm s⁻¹ on the edge-plane sites and close to zero on pristine basal-plane sites for MoS₂.¹³⁵ Mechanical damage induced on the

basal plane significantly improved HET rates, further supporting the above proposition.

Exfoliation of bulk TMDs has been reported to produce single or few layers with large compositions of the highly conductive, metallic 1T phase.¹³⁶ This is mainly true in the case of group 6 TMDs.¹²⁴ While no correlation has been established between the HET and the extent of exfoliation or phase composition of TMDs, Zhang and co-workers demonstrated the superior HET of Li-exfoliated MoS₂ after reductive treatment.¹²⁷ This observation was subsequently reproduced by Pumera and co-workers, who then performed density function theoretical (DFT) calculations and attributed the enhanced HET to the stabilization of the metallic 1T phase by electron doping.¹³¹

3.3.3.2 Transition Metal Dichalcogenides for Electrochemical Energy

Applications: Electrocatalysts for the HER

In an attempt to move away from the reliance on expensive Pt-based catalysts, transition metal dichalcogenides (TMDs) present as promising alternatives with their high electrocatalytic performance towards the HER. A vast amount of literature has been dedicated to investigate the production of hydrogen at various TMDs and the factors that govern their HER performance. TMDs of differing structures have been reported to display varied HER electrocatalytic activity, demonstrating the importance of identifying their active sites for the HER. Moreover, the electrical conductivity and atomic doping of TMDs have been pointed out to influence their HER performance.

While earlier studies have suggested that the TMD edge-plane sites are the catalytic active sites, a theoretical study by Nørskov and co-workers highlighted

the key role played by sulphur-terminated edges of MoS₂ as active sites for the HER.¹³⁷ Based on DFT calculations, the ΔG_H at the MoS₂ edges is close to zero. Subsequently, superior electrochemical and catalytic activity was demonstrated at edge-sites not only for MoS₂, but also for other TMD materials like WS₂, MoSe₂ and WSe₂.^{138,139} Hence, fabrication of TMDs with increased edge-exposed surface with respect to the basal portion is one of the strategies to enhance the electrocatalytic properties.^{140,141}

In order to enhance the electrocatalytic properties of TMD materials, a commonly employed method is splitting of the layers of bulk TMD materials into single or few layer sheets via Li intercalation with organolithium compounds.¹⁴²⁻¹⁴⁴ Moreover, it is a well-known fact that the properties of TMD materials in their bulk states vastly differ from their exfoliated counterparts. This arises from the chemical nature of TMDs when existing in various polymorphs – 2H and 1T phases as the most frequently encountered.¹²⁴ In the case of MoS₂, the reducing agent *n*-butyllithium destabilises the thermodynamically favoured 2H polymorph into the octahedral 1T structure. Jin and co-workers demonstrated the conversion of bulk, semiconducting 2H phase MoS₂ nanostructures directly grown on graphite to the exfoliated, metallic 1T phase by exfoliation using lithium intercalation.¹⁴⁵ This phase conversion in turn improved the HER performance with the 1T phase MoS₂ nanosheets yielding an overpotential of -0.19 V vs RHE at a current density of -10 mA cm⁻¹ and a low Tafel slope of 43 mV dec⁻¹.

The above observations prompted Chhowalla and co-workers to work on elucidating the mechanism behind the enhanced HER performance of 1T phase MoS₂.¹⁴⁴ A solvent-free intercalation method was used to produce high

concentrations of metallic 1T phase MoS₂ nanosheets with stellar HER performance (Tafel slope of 40 mV dec⁻¹) as expected. They reported that while edge oxidation of 2H phase MoS₂ significantly reduced the HER electrocatalytic activity, 1T phase MoS₂ remains unaffected after oxidation. Clearly, the HER activity of 2H phase MoS₂ is dependent on the reduced edges as discussed earlier. However, this is not so for 1T phase MoS₂. Electrical conductivity was determined to be an important factor governing the HER performance of both phases. This was further confirmed by using carbon nanotubes to increase the conductivity of 2H phase MoS₂ for enhanced electrocatalytic activity towards the HER.

Moreover, another strategy directed at altering the electrocatalytic properties of TMDs is atomic doping where dopant atoms can directly substitute the atoms in the lattice or into the interstitial sites between atoms in the crystal lattice. Moreover, it is also possible for dopant atoms to intercalate between layers in the context of TMDs.¹⁴⁶ Hitherto, the strategy of atomic doping has garnered some success in enhancing the HER catalytic activity of TMDs. Specifically, previous reports on MoS₂ doped with vanadium,¹⁴⁷ rhenium¹⁴⁸ and cobalt¹⁴⁹ resulted in higher HER efficiencies. Computational studies also determined the potential positive effects on HER catalytic activity of various transition metal-doped (i.e. Pt, Ni, Ru, Rh, Co, Fe, Mn, Ta, V, Nb, Cr, Os, Ir and Re) MoS₂ through tuning the adsorption free energy for hydrogen (ΔG_H) on the edges closer to the thermoneutral value.¹⁵⁰

3.3.3.3 Transition Metal Dichalcogenides for Electrochemical Sensing

Transition metal dichalcogenides (TMDs), especially when exfoliated to single or few layer sheets, or nanoparticles, are starting to be recognised and

exploited for electrochemical sensing applications. Owing to their desirable properties like high conductivity, high surface-to-volume ratio and ease of functionalization, TMDs have been demonstrated for the electrochemical detection of important biomarkers.

Among the TMD materials, MoS₂ is the most highly explored for electrochemical sensing applications to date. Zhang and co-workers prepared single or few layer MoS₂ nanosheets by electrochemical Li intercalation followed by exfoliation in water. An APTES-MoS₂ film was deposited on a glassy carbon (GC) electrode for the detection of ascorbic acid (AA), dopamine (DA) and uric acid (UA).¹²⁷ The constructed electrode platform outperformed the bare GC electrode and was able to simultaneously detect and distinguish the three biomolecules. Moreover, a prominent work by Li and co-workers employed ultrasmall MoS₂ nanoparticles (< 2nm) for the electrocatalytic reduction of H₂O₂.¹⁵¹ This resulted in an extremely sensitive H₂O₂ sensor with a detection limit as low as 2.5 nM, accompanied by a wide linear range of 5 orders of magnitude. The MoS₂-based sensor was able to detect trace amount of H₂O₂ released from Raw 264.7 cells and subsequently, used for the construction of an efficient glucose biosensor by the immobilization of glucose oxidase on the MoS₂ nanoparticles films.

Furthermore, Pumera and co-workers demonstrated the different affinities of MoS₂ towards single- and double-stranded DNA.¹⁵² MoS₂ nanoflakes were synthesised and utilised as electroactive labels for probing DNA hybridization events. By taking advantage of the electrochemical behaviours of MoS₂, the inherent oxidation of MoS₂ served as the analytical signal. A wide range of detection was established from 0.03 nM to 300 nM. Moving beyond MoS₂, Pumera

and co-workers demonstrated the use of WS₂ as an electroactive label in another work.¹⁵³ Preparation of WS₂ nanoparticles was achieved by solution-based electrochemical exfoliation. The high electrocatalytic activity for hydrogen evolution of these WS₂ nanoparticles allowed them to be used as labels in a standard magneto-immunosandwich assay for protein detection through the HER. The system produced high analytical performance with a low detection limit of 2 ng mL⁻¹ and a wide range of detection from 2 to 500 ng mL⁻¹.

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Part I

Chapter 4

Transition Metal Oxides for the Oxygen Reduction Reaction

4.1 *Introduction*

4.2 *Results and Discussion*

4.3 *Conclusion*

4.4 *Experimental*

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

1. **Rou Jun Toh**, Zdenek Sofer, Martin Pumera. Transition Metal Oxides for the Oxygen Reduction Reaction: Influence of the Oxidation States of the Metal and its Position on the Periodic Table. *ChemPhysChem* **2015**, 16, 3527-3531.

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4.1 Introduction

Electrocatalysts have been developed to meet the needs and requirements of renewable energy applications. In this aspect, transition metal oxides have been well explored and are promising for the electrocatalysis of the oxygen reduction reaction (ORR).¹⁻⁴ However, little attention has been paid to studying their electrocatalytic performance with respect to the position of the metal on the periodic table and the metal oxidation state. In this chapter, we address the following basic questions: 1) are there trends in electrocatalytic performance to be observed across the periodic table or down a group; 2) how would the metal oxidation states affect the catalytic activity of their respective transition metal oxides towards ORR.

Moreover, many reports focus on only one or a few metal oxides at a time. Herein, thirty transition metal oxides were selected for investigation: Sc_2O_3 , TiO , TiO_2 , V_2O_3 , V_2O_5 , Cr_2O_3 , MnO , Mn_3O_4 , Mn_2O_3 , MnO_2 , Fe_2O_3 , CoO , Co_3O_4 , Co_2O_3 , NiO , Cu_2O , CuO , ZnO , Y_2O_3 , ZrO_2 , Nb_2O_5 , MoO_2 , MoO_3 , CdO , HfO_2 , Ta_2O_5 , WO_2 , WO_3 , HgO and La_2O_3 . The transition metal oxides were first prepared following a simple and scalable method. Characterisation of the prepared materials was achieved with scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDS) and linear scan voltammetry (LSV). Elaborating on the electrochemical responses originating from oxygen (O_2) reduction on the materials, we found no clear or obvious correlation between the position of a metal and the electrocatalytic performance of its respective transition metal oxides. In addition, the mixed-valence (+2,+3) oxides performed the poorest compared to their respective pure transition metal oxides.

4.2 Results and Discussion

SEM images of the synthesised transition metal oxides are illustrated in Figure 4.1. It can be observed that most of the metal oxides adopt characteristic microstructures, with the exception of V_2O_3 and MoO_3 where it was observed that the materials took a congeal form. The particle size of each individual metal oxide are observed from the SEM images and the materials may be categorised into three distinct groups: 1) particle size $< 1\ \mu\text{m}$; 2) particle size ranging from $1 - 10\ \mu\text{m}$; 3) particle size $> 10\ \mu\text{m}$. Metal oxides which fall into the first group, with particle size less than $1\ \mu\text{m}$, are Fe_2O_3 , Co_2O_3 , NiO , CuO , ZnO , CdO , HfO_2 , Ta_2O_5 and WO_3 . Metal oxides with particle size ranging from $1 - 10\ \mu\text{m}$ consist of Sc_2O_3 , TiO , TiO_2 , Cr_2O_3 , MnO , Mn_3O_4 , Mn_2O_3 , MnO_2 , CoO , Co_3O_4 , Cu_2O , Y_2O_3 , ZrO_2 , Nb_2O_5 , WO_2 , HgO and La_2O_3 . Metal oxides with larger particle size of more than $10\ \mu\text{m}$ include V_2O_5 and MoO_2 . Further analysis with EDS provided purity data for the studied materials (Figure 4.2).

Figure 4.1 Scanning electron microscopy (SEM) images of the transition metal oxides.

Figure 4.2 Energy-dispersive X-ray spectroscopy (EDS) images of the transition metal oxides.

Figure 4.2 (Continued) Energy-dispersive X-ray spectroscopy (EDS) images of the transition metal oxides.

Figure 4.3A illustrates the linear scan voltammograms obtained from the electrochemical reduction of oxygen in air-saturated 0.1 M KOH using a bare GC, and GC electrodes modified with transition metal oxides and Pt/C. With that, a scatter plot depicting the reduction onset potentials of bare GC, Pt/C and transition metal oxides according to their positions on the periodic table is represented in Figure 4.3B. As observed in Figure 4.3B, Pt/C continues to exhibit superior electrocatalytic performance with the earliest onset potential (-161 mV). Moreover, when comparing the transition metal oxides with bare GC, manganese (Mn), iron (Fe), cobalt (Co) and nickel (Ni) oxides displayed onset potentials higher than that of bare GC (-300 mV) and hence, better electrocatalytic activity towards ORR. On the other hand, most of the other transition metal oxides exhibited similar or even more negative onset potentials than that of bare GC. However, there are no obvious trends observed from the results obtained. Hence, this may suggest that electrocatalytic performance of the metal oxide towards ORR is not significantly affected by the position of its metal on the periodic table. As a control, the currents associated with electrochemical reduction of oxygen were

verified by comparing linear scan voltammograms obtained in air-saturated 0.1 M KOH, and nitrogen-saturated 0.1 M KOH where oxygen is absent (Figure 4.4).

Figure 4.3 (A) Linear scan voltammograms for ORR in air-saturated, 0.1 M KOH using a bare glassy carbon electrode, and GC electrodes modified with MnO₂, NiO and Pt/C. Scan rate, 5 mV s⁻¹. (B) Reduction onset potentials of transition metal oxides from linear scan voltammograms.

Figure 4.4 Linear scan voltammograms in air-saturated (black line) and nitrogen-saturated (red line) 0.1 M KOH using bare GC electrodes and GC electrodes modified with Pt/C and the transition metal oxides.

Subsequently, it would be of interest to also investigate the effect of metal oxidation states on the electrocatalytic performance of the metal oxide towards ORR. For this purpose, several transition metals were studied and their different oxides compared. Figure 4.5A shows the linear scan voltammograms obtained from the electrochemical reduction of oxygen in air-saturated 0.1 M KOH using GC electrodes modified with different cobalt oxides (CoO, Co₂O₃ and Co₃O₄), and Figure 4.5B displays the relationship between the ORR onset potential and metal oxidation states of different elements. From Figure 4.5B, no general trend between the metal oxidation state and their pure transition metal oxides' ORR electrocatalytic performance may be established. For vanadium (V), cobalt (Co), copper (Cu) and molybdenum (Mo) oxides, an enhancement in the kinetics of ORR was observed as the metal oxidation state increases. However, the opposite is true for manganese (Mn), titanium (Ti) and tungsten (W) oxides.

Moreover, the mixed-valence (+2,+3) oxides consistently performed the poorest compared to their respective pure transition metal oxides. From Figure 4.5B, it is observed that Mn₃O₄ (-308 mV) and Co₃O₄ (-301 mV) have more negative onset potentials than MnO (-213 mV) and Mn₂O₃ (-225 mV), and CoO (-296 mV) and Co₂O₃ (-291 mV) respectively. Hence, the importance of the purity of transition metal oxides for the application of ORR catalysis may be highlighted from these findings. This is noteworthy as mixed-valence oxides are frequently used in catalysis due to convenience of synthesis.

Figure 4.5 (A) Linear scan voltammograms for ORR in air-saturated, 0.1 M KOH using GC electrodes modified with CoO, Co₂O₃ and mixed-valence (+2,+3) oxide, Co₃O₄. Scan rate, 5 mV s⁻¹. (B) Reduction onset potentials of metals oxides with multiple oxidation states. Error bars represent RSD ($n=3$, 95% confidence interval).

In addition, a comparison of the morphology and particle size of the studied materials with their corresponding electrocatalytic activity towards ORR showed no strong correlation as expected. This is because electrochemistry is happening on the surface and hence, there is no quantum confinement effect at the size of particles used.

4.3 Conclusion

After screening a sufficiently large sample size of transition metal oxides, we show that there is no significant correlation between the position of a metal on the periodic table and the electrocatalytic performance of its respective transition metal oxides. The electrocatalytic activities of manganese (Mn), iron (Fe), cobalt (Co) and nickel (Ni) oxides were revealed in the enhancement of oxygen reduction kinetics under alkaline conditions. While these transition metal oxides generally performed better than the bare glassy carbon, it was also observed that the mixed-valence (+2,+3) oxides performed the poorest compared to their respective pure transition metal oxides. These findings are of profound importance to the development and choice of electrocatalysts for renewable energy applications.

4.4 Experimental

4.4.1 Materials

N,N-dimethylformamide (DMF) was purchased from Merck. Tungsten (VI) oxide (WO_3) was purchased from Sigma Aldrich. Glassy carbon (GC, 3 mm diameter) working, Ag/AgCl reference, and Pt counter electrodes were acquired from Autolab (Japan). Sc_2O_3 , Y_2O_3 , La_2O_3 , TiO_2 , ZrO_2 , HfO_2 , Nb_2O_5 , Ta_2O_5 , Cr_2O_3 , MoO_3 , WO_3 , Ti and W were obtained from Sigma-Aldrich, Czech Republic. Co_2O_3 was obtained from Riedel-deHaën (Germany). V_2O_5 , MnO_2 , Fe_2O_3 , NiO, Cu_2O , CuO, ZnO, CdO and HgO were obtained from Lach-Ner, Czech Republic. Hydrazine hydrate, cobalt carbonate, ammonium vanadate and cobalt nitrate hexahydrate was obtained from Penta, Czech Republic. Hydrogen (99.9999%) and argon (99.996%) were obtained from SIAD, Czech Republic.

4.4.2 Apparatus

Scanning electron microscopy (SEM) images and energy-dispersive X-ray spectroscopy (EDS) were acquired using a Joel 7600F instrument (Joel, Japan). The instrument was operated at 1 or 2 kV for SEM and 20 kV for EDS measurements. For SEM and EDS measurements, samples preparation was achieved by coating a uniform layer of the materials of interest on a silicon wafer. All voltammetric experiments were performed using a μ Autolab type III electrochemical analyser (Eco Chemie, The Netherlands) connected to a computer and controlled by NOVA, Version 1.8.17 software. Electrochemical experiments were carried out at room temperature in an electrochemical cell (5 mL) with the employment of a three electrode configuration. A platinum electrode functioned as the counter electrode,

and an Ag/AgCl electrode served as the reference electrode. All electrochemical potentials in this chapter are indicated as versus the Ag/AgCl reference electrode.

4.4.3 Procedures

Titanium (II) oxide was prepared by the reaction of TiO_2 with Ti. The stoichiometric mixture of Ti and TiO_2 for the formation of 3 g of TiO was homogenised in agate mortar and pressed in the pellets. The pellets were heated under vacuum on 1600°C for 4 hours.

Vanadium (III) oxide was prepared by reduction of NH_4VO_3 with hydrazine. 10 g of ammonium vanadate was dissolved in 150 ml of water. To the reaction mixture was added 6 ml of hydrazine hydrate and reaction mixture was heated under reflux for 30 minutes. Suction filtration was employed to separate the formed vanadium (III) oxide. After repeated washing with water, the oxide was dried in vacuum oven (200°C , 48 hours).

Molybdenum (IV) oxide was synthesised by reduction of molybdenum (VI) oxide with hydrazine hydrate. 10 g of MoO_3 was dispersed in 50 mL of water by ultrasonication. To the reaction mixture was added 10 mL of hydrazine hydrate and the reaction mixture was heated stirred at room temperature for 12 hours. Formed molybdenum(IV) oxide was separated by suction filtration, repeatedly washed with water and dried in vacuum oven (200°C , 48 hours).

Tungsten (IV) oxide was prepared by heating a stoichiometric mixture of W and WO_3 in evacuated ampoule. The stoichiometric mixture of W and WO_3 for formation of 4 g of WO_2 was homogenised in agate mortar and pressed in the

pellet form. The pellets were sealed under vacuum in quartz ampoules by oxygen-hydrogen torch and heated on 950 °C for 40 hours.

Manganese (II) oxide was prepared by reduction of MnO_2 with hydrogen. 3 g of MnO_2 was placed in alumina boat inside tube furnace. Tube furnace was repeatedly flushed with argon and subsequently heated in hydrogen atmosphere on 800 °C for 5 hours with hydrogen flow of 100 mL min⁻¹. The heating and cooling rate was 5 °C min⁻¹ and

Manganese (III) oxide was prepared by thermal decomposition of MnO_2 . 5 g of MnO_2 was placed in alumina crucible and heated on 700 °C for 24 hours. The heating and cooling rate was 5 °C min⁻¹.

Manganese (II,III) oxide was prepared by thermal decomposition of MnO_2 . 5 g of MnO_2 was placed in alumina crucible with lid and heated on 1200 °C for 24 hours. The heating rate was 5 °C. The rapid cooling by air quenching was applied to avoid oxidation.

Cobalt (II) oxide was prepared by thermal decomposition of cobalt carbonate. 5 g of cobalt carbonate was heated under vacuum on 350 °C for 10 hours.

Cobalt (II,III) oxide was prepared by thermal decomposition of cobalt nitrate. 5 g of cobalt nitrate hexahydrate was heated in alumina crucible on 700 °C for 2 hours.

Oxygen Reduction Reaction (ORR) Studies on Transition Metal Oxides. ORR efficiency of transition metal oxides were performed on glassy carbon (GC) electrodes. GC electrodes were first renewed on a polishing pad using alumina particle (0.05 µm) slurry and washed with ultrapure water. The transition metal

oxides (5 mg mL^{-1}) were dispersed in DMF. Next, the suspension was subjected to at least 60 min of ultrasonication treatment to form a homogenous ink. Subsequently, the suspension ($1 \text{ }\mu\text{L}$) was pipetted onto the GC electrode surface, and left to evaporate at room temperature. A film of randomly distributed material is thus produced on the GC electrode surface. Linear scan voltammetry (LSV) experiments were conducted at a scan rate of 5 mV s^{-1} in KOH solution (0.1 M) saturated with air for the oxygen reduction reaction. Reduction onset potentials were standardised and determined as the potential where current is 10% of the peak current.

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Part I

Chapter 5

Ternary Transition Metal Oxide Nanoparticles with
Spinel Structure for the Oxygen Reduction Reaction

5.1 *Introduction*

5.2 *Results and Discussion*

5.3 *Conclusion*

5.4 *Experimental*

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

1. **Rou Jun Toh**; Alex Yong Sheng Eng, Zdenek Sofer, David Sedmidubsky, Martin Pumera. Ternary Transition Metal Oxide Nanoparticles with Spinel Structure for the Oxygen Reduction Reaction. *ChemElectroChem* **2015**, 2, 982-987.

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5.1 Introduction

Mixed-valence transition-metal oxides with a spinel structure were identified as active catalysts for the oxygen reduction reaction (ORR) in alkaline conditions.¹⁻³ In particular, it has been reported that Ni, Cu and Mn substituted Co_3O_4 shows high activity and stability as electrocatalysts for ORR.²⁻⁴ However, little attention has been paid to comparing the effect of different substitutions. Herein, we address the following basic questions: 1) is the improved catalytic activity attributed to the presence of cobalt oxide; 2) how would different substitutions at X (Ni, Zn) or Y (Co, Mn) in a spinel of general form XY_2O_4 , affect the catalytic activity towards ORR.

In this work, four different nanocrystal spinel oxides were selected for investigation: NiCo_2O_4 , NiMn_2O_4 , ZnCo_2O_4 , and ZnMn_2O_4 . For this purpose, mixed-valence transition-metal oxide nanocrystals of XY_2O_4 composition, where X= Ni or Zn and Y= Mn or Co, were first prepared according to a simple and scalable method. Characterisation of the prepared materials were achieved through scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDS), X-ray photoelectron spectroscopy (XPS), X-ray diffraction (XRD) and cyclic voltammetry (CV). The electrochemical responses originating from oxygen (O_2) reduction on the materials will be elaborated. In general, cobalt-based spinel oxides displayed significant electrocatalysis of ORR, with Ni-substituted spinel oxides outperforming their respective Zn-substituted spinel oxides.

5.2 Results and Discussion

The compositions of the synthesised materials are validated with EDS (Figure 5.1). SEM images of the synthesised materials are displayed in Figure 5.2. It can be clearly seen that the individual spinel oxides adopt characteristic microstructures. The synthesised powders consist of small crystallites with the exception of NiMn_2O_4 , where octahedral crystals with surface defects can be identified.

Figure 5.1 Energy-dispersive X-ray spectroscopy (EDS) images of NiCo_2O_4 , NiMn_2O_4 , ZnCo_2O_4 , and ZnMn_2O_4 . Scale bar, 10 μm .

Figure 5.2 Scanning electron microscopy (SEM) images of NiCo_2O_4 , NiMn_2O_4 , ZnCo_2O_4 , and ZnMn_2O_4 . Scale bars, 100 nm (A); 1 μm (B); and 10 μm (C).

Phase purity of the spinels was verified by X-ray diffraction. Whereas both manganite spinels were found as single phase, binary oxides, ZnO and NiO, were formed as impurity phases in the cobaltite spinels in the concentrations 6.1 and 13.6 mol%, respectively. This is due to the high preference of Co^{2+} to occupy the tetrahedral position at the expense of the counter cation resulting in a shift of the respective miscibility range boundary towards $\text{Zn}_{0.87}\text{Co}_{2.13}\text{O}_4$ and $\text{Ni}_{0.69}\text{Co}_{2.31}\text{O}_4$. High oxygen activity (combined with low temperature) during the final heat treatment would be necessary to suppress Co^{2+} formation and to stabilise the spinel phase in stoichiometric composition. All samples except ZnMn_2O_4 exhibit cubic structure with $\text{Fd-}3\text{m}$ space group. The ZnMn_2O_4 has a tetragonal structure with I_{41}/amd space group. All synthesised materials were composed of nanosized crystallites. The average crystallite size calculated using Scherrer formula⁵ was 10 nm for NiCo_2O_4 , 15 nm for ZnCo_2O_4 , 14 nm for ZnMn_2O_4 and 7 nm for NiMn_2O_4 . Such small sizes of the crystallites are in contradiction with the octahedral shape of the observed crystals and can originate from high concentrations of defects present in the material synthesised at very low temperatures. The diffraction patterns are shown in Figure 5.3.

Figure 5.3 X-ray diffractogram of NiCo_2O_4 , NiMn_2O_4 , ZnCo_2O_4 and ZnMn_2O_4 spinel nanocrystals. Impurity phases are marked.

XPS is a surface-sensitive technique with the maximum depth of analysis at about 10 nm. This allows for topography study of the spinel oxides, with detection limits at about 0.1 wt% of the material.⁶⁻⁸ The technique was used to investigate the chemical composition of spinels. The XPS survey (wide scan) spectra (Figure 5.4A) provided information on the metals present in the spinel oxides. Moreover, XPS was used to investigate the $\text{A}^{2+}/\text{B}^{3+}$ elements ratio on the surface of synthesised spinels and the surrounding chemical environment of each ion, where the high-resolution XPS spectra can give information on the site occupancy of a given ion in the lattice and its oxidation state.

Since this technique is sensitive towards the chemical environment of the investigated elements, we can attribute an octahedral or tetrahedral position to a

given cation in the spinel structure. The survey spectra yielded a Co/Ni ratio of 2.1 for NiCo_2O_4 which is in relatively good agreement with the spinel stoichiometry. The high resolution spectra for Ni 2p and Co 2p orbitals in NiCo_2O_4 are shown in Figure 5.4B. The Co $2p_{3/2}$ and Co $2p_{1/2}$ have maximums at 780.7 eV and 796.0 eV, respectively. This is in good agreement with the values reported in the literature.⁹ The chemical shift of $2p_{1/2}$ and $2p_{3/2}$ peaks is not very sensitive towards oxidation.¹⁰ For the investigation of cobalt position within spinel lattice, the energy and intensity of 2p satellite peaks and the separation between $2p_{3/2}$ and $2p_{1/2}$ peaks are very useful.⁹ The presence of satellite peaks is typical for Co^{2+} in octahedral position. The peak position and the separation energy of 15.3 eV between $2p_{1/2}$ and $2p_{3/2}$ indicate that the majority of cobalt is present as Co^{3+} in octahedral sites of the spinel lattice.¹¹ Furthermore, the high resolution XPS spectra for Ni 2p in NiCo_2O_4 displayed maximums at 854.6 eV for $2p_{3/2}$ and 871.9 eV for $2p_{1/2}$. In addition, satellite peaks can be observed at 860.2 eV and 878.0 eV. The obtained energies are comparable with the data found in literature.^{9,11,12} The lower intensity of 2p satellite peaks and asymmetrical shape of $2p_{3/2}$ can indicate the mixed chemical environment of Ni^{2+} ions, which is present in both the octahedral and tetrahedral sites of the spinel lattice. At lower energy, the shoulder of $2p_{3/2}$ peak can be observed at 852.8 eV.¹³ This indicates the trace presence of elemental nickel on the surface. The mechanism of metallic nickel formation on the surface remains unclear; it can however, be related to the formation of CO during oxalate decomposition. Finally, the high resolution O 1s spectrum is shown in Figure 5.5. The maximum of the main peak at 529.5 eV is close to the values typically reported for the transition metal oxides¹⁴. The asymmetrical tail of the O 1s peak with a

maximum at 531.1 eV originates from different surface species such as cobalt oxo-hydroxides, chemisorbed oxygen and carbon dioxide.

The Co/Zn ratio in ZnCo_2O_4 obtained from the XPS survey spectra is 2.38, which is consistent with the excess Co determined by XRD and confirms the non-stoichiometry of the obtained material. The high-resolution XPS spectra of Co 2p and Zn 2p are consistent with these results. The maxima of Co $2p_{3/2}$ and Co $2p_{1/2}$ are located at 780.1 eV and 795.0 eV. These values are slightly lower than the values discussed in the previous paragraph for NiCo_2O_4 and are almost similar to the peak energies reported for Co 2p in NiCo_2O_4 and Co 2p in ZnCo_2O_4 .^{9,15} Moreover, the satellite peaks typical for Co^{2+} in the octahedral position partially substitutes Zn^{2+} were identified. This is a typical behaviour of cobalt–zinc spinels, where a part of the Zn^{2+} tetrahedral sites is substituted by cobalt. The positions of Zn $2p_{1/2}$ and Zn $2p_{3/2}$ at 1043.8 eV and 1020.8 eV, respectively, are only slightly higher compared to the values reported for ZnCo_2O_4 in literature.¹⁵ The high resolution O 1s spectrum is shown on Figure 5.5. The position of the maximum at 529.7 eV is almost identical with the value obtained for NiCo_2O_4 . Moreover, the satellite peak with a maximum at 531.6 eV is observed. Its origin is similar to that discussed for NiCo_2O_4 . However a slightly broader shape may indicate a higher tendency for hydroxide formation as well as carbon dioxide and oxygen adsorption from the surrounding environment.

The Mn/Ni ratio of the NiMn_2O_4 spinel was 3.65. This value is higher compared to both the ideal stoichiometry ratio and that determined from XRD, indicating the presence of Mn rich oxides on the material particles surface. The high resolution XPS spectra of Mn 2p and Ni 2p are shown in Figure 5.4B. The Mn

$2p_{3/2}$ and Mn $2p_{1/2}$ have maxima at 641.5 eV and 653.2 eV respectively. These data were reported for the NiMn_2O_4 stoichiometric spinel.¹⁶ No significant broadening or high intensity satellite peaks indicate the dominant presence of Mn^{3+} in the octahedral sites of the spinel lattice. The Ni $2p_{3/2}$ has a maximum at 854.6 eV and Ni $2p_{1/2}$ at 872.3 eV with the satellite peaks located at 861.3 eV and 878.7 eV. The slight shift towards higher energy levels is observed in comparison with Ni 2p in NiCo_2O_4 . In the high resolution spectra of NiMn_2O_4 , the shoulder located at 852.8 eV, clearly visible in the NiCo_2O_4 sample, has not been observed. The intensity of satellite peaks of Ni^{2+} indicates a partial occupation of the octahedral lattice sites by Ni^{2+} in high spin configuration. The high resolution O 1s peak has a maximum at 529.5 eV which has also been observed in other spinels and is typical for O 1s energy in 3d element oxides (Figure 5.5). The shoulder of O 1s peak is located at 531.2 eV and has significantly lower energy compared to other investigated samples. This can originate from well-developed octahedral particles with lower surface area observed by SEM. This led us to a conclusion that the intensity of the O 1s peak shoulder associated with adsorbed oxygen and surface degradation is strongly dependent on the morphology and surface quality of the synthesised material.

The Mn/Zn ratio in the ZnMn_2O_4 spinel was 5.01, which is more than two-fold of that of the stoichiometric material. This indicates high surface enrichment of Mn oxides on the spinel surface. The high resolution spectra of Mn 2p are shown in Figure 5.4B. The Mn $2p_{3/2}$ and Mn $2p_{1/2}$ are located at 642.4 eV and 653.7 eV, respectively. The slight shift towards higher energy levels can originate from the Mn oxides precipitated on the surface, however, their concentration is very low and no additional phases were detected by X-ray diffraction. These values are

almost similar to the data reported for ZnMn_2O_4 spinel nanostructures.¹⁷ The high resolution XPS spectra of Zn 2p yield the maxima at 1020.6 eV for Zn 2p_{3/2} and 1043.6 eV for Zn 2p_{1/2}. These results are only slightly lower in comparison with the values reported in literature.¹⁷ The peaks originating from Zn 2p states are significantly broader compared to those obtained in ZnCo_2O_4 . This can be related to the surface reaction of Zn^{2+} with humidity in atmosphere, and hence formation of zinc hydroxides or surface states and defected surface states within the Zn-Mn spinel. This interpretation is also supported by the high resolution XPS spectra of O 1s peak (Figure 5.5) where the maximum originating from transition metal oxygen bond (529.5 eV) has an even lower intensity than the O 1s peak resulting from surface states, hydroxides and other hydrolysis products formed on the surface (531.3 eV). This indicates that within the investigated group of mixed oxides, ZnMn_2O_4 exhibits the highest tendency towards surface degradation and decomposition. This is also documented subsequently by electrochemical characterisation using the hydrogen evolution reaction (HER), where no electrocatalytic properties were detected for this material.

Figure 5.4 (A) X-ray photoelectron spectroscopy (XPS) survey spectra of NiCo_2O_4 , NiMn_2O_4 , ZnCo_2O_4 , and ZnMn_2O_4 spinel oxides. (B) X-ray photoelectron spectroscopy (XPS) high resolution spectra of cations in NiCo_2O_4 , NiMn_2O_4 , ZnCo_2O_4 , and ZnMn_2O_4 spinel oxides.

Figure 5.5 High resolution XPS spectra of O 1s peak of ZnCo_2O_4 , NiCo_2O_4 , NiMn_2O_4 , and ZnMn_2O_4 .

Following the detailed characterisations of the mixed-valence transition-metal oxides, it is of equal importance to investigate how the abilities of these materials to catalyse the reduction of O_2 in potassium hydroxide (KOH) correspond to their topographies. Electrocatalytic activities of the spinel oxides towards the oxygen reduction reaction (ORR) were evaluated using cyclic voltammetry (CV). Figure 5.6A illustrates the linear scan voltammograms obtained from the electrochemical reduction of oxygen in air-saturated 0.1 M KOH, while Figure 5.6B compares the reduction onset potentials of bare GC and the spinel oxides. Figure 5.7 is shown for comparison in N_2 purged solution.

It may be observed from Figure 5.6B that manganese-based spinel oxides, $NiMn_2O_4$ and $ZnMn_2O_4$, showed onset potentials similar to that of bare GC (-266 mV). On the other hand, $NiCo_2O_4$ and $ZnCo_2O_4$ displayed much less negative reduction onset potentials of -147 mV and -239 mV, respectively. Hence, the much less negative onset potentials suggest significant electrocatalytic activities of the cobalt-based spinel oxides toward ORR, which may be attributed to the presence of cobalt oxide. Moreover, it is worthy to note that the $NiCo_2O_4$ spinel displayed a greater enhancement in the kinetics of ORR compared to $ZnCo_2O_4$, and exhibited the greatest electrocatalytic activity among the spinel oxides. The difference in electrocatalytic performances of $NiCo_2O_4$ and $ZnCo_2O_4$ may be explained by several factors: 1) differences in the structure and composition where cobalt in $NiCo_2O_4$ is dominantly present as Co^{3+} , whereas a partial substitution of Zn^{2+} by Co^{2+} in tetrahedral sites was observed in $ZnCo_2O_4$; and 2) differences in substitutions of elements in the 2+ oxidation state; it is observed that Ni^{2+} spinel oxides generally

performed better than their respective Zn based counterparts and also 3) phase separation with simultaneous formation of NiO.

Figure 5.6 (A) Linear scan voltammograms for ORR in air-saturated, 0.1 M potassium hydroxide using a bare glassy carbon electrode, and GC electrodes modified with NiCo₂O₄, NiMn₂O₄, ZnCo₂O₄ and ZnMn₂O₄. Scan rate, 0.1 V s⁻¹. **(B)** Reduction onset potentials of bare GC, NiCo₂O₄, NiMn₂O₄, ZnCo₂O₄ and ZnMn₂O₄ from linear scan voltammograms. Error bars represent RSD ($n=3$, 95% confidence interval).

Figure 5.7 Cyclic voltammograms in nitrogen-purged, 0.1 M potassium hydroxide using a bare glassy carbon electrode, and GC electrodes modified with NiCo₂O₄, NiMn₂O₄, ZnCo₂O₄ and ZnMn₂O₄. Scan rate, 0.1 V s⁻¹.

5.3 Conclusion

Mixed-valence transition-metal oxides were prepared and the exceptional catalytic behaviours of cobalt-based spinel oxides (NiCo_2O_4 and ZnCo_2O_4) for ORR were demonstrated providing a promising alternative to noble metals in preparing highly efficient catalysts for ORR. In addition, Ni-substituted spinel oxides generally outperformed their Zn based spinel counterparts. Of the four spinel oxides investigated, NiCo_2O_4 exhibited the greatest electrocatalytic activity toward ORR. Differences in the structure and composition exist where cobalt in NiCo_2O_4 is dominantly present in Co^{3+} , whereas partial substitution of Zn^{2+} by Co^{2+} in the tetrahedral sites in ZnCo_2O_4 was observed. These findings are of paramount importance to the design and development of the advanced ORR electrocatalysts for renewable energy applications.

5.4 Experimental

5.4.1 Materials

Isopropanol (IPA) was purchased from Merck. Glassy carbon (GC), Ag/AgCl reference, and Pt counter electrodes were obtained from Autolab (Japan). Zinc acetate dihydrate, cobalt acetate tetrahydrate and oxalic acid dihydrate was obtained from Penta, Czech Republic. Manganese acetate tetrahydrate and nickel acetate tetrahydrate was obtained from Sigma-Aldrich, Czech Republic.

5.4.2 Apparatus

Scanning electron microscopy (SEM) images and energy-dispersive X-ray spectroscopy (EDS) were acquired using a Joel 7600F instrument (Joel, Japan). The instrument was operated at 2 kV for SEM and 15 kV for EDS measurements. X-ray photoelectron spectroscopy (XPS) measurements were performed with a Phoibos 100 spectrometer and a monochromatic Mg X-ray radiation source (SPECS, Germany). Survey (wide scan) spectra were recorded for each sample. For SEM, EDS, and XPS measurements, samples were prepared by coating a uniform layer of the materials under study on a carbon conductive tape. X-ray powder diffraction data were collected at room temperature with an X'Pert PRO θ - θ powder diffractometer with parafocusing Bragg-Brentano geometry using $\text{CuK}\alpha$ radiation ($\lambda = 1.5418 \text{ \AA}$, $U = 40 \text{ kV}$, $I = 30 \text{ mA}$). Data evaluation was performed in the software package, HighScore Plus 3.0e. All voltammetric experiments were carried out by using a μ Autolab type III electrochemical analyser (Eco Chemie, The Netherlands) connected to a computer and controlled by NOVA, Version 1.8.17 software. Electrochemical experiments were carried out at room temperature in an

electrochemical cell (5 mL) with the employment of a three electrode configuration. A platinum electrode functioned as the counter electrode, and an Ag/AgCl electrode served as the reference electrode. All electrochemical potentials in this chapter are indicated as versus the Ag/AgCl reference electrode.

5.4.3 Procedures

Synthesis of Spinel with the General Formula of XY_2O_4 . Acetates in 0.05 M concentrations, and oxalic acid in 0.05 M concentrations, were used. The oxalates were precipitated by reaction of stoichiometric amount of X and Y elements with oxalic acid in 10% molar excess. The precipitate was formed by slow dropping of oxalic acid solution into vigorously stirred metal acetate solution. The precipitate was filtered out by suction and dried at 100 °C for 24 hours. The spinels were formed by high temperature decomposition of the oxalate precursor. Oxalate precursor was placed in alumina crucible and heated at 450 °C for two hours on air. The rate of heating and cooling was 10 °C min⁻¹.

Oxygen Reduction Reaction (ORR) Studies on Spinel Oxides. ORR efficiency of spinel oxides were performed on glassy carbon (GC) electrodes. GC electrodes were first renewed on a polishing pad using alumina particle (0.05 µm) slurry and washed with ultrapure water. The spinel oxides (1 mg mL⁻¹) were dispersed in 2.5:1 v/v water/isopropanol mixed solvent. The suspension was then subjected to ultrasound treatment for at least 30 min sonication to form a homogenous ink. Next, the suspension (1 µL) was pipetted onto the GC electrode surface, and left to evaporate at room temperature. A film of randomly distributed material is hence generated on the GC electrode surface. Cyclic voltammetry

experiments were performed at a scan rate of 100 mV s^{-1} in KOH solution (0.1 M) saturated with air for the oxygen reduction reaction. In control experiments, cyclic voltammetry measurements (Figure 5.7) were performed in N_2 by bubbling highly purified nitrogen through the experimental solutions for at least 20 min.

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Part I

Chapter 6

Ultrapure MoS_2 Shows Enhanced Catalysis for
Hydrogen Evolution over Impurities-Doped
Counterpart

6.1 *Introduction*

6.2 *Results and Discussion*

6.3 *Conclusion*

6.4 *Experimental*

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

1. **Rou Jun Toh**; Zdenek Sofer, Jan Luxa, Martin Pumera. Ultrapure Molybdenum Disulfide Shows Enhanced Catalysis for Hydrogen Evolution over Impurity-Doped Counterpart. *ChemCatChem* **2017**, 9, 1168-1171.

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6.1 Introduction

Transition metal dichalcogenides (TMDs) have been in the spotlight due to their layered structure akin to graphite. The large variation in band gaps of TMD materials confers a wide spectrum of electronic properties ranging from insulating to metallic.^{1,2} Of particular interest is their outstanding catalytic activity towards the hydrogen evolution reaction (HER), which could support the generation of hydrogen at high efficiencies and low costs in comparison to precious Pt-based catalysts.³⁻⁵

Naturally occurring MoS₂ ore (molybdenite) are found to contain transition metals and semimetals impurities, by substitution and/or intercalation.⁶ This consequently highlights concerns regarding the presence of impurities in MoS₂ arising from its preparation methods and starting materials which may act as dopants to influence its electronic and hence, catalytic properties. Furthermore, Addou *et al.* reported that the presence of impurities in parts per millions (ppm) may be a contributing factor to the variations in electronic property of MoS₂.⁶ Therefore, while it is of paramount importance to investigate the role impurities can play in affecting the HER efficiency of MoS₂, no work has been reported on this thus far.

Herein, we synthesised MoS₂ from its elements which resulted in an ultrapure MoS₂ material. This allowed us to compare the HER catalytic activity with a relatively impure MoS₂ sample obtained commercially. Through this comparison, we wish to address the following basic questions: 1) does the elemental purity of MoS₂ influence its catalytic activity towards HER and if so, 2) can relevance be

drawn to the effects of doping on the electrocatalytic activity of MoS₂. For this purpose, the MoS₂ materials were characterised using scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDS), X-ray diffraction (XRD), laser diffraction, X-ray fluorescence (XRF). Furthermore, linear sweep voltammetry (LSV) was employed to test their HER electrocatalytic performance. We showed that the ultrapure MoS₂ shows enhanced HER catalysis compared to its impurities-doped counterpart. With atomic doping as a strategy generally employed to elicit an improvement in the electrocatalytic activity of TMDs, this work challenges such an existing notion while emphasizing the need for a more in-depth understanding of this strategy.

6.2 Results and Discussion

Considering MoS₂ is one of the most highly investigated transition metal dichalcogenides (TMDs), an ultrapure MoS₂ material was first synthesised by a simple and scalable method from its elements (of high purity), herein denoted as MoS₂-M. A side-by-side comparison of MoS₂-M was performed against MoS₂ obtained from Alfa Aesar (MoS₂-AA).

The platelet structures of MoS₂-AA and MoS₂-M are shown in their SEM images (Figure 6.1) while EDS (Figure 6.2 and Table 6.1) confirms the successful preparation of MoS₂-M from its elements. In order to investigate if the elemental purity of MoS₂ would affect the HER catalytic activity, it is crucial that MoS₂-AA and MoS₂-M are comparable in terms of the other key factors which may influence HER efficiency: particle size and phase purity.^{7,8} Figure 6.3 illustrates the particle size distributions of the MoS₂ materials. Both MoS₂-AA and MoS₂-M are observed to have comparable particle sizes with mean diameters of 24.1 μm and 15.7 μm respectively.

Figure 6.1 Scanning electron microscopy (SEM) images of MoS₂-AA and MoS₂-M. Scale bars, 1 μm .

Figure 6.2 Energy-dispersive X-ray spectroscopy (EDS) elemental mapping for MoS₂-AA and MoS₂-M. Scale bars, 10 µm.

Table 6.1 Energy-dispersive X-ray spectroscopy (EDS) analysis of MoS₂-AA and MoS₂-M; elemental composition represented in atomic percentage.

Materials	X/M ratio	Mo (at%)	S (at%)	C (at%)	O (at%)	Al (at%)	K (at%)	F (at%)
MoS ₂ -AA	1.77	33.45	59.07	-	7.48	-	-	-
MoS ₂ -M	1.80	23.24	41.84	34.43	-	0.49	-	-

Figure 6.3 Particle size distribution of MoS₂-AA and MoS₂-M.

The XRD patterns of MoS₂-AA and MoS₂-M are represented in Figure 6.4. The phase purities of both MoS₂ materials were verified and found to be a single hexagonal 2H phase. Diffraction lines with relative intensity of 1% or higher, and corresponding to the pattern from the PDF card for the 2H phase (P6₃/mmc), are marked. Diffraction patterns with lower intensities are not visible due to the high preferential orientation originating from the layered structure of MoS₂.

Figure 6.4 X-ray diffraction (XRD) patterns of MoS₂-AA and MoS₂-M. Unassigned peak for MoS₂-M at ~15 ° 2θ originates from K-beta line diffraction of MoS₂ (001) reflection.

Subsequently, XRF was carried out to analyse the elemental compositions of MoS₂-AA and MoS₂-M (Table 6.2). In addition to the supposed Mo metal and S chalcogen, traces of W (0.02 wt%) and parts per million amounts of transition metals Y (410 ppm), Mn (65 ppm), Fe (940 ppm) and Zn (168 ppm) and semimetal Si (560 ppm) were shown to be present in the MoS₂-AA sample. K (297 ppm) and Ca (252 ppm) were also detected in MoS₂-AA. In contrast, elemental analysis of the synthesised MoS₂-M sample showed a low concentration of Mg (89 ppm) as the only impurity. Contamination with alkali metals (Na, K) and alkaline earth (Ca, Mg) is very common since it can originate from the vessel, surrounding environment or contamination from the production process (e.g. crucible and vessel material).

Hence, with comparable particle sizes and phase purity but significantly different elemental purities, MoS₂-AA and MoS₂-M serve as excellent candidates for the following investigation of the effects of impurities on HER catalytic activity of MoS₂.

Table 6.2 X-ray fluorescence (XRF) analysis of MoS₂-AA and MoS₂-M; elemental composition of Mo, W and S represented in weight percentage. Cd, Mg, Y, Si, K, Ca, V, Mn, Fe, Zn represented in parts per million. Concentration of all other elements are below 0.01 wt%.

	MoS ₂ -AA	MoS ₂ -M
X/M ratio	2.05	1.95
Mo (wt%)	59.2	60.53
W (wt%)	0.02	0
S (wt%)	40.51	39.46
Cd (ppm)	0	0
Mg (ppm)	0	89
Y (ppm)	410	0
Si (ppm)	560	0
K (ppm)	297	0
Ca (ppm)	252	0
V (ppm)	0	0
Mn (ppm)	65	0
Fe (ppm)	940	0
Zn (ppm)	168	0

The HER catalytic activities of MoS₂-AA and MoS₂-M were studied with LSV in sulphuric acid (0.5 M) at a low scan rate (2 mV s⁻¹) and the obtained HER polarization curves and Tafel plots are displayed in Figure 6.5 accordingly. Bare glassy carbon (GC) served as a reference material.

For the purpose of comparison, the discussion will revolve around three commonly adopted HER performance indices: overpotential, onset potential and Tafel slope. Overpotentials were measured at 10 mA cm⁻² while the onset potential is defined as the potential at which the current density is -0.07 mA cm⁻². In general, higher HER catalytic efficiencies are characterised by lower overpotentials and less negative onset potentials as less energy is necessary to drive the reaction. Moreover, Tafel slope analysis helps us to understand the mechanism of hydrogen evolution on the MoS₂ surfaces. The rate-limiting steps may be as follow:⁹

a. Volmer adsorption step:

b. Heyrovsky desorption step:

c. Tafel desorption step:

From Figure 6.5A, it is observed that both MoS₂-AA and MoS₂-M displayed significantly superior HER catalytic activities over the bare GC control. However, due to their bulk nature, the MoS₂ materials generally present lower HER activity compared to the commercially available Pt-based catalysts, which have been reported to demonstrate a Tafel slope of ~30 mV dec⁻¹ and overpotential of ~0.05 V

vs RHE.^{4,5,8} Tafel slope analysis (Figure 6.5B) showed that MoS₂-AA and MoS₂-M gave similar values of 172 mV dec⁻¹ and 175 mV dec⁻¹ respectively. Since the Tafel slopes are higher than the 120 mV dec⁻¹ theoretical value, it is implied that the rate-limiting step of HER on both MoS₂ surfaces is the Volmer adsorption. Moreover, a more comprehensive comparison in terms of the overpotentials and onset potentials reveals that MoS₂-AA displayed an overpotential of 0.82 V vs RHE and onset potential of -0.41 V vs RHE while MoS₂-M afforded a lower overpotential of 0.77 V vs RHE and a less negative onset potential of -0.38 V vs RHE. From these results, we conclude that the ultrapure MoS₂-M elicited a higher HER efficiency than its impurities-doped counterpart. Considering the relatively significant amounts of transition metals Fe, Zn and Mn present in MoS₂-AA compared to in MoS₂-M, this work presents a surprising yet profound observation. Furthermore, our results are in agreement with a recent report that Nb and Ta-doped bulk MoS₂ displayed inferior HER catalytic activity compared to its undoped counterpart.¹⁰

Figure 6.5 HER polarization curves. (A) Linear sweep voltammograms for HER in acidic electrolyte on MoS₂-AA and MoS₂-M and their respective Tafel plots (B). Bare GC serves as a control experiment. Conditions: background electrolyte, 0.5 M H₂SO₄; scan rate, 2 mV s⁻¹.

6.3 Conclusion

In this work, the effect of elemental purity on HER catalytic activity was investigated for MoS₂, one of the most representative TMD materials. We report a simple and scalable preparation method of an ultrapure MoS₂ material from its elements. When contrasted with the synthesised MoS₂-M, commercially obtained MoS₂-AA contained relatively significant amounts of elemental impurities. Despite the presence of transition metals impurities Fe, Zn and Mn, superior HER catalytic activity is observed for the MoS₂ material with a higher elemental purity (MoS₂-M). We provide an insight into the influence of impurities on the catalytic activity of TMD materials. While results from previous computational studies point to the possible enhancement of HER catalytic activity by doping with these transition metals, our investigation emphasises the importance of intentional and proper design of this strategy to realise its true effect in the experimental setting. With many factors to consider in the experimental context, elemental purity as one, we believe that our work highlights the need for a more in-depth understanding of the atomic doping strategy and its true benefits for harnessing the endless potential of TMDs as catalysts for the hydrogen evolution.

6.4 Experimental

6.4.1 Materials

Mo (99.999%) was obtained from Alfa Aesar, Germany. Sulfur (99.999%) was obtained from Strem, Germany. Sulphuric acid was purchased from Sigma Aldrich, Singapore. MoS₂-AA was purchased from Alfa Aesar, Czech Republic. Glassy carbon (GC), the Ag/AgCl reference and Pt counter electrodes were acquired from Autolab (The Netherlands).

6.4.2 Apparatus

Scanning electron microscopy (SEM) images and energy-dispersive X-ray spectroscopy (EDS) data were acquired by using a Jeol 7600F SEM instrument (Jeol, Japan) operating at 5 kV and 15 kV respectively. X-ray powder diffraction (XRD) data were collected with a *Bruker D8 Discoverer* powder diffractometer (Bruker AXS, Germany) at room temperature with parafocusing Bragg-Brentano geometry using CuK α radiation ($\lambda = 1.5418 \text{ \AA}$, $U = 40 \text{ kV}$, $I = 40 \text{ mA}$). Data were scanned with an ultrafast LYNXEYE XE detector over the angular range of 5° to 80° (2θ) with a step size of 0.019° (2θ) and a counting time of 1 s step^{-1} . Detection limit of the XRD analysis is about 0.5 at%. The software package HighScore Plus 3.0e was employed for subsequent data evaluation. The XRF analysis was performed by ARL PERFORM'X sequential WD-XRF spectrometer equipped with an Rh anode end-window X-ray tube type 4GN fitted with $50 \text{ }\mu\text{m}$ Be window. All peak intensity data were collected by software Oxsas in vacuum. The generator settings-collimator-crystal-detector combinations were optimised for all 82 measured elements with analysis time of 6s per element. Detection limit of the XRF analysis is in the range

of 1 to 10 ppm depending on the element. The obtained data were evaluated by standardless software Uniquant 5 integrated in Oxsas. Concentration estimations can be made for trace concentrations of about 0.01% of the absolute value, for trace concentrations (below 1000 ppm) up to about 0.2% of the absolute value, and for concentration exceeding 10 wt%. The obtained data were evaluated by standardless software Uniquant 5 integrated in Oxsas. The analysed powders were pressed into pellets about 5 mm thick and diameter of 40 mm without any binding agent and covered with 4 µm supporting polypropylene (PP) film. All voltammetric experiments were carried out using a µAutolab type III electrochemical analyser (Eco Chemie, The Netherlands) connected to a computer and controlled by the NOVA software, Version 1.8.17. Electrochemical experiments were carried out at room temperature in an electrochemical cell (5 mL) with the employment of a three electrode configuration. A platinum electrode functioned as the counter electrode, and an Ag/AgCl electrode served as the reference electrode.

6.4.3 Procedures

Synthesis of MoS₂-M. Stoichiometric amounts of molybdenum and sulfur corresponding to the transition metal dichalcogenide (5 g) were placed in a quartz glass ampoule. The ampoule was evacuated to a base pressure below 5×10^{-3} Pa and melt sealed by oxygen-hydrogen torch. Following that, the ampoule was subjected to heating at 500 °C for 24 h and then at 800 °C for 48 h. The rate of heating and cooling was 5 °C min⁻¹.

Hydrogen Evolution Reaction (HER) Studies on MoS₂. HER catalytic performance of the MoS₂ materials were carried out on glassy carbon (GC)

electrodes. GC electrodes were first renewed on a polishing pad using alumina particle (0.05 μm) slurry and washed with ultrapure water. Subsequently, 1 mg mL⁻¹ suspensions of TMD materials were prepared in ultrapure water and ultrasonicated for a period of 10 min prior to each electrochemical measurement to ensure homogeneity of the material under study. An aliquot (4 μL) was drop casted on the electrode surface and dried at room temperature to yield a randomly distributed film of desired material (4.0 μg) on the GC electrode surface. The materials of interest include MoS₂-AA and MoS₂-M. HER measurements were performed in a 0.5 M H₂SO₄ electrolyte using linear sweep voltammetry (LSV) at a scan rate of 2 mV s⁻¹. Measurements were first performed relative to the Ag/AgCl reference electrode. Subsequently, the measurements were corrected and the LSVs were represented versus the reversible hydrogen electrode (RHE). The measured potentials were calculated based on the equation $E_{\text{RHE}} = E_{\text{Ag/AgCl}} + 0.059 \times \text{pH} + E^{\circ}_{\text{Ag/AgCl}}$, where $E_{\text{Ag/AgCl}}$ is the measured potential, and pH of H₂SO₄ (0.5 M) is set as zero. The standard potential of Ag/AgCl (1M KCl) at 25 °C, $E^{\circ}_{\text{Ag/AgCl}}$ is then 0.235 V vs RHE.

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Part I

Chapter 7

3R Phase of MoS_2 and WS_2 Outperforms the Corresponding 2H Phase for Hydrogen Evolution

7.1 *Introduction*

7.2 *Results and Discussion*

7.3 *Conclusion*

7.4 *Experimental*

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

1. **Rou Jun Toh**; Zdenek Sofer, Jan Luxa, David Sedmidubsky, Martin Pumera. 3R Phase of MoS₂ and WS₂ Outperforms the Corresponding 2H Phase for Hydrogen Evolution. *Chem. Commun.* **2017**, 53, 3054-3057.

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7.1 Introduction

Transition metal dichalcogenides (TMDs) have gained recognition rapidly due to their high catalytic activity towards the hydrogen evolution reaction (HER),¹⁻³ which could support efficient and low-cost production of hydrogen without the reliance on precious Pt-based catalysts. Such a characteristic points to the importance of understanding the HER catalytic mechanism, and consequently the synthesis of TMDs with different morphologies aimed at fine-tuning and improving their performances.

Considering the key roles played by the edge-sites and electrical conductivity of TMD materials for the HER,⁴⁻⁸ it is critical to take into account the different electronic properties that may arise from variations in transition-metal coordination by the chalcogen and the stacking sequences of multiple layers of TMD materials. The 1T form displays metallic behaviour, while both 2H and 3R forms exhibit semiconducting behaviour. Each TMD polymorph possesses unique structures and electronic properties where different catalytic properties can emerge from. Hence, it is imperative to make a comparison of different TMD polymorphs, aiming to understand the influence of polymorph structures on the catalytic properties of TMD materials. Previous works have reported a 2H → 1T phase transition when TMD materials are subjected to chemical exfoliation⁹ and a comparison of the catalytic properties of 1T- and 2H-TMDs has shown that the exfoliated, metallic 1T phase performs as a better catalyst than their bulk, semiconducting 2H counterparts for HER.¹⁰ This was explained by the increased edge-exposed surface, reduced sheet size and enhanced electrical conductivity of the 1T phase. However, there has been no previous study, resulting in a lack of

understanding of the catalytic properties of 3R-TMDs and comparison with their semiconducting 2H counterparts.

In this work, we wish to address the following basic questions: 1) is the catalytic activity towards HER influenced by polymorph structure and if so, 2) is an additional chemical exfoliation step necessary for producing an efficient HER catalyst. For this purpose, we synthesised TMD materials (MoS₂ and WS₂) in their 3R phase, which allows us to perform a comparison of HER catalytic activity with their corresponding 2H phase. The synthesis was performed from oxides using alkali metal carbonate/sulphur melt. Based on X-ray diffraction (XRD), high resolution transmission electron microscopy (HR-TEM), scanning electron microscopy (SEM), laser diffraction, energy dispersive X-ray spectroscopy (EDS), X-ray fluorescence (XRF), X-ray photoelectron spectroscopy (XPS) and electrochemical analyses, we show that the 3R phase outperforms the 2H phase in HER catalysis. However, this improvement in HER catalytic property presents in varying magnitudes for MoS₂ and WS₂, and at different synthesis temperatures. Comparison with existing literature shows that in the case of MoS₂, catalytic performance towards HER for the bulk, semiconducting 3R phase is on par with the exfoliated, metallic 1T phase.

7.2 Results and Discussion

Considering the two highly studied transition metal dichalcogenides (TMDs), *i.e.* MoS₂ and WS₂, we first synthesised the 3R phase from their respective oxides with sulphur in carbonate flux according to previously established protocols at 550 °C.^{11,12} Herein, they are denoted as 3R-MoS₂ (550 °C) and 3R-WS₂ (550 °C). We studied their HER catalytic properties, and further investigated if the polymorph structure influences HER catalytic performances of MoS₂ and WS₂ by making a comparison of the 3R phase TMDs with their corresponding 2H phases; 2H-MoS₂ (AA) or 2H-WS₂ (AA). The materials were fully characterised by using XRD, HR-TEM, SEM, laser diffraction, EDS, XRF, XPS and electrochemically tested as catalysts for hydrogen evolution.

The phase purity of each material was verified by X-ray diffraction (XRD). Figure 7.1 represents the XRD patterns of the TMD materials. The 2H-MoS₂ (AA) (Figure 7.1A) and 2H-WS₂ (AA) (Figure 7.1B) were found as a single 2H phase and exhibiting a hexagonal structure, while 3R-MoS₂ (550 °C) (Figure 7.1C) and 3R-WS₂ (550 °C) (Figure 7.1D) were formed as a mixture of rhombohedral 3R phase and hexagonal 2H phase. The ratio evaluated from XRD gives a composition of about 1:1 of 3R to 2H phases. Presence of the desired 3R stacking of the atomic layers in 3R-MoS₂ (550 °C) and 3R-WS₂ (550 °C) were further confirmed by HR-TEM analyses in Figure 7.2 and 7.3 respectively. Further characterisation of the materials was performed using SEM (Figure 7.4), which indicated the homogeneous formation of platelet structures and laser diffraction measurements revealed comparable particle sizes of the TMD materials in their 2H phase and respective 3R phase (Figure 7.5). 2H-MoS₂ (AA) and 3R-MoS₂ (550 °C) have mean particle diameters of

24.2 μm and 12.9 μm respectively while 2H-WS₂ (AA) and 3R-WS₂ (550 °C) exhibited mean particle diameters of 11.1 μm and 10.4 μm respectively.

Figure 7.1 X-ray diffraction (XRD) patterns of (A) 2H-MoS₂ (AA), (B) 2H-WS₂ (AA), (C) 3R-MoS₂ (550 °C), and (D) 3R-WS₂ (550 °C).

Figure 7.2 Transmission electron microscopy (TEM) image of 3R-MoS₂ (550 °C) sheets with corresponding selective area electron diffraction (SEAD) pattern. High resolution TEM images show the top and cross-sectional views of 3R-MoS₂ (550 °C), indicating the desired 3R stacking of the atomic layers. Scale bars represent 80 nm, 10 nm, 2 nm and 0.5 nm respectively.

Figure 7.3 Transmission electron microscopy (TEM) image of 3R-WS₂ (550 °C) sheets with corresponding selective area electron diffraction (SEAD) pattern. High resolution TEM images show the top and cross-sectional views of 3R-WS₂ (550 °C), indicating the desired 3R stacking of the atomic layers. Scale bars represent 100 nm, 10 nm, 2 nm and 0.5 nm respectively.

Figure 7.4 Scanning electron microscopy (SEM) images of the transition metal dichalcogenides; 2H-MoS₂ (AA), 3R-MoS₂ (550 °C), 3R-MoS₂ (650 °C), 3R-MoS₂ (750 °C), 2H-WS₂ (AA), 3R-WS₂ (550 °C), 3R-WS₂ (650 °C) and 3R-WS₂ (750 °C). Scale bars, 1 μm.

Figure 7.5 Particle size distributions of 2H-MoS₂ (AA), 3R-MoS₂ (550 °C), 3R-MoS₂ (650 °C), 3R-MoS₂ (750 °C), 2H-WS₂ (AA), 3R-WS₂ (550 °C), 3R-WS₂ (650 °C) and 3R-WS₂ (750 °C).

Moreover, the composition of each material was confirmed via EDS (Figure 7.6-7.9) and XRF (Table 7.1). The concentration of potassium originating from the potassium carbonate flux was 1.2 wt% and 1.0 wt% in 3R-MoS₂ (550 °C) and 3R-WS₂ (550 °C) respectively. In 3R-MoS₂ (550 °C), the concentration of tungsten lies below 0.01 wt%. However, in 3R-WS₂ (550 °C), the concentration of molybdenum is 0.15 wt%. In addition, traces of Zn were detected in 3R-MoS₂ (550 °C) (~0.01 wt%), and traces of Fe were found in 3R-WS₂ (550 °C) (~0.02 wt%). The concentrations of all other impurities were below 0.01 wt%. Overall, it was determined that the presence of impurities is insignificant in these samples and would not interfere with the electrochemical signals of the TMD materials.

Figure 7.6 Energy-dispersive X-ray spectroscopy (EDS) data for 2H-MoS₂ (AA). Scale bar, 10 μm.

Figure 7.7 Energy-dispersive X-ray spectroscopy (EDS) data for 2H-WS₂ (AA). Scale bar, 10 μm.

Figure 7.8 Energy-dispersive X-ray spectroscopy (EDS) data for 3R-MoS₂ (550 °C). Scale bar, 10 μm.

Figure 7.9 Energy-dispersive X-ray spectroscopy (EDS) data for 3R-WS₂ (550 °C). Scale bar, 10 μm.

Table 7.1 X-ray fluorescence analysis (XRF) of materials; elemental composition of Mo, W, S and K represented in weight percentage (wt%). Concentration of all other elements are below 0.01 wt%.

	Mo (wt%)	W (wt%)	S (wt%)	K (wt%)	Zn (wt%)	Fe (wt%)
3R-MoS₂ (550 °C)	59.18	0	39.61	1.19	0.012	0
3R-MoS₂ (650 °C)	53.62	0.012	45.76	0.592	0.012	0
3R-MoS₂ (750 °C)	60.14	0	39.68	0.162	0.014	0
3R-WS₂ (550 °C)	0.149	68.25	30.45	1.04	0	0.018
3R-WS₂ (650 °C)	0.169	68.06	31.15	0.598	0	0.018
3R-WS₂ (750 °C)	0.387	70.08	29.46	0.053	0	0.011

Moving on with investigating the HER efficiency of the TMD materials, linear sweep voltammetry (LSV) was performed in 0.5 M sulphuric acid at a low scan rate of 2 mV s⁻¹. We studied the HER catalytic performances of the 3R phase and compared them to that of the corresponding 2H phase.

The polarization curves and Tafel plots of 3R-MoS₂ (550 °C) and 2H-MoS₂ (AA) are represented in Figure 7.10A and 7.10B, and that of 3R-WS₂ (550 °C) and 2H-WS₂ (AA) and are shown in Figure 7.10C and 7.10D. The bare glassy carbon (GC) was included as a material of reference. We adopted three commonly used HER performance indices, namely the overpotential, onset potential and Tafel slope. Overpotentials were taken at -10 mA cm⁻², and the onset potential is defined as the potential at which the current density is -0.07 mA cm⁻². In general, lower overpotentials and less negative onset potentials indicate higher HER catalytic

efficiencies as less energy is required for the reaction to take place. Analysis of the Tafel slope gives us an idea of the mechanism of the hydrogen evolution on the different TMD surfaces, where the rate-determining steps may be accounted as such:⁸

1. Volmer adsorption step:

2. Heyrovsky desorption step:

3. Tafel desorption step:

Figure 7.10 HER polarization curves of 2H and 3R phase TMDs. Linear sweep voltammograms for HER in acidic electrolyte on (A) MoS₂ and (C) WS₂ compounds. Tafel plots for (B) MoS₂ and (D) WS₂ compounds. Conditions: background electrolyte, H₂SO₄ (0.5 M); scan rate, 2 mV s⁻¹.

We observed similar and outstanding HER catalytic performances for both materials in the 3R phase. 3R-MoS₂ (550 °C) displayed an overpotential of 0.52 V vs RHE, onset potential of -0.20 V vs RHE, while 3R-WS₂ (550 °C) showed an overpotential of 0.56 V vs RHE, onset potential of -0.23 V vs RHE. Tafel analysis of 3R-MoS₂ (550 °C) (113 mV dec⁻¹) and 3R-WS₂ (550 °C) (111 mV dec⁻¹), which is close to the 120 mV dec⁻¹ theoretical value, generally points to the Volmer adsorption as the limiting step. When compared with 2H-MoS₂ (AA), 3R-MoS₂ (550 °C) exhibited an overpotential which was a significant 0.29 V lower. The relatively more negative onset potential of 2H-MoS₂ (AA) (-0.41 V vs RHE) further exemplifies its poorer HER catalytic performance. In the case of WS₂, the 2H phase also displayed a poorer HER catalytic performance than the 3R phase, even though to a lesser extent. 2H-WS₂ (AA) displayed an overpotential (0.73 V vs RHE) which is 0.17 mV less than that of 3R-WS₂ (550 °C), and a more negative onset potential of -0.30 V vs RHE. In general, the 2H phase has poorer HER efficiencies than the 3R phase for MoS₂ and WS₂, with the Tafel slopes of 2H-MoS₂ (AA) (172 mV dec⁻¹) and 2H-WS₂ (AA) (151 mV dec⁻¹) similarly directing us to the Volmer adsorption as the HER rate-determining step. Moreover, a second TMD material in 2H phase with different particle sizes was included as an additional control for both MoS₂ and WS₂. The additional materials are denoted as 2H-MoS₂ (SA) and 2H-WS₂ (SA) respectively. 2H-MoS₂ (SA) and 2H-WS₂ (SA) both exhibit a single hexagonal 2H phase (Figure 7.11) and their particle sizes are 1.8 μm and 4.1 μm respectively (Figure 7.12). 2H-MoS₂ (SA) displayed the Volmer adsorption as the HER rate-determining step, an overpotential of 0.56 V vs RHE and an onset potential of -0.22 V vs RHE. Despite having particle sizes of ~7 fold smaller, 2H-MoS₂ (SA) conferred an inferior HER

catalytic performance compared to 3R-MoS₂ (550 °C). A similar observation is made with WS₂. The additional control experiment further confirms the notion that the 3R phase outperforms its corresponding 2H phase for hydrogen evolution.

Figure 7.11 X-ray diffraction spectra of 2H-MoS₂ (SA) and 2H-WS₂ (SA).

Figure 7.12 Particle size distributions of 2H-MoS₂ (SA) and 2H-WS₂ (SA).

The stellar HER efficiency of the 3R phase prompted us to make a comparison with the exfoliated, 1T phase, studied previously by our group.^{10,13} 1T-MoS₂ and 1T-WS₂ were prepared by the exfoliation of 2H-MoS₂ (SA) and 2H-WS₂ (SA) respectively which would result in particle sizes of the 1T phase TMDs being similar or slightly smaller than their 2H counterparts. While the 3R-MoS₂ (550 °C) exhibited an overpotential of 0.52 V vs RHE and a Tafel slope of 113 mV dec⁻¹, 1T-MoS₂ was reported to display a similar overpotential of about 0.55 V vs RHE and a Tafel slope of 99 mV dec⁻¹. Despite being in its bulk form and with bigger particle

sizes, it was surprising to note that the HER catalytic performance of 3R-MoS₂ (550 °C) is comparable to that of the exfoliated, 1T-MoS₂. On the contrary, 3R-WS₂ (550 °C) did not perform as well as the exfoliated, 1T-WS₂; 1T-WS₂ reported an overpotential of 0.35 V vs RHE and a Tafel slope of 85 mV dec⁻¹.

In an attempt to optimise the synthesis conditions of 3R phase MoS₂ and WS₂ for HER applications, the study was further extended to include two other synthesis temperatures; 650 °C and 750 °C. A comparison of the HER catalytic performances of the 3R phases synthesised at different temperatures was performed, and their polarization curves and Tafel plots are represented in Figure 7.13. A careful analysis of their HER performance indicators (Figure 7.14) disclosed a general trend, applicable for both MoS₂ and WS₂ materials. In both cases of MoS₂ and WS₂, the 3R phase displayed superior or comparable HER performance than the 2H phase regardless of synthesis temperatures. However, as the synthesis temperature for the 3R phase is increased from 550 °C to 750 °C, we observed a decreasing trend in HER efficiency. Hence, we report that the optimal temperature for synthesis of 3R phase MoS₂ and WS₂ for HER applications is 550 °C.

Figure 7.13 HER polarization curves of 2H and 3R phase TMDs. Linear sweep voltammograms for HER in acidic electrolyte on (A) MoS₂ and (C) WS₂ compounds. Tafel plots for (B) MoS₂ and (D) WS₂ compounds. Conditions: background electrolyte, H₂SO₄ (0.5 M); scan rate, 2 mV s⁻¹.

Figure 7.14 Bar charts illustrate the averages of the (A) overpotential at -10 mA cm⁻² current density, (B) HER onset potential and (C) Tafel slopes for bare glassy carbon, MoS₂ and WS₂ in 2H and 3R phases; 3R phase MoS₂ and WS₂ were prepared at 550 °C, 650 °C or 750 °C.

Delving deeper to understand the trend observed with different synthesis temperatures for 3R phase MoS₂ and WS₂, we consider four possible factors that may affect the HER efficiency of the materials; 1) 3R phase concentration, 2) crystallite size, 3) presence of impurities and 4) particle size. For this purpose, characterisation of the materials was carried out using XPS, XRD, EDS, XRF, SEM and laser diffraction. Figure 7.15 shows the high resolution XPS spectra of 3R phase MoS₂ and WS₂ synthesised at 550 °C, 650 °C and 750 °C. Deconvolution analyses of the Mo bonding modes show the presence of 2H and/or 3R phases in the 3R-MoS₂ (550 °C), 3R-MoS₂ (650 °C) and 3R-MoS₂ (750 °C) materials with Mo 3d_{5/2} and Mo 3d_{3/2} at binding energies of ~229.0 and 232.5 eV respectively. Similarly, deconvolution analyses of the W bonding modes show the presence of 2H and/or 3R phases in the 3R-WS₂ (550 °C), 3R-WS₂ (650 °C) and 3R-WS₂ (750 °C) materials with W 4f_{7/2} and Mo 4f_{5/2} at binding energies of ~33.2 and 35.3 eV respectively. In order to distinguish the 2H and 3R phases, X-ray diffraction was employed. The X-ray diffractogram of 3R phase MoS₂ and WS₂ synthesised at 550 °C, 650 °C and 750 °C are shown in Figure 7.16, revealing that the 3R phase was successfully produced at all three temperatures. However, we observed that an increase in synthesis temperature generally led to a decrease in 3R phase concentration. This is most significant for MoS₂, where the concentration of 3R phase was only 30 wt% for the material synthesised at 750 °C. On the other hand, the concentration of 3R-WS₂ was still about 45 wt% for the material synthesised at 750 °C. Moreover, decreasing FWHM of the reflection indicates improvement of crystallinity and increase of crystallite size with increasing synthesis temperature. Therefore, a reduction in 3R phase concentration and increase in crystallite size account for the

decreasing trend in HER catalytic performance of 3R phase MoS₂ and WS₂ with increasing synthesis temperature.

Figure 7.15 X-ray photoelectron spectroscopy (XPS) of (A) 3R-MoS₂ (550 °C), 3R-MoS₂ (650 °C) and 3R-MoS₂ (750 °C), and (B) 3R-WS₂ (550 °C), 3R-WS₂ (650 °C) and 3R-WS₂ (750 °C).

Figure 7.16 X-ray diffraction (XRD) patterns of (A) 3R-MoS₂ (550 °C), 3R-MoS₂ (650 °C) and 3R-MoS₂ (750 °C), and (B) 3R-WS₂ (550 °C), 3R-WS₂ (650 °C) and 3R-WS₂ (750 °C).

Further characterization was performed with EDS (Figure 7.17-7.20), which presented no obvious changes in elemental composition regardless of the synthesis temperature. More in-depth analyses with XRF (Table 7.1) reported that the concentration of potassium originating from potassium carbonate flux ranges from 0.05-1.2 wt% and decreases with increasing synthesis temperature. While only traces of tungsten were detected in 3R-MoS₂, the concentration of molybdenum ranges from 0.15-0.39 wt% in 3R-WS₂ and this concentration increased with synthesis temperature. Furthermore, similar traces of Zn and traces of Fe were found in 3R-MoS₂ and 3R-WS₂ respectively regardless of synthesis temperature. The concentrations of all other impurities were below 0.01 wt%. It can be concluded that the changes in elemental composition of 3R-MoS₂ and 3R-WS₂ synthesised at different temperatures were not significant enough to interfere with the electrochemical signals. Next, to study the relation between particle size and HER catalytic activity, we performed SEM (Figure 7.4) and laser diffraction measurements for particle size analysis (Figure 7.5). All samples exhibit highly polydisperse with particle sizes in the range of 0.2 – 50 µm. However, a closer observation of the particle size distribution curves from laser diffraction measurements does not reveal any correlation with synthesis temperature. From these results, we can conclude that the main influence on electrocatalytic activity will be related to the concentration of 3R phase in the TMD materials and the size of individual crystallites.

Figure 7.17 Energy-dispersive X-ray spectroscopy (EDS) data for 3R-MoS₂ (650 °C). Scale bar, 10 μm.

Figure 7.18 Energy-dispersive X-ray spectroscopy (EDS) data for 3R-WS₂ (650 °C). Scale bar, 10 μm.

Figure 7.19 Energy-dispersive X-ray spectroscopy (EDS) data for 3R-MoS₂ (750 °C). Scale bar, 10 μm.

Figure 7.20 Energy-dispersive X-ray spectroscopy (EDS) data for 3R-WS₂ (750 °C). Scale bar, 10 μm.

7.3 Conclusion

A comprehensive study and comparison of the 3R and 2H phases of the two most representative TMD materials, MoS₂ and WS₂, has been executed in relation to their catalytic properties towards the hydrogen evolution reaction. We report the synthesis of 3R-MoS₂ and 3R-WS₂ materials via a simple and scalable method at three different temperatures (550 °C, 650 °C and 750 °C). The optimal synthesis temperature was determined to be 550 °C for applications in industrially important electrocatalysis like HER, where high 3R phase concentration and small crystallite sizes were obtained. It was demonstrated that the 3R phase displayed superior HER efficiencies compared to the 2H phase. Additionally, a more significant improvement in HER catalytic activity was observed with 3R-MoS₂ than 3R-WS₂. Specifically, it is surprising to note that the bulk, 3R-MoS₂ (550 °C) displayed comparable HER efficiency to that of the previously studied exfoliated, 1T-MoS₂. These results highlight the profound impact different polymorph structures of TMD materials have on their HER efficiencies, and further challenges the need for the commonly employed technique of exfoliation to enhance the catalytic properties of TMD materials. The insights from this work not only provide an understanding of the underlying electrochemical activity of TMD materials, but are also particularly important in the search for highly efficient catalysts for the hydrogen evolution.

7.4 Experimental

7.4.1 Materials

WO₃ (99.9%) and MoO₃ (99.9%) were obtained from Sigma-Aldrich, Czech Republic. 2H-MoS₂ (AA) and 2H-WS₂ (AA) were purchased from Alfa Aesar, Czech Republic. 2H-MoS₂ (SA) and 2H-WS₂ (SA) were purchased from Sigma Aldrich, Czech Republic. Sulphuric acid was purchased from Sigma Aldrich, Singapore. K₂CO₃ (>99%), S (>99%) and toluene were obtained from PENTA, Czech Republic. Glassy carbon (GC), the Ag/AgCl reference and Pt counter electrodes were acquired from Autolab (The Netherlands).

7.4.2 Apparatus

X-ray diffraction (XRD) was done using a Bruker D8 Discoverer diffractometer in Bragg–Brentano parafocusing geometry. A Cu K_α radiation was used. Diffraction patterns were measured between 10° and 80° of 2θ. The obtained data were evaluated using HighScore Plus 3.0e software. High resolution transmission electron microscopy (HR-TEM) was performed using an EFTEM Jeol 2200 FS microscope (Jeol, Japan). A 200 keV acceleration voltage was used for measurement. Sample preparation was attained by drop casting the suspension (1 mg mL⁻¹ in water) on a TEM grid (Cu; 200 mesh; Formvar/carbon) and drying at 60 °C for 12 h. X-ray fluorescence (XRF) analysis was performed by ARL PERFORM'X sequential WD-XRF spectrometer equipped with an Rh anode end-window X-ray tube type 4GN fitted with 50 μm Be window. All peak intensity data were collected by software Oxsas in vacuum. The generator settings-collimator-crystal-detector combinations were optimised for all 82 measured elements with

analysis time of 6s per element. The obtained data were evaluated by standardless software Uniquant 5 integrated in Oxsas. The analysed powders were pressed into pellets about 5 mm thick and diameter of 40 mm without any binding agent and covered with 4 µm supporting polypropylene (PP) film. Scanning electron microscopy (SEM) was performed using a Jeol 7600F SEM instrument (Jeol, Japan) operating at 5 kV. Energy-dispersive X-ray spectroscopy (EDS) data were acquired on a Jeol 7600F instrument (Jeol, Japan) at 15 kV. X-ray photoelectron spectroscopy (XPS) measurements were performed using a Phoibos 100 spectrometer and a monochromatic Mg X-ray radiation source (SPECS, Germany). Survey (wide-scan) and high resolution spectra were recorded for each sample. For SEM, EDS, and XPS measurements, samples were prepared by coating a uniform layer of the materials under study onto carbon conductive tape. All voltammetric experiments were carried out using a µAutolab type III electrochemical analyser (Eco Chemie, The Netherlands) connected to a computer and controlled by the NOVA, Version 1.8.17, software. Electrochemical experiments were carried out at room temperature in an electrochemical cell (5 mL) with the employment of a three electrode configuration. A platinum electrode functioned as the counter electrode, and an Ag/AgCl electrode served as the reference electrode.

7.4.3 Procedures

Synthesis of 3R Phase MoS₂. A mixture of K₂CO₃ (15 g), S (31 g) and MoO₃ (21 g) was heated at temperatures of 550 °C, 650 °C or 750 °C. The heating was performed for 8 h in quartz glass tube loosely capped with quartz lid. The heating and cooling rate was 5 °C min⁻¹. Synthesised MoS₂ was leached with hot water and

unreacted sulfur was extracted with toluene using Soxhlet extractor. Finally, the product was ultrasonicated with hot water and separated by suction filtration.

Synthesis of 3R Phase WS₂. A mixture of K₂CO₃ (15 g), S (41 g) and WO₃ (33 g) was heated at temperatures of 550 °C, 650 °C or 750 °C. The heating was performed for 8 h in quartz glass tube loosely capped with quartz lid. The heating and cooling rate was 5 °C min⁻¹. Synthesised WS₂ was leached with hot water and unreacted sulfur was extracted with toluene using Soxhlet extractor. Finally, the product was ultrasonicated with hot water and separated by suction filtration.

Hydrogen Evolution Reaction (HER) Studies on 2H and 3R Phase Transition Metal Dichalcogenides. HER efficiency of TMD materials were performed on glassy carbon (GC) electrodes. GC electrodes were first renewed on a polishing pad using alumina particle (0.05 µm) slurry and washed with ultrapure water. Subsequently, 1 mg mL⁻¹ suspensions of TMD materials were prepared in ultrapure water and ultrasonicated for a period of 10 min prior to each electrochemical measurement to ensure homogeneity of the material under study. An aliquot (4 µL) was deposited on the electrode surface and left to dry at room temperature to yield a randomly distributed film of 4.0 µg of desired material on the GC electrode surface. The materials of interest include 2H-MoS₂ (AA), 2H-MoS₂ (SA), 3R-MoS₂ (550 °C), 3R-MoS₂ (650 °C), 3R-MoS₂ (750 °C), 2H-WS₂ (AA), 2H-WS₂ (SA), 3R-WS₂ (550 °C), 3R-WS₂ (650 °C) and 3R-WS₂ (750 °C). HER measurements were performed in a 0.5 M H₂SO₄ electrolyte using linear sweep voltammetry (LSV) at a scan rate of 2 mV s⁻¹. Measurements were first performed relative to the Ag/AgCl reference electrode. Subsequently, the measurements were corrected and the LSVs were represented versus the reversible hydrogen electrode (RHE). The

measured potentials were calculated based on the equation $E_{\text{RHE}} = E_{\text{Ag/AgCl}} + 0.059 \times \text{pH} + E^{\circ}_{\text{Ag/AgCl}}$, where $E_{\text{Ag/AgCl}}$ is the measured potential, and pH of H₂SO₄ (0.5 M) is set as zero. The standard potential of Ag/AgCl (1M KCl) at 25 °C, $E^{\circ}_{\text{Ag/AgCl}}$ is then 0.235 V vs RHE.

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Part I

Chapter 8

Catalytic Properties of Group 4 Transition Metal
Dichalcogenides: Effect of Electrochemical
Pretreatment

8.1 Introduction

8.2 Results and Discussion

8.2.1 Inherent Electrochemistry of Group 4 TMDs

8.2.2 Characterisation of Electrochemically Treated Group 4 TMDs

8.2.3 Electrochemically Treated Group 4 TMDs for HER

8.3 Conclusion

8.4 Experimental

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

1. **Rou Jun Toh**, Zdenek Sofer, Martin Pumera. Catalytic Properties of Group 4 Transition Metal Dichalcogenides (MX_2 ; M = Ti, Zr, Hf; X = S, Se, Te): Effect of Electrochemical Pretreatment. *J. Mater. Chem. A* **2016**, 4, 18322-18334.

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8.1 Introduction

Interest in layered transition metal dichalcogenides (TMDs) has proliferated due to their high electrocatalytic activity towards hydrogen production.¹⁻³ In particular, enormous attention has been placed on studying the electrochemical behaviour of TMDs in order to advance their development for various electrochemical applications. However, it is astonishing to learn that a substantial percentage of existing literature has been dedicated exclusively to the study of group 6 TMDs (i.e., MoS₂, WS₂, MoSe₂ and WSe₂), and therefore, little is known and understood about the electrochemical behaviour of other TMD materials.

Considering the current upsurge of interest to exploit TMDs for a multitude of electrochemical applications, we believe that an electrochemical study of TMD materials outside of the group 6 is necessary and opportune. Previous studies on the electronic character of group 4 TMDs (MX₂; M = Ti, Zr, Hf; X = S, Se, Te) reveal that the predominantly layered materials are semiconductors,⁴⁻⁶ making them promising candidates for electrochemical applications. Nonetheless, there is limited understanding of the electrochemical behaviour of group 4 TMDs. Herein, we wish to address the gap in literature by conducting a comprehensive study of the electrochemical behaviour of group 4 TMDs in order to provide a first insight and extensive comparison of group 4 TMD materials.

We characterised the materials using scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS), and phase purity was verified by X-ray diffraction (XRD). Voltammetric characterizations of the

inherent electrochemistry of the materials with solid-state electrode modification are then performed. This serves to demonstrate the often overlooked, yet important and well-defined redox properties of group 4 TMDs. Subsequently, electrochemical treatments of the materials are performed according to their observed redox properties. In addition, X-ray photoelectron spectroscopy (XPS) is employed to investigate the surface composition of the materials before and after electrochemical treatment. Finally, the impact of electrochemical treatment on surface composition and electrocatalytic applications are explored in detail for the hydrogen evolution reaction (HER).

8.2 Results and Discussion

In order to characterise the materials of interest, we examine the surface morphologies of the group 4 TMDs by SEM. Figure 8.1A-C show the electron micrographs of the group 4 TMDs, which in their bulk states, are observed as large polygonal stacks up to tens of micrometers across and thick. Moreover, these materials form well-dispersed suspensions in water (Figure 8.1D) which are used for subsequent investigations. We further studied the surface elemental composition of the group 4 TMDs using EDS (Figure 8.2 and Table 8.1), where the presence of their corresponding metal (Ti, Zr or Hf) and chalcogen (S, Se or Te) are confirmed. Furthermore, the presence of surface oxides has been determined in the materials and would be an important point of consideration in later experiments and discussions.

We further verified the purity of the synthesised TMDs by X-ray diffraction (XRD). Since EDS analysis is not sufficiently accurate and XPS is highly surface sensitive, XRD would be able to give information about phase purity and the possible formation of other phases with sensitivity below 1 wt%. The obtained diffractograms are shown in Figure 8.3 together with their corresponding PDF cards. All synthesised samples were found to be pure and without any additional phases.

Figure 8.1 (A-C) Scanning electron microscopy (SEM) images of group 4 TMDs. Scale bars: 10 μm (A); 10 μm (B); and 100 μm (C). (D) Suspensions of group 4 TMD materials in ultrapure water at a concentration of 1 mg mL⁻¹.

Figure 8.2 Scanning electron micrographs and corresponding energy-dispersive X-ray maps of group 4 TMDs. Scale bars represent 10 μm .

Table 8.1 Elemental compositions of group 4 TMD materials based on energy-dispersive X-ray spectroscopy.

Material	Ti (at%)	Zr (at%)	Hf (at%)	S (at%)	Se (at%)	Te (at%)	C (at%)	O (at%)	Al (at%)	Si (at%)
TiS ₂	27.19	-	-	41.07	-	-	14.23	16.95	0.32	0.24
ZrS ₂	-	27.00	-	29.47	-	-	28.70	14.43	0.39	-
HfS ₂	-	-	20.22	15.76	-	-	23.06	40.97	-	-
TiSe ₂	17.06	-	-	-	25.55	-	19.21	36.36	-	1.82
ZrSe ₂	-	9.91	-	-	21.40	-	50.94	17.75	-	-
HfSe ₂	-	-	10.34	-	20.61	-	47.35	21.70	-	-
TiTe ₂	23.30	-	-	-	-	34.65	-	41.08	0.98	-
ZrTe ₂	-	12.66	-	-	-	30.30	19.15	37.88	-	-
HfTe ₂	-	-	11.25	-	-	25.34	23.96	39.45	-	-

Figure 8.3 Diffractograms of group 4 TMDs with corresponding PDF cards.

8.2.1 Inherent Electrochemistry of Group 4 TMDs

In current literature, fundamental understanding of the inherent electrochemistry of group 4 TMDs is inadequate. In order to address the gap in literature, we conducted cyclic voltammetry (CV) in two scan directions: cathodic and anodic. CV is performed in pH 7.2 PBS electrolyte and scanned over the potential range of -1.8 V to +1.8 V. The acquired voltammetric profiles of the respective scans reveal information about the electroactive surface moieties of the TMD materials. Furthermore, three consecutive scans were recorded to determine the nature of these electrochemical processes in the TMDs. The redox peaks observed arise from the type and number of electroactive groups present on the surface of the TMDs, and are characteristic of various TMD materials.

Figure 8.4 illustrates the cyclic voltammograms derived from the cathodic and anodic scans of TMD materials bearing the S chalcogen; TiS_2 , ZrS_2 and HfS_2 . The first scan of TiS_2 in the cathodic direction resulted in a reduction peak at -1.2 V (Figure 8.4A) while that in the anodic direction showed a small oxidation peak at +0.4 V, followed by a mild reduction peak at -1.2V (Figure 8.4B). During the first cathodic scan, ZrS_2 showed a small reduction peak at -0.9 V (Figure 8.4C) and no significant peaks when cyclic voltammetry was performed in the anodic direction (Figure 8.4D). This may indicate that an initial oxidative treatment renders the electroactive moieties on ZrS_2 less susceptible to reduction. Observation from both cathodic and anodic scans of HfS_2 indicates no significant redox peaks and hence, indicates the lack of electroactive moieties on the surface of the material

(Figure 8.4E and 8.4F). In all cases, any redox electroactivity present is only observed in the first scans and not subsequent scans, pointing toward the chemical irreversibility of these reactions.

Figure 8.4 Cyclic voltammograms derived from reduction and oxidation of MS_2 ; (A) Cathodic scan and (B) anodic scan of TiS_2 ; (C) cathodic scan and (D) anodic scan of ZrS_2 ; (E) cathodic scan and (F) anodic scan of HfS_2 . Measurement of three scans is represented accordingly: scan 1 (black), scan 2 (red) and scan 3 (blue). Conditions: background electrolyte, PBS (50 mM), pH 7.2; scan rate, 100 mV s^{-1} ; measurements are conducted with reference to the Ag/AgCl electrode.

Figure 8.5 illustrates the cyclic voltammograms acquired from the cathodic and anodic scans of TMD materials bearing the Se chalcogen; TiSe_2 , ZrSe_2 and HfSe_2 . The first scan of TiSe_2 in the cathodic direction shows a shoulder reduction peak at -1.25 V, followed by a shoulder oxidation peak at $+1.25$ V (Figure 8.5A). In contrast, the first scan in the anodic direction showed an intense oxidation peak at $+1.0$ V, followed by an absence of reduction peaks (Figure 8.5B). This suggests that an initial oxidative treatment could provide a protective effect for the electroactive moieties towards reduction. Observation of the cathodic scan of ZrSe_2 shows a small reduction peak at -0.9 V (Figure 8.5C) while that of the anodic scan shows an oxidation peak at $+1.1$ V (Figure 8.5D). Similar observations were made in the case of HfSe_2 (Figure 8.5E and 8.5F). A point worth noting is that all the TMD materials bearing the Se chalcogen display a distinct oxidation peak at about $+1.0$ V where previous research on selenium electrochemistry points to the possibility of oxidation to Se^{6+} .^{7,8} Moreover, in all cases, any redox electroactivity present is only observed in the first scans and not subsequent scans, pointing toward the chemical irreversibility of these reactions.

Figure 8.5 Cyclic voltammograms derived from reduction and oxidation of MSe_2 ; (A) Cathodic scan and (B) anodic scan of $TiSe_2$; (C) cathodic scan and (D) anodic scan of $ZrSe_2$; (E) cathodic scan and (F) anodic scan of $HfSe_2$. Measurement of three scans is represented accordingly: scan 1 (black), scan 2 (red) and scan 3 (blue). Conditions: background electrolyte, PBS (50 mM), pH 7.2; scan rate, 100 mV s^{-1} ; measurements are conducted with reference to the Ag/AgCl electrode.

Figure 8.6 illustrates the cyclic voltammograms derived from the cathodic and anodic scans of the TMD materials bearing the Te chalcogen; TiTe_2 , ZrTe_2 and HfTe_2 . An interesting observation is made when TMD materials containing the Te chalcogen were compared with each other. Distinctive oxidation peaks were observed at about +0.25 V and +0.5 V, while reduction peaks were seen at about -0.75 V, -1.0 V and -1.25 V. These peaks is rationalised by looking at the electrochemistry of the Te chalcogen component of the materials, supported by existing literature.⁹⁻¹¹ The initial cathodic peak at about -1.25 V is likely due to reduction to Te^0 . The anodic peak at about +0.25V is attributed to the oxidation of Te^0 to Te^{4+} , while that at about +0.5 V may correspond to the oxidation of Te^{2+} to Te^{4+} . The prominent peaks observed in subsequent cathodic scans at about -0.75 V and -1.0 V are likely to be due to the reduction of Te^{4+} to Te^0 , and Te^0 to Te^{2-} respectively.

Figure 8.6 Cyclic voltamograms derived from reduction and oxidation of MTe_2 ; (A) Cathodic scan and (B) anodic scan of TiTe_2 ; (C) cathodic scan and (D) anodic scan of ZrTe_2 ; (E) cathodic scan and (F) anodic scan of HfTe_2 . Measurement of three scans is represented accordingly: scan 1 (black), scan 2 (red) and scan 3 (blue). Conditions: background electrolyte, PBS (50 mM), pH 7.2; scan rate, 100 mV s^{-1} ; measurements are conducted with reference to the Ag/AgCl electrode.

8.2.2 Characterisation of Electrochemically Treated Group 4 TMDs

Earlier, the investigation of inherent electrochemistry of the group 4 TMDs revealed their characteristic oxidation and reduction peaks. By careful observation, we identified a suitable oxidation and reduction potential for the electrochemical treatment of each TMD material. As a general rule, these potentials lie marginally beyond the characteristic redox potentials of the corresponding TMD material. The oxidation and reduction treatment potentials are determined to be + 0.7 V and -1.5 V for TiS_2 , +1.5 V and -1.6 V for TiSe_2 , +1.5 V and -1.25 V for ZrSe_2 and HfSe_2 , +1.1 V and -1.6V for TiTe_2 , ZrTe_2 and HfTe_2 . ZrS_2 was subjected to only reductive treatment at -1.1 V due to the absence of a characteristic oxidation peak. On the other hand, no oxidative or reductive treatment was performed on HfS_2 as it is electrochemically inert within the potential range of -1.8 V to +1.8 V.

At these potentials, we would expect that there will be chemical alterations to the surface structure of the group 4 TMDs as a consequence of redox reactions of the materials. In order to confirm this, the technique of X-ray photoelectron spectroscopy (XPS) was employed to characterise the surface composition of the TMD materials before and after their respective electrochemical treatments. As a surface-sensitive technique with a maximum depth of analysis at about 10 nm and detection limits at approximately 0.1 wt%, the XPS high resolution spectra allow us to examine the surface elemental compositions, where chalcogen-to-metal ratios are reported in Table 8.2, and bonding information of the individual materials before and after electrochemical treatment.

Table 8.2 Tabulated chalcogen-to-metal ratios of group 4 TMD materials before and electrochemical treatments based on high resolution X-ray photoelectron spectroscopy.

Material	Surface Chalcogen / Metal Ratio		
	Before electrochemical treatment	After reductive treatment	After oxidative treatment
TiS₂	1.41	Loss of TiS ₂	Loss of TiS ₂
ZrS₂	0.77	Loss of ZrS ₂	-
HfS₂	0.43	-	-
TiSe₂	1.67	Loss of TiSe ₂	Loss of TiSe ₂
ZrSe₂	0.88	Loss of ZrSe ₂	Loss of ZrSe ₂
HfSe₂	0.80	1.15	0.99
TiTe₂	3.78	Loss of TiTe ₂	Loss of TiTe ₂
ZrTe₂	5.08	Loss of ZrTe ₂	Loss of ZrTe ₂
HfTe₂	4.16	Loss of HfTe ₂	Loss of HfTe ₂

The high resolution XPS spectra of Ti 2p, Zr 3d and Hf 4f orbitals and S 2p orbitals of TMD materials bearing the S chalcogen before and after electrochemical treatment are displayed in Figure 8.7. Deconvolution analysis of the bonding modes are performed based on previous XPS studies of titanium sulphides and their corresponding oxides.^{12 - 16} Before electrochemical treatment, it is observed from Figure 8.7A the presence of both Ti and S. The three pairs of peaks revealed in the Ti 2p spectra of TiS₂ indicate the presence of Ti⁴⁺ as in TiO₂ (458.0 and 463.7 eV), Ti⁴⁺ as in TiS₂ (455.0 and 460.2 eV) and Ti⁶⁺ as in TiS₃ (456.0 and 461.6 eV). S²⁻ as in TiS₂ (160.2 and 161.3 eV) and S₂²⁻ as in TiS₃ (161.2 and 162.4 eV) are further confirmed in the analysis of the S 2p spectrum. The presence of the sulphur is no longer significant after both reductive (-1.5 V) and oxidative (+0.7 V) treatment of TiS₂, with significant changes to the Ti 2p spectra as well. Fitting of the Ti 2p spectra indicate the absence of initial TiS₂ and TiS₃

species, leaving behind only Ti oxides after electrochemical treatment. Ti^{4+} in an oxide environment is observed after both reductive and oxidative treatment. A similar observation is made with the ZrS_2 material after reductive (-1.1 V) treatment (Figure 8.7B). This is in comparison with an obvious presence of both the Zr and S before electrochemical treatment where the Zr 3d peak shows two distinct pairs of peaks originating from Zr^{4+} in a sulphide environment as in ZrS_2 (181.1 and 183.5 eV)^{17,18} and Zr^{4+} in an oxide environment as in ZrO_2 (182.2 and 184.6 eV).¹⁹

Moreover, the high resolution XPS spectra of Hf 4f orbitals and S 2p orbitals (Figure 8.7C) indicate the presence of both Hf and S in the HfS_2 material. The Hf 4f and S 2p spectra of HfS_2 confirm the presence of the metal dichalcogenide with Hf^{4+} (15.8 and 17.5 eV) and S^{2-} (161.0 and 162.2 eV) as in HfS_2 .²⁰ Partial oxidation of the material is reported as Hf^{4+} in an oxide environment (17.3 and 19.0 eV)^{21,22} is observed in the Hf 4f peak.

Figure 8.7 High resolution X-ray photoelectron spectra of the metal (Ti 2p, Zr 3d and Hf 4f regions) and S chalcogen (S 2p region) of the (A) TiS_2 , (B) ZrS_2 and (C) HfS_2 materials prior to and after electrochemical treatment.

The high resolution XPS spectra of Ti 2p, Zr 3d and Hf 4f orbitals and Se 3d orbitals of TMD materials bearing the Se chalcogen before and after electrochemical treatment are displayed in Figure 8.8. The presence of both Ti and Se are established from the XPS spectra (Figure 8.8A). Fitting of the Ti 2p spectra reveals the strong presence of Ti oxides; Ti^{4+} as in TiO_2 (458.8 and 464.5 eV) and Ti^{3+} as in Ti_2O_3 (457.3 and 462.5 eV)^{14,16}, and also Ti^{4+} as in TiSe_2 (456.2 and 462.1 eV)²³ in lesser concentrations. The Se 3d spectrum confirms the presence of the metal dichalcogenide with a doublet attributing to Se^{2-} as in TiSe_2 (54.1 and 54.9 eV)²⁴ as well as reveals the presence of Se^0 (55.8 and 56.6 eV).²⁵ Both reductive (-1.6 V) and oxidative (+1.5 V) treatments result in loss of the metal dichalcogenide. TiO_2 remains after oxidative treatment, while TiO_2 and Se^0 are detected after reductive treatment.

From Figure 8.8B, Zr^{4+} (181.9 and 184.4 eV) and Se^{2-} (52.7 and 53.5 eV) as in ZrSe_2 are observed before electrochemical treatment of the ZrSe_2 material.^{26,27} In addition, Se^0 (54.7 and 55.6 eV) is found present from the Se 3d peak.²⁵ Both reductive (-1.25 V) and oxidative (+1.5 V) treatments result in the loss of the metal dichalcogenide and the formation of Zr^{4+} in an oxide environment as in ZrO_2 .

Moreover, it is observed that both Hf and Se are present before and after electrochemical treatment of the HfSe_2 material (Figure 8.8C). From the Hf 4f peak fitting, the major presence of Hf^{4+} as in HfSe_2 (16.8 and 18.5 eV)²⁰ is observed, and Hf^0 (14.5 and 16.2 eV)²⁸ at a lower concentration. From the Se 3d peak, doublets attributing to Se^{2-} as in HfSe_2 (52.1 and 53.0 eV)²⁰ and Se^0 (54.5 and 55.4 eV)²⁵ are assigned. The presence of HfSe_2 remains even after reductive (-1.25 V) and oxidative (+1.5 V) treatments. However, relative

concentrations of HfSe_2 markedly diminished with reductive treatment compared to that of oxidative treatment.

Figure 8.8 High resolution X-ray photoelectron spectra of the metal (Ti 2p, Zr 3d and Hf 4f regions) and Se chalcogen (Se 3d region) of the (A) TiSe_2 , (B) ZrSe_2 and (C) HfSe_2 materials prior to and after electrochemical treatment.

The high resolution XPS spectra of Ti 2p (Figure 8.9A), Zr 3d (Figure 8.9B) and Hf 4f (Figure 8.9C) orbitals and Te 3d orbitals of TMD materials bearing the Te chalcogen before and after electrochemical treatment are displayed in Figure 8.9. Fitting of the Ti 2p peak displayed the presence of Ti^{4+} as in TiTe_2 (455.0 and 41.0 eV) in the TiTe_2 material before electrochemical treatment.²⁹ However, the major presence of Ti oxides are revealed from two doublets arising due to Ti^{4+} as in TiO_2 (458.0 and 263.7 eV) and Ti^{3+} as in Ti_2O_3 (457.0 and 462.2 eV).^{14,16} Analysis of the Te 3d peak shows the presence of Te^{2-} as in TiTe_2 (572.0 and 582.5 eV)³⁰, Te^0 (573.4 and 583.8 eV) and Te^{4+} as in TeO_2 (575.5 and 585.8 eV).^{31,32} It should be brought to attention that upon reductive treatment (-1.6 V), the presence of Ti is no longer significant. Deconvolution analysis of the Te bonding modes indicates loss of the metal dichalcogenide. Likewise, the loss of TiTe_2 is observed with oxidative treatment (+1.1 V). However, the presence of Ti oxides remains.

Similar patterns are observed subsequently in both cases of ZrTe_2 and HfTe_2 . From Figure 8.9B, the doublets at 181.8 and 184.2 eV, and 571.9 and 582.2 eV can be attributed to the Zr^{4+} and Te^{2-} as in ZrTe_2 respectively. Loss of the metal dichalcogenide is observed upon both reductive (-1.6 V) and oxidative treatment (+1.1 V). Moreover, the loss of zirconium is evident with reductive treatment while Zr oxides remain detected with oxidative treatment. In the case of HfTe_2 , analysis of the Hf 4f bonding modes in Figure 8.9C shows a doublet at 16.2 and 17.9 eV corresponding to Hf^{4+} as in HfTe_2 . The doublet at 571.4 and 581.8 eV originates from Te^{2-} as in HfTe_2 . The presence of HfTe_2 is insignificant while Hf oxides are present after reductive (-1.6 V) and oxidative (+1.1 V) treatments.

Figure 8.9 High resolution X-ray photoelectron spectra of the metal (Ti 2p, Zr 3d and Hf 4f regions) and Te chalcogen (Te 3d region) of the (A) TiTe_2 , (B) ZrTe_2 and (C) HfTe_2 materials prior to and after electrochemical treatment.

Overall, a general trend is observed that electrochemical treatment elicits the dissolution of group 4 metal dichalcogenides. In most cases, the presence of metal dichalcogenide is no longer detected or has depleted greatly. The only exception is in the case of HfSe_2 where after oxidative treatment at +1.5 V, HfSe_2 remained as the major component. Although XPS is an indispensable technique for analysis of TMD elemental compositions, it is also important to emphasise that it may not provide the full picture of the effects of electrochemical treatment on group 4 TMDs. As discussed earlier, it may be expected that oxidative treatment of the metal diselenides would lead to the formation of Se^{6+} , which may be in the form of selenate anion or its oxide.^{7,8} Due to the high solubility of SeO_3 , dissolution into the surrounding electrolyte upon formation means that it would escape detection via XPS. Moreover, the possibility of oxidation in air may present much higher amounts of surface oxides than is true for HER catalytic studies discussed subsequently. Hence, XPS analysis allows us to postulate the effects of electrochemical treatment on group 4 TMDs, but should not be interpreted as absolute.

8.2.3 Electrochemically Treated Group 4 TMDs for HER

Following characterisation of the TMD materials, it is of equal significance to investigate how the abilities of these materials to catalyse the hydrogen evolution reaction (HER) in acidic medium (0.5 M H_2SO_4) correspond to their surface composition before and after electrochemical treatment. The HER performances of the materials were evaluated using linear sweep voltammetry (LSV).

Figure 8.10A-C illustrates the linear sweep voltammograms (LSV) obtained from the evolution of hydrogen in air-saturated 0.5 M H_2SO_4 . TMD materials are grouped according to the chalcogen they contain; S (Figure 8.10A), Se (Figure 8.10B) or Te (Figure 8.10C), in order to study any trends on HER performance down group 4 metals (Ti, Zr, Hf). From the cathodic scan of TiSe_2 , a shoulder peak was observed at -0.75 V. This could be due to the inherent electrochemistry of the TiSe_2 material, matching the reduction peak observed in the study of its inherent electrochemistry. Similarly, reduction peaks are observed in the cathodic scans for TMD materials containing the Te chalcogen, which matches the observations made in the study of their inherent electrochemistry.

In addition, three commonly adopted HER performance indicators namely the overpotential (Figure 8.10D), Tafel slope (Figure 8.10E) and onset potential (Figure 8.10F) were plotted for bare GC and the untreated TMD materials for comparison. The onset potential was determined at the potential where a steep decrease is observed for the current density corresponding to proton reduction. Overpotentials were taken at -10 mA cm^{-2}

². Moreover, the Tafel slope is determined by the increase in overpotential required to bring about a unit increase in current density. Tafel slope analysis can give us an understanding of the hydrogen evolution mechanism on the different TMD surfaces, where the rate-determining steps may be accounted as follows:³³

1. Volmer adsorption step:

2. Heyrovsky desorption step:

3. Tafel desorption step

In general, group 4 TMDs exhibit a Tafel slope close to or higher than the 120 mV dec^{-1} theoretical value before and after electrochemical treatment. This observation points to the adsorption step as the slow step for all the materials of interest. For this reason, Tafel analysis of HER mechanism will not be discussed in detail. Instead, desirable HER catalytic activities will be determined and discussed based on lower overpotentials, smaller Tafel slopes and less negative onset potentials as less energy is required for the reaction to take place.

As illustrated in Figure 8.10D- 8.10F, the bare glassy carbon (GC) has an overpotential of 1.12 V at -10 mA cm^{-2} , a Tafel slope of 158 mV dec^{-1} , and an onset potential of -0.96 V . In general, the TMD materials have lower overpotentials, gentler Tafel slopes, and less negative onset potentials than

that of the bare GC. Particularly, HfS_2 displayed the best HER performance with the smallest overpotential at -10 mA cm^{-2} and onset potential.

Figure 8.10 The effect of metal (Ti, Zr or Hf) on HER performance of group 4 TMDs. Linear sweep voltammograms for HER in acidic electrolyte on (A) MS_2 , (B) MSe_2 and (C) MTe_2 deposited on a GC electrode. Bar charts illustrate the averages and corresponding error bars of the (D) overpotential at -10 mA cm^{-2} current density, (E) Tafel slopes and (F) HER onset potential for bare GC and the TMD materials. Conditions: background electrolyte, H_2SO_4 (0.5 M); scan rate, 2 mV s^{-1} .

For TMD materials containing the S chalcogen, overpotentials at -10 mA cm^{-2} decrease down group 4 metals from TiS_2 (1.13 V) > ZrS_2 (1.04 V) > HfS_2 (0.99 V) (Figure 8.10D). A similar trend is observed in Figure 8.10F where the onset potentials become less negative down the group; TiS_2 (-0.95 V) > ZrS_2 (-0.85 V) > HfS_2 (-0.81 V). While the Tafel slopes of TiS_2 (147 mV dec^{-1}), ZrS_2

(138 mV dec⁻¹) and HfS₂ (143 mV dec⁻¹) are similar (Figure 8.10E), there is clear trend of an increasing HER performance down Group 4 metals for TMD materials containing the S chalcogen; TiS₂ < ZrS₂ < HfS₂.

For TMD materials containing the Se chalcogen, overpotentials at -10 mA cm⁻² are similar with 1.05 V for TiSe₂, 1.06 V for ZrSe₂, and 1.03 V for HfSe₂ (Figure 8.10D). Among the three, TiSe₂ has the gentlest Tafel slope of 107 mV dec⁻¹, while that of ZrSe₂ (159 mV dec⁻¹) and HfSe₂ (159 mV dec⁻¹) are similar (Figure 8.10E). From Figure 8.10F, similar onset potentials were reported for TiSe₂ (-0.84 V), ZrSe₂ (-0.86 V) and HfSe₂ (-0.85 V). Hence, we may conclude that the HER performances down group 4 metals for TMD materials containing the Se chalcogen are similar; TiSe₂ ≈ ZrSe₂ ≈ HfSe₂. However, TiSe₂ may have a slight advantage over the other two materials due to its gentler Tafel slope.

For TMD materials containing the Te chalcogen, overpotentials at -10 mA cm⁻² are similar for TiTe₂ (1.13 V), ZrTe₂ (1.11 V) and HfTe₂ (1.11 V). A comparable pattern is observed in Figure 8.10F where the onset potentials of TiTe₂ (-0.95 V), ZrTe₂ (-0.95 V) and HfTe₂ (-0.96 V) are similar as well. Figure 8.10D shows that the Tafel slope of TiTe₂ (233 mV dec⁻¹) is much larger than that of HfTe₂ (145 mV dec⁻¹) and ZrTe₂ (152 mV dec⁻¹). Hence, it is resolved that the HER performances down the group 4 metals for TMD materials containing the Te chalcogen are similar (TiTe₂ ≈ ZrTe₂ ≈ HfTe₂), with TiTe₂ being slightly inferior due to its large Tafel slope.

Figure 8.11A-C illustrates the linear sweep voltammograms (LSV) obtained from the evolution of hydrogen in 0.5 M H₂SO₄. TMD materials are grouped according to the metal they contain; Ti (Figure 8.11A), Zr (Figure

8.11B), Hf (Figure 8.11C), in order to study how HER performance is affected by group 16 chalcogens (S, Se, Te) as we go down the group.

Figure 8.11 The effect of chalcogen (S, Se or Te) on HER performance of group 4 transition metal dichalcogenides. Linear sweep voltammograms for HER in acidic electrolyte on (A) TiX₂, (B) ZrX₂ and (C) HfX₂ deposited on a GC electrode. Bar charts illustrate the averages and corresponding error bars of the (D) overpotential at -10 mA cm⁻² current density, (E) Tafel slopes and (F) HER onset potential for bare GC and the TMD materials. Conditions: background electrolyte, H₂SO₄ (0.5 M); scan rate, 2 mV s⁻¹.

For TMD materials containing the Ti metal, TiSe₂ (1.05 V) exhibited the lowest overpotential at -10 mA cm⁻² compared to that of TiS₂ (1.13 V) and TiTe₂ (1.13 V) (Figure 8.11D). Tafel slope calculations in Figure 8.11E matched this observation, showing that TiSe₂ has the gentlest Tafel slope (107 mV dec⁻¹) in comparison to that of TiS₂ (147 mV dec⁻¹) and TiTe₂ (233 mV dec⁻¹).

Likewise, it is observed in Figure 8.11F that TiSe_2 has the least negative onset potential (-0.84 V) compared to that of TiS_2 (-0.95 V) and TiTe_2 (-0.95 V). Overall, we may say that the HER performance of TiSe_2 is superior among the TMD materials containing the Ti metal.

For TMD materials containing the Zr metal, overpotentials at -10 mA cm^{-2} increase down group 16 chalcogens from ZrS_2 (1.04 V) $<$ ZrSe_2 (1.06 V) $<$ ZrTe_2 (1.11 V) (Figure 8.11B). This observation was backed up by the data in Figure 8.11F, showing that the onset potential becomes more negative down group 16 chalcogens; ZrS_2 (-0.85 V) $>$ ZrSe_2 (-0.86 V) $>$ ZrTe_2 (-0.95 V). While ZrSe_2 displayed the steepest Tafel slope (159 mV dec^{-1}) in relation to ZrS_2 (138 mV dec^{-1}) and ZrTe_2 (145 mV dec^{-1}), it is deduced that the HER performance generally decreases down group 16 chalcogens for TMD materials containing the Zr metal; $\text{ZrS}_2 > \text{ZrSe}_2 > \text{ZrTe}_2$.

For TMD materials containing the Hf metal, an increasing trend for overpotential at -10 mA cm^{-2} down group 16 chalcogens is illustrated in Figure 8.11C; HfS_2 (0.99 V) $<$ HfSe_2 (1.03 V) $<$ HfTe_2 (1.11 V) (Figure 8.11D). Moreover, it is observed from Figure 8.11F that the onset potential gets increasingly negative down group 16 chalcogens; HfS_2 (-0.81 V) $>$ HfSe_2 (-0.85 V) $>$ HfTe_2 (-0.96 V). While the Tafel slopes of HfS_2 (143 mV dec^{-1}), HfSe_2 (156 mV dec^{-1}) and HfTe_2 (152 mV dec^{-1}) are similar (Figure 8.11E), we can deduce that there is a decreasing trend for HER performance down Group 16 chalcogens for TMD materials containing the Hf metal; $\text{HfS}_2 > \text{HfSe}_2 > \text{HfTe}_2$.

Next, we discuss the influence of electrochemical treatment on the catalytic activity of group 4 TMDs towards HER. In particular, we would like to explore the possibility of tuning the catalytic properties of group 4 TMD

materials by the application of an oxidative or reductive electrochemical treatment, which would cause chemical alterations and also the possibility of removing surface impurities. Such a prospect has been displayed in a work by Chia *et al.* as they show the activation of t-butyllithium exfoliated MoS₂, MoSe₂ and WSe₂ for HER catalysis by reductive treatment.³⁴ Linear sweep voltammograms (LSV) were obtained from the evolution of hydrogen in air-saturated 0.5 M H₂SO₄ before and after electrochemical treatment of the TMD materials, and their HER performance indices are analysed.

Figure 8.12 shows the HER performance of TiS₂ before and after electrochemical treatment. From Figure 8.12B, it can be seen that the overpotential of the TiS₂ material (1.12 V) did not change significantly with reductive treatment. Conversely, oxidative treatment caused an increase in overpotential from 1.13 V to 1.23 V. Tafel slopes increased from 147 mV dec⁻¹ to 155 mV dec⁻¹ and 171 mV dec⁻¹ after reductive and oxidative treatment respectively (Figure 8.12C). Moreover, Figure 8.12D illustrates that the onset potentials are more negative with electrochemical treatment. Onset potential is reported as -0.98 V with reductive treatment, and -1.0 V with oxidative treatment. We conclude that the HER performance of TiS₂ became poorer after electrochemical treatment.

Figure 8.12 The effect of electrochemical treatment on HER performance of TiS_2 . (A) Linear sweep voltammograms for HER in acidic electrolyte on TiS_2 , and TiS_2 after -1.5 V and +0.7 V treatment deposited on a GC electrode. Bar charts represent the averages and corresponding error bars of the (B) overpotential at -10 mA cm^{-2} current density, (C) Tafel slopes and (D) HER onset potential for bare GC and TiS_2 prior to and after electrochemical treatment.

Moreover, the ZrS_2 material was subjected to a reductive treatment where the overpotential at -10 mA cm^{-2} increased from 1.04 V to 1.24 V after reductive treatment (Figure 8.13B). Furthermore, the Tafel slope increased slightly by 8 mV dec^{-1} to 146 mV dec^{-1} with reductive treatment (Figure 8.13C). It is also illustrated that reductive treatment yields a material with a more negative onset potential of -1.01 V compared to that of the starting ZrS_2 material (-0.85 V) (Figure 8.13D). These observations combined to resolve that the electrochemical treatment of the material decreased its HER performance.

Figure 8.13 The effect of electrochemical treatment on HER performance of ZrS₂. (A) Linear sweep voltammograms for HER in acidic electrolyte on ZrS₂, and ZrS₂ after -1.1 V treatment deposited on a GC electrode. Bar charts represent the averages and corresponding error bars of the (B) overpotential at -10 mA cm⁻² current density, (C) Tafel slopes and (D) HER onset potential for bare GC and ZrS₂ prior to and after electrochemical treatment.

In both cases of TiS₂ and ZrS₂, it is seen that electrochemical treatment yields materials which are less active towards HER catalysis. An insight into this behaviour is brought by studying the XPS characterisations of these materials (Figure 8.7A and 8.7B) where the loss of metal disulphide is observed in both cases upon electrochemical treatment. It can be concluded that the metal disulphides are responsible for the catalysis of HER.

Furthermore, Figure 8.14 shows the HER performance of TiSe₂. It is illustrated in Figure 8.14B that the overpotential of TiSe₂ at -10 mA cm⁻² increased from 1.05 V to 1.10 V after reductive treatment, and remained relatively constant after oxidative treatment (1.05 V). A similar observation is made in Figure 8.14C, where the Tafel slope (107 mV dec⁻¹) significantly

increased after reductive treatment (176 mV dec^{-1}) while oxidative treatment (143 mV dec^{-1}) saw a slight increase in the Tafel slope. In addition, the onset potential became more negative after reductive treatment of the TiSe_2 material (-0.92 V). On the other hand, the onset potential remained relatively stable (-0.82 V) after oxidative treatment (Figure 8.14D). Hence, we conclude that HER performance of the TiSe_2 material decreased slightly upon oxidative treatment while reductive treatment yields a material with an even more considerable decrease in HER catalytic activity. This can be accounted for by the depletion of the metal diselenide as observed in earlier XPS studies (Figure 8.8A), pointing to the HER catalytic activity of the metal diselenide.

Figure 8.14 The effect of electrochemical treatment on HER performance of TiSe_2 . (A) Linear sweep voltammograms for HER in acidic electrolyte on TiSe_2 , and TiSe_2 after -1.6 V and $+1.5 \text{ V}$ treatment deposited on a GC electrode. Bar charts represent the averages and corresponding error bars of the (B) overpotential at -10 mA cm^{-2} current density, (C) Tafel slopes and (D) HER onset potential for bare GC and TiSe_2 prior to and after electrochemical treatment.

Investigation of the HER activity of ZrSe_2 before and after electrochemical treatment shows that the overpotential of ZrSe_2 at -10 mA cm^{-2} decreased from 1.06 V to 0.94 V and 1.00 V upon reductive and oxidative treatment respectively (Figure 8.15B). Moreover, the material displayed a high Tafel slope with reductive treatment (177 mV dec^{-1}). On the other hand, oxidative treatment resulted in a material with a lower Tafel slope of 138 mV dec^{-1} compared to that of the starting ZrSe_2 material (159 mV dec^{-1}) (Figure 8.15C). From Figure 8.15D, it is observed that the onset potentials are less negative with electrochemical treatment of ZrSe_2 . The onset potentials of the materials are -0.76 V and -0.78 V after reductive and oxidative treatment respectively. Hence, observations of the overpotentials and onset potentials agreed to conclude that electrochemical treatment of the ZrSe_2 material yields materials possessing superior HER performance, with reductive treatment showing a better overall advantage. Matching the XPS results of the ZrSe_2 material before and after electrochemical treatment (Figure 8.8B), we are able to explain the above observations. The loss of the metal diselenide is observed upon both reductive and oxidative treatment of the ZrSe_2 material. This could result in the availability of Zr which displays a superior HER performance. With reductive treatment, the formation of an oxide passivating layer³⁵ is less likely and hence, resulting in a higher increase in HER performance.

Figure 8.15 The effect of electrochemical treatment on HER performance of ZrSe₂. (A) Linear sweep voltammograms for HER in acidic electrolyte on ZrSe₂, and ZrSe₂ after -1.25 V and +1.5 V treatment deposited on a GC electrode. Bar charts represent the averages and corresponding error bars of the (B) overpotential at -10 mA cm⁻² current density, (C) Tafel slopes and (D) HER onset potential for bare GC and ZrSe₂ prior to and after electrochemical treatment.

It is displayed in Figure 8.16B that the overpotential of HfSe₂ at -10 mA cm⁻² decreased slightly from 1.03 V to 1.02 V and 0.99 V after reductive and oxidative treatment respectively. Similarly, Figure 8.16C shows that the Tafel slope decreased with both reductive (145 mV dec⁻¹) and oxidative treatment (133 mV dec⁻¹). Furthermore, the onset potential became less negative after electrochemical treatment of the HfSe₂ material (Figure 8.16D). The onset potential of the starting HfSe₂ material (-0.85 V) improved to -0.84 V and -0.77 V after reductive and oxidative treatment respectively. Hence, it is concluded that HER performance of the HfSe₂ material improved with electrochemical treatment, with oxidative treatment yielding a material with better HER catalytic activity. Matching the XPS results of the HfSe₂ material

before and after electrochemical treatment (Figure 8.8C), we explain the above observations. In contrast to most of the other TMD materials, the presence of HfSe_2 can still be observed from XPS after electrochemical treatment. While the relative amounts of HfSe_2 is greatly reduced after reductive treatment, HfSe_2 remains relatively stable with oxidative treatment and remains the major component. Moreover, the depletion of Se^0 is noted with oxidative treatment.^{7,8} The removal of Se^0 as an impurity is determined to be crucial for enhancing HER catalytic activity.

Figure 8.16 The effect of electrochemical treatment on HER performance of HfSe_2 . (A) Linear sweep voltammograms for HER in acidic electrolyte on HfSe_2 , and HfSe_2 after -1.25 V and $+1.5\text{ V}$ treatment deposited on a GC electrode. Bar charts represent the averages and corresponding error bars of the (B) overpotential at -10 mA cm^{-2} current density, (C) Tafel slopes and (D) HER onset potential for bare GC and HfSe_2 prior to and after electrochemical treatment.

Figure 8.17A shows the LSV obtained from the evolution of hydrogen in air-saturated $0.5\text{ M H}_2\text{SO}_4$ before and after electrochemical treatment of TiTe_2 . Figure 8.17B illustrates a decrease in the overpotential of TiTe_2 at -10

mA cm^{-2} from 1.13 V to 1.10 V upon reductive treatment, and increased to 1.16 V upon oxidative treatment. Moreover, the material displayed a much gentler Tafel slope with reductive treatment (113 mV dec^{-1}). On the other hand, oxidative treatment resulted in a material with a much higher Tafel slope of 461 mV dec^{-1} compared to that of the starting TiTe_2 material (232 mV dec^{-1}) (Figure 8.17C). From Figure 8.17D, it is observed that the onset potentials remained relatively similar after reductive treatment (-0.96 V) and oxidative treatment (-0.94 V) of TiTe_2 . Overall, we may conclude that reductive treatment of the TiTe_2 material yields materials possessing superior HER performance, while the opposite is true with oxidative treatment.

Figure 8.17 The effect of electrochemical treatment on HER performance of TiTe_2 . (A) Linear sweep voltammograms for HER in acidic electrolyte on TiTe_2 , and TiTe_2 after -1.6 V and $+1.1 \text{ V}$ treatment deposited on a GC electrode. Bar charts represent the averages and corresponding error bars of the (B) overpotential at -10 mA cm^{-2} current density, (C) Tafel slopes and (D) HER onset potential for bare GC and TiTe_2 prior to and after electrochemical treatment.

Moreover, it is displayed in Figure 8.18B that the overpotential of ZrTe_2 at -10 mA cm^{-2} increased slightly from 1.11 V to 1.12 V and 1.15 V after reductive and oxidative treatment respectively. Correspondingly, it is observed in Figure 8.18C that the Tafel slope increased with both reductive (227 mV dec^{-1}) and oxidative treatment (197 mV dec^{-1}). Moreover, the onset potential became more negative after electrochemical treatment of the ZrTe_2 material (Figure 8.18D). The onset potential of the starting ZrTe_2 material (-0.95 V) became more negative after both reductive and oxidative treatment (-0.99 V). Hence, it is resolved that the ZrTe_2 material became less active towards HER catalysis after electrochemical treatment.

Figure 8.18 The effect of electrochemical treatment on HER performance of ZrTe_2 . (A) Linear sweep voltammograms for HER in acidic electrolyte on ZrTe_2 , and ZrTe_2 after -1.6 V and $+1.1 \text{ V}$ treatment deposited on a GC electrode. Bar charts represent the averages and corresponding error bars of the (B) overpotential at -10 mA cm^{-2} current density, (C) Tafel slopes and (D) HER onset potential for bare GC and ZrTe_2 prior to and after electrochemical treatment.

Additionally, Figure 8.19B shows that the overpotential of HfTe_2 at -10 mA cm^{-2} increased from 1.11 V to 1.19 V and 1.39 V after reductive and oxidative treatment respectively. It is also observed in Figure 8.19C that the Tafel slope was gentler with both reductive (127 mV dec^{-1}) and oxidative treatment (159 mV dec^{-1}). Moreover, the onset potential became more negative after electrochemical treatment of the HfTe_2 material (Figure 8.19D). The onset potential of the starting HfTe_2 material (-0.96 V) became more negative after both reductive (-1.01 V) and oxidative treatment (-1.02 V). Overall, it is concluded that the HfTe_2 material became less active towards HER catalysis after electrochemical treatment.

Figure 8.19 The effect of electrochemical treatment on HER performance of HfTe_2 . (A) Linear sweep voltammograms for HER in acidic electrolyte on HfTe_2 , and HfTe_2 after -1.6 V and $+1.1 \text{ V}$ treatment deposited on a GC electrode. Bar charts represent the averages and corresponding error bars of the (B) overpotential at -10 mA cm^{-2} current density, (C) Tafel slopes and (D) HER onset potential for bare GC and HfTe_2 prior to and after electrochemical treatment.

Comparing the XPS results of the TiTe_2 (Figure 8.9A), ZrTe_2 (Figure 8.9B) and HfTe_2 (Figure 8.9C) materials before and after electrochemical treatment, similar changes in surface composition are observed upon electrochemical treatment. This is well matched with the similar alterations to their HER performances. The loss of metal ditelluride is evident upon both reductive and oxidative treatment, where metal and tellurium oxides remain. This resulted in the general decrease in catalytic activity towards HER. However, an exception is noted in the case of TiTe_2 with reductive treatment, where an improvement of HER performance is observed. A possible explanation is the relatively high concentrations of Te^0 compared to oxides contributing to the increased catalytic activity of HER.

Table 8.3 Observed trends of groups 4 (this work), 5 and 6 TMDs for electrocatalysis of hydrogen evolution.³⁶⁻³⁸

Group	Metal Effect		
	S Chalcogen	Se Chalcogen	Te Chalcogen
4	$\text{TiS}_2 < \text{ZrS}_2 < \text{HfS}_2$	$\text{TiSe}_2 \approx \text{ZrSe}_2 \approx \text{HfSe}_2$	$\text{TiTe}_2 \approx \text{ZrTe}_2 \approx \text{HfTe}_2$
5	$\text{VS}_2 \approx \text{NbS}_2 \approx \text{TaS}_2$	$\text{VSe}_2 \approx \text{NbSe}_2 \approx \text{TaSe}_2$	$\text{VTe}_2 < \text{NbTe}_2 < \text{TaTe}_2$
6	$\text{MoS}_2 < \text{WS}_2$	$\text{MoSe}_2 > \text{WSe}_2$	-
	Chalcogen Effect		
	Period 4 Metal	Period 5 Metal	Period 6 Metal
4	$\text{TiS}_2 < \text{TiSe}_2 > \text{TiTe}_2$	$\text{ZrS}_2 > \text{ZrSe}_2 > \text{ZrTe}_2$	$\text{HfS}_2 > \text{HfSe}_2 > \text{HfTe}_2$
5	$\text{VS}_2 > \text{VSe}_2 > \text{VTe}_2$	$\text{NbS}_2 \approx \text{NbSe}_2 \approx \text{NbTe}_2$	$\text{TaS}_2 \approx \text{TaSe}_2 \approx \text{TaTe}_2$
6	-	$\text{MoS}_2 < \text{MoSe}_2$	$\text{WS}_2 > \text{WSe}_2$

A comparison of the observed trends for group 4 TMDs (in this work), with previously reported trends of groups 5 and 6 TMDs, is established in Table 8.3. Few similarities are observed between group 4 and 6; HER activity increased down the groups for TMDs bearing the S chalcogen, and decreased down group 16 for hafnium dichalcogenides and tungsten dichalcogenides. This is in contrast to the

lack of similar trends matching to that for group 5 TMDs. A possible explanation could be that both group 4 and 6 TMDs are semiconducting while group 5 TMDs are true metals in their bulk forms.³⁹ On the other hand, the effect of electrochemical treatment differed greatly from the trends observed in the work of Chia et al. where group 6 TMDs mostly exhibited improved HER efficiencies with reductive treatment.³⁴ A decline in HER efficiencies was generally observed for group 4 metal dichalcogenides due their instability towards electrochemical treatment. Exceptions were noted where electrochemical activation towards HER catalysis was achieved with ZrSe₂ and HfSe₂ via both electrochemical oxidation and reduction, and TiTe₂ via electrochemical reduction. Exceptions noted with ZrSe₂ and TiTe₂ is likely due to Zr and Te being made available respectively for enhanced HER catalytic performance. HfSe₂ is the only group 4 TMD which was relatively stable under electrochemical treatment which served to remove possible impurities to improve the catalytic activity towards HER. Hence, even though some similarities are recognised for group 4 and 6 bulk TMDs due to their semiconductor properties, electrochemical treatment may not be as suitable for group 4 TMDs.

8.3 Conclusion

The inherent electrochemistry of layered group 4 transition metal dichalcogenide (TMD) materials has been explored, where distinct redox properties of Se and Te chalcogens are well perceived in the corresponding TMD materials. Notably, HfS_2 is electrochemically inert within a wide potential window of -1.8 V to +1.8 V and displayed superior HER activity compared to the other group 4 TMDs, making it a promising candidate for electrochemical sensing applications. Trends in HER activity down group 4 metals and group 16 chalcogens are observed; increased activity down group 4 metal disulphides, and decreased activity down group 16 zirconium dichalcogenides and hafnium dichalcogenides. Efforts to tune the catalytic properties of group 4 TMDs disclosed many perturbations by electrochemical oxidation or reduction. We highlighted the marked response of each group 4 TMD material to electrochemical treatment upon cross reference to the surface compositions of these materials. Out of the nine materials, electrochemical activation towards HER catalysis was exhibited for ZrSe_2 and HfSe_2 via both electrochemical oxidation and reduction, and TiTe_2 via electrochemical reduction. In other cases, the HER activities deteriorated with electrochemical treatment. In general, it was revealed that the metal dichalcogenides were mostly unstable upon electrochemical treatment, causing their decomposition and partial dissolution. This accounts for the decrease in HER catalytic activity in most cases. Therefore, even as we show that electrochemical treatment may not be suitable for tuning the HER catalytic activity for group 4 TMDs, with the exception of HfSe_2 , due to their

instability upon electrochemical treatment, the results point towards the importance of purity of the materials for high catalytic performance. This first insight into the electrochemical behaviour of group 4 TMDs and understanding of the impact of electrochemical treatment on these materials will be pivotal towards achieving desired goals in electrochemical applications.

8.4 Experimental

8.4.1 Materials

Sulphur (99.999%), selenium (99.999%) and tellurium (99.999%) were purchased from STREM, Germany. Titanium (-150 mesh, 99.9%), zirconium (sponge, 99.5%) and hafnium (-325 mesh, 99.5%) were obtained from Alfa Aesar, Germany. Potassium phosphate dibasic, sodium phosphate monobasic, sodium chloride, sulphuric acid were purchased from Sigma Aldrich. Glassy carbon (GC), the Ag/AgCl reference and Pt counter electrodes were obtained from Autolab (The Netherlands).

8.4.2 Apparatus

Scanning electron microscopy (SEM) images and energy-dispersive X-ray spectroscopy (EDS) were acquired using a Joel 7600F instrument (Joel, Japan) operating at 5 kV and 15 kV respectively. X-ray diffraction was done using a Bruker D8 diffractometer in Bragg–Brentano parafocusing geometry. A $\text{CuK}\alpha$ radiation was used. Diffraction patterns were measured between 10° and 80° of 2θ . The obtained data were evaluated using HighScore Plus 3.0e software. X-ray photoelectron spectroscopy (XPS) measurements were performed using a Phoibos 100 spectrometer and a monochromatic Mg X-ray radiation source (SPECS, Germany). Survey (wide-scan) and high resolution spectra were recorded for each sample. For the XPS measurements of the untreated samples, sample preparation was done by coating a uniform layer of the materials under study on a carbon conductive tape. On the other hand, electrochemically treated samples were obtained by coating four

consecutive 8.0 μL aliquots of 1 mg mL^{-1} concentration on a Zensor screen printed electrode (eDAQ, Australia) and subjected to their respective oxidation or reduction potential. After electrochemical treatment, the screen printed electrode was directly mounted on the XPS holder for characterisation. The relative sensitivity factors were also adopted for the calculation of chalcogen-to-metal ratios. All voltammetric experiments were carried out using a $\mu\text{Autolab}$ type III electrochemical analyser (Eco Chemie, The Netherlands) connected to a computer and controlled by the NOVA, Version 1.8.17, software. Electrochemical experiments were carried out at room temperature in an electrochemical cell (5 mL) with the employment of a three electrode configuration. A platinum electrode served as the auxiliary electrode, an Ag/AgCl electrode functioned as the reference electrode and a working electrode made of glassy carbon (GC, 3 mm diameter) was employed.

8.4.3 Procedures

Synthesis Procedure of TMDs. Stoichiometric amounts of metal and chalcogen forming 6 g of transition metal dichalcogenide were put in a quartz glass ampoule (18 mm x 100 mm; wall thickness 1.5 mm). Evacuation of the ampoule was carried out at base pressure below 5×10^{-3} Pa and melt sealed using oxygen/hydrogen welding torch. The ampoule was placed in muffle furnace and heated on 420 $^{\circ}\text{C}$ for 48 hours, 24 hours on 600 $^{\circ}\text{C}$ and 48 hours on 800 $^{\circ}\text{C}$. The heating and cooling rate was in all cases 5 $^{\circ}\text{C min}^{-1}$.

Inherent Electrochemistry of Group 4 TMDs. The TMD materials were dispersed in ultrapure water with a concentration of 1 mg mL^{-1} . Initially, the

suspensions were subjected to ultrasonication for 1.5 h to achieve homogeneous dispersions. Subsequently, the samples were ultrasonicated for 10 min durations prior to each electrochemical measurement in order to maintain the well-dispersed suspension of the material under study. The suspension (4 μL) was pipetted onto the GC electrode surface and left to evaporate at room temperature. A film of randomly distributed material is yield on the GC electrode surface. The materials of interest include TiS_2 , ZrS_2 , HfS_2 , TiSe_2 , ZrSe_2 , HfSe_2 , TiTe_2 , ZrTe_2 and HfTe_2 . Fundamental electrochemical studies were carried out in 50 mM, pH 7.2 phosphate buffered saline (PBS) as the background electrolyte. Deoxygenation was achieved by bubbling highly purified nitrogen through the experimental solutions for at least 20 min. Cyclic voltammetry (CV) experiments were performed at a scan rate of 100 mV s^{-1} . The CV scan was set to begin at 0 V where redox processes were not probable. Next, the cathodic study was performed by scanning towards -1.8 V followed by a reverse sweep of potential to +1.8 V. A reverse sequence was applied for the anodic study, before returning to 0 V.

Hydrogen Evolution Reaction (HER) Studies on Group 4 TMDs and the Effect of Electrochemical Treatment. HER efficiency of TMD materials were performed on glassy carbon (GC) electrodes. GC electrodes were first renewed on a polishing pad using alumina particle (0.05 μm) slurry and washed with ultrapure water. Subsequently, 1 mg mL^{-1} suspensions of TMD materials were prepared in ultrapure water and ultrasonicated for a period of 10 min prior to each electrochemical measurement to ensure homogeneity of the material under study. A 4 μL aliquot was deposited on the electrode surface and left to dry at room temperature to yield a randomly distributed film of 4.0 μg of desired material on the GC electrode surface. The materials of interest include TiS_2 , ZrS_2 , HfS_2 , TiSe_2 ,

ZrSe₂, HfSe₂, TiTe₂, ZrTe₂, HfTe₂, and their respective pretreated counterparts. HER measurements were performed in a 0.5 M H₂SO₄ electrolyte using linear sweep voltammetry (LSV) at a scan rate of 2 mV s⁻¹. Measurements were first performed relative to the Ag/AgCl reference electrode. Subsequently, the measurements were corrected and the LSVs were represented versus the reversible hydrogen electrode (RHE). The measured potentials were calculated based on the equation $E_{\text{RHE}} = E_{\text{Ag/AgCl}} + 0.059 \times \text{pH} + E^{\circ}_{\text{Ag/AgCl}}$, where $E_{\text{Ag/AgCl}}$ is the measured potential, and pH of H₂SO₄ (0.5 M) is set as zero. The standard potential of Ag/AgCl (1M KCl) at 25 °C, $E^{\circ}_{\text{Ag/AgCl}}$ is then 0.235 V vs RHE.

Electrochemical Treatment of Group 4 TMDs. In order to obtain the oxidised or reduced forms of TMDs, an oxidation or reduction potential is applied for a period of 300 s in 50 mM, pH 7.2 PBS. Specific to the TMD materials, electrochemical treatment was performed at different potentials based on the redox peaks observed earlier in the study of their inherent electrochemistry. TiS₂ was treated at +0.7 V (oxidation) or -1.5 V (reduction), ZrS₂ was treated at -1.1 V (reduction), TiSe₂ was treated at +1.5 V (oxidation) or -1.6 V (reduction), ZrSe₂ and HfSe₂ were treated at +1.5 V (oxidation) or -1.25 V (reduction), TiTe₂, ZrTe₂ and HfTe₂ were treated at +1.1 V (oxidation) or -1.6 V (reduction). Following the electrochemical treatment, the electrode was gently immersed in distilled water to remove any adsorbed materials before testing for HER efficiency.

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Part II

Chapter 9

Haemoglobin Electrochemical Detection on Various
Reduced Graphene Surfaces: Well-Defined Glassy
Carbon Outperforms the Graphenoids

9.1 *Introduction*

9.2 *Results and Discussion*

9.3 *Conclusion*

9.4 *Experimental*

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

1. **Rou Jun Toh**, Weng Kung Peng, Jongyoon Han, Martin Pumera. Haemoglobin Electrochemical Detection on Various Reduced Graphene Surfaces: Well-Defined Glassy Carbon Electrode Outperforms the Graphenoids. *RSC Adv.* **2014**, 4, 8050-8054.

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9.1 Introduction

Haemoglobin (Hb) functions physiologically in the storage and transport of molecular oxygen (O_2) in the blood of humans. The metalloprotein has a molecular weight of 64.5 kDa and comprises four polypeptide subunits (two α and two β subunits) of similar structure, each of which has an iron-bearing heme within molecularly accessible crevices.^{1,2} The iron ion may be in either the Fe^{II} or Fe^{III} oxidation state. In its Fe^{II} state, haemoglobin is able to bind oxygen to form oxyhaemoglobin (Oxy-Hb), but in its Fe^{III} state, methemoglobin (Met-Hb) is not able to bind oxygen. Fe^{II} is converted to Fe^{III} spontaneously in trace amounts (1–2 % of Hb), and if not mitigated by internal reduction mechanisms within the red blood cell, high levels of Met-Hb (methemoglobinemia) could result in fatality.³⁻⁵

The iron ion in haemoglobin acts as a redox-active center. The direct electron transfer reactions of proteins with redox-active centers may be used to probe the nature of many natural energy conversion processes, from photosynthesis to respiration, most of which are mediated by these protein molecules.^{6,7} Hence, the direct electrochemistry of haemoglobin may provide fundamental knowledge of its redox behaviour within the red blood cell. Accurate monitoring of such reactions is critical as chemical changes in the red blood cell usually take place due to primary hematologic disorders like anemia, or a wider variety of other diseases which produces secondary abnormalities of the blood.^{8,9} While the electrochemistry of heme within red blood cells may be complicated due to the presence of cellular membrane, electrochemical measurement of cell-free haemoglobin, resulting from red blood cell lysis, is also important since they are directly related to various pathophysiological conditions.^{10,11} However, the facilitation of electron transfer

between the heme center in the large three-dimensional structure of haemoglobin and bare solid electrodes poses a challenge. Poor electrochemical property is observed at bare glassy carbon electrodes as the heme groups are deeply concealed in the hydrophobic pockets of haemoglobin, and hence resulting in inaccessibility of the redox centers.¹²⁻¹⁴ In order to improve the electrochemical property of haemoglobin, its direct electron transfer has been investigated at a variety of modified electrodes.¹³⁻¹⁸

In recent years, much attention has been placed on carbon-based nanomaterials like graphenes due to their high surface area, outstanding electrical conductivity, high mechanical strength and structural flexibility.^{19,20} There have been many reported advantageous application of graphene for electrochemical sensing;^{21,22} however, there have been reports showing that graphene may not be that advantageous when compared to its parent i.e. graphite.²³⁻²⁶ Given the importance of haemoglobin detection, and previous reports claiming enhanced direct electron transfer characteristics with the utilization of carbon nanomaterials,^{13,14,27-32} we investigate whether graphene, and in what form may influence the electron transfer characteristics of the $\text{Fe}^{\text{III}}/\text{Fe}^{\text{II}}$ redox couple of haemoglobin in solution. For this purpose, graphite oxide (GO), chemically reduced graphene oxide (CRGO), graphene oxide (GO'), electrochemically reduced graphene oxide (ERGO) and edge plane pyrolytic graphite (EPPG) (all these materials were termed as graphenoids)³³ electrodes were used. Bare glassy carbon (GC) electrodes served as control experiments. We found that there were no major enhancements to electron transfer with use of the carbon nanomaterials.

9.2 Results and Discussion

Figure 9.1 (A) Cyclic voltammograms and (B) differential pulse voltammograms obtained in 50 mM phosphate buffer at pH 3.5 for the electrochemical reduction of human Hb (0.05 mg mL^{-1} , 0.78 μM) at GC electrodes modified with graphene materials. Scan rate, 0.01 V s^{-1} Insets: Peak heights of $\text{Fe}^{\text{III}}/\text{Fe}^{\text{II}}$ redox couple corresponding to different graphene modified electrode surfaces. Error bars represent RSD ($n=3$; 95% confidence interval).

Cyclic voltammetry (Figure 9.1A) and differential pulse voltammetry (Figure 9.1B) were used to investigate the electrochemistry of haemoglobin in solution. From Figure 9.1A, the reduction of haemoglobin started at about -0.05 V and showed a peak at -0.15 V . The reduction peak height on each of the modified electrodes were measured and represented in bar graphs (Figure 9.1 insets). With CV, EPPG electrodes gave a high peak current magnitude of 6.86 μA , and performed superior to the other carbon nanomaterials investigated. On the other hand, with DPV, the reduction of haemoglobin started at $+0.05 \text{ V}$ and peaked at -0.15 V . Bare GC electrodes gave the highest peak current magnitude of 722 nA . However, EPPG electrodes continued to show excellent performance with a peak current magnitude of 714 nA . Overall, it may be inferred that EPPG has the highest capacity to improve the electron transfer characteristics of the $\text{Fe}^{\text{III}}/\text{Fe}^{\text{II}}$ redox couple of haemoglobin, followed closely by bare GC electrodes. It is worthy to note

that no significant enhancement of electron transfer characteristics was observed with GO, CRGO, GO' and ERGO electrodes. Even more surprising is that GO, CRGO, GO' and ERGO electrodes observed lower peak current magnitudes than that with bare GC electrodes. Note that large reduction currents at potentials $< -0.5\text{V}$ are due to the inherent electrochemical reduction of GO and GO'.³⁴⁻³⁶

It would be interesting to investigate the superior performance of bare GC compared to GO, CRGO, GO' and ERGO electrodes. A possible reason for GO, CRGO, GO' and ERGO electrodes to see lower peak current magnitudes for the reduction of haemoglobin than that with bare GC electrodes could be that they possess different linear response ranges. In order to do so, it is crucial to understand the how the peak current is dependent on the concentration of haemoglobin. For this purpose, cyclic voltammetry and differential pulse voltammetry were performed on bare GC and CRGO electrodes.

For bare GC electrodes, both CV (Figure 9.2A) and DPV (Figure 9.2B) gave agreeable results. The reduction of haemoglobin was observed to peak at -0.15 V . It is observed that the higher the haemoglobin concentration, the higher the peak current magnitude. On the CRGO electrode, the reduction of haemoglobin was observed to peak at about -0.20 V using CV (Figure 9.3A), and -0.15 V using DPV (Figure 9.3B). Similarly, the higher the concentration of haemoglobin, the higher the magnitude of peak current observed.

Figure 9.2 (A) Cyclic voltammograms and (B) differential pulse voltammograms obtained in aqueous 50 mM phosphate buffer at pH 3.5 for the electrochemical reduction of human haemoglobin of different concentrations at GC electrodes. Scan rate, 0.01 V s⁻¹

Figure 9.3 (A) Cyclic voltammograms and (B) differential pulse voltammograms obtained in 50 mM phosphate buffer at pH 3.5 for the electrochemical reduction of human haemoglobin of different concentrations at GC electrodes modified with CRGO. Scan rate, 0.1 V s⁻¹.

The calibration plots of bare GC electrodes were then compared to that of CRGO electrodes (Figure 9.4). Figure 9.4A illustrates the calibration plots derived from CV measurements, whereas Figure 9.4B demonstrates the calibration plots resulting from DPV measurements. The linear response range on bare GC electrodes may be estimated to be from 0.0 to 1.55 μM concentrations of haemoglobin. Saturation of concentration dependence is evident with haemoglobin concentrations higher than 1.55 μM. On the other hand, the

haemoglobin concentration range over which peak current is proportional to concentration may be observed to be from 0.0 to 1.55 μM for CRGO electrodes. Saturation of concentration dependence is evident with haemoglobin concentrations higher than 1.55 μM . Hence, the linear response ranges on both bare GC and CRGO electrodes are the same.

From CV measurements (Figure 9.4A), at 1.55 μM concentration of haemoglobin, peak current of -6.96 μA corresponding to the $\text{Fe}^{\text{III}}/\text{Fe}^{\text{II}}$ redox couple was observed on bare GC electrodes. This is approximately 1.4 fold more than that on CRGO electrodes which gave a peak current of -4.83 μA . Similarly, a peak current of 878 nA corresponding to the $\text{Fe}^{\text{III}}/\text{Fe}^{\text{II}}$ redox couple was observed on bare GC electrodes from DPV measurements (Figure 9.4B). This is 1.3 fold more than that on CRGO electrodes which gave a peak current of 646 nA. Therefore, we may conclude that bare GC measurements gave higher sensitivity, demonstrating again its superior performance compared to GO, CRGO, GO' and ERGO electrodes.

Figure 9.4 Reduction peak height of $\text{Fe}^{\text{III}}/\text{Fe}^{\text{II}}$ redox couple of cyclic voltammograms (A) and differential pulse voltammograms (B) at different concentrations of human Hb at GC electrodes and GC electrodes modified with 1 mg mL^{-1} CRGO. The calibration curve observed concentration saturation starting at 1.55 μM . Error bars represent RSD ($n=3$; 95% confidence interval).

9.3 Conclusion

We have demonstrated that bare glassy carbon remains as an appropriate electrode material for further probing into the electrochemical sensing of haemoglobin in solution. Surprisingly, most of the carbon nanomaterials investigated, including GO, CRGO, GO' and ERGO, did not display improvements to the electron transfer characteristics of the $\text{Fe}^{\text{III}}/\text{Fe}^{\text{II}}$ redox couple of haemoglobin in solution. These findings are in contrast to previous studies, which report an enhancement in direct electron transfer characteristics with the utilization of carbon nanomaterials. However, most of the previous studies relate to electrochemical sensing of haemoglobin immobilised on the electrode surface.^{13,14,27,29-32} Our findings are of high importance for the further investigation of the electron transfer characteristics of the $\text{Fe}^{\text{III}}/\text{Fe}^{\text{II}}$ redox couple of haemoglobin, and the differences between solution-based and solid-state electrochemical sensing of the protein.

9.4 Experimental

9.4.1 Materials

N,N-dimethylformamide (DMF) was purchased from Merck. Potassium phosphate dibasic (K_2HPO_4), phosphoric acid (H_3PO_4 , ≥ 85 wt% in H_2O), haemoglobin human (lyophilised powder) were obtained from Sigma-Aldrich. Glassy carbon (GC), edge plane pyrolytic graphite (EPPG), Ag/AgCl reference, and Pt counter electrodes were obtained from Autolab (Japan).

9.4.2 Apparatus

All voltammetric experiments were carried out by using a μ Autolab type III electrochemical analyser (Eco Chemie, The Netherlands) connected to a computer and controlled by General Purpose Electrochemical Systems, Version 4.9 software (GPES). Electrochemical experiments were carried out at room temperature in an electrochemical cell (5 mL) with the employment of a three electrode configuration. A platinum electrode functioned as the counter electrode, and an Ag/AgCl electrode served as the reference electrode. All electrochemical potentials in this chapter are indicated as versus the Ag/AgCl reference electrode.

9.4.3 Procedures

Graphite Oxide Preparation with the Staudenmaier Method. Graphite oxide was prepared using graphite according to the Staudenmaier³⁷ method whereby 17.5 mL of H_2SO_4 (95–98 %) and 9 mL of HNO_3 (fuming) were stirred in a round bottom flask at 0 °C for 15 mins. 1 g of graphite was then added to the mixture under vigorous stirring to obtain a homogeneous dispersion. 11 g of $KClO_3$

was added into the mixture over 15 mins in order to avoid temperature spike and formation of ClO_2 gas, which is explosive at high concentrations. The mixture was left to stir vigorously for 96 hrs at room temperature. On completion, the mixture was then poured into 1 L of deionised water and filtered. Graphite oxide was then redispersed and washed repeatedly with 5 % HCl solutions to remove sulphate ions. Graphite oxide was finally washed with deionised water under centrifugation until the filtrate was observed to be of neutral pH. Slurry graphite oxide was then dried in a vacuum oven at 60 °C for 48 hrs before use.

Graphene Oxide Preparation from Graphite Oxide. Graphite oxide was placed in an ultrasonic bath (150 W) for 2h to give a uniform dispersion of graphene oxide.

CRGO Preparation from Graphite Oxide. Graphite oxide (50 mg) was dispersed in ultrapure water to give a 1.0 mg/mL colloidal solution and ultrasonicated (150 W) for 3 h. Hydrazine monohydrate (2 mL, 32.1 mmol) was added dropwise and the reaction mixture was left to stir under reflux for 24 h. The mixture was cooled to room temperature and filtered/washed over a PTFE membrane (0.2 μm) with methanol and ultrapure water to give CRGO upon drying at 50 °C for 5 days.

ERGO Preparation from Graphite Oxide. Electrochemically reduced graphene was obtained by applying a potential of -1.2 V (vs Ag/AgCl) for 900 s to a graphene-oxide modified GC electrode in a phosphate buffer solution (pH 3.5).³⁸

Electrochemical Measurements. Cyclic voltammetry experiments were performed at a scan rate of 100 mV s⁻¹ in phosphate buffer solution (50 mM), and

haemoglobin human with phosphate buffer solution as the supporting electrolyte. The reaction system was allowed to equilibrate for 5 s before differential pulse voltammetry measurements were performed under the same experimental conditions. Deoxygenation was achieved by the bubbling highly purified nitrogen through the experimental solutions for at least 20 min.

Preparation of Electrode Surfaces. Glassy carbon electrodes were first renewed on a polishing pad using alumina particle ($0.05\ \mu\text{m}$) slurry and washed with ultrapure water. The graphene materials ($1.0\ \text{mg mL}^{-1}$) were dispersed in DMF. Subsequently, the suspensions were subjected to an ultrasound bath treatment ($37\ \text{kHz}$, $3\ \text{W/cm}^2$) for 60 s. Next, the suspension ($1\ \mu\text{L}$) was pipetted onto the GC electrode surface, and left to evaporate at room temperature. We optimised the loading concentration of graphene from $0.1\ \text{mg/mL}$ to $2\ \text{mg mL}^{-1}$. We have performed electrochemical characterization of the modified electrodes (CRGO, GO, GO', ERGO) with ferry/ferrocyanide redox probe. We calculated the ΔE for four concentrations of graphene materials (0.1 , 0.5 , 1.0 , $2.0\ \text{mg mL}^{-1}$). ΔE is a representation of the heterogeneous electron transfer (HET) rate. The lower the ΔE value, the higher the HET rate. We found that the HET increases when graphene loading increases from $0.1\ \text{mg mL}^{-1}$ to $0.5\ \text{mg mL}^{-1}$ and after that it levels off in the range 0.5 - $2\ \text{mg mL}^{-1}$. We thus chose $1\ \text{mg mL}^{-1}$ as robust value for graphene loading.

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Part II

Chapter 10

Direct Electrochemical Detection of Haemoglobin in
Red Blood Cells

10.1 *Introduction*

10.2 *Results and Discussion*

10.3 *Conclusion*

10.4 *Experimental*

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

1. **Rou Jun Toh**, Weng Kung Peng, Jongyoon Han, Martin Pumera. Direct In Vivo Electrochemical Detection of Haemoglobin in Red Blood Cells. *Sci. Rep.* **2014**, 28, 4:6209.

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10.1 Introduction

As discussed in **Chapter 9**, activity measurement of proteins such as haemoglobin may be crucial to investigate the nature of many *in vivo* energy conversion processes, from photosynthesis to respiration, most of which are facilitated by these protein molecules.¹⁻⁴ In particular, the direct electrochemical detection of metalloproteins has been applied to bioanalysis.^{5,6} The iron ion in haemoglobin serves as a redox-active center. Hence, the direct electron transfer reactions of haemoglobin may provide fundamental understanding of its redox behaviour within the red blood cell. It is important to monitor accurately such reactions because abnormal shift in chemical activity in the red blood cell usually occurs due to primary hematologic disorders like anemia, or a wider variety of other diseases which produces secondary abnormalities of the blood.^{7,8} Moreover, it is essential to determine the concentration of haemoglobin in blood samples as an indication of the red blood cell count in blood and hence, the oxygen transport ability of blood.

As an extension to the work in **Chapter 9**, our goal in this chapter is to probe into the electrochemical behaviour of the iron ion of haemoglobin contained in red blood cells (RBCs). Previous studies have shown that direct electron transfer to cell-free haemoglobin redox center is possible.⁹⁻¹² However, little is studied about the voltammetric responses obtained directly from RBCs as the electrochemistry of haemoglobin within RBCs may be complicated due to the presence of the cellular membrane and complex biological environment. Herein, we would like to address the following basic questions: 1) is the electrochemical activity of iron ion in haemoglobin observed with the chemical systems of RBCs and blood; and 2) is the

electrochemical activity of iron ion in haemoglobin interfered by the biological complexity of RBCs. If so, what is happening at the iron centre of haemoglobin in these systems? The commonly used glassy carbon electrode was established as an appropriate surface for the investigation of such electrochemical behaviour directly from RBCs and blood.

10.2 Results and Discussion

Experimental conditions were first optimised by investigating the reduction of haemoglobin in solution at different pH values on bare GC electrodes using cyclic voltammetry (CV). A marked effect of pH on the voltammetric response for haemoglobin may be observed in Figure 10.1A. The reduction of haemoglobin shows a peak at approximately -0.3 V and -0.15 V at pH 5.5 and pH 3.5 respectively. A decrease in pH shows a shift in reduction peak potential to less negative values. Accompanying this observation, a decrease from pH 5.5 to pH 3.5 saw an almost eleven-fold increase in the charge under the voltammetric response (Figure 10.1 inset). The observed pH dependence of the redox process is in agreement with previously reported works,^{12,13} suggesting the involvement of H⁺. Similarly, a study on pH dependence was carried out for blood at GC-Nf-B-3Nf electrodes using cyclic voltammetry (Figure 10.1B). A significant effect of pH on the voltammetric response for blood was noted. The reduction of blood shows a peak at about -0.3 V at both pH 3.5 and pH 7.4. A decrease from pH 7.4 to pH 3.5 saw an almost four-fold increase in the charge under the voltammetric response (Figure 10.1B inset). Hence, an acidic pH of 3.5 was chosen as an experimental condition for subsequent measurements in order to work with more defined voltammetric signature of haemoglobin.

Figure 10.1 (A) Cyclic voltammograms obtained in aqueous 50 mM phosphate buffer at pH 5.5 and 3.5 for the electrochemical reduction of human Hb (0.05 mg mL^{-1} , $0.78 \text{ }\mu\text{M}$) at GC electrodes. Reduction peak potential of haemoglobin decreases with an increase in pH. Scan rate, 0.1 Vs^{-1} . Inset: Reduction peak height of cyclic voltammograms at different pH values. Error bars represent RSD ($n=3$; 95% confidence interval). (B) Cyclic voltammograms obtained in aqueous 50 mM phosphate buffer for the electrochemical reduction of blood at pH 3.5 and pH 7.4. Blood concentration was chosen to be $1 \times 10^3 \text{ cells }\mu\text{L}^{-1}$. Reduction peak current magnitudes decreases with an increase in pH. Scan rate, 0.1 V s^{-1} . Inset: Reduction peak height of cyclic voltammograms at different pH values. Error bars represent RSD ($n=3$; 95% confidence level)

A step forward from the investigation of electrochemistry of cell-free haemoglobin would be the measurement of such directly from intact red blood cells (RBCs) and blood. Such a measurement could provide better insights on actual electrochemical activity of iron ions in red blood cells *in vivo*. However, to eliminate the complications of biological environments, we began by studying these systems from a chemical point of view by obtaining the lyophilised forms of red blood cells and blood, and re-suspending them in phosphate buffers accordingly.

We investigated a solid state approach by sandwiching the red blood cell or blood layer between layers of Nafion that were deposited on the surface of a GC electrode to give GC-Nf-RBC-3Nf and GC-Nf-B-3Nf electrode surfaces respectively,

as illustrated in Figure 10.2. Nafion contains a highly acidic sulfonate group, and is employed as a cation-exchange polymer. Electrodes coated with Nafion (and other surface confined molecules) have commonly been used to trap or concentrate solution phase species and thus improve their analytical detection.¹⁴⁻¹⁶ In this work, the Nafion layers are thought to have two functions; (1) stabilizing matrix for RBCs on the GC electrode, and (2) to provide a readily exchangeable source of H^+ to molecules within the membranes to balance the charge as a result of electrochemical reductions.

Figure 10.2 Schematic diagram of the proposed structures of GC-Nf-RBC-3Nf and GC-Nf-B-3Nf electrode surfaces.

To determine if it is possible to probe iron redox activity of haemoglobin directly from RBCs (lyophilised) and blood (lyophilised), cyclic voltammetry was used to study the reduction of red blood cells and blood on GC-Nf-RBC-3Nf and GC-Nf-B-3Nf electrodes, respectively. Figure 10.3A compares the reduction of red blood cells to that of haemoglobin using the solid state approach. The reduction of haemoglobin started at -0.05 V and showed a peak at -0.30 V. The same is observed for the reduction of red blood cells at concentrations 0.145 mg/mL RBCs and below. However, higher concentrations of red blood cells see a reduction starting at -0.05

V and showing a peak at -0.15 V. The reduction peak potential is shifted to less negative values. It is interesting to note that the reduction peak potential for higher concentrations of red blood cells correspond to that of reduction of haemoglobin in solution (-0.15 V). Similarly, Figure 10.3B compares the reduction of blood to that of haemoglobin. A reduction peak potential of -0.30 V was observed for blood concentrations of 1×10^3 cells/ μL and below. At higher concentrations of blood, shifts of reduction peak potentials to less negative values were observed. Hence, it may be concluded that reduction peaks corresponding to haemoglobin were obtained directly from the (lyophilised) red blood cells and blood, despite the possible complications due to the presence of the cellular membrane and complex biological environments. Calibration to quantify the signal magnitude against different concentrations of RBCs was conducted in **Chapter 9**.

Figure 10.3 (A) Cyclic voltammograms obtained in aqueous 50 mM phosphate buffer at pH 3.5 for the electrochemical reduction of red blood cells (powder) at GC-Nf-RBC-3Nf electrodes. Scan rate, 0.1 Vs^{-1} . Control experiments were performed using Hb and pH 3.5 PBS. (B) Cyclic voltammograms (from $+0.5$ V to -1.0 V) were obtained in aqueous 50 mM phosphate buffer at pH 3.5 for the electrochemical reduction of blood at GC-Nf-B-3Nf electrodes. Legend shows concentrations of blood (Cells ($\times 10^3$)/ μL). Scan rate, 0.1 Vs^{-1} . Control experiments were performed using Hb and pH 3.5 PBS.

Having established that the reduction signals from red blood cells (lyophilised) and blood (lyophilised) correspond to that of reduction of haemoglobin, it is equally important to determine if the source of these signals come: 1) directly from red blood cells or 2) free haemoglobin released into the supernatant due to lysis of the red blood cells. For this purpose, a lysis assessment was conducted. Scanning electron microscopy (SEM) images of red blood cells in powder form are shown in Figure 10.4. The red blood cells exhibited mostly biconcave structures with diameters of about 3 μm . Crystals are observed on the membranes of the red blood cells. These crystals may be derived from the preparation process.

Figure 10.4 Scanning electron microscopy (SEM) of red blood cells (lyophilised) at 5.0 kV. Red blood cells have diameters of about 3 μm .

Cyclic voltammetry was used to investigate the reduction of red blood cells (lyophilised) before and after filtration (Figure 10.5). The filtration process would aid in removing any free haemoglobin released into the supernatant due to lysis of the red blood cells. It may be observed that the peak current magnitude dropped about four-fold from about 13 μA before filtration to approximately 3 μA from the filtrate or supernatant of the red blood cell suspension. The residue red blood cells

obtained after filtration were re-suspended in pH 3.5 phosphate buffer. The peak current magnitude observed was similar to that of the red blood cell suspension before filtration. Hence, the reduction signals observed may be derived directly from the red blood cells. To further support such a claim, optical microscopy of the red blood cells was performed before and after filtration (Figure 10.6). The images obtained were similar before and after filtration, showing no substantial lysis of the red blood cells.

Figure 10.5 Cyclic voltammograms were obtained in aqueous 50 mM phosphate buffer at pH 3.5 for the electrochemical reduction of red blood cells before filtration and after filtration; filtrate and re-suspended residue at GC-Nf-RBC-3Nf electrodes. Red blood cell concentration = 72.5 mg mL^{-1} ($2500 \times 10^3 \text{ cells}/\mu\text{L}$). Scan rate is 0.1 V s^{-1} .

Figure 10.6 Optical microscopy of red blood cells before and after filtration.

Moreover, it is crucial to understand the concentration dependence of the voltammetric signals. Cyclic voltammetry (CV) was used to study the reduction of red blood cells (Figure 10.7A) and blood (Figure 10.7B) on GC-Nf-RBC-3Nf and GC-Nf-B-3Nf electrodes respectively and the insets illustrate the calibration plot obtained correspondingly. Calibration of the signal response against red blood cell concentration may act as a crucial clinical diagnosis of the oxygen transport ability of a blood sample. From Figure 10.7A, the red blood cell concentration range over which peak current is proportional to concentration ranges from 0 to 1.45 mg/mL. Saturation of concentration dependence is observed with red blood cell concentrations higher than 1.45 mg/mL (50×10^3 cells/ μ L). Figure 10.7B shows that the blood concentration range over which the peak current is proportional to concentration may be estimated to be from 0 to 5×10^3 cells/ μ L. At blood concentrations higher than 5×10^3 cells/ μ L, peak current magnitude decreases with an increase in blood concentration. Red blood cells in blood samples were quantified by taking reference to a standard average RBC count of 5000×10^3 cells per microliter of blood in a healthy individual.¹⁷ The linear response ranges obtained in both cases are very narrow. This would imply the need for high dilutions during sample preparation. However, such an observation would mean that the volume of blood sample required may be minimised. This is a critical advantage in clinical studies where volume and frequency of blood withdrawal are of concern.

Figure 10.7 (A) Cyclic voltammograms obtained in aqueous 50 mM phosphate buffer at pH 3.5 for the electrochemical reduction of red blood cells at GC-Nf-RBC-3Nf electrodes. Legend shows concentrations of RBC (mg mL⁻¹). Scan rate, 0.1 V s⁻¹. Inset: Reduction peak height corresponding to different concentrations of RBC. The calibration curve observed concentration saturation starting at 1.45 mg mL⁻¹ (50×10³ cells μL⁻¹). Error bars represent RSD ($n=3$; 95% confidence interval). (B) Cyclic voltammograms obtained in aqueous 50 mM phosphate buffer at pH 3.5 for the electrochemical reduction of blood at GC-Nf-B-3Nf electrodes. Legend shows concentrations of blood (Cells (x10³)/μL). Scan rate, 0.1 Vs⁻¹. Inset: Reduction peak height corresponding to different concentrations of blood. The calibration curve observed concentration saturation starting at 5×10³ cells/μL. Error bars represent RSD ($n=3$; 95% confidence interval).

Our experiments hitherto investigated the chemical systems of red blood cells and blood. However, these systems do not completely replicate that of real biological systems as they do not have regulation and feedback mechanisms. In biological systems, most parameters are required to stay within a narrow range around a certain optimal level under certain environmental conditions. The maintenance of this optimal level for a parameter is known as homeostasis. One of the challenges faced would be the uncertainty in behaviour of voltammetric responses from real blood samples due to homeostatic processes.

In order to study the voltammetric responses from real biological systems, we employed human red blood cells (live) derived directly from real blood samples. Similar to experiments carried out previously, a solid state approach was taken by

sandwiching the red blood cell layer between layers of Nafion that were deposited on the surface of a GC electrode (GC-Nf-RBC-3Nf) as illustrated in Figure 10.2. Cyclic voltammetry (CV) was used to study the reduction of red blood cells (live) obtained directly from real blood samples on GC-Nf-RBC-3Nf electrodes (Figure 10.8). Comparison was made between the voltammetric responses derived from the electrochemical reduction of red blood cells (live) and red blood cells (lyophilised). The reduction of red blood cells in both cases starts at +0.05 V and shows a peak at -0.15 V. Therefore, the similar reduction potentials and shape of cyclic voltammograms observed points to the possibility of probing haemoglobin activity from a real biological system without much interference.

Figure 10.8 Cyclic voltammograms obtained in aqueous 50 mM phosphate buffer at pH 3.5 for the electrochemical reduction of red blood cells (live) at GC-Nf-RBC-3Nf electrodes. Comparison was made between red blood cells purchased from Sigma Aldrich in powder form (RBCs suspended in pH 3.5 PBS, RBC concentration of 1.45 mg mL^{-1} , $50 \times 10^3 \text{ cells } \mu\text{L}^{-1}$) and red blood cells derived directly from blood samples (RBCs suspended in pH 7.4, 1x PBS, RBC concentration of approximately 10% vol.). Scan rate, 0.1 V s^{-1} .

The reproducibility of the proposed biosensor for haemoglobin in red blood cells (live) was investigated at 50 mM phosphate buffer solution. The relative standard deviation was 4.3 % ($n=3$) for three successive measurements

demonstrated that the electrode-to-electrode reproducibility is acceptable in the response of three biosensors prepared under the same conditions independently. Hence, it may be established that the analyte is stable during electrode modification and electrochemical measurements.

10.3 Conclusion

In conclusion, a simple protocol was developed for the immobilization of RBCs or blood on a glassy carbon electrode using Nafion. On the modified electrode, direct detection of haemoglobin from RBCs and blood samples were realised at pH 3.5 PBS, which may allow for quantitative analysis of red blood cells in blood samples for clinical diagnosis. Stability of the analyte under the proposed experimental conditions was demonstrated by the high reproducibility of the experiments. Moreover, it is important to consider what is happening at the iron centre of haemoglobin during measurements. For this purpose, we consider the experimental conditions employed. The above experiments were performed under aerobic conditions. HbFe^{II} reacts rapidly with oxygen to give $\text{HbFe}^{\text{II}}\text{-O}_2$.¹⁸ With comparison to previous research, it is most probable that the reduction peak from haemoglobin in the range of -0.15 V to -0.3 V corresponds to the electrochemical reduction of the $\text{HbFe}^{\text{II}}\text{-O}_2$ complex to give HbFe^{II} and hydrogen peroxide.^{19,20} There leaves much to be studied about the mechanism involved but we believe that such a biosensor shows great promise for the study of the physiological status of RBCs in real blood samples for scientific and clinical purposes.

10.4 Experimental

10.4.1 Materials

N,N-dimethylformamide (DMF) was purchased from Merck. Potassium phosphate dibasic (K_2HPO_4), phosphoric acid (H_3PO_4 , ≥ 85 wt% in H_2O), Nafion 117 solution (~ 5 wt% in a mixture of lower aliphatic alcohols and water), haemoglobin human (lyophilised powder), red blood cells from sheep (dry powder, glutaraldehyde treated) were obtained from Sigma-Aldrich. Lyophilised human whole blood was obtained from the Institute for Reference Materials and Measurements and reconstituted as described in the instructions for use. Human red blood cells (live) were obtained from i-DNA Biotechnology Pte Ltd. Glassy-carbon (GC), Ag/AgCl reference, and Pt counter electrodes were obtained from Autolab (Japan).

10.4.2 Apparatus

All voltammetric experiments were carried out by using a μ Autolab type III electrochemical analyser (Eco Chemie, The Netherlands) connected to a computer and controlled by General Purpose Electrochemical Systems, Version 4.9 software (GPES). Electrochemical experiments were carried out at room temperature in an electrochemical cell (5 mL) with the employment of a three electrode configuration. A platinum electrode functioned as the counter electrode, and an Ag/AgCl electrode served as the reference electrode. All electrochemical potentials in this chapter are indicated as versus the Ag/AgCl reference electrode.

10.4.3 Procedures

Preparation of Electrode Surface. Glassy carbon electrodes were first renewed on a polishing pad using alumina particle (0.05 μm) slurry and washed with ultrapure water. A 1 μL droplet of Nafion solution was placed on the electrode surface with a micropipette. The solvent was then allowed to dry in air for 5 min. Subsequently, a 1 μL aliquot of the analyte solution was deposited on top of the Nafion layer. 3 μL droplets of the Nafion solution were then deposited on top of the analyte layer in three separate 1 μL deposition steps. The solvent was allowed to evaporate for 5 min after each addition. The optimal coating procedure described above was determined by a number of trial and error experiments, and further supported by a previous research²¹. The resulting modified electrode was abbreviated as GC-Nf-RBC-3Nf or GC-Nf-B-3Nf with red blood cells or blood as the analyte respectively.

Cyclic Voltammetry. Experiments were performed at a scan rate of 100 mV s^{-1} in phosphate buffer solution (pH 3.5, 50 mM) as supporting electrolyte.

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Part II

Chapter 11

MoSe₂ Nanolabels for Electrochemical Immunoassays

11.1 *Introduction*

11.2 *Results and Discussion*

11.3 *Conclusion*

11.4 *Experimental*

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

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11.1 Introduction

When exfoliated to single layer, transition metal dichalcogenides (TMDs) demonstrate high surface-to-volume ratios, layered structures that can accommodate ionic or organic compounds, electronic properties ranging from insulating to metallic, versatility in functionalization, and advantageous optical properties in the range of visible wavelength.¹⁻⁵ These properties allow them to be useful for advanced energy storage and conversion⁶, electrochemical catalysis^{7,8} and in particular, sensing applications.^{9,10}

Selective and sensitive detection of biomolecules is one of the critical foundations of biomedical research. In order to achieve sensitive detection, a signal enhancing element is typically employed to label target molecules. Where the catalytic detection mode is employed, a characteristic of the signal enhancing element would be that it possesses catalytic activity towards a reaction to be probed. Specifically, the hydrogen evolution reaction (HER) has been probed for such a purpose^{11,12} where gold nanoparticles (NPs)^{13,14} are commonly used as labels due to their long-term stability. With a long shelf life and high catalytic activity towards the HER,¹⁵⁻¹⁷ TMDs present as promising low costs and efficient alternative labels for biomolecular detection.

A solution-based electrochemical exfoliation method (Figure 11.1A) has previously been developed for the fabrication of WS₂ nanoparticles,¹² and herein, we investigate if such a technique can be extensively used with other TMD materials for producing nanolabels for magneto-immunoassays. For this purpose, MoSe₂ was chosen as the material of interest. MoSe₂ has been demonstrated to be most

catalytically active for the HER amongst the highly studied group 6 *t*-BuLi exfoliated TMD materials.^{16,18–21}

Synthesised MoSe₂ NPs were tagged to rabbit IgG proteins and captured by anti rabbit IgG attached to magnetic beads (Figure 11.1B). Consequently, the advantageous use of MoSe₂ nanolabels for magneto-immunoassays was demonstrated via HER catalysis. Furthermore, we showed the viability and reproducibility of the solution-based exfoliation technique for producing TMD nanoparticles as labels for biomolecular detection which can be applied in the mass production and development of low-cost immunosensors.

Figure 11.1 (A) Solution-based electrochemical exfoliation with bipolar electrodes of MoSe₂ (*t*-BuLi) sheets to MoSe₂ NPs. (B) Preparation of magneto-immunoassay for detection of rabbit IgG using MoSe₂ nanolabels via HER catalysis: (i) Incubation of magnetic bead (MB) with anti rabbit IgG. (ii) Incubation of desired concentration of rabbit IgG with MoSe₂ NPs. (iii) Final conjugation of anti rabbit IgG with labelled rabbit IgG and electrochemical detection through HER.

11.2 Results and Discussion

Herein, we investigate the interactions between MoSe₂ NPs (synthesised by solution-based electrochemical exfoliation method with bipolar electrodes) and rabbit IgG for the quantitative determination of the protein. Characterization of the prepared MoSe₂ NPs was first performed using X-ray photoelectron spectroscopy (XPS), ultraviolet visible (UV-Vis) absorption spectroscopy, scanning transmission electron microscopy (STEM), Raman spectroscopy, transmission electron microscopy (TEM) and electron dispersive X-ray spectroscopy (EDS). The electrocatalytic activity of MoSe₂ NPs towards hydrogen evolution reaction (HER) was probed via linear sweep voltammetry (LSV) and chronoamperometry. Finally, STEM and ion-coupled plasma mass spectrometry (ICP-MS) analysis verified the attachment of MoSe₂ NPs as labels for magneto-immunoassays. Herein, we wish to address the following questions: i) are MoSe₂ NPs obtained by solution-based electrochemical exfoliation method with bipolar electrodes, and ii) is a selective and sensitive immunoassay achieved with MoSe₂ NPs.

The high availability, low cost and promising catalytic activity of transition metal dichalcogenides make them promising signal enhancing elements for biomolecular detection. Considering the highly active MoSe₂ for HER catalysis, we first synthesised the microparticles (MPs) by exfoliating the bulk 2H-MoSe₂ to single or few sheets material with lateral dimensions in the micrometer range and a dominant 1T phase crystalline structure¹⁶; denoted as MoSe₂ (*t*-BuLi). In order to exfoliate the MoSe₂ (*t*-BuLi) sheets to MoSe₂ NPs, a solution-based electrochemical exfoliation method as reported previously with WS₂ was employed, where a constant DC potential of 10 V was applied across two Pt driving electrodes.¹² The main

advantage of this technique is that the size reduction of MoSe₂ (*t*-BuLi) was carried out in solution, where direct contact with the Pt driving electrodes is not required. Hence, the starting material is a powder and the modification of electrodes is not necessary, making this exfoliation method a simple and low cost one.

XPS was performed to examine the surface elemental compositions and bonding information, and hence the chemical changes associated with the electrochemical exfoliation of MoSe₂ (*t*-BuLi) sheets. The high resolution XPS spectra of MoSe₂ (*t*-BuLi) and MoSe₂ NPs were compared in Figure 11.2A. Deconvolution analysis of the Mo bonding modes²²⁻²⁴ reveals the presence of 1T-MoSe₂ and partial oxidation of the nanoparticles. This may be explained by the high positive potential applied, causing partial oxidation of the MoSe₂ NPs. Figure 11.2B shows the UV-Vis spectra of MoSe₂ (*t*-BuLi) and the resulting MoSe₂ NPs. The MoSe₂ (*t*-BuLi) sheets absorb in the entire range of visible light.^{25,26} On the other hand, MoSe₂ NPs exhibit a single absorption band corresponding to excitonic features in the UV region at $\lambda \approx 200$ nm.²⁷ In addition, Raman spectrum (Figure 11.3) of MoSe₂ (*t*-BuLi) shows the two peaks characteristic of layered MoSe₂; 238.8 cm⁻¹ and 282.8 cm⁻¹ correspond to the out-of-plane mode of A_{1g} and in-plane mode of E_{2g}¹ respectively. In contrast, no characteristic peaks were observed from the Raman spectrum of the MoSe₂ NPs. This is in agreement with previously reported observations of decreased Raman intensity with reduced particle sizes.²⁷ In order to further verify the formation and crystal phase of the MoSe₂ NPs, STEM and TEM are employed. From the STEM image the MoSe₂ NPs (Figure 11.2C), it was illustrated that the particle sizes are below 100 nm. Moreover, TEM characterisation (Figure 11.4A) of the exfoliated MoSe₂ NPs shows the platelet particle morphology typical for layered compounds with particle sizes below 100 nm. The high-resolution TEM

images (Figure 11.4B and 11.4C) show atomic arrangement characteristic for 1T phase.¹⁶ The composition of the MoSe₂ NPs were also confirmed with elemental mapping via EDS (Figure 11.5).

Figure 11.2 (A) High-resolution XPS spectra of Mo 3d regions of MoSe₂ (*t*-BuLi) and MoSe₂ NPs. Deconvoluted peaks correspond to the metallic 1T phase, semiconducting 2H phase, oxidised (IV) state, or oxidised (VI) state. (B) UV-Vis absorption spectra of MoSe₂ (*t*-BuLi) and MoSe₂ NPs. (C) STEM image of MoSe₂ NPs.

Figure 11.3 Raman spectra of MoSe₂ (*t*-BuLi) and MoSe₂ NPs.

Figure 11.4 (A) TEM and (B-C) high-resolution TEM images of MoSe₂ NPs.

Figure 11.5 Elemental mapping of MoSe₂ NPs via electron dispersive X-ray spectroscopy (EDS).

We evaluated the HER catalytic efficiency of MoSe₂ NPs against the MoSe₂ (*t*-BuLi) starting material via LSV measurements in 0.5 M H₂SO₄ on glassy carbon (GC) electrode (Figure 11.6A). The lowest overpotential of -0.524 V vs RHE at a current density of -10 mA/cm² was reported for MoSe₂ (*t*-BuLi). After solution-based exfoliation of the MoSe₂ (*t*-BuLi) sheets, the overpotential reported for MoSe₂ NPs shifted to -0.722 V vs RHE. This can be accounted by the partial oxidation of the MoSe₂ NPs as illustrated in the XPS spectrum (Figure 11.2A). Nonetheless, the HER catalytic activity of MoSe₂ NPs has been established to be much higher than that of the control GC electrode. The reason for working with MoSe₂ NPs as labels instead of MoSe₂ (*t*-BuLi) large sheets^{8,28} is that MoSe₂ NPs labels are of comparable sizes to proteins and much smaller than the paramagnetic beads which would lead to better reproducibility of assays compared to if MoSe₂ (*t*-BuLi) labels were used.

Tafel analysis was executed for the HER polarization curve of the MoSe₂ NPs to determine the electrochemical mechanism involved. The mechanism of hydrogen evolution on the MoSe₂ NPs can be understood from the following rate-determining steps:²⁹

1. Volmer adsorption step

2. Heyrovsky desorption step

3. Tafel desorption step

We observed a Tafel slope of 138 mV dec⁻¹ on the MoSe₂ NPs and further identified the Volmer adsorption mechanism as the rate-determining step as the slope is close to the theoretical value of 120 mV/dec.

Figure 11.6 Optimisation of HER conditions for MoSe₂ NPs as an electrochemical label; (A) Linear sweep voltammograms derived from HER in acidic electrolyte on bare GC, and MoSe₂ (*t*-BuLi) and MoSe₂ NPs deposited on a GC electrode. Conditions: 0.5 M H₂SO₄, scan rate 2 mV s⁻¹. B) Bar chart illustrates the obtained currents at 200 s from chronoamperometry at MoSe₂ NPs on an SP electrode. Error bars represent RSD (n = 3; 95% confidence interval). Inset: Recorded chronoamperograms. Conditions: 0.5 M H₂SO₄, applied potential of -0.77 V, -0.87 V and -0.97 V vs RHE.

Moreover, the chronoamperometry technique was employed to investigate the utility of MoSe₂ NPs as viable labels. The obtained MoSe₂ NPs were subjected to three different potentials (-0.77 V, -0.87 V and -0.97 V vs RHE) on an SP electrode for HER catalysis. The current outputs were recorded for each potential in Figure 11.6B, illustrating that the current output at 200 s is highest with an applied potential of -0.97 V vs RHE and decreased with lower applied potentials. Furthermore, at -0.97 V vs RHE, the current output on a bare SP electrode was negligible compared to that with the MoSe₂ NPs (Figure 11.7). As such, in order to optimise experimental conditions so that MoSe₂ NPs can serve as an effective

electrochemical label, an applied potential of -0.97 V vs RHE was chosen for subsequent experiments and the current response at 200 s was evaluated.

Subsequently, magnetic beads (MBs) modified with anti-rabbit IgG were used as platforms for capture, pre-concentration and detection of rabbit IgG. For this purpose, MBs were conjugated with anti-rabbit IgG. On the other end, the rabbit IgG was conjugated with MoSe₂ NPs. In this way, rabbit IgG is recognised by the anti-rabbit IgG, forming IgG-MoSe₂ NPs/anti-IgG-MB complex (Figure 11.1B). The final amount of MoSe₂ NPs will be representative of the concentration of rabbit IgG in the sample

Figure 11.7 Chronoamperometric responses resulting from the electrocatalysis of hydrogen evolution at bare SPE and MoSe₂ NPs.

In order to confirm that the MoSe₂ NPs have been attached on the surface of the rabbit IgG in the magneto-immunoassay, scanning transmission electron microscopy (STEM) and ICP-MS analyses were carried out. Figure 11.8 shows the STEM images of the obtained magneto-immunoassay. When MoSe₂ NPs were used as labels (Figure 11.8B), an obvious presence of nanoparticles were observed on the surface of the magnetic beads. The size of these particles matches that of smaller sized fraction (~100 nm) of MoSe₂ NPs observed earlier in Figure 11.2C. This observation, however, is not significant in the control where no label was used

(Figure 11.8A). Furthermore, ICP-MS was used to quantify the amount of MoSe₂ present in the final IgG-MoSe₂ NPs/anti rabbit IgG-MB complex. The magneto-immunoassay complex contained 0.14 μg of MoSe₂ per gram of MBs, proportional to a rabbit IgG protein concentration of 500 ng mL^{-1} .

Figure 11.8 STEM images of magneto-immunoassay: A) without MoSe₂ NPs (IgG/anti rabbit IgG-MB complex) and (B) with MoSe₂ NPs as labels (rabbit IgG-MoSe₂ NPs/anti rabbit IgG-MB complex).

Following the successful characterization of the desired IgG-MoSe₂ NPs/anti rabbit IgG-MB complex, we analysed the performance parameters of the magneto-immunoassay. Selectivity of the immunoassay was evaluated in Figure 11.9A. Two different control experiments were carried out for this purpose: 1) a magneto-immunoassay without MoSe₂ NPs as labels, and 2) a non-specific protein, human haemoglobin (Hb) was used to replace the rabbit IgG protein. High selectivity was evident with depressed current responses from both control assays. This high selectivity is a result of the immunoassay protocol which involves multiple washing steps to remove any interfering species and non-complementary proteins in the real samples, leading to a depressed current response. In this Way, our method would mainly be used in the analysis of proteins pre-extracted from real samples.

Moreover, sensitivity of the immunoassay was investigated. Figure 11.9B shows a strong concentration dependence of the current response at 200 s over a wide

range where the current decreased as rabbit IgG protein concentrations increased from 2 to 500 ng mL⁻¹. A low limit of detection (LOD) of 1.23 ng mL⁻¹ and good linearity ($r = 0.95$) were demonstrated. The mean relative standard deviation (RSD) from five different protein concentrations was reported to be 9.7%, where triplicates were ran for each concentration. This indicates a high reproducibility of the system.

Figure 11.9 Evaluation of magneto-immunoassay performance; (A) Selectivity of immunoassay was demonstrated in chronoamperometric responses. Analytical signal obtained for immunoassay with complementary rabbit IgG protein is compared with non-complementary Hb protein. Control experiments were carried out with bare SP electrode (black), immunoassay with no protein and no label. Conditions: 0.5 M H₂SO₄, applied potential -0.97 V vs RHE. (B) Logarithmic relationship between the concentration of rabbit IgG protein and the value of analytical signal obtained. Error bars represent RSD ($n = 3$; 95% confidence interval). Conditions: 0.5 M H₂SO₄, applied potential -0.97 V vs RHE.

We compared the performance parameters of the magneto-immunoassay with TMD nanolabels obtained by solution-based electrochemical exfoliation with bipolar electrodes. The previously obtained magneto-immunoassay with WS₂ nanolabels gave a similar LOD of 2 ng mL⁻¹ with good linearity ($r = 0.96$) and reproducibility (RSD $\approx 10\%$) as well.¹¹ Furthermore, performance parameters of the magnetic-immunoassay with TMD NPs as labels paralleled that with gold NPs as labels (LOD = 0.31 ng mL⁻¹)³⁰, highlighting the potential of TMD NPs prepared with

solution-based electrochemical exfoliation with bipolar electrodes as low cost alternative labels. Hence, the simple exfoliation technique is viable and reproducible for producing TMD nanoparticles as low cost alternative labels for biomolecular detection.

11.3 Conclusion

Overall, we demonstrated the preparation of MoSe₂ NPs by a simple solution-based electrochemical exfoliation method and their application as promising nanolabels for biomolecular detection via HER by means of chronoamperometry as a transduction method. Characterization of the MoSe₂ NPs suggested a significant reduction in size of the final NPs (~100 nm) compared to the starting material, which allows it to be an efficient label for the magneto-immunoassay. Performance parameters of the immunoassay demonstrated a wide range of detection for rabbit IgG protein, high selectivity, sensitivity and reproducibility. These findings have strong implications as we demonstrate the viability and reproducibility of solution-based electrochemical synthesis of TMD nanoparticles as labels for the development of low costs and efficient biosensing systems for several applications ranging from diagnostics to environmental control.

11.4 Experimental

11.4.1 Materials

Molybdenum selenide MoSe₂ (99.9%) and tert-butyllithium (1.7 M in pentane) were acquired from Alfa Aesar (Germany) and Sigma-Aldrich (Czech Republic) respectively. Magnetic beads (Dynabeads M-280 Tosylactivated) were obtained from Invitrogen™ (Singapore). Boric acid, sodium hydroxide, phosphate buffered saline tablet, albumin from bovine serum (BSA), TWEEN® 20, rabbit IgG from serum, anti-rabbit IgG produced in goat and haemoglobin (lyophilised powder) were obtained from Sigma Aldrich (Singapore).

11.4.2 Apparatus

X-ray photoelectron spectroscopy (XPS) measurements were done using a Phoibos 100 spectrometer and a monochromatic Mg X-ray radiation source (SPECS, Germany). Survey (wide-scan) and high resolution spectra were recorded for each sample. For XPS measurements, sample preparation was done by coating a uniform layer of the materials under study onto a silicon wafer. A UV-vis spectrometer (Shimadzu UV-2500) was employed to obtain the UV-vis spectra. Scanning transmission electron microscopy (STEM) images were acquired by using a Jeol 7600F SEM instrument (Jeol, Japan) operating at 15 kV (MoSe₂ NPs) or 25 kV (magneto-immunoassay). Raman spectra were acquired using a confocal micro-Raman LabRam HR instrument (HORIBA Scientific) in backscattering geometry with a CCD detector, a 514.5 nm Ar laser and a 100 objective attached to an Olympus optical microscope. Prior to measurements, calibration was achieved using an internal silicon reference at 520 cm⁻¹ with a peak position resolution of

less than 1 cm⁻¹. The spectra ranged from 100 to 1000 cm⁻¹. Transmission electron microscopy (TEM), HR-TEM and electron dispersive X-ray spectroscopy (EDS) images were obtained with an EFTEM Jeol 2200 FS HRTEM microscope (Jeol, Japan) operated at 200 keV. EDS mapping was performed with SSD detector from Oxford Instruments (X-MaxN 80 T). Samples were prepared for analysis by drop casting the suspension on a TEM grid (Cu/lacey carbon; 200 mesh) and dried at 60 °C for 2 hours. All voltammetric experiments were performed using a µAutolab type III electrochemical analyser (Eco Chemie, The Netherlands) connected to a computer and controlled by the NOVA, Version 1.8.17, software.

11.4.3 Procedures

Synthesis of Nanolabel. MoSe₂ sheets intercalated by Li using tert-butyllithium were used for the synthesis of MoSe₂ NPs. After intercalation, the MoSe₂ sheets were exfoliated in water according to a previously described protocol⁸ and purification was achieved by centrifugation. The resulting solid powder of MoSe₂ (*t*-BuLi) was dispersed in ultrapure water (0.5 mg mL⁻¹) and subjected to ultrasonication for a duration of 4 h. Subsequently, 0.5 M Na₂SO₄ was added to 4 mL of the MoSe₂ dispersion. The resultant dark suspension was placed into an electrochemical cell. After which, the two driver electrodes of Pt were placed in the electrochemical cell (see Figure 11.1A). The distance between the electrodes is around 2 cm (Figure 11.10). 10 V of DC potential was applied for 30 min, after which a clear suspension was obtained (Figure 11.11). The suspension was allowed to settle for several hours and finally, the supernatant was collected. For XPS, STEM, Raman, TEM and EDS characterisation, dialysis of the supernatant was performed using a membrane with a molecular weight cut-off below 1 kDa.

Figure 11.10 Electrochemical setup of solution-based exfoliation of MoSe₂ (*t*-BuLi) material. Images are taken at 5 min intervals.

Figure 11.11 Comparison of MoSe₂ suspension before and after solution-based electrochemical exfoliation.

Magneto-immunoassay. Based on previously reported procedures, the magneto-immunoassay was prepared.¹² 3 mg mL⁻¹ of magnetic beads (MB) was washed in borate buffer (pH 9.2), and incubated overnight with anti-rabbit IgG at 37 °C and 400 rpm agitation. The final concentration of anti rabbit IgG in the mixture was 40 µg mL⁻¹. Subsequently, the MBs/anti rabbit IgG complex was washed with PBS TWEEN® 20 (0.5% v/v). Next, the blocking step was performed by re-suspending the obtained complex in a PBS solution containing 5% BSA (w/v) at 25 °C and 650 rpm agitation for 40 min. When the blocking step is completed, washing of the samples was done with PBS and BSA (0.1% w/v).

In order to attach the electrochemically active label to the rabbit IgG, the label was incubated with the protein at 25°C with 650 rpm agitation for 2 h. Subsequently, a blocking step was carried out by adding BSA (5% w/v) in ultrapure water to the solution of labelled protein, and extending the incubation for 20 min under the same initial conditions. After the blocking step, the sample was centrifuged at 4 °C

and 14000 rpm for 2 h. 70 μ L PBS was used for the reconstitution of the resulting pallet.

The final incubation step was carried out by first pre-concentrating the magnetic bead/anti rabbit IgG from 70 μ L of the supernatant. Next, 70 μ L of the labelled rabbit IgG was mixed with the magnetic bead/anti-rabbit IgG. The incubation was performed at 25°C under 650 rpm agitation for a duration of 30 min. Subsequently, washing steps were carried out using PBS TWEEN® 20, PBS and ultrapure water. Finally, the assembly was re-suspended in ultrapure water.

Electrochemical Measurements. HER performance was evaluated using linear sweep voltammetry (LSV) where a three electrode electrochemical system was employed. The three electrodes used are a glassy carbon (GC) working electrode (3 mm diameter), a Pt counter electrode and Ag/AgCl reference electrode. Modification of the working electrode was achieved by drop-casting MoSe₂ NP solution (3 μ L) and leaving the solution to dry at room temperature. Moreover, screen printed (SP) electrodes (from eDAQ instruments) composed of a graphitic carbon working electrode (3 mm diameter) and counter electrode with reference electrode of Ag/AgCl were used for the optimisation of experimental conditions and magneto-immunoassays. In all cases, measurements (chronoamperometry) were carried out in H₂SO₄ (0.5 M); 15 μ L of H₂SO₄ (1 M) was mixed with 15 μ L of MoSe₂ NPs solution or magneto-immunoassay on the SP electrode. LSV measurements were performed at a scan rate of 2 mV/s while chronoamperometry was performed at -0.97 V vs RHE for 200 s.

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Part II

Chapter 12

1T Phase WS₂ Protein-based Biosensor

12.1 *Introduction*

12.2 *Results and Discussion*

12.3 *Conclusion*

12.4 *Experimental*

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

1. **Rou Jun Toh**, Carmen C. Mayorga-Martinez, Zdenek Sofer, Martin Pumera. 1T-Phase WS₂ Protein-based Biosensor. *Adv. Func. Mater.* **2017**, 1604923.

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12.1 Introduction

Exfoliation of TMDs using tert-butyllithium (*t*-BuLi) produces single to few layer sheets with large compositions of highly conductive, metallic 1T phase¹ which has been reported to display excellent capacitive behaviour,² electrocatalytic performance towards hydrogen evolution reaction (HER)³ and application for gas sensing.⁴ For these reasons, 1T phase TMD materials possess immense potential to be explored for further sensing applications.

A selective and sensitive detection platform for hydrogen peroxide (H₂O₂) would have profound implications on the fields of bioanalysis, food security and environmental control.⁵⁻⁷ H₂O₂ is generated as a consequence of the incomplete reduction of oxygen in a broad range of biological processes.⁸ As a major reactive oxygen species (ROS) in living organisms, H₂O₂ plays a critical role in the signal transduction and oxidative pathway of cells.⁹⁻¹¹ Moreover, H₂O₂ acts as an oxidative stress marker which is important for the diagnosis of ageing and diseases such as cancer, cardiovascular and neurodegenerative disorders.^{12 - 15} Therefore, the determination of H₂O₂ is necessary and vital, where methods such as chemiluminescence,¹⁶ colorimetry,¹⁷ fluorescence¹⁸ and electrochemistry^{19,20} have been extensively explored. Amongst them, electrochemical methods stand out due to their high sensitivity, rapid response, low cost and easy miniaturization.

The development of electrochemical H₂O₂ biosensors has seen much effort devoted to the design of modified electrodes which can effectively and selectively catalyse the reduction or oxidation of H₂O₂. Heme proteins (haemoglobin, myoglobin, and cytochrome C) are commonly employed in the construction of H₂O₂ biosensors due to their commercial availability and peroxidase activity.

However, facilitation of electron transfer between the heme center and conventional electrode surfaces is physiologically hampered.²¹ In order to enhance the electron transfer rate of the heme proteins, immobilization materials such as metal nanoparticles,²² carbon nanomaterials^{23, 24} and metallic oxide nanostructures^{25,26} have been investigated. Their large surface area, good stability, excellent electrical properties and biocompatibility allow access to the direct electrochemistry of heme proteins and thus, enhance the performance of biosensors.

Hitherto, MoS₂ has been explored for the successful construction of heme-based H₂O₂ biosensors by facilitating the direct electrochemistry of heme proteins.²⁷⁻²⁹ In addition, it has been reported that MoS₂ nanoparticles (NPs) alone display electrocatalytic activity towards the reduction of H₂O₂.³⁰ However, no work has been presented to explore the promising potential of other TMD materials in this aspect. Herein, we demonstrate the advantageous role of 1T phase WS₂ for the construction of a heme-based H₂O₂ biosensor compared to 1T phase MoS₂, MoSe₂ and WSe₂. Consequently, we show the viability of this biosensor for hydrogen peroxide determination in human serum for applications in real sample analysis and the development of low-cost, high sensitivity biosensors.

12.2 Results and Discussion

Figure 12.1 Schematic representation (not to scale) of the construction of GTA/Hb/1T-WS₂/GC electrode.

In this work, we investigated the synergistic interaction between 1T phase WS₂ (1T-WS₂) and haemoglobin (Hb) towards the electrocatalysis of hydrogen peroxide reduction. Characterization of 1T-WS₂ was first performed using scanning electron microscopy (SEM), energy-dispersive spectroscopy (EDS), transmission electron microscopy (TEM), high resolution TEM (HR-TEM), Raman spectroscopy, X-ray photoelectron spectroscopy (XPS) and X-ray diffraction (XRD). Construction of the H₂O₂ biosensor was achieved by the deposition of three consecutive layers on the surface of a glassy carbon (GC) electrode; 1T phase WS₂ (1T-WS₂), followed by haemoglobin (Hb) and finally, glutaraldehyde (GTA) to give GTA/Hb/1T-WS₂/GC electrode, as illustrated in Figure 12.1. Glutaraldehyde is employed in this work to serve two main functions; (1) crosslinking matrix for haemoglobin to maintain the structural rigidity of the protein, and (2) to entrap and stabilise the 1T-WS₂ and Hb layers on the GC electrode.³¹⁻³⁴ Following that, the constructed biosensor was validated by SEM and heterogeneous electron transfer (HET) studies.

Its electrocatalytic activity towards hydrogen peroxide reduction was probed via cyclic voltammetry (CV) and chronoamperometry.

The high availability, low cost and widely reported catalytic and sensing activity of 1T phase WS₂ makes it a promising candidate for the catalytic reduction of hydrogen peroxide.^{1,4} 1T-WS₂ was first prepared by exfoliating the bulk WS₂ material. SEM was performed to compare the surface morphologies of the bulk and exfoliated 1T-WS₂ material (Figure 12.2). Bulk WS₂ showed large polygonal stacks of up to tens of micrometers wide and a few hundred nanometers thick. In contrast, the exfoliated 1T-WS₂ displayed decreased flake sizes, extensive wrinkling of edges and a significant expansion across layers. This is in agreement with Raman analyses shown in Figure 12.3A. The in-plane E_{2g}¹ and out-of-plane A_{1g} modes of WS₂ are observed at 355 cm⁻¹ and 419 cm⁻¹ respectively. The softening of both modes for 1T-WS₂ with respect to bulk WS₂ indicated a decreased lateral size as well as a reduced number of layers.^{1,35} Further characterization of the materials with EDS confirmed their surface elemental compositions (Figure 12.4). Moreover, Figure 12.5 shows TEM image of 1T-WS₂ and the corresponding selective area electron diffraction (SAED) and HR-TEM image. TEM of 1T-WS₂ shows the presence of individual few layer sheets with size of up to one micron while the hexagonal symmetry of WS₂ is displayed in the SAED. HR-TEM was used for identification of the presence of 1T phase WS₂ where the hexagonal arrangement of atoms typical of the 1T phase is observed in the sample.^{36,37} The presence of 1T phase WS₂ was further supported by XPS analyses where Figure 12.3B shows the high resolution XPS spectra of WS₂ after exfoliation and deconvolution analyses of the W and S bonding modes revealed the dominant presence of the metallic, 1T polymorph. Furthermore, XRD shows the

presence of 1T phase WS₂ without additional impurities (Figure 12.3C). The high intensity of (ool) reflections indicated high preferential orientation. Hence, the successful exfoliation of bulk WS₂ to 1T phase WS₂ was demonstrated.

Figure 12.2 Scanning electron microscopy (SEM) images of bulk WS₂ and 1T-WS₂. Scale bars represent 1 μm .

Figure 12.3 (A) Raman spectra of bulk WS₂ and 1T-WS₂. (B) High resolution X-ray photoelectron spectra of W 4f and S 2p regions of 1T-WS₂. Deconvoluted peaks correspond to the metallic 1T phase (red), semiconducting 2H phase (blue), or the oxidised (VI) state (olive). (C) X-ray diffraction (XRD) pattern of 1T-WS₂.

Figure 12.4 Electron dispersive X-ray spectroscopy (EDS) of bulk WS₂ and 1T-WS₂. Scale bars represent 10 μm .

Figure 12.5 Transmission electron microscopy (TEM) image of 1T-WS₂ sheets with corresponding selective area electron diffraction pattern and detail of area used for electron diffraction (red circle). High resolution TEM image of 1T-WS₂ shows dominant presence of 1T phase. Scale bars represent 200 nm, 10 nm and 1 nm, respectively.

Consequently, optical and electrochemical methods were used to monitor the modification process of the GC electrode and construction of the biosensor. Figure 12.6 shows the SEM images of 1T-WS₂, Hb/1T-WS₂ and GTA/Hb/1T-WS₂. A uniform distribution of layered 1T-WS₂ is observed with the first modification step. Subsequently, the layered structure became less distinct as the addition of Hb cast a dark film over the 1T-WS₂ material. The effect of glutaraldehyde as a binding matrix is evident as a smooth film was formed over the surface. Successful

immobilization of each layer of material was further confirmed with HET studies using cyclic voltammetry (Figure 12.7). Oxidation and reduction peaks of the ferri/ferrocyanide redox probe are observed at -0.10 V and +0.46 V respectively at the bare GC electrode and the electrochemical response was measured with each consecutive modification step. An increased redox current and decreased separation between the redox peak potentials is observed at the 1T-WS₂/GC. This can be accounted for by the high conductivity and increased surface area of the 1T phase WS₂, hence increasing the HET rate. With the subsequent immobilization of Hb, a slight decrease in redox current is observed. The less efficient electron transfer is a result of the protein nature of Hb. The final immobilization step of glutaraldehyde resulted in a significant decrease in redox currents, showing successful entrapment of both WS₂ and Hb on the GC electrode.

Figure 12.6 Scanning electron microscopy (SEM) images of 1T-WS₂, Hb/1T-WS₂ and GTA/Hb/1T-WS₂ at high magnification (left side); scale bars represent 100 nm, and low magnification (right side); scale bars represent 10 μ m.

Figure 12.7 Changes in heterogeneous electron transfer rates due to the modification process of the GC electrode. Cyclic voltammograms resulting from electrochemical activity of ferri/ferrocyanide redox probe at GC electrode, 1T-WS₂/GC, Hb/1T-WS₂/GC and GTA/Hb/1T-WS₂/GC. Conditions: 5 mM Fe(CN)₆^{3-/4-} in 0.1 M KCl; scan rate: 100 mV s⁻¹.

Figure 12.8 Cyclic voltammograms resulting from electrochemical reduction of 1 mM H₂O₂ at (a) GTA/Hb/1T-WS₂/GC (black line); (b) GTA/Hb/GC (red line); (c) GTA/1T-WS₂/GC (blue line); (d) GTA/GC (magenta line); (e) bare GC (olive line); and (f) GTA/Hb/B-WS₂/GC (purple dash). Conditions: N₂-saturated, 10 mM PBS at pH 7.4; scan rate: 50 mV s⁻¹.

First, CV was employed to evaluate the electrocatalytic activity of the constructed biosensor towards H₂O₂ reduction in a N₂-saturated phosphate buffered saline (PBS) (10 mM, pH 7.4). Figure 12.8 shows that both GTA/Hb/GC and GTA/WS₂/GC displayed reduction currents higher than that from control experiments at the GTA/GC and bare GC, where current responses were insignificant. Moreover, the highest reduction currents were achieved at the

GTA/Hb/WS₂/GC. In addition, a biosensor was also constructed with bulk WS₂ (B-WS₂) for comparison, where GTA/Hb/B-WS₂/GC performed inferior to its 1T phase WS₂ counterpart. From these, we conclude that both Hb and 1T-WS₂ possess electrocatalytic activity towards hydrogen peroxide reduction, with synergistic effects between 1T-WS₂ and Hb.

In order to understand the synergistic interactions between 1T-WS₂ and Hb, chronoamperometric response of the GTA/Hb/1T-WS₂/GC towards consecutive additions of H₂O₂ was compared with that of GTA/Hb/GC, GTA/1T-WS₂/GC and GTA/Hb/B-WS₂/GC in Figure 12.9A. While GTA/1T-WS₂/GC showed a stable increase in reduction currents with each addition of H₂O₂, the electrochemical response from GTA/Hb/GC appeared otherwise and depleted over time. On the other hand, GTA/Hb/1T-WS₂/GC exhibited a stable and higher electrochemical response than that obtained with GTA/1T-WS₂/GC. The increased sensitivity of the GTA/Hb/1T-WS₂/GC biosensor was achieved as the electrocatalytic activities of both 1T-WS₂ and Hb towards the reduction of H₂O₂ can be exploited. With more catalytically active edge sites exposed, the exfoliated 1T phase WS₂ was able to display excellent electrocatalytic activity towards the reduction of H₂O₂, and at the same time, provided a high surface-to-volume ratio and conductive platform for the uniform immobilization of Hb and effective facilitation of electron transfer between Hb and the electrode surface. This is in contrast to the unstable electrochemical response obtained when bulk WS₂ which consists mainly of the semi-conducting 2H polymorph¹ is used instead.

Figure 12.9 Chronoamperometric responses upon successive additions of 100 μM H₂O₂ at: (A) GTA/Hb/1T-WS₂/GC, GTA/Hb/GC, GTA/1T-WS₂/GC and GTA/Hb/B-WS₂/GC at an applied potential of -400 mV; (B) GTA/Hb/1T-MoS₂/GC, GTA/Hb/1T-MoSe₂/GC, GTA/Hb/1T-WS₂/GC and GTA/Hb/1T-WSe₂/GC at an applied potential of -400 mV; (C) GTA/Hb/1T-WS₂/GC at an applied potential of -400 mV with different 1T-WS₂ loading concentrations: 0.5 mg mL⁻¹, 1.0 mg mL⁻¹ and 5.0 mg mL⁻¹; (D) GTA/Hb/1T-WS₂/GC at different applied potentials: -500 mV, -400 mV, -300 mV, -200 mV and -100 mV. Arrows indicate time points for the addition of H₂O₂. Conditions: stirred, N₂-saturated, 10 mM PBS at pH 7.4.

Moreover, it would be of interest to explore the potential of other 1T phase TMDs prepared by *t*-BuLi exfoliation¹ in the construction of heme-based H₂O₂ biosensors. For this purpose, we constructed biosensors with 1T phase MoS₂ (1T-MoS₂), MoSe₂ (1T-MoSe₂) and WSe₂ (1T-WSe₂) denoted as GTA/Hb/1T-MoS₂/GC, GTA/Hb/1T-MoSe₂/GC and GTA/Hb/1T-WSe₂/GC respectively. The SEM images and EDS elemental mappings of 1T-MoS₂, 1T-MoSe₂ and 1T-WSe₂ are presented in Figure 12.10 and 12.11. Figure 12.9B compares the performances of GTA/Hb/1T-

MoS₂/GC, GTA/Hb/1T-MoSe₂/GC, GTA/Hb/1T-WS₂/GC and GTA/Hb/1T-WSe₂/GC. Chronoamperometric studies showed that the biosensor with 1T-WS₂ outperformed that with 1T-MoS₂, 1T-MoSe₂ and 1T-WSe₂, agreeable with CV investigations (Figure 12.12A). Hence, the GTA/Hb/1T-WS₂/GC serves as a suitable biosensor for further electrochemical characterizations.

Figure 12.10 Scanning electron microscopy (SEM) images of 1T-MoS₂, 1T-MoSe₂ and 1T-WSe₂. Scale bars represent 1 μm.

Figure 12.11 Electron dispersive X-ray spectroscopy (EDS) of 1T-MoS₂, 1T-MoSe₂ and 1T-WSe₂. Scale bars represent 10 μm.

Figure 12.12 Cyclic voltammograms resulting from electrochemical reduction of 650 μM H_2O_2 at (A) GTA/Hb/1T-MoS₂/GC, GTA/Hb/1T-MoSe₂/GC, GTA/Hb/1T-WS₂/GC and GTA/Hb/1T-WSe₂/G; and (B) GTA/Hb/1T-WS₂/GC with 1T-WS₂ loading concentrations of (i) 0.5 mg mL⁻¹, (ii) 1.0 mg mL⁻¹ and (iii) 5.0 mg mL⁻¹. Conditions: N₂-saturated, 10 mM PBS at pH 7.4; scan rate: 50 mV s⁻¹.

Subsequently, experimental conditions for H_2O_2 detection were optimised at the GTA/Hb/1T-WS₂/GC biosensor. The effect of 1T-WS₂ loading concentration on electrocatalytic activity of the biosensor towards H_2O_2 reduction was studied in Figure 12.9C. Reduction currents increased as the loading of 1T-WS₂ increased from 0.5 mg mL⁻¹ to 5.0 mg mL⁻¹. This trend is in line with voltammetric studies (Figure 12.12B). Thus, a loading concentration of 5.0 mg mL⁻¹ was chosen for subsequent experiments. Furthermore, Figure 12.9D shows the chronoamperometric response of GTA/Hb/WS₂/GC with different applied potentials for the electrocatalytic reduction of H_2O_2 . We observed that the reduction currents increased with increasingly negative potentials applied from -0.1 V to -0.5 V. However, the increase in reduction currents is less significant from -0.4 V to -0.5 V at lower concentrations of H_2O_2 . We hence selected -0.4 V as a robust value for the applied potential for further chronoamperometric studies where effects of interference species may also be minimised.

Figure 12.13 (A) Chronoamperometric responses of the GTA/Hb/1T-WS₂/GC upon successive additions of 2, 10, 20, 100, 200, 400 μM H₂O₂ (in order). Four successive additions of H₂O₂ were performed for each concentration from 2 to 200 μM , followed by one final addition of 400 μM H₂O₂. (B) Logarithmic relationship between the concentration of H₂O₂ and the catalytic current was obtained. Error bars represent RSD ($n = 3$; 95% confidence interval). Conditions: stirred, N₂-saturated, 10 mM PBS at pH 7.4; applied potential: -400 mV.

Following the optimisation of experimental conditions, we investigated the analytical parameters of the GTA/Hb/1T-WS₂/GC biosensor. In Figure 12.13A, chronoamperometry was used to probe the reduction currents with successive additions of H₂O₂ into stirred N₂-saturated 10 mM PBS at -0.4 V. An increase in reduction currents is observed with increasing concentrations of H₂O₂ and the concentration dependence of this chronoamperometric signal was evaluated in Figure 12.13B. The GTA/Hb/1T-WS₂/GC biosensor demonstrated a wide linear range over the concentration of H₂O₂ from 2 μM to 38 μM ($r^2 = 0.98$) and from 48 μM to 1728 μM ($r^2 = 0.99$) with good linearity. A low limit of detection of 36 nM was attained and the high reproducibility of the system was reflected in the low relative standard deviation (RSD) of 3.9%. Furthermore, repeatability of the constructed biosensor was tested as shown in Figure 12.14. The average RSD obtained from 5 different H₂O₂ concentrations (20, 40, 60, 80, 100 μM), where three measurements were taken with the same biosensor, over a period of 5 days, is 6.2%. Moreover, we

observed from this data that the GTA/Hb/1T-WS₂/GC biosensor displayed good stability as it retained 81.5% of its initial current response after 5 days (Figure 12.15).

Figure 12.14 Repeatability of GTA/Hb/1T-WS₂/GC biosensor. (A) Chronoamperometric responses upon five successive additions of 20 μM H_2O_2 . Three measurements were performed over 5 days. (B) Catalytic currents obtained at different concentrations of H_2O_2 . Error bars represent RSD ($n = 3$; 95 % confidence interval). Conditions: stirred, N_2 -saturated, 10 mM PBS at pH 7.4; applied potential: -400 mV.

Figure 12.15 Stability of GTA/Hb/1T-WS₂/GC biosensor. Percentage recovery of catalytic current obtained at 40 μM H_2O_2 over 8 days. Conditions: stirred, N_2 -saturated, 10 mM PBS at pH 7.4; applied potential: -400 mV.

The high analytical performance GTA/Hb/1T-WS₂/GC biosensor makes it a promising platform for the detection of H_2O_2 in real samples. For this reason, we investigated the interference effect of the GTA/Hb/1T-WS₂/GC biosensor towards ascorbic acid (AA), dopamine (DA), uric acid (UA) and glucose, which are present

in large quantities in the physiological environment.^{38,39} Figure 12.16A shows the current responses of the biosensor with the successive additions of 50 μM H₂O₂, 500 μM AA, 500 μM DA, 500 μM UA, 500 μM glucose, and 50 μM H₂O₂ into stirred, N₂-saturated PBS. While no significant change in the reduction currents is observed with high concentrations of the interference species, a significant increase is observed with the additions of H₂O₂. This can be attributed to the preferential reduction of H₂O₂ as the negative applied potential allows the detection to be free from interferences by biomolecules.⁴⁰ Moreover, Figure 12.16B shows the measurement upon successive additions of 20 μM H₂O₂ in PBS (black line) and 50% v/v human serum (red line), similar responses were observed in both cases. Hence, we conclude that the GTA/Hb/1T-WS₂/GC biosensor has wide linear range, low detection limit, high reproducibility, repeatability and stability with good selectivity and is a suitable platform for the detection of H₂O₂ in real samples.

Figure 12.16 (A) Chronoamperometric response of the GTA/Hb/1T-WS₂/GC to 50 μM H₂O₂ in the presence of interferences: 500 μM AA, 500 μM DA, 500 μM UA and 500 μM glucose. (B) GTA/Hb/1T-WS₂/GC biosensor for H₂O₂ detection in human serum. Chronoamperometric responses upon successive additions of 20 μM H₂O₂ in PBS and 1:1 PBS:serum. Conditions: stirred, N₂-saturated, 10 mM PBS at pH 7.4; applied potential: -400 mV.

Moreover, the constructed GTA/Hb/1T-WS₂/GC biosensor in this work exhibited one of the best analytical ranges and detection limits when contrasted

with previous reports of H₂O₂ detection with TMD-based sensors (Table 12.1). The use of exfoliated 1T phase WS₂ sheets in our work has resulted in a H₂O₂ biosensor which significantly outperformed previous works focused on the use of MoS₂ sheets.²⁷⁻²⁹ On the other hand, the biosensor based on ultrasmall MoS₂ nanoparticles (MoS₂ NPs/GC)³⁰ performed better than the GTA/Hb/1T-WS₂/GC for H₂O₂ detection. This can be attributed to the smaller MoS₂ nanoparticle sizes (< 2 nm) compared to the larger 1T-WS₂ sheets with micrometer lateral dimensions, which is in good agreement with size-dependent electrocatalytic performance studies reported previously.^{30,41}

Table 12.1 Performance parameters of comparable biosensors for the detection of H₂O₂.

Sensor/ Transduction Method	Analytical Ranges (μM)	Limit of Detection (μM)	Reference
MoS ₂ NPs/GC Chronoamperometry	0.005 – 0.1; 0.1 – 100	0.0025	30
GTA/Hb/1T-WS ₂ /GC Chronoamperometry	2 – 38; 48 – 1728	0.036	This work
MoS ₂ /Graphene-Carbon Nanotubes/GC Chronoamperometry	5 – 145	0.83	27
Hb-AuNPs@MoS ₂ /GC Cyclic voltammetry	10 – 300	4	28
Nafion/Hb/MoS ₂ /GC Chronoamperometry	20 – 180	6.7	29

12.3 Conclusion

We demonstrated the advantageous role of *t*-BuLi exfoliated, 1T phase WS₂ for the construction of a heme-based H₂O₂ biosensor compared to 1T phase MoS₂, MoSe₂ and WSe₂. The GTA/Hb/1T-WS₂/GC biosensor was fabricated based on a layer-by-layer deposition of 1T-WS₂ and Hb, and entrapment by GTA. Synergistic interactions between 1T-WS₂ and Hb resulted in a highly sensitive and selective H₂O₂ biosensor that exhibits a wide range and low detection limit. Consequently, we showed the viability of this biosensor for H₂O₂ detection in human serum. These findings have strong implications for the development of highly sensitive and low-cost H₂O₂ biosensing systems for applications in the fields of bioanalysis, food safety and environmental control.

12.4 Experimental

12.4.1 Materials

Molybdenum disulfide MoS₂ (99.9%), molybdenum diselenide MoSe₂ (99.9%), tungsten disulfide WS₂ (99.8%) and tungsten diselenide WSe₂ (99.8%) were acquired from Alfa Aesar, Germany. Tert-butyllithium (1.7 M in pentane) was purchased from Sigma-Aldrich, Czech Republic. Hexane was acquired from Lach-ner, Czech Republic. Argon (99.9999% purity) was obtained from SIAD, Czech Republic. Phosphate buffered saline tablet, potassium chloride (max 0.0001% Al), Potassium hexacyanoferrate (II) trihydrate ($\geq 98.5\%$), potassium hexacyanoferrate (III) (~99%), glutaraldehyde solution (~50% in water), haemoglobin human (lyophilised powder) and human serum were obtained from Sigma-Aldrich, Singapore. Hydrogen peroxide (35% w/w aq. soln.) was purchased from Alfa Aesar, Singapore. Glassy carbon (GC), Ag/AgCl reference and Pt counter electrodes were obtained from CH Instruments, Inc, USA.

12.4.2 Apparatus

Scanning electron microscopy (SEM) images and energy-dispersive X-ray spectroscopy (EDS) were acquired using a Joel 7600F instrument (Joel, Japan). The instrument was operated at 5 kV for SEM and 15 kV for EDS measurements. X-ray photoelectron spectroscopy (XPS) measurements were performed using a Phoibos 100 spectrometer and a monochromatic Mg X-ray radiation source (SPECS, Germany). Survey

(wide-scan) and high resolution spectra were recorded for each sample. For SEM, EDS, and XPS measurements, sample preparation was performed by coating a uniform layer of the materials under study onto carbon conductive tape. Raman spectra were acquired using a confocal micro-Raman LabRam HR instrument (HORIBA Scientific) in backscattering geometry with a CCD detector, a 514.5 nm Ar laser and a 100 objective attached to an Olympus optical microscope. Prior to measurements, calibration was achieved using an internal silicon reference at 520 cm⁻¹ with a peak position resolution of less than 1 cm⁻¹. The spectra ranged from 100 to 1000 cm⁻¹. High resolution transmission electron microscopy (HR-TEM) was performed with an EFTEM Jeol 2200 FS microscope (Jeol, Japan). An acceleration voltage of 200 keV was used for measurement. The sample was prepared by drop casting the suspension (1 mg mL⁻¹ in water) on a TEM grid (Cu; 200 mesh; Formvar/carbon) and drying at 60 °C for 12 h. X-ray powder diffraction (XRD) data were collected at room temperature on a Bruker D8 Discoverer powder diffractometer with parafocusing Bragg–Brentano geometry using CuK α radiation (λ = 0.15418 nm, U = 40 kV, I = 40 mA). The data were scanned over the angular range of 10 – 80° (2 θ) with a step size of 0.02° (2 θ). Data evaluation was performed in the software package EVA. All voltammetric and chronoamperometric experiments were carried out using a μ Autolab type III electrochemical analyser (Eco Chemie, The Netherlands) connected to a computer and controlled by the NOVA, Version 1.8.17, software. Electrochemical experiments were carried out at room temperature in an electrochemical cell (5 mL) with

the employment of a three electrode configuration. A platinum electrode functioned as the counter electrode, and an Ag/AgCl electrode served as the reference electrode.

12.4.3 Procedures

Synthesis of tert-Butyllithium Exfoliated Transition Metal Dichalcogenides. Exfoliation of bulk MoS₂, MoSe₂, WS₂ materials was performed according to the previously established protocol.⁴ Suspensions were prepared by adding a particular TMD powder (3 g) to tert-butyllithium (20 mL; *t*-BuLi) in pentane (1.7 M). Subsequently, the suspensions were stirred at 25 °C under argon atmosphere for 72 h. The resulting Li-intercalated materials were separated by suction filtration under argon atmosphere and washed three times with hexane (dried over sodium). After which, the exfoliated material was dispersed in water (100 mL) and subjected to ultrasonication for 15 min. Centrifugation at 18000 g and re-dispersion in water was carried out in order to achieve purification of the samples. This process was repeated until the conductivity falls below 20 µS. Finally, the exfoliated TMD materials were dried in a vacuum oven at 25 °C for 48 h prior to further use.

Electrode Preparation. Prior to use, the glassy carbon (GC) electrodes were first renewed using a 5 µm followed by a 0.05 µm alumina particle slurry on a polishing pad. Subsequently, the electrode was thoroughly rinsed with ultrapure water and dried under a flow of nitrogen gas.

Suspensions of *t*-BuLi exfoliated 1T phase WS₂ (5 mg mL⁻¹) were prepared in ultrapure water and ultrasonicated for a period of 5 min before each electrochemical measurement to ensure homogeneity of the material under study. An aliquot (4 µL) was then deposited on the electrode surface and left to dry at room temperature. This yields a uniformly distributed film of 1T phase WS₂ (20 µg; 1T-WS₂) on the GC electrode surface. The resultant electrode is denoted as 1T-WS₂/GC. Following this, haemoglobin (0.5 mg mL⁻¹; Hb) was prepared in ultrapure water. The Hb solution (4 µL) was pipetted onto the surface of the electrode and left to dry at room temperature; resulting in Hb/1T-WS₂/GC. Subsequently, a solution of glutaraldehyde (0.25% v/v; GTA) in ultrapure water was prepared. Crosslinking of the Hb protein was achieved by spreading a volume of GTA solution (4 µL) over the electrode surface and left to dry in an oven at 35 °C. The final modified electrode is termed here GTA/Hb/1T-WS₂/GC. The electrode was stored at 4 °C when not in use.

A similar procedure was used in the fabrication of different types of modified electrodes; GTA/Hb/1T-MoS₂/GC, GTA/Hb/1T-MoSe₂/GC, GTA/Hb/1T-WSe₂/GC, GTA/Hb/B-WS₂/GC, GTA/Hb/1T-WS₂/GC, Hb/1T-WS₂/GC, GTA/Hb/GC and GTA/GC. Pre-treatment of GTA/Hb/1T-MoS₂/GC, GTA/Hb/1T-MoSe₂/GC, GTA/Hb/1T-WS₂/GC, GTA/Hb/1T-WSe₂/GC, Hb/1T-WS₂/GC and GTA/Hb/B-WS₂/GC electrodes was performed with cyclic voltammetry (CV) at a scan rate of 50 mV s⁻¹ between 0.2 and -0.6 V in PBS electrolyte (10 mM) for 2 scans. This served to ensure that the inherent electrochemistry of the TMD materials³ would not interfere with subsequent measurements.

Detection of Hydrogen Peroxide. Cyclic voltammetry (CV) was performed at a scan rate of 50 mV s⁻¹ between 0.2 V and -0.6 V in an N₂ saturated, PBS electrolyte (10 mM, pH 7.4). Chronoamperometry was performed at a constant potential of -0.4 V in an N₂ saturated, PBS electrolyte (10 mM, pH 7.4) under stirring conditions. The resulting chronoamperograms were subjected to baseline correction for further analysis.

Heterogeneous Electron Transfer (HET) Measurement. Cyclic voltammetry (CV) was performed at a scan rate of 100 mV s⁻¹ between -0.3 V and 0.6 V with ferri/ferrocyanide (5 mM)

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Part II

Chapter 13

Group 6 Layered Transition Metal Dichalcogenides in Lab-on-a-Chip Devices: 1T Phase WS₂ for Microfluidics Non-Enzymatic Detection of Hydrogen Peroxide

13.1 Introduction

13.2 Results and Discussion

13.2.1 Lab-on-a-Chip Device Characterization

13.2.2 Optimisation of TMDs-based Lab-on-a-Chip Devices

13.2.3 Performance of WS₂-based Lab-on-a-Chip Device

13.3 Conclusion

13.4 Experimental

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13.1 Introduction

Lab-on-a-chip (LOC) devices have been developed extensively to improve the sensing speed and efficiency, by miniaturizing chemical and biological analytical instruments. They bring benefits such as portability, field deployability, low sample consumption and low costs.¹ However, it remains a challenge in the field of analytical LOC devices to provide a downsized, yet highly sensitive detector for the minute sample amounts often encountered in real-world applications. In this aspect, electrochemical detection is advantageous compared with more conventional optical detection methods, due to its ease of miniaturization, low energy consumption and compatibility with advanced microfabrication schemes.²

For the purpose of improving the sensitivity and detection limits of LOC devices, various nanomaterial-based electrochemical detectors have been integrated within LOC systems.^{3,4} While carbon nanotubes are the most extensively studied due to their excellent mechanical properties, high thermal and electrical conductivity, and electrochemical properties,⁵⁻¹³ the one-dimensional (1D) nature of the material poses a huge challenge in fabrication.^{14,15} On the other hand, increasing attention is being placed on two-dimensional (2D) nanomaterials¹⁶ with advantageous electrochemical properties which can offer easy patternability and device fabrication due to their 2D atomically layered structures.¹⁷

Graphene and graphene oxide-modified transducing platforms have been reported widely to provide highly sensitive detection in LOC systems.¹⁸⁻²¹ Moreover, transition metal dichalcogenides (TMDs), possessing 2D layered structures, similar to that of graphene, and desirable properties such as high

surface-to-volume ratio, excellent conductivity and capacitive behaviour,^{22 - 24} present as promising candidates for enhancing the performance of electrochemical LOC devices. Sarkar *et al.* demonstrated the high performance of a MoS₂-based field effect transistor (FET) biosensor, surpassing the sensitivity of that based on graphene by more than 74-fold, and its ability to be scaled down for integration into LOC systems without compromising its sensitivity.¹⁷ Highlighting the promising potential of TMDs for sensing applications, it is surprising that they remain under-explored in the field of analytical LOC devices with only one significant work published hitherto to the best of our knowledge.

As a major reactive oxygen species (ROS) in living organisms and an oxidative stress marker,²⁵⁻²⁷ highly sensitive and inexpensive point-of-care testing of H₂O₂ is of paramount importance. Considering batch measurements, Wang *et al.* reported the high electrocatalytic performance of MoS₂ nanoparticles towards H₂O₂ reduction.²⁸ In this work, we integrate 2D layered TMDs (MoS₂, MoSe₂, WS₂, WSe₂) as detector surfaces for hydrogen peroxide (H₂O₂) in LOC devices. Moreover, a simple and reproducible method of preparing TMDs-based LOC microfluidic devices is presented. This method allows investigators without access to state-of-the-art fabrication equipment to explore electrochemical LOC microfluidics and diagnostics with ease and at a low cost. Herein, the TMD materials were prepared by tert-butyllithium exfoliation and consequently, we demonstrate the high performance of 2D layered, 1T phase WS₂-based LOC device for the detection of H₂O₂ (Scheme 1) compared to that based on MoS₂, MoSe₂ and WSe₂. The robust 1T phase WS₂-based LOC device is further practical for H₂O₂ determination in human serum, indicating its feasibility for applications in real biosample analysis.

13.2 Results and Discussion

13.2.1 Lab-on-a-Chip Device Characterization

A major step in the development of electrochemical lab-on-a-chip (LOC) devices is the preparation of miniaturised electrodes. In the context of LOC microfluidics, it is crucial for the electrode thickness to be sufficiently low for the efficient attachment of microfluidic channels. Though conventional photolithography techniques can produce electrodes of nanometer thickness, sophisticated facilities and a clean room are required.²⁹ Screen printing has been explored as a cheaper and simpler alternative for the mass printing of conductive inks as electrodes.³⁰ However, high initial cost investment and a well-equipped facility is still necessary. Herein, we introduce a simple and reproducible manual screen printing procedure for the fabrication of low cost, electrochemical lab-on-a-chip (LOC) devices and characterised the LOC device using energy-dispersive spectroscopy (EDS), scanning electron microscopy (SEM), open circuit potential (OCP) determination and contact angle measurements.

The optimised LOC device design is illustrated in Figure 13.1. Linear electrode geometries were chosen for ease of fabrication and channel alignment. A laser cutter is employed to create the electrode stencil on a masked glass substrate. For this reason, an ultrathin polyimide mask with silicone adhesive is used as the stencil for a three-electrode system. The heat resistant and flame retardant properties of polyimide³¹ make it a suitable material for laser cutting while the silicone adhesive ensures that the mask can be removed from the glass substrate without leaving residues.

Figure 13.1 (A and B) Schematic representation (not to scale) and photograph of lab-on-a-chip set-up for the electrocatalytic reduction of H_2O_2 (C).

Figure 13.2 Energy-dispersive X-ray spectroscopy (EDS) data for silver electrode before (Ag) and after (Ag/AgCl) treatment with bleach. Scale bars, 1 μm .

In order to produce the electrodes, carbon ink is used not only because it is relatively inexpensive, but also because of its attractive electrochemical properties for sensing applications such as low background currents and broad potential windows.^{32,33} With carbon ink as a base material, a pseudo reference electrode of silver/ silver chloride (Ag/AgCl) is fabricated by printing a layer of silver conductive ink over the carbon material followed by treatment with bleach.³⁴ The formation of AgCl upon bleach treatment is illustrated in the EDS elemental

mapping (Figure 13.2). OCP determination of three separate pseudo reference electrodes revealed a high reproducibility of the fabrication method with a relative potential of 31.7 ± 0.2 mV vs commercially obtained Ag/AgCl electrode (Figure 13.3).

Figure 13.3 Open circuit potential (OCP) measurements of three pseudo Ag/AgCl vs commercially obtained Ag/AgCl reference electrode. Mean OCP was determined to be 31.7 ± 0.2 mV vs Ag/AgCl. Conditions: 3.5 M KCl.

Moreover, modification of the working electrode with the desired transition metal dichalcogenides (TMDs) was achieved by the drop-casting method. It is postulated that the layered TMD materials are stably attached to the carbon material due to strong hydrophobic interactions.³⁵ 1T phase WS₂-based LOC device as representative, successful modification of the carbon working electrode is observed through EDS elemental mapping (Figure 13.4) and contact angle measurements (Figure 13.5) showed that attachment of WS₂ to the carbon working electrode is realised as the hydrophobicity of WS₂ increased after deposition onto the highly hydrophobic carbon material. This is further verified by EDS elemental mapping of the WS₂-modified carbon working electrode where the presence of WS₂ continued to be observed after calibration experiments were performed on the LOC device (Figure 13.6).

Figure 13.4 Energy-dispersive X-ray spectroscopy (EDS) data for carbon electrode before (C) and after (C/WS₂) deposition of WS₂ material. Scale bars, 1 μm.

Figure 13.5 Contact angle measurements of C and WS₂ on glass substrates and WS₂ deposited on C.

Figure 13.6 Energy-dispersive X-ray spectroscopy (EDS) data for 1T phase WS₂-modified carbon electrode (C/WS₂) after calibration experiments. Scale bar, 1 μm.

Moreover, further observations under a scanning electron microscopy were made to characterise the electrode widths, separation and thicknesses. From Figure 13.7A, it is shown that all three electrodes displayed similar widths of ~ 1.2 mm. The distance between the Ag/AgCl reference electrode (RE) and carbon counter electrode (CE) is ~ 0.2 mm while that between the carbon CE and WS₂ modified (C/WS₂) working electrode (WE) is ~ 1.7 mm (Figure 13.7B). Furthermore, the thin polyimide stencil (total thickness of 25 μm) ensures that the resultant electrode has a sufficiently low thickness for efficient channel bonding. The thicknesses of three electrodes were measured and determined to be 4.1 ± 0.6 μm , 4.0 ± 0.5 μm and 4.0 ± 0.8 μm for the RE, CE and WE respectively (Figure 13.8). The resultant electrode thicknesses were lower than the typical thickness obtained through automated screen-printing on 240 mesh screen (12.7 μm).³⁶ This allowed strong plasma bonding of the polydimethylsiloxane (PDMS) channels over the prepared electrodes. Channel bonding was further enforced using a silicone sealant and appropriate wire connections were made using silver conductive epoxy. Hence, the low cost (<USD 0.50 per device), simple and reproducible production of electrochemical LOC devices is realised and used for subsequent investigations of the performance of different layered TMDs for the detection of H₂O₂.

Figure 13.7 Scanning electron microscopy (SEM) images of the prepared electrodes to illustrate the (A) width of the Ag/AgCl, C and C/WS₂ electrodes, and (B) distance between the Ag/AgCl and C electrodes, and that between the C and C/WS₂ electrodes.

Figure 13.8 Measurements of the thickness of the prepared Ag/AgCl, C and C/WS₂ electrodes under scanning electron microscopy (SEM). Error bars represent RSD ($n = 7$; 95% confidence interval)

13.2.2 Optimisation of TMDs-based Lab-on-a-Chip Devices

Considering the high availability, low cost and highly reported catalytic and sensing activity of group 6 transition metal dichalcogenides (TMDs) (MoS_2 , MoSe_2 , WS_2 , WSe_2), we first synthesised the 2D layered materials by tert-butyllithium (*t*-BuLi) exfoliation of their bulk forms. Characterization of the obtained 2D layered materials was performed with scanning electron microscopy (SEM), X-ray photoelectron spectroscopy (XPS), high resolution transmission electron microscopy (HR-TEM) and Raman spectroscopy. Following that, the respective LOC devices were constructed and their electrocatalytic activities towards the reduction of H_2O_2 were studied using chronoamperometric techniques.

Exfoliation with *t*-BuLi produces single to few layer sheets of TMDs composed of the conductive, metallic 1T phase.³⁷ The successful formation of these 2D layered, 1T phase TMD materials were verified using XPS. The XPS wide scan confirmed the elemental compositions of the individual TMD materials (Figure 13.9). In addition, high resolution XPS spectra of the metals and chalcogens were measured. Figure 13.10 illustrates the deconvolution analyses of Mo 3d bonding modes of MoS_2 and MoSe_2 and W 4f bonding modes of WS_2 and WSe_2 where the 1T phase is present. Likewise, SEM of the 2D layered TMD materials showed extensive wrinkling of edges with significant spacing between the layers (Figure 13.11), typical of the exfoliated materials.

Figure 13.9 X-ray photoelectron spectroscopy (XPS) wide spectra of the *t*-BuLi exfoliated TMD materials.

Figure 13.10 High resolution X-ray photoelectron spectroscopy (XPS) spectra of Mo 3d (top) of MoS₂ and MoSe₂, and W 4f (bottom) of WS₂ and WSe₂.

Figure 13.11 Scanning electron microscopy (SEM) images of the *t*-BuLi exfoliated TMD materials.

Subsequently, the prepared 2D layered TMD materials were integrated into LOC devices and their electrocatalytic performances towards the reduction of H_2O_2 were compared in Figure 13.12A. The reduction currents obtained with the 1T phase WS_2 -based LOC device significantly surpassed that based on MoS_2 , MoSe_2 and WSe_2 . For this reason, an extensive characterization of WS_2 was performed using HR-TEM (Figure 13.13) and Raman spectroscopy (Figure 13.14) which further displayed its metallic 1T phase and 2D layered nature.

Moreover, the 1T phase WS_2 -based LOC device, serving as a suitable H_2O_2 sensor, was optimised for further electrochemical characterizations. The effect of WS_2 loading concentration (Figure 13.12B) was investigated where bare screen printed (SP) electrode with zero WS_2 loading served as a control. Reduction currents were negligible at the bare SP electrode and started to increase as the WS_2 loading is increased to 2.5 mg mL^{-1} and subsequently 10 mg mL^{-1} . However, the reduction currents remained stagnant as WS_2 loading is further increased to 15 mg mL^{-1} . This is likely due to the saturation of the sensor surface at 10 mg mL^{-1} , which was chosen to be the optimal WS_2 loading concentration for subsequent

experiments. Furthermore, different applied potentials were investigated for the electrocatalytic reduction of H_2O_2 . While a low reduction potential is preferred in order to minimise the effects of interference species,³⁸ sensitivity of the LOC device cannot be compromised as well. Figure 13.12C illustrates the increase in reduction currents as the applied potential is increased from -0.4 V to -0.5 V. However, this is not the case as the reduction potential is increased from -0.5 V to -0.6 V. Therefore, a robust value of -0.5 V was chosen as the applied potential for electrocatalytic reduction of H_2O_2 in the WS_2 -based LOC device.

Figure 13.12 Chronoamperometric responses upon injection of $200 \mu\text{M}$ H_2O_2 : (A) Comparison of *t*-BuLi exfoliated TMDs for the electrocatalytic reduction of H_2O_2 in a lab-on-a-chip device. Optimisation of experimental conditions on a 1T phase WS_2 -based lab-on-a-chip device: (B) different WS_2 loading concentrations at an applied potential of -0.5 V and (C) different applied potentials at a WS_2 loading of 10 mg mL^{-1} . Arrows indicate time points for the injection of H_2O_2 .

Figure 13.13 Transmission electron microscopy (TEM) image of WS₂ sheets with corresponding selective area electron diffraction pattern. High resolution TEM image of WS₂ shows presence of 1T phase. Scale bars represent 200 nm and 2 nm respectively.

Figure 13.14 Raman spectrum of 1T phase WS₂.

13.2.3 Performance of WS₂-based Lab-on-a-Chip Device

Figure 13.15 Calibration of 1T phase WS₂-based lab-on-a-chip device. Current spike obtained upon second of the three H₂O₂ injections is chosen for analysis. (A) Chronoamperometric responses at 1T phase WS₂ upon injection of different H₂O₂ concentrations. Applied potential: -0.5 V. (B) Logarithmic relationship between the concentration of H₂O₂ (20 nM to 2 mM) and the obtained catalytic current. Error bars represent RSD (n = 3; 95% confidence interval). Insets: Magnified views for concentrations of H₂O₂ from 20 nM to 20 μM.

Consequently, analytical parameters of the WS₂-based LOC device were examined for the detection of H₂O₂. Figure 13.15A shows the chronoamperometric responses obtained upon injection of different concentrations of H₂O₂. An increase in H₂O₂ concentrations resulted in a corresponding increase in reduction currents. The H₂O₂ concentration dependence of this chronoamperometric signal is illustrated in Figure 13.15B where a wide linear range from 20 nM to 20 μM ($r = 0.92$) and from 100 μM to 2 mM ($r = 0.98$) is observed. The LOC device attained a low limit of detection (LOD) at 2.0 nM and the reproducibility of the system is reflected in the relative standard deviation (RSD) value (17.6%) of 3 different LOC devices. 10 different H₂O₂ concentrations were measured using each LOC device and the second of three measurements was taken as representative. In addition,

the repeatability of the system was determined by taking the average RSD (3.3%) of 10 different H_2O_2 concentrations where three measurements of each concentration were taken using the same LOC device.

Impelled by the high analytical performance of the WS_2 -based LOC device, it was further investigated as a system for H_2O_2 detection in real samples. Interference study of the system was carried out using biomolecules which are present in the physiological environment: ascorbic acid (AA), dopamine (DA), uric acid (UA) and glucose (Glu).^{39,40} Figure 13.16A shows a comparison of the chronoamperometric responses upon injection of the interference species into the LOC device at their respective physiological concentrations.^{41,42} Relatively low or insignificant reduction currents are observed with the interference species, accounted for by the preferential reduction of H_2O_2 at an applied negative reduction potential. Moreover, an 88.5% signal recovery is observed with 10% human serum in PBS (Figure 13.16B). Taking into account that the cytotoxic levels of H_2O_2 are usually high at $\geq 50 \mu\text{M}$,⁴³⁻⁴⁵ our WS_2 -based LOC device proves to be sensitive enough (LOD = 2.0 nM) for the detection of critical H_2O_2 concentrations even after dilution of the real samples.

Figure 13.16 (A) Interference studies of WS_2 -based lab-on-a-chip device. Arrows indicate time points for the injection of H_2O_2 and corresponding interferences. (B) Chronoamperometric responses upon injection of H_2O_2 in PBS and 10% human serum in PBS. Arrows indicate time points for the injection of 200 μM H_2O_2 . Applied potential: -0.5 V.

While the simple design and low sample volumes ($< 20 \mu\text{L}$) of the WS_2 -based LOC device may limit the possibility of additional assay routes for the confirmation of H_2O_2 determination, it must be emphasized that with H_2O_2 being an electroactive molecule, electrochemical techniques have been established as a simple, fast and effective method for H_2O_2 determination^{46,47} in contrast to other techniques such as chemiluminescence,⁴⁸ fluorescence,⁴⁹ and spectrophotometry.⁵⁰ In addition, a comparison with another work highlighted the advantages of our device. Rusling and co-workers demonstrated a detection limit of 5 nM for H_2O_2

detection with a heme protein-based microfluidic electrochemical device using glutathione-decorated gold nanoparticles.⁵¹ While the sensitivity of the heme-based device is similar to that of our WS₂-based LOC device, the non-enzymatic approach employed in this work confers advantages in experimental conditions and sample preparation. The use of a protein would require specific experimental and storage conditions, such as temperature and pH, to ensure the structural and conformational stability of the protein.⁵² Moreover, deoxygenation is a necessary step for the function of heme-based biosensors.^{53, 54} This is an important consideration for the detection of H₂O₂ in a LOC device where the gas permeability of PDMS leading to oxygen leakage into the flow chamber may decrease the signal-to-noise ratio of heme-based biosensors.⁵⁵ Thinking ahead to applications in mass production, the use of gold nanoparticles can incur high costs in contrast to TMDs which are abundant and low-cost. Hence, we present a reproducible and highly sensitive WS₂-based LOC device for H₂O₂ detection with wide linear range, low detection limit, and good selectivity for applications in real sample analysis.

13.3 Conclusion

We demonstrated a low cost, simple and reproducible preparation method for electrochemical LOC microfluidic devices and modified the transducing platform with four *t*-BuLi exfoliated group 6 TMD materials by the drop-casting method. The TMDs-based LOC devices were investigated for the detection of H₂O₂. The 1T phase WS₂-based LOC device exhibited a wide linear range, low detection limit and feasibility for H₂O₂ determination in human serum. This work not only has strong implications for investigators looking to explore the field of electrochemical LOC microfluidics without sophisticated facilities, it also represents one of the firsts in exploring and exploiting the desirable electrochemical properties of TMDs for sensing applications in LOC devices. We believe that these findings will serve to spur the development of high performance point-of-care H₂O₂ sensing systems for bioanalysis, food safety and environmental applications. In addition, this work demonstrates a low-cost fabrication of electrochemically superior electrodes for other BioMEMS and microfluidic systems.

13.4 Experimental

13.4.1 Materials

Carbon conductive paste (BQ242) and polyimide tape (0.5 mil) were purchased from DuPont, USA and CAPLINQ, Canada respectively. Conductive silver epoxy (CW2400) was acquired from Chemtronics, USA. SU-8 2035 photoresist and hexamethyldisilane were obtained from MicroChem, USA and Sigma-Aldrich, St. Louis, MO respectively. Harris Uni-Core cutting tip (2 mm) was obtained from Ted Pella, Inc., USA. Polydimethylsiloxane (Sylgard 184) was acquired from Dow Corning Inc., USA. Liquid chlorine bleach was purchased from GB Chemicals Pte. Ltd., Singapore. Clear silicone sealant was obtained from Sellys, Australia. MoS₂, MoSe₂, WS₂, WSe₂ (< 2 μ m) bulk powders were obtained from Alfa Aesar, Germany and tert-butyllithium (1.7 M in pentane) was purchased from Sigma-Aldrich, Czech Republic. Hexane was acquired from Lach-ner, Czech Republic. Argon (99.9999% purity) was obtained from SIAD, Czech Republic. Phosphate buffered saline tablet and human serum were purchased from Sigma-Aldrich, Singapore. Hydrogen peroxide (35% w/w aq. soln.) and silver conductive ink (S-020) were acquired from Alfa Aesar, Singapore. Ag/AgCl electrode was acquired from CH Instruments, Inc., Singapore.

13.4.2 Apparatus

Electrode stencils were created by a laser cutter (Universal Laser Systems, Inc., USA). Plasma bonding of PDMS channels to the glass substrates was achieved using a Covance-MPR plasma system (FemtoScience, Korea). Scanning electron microscopy (SEM) images and energy-dispersive X-ray spectroscopy (EDS) were

acquired using a Joel 7600F instrument (Joel, Japan) operating at 5 kV and 15 kV respectively. X-ray photoelectron spectroscopy (XPS) measurements were performed using a Phoibos 100 spectrometer and a monochromatic Mg X-ray radiation source (SPECS, Germany). Survey (wide-scan) and high resolution spectra were recorded for each sample. For SEM, EDS, and XPS measurements, sample preparation was performed by coating a uniform layer of the materials under study onto carbon conductive tape. Contact angle measurements were performed using an Attension Theta system (Biolin Scientific, Sweden). Injection of phosphate buffer saline (PBS) and hydrogen peroxide into the microfluidic device was achieved using a Fusion 200 syringe pump (Chemxy Inc, USA) and a Pump 11 Elite syringe pump respectively. The Fusion 200 syringe pump has a flow performance with accuracy and smooth flow ranging from 100 pL min⁻¹ to 128 mL min⁻¹ while the Pump 11 Elite syringe pump performs in the range of 1.28 pL min⁻¹ to 88.28 mL min⁻¹. Flow rates were monitored with a SLI-0430 liquid flow meter (Sensirion AG, Switzerland) which works in a flow range of -80 µL min⁻¹ to 80 µL min⁻¹. Chronoamperometric experiments were carried out using a µAutolab type III electrochemical analyser (Eco Chemie, The Netherlands) connected to a computer and controlled by the NOVA, Version 1.8.17, software.

13.4.3 Procedures

Synthesis of 2D Layered Transition Metal Dichalcogenides. Exfoliation of bulk MoS₂, MoSe₂, WS₂ and WSe₂ materials was performed by tert-butyllithium exfoliation. Suspensions were prepared by adding a particular TMD powder (3 g) to tert-butyllithium (20 mL; *t*-BuLi) in pentane (1.7 M). Subsequently, the suspensions

were stirred at 25 °C under argon atmosphere for 72 h. The resulting Li-intercalated materials were separated by suction filtration under argon atmosphere and washed three times with hexane (dried over sodium). After which, the exfoliated material was dispersed in water (100 mL) and subjected to ultrasonication for 15 min. Centrifugation at 18000 g and re-dispersion in water was carried out in order to achieve purification of the samples. This process was repeated until the conductivity falls below 20 μ S. Finally, the exfoliated TMD materials were dried in a vacuum oven at 25 °C for 48 h prior to further use.

Electrode Preparation. The electrodes were designed using AutoCAD (Autodesk, USA) and patterned on a glass substrate by laser cutting a polyimide tape mask. Next, carbon conductive paste was spread evenly over the patterned stencil and allowed to cure for 1 h at 100 °C. A three electrode setup was employed. The carbon electrode serves as the counter and working electrode, as well as a base material for obtaining the pseudo Ag/AgCl reference electrode. The pseudo Ag/AgCl reference electrode was obtained by depositing a thin layer of Ag conductive ink over the carbon material. The Ag conductive ink was allowed to cure for 30 min at 100 °C. Subsequently, the Ag electrode was treated with bleach for 1 min to produce the pseudo Ag/AgCl electrode. Modification of the working electrode was performed by drop-casting a suspension of the exfoliated TMD materials (5 μ L) onto the carbon electrode and left to dry for 30 min at room temperature.

Chip Fabrication. All experiments were carried out in microfluidic chips made of polydimethylsiloxane (PDMS). The fabrication process can be broken down into three major steps: (1) fabrication of the SU-8 master, (2) casting of

PDMS, and (3) plasma bonding of PDMS over the prepared electrodes on the glass substrate. The master for the PDMS device was fabricated using standard photolithography techniques with SU-8 2035 photoresist. The positive master mold for the device contained channels that were 49 μm deep. In order to prevent adhesion with PDMS, the master mold was treated with hexamethyldisilane for 1 h. After the silane treatment, PDMS was poured onto the master mold, which was degassed in a desiccator with a ~ 5 psi vacuum for 30 min before pouring. After curing in an oven at 80 $^{\circ}\text{C}$ for 3 h, the PDMS layer was peeled from the silicon master. Inlet and outlet ports were created with a 2 mm cutting tip. It was treated with oxygen plasma in a plasma cleaner for 40 s before it was bonded to the patterned glass substrate. To ensure strong bonding between the PDMS channel and the glass substrate, curing is performed in an oven at 80 $^{\circ}\text{C}$ for 1 h and silicone sealant is applied. Subsequently, wires were attached for connection with silver conductive epoxy to the three-electrode system accordingly.

Microfluidic Setup. Two syringe pumps were connected to a Y-connector at the inlet of the microfluidic device. PBS (10 mM, pH 7.4) was injected into the microfluidic chips at a flow rate of 40 $\mu\text{L min}^{-1}$. At ~ 100 s intervals, the flow of PBS was stopped and simultaneously, hydrogen peroxide (5 μL) was injected into the channel at 50 $\mu\text{L min}^{-1}$. Waste liquid is collected at the outlet of the microfluidic device and *via* a Teflon tubing to a flow meter to measure the flow rates and monitor any possible leakages in the system.

Potential Determination of Pseudo Ag/AgCl Reference Electrode. Open circuit potential (OCP) determination of the prepared pseudo Ag/AgCl reference electrode was done with reference to a commercially obtained Ag/AgCl electrode.

The relative potential was measured in KCl (3.5 M) over a period of 120 s and the average OCP was determined for each electrode.

Detection of Hydrogen Peroxide. Chronoamperometry was performed at a constant applied potential of -0.5 V and the resulting chronoamperograms were subjected to baseline correction before further analysis.

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Chapter 14

Conclusion and Epilogue

14.1 Conclusion

14.1.1 Part I: Electrocatalytic Properties of Materials for Energy Applications

14.1.2 Part II: Nanomaterial-based Electrochemical Sensors

14.2 Epilogue

14.1 Conclusion

This thesis titled “Materials for Electrochemical Energy and Sensing Applications” emphasises the use of functional materials and electrochemical methods in addressing the challenges faced in energy and sensing applications.

The oxygen reduction reaction (ORR) and hydrogen evolution reaction (HER) are critical energy-related processes. While current state-of-art Pt and Pt-based electrocatalysts used are expensive and low in abundance, transition metal oxides and transition metal dichalcogenides have been identified as promising, low-cost alternatives for the ORR and HER respectively. Despite a surge in efforts and promising reports on the development of alternative electrocatalysts, a lack of comprehensive understanding of the factors that govern the electrocatalytic properties of these materials poses as a limitation.

Moreover, there is a constant need to develop highly selective and sensitive sensors to meet the demands and future needs in the diverse fields of bio-diagnostics, security and environmental control. While nanomaterials such as graphene and transition metal dichalcogenides have the potential to greatly enhance sensor performance, they have yet been explored for certain sensing applications.

Therefore, two main focus of research are highlighted in this thesis. The first focus, covered in **Part I**, is to investigate and better understand the factors that may affect the electrocatalytic properties of transition metal oxides and transition metal dichalcogenides for the oxygen reduction reaction and hydrogen evolution reaction respectively. The second focus is addressed in **Part II**, where

opportunities are explored for the application of graphene and transition metal dichalcogenides in the electrochemical sensing of biomarkers towards improved sensor performance and point-of-care diagnostics.

14.1.1 Part I: Electrocatalytic Properties of Materials for Energy Applications

Towards the design and development of advanced electrocatalysts for the oxygen reduction reaction (ORR) and hydrogen evolution reaction (HER), many reports focus on exploring only one or a few materials. While this provides clear and in-depth information about the electrocatalytic activity of individual materials, it is also crucial to take the big picture approach to identify and understand factors that may govern their electrocatalytic properties.

With regards to the electrocatalytic activity of transition metal oxides towards the ORR, the screening and comparison of thirty simple transition metal oxides in **Chapter 4** showed no significant correlation between the position of a metal on the periodic table and the electrocatalytic performance of its corresponding metal oxide. It was however established that Mn-, Fe-, Co- and Ni-based oxides generally displayed enhanced ORR kinetics under alkaline conditions compared to the bare glassy carbon. Also surprisingly, the commonly used mixed valence (+2,+3) oxides performed poorly against their single valence counterparts. Subsequently, in **Chapter 5**, a study of the influence of different substitutions of ternary transition metal oxides revealed the exceptional electrocatalytic behaviour of Co-based and/or Ni-substituted spinel oxides.

On the other hand, **Chapter 6** demonstrated the enhanced HER electrocatalysis of ultrapure MoS₂ compared to its impurities-doped counterpart. With atomic doping known as a general strategy employed to enhance the HER electrocatalytic activity of transition metal dichalcogenides, we emphasised the

importance of intentional and properly designed atomic doping to realise its true effect in the experimental setting while highlighting the need for a more in-depth understanding of such a strategy. Moreover, the influence different polymorph structures of transition metal dichalcogenides have on their HER efficiencies was stressed in **Chapter 7**. In particular, the observation that bulk 3R-MoS₂ displayed comparable HER performance to the exfoliated 1T-MoS₂ further challenges the need for the commonly employed technique of exfoliation to enhance the electrocatalytic properties of transition metal dichalcogenides. Stepping into unexplored territories, the electrochemical and HER electrocatalytic properties of group 4 transition metal dichalcogenides were characterised in **Chapter 8** where HfS₂ was determined to be electrochemically inert and displayed the highest HER activity. While electrochemical treatment has been reported to successfully activate group 6 transition metal dichalcogenides for HER electrocatalysis, such a strategy was determined to be unsuitable for group 4 transition metal dichalcogenides, with the exception of HfSe₂.

14.1.2 Part II: Nanomaterial-based Electrochemical Sensors

The enhanced electronic and bio-compatibility properties of graphene and transition metal dichalcogenides have made them suitable candidates for electrochemical sensing applications. Nonetheless, the work highlighted in **Chapter 9** demonstrates the lack of advantage in using graphene and graphene-related materials for the electrochemical sensing of haemoglobin in solution. As the bare glassy carbon remains as an appropriate electrode material, further work discussed in **Chapter 10** shows the direct determination of haemoglobin from red blood cells and blood samples at pH 3.5, which may allow for quantitative analysis of red blood cells in blood samples for scientific and clinical purposes.

In **Chapter 11**, the feasibility and reproducibility of a simple solution-based electrochemical exfoliation method for transition metal dichalcogenide nanoparticles was highlighted. Synthesised MoSe₂ nanoparticles served as efficient labels in a magneto-immunoassay for the detection of rabbit IgG protein via HER. Moreover, the later chapters demonstrate the advantageous role of *t*-BuLi exfoliated WS₂ in the construction of H₂O₂ sensors viable for real samples analysis. The synergistic interactions between 1T-WS₂ and haemoglobin shown in **Chapter 12** resulted in high sensor performance while **Chapter 13** represents one of the first works to exploit the desirable electrochemical properties of transition metal dichalcogenides for sensing applications in lab-on-a-chip (LOC) devices. Furthermore, we believe that the simple and low cost preparation method of these devices would allow investigators without access to state-of-the-art fabrication equipment to explore electrochemical LOC microfluidic and diagnostics with ease in order to spur the development of high performance point-of-care sensors.

14.2 Epilogue

Perspective

As we entered into the 21st century, our technology driven society has highlighted the need to make research more responsive to the needs of business and civil society. For this reason, research activities on renewable energy sources as well as chemical sensors are emerging rapidly. Renewable energy sources like hydrogen fuel are demanded worldwide to drive sustainable development and growth while keeping within the context of environmental conservation. On the other hand, sensors are indispensable in the diverse fields of bioanalysis, security and environmental control.

The constant search and discovery for potential materials towards their utility in energy and sensing applications have never failed to surprise and encourage the scientific community. Transition metal oxides, though with a long and distinguished history, remain fiercely investigated as strong contenders for oxygen reduction reaction electrocatalysis. It is envisaged that broader studies of these materials to identify trends and factors that govern their electrocatalytic activity can spur the development of advanced electrocatalysts, which can subsequently replace the expensive state-of-the-art platinum-based materials.

The excitement that came from the discovery of graphene materials due to their outstanding electrical, mechanical and optical properties was followed by close scrutiny of their use in applications that may prove to be revolutionary. While graphene has presented promising results as a nanomaterial for electrochemical sensing, it is equally important to consider reports that show

otherwise. There remains much room for the exploration and understanding of graphene for electrochemical sensing in order to provide accurate information for the development of new electrode materials and graphene-based sensors.

Moreover, mounted interests in 2D layered nanomaterials since the discovery of graphene have reignited the application of transition metal dichalcogenides towards the development of energy-converting systems and sensors with their unique properties. Research on this emerging class of new material is still in its early stage. Nonetheless, spectacular progress in the fundamental studies of transition metal dichalcogenides in the past few years has opened up boundless opportunities for the development of next generation technologies.

The demand for materials aimed at energy and sensing applications will only grow and not cease to exist with time. Collective and continuous efforts from the scientific community as a whole will prove to be pivotal in understanding and fulfilling the myriad of potentials these materials can bring.

Appendices

Transition Metal Oxides for the Oxygen Reduction Reaction: Influence of the Oxidation States of the Metal and its Position on the Periodic Table

Rou Jun Toh,^[a] Zdeněk Sofer,^[b] and Martin Pumera^{*[a]}

Electrocatalysts have been developed to meet the needs and requirements of renewable energy applications. Metal oxides have been well explored and are promising for this purpose, however, many reports focus on only one or a few metal oxides at once. Herein, thirty metal oxides, which were either commercially available or synthesized by a simple and scalable method, were screened for comparison with regards to their electrocatalytic activity towards the oxygen reduction reaction (ORR). We show that although manganese, iron, cobalt, and

nickel oxides generally displayed the ability to enhance the kinetics of oxygen reduction under alkaline conditions compared with bare glassy carbon, there is no significant correlation between the position of a metal on the periodic table and the electrocatalytic performance of its respective metal oxides. Moreover, it was also observed that mixed valent (+2, +3) oxides performed the poorest, compared with their respective pure metal oxides. These findings may be of paramount importance in the field of renewable energy.

1. Introduction

The reduction of oxygen to water serves as a vital reaction in many renewable-energy applications, such as metal–air batteries^[1] and fuel cells.^[2] The oxygen reduction reaction (ORR) may be performed in both acidic and alkaline media; in alkaline medium, molecular oxygen is reduced through either a four-electron ($\text{O}_2 + 2\text{H}_2\text{O} + 4\text{e}^- \rightarrow 4\text{OH}^-$) process or two two-electron ($\text{O}_2 + \text{H}_2\text{O} + 2\text{e}^- \rightarrow \text{HO}_2^- + \text{OH}^-$; $\text{HO}_2^- + \text{H}_2\text{O} + 2\text{e}^- \rightarrow 3\text{OH}^-$) processes.^[3] Despite the abundant benefits the oxygen reduction reaction can potentially bring forth, the sluggish kinetics of this reaction hampers the rate of reaction and has been a major limiting factor for energy-conversion efficiency, stability, and the bulk commercialization of related energy devices.^[4]

In the past decades, one of the focuses of electrochemistry has been exploring new electrocatalysts to enhance the kinetics of the otherwise sluggish ORR. Hitherto, Pt and Pt-based materials are recognized to be the most active ORR catalysts.^[5–8] However, the rarity of Pt in the Earth's crust, and thus, its low availability and high costs are major bottlenecks to large-scale commercial application. As such, alternative ORR catalysts such as nonprecious metals,^[9–11] transition metals,^[12,13] doped carbon materials,^[14,15] and nitrogen-coordinated metal on carbon matrices^[16,17] have been investigated under alkaline

conditions. Moreover, transition metal oxides have been explored with promising advances. Previous studies have reported the electrocatalytic performance of individual transition metal oxides.^[18–21] However, little attention has been paid to studying their electrocatalytic performance with respect to the position of the metal on the periodic table and the metal oxidation state.

In this paper, we address the following basic questions: 1) Are there trends in electrocatalytic performance to be observed across the periodic table or down a group? 2) How would the metal oxidation states affect the catalytic activity of their respective transition metal oxides towards ORR? Thirty transition metal oxides were selected for investigation: Sc_2O_3 , TiO , TiO_2 , V_2O_3 , V_2O_5 , Cr_2O_3 , MnO , Mn_3O_4 , Mn_2O_3 , MnO_2 , Fe_2O_3 , CoO , Co_3O_4 , Co_2O_3 , NiO , Cu_2O , CuO , ZnO , Y_2O_3 , ZrO_2 , Nb_2O_5 , MoO_2 , MoO_3 , CdO , HfO_2 , Ta_2O_5 , WO_2 , WO_3 , HgO , and La_2O_3 . We found no clear or obvious correlation between the position of a metal and the electrocatalytic performance of its respective transition metal oxides. In addition, the mixed valent (+2, +3) oxides performed the poorest, compared with their respective pure transition metal oxides.

2. Results and Discussion

The high availability, low cost, and promising catalytic activity of transition metal oxides make them desirable alternatives to noble metals for electrocatalysis. Hence, it is of importance and interest to screen a sufficient sample size of transition metal oxides to understand how the position on the periodic table and oxidation state of their respective metals would affect electrocatalytic performance towards oxygen reduction reaction (ORR). For this purpose, transition metal oxides were first prepared by using a simple and scalable method, and

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Figure 1. Scanning electron microscopy (SEM) images of the transition metal oxides; Sc_2O_3 , TiO , TiO_2 , V_2O_3 , V_2O_5 , Cr_2O_3 , MnO , Mn_3O_4 , Mn_2O_3 , MnO_2 , Fe_2O_3 , CoO , Co_3O_4 , Co_2O_3 , NiO , Cu_2O , CuO , ZnO , Y_2O_3 , ZrO_2 , Nb_2O_5 , MoO_2 , MoO_3 , CdO , HfO_2 , Ta_2O_5 , WO_2 , WO_3 , HgO , and La_2O_3 .

then characterized by using scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDX), and linear scan voltammetry (LSV). The electrochemical responses originating from the reduction of oxygen (O_2) on the materials will be elaborated.

SEM images of the synthesized transition metal oxides are shown in Figure 1. It can be observed that most of the metal oxides adopt characteristic microstructures, with the exception of V_2O_3 and MoO_3 , where it was observed that the materials took a congeal form. The particle size of each individual metal oxide was obtained from the SEM images and the materials may be categorized into three distinct groups: 1) particle size $< 1 \mu\text{m}$, 2) particle size of $1\text{--}10 \mu\text{m}$, and 3) particle size $> 10 \mu\text{m}$. Metal oxides that fall into the first group, with a particle size less than $1 \mu\text{m}$, are Fe_2O_3 , Co_2O_3 , NiO , CuO , ZnO , CdO , HfO_2 , Ta_2O_5 , and WO_3 . Metal oxides with a particle size ranging from 1 to $10 \mu\text{m}$ consist of Sc_2O_3 , TiO , TiO_2 , Cr_2O_3 , MnO , Mn_3O_4 , Mn_2O_3 , MnO_2 , CoO , Co_3O_4 , Cu_2O , Y_2O_3 , ZrO_2 , Nb_2O_5 , WO_2 , HgO , and La_2O_3 . Metal oxides with a particle size larger than $10 \mu\text{m}$ include V_2O_5 and MoO_2 . Further analysis with EDX provided purity data for the studied materials (Figures S1 and S2 in the Supporting Information).

Figure 2A illustrates the linear scan voltammograms obtained from the electrochemical reduction of oxygen in air-sa-

turated 0.1 M KOH by using a bare glassy carbon (GC) electrode and GC electrodes modified with transition metal oxides and Pt/C. The currents associated with the electrochemical reduction of oxygen were identified by comparing linear scan voltammograms obtained in air-saturated 0.1 M KOH , and nitrogen-saturated 0.1 M KOH , where oxygen is absent (Figure S3 in the Supporting Information). A scatter plot depicting the reduction onset potentials of bare GC, Pt/C, and transition metal oxides according to their positions on the periodic table is represented in Figure 2B. As observed in Figure 2B, Pt/C continues to exhibit superior electrocatalytic performance with the earliest onset potential (-161 mV). Moreover, when comparing the transition metal oxides with bare GC, manganese, iron, cobalt, and nickel oxides display onset potentials that are higher than that of bare GC (-300 mV) and hence, better electrocatalytic activity towards ORR. On the other hand, most of the other transition metal oxides exhibited similar or even more negative onset potentials than that of bare GC. However, there are no obvious trends observed from the results obtained. Hence, this may suggest that electrocatalytic performance of the metal oxide towards ORR is not significantly affected by the position of its metal on the periodic table.

It would be of interest to also investigate the effect of the metal oxidation states on the electrocatalytic performance of

Figure 2. A) Linear scan voltammograms resulting from the electrochemical reduction of oxygen in air-saturated potassium hydroxide (0.1 M) using a bare glassy carbon electrode (black line), and GC electrodes modified with MnO₂ (red line), NiO (blue line), and Pt/C (pink line). Scan rate is 5 mV s⁻¹. B) Reduction onset potentials of transition metal oxides from linear scan voltammograms.

the metal oxide towards the ORR. For this purpose, several transition metals were studied and their different oxides compared. Figure 3A shows the linear scan voltammograms obtained from the electrochemical reduction of oxygen in air-saturated 0.1 M KOH with GC electrodes modified with different cobalt oxides (CoO, Co₂O₃ and Co₃O₄), and Figure 3B displays the relationship between the ORR onset potential and metal oxidation states of different elements. From Figure 3B, no general trend between the metal oxidation state and their pure transition metal oxides' ORR electrocatalytic performance may be established. For vanadium, cobalt, copper, and molybdenum oxides, an enhancement in the kinetics of the ORR was observed as the metal oxidation state increases. However, the opposite is true for manganese, titanium, and tungsten oxides.

Moreover, the mixed valent (+2, +3) oxides consistently performed the poorest compared with their respective pure transition metal oxides. From Figure 3B, it is observed that Mn₃O₄ (−308 mV) and Co₃O₄ (−301 mV) have more negative onset potentials than MnO (−213 mV) and Mn₂O₃ (−225 mV), and CoO (−296 mV) and Co₂O₃ (−291 mV) respectively. Hence, the importance of the purity of transition metal oxides for the application of ORR catalysis may be highlighted from these findings. This is noteworthy as mixed valent oxides are frequently used in catalysis due to convenience of synthesis.

In addition, a comparison of the morphology and particle size of the studied materials with their corresponding electrocatalytic activity towards ORR showed no strong correlation as expected. This is because electrochemistry is happening on

Figure 3. A) Linear scan voltammograms resulting from the electrochemical reduction of oxygen in air-saturated potassium hydroxide (0.1 M) using GC electrodes modified with CoO (black line), Co₂O₃ (red line) and mixed valent (+2, +3) oxide, Co₃O₄ (blue line). Scan rate is 5 mV s⁻¹. B) Reduction onset potentials of metal oxides with multiple oxidation states. Error bars represent RSD (n=3, 95% confidence interval).

the surface and hence, there is no quantum confinement effect at the size of particles used.

3. Conclusions

We showed that there is no significant correlation between the position of a metal on the periodic table and the electrocatalytic performance of the respective transition metal oxides. The electrocatalytic activities of manganese, iron, cobalt, and nickel oxides were reflected in the enhancement of the kinetics of oxygen reduction under alkaline conditions. Whereas these transition metal oxides generally performed better than the bare glassy carbon, it was also observed that the mixed valent (+2, +3) oxides performed the poorest compared with their respective pure transition metal oxides. These findings have strong implications in the development and choice of electrocatalysts for renewable energy applications.

Experimental Section

Materials

N,N-dimethylformamide (DMF) was purchased from Merck. Tungsten(VI) oxide (WO_3) was purchased from Sigma Aldrich. Glassy carbon (GC, 3 mm diameter), Ag/AgCl reference, and Pt counter electrodes were obtained from Autolab (Japan). Sc_2O_3 , Y_2O_3 , La_2O_3 , TiO_2 , ZrO_2 , HfO_2 , Nb_2O_5 , Ta_2O_5 , Cr_2O_3 , MoO_3 , WO_3 , Ti, and W were obtained from Sigma-Aldrich, Czech Republic. Co_2O_3 was obtained from Riedel-deHaën, Germany. V_2O_5 , MnO_2 , Fe_2O_3 , NiO, Cu_2O , CuO, ZnO, CdO, and HgO were obtained from Lach-Ner, Czech Republic. Hydrazine hydrate, cobalt carbonate, ammonium vanadate, and cobalt nitrate hexahydrate was obtained from Penta, Czech Republic. Hydrogen (99.9999%) and argon (99.996%) were obtained from SIAD, Czech Republic.

Synthesis

Titanium(III) oxide was prepared by the reaction of TiO_2 with Ti. A stoichiometric mixture of Ti (1.124 g) and TiO_2 (1.876 g) for the formation of 3 g of TiO was homogenized in agate mortar and pressed into pellets. The pellets were heated under vacuum on 1600°C for 4 h.

Vanadium(III) oxide was prepared by reduction of NH_4VO_3 with hydrazine. Ammonium vanadate (10 g) was dissolved in water (150 mL). Hydrazine hydrate (6 mL) was added to the reaction mixture and it was heated under reflux for 30 min. The vanadium(III) oxide formed was separated by suction filtration, repeatedly washed with water, and finally dried in vacuum oven (200°C , 48 h).

Molybdenum(IV) oxide was synthesized by the reduction of molybdenum(VI) oxide with hydrazine hydrate. MoO_3 (10 g) was dispersed in water (50 mL) by ultrasonication. Hydrazine hydrate (10 mL) was added to the reaction mixture and it was stirred at room temperature for 12 h. Formed molybdenum(IV) oxide was separated by suction filtration, repeatedly washed with water, and dried in vacuum oven (200°C , 48 h).

Tungsten(IV) oxide was prepared by heating a stoichiometric mixture of W and WO_3 in evacuated ampoule. The stoichiometric mixture of W (1.136 g) and WO_3 (2.864 g) for formation of 4 g of WO_2 was homogenized in an agate mortar and pressed in the pellet form. The pellet were sealed under vacuum in a quartz ampoule by using an oxygen-hydrogen torch and heated at 950°C for 40 h.

Manganese(II) oxide was prepared by the reduction of MnO_2 with hydrogen. MnO_2 (3 g) was placed in an alumina boat inside a tube furnace. The tube furnace was repeatedly flushed with argon and subsequently heated under an hydrogen atmosphere at 800°C for 5 h with a hydrogen flow of 100 mL min^{-1} . The heating and cooling rate was 5°C min^{-1} .

Manganese(III) oxide was prepared by the thermal decomposition of MnO_2 . MnO_2 (5 g) was placed in an alumina crucible and heated at 700°C for 24 h. The heating and cooling rate was 5°C min^{-1} .

Manganese(II, III) oxide was prepared by thermal decomposition of MnO_2 . MnO_2 (5 g) was placed in an alumina crucible with lid and heated at 1200°C for 24 h. The heating rate was 5°C . Rapid cooling by air quenching was applied to avoid oxidation.

Cobalt(II) oxide was prepared by thermal decomposition of cobalt carbonate. Cobalt carbonate (5 g) was heated under vacuum at 350°C for 10 h.

Cobalt(III) oxide was prepared by thermal decomposition of cobalt nitrate. Cobalt nitrate hexahydrate (5 g) was heated in an alumina crucible at 700°C for 2 h.

Instrumentation

SEM images were acquired by using a Joel 7600F SEM instrument (Joel, Japan) operating at 1 or 2 kV. EDX data were recorded on a Joel 7600F instrument (Joel, Japan) at 20 kV. For SEM and SEM-EDX measurements, samples were prepared by coating a uniform layer of the materials under study onto a silicon wafer. All voltammetric experiments were performed using a μ Autolab type III electrochemical analyser (Eco Chemie, The Netherlands) connected to a computer and controlled by NOVA, Version 1.8.17 software. Electrochemical experiments were conducted in an electrochemical cell (5 mL) at room temperature. A three electrode configuration was used. A platinum electrode functioned as the auxiliary electrode, and an Ag/AgCl electrode served as the reference electrode. All electrochemical potentials in this paper are stated versus a Ag/AgCl reference electrode.

Cyclic Voltammetry

GC working electrode surfaces were renewed by polishing with $0.05\text{ }\mu\text{m}$ alumina particles on a polishing pad. The transition metal oxides (5 mg mL^{-1}) were dispersed in DMF. The suspension was then subjected to ultrasound treatment for at least 60 min sonication to form a homogenous ink. Next, the suspension ($1\text{ }\mu\text{L}$) was pipetted onto the GC electrode surface, and left to evaporate at room temperature. A film of randomly distributed material is, hence, generated on the GC electrode surface. LSV experiments were performed at a scan rate of 5 mVs^{-1} in potassium hydroxide solution (0.1 M) saturated with air for the oxygen reduction reaction. Reduction onset potentials were standardised and determined as the potential where current is 10% of the peak current.

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Ternary Transition Metal Oxide Nanoparticles with Spinel Structure for the Oxygen Reduction Reaction**

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It is crucial to develop electrocatalysts for the oxygen reduction reaction (ORR) for renewable energy applications. Mixed-valence transition-metal oxides with a spinel structure have been explored for this purpose. In this work, we study the influence of the composition of the catalysts (XY_2O_4 , $X = \text{Ni, Zn}$ and $Y = \text{Co, Mn}$) on the ORR. The four different types of spinel oxide nanocrystals (NiCo_2O_4 , NiMn_2O_4 , ZnCo_2O_4 and ZnMn_2O_4) were synthesized by a simple and scalable method. This allowed for a systematic investigation of the transition-metal influence on the performance of the ORR. Given the general spinel structure of XY_2O_4 , we systematically changed X (Ni, Zn)

and Y (Co, Mn). The effects of the presence of different metals in the spinel oxide on the morphology and electrocatalytic properties of the materials toward the ORR were investigated and compared by using scanning electron microscopy, energy-dispersive X-ray spectroscopy, X-ray photoelectron microscopy, and voltammetry. In general, cobalt-based spinel oxides displayed a significant electrocatalytic activity towards the ORR, with Ni-substituted spinel oxides outperforming their respective Zn-substituted congeners. These findings may have great impact in the research field of renewable energy.

1. Introduction

The oxygen reduction reaction (ORR) plays an important role in a variety of energy applications, including fuel cells^[1] and metal–air batteries.^[2] One of the focuses of electrochemistry in the past decades was to explore new electrocatalysts to enhance the kinetics of the otherwise sluggish ORR. The oxygen electrocatalyst at the cathode has been a major limiting factor for energy-conversion efficiency, stability, and cost of these energy devices. Thus far, Pt and Pt-based materials are recognized to be the most active ORR catalysts.^[3–5] However, the rarity of Pt in the Earth's crust, low availability and high costs of Pt pose as major bottlenecks to large-scale commercial application. As such, alternative ORR catalysts such as non-precious metals^[6,7] or metal oxides,^[8,9] nitrogen-coordinated metal on carbon matrices,^[10,11] and doped carbon materials^[12] have been investigated in alkaline conditions. Nonetheless, it remains a challenge to develop an ORR catalyst with high activity, high availability and low cost compared to Pt. Transition

metals fulfil these requirements, and have been explored with promising advances.^[13,14]

Mixed-valence transition-metal oxides with a spinel structure were identified as active catalysts for the ORR in alkaline conditions.^[15,16,17] Cobalt-based spinel oxides have advantages of activity, low cost, simple preparation and high stability.^[18] In particular, it has been reported that Ni-, Cu- and Mn-substituted Co_3O_4 have shown high activity and stability as electrocatalysts for the ORR.^[16,17,19] However, little attention has been paid to a comparison of the effect of different substitutions. Herein, we addressed the following basic questions: 1) Is the improved catalytic activity attributed to the presence of cobalt oxide? 2) How would different substitutions at X (Ni, Zn) or Y (Co, Mn) in a spinel of general form XY_2O_4 affect the catalytic activity towards the ORR? In this work, four different types of nanocrystal spinel oxides were selected for investigation, namely, NiCo_2O_4 , NiMn_2O_4 , ZnCo_2O_4 and ZnMn_2O_4 .

2. Results and Discussion

The high availability, low cost, and promising catalytic activity of transition metals make them desirable alternatives to noble metals for electrocatalysis. Hence, it is of paramount importance to investigate how mixed-valence transition-metal oxides would affect the electrocatalysis of the ORR. For this purpose, mixed-valence transition-metal oxide nanocrystals of XY_2O_4 composition, where $X = \text{Ni}$ or Zn and $Y = \text{Mn}$ or Co , were first prepared using a simple and scalable method, and then characterized by using scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDX, Figure S1 of the Supporting Information, SI), X-ray photoelectron spectroscopy

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[**] Influence of the XY_2O_4 Spinel Catalyst Composition ($X = \text{Ni, Zn}$ and $Y = \text{Co, Mn}$) on the ORR

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Figure 1. SEM images of NiCo_2O_4 , NiMn_2O_4 , ZnCo_2O_4 , and ZnMn_2O_4 . Scale bars: 100 nm (A); 1 μm (B); and 10 μm (C).

(XPS), X-ray diffraction (XRD) and linear-scan voltammetry (LSV). The electrochemical responses originating from the reduction of oxygen (O_2) on the materials will be elaborated.

SEM images of the synthesized materials are shown in Figure 1. It can be clearly seen that the individual spinel oxides adopt characteristic microstructures. The synthesized powders consist of small crystallites with the exception of NiMn_2O_4 , where octahedral crystals with surface defects can be identified.

The phase purity of the spinels was verified by X-ray diffraction. Whereas both manganite spinels were found as a single phase, the binary oxides ZnO and NiO were formed as impurity phases in the cobaltite spinels at concentrations of 6.1 and 13.6 mol%, respectively. This is due to the high preference of Co^{2+} to occupy the tetrahedral position at the expense of the counter cation resulting in a shift of the respective miscibility range boundary towards $\text{Zn}_{0.87}\text{Co}_{2.13}\text{O}_4$ and $\text{Ni}_{0.69}\text{Co}_{2.31}\text{O}_4$. A high oxygen activity (combined with a low temperature) during the final heat treatment would be necessary to suppress Co^{2+} formation and stabilize the spinel phase in stoichiometric composition. All the samples except ZnMn_2O_4 exhibit a cubic structure with Fd-3 m space group. ZnMn_2O_4 has a tetragonal structure with $I4_1/\text{amd}$ space group. All synthesized materials were composed of nanosized crystallites. The average crystallite size calculated using the Scherrer formula^[20] was 10 nm for NiCo_2O_4 , 15 nm for ZnCo_2O_4 , 14 nm for ZnMn_2O_4 and 7 nm for NiMn_2O_4 . Such small sizes of the crystallites are in contradiction with the octahedral shape of the observed crystals and can originate from high concentrations of defects present in the material synthesized at very low temperatures. The following lattice parameters were determined for the respective spinels: $a = 0.812434 \pm 0.000073$ nm for NiCo_2O_4 , $a = 0.83835 \pm 0.00034$ nm for NiMn_2O_4 , $a = 8.11579 \pm 0.00005$ nm for ZnCo_2O_4

and $a = 0.57310 \pm 0.00007$ nm, $c = 0.92371 \pm 0.00013$ nm for ZnMn_2O_4 . The diffraction patterns are shown in Figure 2.

BET measurements were performed to obtain the surface area of the prepared nanoparticles. The ZnMn_2O_4 particles have a surface area of $28.7 \text{ m}^2 \text{ g}^{-1}$, the ZnCo_2O_4 structures have a surface area of $35.6 \text{ m}^2 \text{ g}^{-1}$, the NiMn_2O_4 particles have a surface area of $93.3 \text{ m}^2 \text{ g}^{-1}$ and the NiCo_2O_4 nanoparticles have a surface area of $31.2 \text{ m}^2 \text{ g}^{-1}$.

The spinel nanoparticles were further characterized by resistance measurements. Materials with spinel structure are generally insulating, however, defect formation and phase separation led to a population of itinerant charge carriers in the conductive band and to resistivity lowering. We measured the resistance using the two-point probe method due to its relatively high magnitude. NiCo_2O_4 had a resistivity of 0.83 k Ω , ZnCo_2O_4 of 320 k Ω , and NiMn_2O_4 of 8.8 M Ω ; the resistance of ZnMn_2O_4 was over 10 M Ω . For all the materials, the resistance was sufficiently low to avoid charging effects during the XPS and SEM measurements. The obtained results are in good agreement with the XRD measurements, where the lowest resistance was observed on the samples revealing phase separation.

XPS is a surface-sensitive technique with a maximum depth of approximately 10 nm, which allows the study of the topography of the spinel oxides, with detection limits at about 0.1 wt% of the material.^[21–23] XPS was applied to investigate the $\text{A}^{2+}/\text{B}^{3+}$ element ratio on the surface of the synthesized spinels and investigate the surrounding chemical environment of each ion, where high-resolution XPS spectra can give information on the site occupancy of a given ion in the lattice and its oxidation state. XPS was also applied to investigate the chemical composition of the spinels. The XPS survey (wide-scan) spectra (Figure 3) provided information on the metals present in the spinel oxides.

Figure 2. X-ray diffraction patterns of NiCo_2O_4 , NiMn_2O_4 , ZnCo_2O_4 , and ZnMn_2O_4 spinel nanocrystals. The impurity phases are marked.

Figure 3. XPS survey spectra of NiCo_2O_4 , NiMn_2O_4 , ZnCo_2O_4 , and ZnMn_2O_4 spinel oxides.

The high-resolution XPS spectra of 2p orbitals are shown in Figure 4. Since this technique is sensitive towards the chemical environment of the investigated elements, we can attribute an octahedral or tetrahedral position to a given cation in the spinel structure. The survey spectra yielded a Co/Ni ratio of 2.1 which is in relative good agreement with the spinel stoichiometry. The high-resolution spectra for the Ni2p and Co2p states in NiCo_2O_4 are shown in Figure 4. Co2p_{3/2} and Co2p_{1/2} have

maxima at 780.7 and 796.0 eV, respectively. This is in good agreement with the values reported in the literature.^[24] The chemical shift of the 2p_{1/2} and 2p_{3/2} peaks is not very sensitive towards oxidation.^[25] For the investigation of the cobalt position within the spinel lattice, the energy and intensity of the 2p satellite peaks and the separation between the 2p_{3/2} and 2p_{1/2} peaks are very useful.^[24] The presence of satellite peaks is typical for Co^{2+} in the octahedral position. The peak position and the separation energy of 15.3 eV between 2p_{1/2} and 2p_{3/2} indicate that the majority of cobalt is present as Co^{3+} at octahedral sites of the spinel lattice.^[26] The high-resolution XPS spectra for Ni2p in NiCo_2O_4 are shown in Figure 4 with maxima at

854.6 eV for 2p_{3/2} and 871.9 eV for 2p_{1/2}. In addition, satellite peaks can be observed at 860.2 and 878.0 eV. The obtained energies are comparable with the data found in the literature.^[24,26,27] The lower intensity of the 2p satellite peaks and the asymmetrical shape of 2p_{3/2} indicate the mixed chemical environment of the Ni^{2+} ions, which is present in both the octahedral and tetrahedral sites of the spinel lattice. At lower energy, the shoulder of the 2p_{3/2} peak can be observed at 852.8 eV.^[28] This indicates the presence of traces of elemental nickel on the surface. The mechanism of metallic nickel formation on the surface remains unclear; it can however, be related to the formation of CO during oxalate decomposition. In addition, the high-resolution O1s spectrum is shown in Figure S2. The maximum of the main peak at 529.5 eV is close to the values typically reported for the transition metal oxides.^[29] The asymmetrical tail of the O1s peak with a maximum at 531.1 eV originates from different surface species such as cobalt oxo-hydroxides, chemisorbed oxygen and carbon dioxide. The Co/Zn ratio in ZnCo_2O_4 obtained from the XPS survey spectra is 2.38, which is consistent with the excess Co determined by XRD and confirms the non-stoichiometry of the obtained material. The high-resolution XPS spectra of Co2p and Zn2p are consistent with these results. The maxima of Co2p_{3/2} and Co2p_{1/2} are located at 780.1 and 795.0 eV. These values are slightly lower than the values discussed in the previous paragraph for NiCo_2O_4 and are almost similar to the peak energies reported for Co2p in NiCo_2O_4 and Co2p in ZnCo_2O_4 .^[24,30] In addition, satellite peaks that are typical for Co^{2+} at the tetrahedral position were observed, indicating a partial substitution of Zn^{2+} . This is a typical behavior of cobalt–zinc spinels, where a part of the Zn^{2+} tetrahedral sites is substituted by cobalt. The positions of Zn2p_{1/2} and Zn2p_{3/2} at 1043.8 and 1020.8 eV, respectively, are only slightly higher compared to the values reported for

Figure 4. XPS high-resolution spectra of cations in NiCo_2O_4 , NiMn_2O_4 , ZnCo_2O_4 , and ZnMn_2O_4 spinel oxides.

ZnCo_2O_4 in the literature.^[30] The high-resolution O 1s spectrum is shown in Figure S2. The position of the maximum at 529.7 eV is almost identical with the value obtained for NiCo_2O_4 . Moreover, a satellite peak with a maximum at 531.6 eV is observed. Its origin is similar to that discussed for NiCo_2O_4 . However, a slightly broader shape may indicate a higher tendency to hydroxide formation as well as carbon dioxide and oxygen adsorption from the surrounding environment. The Mn/Ni ratio in the NiMn_2O_4 spinel was 3.65, which is higher compared to both the ideal stoichiometry ratio and that determined from XRD, indicating the presence of Mn-rich oxides on the material particles surface. The high-resolution XPS spectra of Mn 2p and Ni 2p are shown in Figure 4. $\text{Mn}2\text{p}_{3/2}$ and $\text{Mn}2\text{p}_{1/2}$ have maxima at 641.5 and 653.2 eV respectively. These data were reported for the NiMn_2O_4 stoichiometric spinel.^[31] No significant broadening or high-intensity satellite

peaks indicate the dominant presence of Mn^{3+} in the octahedral sites of the spinel lattice. The high-resolution XPS spectra of Ni 2p are shown in Figure 4. The $\text{Ni}2\text{p}_{3/2}$ has a maximum at 854.6 eV and $\text{Ni}2\text{p}_{1/2}$ at 872.3 eV, with the satellite peaks located at 861.3 and 878.7 eV. A slight shift towards higher energy levels is observed in comparison with Ni 2p in NiCo_2O_4 . In the high-resolution spectra of NiMn_2O_4 , the shoulder located at 852.8 eV, clearly visible in the NiCo_2O_4 sample, has not been observed. The intensity of the satellite peaks of Ni^{2+} indicates a partial occupation of the octahedral lattice sites by Ni^{2+} in high spin configuration. The high-resolution O 1s peak has a maximum at 529.5 eV, which has been also observed in other spinels and is typical for the O 1s energy in 3d element oxides. The shoulder of the O 1s peak is located at 531.2 eV and has a significantly lower energy compared to the other investigated samples. This can originate from well-developed octahedral particles with lower surface area observed by SEM. This led us to a conclusion that the intensity of the O 1s peak shoulder associated with adsorbed oxygen and surface degradation is strongly dependent on the morphology and surface quality of the synthesized material. The high-resolution O 1s spectrum is shown in Figure S2. The Mn/Zn ratio in the ZnMn_2O_4 spinel was 5.01, which is more than two times that of the stoichiometric material. This indicates a high surface enrichment of Mn oxides on the spinel surface. The high-resolution spectra of Mn 2p are shown in Figure 4. The $\text{Mn}2\text{p}_{3/2}$ and $\text{Mn}2\text{p}_{1/2}$ are located at 642.4 and 653.7 eV, respectively. The slight shift towards higher energy levels can originate from the Mn oxides precipitated on the surface; however, their concentration is very low and no additional phases were detected by X-ray diffraction. These values are almost similar to the data reported for ZnMn_2O_4 spinel nanostructures.^[32] The high-resolution XPS spectra of Zn 2p yield maxima at 1020.6 eV for $\text{Zn}2\text{p}_{3/2}$ and 1043.6 eV for $\text{Zn}2\text{p}_{1/2}$. These results are only slightly lower in comparison with the values reported in the literature.^[32] The peaks originating from the Zn 2p states are significantly broader compared to those obtained in ZnCo_2O_4 . This can be related to the surface reaction of Zn^{2+} with humidity in the atmosphere, and hence to the formation of zinc hydroxides or surface states and defected surface states within the Zn–Mn spinel. This interpretation is also supported by the high-resolution XPS spectra of the O 1s peak (Figure S2) where the maximum originating from a transition metal oxygen bond (529.5 eV) has an even lower intensity than the O 1s peak resulting from surface states, hydroxides and other hydrolysis products formed on the surface (531.3 eV). This indicates that within the investigated group of mixed oxides, ZnMn_2O_4 exhibits the highest tendency towards surface degradation and decomposition. This is also documented by electrochemical characterization using the hydrogen evolution reaction (HER), where no electrocatalytic properties were detected for this material.

Following the detailed characterizations of the mixed-valence transition-metal oxides, it is of equal importance to investigate how the abilities of these materials to catalyze the reduction of O_2 in potassium hydroxide (KOH) correspond to their topographies. The ORR catalytic activities of the spinel

Figure 5. A) Linear-scan voltammograms resulting from the electrochemical reduction of oxygen in air-saturated potassium hydroxide (0.1 M) using a bare glassy carbon electrode (black dashed line), and GC electrodes modified with NiCo₂O₄ (red line), NiMn₂O₄ (blue line), ZnCo₂O₄ (pink line) and ZnMn₂O₄ (green line). Scan rate: 0.1 V s⁻¹. B) Reduction onset potentials of bare GC, NiCo₂O₄, NiMn₂O₄, ZnCo₂O₄ and ZnMn₂O₄ from linear-scan voltammograms. The error bars represent RSD ($n=3$, 95% confidence interval).

oxides were evaluated by linear-scan voltammetry (LSV). Figure 5A illustrates the linear-scan voltammograms obtained from the electrochemical reduction of oxygen in air-saturated 0.1 M KOH, while Figure 5B compares the reduction onset potentials of bare glassy carbon (GC) and the spinel oxides. Figure S3 is shown for comparison in an N₂-purged solution.

It may be observed from Figure 5B that the manganese-based spinel oxides NiMn₂O₄ and ZnMn₂O₄ showed onset potentials similar to that of bare GC (−266 mV). On the other hand, NiCo₂O₄ and ZnCo₂O₄ displayed much less negative reduction onset potentials of −147 and −239 mV, respectively. Hence, the much less negative onset potentials suggest significant electrocatalytic activities of the cobalt-based spinel oxides toward the ORR, which may be attributed to the presence of cobalt oxide. Moreover, it is worth to note that the NiCo₂O₄ spinel displayed a greater enhancement in the kinetics of ORR compared to ZnCo₂O₄, and exhibited the greatest electrocatalytic activity among the spinel oxides. The difference in electrocatalytic performances of NiCo₂O₄ and ZnCo₂O₄ may be explained by several factors: 1) Differences in the structure and composition, where the cobalt in NiCo₂O₄ is dominantly present as Co³⁺, whereas a partial substitution of Zn²⁺ by Co²⁺ at the tetrahedral sites was observed in ZnCo₂O₄. 2) Differences in the substitutions of 2+ elements; it is observed that Ni²⁺

spinel oxides generally performed better than their respective Zn-based counterparts and also phase separation with simultaneous formation of NiO.

3. Conclusions

Mixed-valence transition-metal oxides were prepared and the exceptional catalytic behavior of cobalt-based spinel oxides (NiCo₂O₄ and ZnCo₂O₄) for the ORR was demonstrated providing a promising alternative to noble metals in preparing highly efficient catalysts for the ORR. In addition, the Ni-substituted spinel oxides generally outperformed their Zn-based spinel counterparts. Of the four spinel oxides investigated, NiCo₂O₄ exhibited the greatest electrocatalytic activity toward the ORR. Differences in the structure and composition exist where the cobalt in NiCo₂O₄ is dominantly present as Co³⁺, whereas a partial substitution of Zn²⁺ by Co²⁺ at the tetrahedral sites in ZnCo₂O₄ was observed. These findings have strong implications in the design and development of advanced ORR electrocatalysts for renewable energy applications.

Experimental Section

Materials

Isopropanol (IPA) was purchased from Merck. Glassy carbon (GC) as well as the Ag/AgCl reference and Pt counter electrodes were obtained from Autolab (The Netherlands). Zinc acetate dihydrate, cobalt acetate tetrahydrate and oxalic acid dihydrate were obtained from Penta, Czech Republic. Manganese acetate tetrahydrate and nickel acetate tetrahydrate were obtained from Sigma-Aldrich, Czech Republic.

Synthesis

For the synthesis of spinels with the general formula XY₂O₄, acetates in 0.05 mol L⁻¹ concentrations, and oxalic acid in 0.05 mol L⁻¹ concentrations, were used. The oxalates were precipitated by reaction of stoichiometric amounts of X and Y elements with oxalic acid in 10% molar excess. The precipitate was formed by slow dropping of an oxalic acid solution into a vigorously stirred metal acetate solution. The precipitate was filtered out by suction and dried at 100 °C for 24 h. The spinels were formed by high-temperature decomposition of the oxalate precursor. The oxalate precursor was placed in alumina crucible and heated at 450 °C for 2 h in air. The rate of heating and cooling was 10 °C min⁻¹.

Instrumentation

Scanning electron microscopy (SEM) images were acquired using a Joel 7600F SEM instrument (JEOL, Japan) operating at 2 kV. Energy-dispersive X-ray spectroscopy (EDX) data were recorded (JEOL, Japan) at 15 kV. X-ray photoelectron spectroscopy (XPS) measurements were performed using a Phoibos 100 spectrometer and a monochromatic Mg X-ray radiation source (SPECS, Germany). Survey (wide-scan) spectra were recorded for each sample. For the SEM, SEM-EDX, and XPS measurements, the samples were prepared by coating a uniform layer of the materials under study on a carbon conductive tape. All voltammetric experiments were carried out using a μAutolab type III electrochemical analyzer (Eco

Chemie, The Netherlands) connected to a computer and controlled by the NOVA, Version 1.8.17, software. Electrochemical experiments were conducted in an electrochemical cell (5 mL) at room temperature. A three-electrode configuration was employed. A platinum electrode served as the auxiliary electrode, and an Ag/AgCl electrode functioned as the reference electrode. All electrochemical potentials in this paper are stated versus the Ag/AgCl reference electrode. X-ray powder diffraction data were collected at room temperature with an X'Pert PRO θ - θ powder diffractometer with parafocusing Bragg-Brentano geometry using CuK α radiation ($\lambda = 1.5418$ Å, $U = 40$ kV, $I = 30$ mA). Data evaluation was performed using the software package HighScore Plus 3.0e. The surface area was measured by a sorption analyzer Coulter SA 3100 (Backman Coulter). The samples were outgassed for 4 h at 150 °C under high vacuum prior to the sorption experiments. A TCD nitrogen-cooled (77 K) detector was used for the evaluation of the results using the BET and Kelvin equations. To measure the electrical resistivity of the spinel nanoparticles, the powder (100 mg) was compressed into a capsule ($1/4$ inch diameter) at a pressure of 400 MPa for 30 s. The resistivity of the capsules was measured by a two-point technique using an Agilent 34970 A data acquisition/switch unit. The contact distance on the pellets with a thickness of about 0.5 mm was 3 mm.

Cyclic Voltammetry

The GC working electrode surfaces were renewed by polishing with 0.05 μm alumina particles on a polishing pad. The spinel oxides (1 mg mL $^{-1}$) were dispersed in 2.5:1 v/v water/isopropanol mixed solvent. The suspension was then subjected to ultrasound treatment for at least 30 min sonication to form a homogenous ink. Next, the suspension (1 μL) was pipetted onto the GC electrode surface and left to evaporate at room temperature. A film of randomly distributed material is hence generated on the GC electrode surface. Cyclic voltammetry experiments were performed at a scan rate of 100 mV s $^{-1}$ in a potassium hydroxide solution (0.1 M) saturated with air for the ORR. In control experiments, the cyclic voltammetry measurements (Figure S2) were performed in N $_2$ by bubbling highly purified nitrogen through the experimental solutions for at least 20 min.

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Ultrapure Molybdenum Disulfide Shows Enhanced Catalysis for Hydrogen Evolution over Impurities-Doped Counterpart

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The development of electrocatalysts to meet the requirements of renewable energy applications has seen much attention placed on transition-metal dichalcogenide (TMD) materials owing to their promising properties. In particular, the strategy of atomic doping has garnered some success in tuning the electronic properties and harnessing the vast potential that TMDs can offer in the catalysis of the hydrogen evolution reaction (HER). Moreover, with computational studies reporting the promising effects of transition-metal doping, such a strategy has been adopted with much enthusiasm. Herein, we consider one of the most prevalent TMDs, that is, MoS₂, and the possible presence of impurities arising from its preparation method and starting materials that may act as dopants to affect its electronic and catalytic properties. An ultrapure MoS₂ material was synthesized and compared with a relatively impure MoS₂ sample obtained commercially. Ultrapure MoS₂ was found to outperform its impurities-doped counterpart in HER catalysis. These findings not only provide valuable insight into the influence of parts-per-million concentrations of impurities on the catalytic activity of TMD materials but also highlight the importance of the intentional and proper design of atomic doping to realize its true effects. At the same time, the need for a more in-depth understanding and evaluation of the benefits of the atomic-doping strategy in the experimental setting as a means to harness the potential of TMDs as catalysts for hydrogen evolution is also revealed.

Transition-metal dichalcogenides (TMDs) have been placed in the spotlight for their layered structure, which is akin to that of graphite. Large variations in the band gaps of TMD materials confer a wide spectrum of electronic properties ranging from insulating to metallic.^[1,2] For this reason, they have been extensively explored for applications such as energy storage and conversion,^[3,4] lubrication,^[5] sensing,^[6,7] and catalysis.^[8,9] Of par-

ticular interest is their outstanding catalytic activity towards the hydrogen evolution reaction (HER), which could support the generation of hydrogen at high efficiencies and low costs in comparison to precious Pt-based catalysts.^[10–12]

To harness the vast potential that TMDs can offer in HER catalysis, significant effort has been directed to altering the structure and electronic properties of TMD materials. One of the strategies employed is atomic doping, for which dopant atoms can directly substitute the atoms in the lattice or in the interstitial sites between atoms in the crystal lattice. Moreover, it is also possible for dopant atoms to intercalate between layers in the context of TMDs.^[13] Hitherto, the strategy of atomic doping has garnered some success in enhancing the catalytic activity of TMDs in the HER. Specifically, previous reports on MoS₂ doped with vanadium,^[14] rhenium,^[15] and cobalt^[16] resulted in higher HER efficiencies. Computational studies also determined the potential positive effects on the HER catalytic activity of various transition metal doped (i.e., Pt, Ni, Ru, Rh, Co, Fe, Mn, Ta, V, Nb, Cr, Os, Ir, and Re) MoS₂ through tuning the adsorption free energy for hydrogen (ΔG_H) on the edges closer to the thermoneutral value.^[17]

Naturally occurring MoS₂ ore (molybdenite) is found to contain transition metals and semimetal impurities by substitution and/or intercalation.^[18] This consequently highlights concerns regarding the presence of impurities in MoS₂ arising from its preparation methods and starting materials that may act as dopants to influence its electronic and, hence, catalytic properties. Furthermore, Addou et al. reported that the presence of impurities at the parts-per-million (ppm) level may be a contributing factor to the variations in the electronic properties of MoS₂.^[18] Therefore, although it is of paramount importance to investigate the role that impurities can play in affecting the HER efficiency of MoS₂, no work has been reported on this topic thus far.

Herein, we synthesized MoS₂ from its elements, which resulted in an ultrapure MoS₂ material. This allowed us to compare the HER catalytic activity of ultrapure MoS₂ with that of a relatively impure MoS₂ sample obtained commercially. Through this comparison, we wished to address the following basic questions: 1) Does the elemental purity of MoS₂ influence its catalytic activity towards the HER? 2) If so, can relevance be drawn to the effects of doping on the electrocatalytic activity of MoS₂? For this purpose, the MoS₂ materials were characterized by using scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDS), X-ray diffraction (XRD), laser diffraction, X-ray fluorescence (XRF), and electrochemical techniques. We show that ultrapure MoS₂ shows higher HER cata-

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lytic activity than its impurities-doped counterpart. Atomic doping is a strategy generally employed to elicit an improvement in the electrocatalytic activity of TMDs, and this work challenges such a prevalent notion while emphasizing the need for a more in-depth understanding of this strategy.

Considering MoS₂ as one of the most highly investigated TMDs, an ultrapure MoS₂ material was first synthesized by a simple and scalable method from its elements (of high purity), herein denoted as MoS₂-M. A side-by-side comparison of MoS₂-M was performed against MoS₂ obtained from Alfa Aesar (MoS₂-AA). Characterization of MoS₂-AA and MoS₂-M was done with SEM, laser diffraction, XRD, EDS, and XRF. Furthermore, linear sweep voltammetry (LSV) was employed to test their electrocatalytic performance for the hydrogen evolution reaction (HER).

The platelet structures of MoS₂-AA and MoS₂-M are shown in their SEM images (Figure S1, Supporting Information), whereas EDS (Figure S2 and Table S1) confirms the successful preparation of MoS₂-M from its elements. To investigate if the elemental purity of MoS₂ would affect the HER catalytic activity, it is crucial that MoS₂-AA and MoS₂-M be comparable in terms of other key factors that may influence their HER efficiency: particle size and phase purity.^[19,20] Figure 1 illustrates the particle-size distributions of the MoS₂ materials. Both MoS₂-AA and

Figure 1. Particle-size distributions of MoS₂-AA and MoS₂-M.

MoS₂-M are observed to have comparable particle sizes with mean diameters of 24.1 and 15.7 μm, respectively. The XRD patterns of MoS₂-AA and MoS₂-M are represented in Figure 2. The phase purities of both MoS₂ materials were verified and found to be a single hexagonal 2H phase. Diffraction lines with a relative intensity of 1% or higher, and which correspond to the pattern from the PDF card for the 2H phase (*P6₃/mmc*), are marked. Diffraction patterns with lower intensities are not visible owing to the high preferential orientation originating from the layered structure of MoS₂. Subsequently, XRF was performed to analyze the elemental compositions of MoS₂-AA and MoS₂-M (Table 1). In addition to the expected Mo metal and S chalcogen, trace amounts of W (0.02 wt%) and parts-per-million amounts of transition metals Y (410 ppm), Mn (65 ppm),

Figure 2. X-ray diffraction (XRD) patterns of MoS₂-AA and MoS₂-M. The unsigned peak for MoS₂-M at $2\theta \approx 15^\circ$ originates from K- β line diffraction of MoS₂ (001) reflection.

Fe (940 ppm), and Zn (168 ppm), and semimetal Si (560 ppm) are shown to be present in the MoS₂-AA sample. K (297 ppm) and Ca (252 ppm) are also detected in MoS₂-AA. In contrast, elemental analysis of the synthesized MoS₂-M sample shows a low concentration of Mg (89 ppm) as the only impurity. Contamination with alkali metals (Na, K) and alkaline earths (Ca, Mg) is very common, as they can originate from the vessel, surrounding environment, or contamination from the production process (e.g., crucible and vessel material). Hence, with comparable particle sizes and phase purities but with significantly different elemental purities, MoS₂-AA and MoS₂-M serve as excellent candidates for the investigation of the effects of impurities on the HER catalytic activity of MoS₂.

The HER catalytic activities of MoS₂-AA and MoS₂-M were studied by LSV in sulfuric acid (0.5 M) at a low scan rate (2 mV s⁻¹), and the obtained HER polarization curves and Tafel plots are displayed in Figure 3. Bare glassy carbon (GC) served as a reference material. For the purpose of comparison, the discussion will revolve around three commonly adopted HER performance indices: overpotential (Figure S3 a), onset poten-

Figure 3. HER polarization curves. a) Linear sweep voltammograms for the HER in acidic electrolyte on MoS₂-AA (—) and MoS₂-M (—) and b) their respective Tafel plots. Bare GC (----) serves as a control experiment. Conditions: background electrolyte, H₂SO₄ (0.5 M); scan rate, 2 mV s⁻¹. All measurements were performed relative to the Ag/AgCl reference electrode and are corrected to RHE potentials.

Table 1. X-ray fluorescence analysis of MoS₂-AA and MoS₂-M.^[a]

Sample	S/Mo Ratio	Mo [wt %]	W [wt %]	S [wt %]	Cd [ppm]	Mg [ppm]	Y [ppm]	Si [ppm]	K [ppm]	Ca [ppm]	V [ppm]	Mn [ppm]	Fe [ppm]	Zn [ppm]
MoS ₂ -AA	2.05	59.2	0.02	40.51	0	0	410	560	297	252	0	65	940	168
MoS ₂ -M	1.95	60.53	0	39.46	0	89	0	0	0	0	0	0	0	0

[a] Concentrations of all other elements were below 0.01 wt %.

tial (Figure S3b), and Tafel slope (Figure S3c). The overpotentials were measured at 10 mA cm^{-2} , whereas the onset potential is defined as the potential at which the current density is -0.07 mA cm^{-2} . In general, higher HER catalytic efficiencies are characterized by lower overpotentials and less negative onset potentials, as less energy is necessary to drive the reaction. Moreover, Tafel slope analysis helps us to understand the mechanism of hydrogen evolution on the MoS_2 surfaces. The rate-limiting steps may be as follows:^[21]

Volmer adsorption step [Eq. (1)]:

Heyrovsky desorption step [Eq. (2)]:

Tafel desorption step [Eq. (3)]:

From Figure 3a, it is observed that both MoS_2 -AA and MoS_2 -M display significantly superior HER catalytic activities than the bare GC control. However, owing to their bulk nature, the MoS_2 materials generally present lower HER activity than commercially available Pt-based catalysts with a reported Tafel slope of approximately 30 mV dec^{-1} and an overpotential of approximately 0.05 V versus the reversible hydrogen electrode (RHE).^[11, 12, 20] Tafel slope analysis (Figure 3b) shows that MoS_2 -AA and MoS_2 -M have similar values of 172 and 175 mV dec^{-1} , respectively. Given that the Tafel slopes are higher than the theoretical value of 120 mV dec^{-1} , it is implied that the rate-limiting step of the HER on both MoS_2 surfaces is Volmer adsorption. Moreover, a more comprehensive comparison in terms of the overpotentials and onset potentials reveals that MoS_2 -AA exhibits an overpotential of 0.82 V versus RHE and an onset potential of -0.41 V versus RHE, whereas MoS_2 -M affords a lower overpotential of 0.77 V versus RHE and a less negative onset potential of -0.38 V versus RHE. From these results, we conclude that ultrapure MoS_2 -M elicits a higher HER efficiency than its impurities-doped counterpart. Considering the significant amounts of Fe, Zn, and Mn transition metals present in MoS_2 -AA relative to those in MoS_2 -M, this work presents a surprising yet profound observation. Furthermore, our results are in agreement with a recent report that Nb- and Ta-doped bulk MoS_2 displayed inferior HER catalytic activity than its undoped counterpart.^[22]

In summary, the effect of elemental purity on the catalytic activity of MoS_2 , one of the most representative transition-metal dichalcogenide (TMD) materials, in the hydrogen evolution reaction (HER) was investigated. We reported a simple and scalable preparation method for ultrapure MoS_2 from its elements. Upon comparing the synthesized MoS_2 -M material with commercially obtained MoS_2 -AA, the latter was found to contain significant amounts of elemental impurities. Despite the presence of Fe, Zn, and Mn transition-metal impurities, superi-

or HER catalytic activity was observed for the MoS_2 material with higher elemental purity (MoS_2 -M). We provided insight into the influence of impurities on the catalytic activity of TMD materials. Whereas results from previous computational studies point to the possible enhancement in HER catalytic activity by doping with these transition metals, our investigation emphasizes the importance of the intentional and proper design of this strategy to realize its true effects in an experimental setting. With many factors to consider in the experimental context, elemental purity as one, we believe that our work highlights the need for a more in-depth understanding of the atomic doping strategy and its true benefits for harnessing the endless potential of TMDs as catalysts for hydrogen evolution.

Experimental Section

Materials

Mo (99.999%) was obtained from Alfa Aesar, Germany. Sulfur (99.999%) was obtained from Strem, Germany. Sulfuric acid was purchased from Sigma-Aldrich, Singapore. MoS_2 -AA was purchased from Alfa Aesar, Czech Republic. Glassy carbon (GC), the Ag/AgCl reference, and Pt counter electrodes were acquired from Autolab (The Netherlands).

Instrumentation

Scanning electron microscopy (SEM) images and energy-dispersive X-ray spectroscopy (EDS) data were acquired by using a Jeol 7600F SEM instrument (Jeol, Japan) operating at 5 kV and 15 kV , respectively. X-ray powder diffraction (XRD) data were collected with a Bruker D8 Discoverer powder diffractometer (Bruker AXS, Germany) at room temperature with parafocusing Bragg-Brentano geometry by using CuK_α radiation ($\lambda = 1.5418 \text{ \AA}$, $U = 40 \text{ kV}$, $I = 40 \text{ mA}$). Data were scanned with an ultrafast LYNXEYE XE detector over the angular range of 5 to 80° (2θ) with a step size of 0.019° (2θ) and a counting time of 1 s step^{-1} . The detection limit of the XRD analysis was approximately $0.5 \text{ at\%}$. The software package HighScore Plus 3.0e was employed for subsequent data evaluation. XRF analysis was performed by using an ARL PERFORM'X sequential WD-XRF spectrometer equipped with an Rh anode end-window X-ray tube type 4GN fitted with a $50 \text{ }\mu\text{m}$ Be window. All peak-intensity data were collected by Oxsas software under vacuum. The generator settings-collimator-crystal-detector combinations were optimized for all 82 measured elements with an analysis time of 6 s per element. The detection limit of the XRF analysis was in the range of 1 to 10 ppm depending on the element. The obtained data were evaluated by standardless software Uniquant 5 integrated in Oxsas. Concentration estimations could be made for trace concentrations of approximately $0.01 \text{ \%}$ of the absolute value, for trace concentrations (below 1000 ppm) up to approximately $0.2 \text{ \%}$ of the absolute value, and for concentrations exceeding $10 \text{ wt\%}$. The analyzed powders were pressed into pellets approximately 5 mm thick and with a diameter of 40 mm without any binding agent and covered with a $4 \text{ }\mu\text{m}$ supporting polypropylene (PP) film. All voltammetric experiments were performed by using a $\mu\text{Autolab}$ type III electrochemical analyzer (Eco Chemie, The Netherlands) connected to a computer and controlled by NOVA software, version 1.8.17. Electrochemical experiments were conducted in an electrochemical cell (5 mL) at room temperature. A three-electrode setup was employed. A platinum electrode served as the auxiliary electrode, a Ag/AgCl electrode functioned as the reference electrode, and

a glassy carbon (GC, 3 mm diameter) electrode was employed as the working electrode.

Procedures

Synthesis of MoS₂-M: Stoichiometric amounts of molybdenum and sulfur corresponding to the transition-metal dichalcogenide (5 g) were placed in a quartz glass ampoule. The ampoule was evacuated to a base pressure below 5×10^{-3} Pa and melt-sealed by an oxygen–hydrogen torch. Following that, the ampoule was heated at 500 °C for 24 h and then at 800 °C for 48 h. The rate of heating and cooling was 5 °C min⁻¹.

HER studies on MoS₂: The HER catalytic performance of the MoS₂ materials was performed on glassy carbon (GC) electrodes. GC electrodes were first renewed on a polishing pad by using an alumina particle (0.05 μm) slurry and were then washed with ultrapure water. Subsequently, 1 mg mL⁻¹ suspensions of TMD materials were prepared in ultrapure water and ultrasonicated for 10 min prior to each electrochemical measurement to ensure homogeneity of the material under study. An aliquot (4 μL) was drop-casted on the electrode surface and dried at room temperature to yield a randomly distributed film of desired material (4.0 μg) on the GC electrode surface. The materials of interest included MoS₂-AA and MoS₂-M. HER measurements were performed by using linear sweep voltammetry (LSV) at a scan rate of 2 mV s⁻¹ in a 0.5 M H₂SO₄ electrolyte. Linear sweep voltammograms were represented versus reversible hydrogen electrode (RHE). Calculation of the measured potentials were based on Equation (4):

$$E_{\text{RHE}} = E_{\text{Ag/AgCl}} + 0.059 \times \text{pH} + E_{\text{Ag/AgCl}}^0 \quad (4)$$

in which $E_{\text{Ag/AgCl}}$ is the measured potential and the pH of H₂SO₄ (0.5 M) is set as zero. The standard potential of Ag/AgCl (1 M KCl) at 25 °C, $E_{\text{Ag/AgCl}}^0$ was then 0.235 V versus RHE.

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Conflict of interest

The authors declare no conflict of interest.

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3R phase of MoS₂ and WS₂ outperforms the corresponding 2H phase for hydrogen evolution†

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Herein, we compare the bulk, 2H and 3R phases of two most prevalent TMD materials: MoS₂ and WS₂. The 3R phase outperforms its 2H phase counterpart in hydrogen evolution reaction catalysis and is even comparable with the exfoliated, 1T phase in the case of MoS₂.

Transition metal dichalcogenides (TMDs) have gained recognition rapidly due to their layered structure, which is similar to that of graphite.^{1–3} The properties emerging from the anisotropy of TMD materials have been demonstrated to be useful for advanced energy storage and conversion,⁴ electrochemical catalysis,^{5,6} and sensing^{7–9} applications. Of particular interest is their high catalytic activity towards the hydrogen evolution reaction (HER),^{10–12} which could support efficient and low-cost production of hydrogen without reliance on precious Pt-based catalysts. Such a characteristic points to the importance of understanding the HER catalytic mechanism, and consequently, the synthesis of TMDs with different morphologies aimed at fine-tuning and improving their performances.

The key role played by sulphur-terminated edges of MoS₂ as active sites for the HER has been highlighted in recent theoretical studies.^{13,14} Superior electrochemical and catalytic activity was demonstrated at edge-sites not only for MoS₂, but also for other TMD materials like WS₂, MoSe₂ and WSe₂.^{15,16} Hence, fabrication of TMDs with increased edge-exposed surface with respect to the basal portion^{16,17} is one of the strategies out of the many proposed to enhance catalytic properties.¹⁸ Moreover, it is a well-known fact that the properties of TMD materials in their bulk states vastly differ from their exfoliated counterparts. In order to enhance the catalytic properties of TMD materials, a commonly employed method is thus, splitting the layers of bulk TMD materials into single or few layer sheets *via* Li intercalation

with organolithium compounds.^{19–21} Another key factor influencing the catalytic properties of TMD materials is their electrical conductivity. A material with high electrical conductivity enables better communication between the electron source and the active edge sites.²²

Transition-metal coordination by chalcogens and the stacking sequences of multiple layers of TMD materials can elicit different electronic properties. As illustrated in Fig. 1, a single-layered TMD generally presents either an octahedral or a trigonal prismatic coordination phase. In multi-layered TMDs, a large variety of polymorphic structures arise, as each individual layer can possess any of the two coordination phases. The three commonly found polymorphs are defined as 1T, 2H and 3R, where the digit is indicative of the number of layers in the crystallographic unit cell, and the latter designates the type of symmetry exhibited; T stands for tetragonal (*D*_{3d} group), H represents hexagonal (*D*_{3h} group),

Fig. 1 Metal coordinations and stacking sequences of TMD structural unit cells. Metal coordination can be either octahedral or trigonal prismatic. The octahedral coordination allows stacking sequences, which yield a tetragonal symmetry (1T). Dissimilar stacking sequences of trigonal prismatic single layers can give rise to different symmetries: hexagonal symmetry (2H) and rhombohedral symmetry (3R).

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and R denotes rhombohedral (C_{3v}^5 group). The 1T form displays metallic behaviour, while both 2H and 3R forms exhibit semi-conducting behaviour. Each TMD polymorph possesses unique structures and electronic properties from where different catalytic properties can emerge. Hence, it is imperative to make a comparison of different TMD polymorphs, aiming to understand the influence of polymorph structures on the catalytic properties of TMD materials. Previous works have reported a 2H $\rightarrow$ 1T phase transition when TMD materials are subjected to chemical exfoliation²³ and a comparison of the catalytic properties of 1T- and 2H-TMDs has shown that the exfoliated, metallic 1T phase performs as better catalysts than their bulk, semiconducting 2H counterparts for HER.²⁴ This was explained by the increased edge-exposed surface, reduced sheet size and enhanced electrical conductivity of the 1T phase. However, there has been no previous study and there is a lack of understanding of the catalytic properties of 3R-TMDs or comparison with their semiconducting 2H counterparts.

In this work, we wish to address the following basic questions: (1) is the catalytic activity towards HER influenced by the polymorph structure and if so, (2) is an additional chemical exfoliation step necessary for producing an efficient HER catalyst. For this purpose, we synthesized TMD materials (MoS_2 and WS_2) in their 3R phase, which allows us to perform a comparison of their HER catalytic activity with their corresponding 2H phase. The synthesis was performed from oxides using an alkali metal carbonate/sulphur melt. Based on X-ray diffraction (XRD), high resolution transmission electron microscopy (HR-TEM), scanning electron microscopy (SEM), laser diffraction, energy dispersive X-ray spectroscopy (EDS), X-ray fluorescence (XRF), X-ray photoelectron spectroscopy (XPS) and electrochemical analyses, we show that the 3R phase outperforms the 2H phase in HER catalysis. However, this improvement in the HER catalytic property presents in varying magnitudes for MoS_2 and WS_2 , and at different synthesis temperatures. Comparison with existing literature shows that in the case of MoS_2 , catalytic performance towards HER for the bulk, semiconducting 3R phase is on par with the exfoliated, metallic 1T phase.

Considering the two highly studied transition metal dichalcogenides (TMDs), *i.e.* MoS_2 and WS_2 , we first synthesized the 3R phase from their respective oxides with sulphur in carbonate flux according to previously established protocols at 550 °C.^{25,26} Herein, they are denoted as 3R- MoS_2 (550 °C) and 3R- WS_2 (550 °C). We studied their HER catalytic properties, and further investigated if the polymorph structure influences the HER catalytic performances of MoS_2 and WS_2 by making a comparison of the 3R phase TMDs with their corresponding 2H phases; 2H- MoS_2 (AA) or 2H- WS_2 (AA). The materials were fully characterised by using XRD, HR-TEM, SEM, laser diffraction, EDS, XRF, XPS and electrochemically tested as catalysts for hydrogen evolution.

The phase purity of each material was verified by X-ray diffraction (XRD). Fig. S1 (ESI[†]) represents the XRD patterns of the TMD materials. The 2H- MoS_2 (AA) (Fig. S1A, ESI[†]) and 2H- WS_2 (AA) (Fig. S1B, ESI[†]) were found as a single 2H phase and exhibiting a hexagonal structure, while 3R- MoS_2 (550 °C) (Fig. S1C, ESI[†]) and 3R- WS_2 (550 °C) (Fig. S1D, ESI[†]) were formed as a mixture of the rhombohedral 3R phase and the hexagonal 2H phase. The ratio evaluated from XRD gives a composition of

about 2.3 : 1 using the Rietveld refinement method. More details are given in the ESI[†] (Fig. S2). The presence of the desired 3R stacking of the atomic layers in 3R- MoS_2 (550 °C) and 3R- WS_2 (550 °C) was further confirmed by HR-TEM analyses in Fig. S3 and S4 (ESI[†]), respectively. Further characterisation of the materials was performed using SEM (Fig. S5, ESI[†]), which indicated the homogeneous formation of platelet structures, and laser diffraction measurements revealed comparable particle sizes of the TMD materials in their 2H phase and the respective 3R phase (Fig. S6, ESI[†]). 2H- MoS_2 (AA) and 3R- MoS_2 (550 °C) have mean particle diameters of 24.2 μm and 12.9 μm , respectively, while 2H- WS_2 (AA) and 3R- WS_2 (550 °C) exhibited mean particle diameters of 11.1 μm and 10.4 μm respectively. Moreover, the composition of each material was confirmed *via* EDS (Fig. S7–S10, ESI[†]) and XRF (Table S1, ESI[†]). The concentration of potassium originating from the potassium carbonate flux was 1.2 wt% and 1.0 wt% in 3R- MoS_2 (550 °C) and 3R- WS_2 (550 °C), respectively. In 3R- MoS_2 (550 °C), the concentration of tungsten lies below 0.01 wt%. However, in 3R- WS_2 (550 °C), the concentration of molybdenum is 0.15 wt%. In addition, traces of Zn were detected in 3R- MoS_2 (550 °C) (~ 0.01 wt%), and traces of Fe were found in 3R- WS_2 (550 °C) (~ 0.02 wt%). The concentrations of all other impurities were below 0.01 wt%. Overall, it was determined that the presence of impurities is insignificant in these samples and would not interfere with the electrochemical signals of the TMD materials.

Moving on with investigating the HER efficiency of the TMD materials, linear sweep voltammetry (LSV) was performed in 0.5 M sulphuric acid at a low scan rate of 2 mV s⁻¹. We studied the HER catalytic performances of the 3R phase and compared them to that of the corresponding 2H phase.

The polarization curves and Tafel plots of 3R- MoS_2 (550 °C) and 2H- MoS_2 (AA) are represented in Fig. 2A and B, and those of 3R- WS_2 (550 °C) and 2H- WS_2 (AA) are shown in Fig. 2C and D.

Fig. 2 HER polarization curves of 2H and 3R phase TMDs. Linear sweep voltammograms for HER in acidic electrolyte on (A) MoS_2 and (C) WS_2 compounds. Tafel plots for (B) MoS_2 and (D) WS_2 compounds. Conditions: background electrolyte, H_2SO_4 (0.5 M); scan rate, 2 mV s⁻¹. All measurements were performed relative to the Ag/AgCl reference electrode and are corrected to reversible hydrogen electrode (RHE) potentials.

Bare glassy carbon (GC) was included as a material of reference. We adopted three commonly used HER performance indices, namely the overpotential, onset potential and Tafel slope. Overpotentials were taken at -10 mA cm^{-2} , and the onset potential is defined as the potential at which the current density is -0.07 mA cm^{-2} . In general, lower overpotentials and less negative onset potentials indicate higher HER catalytic efficiencies, as less energy is required for the reaction to take place. An analysis of the Tafel slope gives us an idea of the mechanism of the hydrogen evolution on the different TMD surfaces, where the rate-determining step may be determined.²²

We observed similar and outstanding HER catalytic performances for both materials in the 3R phase. 3R-MoS₂ (550 °C) displayed an overpotential of 0.52 V vs. RHE, onset potential of -0.20 V vs. RHE , while 3R-Ws₂ (550 °C) showed an overpotential of 0.56 V vs. RHE, onset potential of -0.23 V vs. RHE . Tafel analysis of 3R-MoS₂ (550 °C) (113 mV dec^{-1}) and 3R-Ws₂ (550 °C) (111 mV dec^{-1}), which is close to the 120 mV dec^{-1} theoretical value, generally points to the Volmer adsorption as the limiting step. When compared with 2H-MoS₂ (AA), 3R-MoS₂ (550 °C) exhibited an overpotential, which was a significant 0.29 V lower. The relatively more negative onset potential of 2H-MoS₂ (AA) (-0.41 V vs. RHE) further exemplifies its poorer HER catalytic performance. In the case of Ws₂, the 2H phase also displayed a poorer HER catalytic performance than the 3R phase, even though to a lesser extent. 2H-Ws₂ (AA) displayed an overpotential (0.73 V vs. RHE), which is 0.17 mV less than that of 3R-Ws₂ (550 °C), and a more negative onset potential of -0.30 V vs. RHE . In general, the 2H phase has poorer HER efficiencies than the 3R phase for MoS₂ and Ws₂, with the Tafel slopes of 2H-MoS₂ (AA) (172 mV dec^{-1}) and 2H-Ws₂ (AA) (151 mV dec^{-1}) similarly directing us to the Volmer adsorption as the HER rate-determining step. Moreover, a second TMD material in the 2H phase with different particle sizes was included as an additional control for both MoS₂ and Ws₂. The additional materials are denoted as 2H-MoS₂ (SA) and 2H-Ws₂ (SA), respectively. 2H-MoS₂ (SA) and 2H-Ws₂ (SA) both exhibit a single hexagonal 2H phase (Fig. S11, ESI†) and their particle sizes are 1.8 μm and 4.1 μm , respectively (Fig. S12, ESI†). 2H-MoS₂ (SA) displayed Volmer adsorption as the HER rate-determining step, an overpotential of 0.56 V vs. RHE and an onset potential of -0.22 V vs. RHE . Despite having particle sizes ~ 7 fold smaller, 2H-MoS₂ (SA) showed an inferior HER catalytic performance compared to 3R-MoS₂ (550 °C). A similar observation is made with Ws₂. The additional control experiment further confirms the notion that the 3R phase outperforms its corresponding 2H phase for hydrogen evolution.

The stellar HER efficiency of the 3R phase prompted us to make a comparison with the exfoliated, 1T phase, studied previously by our group.^{6,24} 1T-MoS₂ and 1T-Ws₂ were prepared by the exfoliation of 2H-MoS₂ (SA) and 2H-Ws₂ (SA), respectively which would result in particle sizes of the 1T phase TMDs being similar to or slightly smaller than their 2H counterparts. While 3R-MoS₂ (550 °C) exhibited an overpotential of 0.52 V vs. RHE and a Tafel slope of 113 mV dec^{-1} , 1T-MoS₂ was reported to display a similar overpotential of about 0.55 V vs. RHE and a Tafel slope of 99 mV dec^{-1} . Despite being in its bulk form and

with bigger particle sizes, it was surprising to note that the HER catalytic performance of 3R-MoS₂ (550 °C) is comparable to that of the exfoliated, 1T-MoS₂. On the contrary, 3R-Ws₂ (550 °C) did not perform as good as the exfoliated, 1T-Ws₂; 1T-Ws₂ reported an overpotential of 0.35 V vs. RHE and a Tafel slope of 85 mV dec^{-1} .

In an attempt to optimise the synthesis conditions of 3R phase MoS₂ and Ws₂ for HER applications, the study was further extended to include two other synthesis temperatures; 650 °C and 750 °C. A comparison of the HER catalytic performances of the 3R phases synthesized at different temperatures was performed, and their polarization curves and Tafel plots are represented in Fig. S13 (ESI†). A careful analysis of their HER performance indicators (Fig. S14, ESI†) disclosed a general trend, applicable for both MoS₂ and Ws₂ materials. In both cases of MoS₂ and Ws₂, the 3R phase displayed superior or comparable HER performance to the 2H phase regardless of synthesis temperatures. However, as the synthesis temperature for the 3R phase is increased from 550 °C to 750 °C, we observed a decreasing trend in the HER efficiency. Hence, we report that the optimal temperature for the synthesis of 3R phase MoS₂ and Ws₂ for HER applications is 550 °C.

Delving deeper to understand the trend observed with different synthesis temperatures for the 3R phase MoS₂ and Ws₂, we consider four possible factors that may affect the HER efficiency of the materials; (1) 3R phase concentration, (2) crystallite size, (3) presence of impurities and (4) particle size. For this purpose, characterisation of the materials was carried out using XPS, XRD, EDS, XRF, SEM and laser diffraction. Fig. S15 (ESI†) shows the high resolution XPS spectra of the 3R phase MoS₂ and Ws₂ synthesized at 550 °C, 650 °C and 750 °C. Deconvolution analyses of the Mo bonding modes show the presence of 2H and/or 3R phases in the 3R-MoS₂ (550 °C), 3R-MoS₂ (650 °C) and 3R-MoS₂ (750 °C) materials with Mo 3d_{5/2} and Mo 3d_{3/2} at binding energies of ~ 229.0 and 232.5 eV , respectively. Similarly, deconvolution analyses of the W bonding modes show the presence of the 2H and/or 3R phases in the 3R-Ws₂ (550 °C), 3R-Ws₂ (650 °C) and 3R-Ws₂ (750 °C) materials with W 4f_{7/2} and Mo 4f_{5/2} at binding energies of ~ 33.2 and 35.3 eV , respectively. For comparison, the XPS spectra of 2H-MoS₂ and 2H-Ws₂ measured on single crystals are also shown (Fig. S16, ESI†). In order to distinguish between the 2H and 3R phases, X-ray diffraction was employed. The X-ray diffractograms of the 3R phase MoS₂ and Ws₂ synthesized at 550 °C, 650 °C and 750 °C are shown in Fig. S17 and S18 (ESI†), revealing that the 3R phase was successfully produced at all three temperatures. However, we observed that an increase in synthesis temperature generally led to a decrease in the 3R phase concentration. This is most significant for MoS₂, where the concentration of the 3R phase was only 30 wt% for the material synthesized at 750 °C. On the other hand, the concentration of 3R-Ws₂ was still about 45 wt% for the material synthesized at 750 °C. Moreover, decreasing FWHM of the reflection indicates improvement of crystallinity and increase of crystallite size with increasing synthesis temperature. Therefore, a reduction in the 3R phase concentration and increase in the crystallite size account for the decreasing trend in the HER catalytic performance of the 3R phase MoS₂ and Ws₂ with increasing synthesis temperature.

Further characterization was performed with EDS (Fig. S19–S22, ESI[†]), which presented no obvious changes in elemental composition regardless of the synthesis temperature. More in-depth analyses with XRF (Table S1, ESI[†]) reported that the concentration of potassium originating from potassium carbonate flux ranges from 0.05 to 1.2 wt% and decreases with increasing synthesis temperature. While only traces of tungsten were detected in 3R-MoS₂, the concentration of molybdenum ranges from 0.15 to 0.39 wt% in 3R-WS₂ and this concentration increases with synthesis temperature. Furthermore, similar traces of Zn and traces of Fe were found in 3R-MoS₂ and 3R-WS₂, respectively, regardless of synthesis temperature. The concentrations of all other impurities were below 0.01 wt%. It can be concluded that the changes in the elemental composition of 3R-MoS₂ and 3R-WS₂ synthesized at different temperatures were not significant enough to interfere with the electrochemical signals. Next, to study the relation between the particle size and the HER catalytic activity, we performed SEM (Fig. S5, ESI[†]) and laser diffraction measurements for particle size analysis (Fig. S6, ESI[†]). All samples are highly polydispersed with particle sizes in the range of 0.2–50 μm. However, a closer observation of the particle size distribution curves from laser diffraction measurements does not reveal any correlation with the synthesis temperature. From these results, we can conclude that the main influence on electrocatalytic activity will be related to the concentration of the 3R phase in the TMD materials and the size of individual crystallites.

A comprehensive study and comparison of the 3R and 2H phases of the two most representative TMD materials, MoS₂ and WS₂, has been conducted in relation to their catalytic properties towards the hydrogen evolution reaction. We report the synthesis of 3R-MoS₂ and 3R-WS₂ materials *via* a simple and scalable method at three different temperatures (550 °C, 650 °C and 750 °C). The optimal synthesis temperature was determined to be 550 °C for applications in industrially important electrocatalysis like HER, where high 3R phase concentration and small crystallite sizes were obtained. It was demonstrated that the 3R phase displayed superior HER efficiency compared to the 2H phase. Additionally, a more significant improvement in the HER catalytic activity was observed with 3R-MoS₂ than with 3R-WS₂. Specifically, it is surprising to note that the bulk, 3R-MoS₂ (550 °C) displayed comparable HER efficiency to that of the previously studied exfoliated, 1T-MoS₂. These results highlight the profound impact different polymorph structures of TMD materials have on their HER efficiencies, and further challenge the need for the commonly employed technique of exfoliation to enhance the catalytic properties of TMD materials. The insights from this work not only provide an

understanding of the underlying electrochemical activity of TMD materials, but are also particularly important in the search for highly efficient catalysts for hydrogen evolution.

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Catalytic properties of group 4 transition metal dichalcogenides (MX_2 ; $\text{M} = \text{Ti}, \text{Zr}, \text{Hf}$; $\text{X} = \text{S}, \text{Se}, \text{Te}$)†

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Interest in layered transition metal dichalcogenides (TMDs) has proliferated due to their properties, making them promising materials for electrochemical applications. Despite this, almost exclusive attention has been placed on group 6 TMDs. Hitherto, there has been a lack of understanding of the electrochemical behaviour of group 4 TMDs, which could serve as promising materials for electrochemical applications with their semiconducting properties. In this work, we provide a first insight into the inherent electrochemistry of group 4 TMDs (*i.e.*, TiS_2 , TiSe_2 , TiTe_2 , ZrS_2 , ZrSe_2 , ZrTe_2 , HfS_2 , HfSe_2 and HfTe_2) and their catalytic activities towards the hydrogen evolution reaction (HER). In particular, HfS_2 is electrochemically inert within a wide potential range of -1.8 V to $+1.8$ V vs. Ag/AgCl and displays superior HER activity compared to the other group 4 TMDs, making it a promising candidate for electrochemical sensing applications. Towards the aim of tuning their HER catalytic properties, the materials are subjected to electrochemical treatment. Electrochemical activation towards the HER is displayed for ZrSe_2 and HfSe_2 via both electrochemical oxidation and reduction, and TiTe_2 via electrochemical reduction. X-ray photoelectron spectroscopy (XPS) analysis points towards the importance of material purity in tuning the catalytic performances of group 4 TMDs. Such findings provide a foundational understanding of the electrochemistry of group 4 TMDs, which when applied appropriately can springboard this field of research towards achieving aims in electrochemical applications.

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Introduction

Layered inorganic materials, such as transition metal dichalcogenides (TMDs),^{1–7} metal dichalcogenides,^{8,9} transition metal oxides,¹⁰ black phosphorus^{11–13} and metal carbides,¹⁴ exhibit many scientifically and technologically important properties, often related to their anisotropy. Layered transition metal dichalcogenides, which have a general chemical formula MX_2 , where M is a transition metal (*i.e.*, Mo, Nb, and Hf) and X is a chalcogen, generally S, Se, or Te, stand out with their array of electronic and magnetic properties which give rise to a wide range of possible applications such as in batteries, supercapacitors and sensors.^{15–20} In particular, they have been reported to demonstrate high electrocatalytic activity towards hydrogen production^{21–23} and oxygen reduction.⁴ For these

reasons, enormous attention has been placed on studying the electrochemical behaviour of TMDs in order to advance their development for various electrochemical applications. However, it is astonishing to learn that a substantial percentage of the existing literature has been dedicated exclusively to the study of group 6 TMDs (*i.e.*, MoS_2 , WS_2 , MoSe_2 and WSe_2), and therefore, little is known and understood about the electrochemical behaviour of other TMD materials.

Considering the current upsurge of interest in exploiting TMDs for a multitude of electrochemical applications, we believe that an electrochemical study of TMD materials outside of the group 6 would be necessary and opportune. Previous studies on the electronic character of group 4 TMDs reveal that the predominantly layered materials are semiconductors,^{24–26} making them promising candidates for electrochemical applications. Nonetheless, there is limited understanding of the electrochemical behaviour of group 4 TMDs. Herein, we wish to address the gap in the literature by conducting a comprehensive study of the electrochemical behaviour of group 4 TMDs in order to provide a first insight and extensive comparison of group 4 TMD materials.

We characterize the materials using scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS), and phase purity is verified by X-ray diffraction (XRD). Voltammetric characterizations of the inherent electrochemistry of the materials with solid-state electrode modification are

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then performed. This serves to demonstrate the often overlooked, yet important and well-defined redox properties of group 4 TMDs. Subsequently, electrochemical treatments of the materials are performed according to their observed redox properties. In addition, X-ray photoelectron spectroscopy (XPS) is employed to investigate the surface composition of the materials before and after electrochemical treatment. Finally, the impact of electrochemical treatment on their surface composition and electrocatalytic applications is explored in detail for the hydrogen evolution reaction (HER).

Experimental section

Materials

Sulphur (99.999%), selenium (99.999%) and tellurium (99.999%) were obtained from STREM, Germany. Titanium (−150 mesh, 99.9%), zirconium (sponge, 99.5%) and hafnium (−325 mesh, 99.5%) were obtained from Alfa Aesar, Germany. Potassium phosphate dibasic, sodium phosphate monobasic, sodium chloride, and sulphuric acid were purchased from Sigma Aldrich. Glassy carbon (GC) and Ag/AgCl reference and Pt counter electrodes were obtained from Autolab (The Netherlands).

Instrumentation

Scanning electron microscopy (SEM) images were acquired by using a Jeol 7600F SEM instrument (Jeol, Japan) operating at 5 kV. Energy-dispersive X-ray spectroscopy (EDS) data were recorded on a Jeol 7600F instrument (Jeol, Japan) at 15 kV. X-ray diffraction was done with a Bruker D8 diffractometer in the Bragg–Brentano parafocusing geometry. CuK α radiation was used. Diffraction patterns were collected between 2θ of 10° and 80° . The obtained data were evaluated using HighScore Plus 3.0e software. X-ray photoelectron spectroscopy (XPS) measurements were performed using a Phoibos 100 spectrometer and a monochromatic Mg X-ray radiation source (SPECS, Germany). Survey (wide-scan) and high resolution spectra were recorded for each sample. For the XPS measurements of the untreated samples, sample preparation was done by coating a uniform layer of the materials under study on a carbon conductive tape. On the other hand, electrochemically treated samples were obtained by coating four consecutive 8.0 μL aliquots of 1 mg mL $^{-1}$ concentration on a Zensor screen printed electrode (eDAQ, Australia) and subjected to their respective oxidation or reduction potentials. After electrochemical treatment, the screen printed electrode was directly mounted on the XPS holder for characterisation. The relative sensitivity factors were also adopted for the calculation of chalcogen-to-metal ratios. All voltammetric experiments were carried out using a $\mu\text{Autolab}$ type III electrochemical analyser (Eco Chemie, The Netherlands) connected to a computer and controlled by the NOVA, Version 1.8.17, software. Electrochemical experiments were conducted in an electrochemical cell (5 mL) at room temperature. A three-electrode setup was employed. A platinum electrode served as the auxiliary electrode, an Ag/AgCl electrode functioned as the reference electrode and a glassy carbon (GC,

3 mm diameter) electrode was adopted as the working electrode.

Procedures

Synthesis procedure of TMDs. A stoichiometric amount of metal and chalcogen forming 6 g of transition metal dichalcogenide was placed in a quartz glass ampoule (18 mm $\times$ 100 mm; wall thickness 1.5 mm). The ampoule was evacuated to a base pressure below 5×10^{-3} Pa and melt sealed using an oxygen/hydrogen welding torch. The ampoule was placed in a muffle furnace and heated at 420°C for 48 hours, 24 hours at 600°C and 48 hours at 800°C . The heating and cooling rate was in all cases at 5°C min^{-1} .

Inherent electrochemistry of group 4 TMDs. The TMD materials were dispersed in ultrapure water with a concentration of 1 mg mL $^{-1}$. The suspensions were subjected to a first-time ultrasonication of 1.5 h to attain homogeneous dispersions. Subsequently, the samples were ultrasonicated for a period of 10 min prior to each electrochemical measurement in order to maintain the well-dispersed suspension of the material under study. The suspension (4 μL) was pipetted onto the GC electrode surface and left to evaporate at room temperature. A film of the randomly distributed material is produced on the GC electrode surface. The materials of interest include TiS $_2$, ZrS $_2$, HfS $_2$, TiSe $_2$, ZrSe $_2$, HfSe $_2$, TiTe $_2$, ZrTe $_2$ and HfTe $_2$. Fundamental electrochemical studies were carried out in phosphate buffered saline (PBS, 50 mM) as the background electrolyte at pH 7.2. Deoxygenation was achieved by bubbling highly purified nitrogen through the experimental solutions for at least 20 min. Cyclic voltammetry experiments were performed at a scan rate of 100 mV s $^{-1}$. The voltammetric scan was set to begin at 0 V; the potential at which redox processes were not expected to occur, and scanned toward -1.8 V followed by a reverse sweep to $+1.8$ V for the cathodic study and the reverse sequence was applied for the anodic study, before returning to 0 V.

Hydrogen evolution reaction (HER) studies on group 4 TMDs and the effect of electrochemical treatment. Hydrogen evolution reaction (HER) efficiency measurements of TMD materials were performed on glassy carbon (GC) electrodes. GC electrodes were first renewed using a 0.05 μm alumina particle slurry on a polishing pad and washed with ultrapure water. Subsequently, 1 mg mL $^{-1}$ suspensions of TMD materials were prepared in ultrapure water and ultrasonicated for a period of 10 min prior to each electrochemical measurement to ensure the homogeneity of the material under study. A 4 μL aliquot was deposited on the electrode surface and left to dry at room temperature to produce a randomly distributed film of 4.0 μg of desired material on the GC electrode surface. The materials of interest include TiS $_2$, ZrS $_2$, HfS $_2$, TiSe $_2$, ZrSe $_2$, HfSe $_2$, TiTe $_2$, ZrTe $_2$, HfTe $_2$, and their respective pretreated counterparts. HER measurements were carried out using linear sweep voltammetry at a scan rate of 2 mV s $^{-1}$ in a 0.5 M H $_2$ SO $_4$ electrolyte. Linear sweep voltammograms were represented *versus* reversible hydrogen electrode (RHE). The measured potentials are calculated using the equation $E_{\text{RHE}} = E_{\text{Ag/AgCl}} + 0.059 \times$

$\text{pH} + E_{\text{Ag}/\text{AgCl}}^0$, where $E_{\text{Ag}/\text{AgCl}}$ is the measured potential, pH of 0.5 M H_2SO_4 is given as zero, and $E_{\text{Ag}/\text{AgCl}}^0$ is the standard potential of Ag/AgCl (1 M KCl) at 25 °C which is 0.235 V.

Electrochemical treatment of group 4 TMDs. Electrochemical treatment was achieved by applying an oxidation or reduction potential for a period of 300 s to procure their oxidized or reduced forms. For different TMD materials, the treatment was performed in PBS (pH 7.2) at a specific potential determined by the redox peaks in the study of their inherent electrochemistry. TiS_2 was treated at +0.7 V (oxidation) or −1.5 V (reduction), ZrS_2 was treated at −1.1 V (reduction), TiSe_2 was treated at +1.5 V (oxidation) or −1.6 V (reduction), ZrSe_2 and HfSe_2 were treated at +1.5 V (oxidation) or −1.25 V (reduction), and TiTe_2 , ZrTe_2 and HfTe_2 were treated at +1.1 V (oxidation) or −1.6 V (reduction). After the treatment, the electrode was gently immersed in distilled water to remove any adsorbed materials before testing for HER efficiency.

Results and discussion

In order to characterize the materials of interest, we examine the surface morphologies of group 4 TMDs by SEM. Fig. 1a–c show the electron micrographs of group 4 TMDs, which in their bulk states, are observed as large polygonal stacks up to tens of micrometers across and thick. Moreover, these materials form well-dispersed suspensions in water (Fig. 1d) which are used for subsequent investigations. We further studied the surface elemental composition of group 4 TMDs using EDS (Fig. S1 and S2†), where the presence of their corresponding metal (Ti, Zr or Hf) and chalcogen (S, Se or Te) is confirmed. Furthermore, the presence of surface oxides has been determined in the materials and would be an important point of consideration in later experiments and discussions.

We further verified the purity of the synthesized TMDs by X-ray diffraction (XRD). Since EDS analysis is not sufficiently accurate and XPS is highly surface sensitive, XRD would be able to give information about phase purity and the possible formation of other phases with a sensitivity below 1 wt%. The obtained diffractograms are shown in Fig. 2 together with their corresponding PDF cards. All synthesized samples were found to be pure and without any additional phases.

Inherent electrochemistry of group 4 TMDs

In the current literature, fundamental understanding of the inherent electrochemistry of group 4 TMDs is inadequate. In order to address the gap in the literature, we conducted cyclic voltammetry (CV) in two scan directions: cathodic and anodic. CV is performed over a potential window from −1.8 V to +1.8 V in phosphate buffered saline (PBS) as the electrolyte at pH 7.2. The acquired voltammetric profiles of the respective scans reveal information about the electroactive surface moieties of the TMD materials. Furthermore, three consecutive scans were recorded to determine the nature of these electrochemical processes in the TMDs. The redox peaks observed arise from the type and number of electroactive groups present on the surface of the TMDs and are characteristic of various TMD materials.

Fig. 1 (a–c) Scanning electron microscopy (SEM) images of group 4 TMDs. Scale bars: 10 μm (a); 10 μm (b); and 100 μm (c). (d) Suspensions of group 4 TMD materials in ultrapure water at a concentration of 1 mg mL^{-1} .

Fig. 3 illustrates the cyclic voltammograms derived from the cathodic and anodic scans of TMD materials bearing the S chalcogen; TiS_2 , ZrS_2 and HfS_2 . The first scan of TiS_2 in the cathodic direction resulted in a reduction peak at −1.2 V (Fig. 3a) while that in the anodic direction showed a small oxidation peak at +0.4 V, followed by a mild reduction peak at −1.2 V (Fig. 3b). During the first cathodic scan, ZrS_2 showed a small reduction peak at −0.9 V (Fig. 3c) and no significant peaks when cyclic voltammetry was performed in the anodic direction (Fig. 3d). This may indicate that an initial oxidative treatment renders the electroactive moieties on ZrS_2 less susceptible to reduction. Observation from both cathodic and anodic scans of HfS_2 indicates no significant redox peaks and hence, indicates the lack of electroactive moieties on the surface of the material (Fig. 3e and f). In all cases, any redox electroactivity present is only observed in the first scans and not in the

Fig. 2 Diffractograms of group 4 TMDs with their corresponding PDF cards.

Fig. 3 Cyclic voltammograms derived from reduction and oxidation of MS_2 ; (a) cathodic scan and (b) anodic scan of TiS_2 ; (c) cathodic scan and (d) anodic scan of ZrS_2 ; (e) cathodic scan and (f) anodic scan of HfS_2 . Measurement of three scans is represented accordingly: scan 1 (black), scan 2 (red) and scan 3 (blue). Conditions: background electrolyte, PBS (50 mM), pH 7.2; scan rate, 100 mV s^{-1} ; all measurements were performed relative to the Ag/AgCl reference electrode.

Fig. 4 Cyclic voltammograms derived from reduction and oxidation of MSe_2 ; (a) cathodic scan and (b) anodic scan of $TiSe_2$; (c) cathodic scan and (d) anodic scan of $ZrSe_2$; (e) cathodic scan and (f) anodic scan of $HfSe_2$. Measurement of three scans is represented accordingly: scan 1 (black), scan 2 (red) and scan 3 (blue). Conditions: background electrolyte, PBS (50 mM), pH 7.2; scan rate, 100 mV s^{-1} ; all measurements were performed relative to the Ag/AgCl reference electrode.

Fig. 5 Cyclic voltammograms derived from reduction and oxidation of MTe₂: (a) cathodic scan and (b) anodic scan of TiTe₂; (c) cathodic scan and (d) anodic scan of ZrTe₂; (e) cathodic scan and (f) anodic scan of HfTe₂. Measurement of three scans is represented accordingly: scan 1 (black), scan 2 (red) and scan 3 (blue). Conditions: background electrolyte, PBS (50 mM), pH 7.2; scan rate, 100 mV s⁻¹; all measurements were performed relative to the Ag/AgCl reference electrode.

subsequent scans, pointing toward the chemical irreversibility of these reactions.

Fig. 4 illustrates the cyclic voltammograms acquired from the cathodic and anodic scans of TMD materials bearing the Se chalcogen: TiSe₂, ZrSe₂ and HfSe₂. The first scan of TiSe₂ in the cathodic direction shows a shoulder reduction peak at -1.25 V, followed by a shoulder oxidation peak at +1.25 V (Fig. 4a). In contrast, the first scan in the anodic direction showed an intense oxidation peak at +1.0 V, followed by an absence of reduction peaks (Fig. 4b). This is interpreted that an initial oxidative treatment could provide a protective effect for the electroactive moieties towards reduction. The observation of the cathodic scan of ZrSe₂ shows a small reduction peak at -0.9 V (Fig. 4c) while that of the anodic scan shows an oxidation peak at +1.1 V (Fig. 4d). Similar observations were made in the case of HfSe₂ (Fig. 4e and f). A point worth noting is that all the TMD materials bearing the Se chalcogen display a distinct oxidation peak at about +1.0 V where previous research on selenium electrochemistry points to the possibility of oxidation to Se⁶⁺.^{27,28} Moreover, in all cases, any redox electroactivity present is only observed in the first scans and not in the subsequent scans, pointing toward the chemical irreversibility of these reactions.

Fig. 5 illustrates the cyclic voltammograms derived from the cathodic and anodic scans of the TMD materials bearing the Te chalcogen: TiTe₂, ZrTe₂ and HfTe₂. An interesting observation is

made when TMD materials containing the Te chalcogen were compared with each other. Distinctive oxidation peaks were observed at about +0.25 V and +0.5 V, while reduction peaks were seen at about -0.75 V, -1.0 V and -1.25 V. These peaks are rationalised by looking at the electrochemistry of the Te chalcogen component of the materials, supported by the existing literature.^{29–31} The initial cathodic peak at about -1.25 V is likely due to reduction to Te⁰. The anodic peak at about +0.25 V is attributed to the oxidation of Te⁰ to Te⁴⁺, while that at about +0.5 V may correspond to the oxidation of Te²⁺ to Te⁴⁺. The prominent peaks observed in the subsequent cathodic scans at about -0.75 V and -1.0 V are likely to be due to the reduction of Te⁴⁺ to Te⁰, and Te⁰ to Te²⁻ respectively.

Characterisation of electrochemically treated group 4 TMDs

Earlier, the investigation of the inherent electrochemistry of group 4 TMDs revealed their characteristic oxidation and reduction peaks. By careful observation, we identified a suitable oxidation and reduction potential for the electrochemical treatment of each TMD material. As a general rule, these potentials lie marginally beyond the characteristic redox potentials of the corresponding TMD material. The oxidation and reduction treatment potentials are determined to be +0.7 V and -1.5 V for TiSe₂, +1.5 V and -1.6 V for TiSe₂, +1.5 V and -1.25 V for ZrSe₂ and HfSe₂, and +1.1 V and -1.6 V for TiTe₂, ZrTe₂ and HfTe₂. ZrS₂ was subjected to only reductive treatment at -1.1 V due to the absence of a characteristic oxidation peak. On the other hand, no oxidative or reductive treatment was

Fig. 6 High resolution X-ray photoelectron spectra of the metal (Ti 2p, Zr 3d and Hf 4f regions) and S chalcogen (S 2p region) of the (a) TiS₂, (b) ZrS₂ and (c) HfS₂ materials before and after electrochemical treatment.

performed on HfS_2 as it is electrochemically inert within the potential range of -1.8 V to $+1.8$ V.

At these potentials, we would expect that there will be chemical alterations to the surface structure of group 4 TMDs as a consequence of redox reactions of the materials. In order to confirm this, the technique of X-ray photoelectron spectroscopy (XPS) was employed to characterise the surface composition of the TMD materials before and after their respective electrochemical treatments. XPS is a surface-sensitive technique with a maximum depth of approximately 10 nm in the TMD samples, with detection limits at about 0.1 wt% of the material. The XPS high resolution spectra allow us to examine the surface elemental compositions, where chalcogen-to-metal ratios are reported in Fig. S3,[†] and bonding information of the individual materials before and after electrochemical treatment.

The high resolution XPS spectra of Ti 2p, Zr 3d and Hf 4f orbitals and S 2p orbitals of TMD materials bearing the S chalcogen before and after electrochemical treatment are displayed in Fig. 6. Deconvolution analysis of the bonding modes is performed based on previous XPS studies of titanium sulphides and their corresponding oxides.^{32–36} Before electrochemical treatment, the presence of both Ti and S is observed from Fig. 6a. The three pairs of peaks revealed in the Ti 2p spectra of TiS_2 indicate the presence of Ti^{4+} as in TiO_2 (458.0 and 463.7 eV), Ti^{4+} as in TiS_2 (455.0 and 460.2 eV) and Ti^{6+} as in

TiS_3 (456.0 and 461.6 eV). S^{2-} as in TiS_2 (160.2 and 161.3 eV) and S_2^{2-} as in TiS_3 (161.2 and 162.4 eV) are further confirmed in the analysis of the S 2p spectrum. The presence of sulphur is no longer significant after both reductive (-1.5 V) and oxidative ($+0.7$ V) treatment of TiS_2 , with significant changes to the Ti 2p spectra as well. The fitting of the Ti 2p spectra indicates the absence of initial TiS_2 and TiS_3 species, leaving behind only Ti oxides after electrochemical treatment. Ti^{4+} in an oxide environment is observed after both reductive and oxidative treatment. A similar observation is made with the ZrS_2 material after reductive (-1.1 V) treatment (Fig. 6b). This is in comparison with an obvious presence of both Zr and S before electrochemical treatment where the Zr 3d peak shows two distinct pairs of peaks originating from Zr^{4+} in a sulphide environment as in ZrS_2 (181.1 and 183.5 eV)^{37,38} and Zr^{4+} in an oxide environment as in ZrO_2 (182.2 and 184.6 eV).³⁹

Moreover, the high resolution XPS spectra of Hf 4f orbitals and S 2p orbitals (Fig. 6c) indicate the presence of both Hf and S in the HfS_2 material. The Hf 4f and S 2p spectra of HfS_2 confirm the presence of the metal dichalcogenide with Hf^{4+} (15.8 and 17.5 eV) and S^{2-} (161.0 and 162.2 eV) as in HfS_2 .⁴⁰ Partial oxidation of the material is reported as Hf^{4+} in an oxide environment (17.3 and 19.0 eV)^{41,42} is observed in the Hf 4f peak.

The high resolution XPS spectra of Ti 2p, Zr 3d and Hf 4f orbitals and Se 3d orbitals of TMD materials bearing the Se

Fig. 7 High resolution X-ray photoelectron spectra of the metal (Ti 2p, Zr 3d and Hf 4f regions) and Se chalcogen (Se 3d region) of the (a) TiSe_2 , (b) ZrSe_2 and (c) HfSe_2 materials before and after electrochemical treatment.

Fig. 8 High resolution X-ray photoelectron spectra of the metal (Ti 2p, Zr 3d and Hf 4f regions) and Te chalcogen (Te 3d region) of the (a) TiTe_2 , (b) ZrTe_2 and (c) HfTe_2 materials before and after electrochemical treatment.

chalcogen before and after electrochemical treatment are displayed in Fig. 7. The presence of both Ti and Se is established from the XPS spectra (Fig. 7a). The fitting of the Ti 2p spectra reveals the strong presence of Ti oxides: Ti^{4+} as in TiO_2 (458.8 and 464.5 eV), Ti^{3+} as in Ti_2O_3 (457.3 and 462.5 eV),^{34,36} and also Ti^{4+} as in TiSe_2 (456.2 and 462.1 eV)⁴³ in lower concentrations. The Se 3d spectrum confirms the presence of the metal dichalcogenide with a doublet attributing to Se^{2-} as in TiSe_2 (54.1 and 54.9 eV)⁴⁴ as well as reveals the presence of Se^0 (55.8 and 56.6 eV).⁴⁵ Both reductive (−1.6 V) and oxidative (+1.5 V) treatments result in loss of the metal dichalcogenide. TiO_2 remains after oxidative treatment, while TiO_2 and Se^0 are detected after reductive treatment.

From Fig. 7b, Zr^{4+} (181.9 and 184.4 eV) and Se^{2-} (52.7 and 53.5 eV) as in ZrSe_2 are observed before electrochemical treatment of the ZrSe_2 material.^{46,47} In addition, Se^0 (54.7 and 55.6 eV) is found to be present from the Se 3d peak.⁴⁵ Both reductive (−1.25 V) and oxidative (+1.5 V) treatments result in the loss of the metal dichalcogenide and the formation of Zr^{4+} in an oxide environment as in ZrO_2 .

Moreover, it is observed that both Hf and Se are present before and after electrochemical treatment of the HfSe_2 material (Fig. 7c). From the Hf 4f peak fitting, the major presence of Hf^{4+} as in HfSe_2 (16.8 and 18.5 eV)⁴⁰ is observed and Hf^0 (14.5 and 16.2 eV)⁴⁸ at a lower concentration. From the Se 3d peak, doublets attributing to Se^{2-} as in HfSe_2 (52.1 and 53.0 eV)⁴⁰ and Se^0 (54.5 and 55.4 eV)⁴⁵ are assigned. The presence of HfSe_2 remains even after reductive (−1.25 V) and oxidative (+1.5 V) treatments. However, the relative concentrations of HfSe_2 markedly diminished with reductive treatment compared to that of oxidative treatment.

The high resolution XPS spectra of Ti 2p (Fig. 8a), Zr 3d (Fig. 8b) and Hf 4f (Fig. 8c) orbitals and Te 3d orbitals of TMD materials bearing the Te chalcogen before and after electrochemical treatment are displayed in Fig. 8. The fitting of the Ti 2p peak displayed the presence of Ti^{4+} as in TiTe_2 (455.0 and 41.0 eV) in the TiTe_2 material before electrochemical treatment.⁴⁹ However, the major presence of Ti oxides is revealed from two doublets arising due to Ti^{4+} as in TiO_2 (458.0 and 263.7 eV) and Ti^{3+} as in Ti_2O_3 (457.0 and 462.2 eV).^{34,36} Analysis of the Te 3d peak shows the presence of Te^{2-} as in TiTe_2 (572.0 and 582.5 eV),⁵⁰ Te^0 (573.4 and 583.8 eV) and Te^{4+} as in TeO_2 (575.5 and 585.8 eV).^{51,52} It should be brought to attention that upon reductive treatment (−1.6 V), the presence of Ti is no longer significant. Deconvolution analysis of the Te bonding modes indicates the loss of the metal dichalcogenide. Likewise, the loss of TiTe_2 is observed with oxidative treatment (+1.1 V). However, the presence of Ti oxides remains.

Similar patterns are observed subsequently in both cases of ZrTe_2 and HfTe_2 . From Fig. 8b, the doublets at 181.8 and 184.2 eV, and 571.9 and 582.2 eV can be attributed to the Zr^{4+} and Te^{2-} as in ZrTe_2 respectively. The loss of the metal dichalcogenide is observed upon both reductive (−1.6 V) and oxidative treatment (+1.1 V). Moreover, the loss of zirconium is evident with reductive treatment while Zr oxides remain detected with oxidative treatment. In the case of HfTe_2 , analysis of the Hf 4f bonding modes in Fig. 8c shows a doublet at 16.2

and 17.9 eV corresponding to Hf^{4+} as in HfTe_2 . The doublet at 571.4 and 581.8 eV originates from Te^{2-} as in HfTe_2 . The presence of HfTe_2 is insignificant while Hf oxides are present after reductive (−1.6 V) and oxidative (+1.1 V) treatments.

Overall, a general trend is observed that electrochemical treatment elicits the dissolution of group 4 metal dichalcogenides. In most cases, the presence of metal dichalcogenides is no longer detected or has depleted greatly. The only exception is for the case of HfSe_2 where after oxidative treatment at +1.5 V, HfSe_2 remained as the major component. Although XPS is an indispensable technique for the analysis of TMD elemental compositions, it is also important to emphasize that it may not provide the full picture of the effects of electrochemical treatment on group 4 TMDs. As discussed earlier, it may be expected that oxidative treatment of the metal diselenides would lead to the formation of Se^{6+} , which may be in the form of the selenate anion or its oxide.^{27,28} Due to the high solubility of SeO_3 , dissolution into the surrounding electrolyte upon formation means that it would escape detection *via* XPS. Moreover, the possibility of oxidation in air may present much higher amounts of surface oxides than is true for HER catalytic studies

Fig. 9 The effect of the metal (Ti, Zr or Hf) on the HER performance of group 4 TMDs. Linear sweep voltammograms for the HER in an acidic electrolyte on (a) MS_2 , (b) MSe_2 and (c) MTe_2 deposited on a GC electrode. Bar charts illustrate the averages of the (d) overpotential at -10 mA cm^{-2} current density, (e) Tafel slopes and (f) HER onset potential, with their corresponding error bars for bare GC and the TMD materials. Conditions: background electrolyte, H_2SO_4 (0.5 M); scan rate, 2 mV s^{-1} . All measurements were performed relative to the Ag/AgCl reference electrode and are corrected to reversible hydrogen electrode (RHE) potentials.

discussed subsequently. Hence, XPS analysis allows us to postulate the effects of electrochemical treatment on group 4 TMDs, but should not be interpreted as absolute.

Electrochemically treated group 4 TMDs for the hydrogen evolution reaction (HER)

Following the characterisation of the TMD materials, it is of equal significance to investigate how the abilities of these materials to catalyze the hydrogen evolution reaction (HER) in an acidic medium (0.5 M H₂SO₄) correspond to their surface composition before and after electrochemical treatment. The HER performances of the materials were evaluated using linear sweep voltammetry (LSV).

Fig. 9a–c illustrate the linear sweep voltammograms (LSVs) obtained from the evolution of hydrogen in air-saturated 0.5 M H₂SO₄. TMD materials are grouped according to the chalcogen they contain: S (Fig. 9a), Se (Fig. 9b) or Te (Fig. 9c), in order to study any trends in the HER performance down group 4 metals (Ti, Zr, and Hf). From the cathodic scan of TiSe₂, a shoulder peak was observed at −0.75 V. This could be due to the inherent electrochemistry of the TiSe₂ material, matching the reduction peak observed in the study of its inherent electrochemistry. Similarly, reduction peaks are observed in the cathodic scans for TMD materials containing the Te chalcogen, which matches the observations made in the study of their inherent electrochemistry.

In addition, three commonly adopted HER performance indicators namely the overpotential (Fig. 9d), Tafel slope (Fig. 9e) and onset potential (Fig. 9f) were plotted for bare GC and the untreated TMD materials for comparison. The onset potential was determined at the potential where a steep decrease is observed for the current density corresponding to proton reduction. Overpotentials were taken at −10 mA cm^{−2}. Moreover, the Tafel slope is determined by the increase in the overpotential required to bring about a unit increase in the current density. Tafel slope analysis can give us an understanding of the hydrogen evolution mechanism on different TMD surfaces, where the rate-determining steps may be accounted as follows:⁵³

1. Volmer adsorption step:

2. Heyrovsky desorption step:

3. Tafel desorption step

In general, group 4 TMDs exhibit a Tafel slope close to or higher than the 120 mV dec^{−1} theoretical value before and after electrochemical treatment. This observation points to the adsorption step as the slow step for all the materials of interest.

For this reason, Tafel analysis of the HER mechanism will not be discussed in detail. Instead, desirable HER catalytic activities will be determined and discussed based on lower overpotentials, smaller Tafel slopes and less negative onset potentials as less energy is required for the reaction to take place.

As illustrated in Fig. 9d–f, the bare glassy carbon (GC) has an overpotential of 1.12 V at −10 mA cm^{−2}, a Tafel slope of 158 mV dec^{−1}, and an onset potential of −0.96 V. In general, the TMD materials have lower overpotentials, gentler Tafel slopes, and less negative onset potentials than that of the bare GC. Particularly, HfS₂ displayed the best HER performance with the smallest overpotential at −10 mA cm^{−2} and onset potential.

For TMD materials containing the S chalcogen, overpotentials at −10 mA cm^{−2} decrease down group 4 metals: TiS₂ (1.13 V) > ZrS₂ (1.04 V) > HfS₂ (0.99 V) (Fig. 9d). A similar trend is observed in Fig. 9f where the onset potentials become less negative down the group: TiS₂ (−0.95 V) > ZrS₂ (−0.85 V) > HfS₂ (−0.81 V). While the Tafel slopes of TiS₂ (147 mV dec^{−1}), ZrS₂ (138 mV dec^{−1}) and HfS₂ (143 mV dec^{−1}) are similar (Fig. 9e), there is a clear trend of an increasing HER performance down group 4 metals for TMD materials containing the S chalcogen: TiS₂ < ZrS₂ < HfS₂.

Fig. 10 The effect of the chalcogen (S, Se or Te) on the HER performance of group 4 transition metal dichalcogenides. Linear sweep voltammograms for the HER in an acidic electrolyte on (a) TiX₂, (b) ZrX₂ and (c) HfX₂ deposited on a GC electrode. Bar charts illustrate the averages of the (d) overpotential at −10 mA cm^{−2} current density, (e) Tafel slopes and (f) HER onset potential, with their corresponding error bars for bare GC and the TMD materials. Conditions: background electrolyte, H₂SO₄ (0.5 M); scan rate, 2 mV s^{−1}. All measurements were performed relative to the Ag/AgCl reference electrode and are corrected to reversible hydrogen electrode (RHE) potentials.

For TMD materials containing the Se chalcogen, overpotentials at -10 mA cm^{-2} are similar with 1.05 V for TiSe_2 , 1.06 V for ZrSe_2 , and 1.03 V for HfSe_2 (Fig. 9d). Among the three, TiSe_2 has the gentlest Tafel slope of 107 mV dec^{-1} , while those of ZrSe_2 (159 mV dec^{-1}) and HfSe_2 (159 mV dec^{-1}) are similar (Fig. 9e). From Fig. 9f, similar onset potentials were reported for TiSe_2 (-0.84 V), ZrSe_2 (-0.86 V) and HfSe_2 (-0.85 V). Hence, we may conclude that the HER performances down group 4 metals for TMD materials containing the Se chalcogen are similar: $\text{TiSe}_2 \approx \text{ZrSe}_2 \approx \text{HfSe}_2$. However, TiSe_2 may have a slight advantage over the other two materials due to its gentler Tafel slope.

For TMD materials containing the Te chalcogen, overpotentials at -10 mA cm^{-2} are similar for TiTe_2 (1.13 V), ZrTe_2 (1.11 V) and HfTe_2 (1.11 V). A comparable pattern is observed in Fig. 9f where the onset potentials of TiTe_2 (-0.95 V), ZrTe_2 (-0.95 V) and HfTe_2 (-0.96 V) are similar as well. Fig. 9d shows that the Tafel slope of TiTe_2 (233 mV dec^{-1}) is much larger than that of HfTe_2 (145 mV dec^{-1}) and ZrTe_2 (152 mV dec^{-1}). Hence, it is resolved that the HER performances down the group 4 metals for TMD materials containing the Te chalcogen are similar ($\text{TiTe}_2 \approx \text{ZrTe}_2 \approx \text{HfTe}_2$), with TiTe_2 being slightly inferior due to its large Tafel slope.

Fig. 10a–c illustrate the linear sweep voltammograms (LSVs) obtained from the evolution of hydrogen in $0.5 \text{ M H}_2\text{SO}_4$. TMD materials are grouped according to the metal they contain: Ti (Fig. 10a), Zr (Fig. 10b), and Hf (Fig. 10c), in order to study how HER performance is affected by group 16 chalcogens (S, Se, Te) as we go down the group.

For TMD materials containing the Ti metal, TiSe_2 (1.05 V) exhibited the lowest overpotential at -10 mA cm^{-2} compared to that of TiS_2 (1.13 V) and TiTe_2 (1.13 V) (Fig. 10d). Tafel slope calculations in Fig. 10e matched this observation, showing that TiSe_2 has the gentlest Tafel slope (107 mV dec^{-1}) in comparison to that of TiS_2 (147 mV dec^{-1}) and TiTe_2 (233 mV dec^{-1}). Likewise, it is observed in Fig. 10f that TiSe_2 has the least negative onset potential (-0.84 V) compared to that of TiS_2 (-0.95 V) and TiTe_2 (-0.95 V). Overall, we may say that the HER performance of TiSe_2 is superior among the TMD materials containing the Ti metal.

For TMD materials containing the Zr metal, overpotentials at -10 mA cm^{-2} increase down group 16 chalcogens: ZrS_2 (1.04 V) < ZrSe_2 (1.06 V) < ZrTe_2 (1.11 V) (Fig. 10b). This observation was backed up by the data in Fig. 10f, showing that the onset potential becomes more negative down group 16 chalcogens: ZrS_2 (-0.85 V) > ZrSe_2 (-0.86 V) > ZrTe_2 (-0.95 V). While ZrSe_2 displayed the steepest Tafel slope (159 mV dec^{-1}) in relation to ZrS_2 (138 mV dec^{-1}) and ZrTe_2 (145 mV dec^{-1}), it is deduced that the HER performance generally decreases down group 16 chalcogens for TMD materials containing the Zr metal: $\text{ZrS}_2 > \text{ZrSe}_2 > \text{ZrTe}_2$.

For TMD materials containing the Hf metal, an increasing trend for the overpotential at -10 mA cm^{-2} down group 16 chalcogens is illustrated in Fig. 10c: HfS_2 (0.99 V) < HfSe_2 (1.03 V) < HfTe_2 (1.11 V) (Fig. 10d). Moreover, it is observed from Fig. 10f that the onset potential gets increasingly negative down group 16 chalcogens: HfS_2 (-0.81 V) > HfSe_2 (-0.85 V) > HfTe_2

(-0.96 V). While the Tafel slopes of HfS_2 (143 mV dec^{-1}), HfSe_2 (156 mV dec^{-1}) and HfTe_2 (152 mV dec^{-1}) are similar (Fig. 10e), we can deduce that there is a decreasing trend for HER performance down group 16 chalcogens for TMD materials containing the Hf metal: $\text{HfS}_2 > \text{HfSe}_2 > \text{HfTe}_2$.

Next, we discuss the influence of electrochemical treatment on the catalytic activity of group 4 TMDs towards the HER. In particular, we would like to explore the possibility of tuning the catalytic properties of group 4 TMD materials by the application of an oxidative or reductive electrochemical treatment which would cause chemical alterations and also the possibility of removing surface impurities. Such a prospect has been displayed in a study by Chia *et al.* as they show the activation of *t*-butyllithium exfoliated MoS_2 , MoSe_2 and WSe_2 for HER catalysis by reductive treatment.⁵⁴ Linear sweep voltammograms (LSVs) were obtained from the evolution of hydrogen in air-saturated $0.5 \text{ M H}_2\text{SO}_4$ before and after electrochemical treatment of the TMD materials, and their HER performance indices are analysed.

Fig. S4† shows the HER performance of TiS_2 before and after electrochemical treatment. From Fig. S4b,† it can be seen that the overpotential of the TiS_2 material (1.12 V) did not change significantly with reductive treatment. Conversely, oxidative treatment caused an increase in the overpotential from 1.13 V to 1.23 V. Tafel slopes increased from 147 mV dec^{-1} to 155 mV dec^{-1} and 171 mV dec^{-1} after reductive and oxidative treatment respectively (Fig. S4c†). Moreover, Fig. S4d† illustrates that the onset potentials are more negative with electrochemical treatment. The onset potential is reported to be -0.98 V with reductive treatment and -1.0 V with oxidative treatment. We conclude that the HER performance of TiS_2 became poorer after electrochemical treatment. Moreover, the ZrS_2 material was subjected to a reductive treatment where the overpotential at -10 mA cm^{-2} increased from 1.04 V to 1.24 V after reductive treatment (Fig. S5b†). Furthermore, the Tafel slope increased slightly by 8 mV dec^{-1} to 146 mV dec^{-1} with reductive treatment (Fig. S5c†). It is also illustrated that reductive treatment yields a material with a more negative onset potential of -1.01 V compared to that of the starting ZrS_2 material (-0.85 V) (Fig. S5d†). These observations combined to resolve that the electrochemical treatment of the material decreased its HER performance.

In both cases of TiS_2 and ZrS_2 , it is seen that electrochemical treatment yields materials which are less active towards HER catalysis. An insight into this behaviour is obtained by studying the XPS characterisations of these materials (Fig. 6a and b) where the loss of metal disulphides is observed in both cases upon electrochemical treatment. It can be concluded that the metal disulphides are responsible for the catalysis of the HER.

Furthermore, Fig. S6† shows the HER performance of TiSe_2 . It is illustrated in Fig. S6b† that the overpotential of TiSe_2 at -10 mA cm^{-2} increased from 1.05 V to 1.10 V after reductive treatment and remained relatively constant after oxidative treatment (1.05 V). A similar observation is made in Fig. S6c,† where the Tafel slope (107 mV dec^{-1}) significantly increased after reductive treatment (176 mV dec^{-1}) while oxidative treatment (143 mV dec^{-1}) saw a slight increase in the Tafel slope. In

addition, the onset potential became more negative after reductive treatment of the TiSe_2 material (-0.92 V). On the other hand, the onset potential remained relatively stable (-0.82 V) after oxidative treatment (Fig. S6d†). Hence, we conclude that the HER performance of the TiSe_2 material decreased slightly upon oxidative treatment while reductive treatment yields a material with an even more considerable decrease in HER catalytic activity. This can be accounted for by the depletion of the metal diselenide as observed in earlier XPS studies (Fig. 7a), pointing to the HER catalytic activity of the metal diselenide.

Investigation of the HER activity of ZrSe_2 before and after electrochemical treatment shows that the overpotential of ZrSe_2 at -10 mA cm^{-2} decreased from 1.06 V to 0.94 V and 1.00 V upon reductive and oxidative treatment respectively (Fig. S7b†). Moreover, the material displayed a high Tafel slope with reductive treatment (177 mV dec^{-1}). On the other hand, oxidative treatment resulted in a material with a lower Tafel slope of 138 mV dec^{-1} compared to that of the starting ZrSe_2 material (159 mV dec^{-1}) (Fig. S7c†). From Fig. S7d,† it is observed that the onset potentials are less negative with electrochemical treatment of ZrSe_2 . The onset potentials of the materials are -0.76 V and -0.78 V after reductive and oxidative treatment respectively. Hence, the observations of the overpotentials and onset potentials are in agreement to conclude that electrochemical treatment of the ZrSe_2 material yields materials possessing superior HER performance, with reductive treatment showing a better overall advantage. Matching the XPS results of the ZrSe_2 material before and after electrochemical treatment (Fig. 7b), we are able to explain the above observations. The loss of the metal diselenide is observed upon both reductive and oxidative treatment of the ZrSe_2 material. This could result in the availability of Zr which displays a superior HER performance. With reductive treatment, the formation of an oxide passivating layer⁵⁵ is less likely and hence, results in a higher increase in HER performance.

It is displayed in Fig. S8b† that the overpotential of HfSe_2 at -10 mA cm^{-2} decreased slightly from 1.03 V to 1.02 V and 0.99 V after reductive and oxidative treatment respectively. Similarly, Fig. S8c† shows that the Tafel slope decreased with

both reductive (145 mV dec^{-1}) and oxidative treatment (133 mV dec^{-1}). Furthermore, the onset potential became less negative after electrochemical treatment of the HfSe_2 material (Fig. S5d†). The onset potential of the starting HfSe_2 material (-0.85 V) improved to -0.84 V and -0.77 V after reductive and oxidative treatment respectively. Hence, it is concluded that the HER performance of the HfSe_2 material improved with electrochemical treatment, with oxidative treatment yielding a material with better HER catalytic activity. Matching the XPS results of the HfSe_2 material before and after electrochemical treatment (Fig. 7c), we explain the above observations. In contrast to most of the other TMD materials, the presence of HfSe_2 can still be observed from XPS after electrochemical treatment. While the relative amounts of HfSe_2 are greatly reduced after reductive treatment, HfSe_2 remains relatively stable with oxidative treatment and remains to be the major component. Moreover, the depletion of Se^0 is noted with oxidative treatment.^{27,28} The removal of Se^0 as an impurity is determined to be crucial for enhancing HER catalytic activity.

Fig. S9a† shows the LSV obtained from the evolution of hydrogen in air-saturated 0.5 M H_2SO_4 before and after electrochemical treatment of TiTe_2 . Fig. S9b† illustrates a decrease in the overpotential of TiTe_2 at -10 mA cm^{-2} from 1.13 V to 1.10 V upon reductive treatment and increased to 1.16 V upon oxidative treatment. Moreover, the material displayed a much gentler Tafel slope with reductive treatment (113 mV dec^{-1}). On the other hand, oxidative treatment resulted in a material with a much higher Tafel slope of 461 mV dec^{-1} compared to that of the starting TiTe_2 material (232 mV dec^{-1}) (Fig. S9c†). From Fig. S9d,† it is observed that the onset potentials remained relatively similar after reductive treatment (-0.96 V) and oxidative treatment (-0.94 V) of TiTe_2 . Overall, we may conclude that reductive treatment of the TiTe_2 material yields materials possessing superior HER performance, while the opposite is true with oxidative treatment.

Moreover, it is displayed in Fig. S10b† that the overpotential of ZrTe_2 at -10 mA cm^{-2} increased slightly from 1.11 V to 1.12 V and 1.15 V after reductive and oxidative treatment respectively. Correspondingly, it is observed in Fig. S10c† that the Tafel slope increased with both reductive (227 mV dec^{-1}) and oxidative

Table 1 Observed trends of group 4 (this work), 5 and 6 TMDs for electrocatalysis of hydrogen evolution.^{7,56,57}

Group	Metal effect		
	S chalcogen	Se chalcogen	Te chalcogen
4	$\text{TiS}_2 < \text{ZrS}_2 < \text{HfS}_2$	$\text{TiSe}_2 \approx \text{ZrSe}_2 \approx \text{HfSe}_2$	$\text{TiTe}_2 \approx \text{ZrTe}_2 \approx \text{HfTe}_2$
5	$\text{VS}_2 \approx \text{NbS}_2 \approx \text{TaS}_2$	$\text{VSe}_2 \approx \text{NbSe}_2 \approx \text{TaSe}_2$	$\text{VTe}_2 < \text{NbTe}_2 < \text{TaTe}_2$
6	$\text{MoS}_2 < \text{WS}_2$	$\text{MoSe}_2 > \text{WSe}_2$	—
Group	Chalcogen effect		
	Period 4 metal	Period 5 metal	Period 6 metal
4	$\text{TiS}_2 < \text{TiSe}_2 > \text{TiTe}_2$	$\text{ZrS}_2 > \text{ZrSe}_2 > \text{ZrTe}_2$	$\text{HfS}_2 > \text{HfSe}_2 > \text{HfTe}_2$
5	$\text{VS}_2 > \text{VSe}_2 > \text{VTe}_2$	$\text{NbS}_2 \approx \text{NbSe}_2 \approx \text{NbTe}_2$	$\text{TaS}_2 \approx \text{TaSe}_2 \approx \text{TaTe}_2$
6	—	$\text{MoS}_2 < \text{MoSe}_2$	$\text{WS}_2 > \text{WSe}_2$

treatment (197 mV dec^{-1}). Moreover, the onset potential became more negative after electrochemical treatment of the ZrTe_2 material (Fig. S10d†). The onset potential of the starting ZrTe_2 material (-0.95 V) became more negative after both reductive and oxidative treatment (-0.99 V). Hence, it is resolved that the ZrTe_2 material became less active towards HER catalysis after electrochemical treatment.

Additionally, Fig. S11b† shows that the overpotential of HfTe_2 at -10 mA cm^{-2} increased from 1.11 V to 1.19 V and 1.39 V after reductive and oxidative treatment respectively. It is also observed in Fig. S11c† that the Tafel slope was gentler with both reductive (127 mV dec^{-1}) and oxidative treatment (159 mV dec^{-1}). Moreover, the onset potential became more negative after electrochemical treatment of the HfTe_2 material (Fig. S11d†). The onset potential of the starting HfTe_2 material (-0.96 V) became more negative after both reductive (-1.01 V) and oxidative treatment (-1.02 V). Overall, it is concluded that the HfTe_2 material became less active towards HER catalysis after electrochemical treatment.

Comparing the XPS results of the TiTe_2 (Fig. 8a), ZrTe_2 (Fig. 8b) and HfTe_2 (Fig. 8c) materials before and after electrochemical treatment, similar changes in their surface composition are observed upon electrochemical treatment. This is well matched with the similar alterations to their HER performances. The loss of metal ditellurides is evident upon both reductive and oxidative treatment, where metal and tellurium oxides remain. This resulted in the general decrease in catalytic activity towards the HER. However, an exception is noted in the case of TiTe_2 with reductive treatment, where an improvement of HER performance is observed. A possible explanation is the relatively high concentrations of Te^0 compared to oxides contributing to the increased catalytic activity of the HER.

A comparison of the observed trends for group 4 TMDs (in this work) with previously reported trends of group 5 and 6 TMDs is established in Table 1. Few similarities are observed between groups 4 and 6; HER activity increased down the groups for TMDs bearing the S chalcogen and decreased down group 16 for hafnium dichalcogenides and tungsten dichalcogenides. This is in contrast with the lack of similar trends matching to that of group 5 TMDs. A possible explanation could be that both group 4 and 6 TMDs are semiconducting while group 5 TMDs are true metals in their bulk forms.¹⁵ On the other hand, the effect of electrochemical treatment differed greatly from the trends observed in the work of Chia *et al.* where group 6 TMDs mostly exhibited improved HER efficiencies with reductive treatment.³⁴ A decline in HER efficiencies was generally observed for group 4 metal dichalcogenides due their instability towards electrochemical treatment. Exceptions were noted where electrochemical activation towards HER catalysis was achieved with ZrSe_2 and HfSe_2 via both electrochemical oxidation and reduction and TiTe_2 via electrochemical reduction. Exceptions noted with ZrSe_2 and TiTe_2 are likely due to Zr and Te being made available respectively for enhanced HER catalytic performance. HfSe_2 is the only group 4 TMD which was relatively stable under electrochemical treatment which served to remove possible impurities to improve the catalytic activity towards the HER. Hence, even though some similarities are

recognised for group 4 and 6 bulk TMDs due to their semiconductor properties, electrochemical treatment may not be as suitable for group 4 TMDs.

Conclusions

We have explored the inherent electrochemistry of layered group 4 transition metal dichalcogenide (TMD) materials, where the distinct redox properties of Se and Te chalcogens are well perceived in the corresponding TMD materials. Notably, HfS_2 is electrochemically inert within a wide potential window of -1.8 V to $+1.8 \text{ V}$ and displayed superior HER activity compared to the other group 4 TMDs, making it a promising candidate for electrochemical sensing applications. Trends in HER activity down group 4 metals and group 16 chalcogens are observed: increased activity down group 4 metal disulphides and decreased activity down group 16 zirconium dichalcogenides and hafnium dichalcogenides. Efforts to tune the catalytic properties of group 4 TMDs disclosed many perturbations by electrochemical oxidation or reduction. We highlighted the marked response of each group 4 TMD material to electrochemical treatment upon cross reference to the surface compositions of these materials. Out of the nine materials, electrochemical activation towards HER catalysis was exhibited for ZrSe_2 and HfSe_2 via both electrochemical oxidation and reduction and TiTe_2 via electrochemical reduction. In other cases, the HER activities deteriorated with electrochemical treatment. In general, it was revealed that metal dichalcogenides were mostly unstable upon electrochemical treatment, which causes their decomposition and partial dissolution. This accounts for the decrease in HER catalytic activity in most cases. Therefore, even as we show that electrochemical treatment may not be suitable for tuning the HER catalytic activity for group 4 TMDs, with the exception of HfSe_2 , due to their instability upon electrochemical treatment, the results point towards the importance of the purity of the materials for high catalytic performance. This first insight into the electrochemical behaviour of group 4 TMDs and understanding of the impact of electrochemical treatment on these materials will be pivotal towards achieving desired goals in electrochemical applications.

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Haemoglobin electrochemical detection on various reduced graphene surfaces: well-defined glassy carbon electrode outperforms the graphenoids

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Monitoring the direct electron transfer reactions of haemoglobin is important for understanding of chemical changes within the red blood cell for haematological studies. However, the facilitation of electron transfer between haemoglobin and bare solid electrodes is challenging. Herein, the influence of carbon nanomaterials; graphite oxide (GO), chemically reduced graphene oxide (CRGO), graphene oxide (GO'), electrochemically reduced graphene oxide (ERGO) and edge plane pyrolytic graphite (EPPG); on the electron transfer between haemoglobin in solution and solid electrodes was investigated. We showed that GO, CRGO, GO' and ERGO did not exhibit improvement to the electron transfer characteristics of haemoglobin in solution, and bare glassy carbon remains an appropriate electrode material for such application in electrochemical sensing. These findings are in contrast to previous studies, which report an enhancement in direct electron transfer characteristics of haemoglobin immobilized on the electrode surface with employment of carbon nanomaterials, and will have profound impact on further investigation of the electron transfer characteristics of hemoglobin.

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Introduction

Haemoglobin (Hb) functions physiologically in the storage and transport of molecular oxygen (O₂) in the blood of humans. This metalloprotein has a molecular weight of 64.5 kDa and comprises four polypeptide subunits (two α and two β subunits) of similar structure, each of which has an iron-bearing heme within molecularly accessible crevices.^{1,2} The iron ion may be in either the Fe^{II} or Fe^{III} oxidation state. In its Fe^{II} state, haemoglobin is able to bind oxygen to form oxyhaemoglobin (Oxy-Hb), but in its Fe^{III} state, methemoglobin (Met-Hb) is not able to bind oxygen. Fe^{II} is converted to Fe^{III} spontaneously in trace amounts (1–2% of Hb), and if not mitigated by internal reduction mechanisms within the red blood cell, high levels of Met-Hb (methemoglobinemia) could result in fatality.^{3–5}

The iron ion in haemoglobin acts as a redox-active center. The direct electron transfer reactions of proteins with redox-active centers may be used to probe the nature of many natural energy conversion processes, from photosynthesis to respiration, most of which are mediated by these protein molecules.^{6,7} Hence, the direct electrochemistry of haemoglobin may provide

fundamental knowledge of its redox behaviour within the red blood cell. Accurate monitoring of such reactions is critical as chemical changes in the red blood cell usually take place due to primary hematologic disorders like anemia, or a wider variety of other diseases which produces secondary abnormalities of the blood.^{8,9} While the electrochemistry of heme within red blood cells may be complicated due to the presence of a cellular membrane, electrochemical measurement of cell-free haemoglobin, resulting from red blood cell lysis, is also important since they are directly related to various pathophysiological conditions.^{10,11} However, the facilitation of electron transfer between the heme center in the large three-dimensional structure of haemoglobin and bare solid electrodes poses a challenge. Poor electrochemical property is observed at bare glassy carbon electrodes as the heme groups are deeply concealed in the hydrophobic pockets of haemoglobin, and hence resulting in inaccessibility of the redox centers.^{12–14} In order to improve the electrochemical property of haemoglobin, its direct electron transfer has been investigated at a variety of modified electrodes.^{13–18}

In recent years, carbon-based nanomaterials like graphenes have been placed in the spotlight of attention due to their high surface area, outstanding electrical conductivity, structural flexibility and high mechanical strength.^{19,20} These desirable properties have allowed the two-dimensional sheet of sp² bonded carbon atoms to find its way into electrochemical sensing applications.^{21,22} There have been reports of many advantageous applications of graphene for electrochemical sensing; however, some reports have also shown that graphene may not be such an advantageous material if compared to *i.e.* its

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parent graphite. Notably, Banks and co-workers demonstrated that graphene electrodes may not be that advantageous for electrochemistry of ferrocyanide(II) and hexaammine-ruthenium(III) chloride, in addition to L-ascorbic acid and β -nicotinamide adenine dinucleotide.²³ Recently, the same group demonstrated that graphene-based carbon paste electrode does not exhibit any advantageous properties for detection of ascorbic and uric acid.²⁴ We have found similar conclusions for graphene modified electrodes when compared to its parent materials, showing that graphene materials of various numbers of layers (up to graphite) behave almost identically in case of uric acid and ascorbic acid,²⁵ as well as in case of trinitrotoluene (TNT).²⁶ Given the importance of haemoglobin detection, and previous reports claiming enhancement in direct electron transfer characteristics with utilization of carbon nanomaterials,^{13,14,27–32} we have decided to investigate whether graphene, and in what form (chemically, electrochemically reduced graphene oxide, graphite oxide and graphene oxide; all these materials were termed graphenoids³³) may influence the electron transfer characteristics of the $\text{Fe}^{\text{III}}/\text{Fe}^{\text{II}}$ redox couple of haemoglobin in solution.

Experimental

Materials

N,N-Dimethylformamide (DMF) was purchased from Merck. Potassium phosphate dibasic (K_2HPO_4), phosphoric acid (H_3PO_4 , ≥ 85 wt% in H_2O), haemoglobin human (lyophilized powder) were obtained from Sigma-Aldrich. Glassy carbon (GC), edge plane pyrolytic graphite (EPPG), Ag/AgCl reference, and Pt counter electrodes were obtained from Autolab (Japan).

Apparatus

All voltammetric experiments were carried out using a μ Autolab type III electrochemical analyser (Eco Chemie, The Netherlands) connected to a computer and controlled by General Purpose Electrochemical Systems, Version 4.9 software (GPES). Electrochemical experiments were conducted in an electrochemical cell (5 mL) at room temperature. A three-electrode configuration was employed. A platinum electrode served as the auxiliary electrode, and an Ag/AgCl electrode functioned as the reference electrode. All electrochemical potentials in this paper are stated *versus* Ag/AgCl reference electrode.

Procedures

Graphite oxide preparation with the Staudenmaier method. Graphite oxide was prepared using graphite according to the Staudenmaier³⁴ method whereby 17.5 mL of H_2SO_4 (95–98%) and 9 mL of HNO_3 (fuming) were stirred in a round bottom flask at 0 °C for 15 min. 1 g of graphite was then added to the mixture under vigorous stirring to obtain homogeneous dispersion. 11 g of KClO_3 was added to the mixture over 15 min in order to avoid temperature spike and formation of ClO_2 gas, which is explosive at high concentrations. The mixture was stirred vigorously for 96 h at room temperature. On completion, the mixture was poured into 1 L of deionized water and filtered. Graphite oxide

was then redispersed and washed repeatedly with 5% HCl solutions to remove sulphate ions. Graphite oxide was finally washed with deionized water under centrifugation until the filtrate was observed to be of neutral pH. Slurry graphite oxide was then dried in a vacuum oven at 60 °C for 48 h before use.

Graphene oxide preparation from graphite oxide from graphite oxide. Graphite oxide was placed in an ultrasonic bath (150 W) for 2 h to give uniform dispersion of graphene oxide.

Chemically reduced graphene oxide (CRGO) preparation from graphite oxide. Graphite oxide (50 mg) was dispersed in ultrapure water to give a 1.0 mg mL^{-1} colloidal solution and ultrasonicated (150 W) for 3 h. Hydrazine monohydrate (2 mL, 32.1 mmol) was added dropwise and the reaction mixture was left to stir under reflux for 24 h. The mixture was cooled to room temperature and filtered/washed over a PTFE membrane (0.2 μm) with methanol and ultrapure water to give CRGO upon drying at 50 °C for 5 days.

Electrochemically reduced graphene oxide (ERGO) preparation from graphite oxide. Electrochemically reduced graphene was obtained by applying a potential of -1.2 V (vs. Ag/AgCl) for 900 s to a graphene-oxide modified GC electrode in a phosphate buffer solution (pH 3.5).³⁵

Electrochemical measurements. Cyclic voltammetry experiments were performed at a scan rate of 100 mV s^{-1} in phosphate buffer solution (50 mM), and haemoglobin human with phosphate buffer solution as the supporting electrolyte. The reaction system was allowed to equilibrate for 5 s before differential pulse voltammetry measurements were performed under the same experimental conditions. Deoxygenation was achieved by bubbling highly purified nitrogen through the experimental solutions for at least 20 min.

Preparation of electrode surfaces. Glassy carbon (GC) working electrode surfaces were renewed by polishing with 0.05 μm alumina particles on a polishing pad. The graphene materials (1.0 mg mL^{-1}) were first dispersed in DMF. The suspensions were then subjected to an ultrasound bath treatment (37 kHz, 3 W cm^{-2}) for 60 s. Next, the suspension (1 μL) was pipetted onto the GC electrode surface, and left to evaporate at room temperature. We optimized the loading concentration of graphene from 0.1 mg mL^{-1} to 2 mg mL^{-1} . We have performed electrochemical characterization of the modified electrodes (CRGO, GO, GO', ERGO) with ferryl/ferrocyanide redox probe. We calculated the ΔE for four concentrations of graphene materials (0.1, 0.5, 1.0, 2.0 mg mL^{-1}). ΔE is a representation of the heterogeneous electron transfer (HET) rate. The lower the ΔE value, the higher the HET rate. We found that the HET increases when graphene loading increases from 0.1 mg mL^{-1} to 0.5 mg mL^{-1} and after that it levels off in the range 0.5 – 2 mg mL^{-1} . We thus chose 1 mg mL^{-1} as a robust value for graphene loading.

Results and discussion

Considering the profound influence carbon nanomaterials may have on the electron transfer between haemoglobin in solution and solid electrodes, it is of paramount importance to investigate if and how different carbon nanomaterials on the $\text{Fe}^{\text{III}}/\text{Fe}^{\text{II}}$ redox activity of haemoglobin. For this purpose, graphite oxide

(GO), chemically reduced graphene oxide (CRGO), graphene oxide (GO'), electrochemically reduced graphene oxide (ERGO) and edge plane pyrolytic graphite (EPPG) electrodes were used. Bare glassy carbon (GC) electrodes served as control experiments. We found that there were no major enhancements to electron transfer with use of the carbon nanomaterials.

Cyclic voltammetry (Fig. 1A) and differential pulse voltammetry (Fig. 1B) were used to investigate the electrochemistry of haemoglobin in solution. From Fig. 1A, the reduction of haemoglobin started at about -0.05 V and showed a peak at -0.15 V. The reduction peak height on each of the modified electrodes were measured and represented in bar graphs (Fig. 1 insets). With CV, EPPG electrodes gave a high peak current magnitude of 6.86 μ A, and performed superior to the other carbon nanomaterials investigated. On the other hand, with DPV, the

reduction of haemoglobin started at $+0.05$ V and peaked at -0.15 V. Bare GC electrodes gave the highest peak current magnitude of 722 nA. However, EPPG electrodes continued to show excellent performance with a peak current magnitude of 714 nA. Overall, it may be inferred that EPPG has the highest capacity to improve the electron transfer characteristics of the $\text{Fe}^{\text{III}}/\text{Fe}^{\text{II}}$ redox couple of haemoglobin, followed closely by bare GC electrodes. It is worthy of note that no significant enhancement of electron transfer characteristics was observed with GO, CRGO, GO' and ERGO electrodes. Even more surprising is that GO, CRGO, GO' and ERGO electrodes observed lower peak current magnitudes than that with bare GC electrodes. Note that large reduction currents at potentials < -0.5 V are due to the inherent electrochemical reduction of GO and GO'.^{36–38}

It would be interesting to investigate the superior performance of bare GC as compared to GO, CRGO, GO' and ERGO electrodes. A possible reason for GO, CRGO, GO' and ERGO electrodes to see lower peak current magnitudes for the

Fig. 1 (A) Cyclic voltammograms (scan rate 0.1 V s^{-1} , $+0.5 \text{ V}$ to -1.0 V) were obtained in aqueous 50 mM phosphate buffer at pH 3.5 for the electrochemical reduction of human haemoglobin (0.05 mg mL^{-1} , $0.78 \text{ } \mu\text{M}$) at GC electrodes modified with graphene materials (1 mg mL^{-1}). Inset: reduction peak height of $\text{Fe}^{\text{III}}/\text{Fe}^{\text{II}}$ redox couple corresponding to different graphene modified electrode surfaces. Error bars represent RSD ($n = 3$; 95% confidence interval). (B) Differential pulse voltammograms (scan rate 0.01 V s^{-1} , $+0.5 \text{ V}$ to -1.0 V) were obtained in aqueous 50 mM phosphate buffer at pH 3.5 for the electrochemical reduction of human haemoglobin (0.05 mg mL^{-1} , $0.78 \text{ } \mu\text{M}$) at GC electrodes modified with graphene materials (1 mg mL^{-1}). Inset: peak heights of $\text{Fe}^{\text{III}}/\text{Fe}^{\text{II}}$ redox couple corresponding to different graphene modified electrode surfaces. Error bars represent RSD ($n = 3$; 95% confidence interval).

Fig. 2 (A) Cyclic voltammograms (scan rate 0.1 V s^{-1} , $+0.5 \text{ V}$ to -1.0 V) were obtained in aqueous 50 mM phosphate buffer at pH 3.5 for the electrochemical reduction of human haemoglobin of different concentrations at GC electrodes. (B) Differential pulse voltammograms (scan rate 0.01 V s^{-1} , $+0.5 \text{ V}$ to -1.0 V) were obtained in aqueous 50 mM phosphate buffer at pH 3.5 for the electrochemical reduction of human haemoglobin of different concentrations at GC electrodes.

reduction of haemoglobin than that with bare GC electrodes could be that they possess different linear response ranges. In order to do so, it is crucial to understand the how the peak current is dependent on the concentration of haemoglobin. For this purpose, cyclic voltammetry and differential pulse voltammetry were performed on bare GC (Fig. 2) and CRGO (Fig. 3) electrodes.

For bare GC electrodes, both CV (Fig. 2A) and DPV (Fig. 2B) gave agreeable results. The reduction of haemoglobin was observed to peak at -0.15 V. It is observed that the higher the haemoglobin concentration, the higher the peak current magnitude. On the CRGO electrode, the reduction of haemoglobin was observed to peak at about -0.20 V using CV (Fig. 3A), and -0.15 V using DPV (Fig. 3B). Similarly, the higher the concentration of haemoglobin, the higher the magnitude of peak current observed.

The calibration plots of bare GC electrodes were then compared to that of CRGO electrodes (Fig. 4). Fig. 4A illustrates the calibration plots derived from CV measurements, whereas Fig. 4B demonstrates the calibration plots resulting from DPV

Fig. 3 (A) Cyclic voltammograms (scan rate 0.1 V s^{-1} , $+0.5 \text{ V}$ to -1.0 V) were obtained in aqueous 50 mM phosphate buffer at $\text{pH } 3.5$ for the electrochemical reduction of human haemoglobin of different concentrations at GC electrodes modified with CRGO. (B) Differential pulse voltammograms (scan rate 0.01 V s^{-1} , $+0.5 \text{ V}$ to -1.0 V) were obtained in aqueous 50 mM phosphate buffer at $\text{pH } 3.5$ for the electrochemical reduction of human haemoglobin of different concentrations at GC electrodes modified with CRGO.

Fig. 4 (A) Reduction peak height of $\text{Fe}^{\text{III}}/\text{Fe}^{\text{II}}$ redox couple of cyclic voltammograms at different concentrations of human haemoglobin at GC electrodes (black line) and GC electrodes modified with 1 mg mL^{-1} CRGO (red line). The calibration curve observed concentration saturation starting at $1.55 \text{ } \mu\text{M}$. Error bars represent RSD ($n = 3$; 95% confidence interval). (B) Reduction peak height of $\text{Fe}^{\text{III}}/\text{Fe}^{\text{II}}$ redox couple of differential pulse voltammograms corresponding to different concentrations of human haemoglobin at GC electrodes (black line) and GC electrodes modified with 1 mg mL^{-1} CRGO (red line). The calibration curve observed concentration saturation starting at about $1.55 \text{ } \mu\text{M}$. Error bars represent RSD ($n = 3$; 95% confidence interval).

measurements. The linear response range on bare GC electrodes may be estimated to be from 0.0 to $1.55 \text{ } \mu\text{M}$ concentrations of haemoglobin. Saturation of concentration dependence is evident with haemoglobin concentrations higher than $1.55 \text{ } \mu\text{M}$. On the other hand, the haemoglobin concentration range over which peak current is proportional to concentration may be observed to be from 0.0 to $1.55 \text{ } \mu\text{M}$ for CRGO electrodes. Saturation of concentration dependence is evident with haemoglobin concentrations higher than $1.55 \text{ } \mu\text{M}$. Hence, the linear response ranges on both bare GC and CRGO electrodes are the same.

From CV measurements (Fig. 4A), at $1.55 \text{ } \mu\text{M}$ concentration of haemoglobin, peak current of $-6.96 \text{ } \mu\text{A}$ corresponding to the $\text{Fe}^{\text{III}}/\text{Fe}^{\text{II}}$ redox couple was observed on bare GC electrodes. This is approximately 1.4 fold more than that on CRGO electrodes which gave a peak current of $-4.83 \text{ } \mu\text{A}$. Similarly, a peak current

of 878 nA corresponding to the $\text{Fe}^{\text{III}}/\text{Fe}^{\text{II}}$ redox couple was observed on bare GC electrodes from DPV measurements (Fig. 4B). This is 1.3 fold more than that on CRGO electrodes which gave a peak current of 646 nA. Therefore, we may conclude that bare GC measurements gave higher sensitivity, demonstrating again its superior performance as compared to GO, CRGO, GO' and ERGO electrodes.

Conclusions

We have demonstrated that bare glassy carbon remains an appropriate electrode material for further probing into the electrochemical sensing of haemoglobin in solution. Surprisingly, most of the carbon nanomaterials investigated, including GO, CRGO, GO' and ERGO, did not display improvement to the electron transfer characteristics of the $\text{Fe}^{\text{III}}/\text{Fe}^{\text{II}}$ redox couple of haemoglobin in solution. These findings are in contrast to previous studies, which report an enhancement in direct electron transfer characteristics with utilization of carbon nanomaterials. However, most of the previous studies relate to electrochemical sensing of haemoglobin immobilized on the electrode surface.^{13,14,27,29–32} Our findings are of high importance for further investigation of the electron transfer characteristics of the $\text{Fe}^{\text{III}}/\text{Fe}^{\text{II}}$ redox couple of haemoglobin, and the differences between solution-based and solid-state electrochemical sensing of the protein.

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Direct In Vivo Electrochemical Detection of Haemoglobin in Red Blood Cells

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The electrochemical behavior of iron ion in haemoglobin provides insight to the chemical activity in the red blood cell which is important in the field of hematology. Herein, the detection of haemoglobin in human red blood cells on glassy carbon electrode (GC) was demonstrated. Red blood cells or raw blood cells was immobilized on a glassy carbon electrode surface with Nafion films employed to sandwich the layer of biological sample firmly on the electrode surface. Cyclic voltammetry (CV) analyses revealed a well-defined reduction peak for haemoglobin at about -0.30 V (vs. Ag/AgCl) at the red blood cell (GC-Nf-RBC-3Nf) and blood (GC-Nf-B-3Nf) film modified GCE in a pH 3.5 phosphate buffer solution. We further demonstrated that the complex biological conditions of a human red blood cell displayed no interference with the detection of haemoglobin. Such findings shall have an implication on the possibilities of studying the electrochemical behaviour of haemoglobin directly from human blood, for various scientific and clinical purposes.

In human blood, the physiological role of haemoglobin (Hb) is the storage and transport of molecular oxygen (O_2) and carbon dioxide (CO_2). The metalloprotein has a molecular weight of 64.5 kDa and is the majority substance in red blood cells (RBCs). Haemoglobin contains four polypeptide subunits (two α and two β subunits) of similar structure, each of which has an iron-bearing heme within molecularly accessible crevices^{1,2}. The iron ion may be in either the II or III oxidation state. In its ferrous state ($HbFe^{II}$), haemoglobin binds oxygen to form oxyhaemoglobin (Oxy-Hb; $HbFe^{II}-O_2$). However, in its ferric state ($HbFe^{III}$), methaemoglobin (Met-Hb) loses oxygen-binding capacity. Fe^{II} is oxidized to Fe^{III} spontaneously in small amounts in human red blood cells (1–2% of Hb), and if not moderated by internal reduction mechanisms within the red blood cell, high levels of Met-Hb (methemoglobinemia) could result in fatality^{3–5}.

Activity measurement of proteins such as haemoglobin may be crucial to investigate the nature of many *in vivo* energy conversion processes, from photosynthesis to respiration, most of which are facilitated by these protein molecules^{6–9}. In particular, the direct electrochemical detection of metalloproteins has been applied to bioanalysis^{10,11}. The iron ion in haemoglobin serves as a redox-active center. Hence, the direct electron transfer reactions of haemoglobin may provide fundamental understanding of its redox behaviour within the red blood cell. It is important to monitor accurately such reactions because abnormal shift in chemical activity in the red blood cell usually occurs due to primary hematologic disorders like anemia, or a wider variety of other diseases which produces secondary abnormalities of the blood^{12,13}. Moreover, it is essential to determine the concentration of haemoglobin in blood samples as an indication of the red blood cell count in blood and hence, the oxygen transport ability of blood.

In this paper, our goal is to probe into the electrochemical behaviour of the iron ion of haemoglobin contained in RBCs. Previous studies have shown that direct electron transfer to cell-free haemoglobin redox center is possible^{14–17}. However, little is studied about the voltammetric responses obtained directly from red blood cells as the electrochemistry of haemoglobin within red blood cells may be complicated due to the presence of the cellular membrane and complex biological environment. Herein, we would like to address the following basic questions: 1) is the electrochemical activity of iron ion in haemoglobin observed with the chemical systems of red blood cells and blood; and 2) is the electrochemical activity of iron ion in haemoglobin interfered by the biological complexity of red blood cells. If so, what is happening at the iron centre of haemoglobin in these systems. The commonly used glassy carbon electrode would serve as the surface for the investigation of such electrochemical behaviour.

Figure 1 | (A) Cyclic voltammograms (from +0.5 V to −1.0 V) were obtained in aqueous 50 mM phosphate buffer at pH 5.5 and 3.5 for the electrochemical reduction of human haemoglobin (0.05 mg/mL, 0.78 μM) at GC electrodes. Reduction peak potential of haemoglobin decreases with an increase in pH. Scan rate is 0.1 Vs^{−1}. Inset: Reduction peak height of cyclic voltammograms at different pH values. Error bars represent RSD ($n = 3$; 95% confidence interval). (B) Cyclic voltammograms (from +0.5 V to −1.0 V) were obtained in aqueous 50 mM phosphate buffer for the electrochemical reduction of blood at pH 3.5 and pH 7.4. Blood concentration was chosen to be 1×10^3 cells/μL. Reduction peak current magnitudes decreases with an increase in pH. Scan rate is 0.1 Vs^{−1}. Inset: Reduction peak height of cyclic voltammograms at different pH values. Error bars represent RSD ($n = 3$; 95% confidence level).

Results and Discussion

Experimental conditions were first optimised by investigating the reduction of haemoglobin in solution at different pH values on bare GC electrodes using cyclic voltammetry (CV). A marked effect of pH on the voltammetric response for haemoglobin may be observed in Figure 1A. The reduction of haemoglobin shows a peak at approximately −0.3 V and −0.15 V at pH 5.5 and pH 3.5 respectively. A decrease in pH shows a shift in reduction peak potential to less negative values. Accompanying this observation, a decrease from pH 5.5 to pH 3.5 saw an almost eleven-fold increase in the charge under the voltammetric response (Figure 1 inset). The observed pH dependence of the redox process is in agreement with previously reported works^{17,18}, suggesting the involvement of H⁺. Similarly, a study on pH dependence was carried out for blood at GC-Nf-B-3Nf electrodes using cyclic voltammetry (Figure 1B). A significant effect of pH on the voltammetric response for blood was noted. The reduction of blood shows a peak at about −0.3 V at both pH 3.5 and pH 7.4. A decrease from pH 7.4 to pH 3.5 saw an almost four-fold increase in the charge under the voltammetric response (Figure 1B inset). Hence, an acidic pH of 3.5 was chosen as an experimental condition for subsequent measurements in order to work with more defined voltammetric signature of haemoglobin.

A step forward from the investigation of electrochemistry of cell-free haemoglobin would be the measurement of such directly from intact red blood cells (RBCs) and blood. Such a measurement could provide better insights on actual electrochemical activity of iron ions in red blood cells *in vivo*. However, to eliminate the complications of biological environments, we began by studying these systems from a chemical point of view by obtaining the lyophilized forms of red blood cells and blood, and re-suspending them in phosphate buffers accordingly.

We investigated a solid state approach by sandwiching the red blood cell or blood layer between layers of Nafion that were deposited on the surface of a GC electrode to give GC-Nf-RBC-3Nf and GC-Nf-B-3Nf electrode surfaces respectively, as illustrated in Figure 2. Nafion contains a highly acidic sulfonate group, and is employed as a cation-exchange polymer. Electrodes coated with Nafion (and other surface confined molecules) have commonly been used to trap or concentrate solution phase species and thus improve their analytical detection^{19–21}. In this work, the Nafion layers are thought to have two functions; (1) stabilizing matrix for RBCs on the GC electrode, and (2) to provide a readily exchangeable source of H⁺ to molecules

within the membranes to balance the charge as a result of electrochemical reductions.

To determine if it is possible to probe iron redox activity of haemoglobin directly from RBCs (lyophilized) and blood (lyophilized), cyclic voltammetry was used to study the reduction of red blood cells and blood on GC-Nf-RBC-3Nf and GC-Nf-B-3Nf electrodes, respectively. Figure 3A compares the reduction of red blood cells to that of haemoglobin using the solid state approach. The reduction of haemoglobin started at −0.05 V and showed a peak at −0.30 V. The same is observed for the reduction of red blood cells at concentrations 0.145 mg/mL RBCs and below. However, higher concentrations of red blood cells see a reduction starting at −0.05 V and showing a peak at −0.15 V. The reduction peak potential is shifted to less negative values. It is interesting to note that the reduction peak potential for higher concentrations of red blood cells correspond to that of reduction of haemoglobin in solution (−0.15 V). Similarly, Figure 3B compares the reduction of blood to that of haemoglobin. A reduction peak potential of −0.30 V was observed for blood concentrations of 1×10^3 cells/μL and below. At higher concentrations of blood, shifts of reduction peak potentials to less negative values were observed. Hence, it may be concluded that reduction peaks corresponding to haemoglobin were obtained directly from the (lyophilized) red blood cells and blood, despite the possible complications due to the presence of the cellular membrane and complex biological environments. Calibration to quantify the signal mag-

Figure 2 | Schematic diagram of the proposed structures of GC-Nf-RBC-3Nf and GC-Nf-B-3Nf electrode surfaces.

Figure 3 | (A) Cyclic voltammograms (from +0.5 V to −1.0 V) were obtained in aqueous 50 mM phosphate buffer at pH 3.5 for the electrochemical reduction of red blood cells (powder) at GC-Nf-RBC-3Nf electrodes. Scan rate is 0.1 Vs^{-1} . Control experiments were performed using Hb (blue dotted line) and pH 3.5 PBS (black dotted line). (B) Cyclic voltammograms (from +0.5 V to −1.0 V) were obtained in aqueous 50 mM phosphate buffer at pH 3.5 for the electrochemical reduction of blood at GC-Nf-B-3Nf electrodes. Legend shows concentrations of blood (Cells ($\times 10^3$)/ μL). Scan rate is 0.1 Vs^{-1} . Control experiments were performed using Hb (blue dotted line) and pH 3.5 PBS (black dotted line).

nitude against different concentrations of RBCs was conducted in a previous study of ours²².

Having established that the reduction signals from red blood cells (lyophilized) and blood (lyophilized) correspond to that of reduction of haemoglobin, it is equally important to determine the source of these signals: 1) directly from red blood cells or 2) free haemoglobin released into the supernatant due to lysis of the red blood cells. For this purpose, a lysis assessment was conducted. Scanning electron microscopy (SEM) images of red blood cells in powder form are shown in Figure 4. The red blood cells exhibited mostly biconcave structures with diameters of about $3 \mu\text{m}$. Crystals are observed on the membranes of the red blood cells. These crystals may be derived from the preparation process.

Cyclic voltammetry was used to investigate the reduction of red blood cells (lyophilized) before and after filtration (Figure 5). The filtration process would aid in removing any free haemoglobin released into the supernatant due to lysis of the red blood cells. It may be observed that the peak current magnitude dropped about four-fold from about $13 \mu\text{A}$ before filtration to approximately $3 \mu\text{A}$ from the filtrate or supernatant of the red blood cell suspension. The residue red blood cells obtained after filtration were re-suspended in pH 3.5 phosphate buffer. The peak current magnitude observed was similar to that of the red blood cell suspension before filtration.

Hence, the reduction signals observed may be derived directly from the red blood cells. To further support such a claim, optical microscopy of the red blood cells was performed before (Figures 6A–C) and after (Figure 6D–F) filtration. The images obtained were similar before and after filtration, showing no substantial lysis of the red blood cells.

Moreover, it is crucial to understand the concentration dependence of the voltammetric signals. Cyclic voltammetry (CV) was used to study the reduction of red blood cells (Figure 7A) and blood (Figure 7B) on GC-Nf-RBC-3Nf and GC-Nf-B-3Nf electrodes respectively and the insets illustrates the calibration plot obtained correspondingly. Calibration of the signal response against red blood cell concentration may act as a crucial clinical diagnosis of the oxygen transport ability of a blood sample. From Figure 7A, the red blood cell concentration range over which peak current is proportional to concentration ranges from 0 to 1.45 mg/mL . Saturation of concentration dependence is observed with red blood cell concentrations higher than 1.45 mg/mL ($50 \times 10^3 \text{ cells}/\mu\text{L}$). Figure 7B shows that the blood concentration range over which the peak current is proportional to concentration may be estimated to be from 0 to $5 \times 10^3 \text{ cells}/\mu\text{L}$. At blood concentrations higher than $5 \times 10^3 \text{ cells}/\mu\text{L}$, peak current magnitude decreases with an increase in blood concentration. Red blood cells in blood samples were quantified by taking

Figure 4 | Scanning electron microscopy (SEM) of red blood cells (lyophilized) at 5.0 kV. Red blood cells have diameters of about $3 \mu\text{m}$.

Figure 5 | Cyclic voltammograms (from +0.5 V to −1.0 V) were obtained in aqueous 50 mM phosphate buffer at pH 3.5 for the electrochemical reduction of red blood cells before filtration (black line) and after filtration; filtrate (red line) and re-suspended residue (blue line) at GC-Nf-RBC-3Nf electrodes. Red blood cell concentration = 72.5 mg/mL (2500×10^3 cells/ μL). Scan rate is 0.1 V s^{-1} .

reference to a standard average RBC count of 5000×10^3 cells per microliter of blood in a healthy individual²³. The linear response ranges obtained in both cases are very narrow. This would imply the need for high dilutions during sample preparation. However, such an observation would mean that the volume of blood sample required may be minimized. This is a critical advantage in clinical studies where volume and frequency of blood withdrawal are of concern.

Our experiments hitherto investigated the chemical systems of red blood cells and blood. However, these systems do not completely replicate that of real biological systems as they do not have regulation and feedback mechanisms. In biological systems, most parameters are required to stay within a narrow range around a certain optimal level under certain environmental conditions. The maintenance of this optimal level for a parameter is known as homeostasis. One of

the challenges faced would be the uncertainty in behaviour of voltammetric responses from real blood samples due to homeostatic processes.

In order to study the voltammetric responses from real biological systems, we employed human red blood cells (live) derived directly from real blood samples. Similar to experiments carried out previously, a solid state approach was taken by sandwiching the red blood cell layer between layers of Nafion that were deposited on the surface of a GC electrode (GC-Nf-RBC-3Nf) as illustrated in Figure 2. Cyclic voltammetry (CV) was used to study the reduction of red blood cells (live) obtained directly from real blood samples on GC-Nf-RBC-3Nf electrodes (Figure 8). Comparison was made between the voltammetric responses derived from the electrochemical reduction of red blood cells (live) and red blood cells (lyophilized). The reduction of red blood cells in both cases starts at +0.05 V and shows a peak at −0.15 V. Therefore, the similar reduction potentials and shape of cyclic voltammograms observed points to the possibility of probing haemoglobin activity from a real biological system without much interference.

The reproducibility of the proposed biosensor for haemoglobin in red blood cells (live) was investigated at 50 mM phosphate buffer solution. The relative standard deviation was 4.3% ($n = 3$) for three successive measurements demonstrated that the electrode-to-electrode reproducibility is acceptable in the response of three biosensors prepared under the same conditions independently. Hence, it may be established that the analyte is stable during electrode modification and electrochemical measurements.

In conclusion, a simple protocol was developed for the immobilization of RBCs or blood on a glassy carbon electrode using Nafion. On the modified electrode, direct detection of haemoglobin from RBCs and blood samples were realized at pH 3.5 PBS, which may allow for quantitative analysis of red blood cells in blood samples for clinical diagnosis. Stability of the analyte under the proposed experimental conditions was demonstrated by the high reproducibility of the experiments. Moreover, it is important to consider what is happening at the iron centre of haemoglobin during measurements. For this purpose, we consider the experimental conditions employed. The above experiments were performed under aerobic conditions.

Figure 6 | Optical microscopy of red blood cells before (A–C) and after (D–F) filtration.

Figure 7 | (A) Cyclic voltammograms (from +0.5 V to −1.0 V) were obtained in aqueous 50 mM phosphate buffer at pH 3.5 for the electrochemical reduction of red blood cells at GC-Nf-RBC-3Nf electrodes. Legend shows concentrations of RBC (mg/mL). Scan rate is 0.1 Vs^{−1}. Inset: Reduction peak height corresponding to different concentrations of RBC. The calibration curve observed concentration saturation starting at 1.45 mg/mL (50×10^3 cells/μL). Error bars represent RSD ($n = 3$; 95% confidence interval). (B) Cyclic voltammograms (from +0.5 V to −1.0 V) were obtained in aqueous 50 mM phosphate buffer at pH 3.5 for the electrochemical reduction of blood at GC-Nf-B-3Nf electrodes. Legend shows concentrations of blood (Cells ($\times 10^3$)/μL). Scan rate is 0.1 Vs^{−1}. Inset: Reduction peak height corresponding to different concentrations of blood. The calibration curve observed concentration saturation starting at 5×10^3 cells/μL. Error bars represent RSD ($n = 3$; 95% confidence interval).

HbFe^{II} reacts rapidly with oxygen to give HbFe^{II}-O₂²⁴. With comparison to previous research, it is most probable that the reduction peak from haemoglobin in the range of −0.15 V to −0.3 V corresponds to the electrochemical reduction of the HbFe^{II}-O₂ complex to give HbFe^{II} and hydrogen peroxide^{25,26}. Obviously, there leaves much to be studied about the mechanism involved. We believe that such a biosensor shows great promise for the study of the physiological status of RBCs in real blood samples for scientific and clinical purposes.

Methods

Materials. *N,N*-dimethylformamide (DMF) was purchased from Merck. Potassium phosphate dibasic (K₂HPO₄), phosphoric acid (H₃PO₄, ≥85wt.% in H₂O), Nafion 117 solution (~5 wt% in a mixture of lower aliphatic alcohols and water), haemoglobin human (lyophilized powder), red blood cells from sheep (dry powder, glutaraldehyde treated) were obtained from Sigma-Aldrich. Lyophilized human

whole blood was obtained from the Institute for Reference Materials and Measurements and reconstituted as described in the instructions for use. Human red blood cells (live) were obtained from i-DNA Biotechnology Pte Ltd. Glassy-carbon (GC), Ag/AgCl reference, and Pt counter electrodes were obtained from Autolab (Japan).

Apparatus. All voltammetric experiments were carried out by using a μAutolab type III electrochemical analyser (Eco Chemie, The Netherlands) connected to a computer and controlled by General Purpose Electrochemical Systems, Version 4.9 software (GPES). Electrochemical experiments were conducted in an electrochemical cell (5 mL) at room temperature. A three-electrode configuration was employed. A platinum electrode served as the auxiliary electrode, and an Ag/AgCl electrode functioned as the reference electrode. All electrochemical potentials in this paper are stated versus Ag/AgCl reference electrode.

Preparation of Electrode Surfaces. Glassy carbon (GC) working electrode surfaces were renewed by polishing with 0.05 μm alumina particles on a polishing pad. A 1 μL droplet of Nafion solution was placed on the electrode surface with a micropipette. The solvent was then allowed to dry in air for 5 min. Subsequently, a 1 μL aliquot of the analyte solution was deposited on top of the Nafion layer. 3 μL droplets of the Nafion solution were then deposited on top of the analyte layer in three separate 1 μL deposition steps. The solvent was allowed to evaporate for 5 min after each addition. The optimal coating procedure described above was determined by a number of trial and error experiments, and further supported by a previous research²⁷. The resulting modified electrode was abbreviated as GC-Nf-RBC-3Nf or GC-Nf-B-3Nf with red blood cells or blood as the analyte respectively.

Electrochemical measurements. Cyclic voltammetry experiments were performed at a scan rate of 100 mV s^{−1} in phosphate buffer solution (pH 3.5, 50 mM) as supporting electrolyte.

Figure 8 | Cyclic voltammograms (from +0.5 V to −1.0 V) were obtained in aqueous 50 mM phosphate buffer at pH 3.5 for the electrochemical reduction of red blood cells (live) at GC-Nf-RBC-3Nf electrodes. Comparison was made between red blood cells purchased from Sigma Aldrich in powder form (black line, RBCs suspended in pH 3.5 PBS, RBC concentration of 1.45 mg/mL, 50×10^3 cells/μL) and red blood cells derived directly from blood samples (red line, RBCs suspended in pH 7.4 1× PBS, RBC concentration of approximately 10% vol.). Scan rate is 0.1 V s^{−1}.

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Author contributions

M.P. and J.H. devised experiments, R.J.T. performed measurements, R.J.T. and M.P. analyzed data. M.P., R.J.T., J.H. and W.K.P. discussed the data and contributed writing the manuscript.

Additional information

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MoSe₂ Nanolabels for Electrochemical ImmunoassaysRou Jun Toh,[†] Carmen C. Mayorga-Martinez,[†] Zdeněk Sofer,[‡] and Martin Pumera^{*,†}[†]Division of Chemistry and Biological Chemistry, School of Physical and Mathematical Sciences, Nanyang Technological University, Singapore 637371[‡]Department of Inorganic Chemistry, University of Chemistry and Technology Prague, Technická 5, 166 28 Prague 6, Czech Republic

S Supporting Information

ABSTRACT: There is huge interest in biosensors as a result of the demand for personalized medicine. In biomolecular detection, transition-metal dichalcogenides (TMDs) can be used as signal-enhancing elements. Herein, we utilize a solution-based electrochemical exfoliation technique with bipolar electrodes to manufacture MoSe₂ nanolabels for biomolecular detection. Prepared MoSe₂ nanoparticles (NPs) exhibit electrocatalytic activity toward the hydrogen evolution reaction (HER), and such a property allows it to act as a robust label for magneto-immunoassays toward protein detection. The magneto-immunoassay also displayed good selectivity, a wide linear range of 2 to 500 ng mL⁻¹, high sensitivity (LOD = 1.23 ng mL⁻¹) and reproducibility (RSD = 9.7%). These findings establish the viability and reproducibility of such an exfoliation technique for TMD nanolabels for the development of low costs and efficient biosensing systems.

Transition-metal dichalcogenides (TMDs) are layered materials with a general chemical formula MX₂, where M is a transition metal (i.e., W, Mo, Hf) and X is a chalcogen, generally S, Se, or Te. When exfoliated to single layer, they demonstrate high surface-to-volume ratios, layered structures that can accommodate ionic or organic compounds, electronic properties ranging from insulating to metallic, versatility in functionalization, and advantageous optical properties in the range of visible wavelength.^{1–5} These properties allow them to be useful for advanced energy storage and conversion,⁶ electrochemical catalysis,^{7,8} and, in particular, sensing applications.^{9,10}

Selective and sensitive detection of biomolecules is one of the critical foundations of biomedical research. In order to achieve sensitive detection, a signal-enhancing element is typically employed to label target molecules. Where the catalytic detection mode is employed, a characteristic of the signal-enhancing element would be that it possesses catalytic activity toward a reaction to be probed. Specifically, the hydrogen evolution reaction (HER) has been probed for such a purpose^{11,12} where gold nanoparticles (NPs)^{13,14} are commonly used as labels due to their long-term stability. With a long shelf life and high catalytic activity toward the HER,^{15–17} TMDs present as promising low costs and efficient alternative labels for biomolecular detection.

A solution-based electrochemical exfoliation method (Scheme 1A) has previously been developed for the fabrication of WS₂ nanoparticles,¹² and herein, we investigate if such a technique can be extensively used with other TMD materials for producing nanolabels for magneto-immunoassays. For this

purpose, MoSe₂ was chosen as the material of interest. MoSe₂ has been demonstrated to be most catalytically active for the HER among the highly studied group VI *t*-BuLi exfoliated TMD materials.^{16,18–21}

Synthesized MoSe₂ NPs were tagged to rabbit IgG proteins and captured by anti rabbit IgG attached to magnetic beads (Scheme 1B). Consequently, the advantageous use of MoSe₂ nanolabels for magneto-immunoassays was demonstrated via HER catalysis. Furthermore, we showed the viability and reproducibility of the solution-based exfoliation technique for producing TMD nanoparticles as labels for biomolecular detection, which can be applied in the mass production and development of low-cost immunosensors.

■ EXPERIMENTAL SECTION

Reagents. Molybdenum selenide MoSe₂ (99.9%) and *tert*-butyllithium (1.7 M in pentane) were acquired from Alfa Aesar (Germany) and Sigma-Aldrich (Czech Republic) respectively. Magnetic beads (Dynabeads M-280 Tosylactivated) were obtained from Invitrogen (Singapore). Boric acid, sodium hydroxide, phosphate-buffered saline tablet, albumin from bovine serum (BSA), TWEEN 20, rabbit IgG from serum, antirabbit IgG produced in goat, and hemoglobin (lyophilized powder) were obtained from Sigma-Aldrich (Singapore).

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Scheme 1. (A) Solution-Based Electrochemical Exfoliation with Bipolar Electrodes of MoSe₂ (*t*-BuLi) Sheets to MoSe₂ NPs; (B) Preparation of Magneto-Immunoassay for Detection of Rabbit IgG Using MoSe₂ Nanolabels via HER Catalysis^a

^aSteps in panel B: (i) Incubation of magnetic bead (MB) with anti rabbit IgG. (ii) Incubation of desired concentration of rabbit IgG with MoSe₂ NPs. (iii) Final conjugation of anti rabbit IgG with labeled rabbit IgG and electrochemical detection through HER.

Synthesis of Label. MoSe₂ sheets intercalated by Li using *tert*-butyllithium were used for the synthesis of MoSe₂ NPs. After intercalation, the MoSe₂ sheets were exfoliated in water according to a previously described protocol,⁸ and purification was achieved by centrifugation. The resulting solid powder of MoSe₂ (*t*-BuLi) was dispersed in ultrapure water (0.5 mg mL⁻¹) and subjected to ultrasonication for a duration of 4 h. Subsequently, 0.5 M Na₂SO₄ was added to 4 mL of the MoSe₂ dispersion. The resultant dark suspension was placed into an electrochemical cell. After which, the two driver electrodes of Pt were placed in the electrochemical cell (see Scheme 1A). The distance between the electrodes is around 2 cm (Figure S1). Ten volts of DC potential was applied for 30 min, after which a clear suspension was obtained (Figure S2). The suspension was allowed to settle for several hours, and finally, the supernatant was collected. For XPS, STEM, Raman, TEM, and EDS characterization, dialysis of the supernatant was performed using a membrane with a molecular weight cutoff below 1 kDa.

Magneto-Immunoassay. On the basis of previously reported procedures, the magneto-immunoassay was prepared.¹² Initially, 3 mg mL⁻¹ of magnetic beads (MB) was washed in borate buffer (pH 9.2) and incubated overnight with anti-rabbit IgG at 37 °C and 400 rpm agitation. The final concentration of anti rabbit IgG in the mixture was 40 μg mL⁻¹. Subsequently, the MBs/anti rabbit IgG complex was washed with PBS TWEEN 20 (0.5% v/v). Next, the blocking step was performed by resuspending the obtained complex in a PBS solution containing 5% BSA (w/v) at 25 °C and 650 rpm agitation for 40 min. When the blocking step is completed,

washing of the samples was done with PBS and BSA (0.1% w/v).

In order to attach the electrochemically active label to the rabbit IgG, the label was incubated with the protein at 25 °C with 650 rpm agitation for 2 h. Subsequently, a blocking step was carried out by adding BSA (5% w/v) in ultrapure water to the solution of labeled protein and extending the incubation for 20 min under the same initial conditions. After the blocking step, the sample was centrifuged at 4 °C and 14 000 rpm for 2 h. Then, 70 μL of PBS was used for the reconstitution of the resulting pallet.

The final incubation step was carried out by first preconcentrating the magnetic bead/anti rabbit IgG from 70 μL of the supernatant. Next, 70 μL of the labeled rabbit IgG was mixed with the magnetic bead/anti-rabbit IgG. The incubation was performed at 25 °C under 650 rpm agitation for a duration of 30 min. Subsequently, washing steps were carried out using PBS TWEEN 20, PBS, and ultrapure water. Finally, the assembly was resuspended in ultrapure water.

Instrumentation. X-ray photoelectron spectroscopy (XPS) measurements were done using a Phoibos 100 spectrometer and a monochromatic Mg X-ray radiation source (SPECS, Germany). Survey (wide-scan) and high-resolution spectra were recorded for each sample. For XPS measurements, sample preparation was done by coating a uniform layer of the materials under study onto a silicon wafer. A UV-vis spectrometer (Shimadzu UV-2500) was employed to obtain the UV-vis spectra. Scanning transmission electron microscopy (STEM) images were acquired by using a Jeol 7600F SEM instrument (Jeol, Japan) operating at 15 kV (MoSe₂ NPs) or 25 kV (magneto-immunoassay). Raman spectra were acquired using a confocal micro-Raman LabRam HR instrument (HORIBA Scientific) in backscattering geometry with a CCD detector, a 514.5 nm Ar laser and a 100 objective attached to an Olympus optical microscope. Prior to measurements, calibration was achieved using an internal silicon reference at 520 cm⁻¹ with a peak position resolution of less than 1 cm⁻¹. The spectra ranged from 100 to 1000 cm⁻¹. Transmission electron microscopy (TEM), HR-TEM, and electron dispersive X-ray spectroscopy (EDS) images were obtained with an EFTEM Jeol 2200 FS HRTEM microscope (Jeol, Japan) operated at 200 keV. EDS mapping was performed with SSD detector from Oxford Instruments (X-MaxN 80 T). Samples were prepared for analysis by drop casting the suspension on a TEM grid (Cu/lacey carbon; 200 mesh) and dried at 60 °C for 2 h. All voltammetric experiments were performed using a μAutolab type III electrochemical analyzer (Eco Chemie, The Netherlands) connected to a computer and controlled by the NOVA, Version 1.8.17, software.

Electrochemical Measurements. HER performance was evaluated using linear sweep voltammetry (LSV) where a three-electrode electrochemical system was employed. The three electrodes used are a glassy carbon (GC) working electrode (3 mm diameter), a Pt counter electrode, and Ag/AgCl reference electrode. Modification of the working electrode was achieved by drop-casting MoSe₂ NP solution (3 μL) and leaving the solution to dry at room temperature. Moreover, screen-printed (SP) electrodes (from eDAQ instruments) composed of a graphitic carbon working electrode (3 mm diameter) and counter electrode with reference electrode of Ag/AgCl were used for the optimization of experimental conditions and magneto-immunoassays. In all cases, measurements (chronoamperometry) were carried out in H₂SO₄ (0.5 M); 15 μL of

H_2SO_4 (1 M) was mixed with 15 μL of MoSe_2 NPs solution or magneto-immunoassay on the SP electrode. LSV measurements were performed at a scan rate of 2 mV/s while chronoamperometry was performed at -0.97 V vs RHE for 200 s.

RESULTS AND DISCUSSION

In this work, we investigate the interactions between MoSe_2 NPs (synthesized by solution-based electrochemical exfoliation method with bipolar electrodes) and rabbit IgG for the quantitative determination of the protein. Characterization of the prepared MoSe_2 NPs was first performed using X-ray photoelectron spectroscopy (XPS), ultraviolet visible (UV-vis) absorption spectroscopy, scanning transmission electron microscopy (STEM), Raman spectroscopy, transmission electron microscopy (TEM), and electron dispersive X-ray spectroscopy (EDS). The electrocatalytic activity of MoSe_2 NPs toward hydrogen evolution reaction (HER) was probed via linear sweep voltammetry (LSV) and chronoamperometry. Finally, STEM and ion-coupled plasma mass spectrometry (ICP-MS) analysis verified the attachment of MoSe_2 NPs as labels for magneto-immunoassays. Herein, we wish to address the following questions: (i) are MoSe_2 NPs obtained by solution-based electrochemical exfoliation method with bipolar electrodes, and (ii) is a selective and sensitive immunoassay achieved with MoSe_2 NPs. The high availability, low cost, and promising catalytic activity of transition-metal dichalcogenides make them promising signal-enhancing elements for biomolecular detection. Considering the highly active MoSe_2 for HER catalysis, we first synthesized the microparticles (MPs) by exfoliating the bulk 2H- MoSe_2 to single or few sheets material with lateral dimensions in the micrometer range and a dominant 1T phase crystalline structure,¹⁶ which is denoted as MoSe_2 (*t*-BuLi). In order to exfoliate the MoSe_2 (*t*-BuLi) sheets to MoSe_2 NPs, a previously reported solution-based electrochemical exfoliation method with WS_2 was employed, where a constant DC potential of 10 V was applied across two Pt driving electrodes.¹² The main advantage of this technique is that the size reduction of MoSe_2 (*t*-BuLi) was carried out in solution, where direct contact with the Pt driving electrodes is not required. Hence, the starting material is a powder, and the modification of electrodes is not necessary, making this exfoliation method a simple and low cost one.

XPS was performed to examine the surface elemental compositions and bonding information, and hence, the chemical changes associated with the electrochemical exfoliation of MoSe_2 (*t*-BuLi) sheets. The high-resolution XPS spectra of MoSe_2 (*t*-BuLi) and MoSe_2 NPs were compared in Figure 1A. Deconvolution analysis of the Mo bonding modes^{22–24} reveals the presence of 1T- MoSe_2 and partial oxidation of the nanoparticles. This may be explained by the high positive potential applied, causing partial oxidation of the MoSe_2 NPs. Figure 1B shows the UV-vis spectra of MoSe_2 (*t*-BuLi) and the resulting MoSe_2 NPs. The MoSe_2 (*t*-BuLi) sheets absorb in the entire range of visible light.^{25,26} On the other hand, MoSe_2 NPs exhibit a single absorption band corresponding to excitonic features in the UV region at $\lambda \approx 200$ nm.²⁷ In addition, Raman spectrum (Figure S3) of MoSe_2 (*t*-BuLi) shows the two peaks characteristic of layered MoSe_2 ; 238.8 and 282.8 cm^{-1} correspond to the out-of-plane mode of A_{1g} and in-plane mode of E_{2g}^1 respectively. In contrast, no characteristic peaks were observed from the Raman spectrum of the MoSe_2 NPs. This is in agreement with previously reported observations of

Figure 1. (A) High-resolution XPS spectra of Mo 3d regions of MoSe_2 (*t*-BuLi) and MoSe_2 NPs. Deconvoluted peaks correspond to the metallic 1T phase (red), semiconducting 2H phase (blue), oxidized (IV) state (magenta), or oxidized (VI) state (olive). (B) UV-vis absorption spectra of MoSe_2 (*t*-BuLi) and MoSe_2 NPs. (C) STEM image of MoSe_2 NPs.

decreased Raman intensity with reduced particle sizes.²⁷ In order to further verify the formation and crystal phase of the MoSe_2 NPs, STEM and TEM are employed. From the STEM image the MoSe_2 NPs (Figure 1C), it was illustrated that the particle sizes are below 100 nm. Moreover, TEM characterization (Figure 2A) of the exfoliated MoSe_2 NPs shows the

Figure 2. (A) TEM and (B,C) high-resolution TEM images of MoSe_2 NPs.

platelet particle morphology typical for layered compounds with particle sizes below 100 nm. The high-resolution TEM images (Figure 2B,C) show atomic arrangement characteristic for 1T phase.¹⁶ The composition of the MoSe_2 NPs were also confirmed with elemental mapping via EDS (Figure S4).

We evaluated the HER catalytic efficiency of MoSe_2 NPs against the MoSe_2 (*t*-BuLi) starting material via LSV measurements in 0.5 M H_2SO_4 on glassy carbon (GC) electrode (Figure 3A). The lowest overpotential of -0.524 V vs RHE at a current density of -10 mA/cm^2 was reported for MoSe_2 (*t*-BuLi). After solution-based exfoliation of the MoSe_2 (*t*-BuLi) sheets, the overpotential reported for MoSe_2 NPs shifted to

Figure 3. Optimization of HER conditions for MoSe₂ NPs as an electrochemical label. (A) Linear sweep voltammograms derived from HER in acidic electrolyte on bare GC (black line), and MoSe₂ (t-BuLi) (red line) and (blue line) MoSe₂ NPs deposited on a GC electrode. Conditions: 0.5 M H₂SO₄, scan rate 2 mV s⁻¹. (B) Bar chart illustrates the obtained currents at 200 s from chronoamperometry at MoSe₂ NPs on an SP electrode. Error bars represent RSD ($n = 3$; 95% confidence interval). Inset: Recorded chronoamperograms. Conditions: 0.5 M H₂SO₄, applied potential -0.77 V (red), -0.87 V (green), and -0.97 V (blue) vs RHE.

-0.722 V vs RHE. This can be accounted by the partial oxidation of the MoSe₂ NPs as illustrated in the XPS spectrum (Figure 1A). Nonetheless, the HER catalytic activity of MoSe₂ NPs has been established to be much higher than that of the control GC electrode. The reason for working with MoSe₂ NPs as labels instead of MoSe₂ (t-BuLi) large sheets^{8,28} is that MoSe₂ NPs labels are of comparable sizes to proteins and much smaller than the paramagnetic beads, which would lead to better reproducibility of assays compared to that of MoSe₂ (t-BuLi) labels.

Tafel analysis was executed for the HER polarization curve of the MoSe₂ NPs to determine the electrochemical mechanism involved. The mechanism of hydrogen evolution on the MoSe₂ NPs can be understood from the following rate-determining steps:²⁹

1. Volmer adsorption step

2. Heyrovsky desorption step

3. Tafel desorption step

We observed a Tafel slope of 138 mV/dec on the MoSe₂ NPs and further identified the Volmer adsorption mechanism as the rate-determining step as the slope is close to the theoretical value of 120 mV/dec.

Moreover, the chronoamperometry technique was employed to investigate the utility of MoSe₂ NPs as viable labels. The obtained MoSe₂ NPs were subjected to three different potentials (-0.77, -0.87, and -0.97 V vs RHE) on an SP electrode for HER catalysis. The current outputs were recorded for each potential in Figure 3B, illustrating that the current output at 200 s is highest with an applied potential of -0.97 V vs RHE and decreased with lower applied potentials. Furthermore, at -0.97 V vs RHE, the current output on a bare SP electrode was negligible compared to that with the MoSe₂ NPs (Figure S5). As such, in order to optimize experimental conditions so that MoSe₂ NPs can serve as an effective electrochemical label, an applied potential of -0.97 V vs RHE was chosen for subsequent experiments, and the current response at 200 s was evaluated.

Subsequently, magnetic beads (MBs) modified with antirabbit IgG were used as platforms for capture, preconcentration, and detection of rabbit IgG. For this purpose, MBs were conjugated with antirabbit IgG. On the other end, the rabbit IgG was conjugated with MoSe₂ NPs. In this way, rabbit IgG is recognized by the antirabbit IgG, forming IgG-MoSe₂ NPs/anti-IgG-MB complex (Scheme 1B). The final amount of MoSe₂ NPs will be representative of the concentration of rabbit IgG in the sample.

In order to confirm that the MoSe₂ NPs have been attached on the surface of the rabbit IgG in the magneto-immunoassay, scanning transmission electron microscopy (STEM) and ICP-MS analyses were carried out. Figure 4 shows the STEM

Figure 4. STEM images of magneto-immunoassay: (A) without MoSe₂ NPs (IgG/anti rabbit IgG-MB complex) and (B) with MoSe₂ NPs as labels (rabbit IgG-MoSe₂ NPs/anti rabbit IgG-MB complex).

images of the obtained magneto-immunoassay. When MoSe₂ NPs were used as labels (Figure 4B), an obvious presence of nanoparticles was observed on the surface of the magnetic beads. The size of these particles matches that of smaller sized fraction (~100 nm) of MoSe₂ NPs observed earlier in Figure 1C. This observation, however, is not significant in the control where no label was used (Figure 4A). Furthermore, ICP-MS was used to quantify the amount of MoSe₂ present in the final IgG-MoSe₂ NPs/anti rabbit IgG-MB complex. The magneto-immunoassay complex contained 0.14 μg of MoSe₂ per gram of MBs, proportional to a rabbit IgG protein concentration of 500 ng mL⁻¹.

Following the successful characterization of the desired IgG-MoSe₂ NPs/anti rabbit IgG-MB complex, we analyzed the

performance parameters of the magneto-immunoassay. Selectivity of the immunoassay was evaluated in Figure 5A. Two

Figure 5. Evaluation of magneto-immunoassay performance; (A) Selectivity of immunoassay was demonstrated in chronoamperometric responses. Analytical signal obtained for immunoassay with complementary rabbit IgG protein (blue) is compared with noncomplementary Hb protein (cyan). Control experiments were carried out with bare SP electrode (black), immunoassay with no protein (red), and no label (green). Conditions: 0.5 M H_2SO_4 , applied potential -0.97 V vs RHE. (B) Logarithmic relationship between the concentration of rabbit IgG protein and the value of analytical signal obtained. Error bars represent RSD ($n = 3$; 95% confidence interval). Conditions: 0.5 M H_2SO_4 , applied potential -0.97 V vs RHE.

different control experiments were carried out for this purpose: (1) a magneto-immunoassay without MoSe_2 NPs as labels; and (2) a nonspecific protein, human hemoglobin (Hb), was used to replace the rabbit IgG protein. High selectivity was evident with depressed current responses from both control assays. This high selectivity is a result of the immunoassay protocol which involves multiple washing steps to remove any interfering species and noncomplementary proteins in the real samples, leading to a depressed current response. In this manner, our method would mainly be used in the analysis of proteins pre-extracted from real samples.

Moreover, sensitivity of the immunoassay was investigated. Figure 5B shows a strong concentration dependence of the current response at 200 s over a wide range where the current decreased as rabbit IgG protein concentrations increased from 2 to 500 ng mL^{-1} . A low limit of detection (LOD) of 1.23 ng mL^{-1} and good linearity ($r = 0.95$) were demonstrated. The mean relative standard deviation (RSD) from five different protein concentrations was reported to be 9.7%, where triplicates were run for each concentration. This indicates a high reproducibility of the system.

We compared the performance parameters of the magneto-immunoassay with TMD nanolabels obtained by solution-based electrochemical exfoliation with bipolar electrodes. The previously obtained magneto-immunoassay with WS_2 nanolabels gave a similar LOD of 2 ng mL^{-1} with good linearity ($r = 0.96$) and reproducibility (RSD $\approx 10\%$) as well.¹¹ Furthermore, performance parameters of the magnetic-immunoassay with

TMD NPs as labels paralleled that with gold NPs as labels (LOD = 0.31 ng mL^{-1}),³⁰ highlighting the potential of TMD NPs prepared with solution-based electrochemical exfoliation with bipolar electrodes as low-cost alternative labels. Hence, the simple exfoliation technique is viable and reproducible for producing TMD nanoparticles as low cost alternative labels for biomolecular detection.

CONCLUSIONS

Overall, we demonstrated the preparation of MoSe_2 NPs by a simple solution-based electrochemical exfoliation method and their application as promising nanolabels for biomolecular detection via HER by means of chronoamperometry as a transduction method. Characterization of the MoSe_2 NPs suggested a significant reduction in size of the final NPs (~ 100 nm) compared to the starting material, which allows it to be an efficient label for the magneto-immunoassay. Performance parameters of the immunoassay demonstrated a wide range of detection for rabbit IgG protein, high selectivity, sensitivity, and reproducibility. These findings have strong implications as we demonstrate the viability and reproducibility of solution-based electrochemical synthesis of TMD nanoparticles as labels for the development of low costs and efficient biosensing systems for several applications ranging from diagnostics to environmental control.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.6b03190.

Electrochemical setup of solution-based exfoliation of MoSe_2 (*t*-BuLi) material, comparison of MoSe_2 suspension before and after solution-based electrochemical exfoliation, Raman spectra of MoSe_2 (*t*-BuLi) and MoSe_2 NPs, elemental mapping of MoSe_2 NPs via electron dispersive X-ray spectroscopy (EDS), and chronoamperometric responses (PDF)

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Notes

The authors declare no competing financial interest.

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1T-Phase WS₂ Protein-Based Biosensor

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Metallic 1T-phase transition metal dichalcogenides have been recognized for their desirable properties like high surface-to-volume ratio, high conductivity, and capacitive behavior, making them outstanding for catalytic and sensing applications. Herein, a hydrogen peroxide (H₂O₂) biosensor is constructed by the immobilization of hemoglobin (Hb) on 1T-phase WS₂ (1T-WS₂) sheets, and entrapment by glutaraldehyde. 1T-WS₂ not only displays electrocatalytic activity toward the reduction of H₂O₂ but also provides a high surface-to-volume ratio and conductive platform for the immobilization of Hb and facilitation of its electron transfer to the electrode surface. The advantageous role of 1T-phase WS₂ is further demonstrated for the construction of a heme-based H₂O₂ biosensor compared to its 1T-phase MoS₂, MoSe₂, and WSe₂ counterparts. Synergistic interactions between 1T-WS₂ and Hb result in a H₂O₂ biosensor with high analytical performance in terms of wide range, sensitivity, selectivity, reproducibility, repeatability, and stability. These findings have profound impact in the research fields of electrochemical sensing and bionanotechnology.

A selective and sensitive detection platform for hydrogen peroxide (H₂O₂) would have profound implications on the fields of bioanalysis, food security, and environmental control.^[9–11] H₂O₂ is generated as a consequence of the incomplete reduction of oxygen in a broad range of biological processes.^[12] As a major reactive oxygen species in living organisms, H₂O₂ plays a critical role in the signal transduction and oxidative pathway of cells.^[13–15] Moreover, H₂O₂ acts as an oxidative stress marker that is important for the diagnosis of aging and diseases such as cancer, and cardiovascular and neurodegenerative disorders.^[16–19] Therefore, the determination of H₂O₂ is definitely necessary and vital, where methods such as chemiluminescence,^[20] colorimetry,^[21] fluorescence,^[22] and electrochemistry^[23,24] have been extensively explored. Amongst them, electrochemical methods stand out

1. Introduction

Layered transition metal dichalcogenides (TMDs) have gained much attention and recognition due to their desirable properties. When exfoliated to single or few-layer sheets, they possess high surface-to-volume ratio, electronic properties ranging from insulating to metallic, and versatility in functionalization.^[1,2] These properties allow them to be useful for electrochemical catalysis and sensing applications.^[3,4] In particular, exfoliation of TMDs using tert-butyllithium (t-BuLi) produces single to few-layer sheets with large compositions of highly conductive, metallic 1T-phase,^[5] which has been reported to display excellent capacitive behavior,^[6] electrocatalytic performance toward hydrogen evolution reaction,^[7] and application for gas sensing.^[8] For these reasons, 1T-phase TMD materials possess immense potential to be explored for further sensing applications.

due to their high sensitivity, rapid response, low cost, and easy miniaturization.

The development of electrochemical H₂O₂ biosensors has seen much effort devoted to the design of modified electrodes that can effectively and selectively catalyze the reduction or oxidation of H₂O₂. Heme proteins (hemoglobin, myoglobin, and cytochrome C) are commonly employed in the construction of H₂O₂ biosensors due to their commercial availability and peroxidase activity. However, facilitation of electron transfer between the heme center and conventional electrode surfaces is physiologically hampered.^[25] In order to enhance the electron transfer rate of the heme proteins, immobilization materials such as metal nanoparticles,^[26] carbon nanomaterials,^[27,28] and metallic oxide nanostructures^[29,30] have been investigated. Their large surface area, good stability, excellent electrical properties, and biocompatibility allow access to the direct electrochemistry of heme proteins and thus enhance the performance of biosensors.

Hitherto, MoS₂ has been explored for the successful construction of heme-based H₂O₂ biosensors by facilitating the direct electrochemistry of heme proteins.^[31–33] In addition, it has been reported that MoS₂ nanoparticles (NPs) alone display electrocatalytic activity toward the reduction of H₂O₂.^[34] However, no work has been presented to explore the promising potential of other TMD materials in this aspect. Herein, we demonstrate the advantageous role of 1T-phase WS₂ for the construction of a heme-based H₂O₂ biosensor compared to 1T-phase MoS₂, MoSe₂, and WSe₂. Consequently, we show the viability of this biosensor for hydrogen peroxide determination in human serum for applications in real sample analysis and the development of low-cost, high sensitivity biosensors.

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2. Results and Discussion

In this work, we investigated the synergistic interaction between 1T-phase WS_2 (1T- WS_2) and hemoglobin (Hb) toward the electrocatalysis of hydrogen peroxide reduction. Characterization of 1T- WS_2 was first performed using scanning electron microscopy (SEM), energy dispersive spectroscopy (EDS), transmission electron microscopy (TEM), high resolution TEM (HR-TEM), Raman spectroscopy, X-ray photoelectron spectroscopy (XPS), and X-ray diffraction (XRD). Construction of the H_2O_2 biosensor was achieved by the deposition of three consecutive layers on the surface of a glassy carbon (GC) electrode: 1T- WS_2 , followed by hemoglobin (Hb), and finally, glutaraldehyde (GTA) to give GTA/Hb/1T- WS_2 /GC electrode, as illustrated in **Figure 1**. Glutaraldehyde is employed in this work to serve two main functions: (1) cross-linking matrix for hemoglobin to maintain the structural rigidity of the protein, and (2) to entrap and stabilize the 1T- WS_2 and Hb layers on the GC electrode.^[35–38] Following this, the constructed biosensor was validated by SEM and heterogeneous electron transfer (HET) studies. Its electrocatalytic activity toward hydrogen peroxide reduction was probed via cyclic voltammetry (CV) and chronoamperometry.

The high availability, low cost, and widely reported catalytic and sensing activity of 1T-phase WS_2 makes it a promising candidate for the catalytic reduction of hydrogen peroxide.^[5,8] 1T- WS_2 was first prepared by exfoliating the bulk WS_2 material. SEM was performed to compare the surface morphologies of the bulk and exfoliated 1T- WS_2 material (Figure S1, Supporting Information). Bulk WS_2 showed large polygonal stacks of up to tens of micrometers wide and a few hundred nanometers thick. In contrast, the exfoliated 1T- WS_2 displayed decreased flake sizes, extensive wrinkling of edges, and a significant expansion across layers. This is in agreement with Raman analyses shown in **Figure 2A**. The in-plane E_{2g}^1 and out-of-plane A_{1g} modes of

WS_2 are observed at 355 and 419 cm^{-1} , respectively. The softening of both modes for 1T- WS_2 with respect to bulk WS_2 indicated a decreased lateral size as well as a reduced number of layers.^[5,39] Further characterization of the materials with EDS confirmed their surface elemental compositions (Figure S2, Supporting Information). Moreover, **Figure 3** shows a TEM image of 1T- WS_2 and the corresponding selective area electron diffraction (SAED) and HR-TEM image. TEM of 1T- WS_2 shows the presence of individual few-layer sheets with sizes of up to one micron, while the hexagonal symmetry of WS_2 is displayed in the SAED. HR-TEM was used for identification of the presence of 1T-phase WS_2 where the hexagonal arrangement of atoms typical of the 1T-phase is observed in the sample.^[40,41] The presence of 1T-phase WS_2 was further supported by XPS analyses where the figure shows the high resolution XPS spectra of WS_2 after exfoliation and deconvolution analyses of the W and S bonding modes revealed the dominant presence of the metallic 1T polymorph. Furthermore, XRD shows the presence of 1T-phase WS_2 without additional impurities (Figure 2C). The high intensity of (00l) reflections indicated high preferential orientation. Hence, the successful exfoliation of bulk WS_2 to 1T-phase WS_2 was demonstrated.

Consequently, optical and electrochemical methods were used to monitor the modification process of the GC electrode and construction of the biosensor. **Figure 4** shows the SEM images of 1T- WS_2 , Hb/1T- WS_2 , and GTA/Hb/1T- WS_2 . A uniform distribution of layered 1T- WS_2 is observed with the first modification step. Subsequently, the layered structure became less distinct as the addition of Hb cast a dark film over the 1T- WS_2 material. The effect of glutaraldehyde as a binding matrix is evident as a smooth film was formed over the surface. Successful immobilization of each layer of material was further confirmed with HET studies using cyclic voltammetry (**Figure 5**). Oxidation and reduction peaks of the ferri/ferrocyanide redox probe are observed at -0.10 V and

Figure 1. Schematic representation (not to scale) of the construction of GTA/Hb/1T- WS_2 /GC electrode.

Figure 2. A) Raman spectra of bulk WS_2 and 1T- WS_2 . B) High resolution X-ray photoelectron spectra of W 4f and S 2p regions of 1T- WS_2 . Deconvoluted peaks correspond to the metallic 1T phase (red), semiconducting 2H phase (blue), or the oxidized (VI) state (olive). C) X-ray diffraction (XRD) pattern of 1T- WS_2 .

Figure 3. Transmission electron microscopy (TEM) image of 1T- WS_2 sheets with corresponding selective area electron diffraction pattern and detail of area used for electron diffraction (circled). High resolution TEM image of 1T- WS_2 shows dominant presence of 1T-phase. Scale bars represent 200, 10, and 1 nm, respectively.

Figure 4. Scanning electron microscopy (SEM) images of 1T-WS₂, Hb/1T-WS₂, and GTA/Hb/1T-WS₂ at high magnification (left side); scale bars represent 100 nm, and low magnification (right side); scale bars represent 10 μ m.

+0.46 V, respectively, at the bare GC electrode and the electrochemical response was measured with each consecutive modification step. An increased redox current and decreased separation between the redox peak potentials is observed at the 1T-WS₂/GC. This can be accounted for by the high conductivity and increased surface area of the 1T-phase WS₂, hence increasing the HET rate. With the subsequent immobilization of Hb, a slight decrease in redox current is observed. The less efficient electron transfer is a result of the protein nature of Hb. The final immobilization step of glutaraldehyde resulted in a significant decrease in redox currents, showing successful entrapment of both WS₂ and Hb on the GC electrode.

First, CV was employed to evaluate the electrocatalytic activity of the constructed biosensor toward H₂O₂ reduction in a N₂-saturated phosphate buffered saline (PBS; 10×10^{-3} M, pH 7.4). Figure S3 (Supporting Information) shows that both GTA/Hb/GC and GTA/WS₂/GC displayed reduction currents higher than that from control experiments at the

GTA/GC and bare GC, where current responses were insignificant. Moreover, the highest reduction currents were achieved at the GTA/Hb/WS₂/GC. In addition, a biosensor was also constructed with bulk WS₂ (B-WS₂) for comparison, where GTA/Hb/B-WS₂/GC performed inferior to its 1T-phase WS₂ counterpart. From these, we conclude that both Hb and 1T-WS₂ possess electrocatalytic activity toward hydrogen peroxide reduction, with synergistic effects between 1T-WS₂ and Hb.

In order to understand the synergistic interactions between 1T-WS₂ and Hb, chronoamperometric response of the GTA/Hb/1T-WS₂/GC toward consecutive additions of H₂O₂ was compared with that of GTA/Hb/GC, GTA/1T-WS₂/GC, and GTA/Hb/B-WS₂/GC in Figure 6A. While GTA/1T-WS₂/GC showed a stable increase in reduction currents with each addition of H₂O₂, the electrochemical response from GTA/Hb/GC appeared otherwise and depleted over time. On the other hand, GTA/Hb/1T-WS₂/GC exhibited a stable and higher electrochemical response than that obtained with GTA/1T-WS₂/GC.

Figure 5. Changes in heterogeneous electron transfer rates due to the modification process of the GC electrode. Cyclic voltammograms resulting from electrochemical activity of ferri/ferrocyanide redox probe at GC electrode (black line), 1T-WS₂/GC (red line), Hb/1T-WS₂/GC (blue line), and GTA/Hb/1T-WS₂/GC (magenta line). Conditions: 5×10^{-3} M Fe(CN)₆^{3-/4-} in 0.1 M KCl; scan rate: 100 mV s⁻¹.

The increased sensitivity of the GTA/Hb/1T-WS₂/GC biosensor was achieved as the electrocatalytic activities of both 1T-WS₂ and Hb toward the reduction of H₂O₂ can be exploited. With more catalytically active edge sites exposed, the exfoliated 1T-phase

WS₂ was able to display excellent electrocatalytic activity toward the reduction of H₂O₂, and at the same time, provided a high surface-to-volume ratio and conductive platform for the uniform immobilization of Hb and effective facilitation of electron transfer between Hb and the electrode surface. This is in contrast with the unstable electrochemical response obtained when bulk WS₂, which consists mainly of the semiconducting 2H polymorph,^[5] is used instead.

Moreover, it would be of interest to explore the potential of other 1T-phase TMDs prepared by t-BuLi exfoliation^[5] in the construction of heme-based H₂O₂ biosensors. For this purpose, we constructed biosensors with 1T-phase MoS₂ (1T-MoS₂), MoSe₂ (1T-MoSe₂), and WSe₂ (1T-WSe₂), denoted as GTA/Hb/1T-MoS₂/GC, GTA/Hb/1T-MoSe₂/GC, and GTA/Hb/1T-WSe₂/GC, respectively. The SEM images and EDS elemental mappings of 1T-MoS₂, 1T-MoSe₂, and 1T-WSe₂ are presented in Figures S4 and S5 (Supporting Information). Figure 6B compares the performances of GTA/Hb/1T-MoS₂/GC, GTA/Hb/1T-MoSe₂/GC, GTA/Hb/1T-WS₂/GC, and GTA/Hb/1T-WSe₂/GC. Chronoamperometric studies showed that the biosensor with 1T-WS₂ outperformed that with 1T-MoS₂, 1T-MoSe₂, and 1T-WSe₂, agreeable with CV investigations (Figure S6A, Supporting Information). Hence, the GTA/Hb/1T-WS₂/GC serves as a suitable biosensor for further electrochemical characterizations.

Subsequently, experimental conditions for H₂O₂ detection were optimized at the GTA/Hb/1T-WS₂/GC biosensor. The effect of 1T-WS₂ loading concentration on electrocatalytic

Figure 6. Chronoamperometric responses upon successive additions of 100×10^{-6} M H₂O₂ at A) GTA/Hb/1T-WS₂/GC, GTA/Hb/GC, GTA/1T-WS₂/GC, and GTA/Hb/B-WS₂/GC at an applied potential of -400 mV; B) GTA/Hb/1T-MoS₂/GC (black line), GTA/Hb/1T-MoSe₂/GC, GTA/Hb/1T-WS₂/GC, and GTA/Hb/1T-WSe₂/GC at an applied potential of -400 mV; C) GTA/Hb/1T-WS₂/GC at an applied potential of -400 mV with different 1T-WS₂ loading concentrations: 0.5, 1.0, and 5.0 mg mL⁻¹; D) GTA/Hb/1T-WS₂/GC at different applied potentials: -500 mV, -400 mV, -300 mV, -200 mV, and -100 mV. Arrows indicate time points for the addition of H₂O₂. Conditions: stirred, N₂-saturated 10×10^{-3} M PBS at pH 7.4.

activity of the biosensor toward H_2O_2 reduction was studied in Figure 6C. Reduction currents increased as the loading of 1T- WS_2 increased from 0.5 to 5.0 mg mL^{-1} . This trend is in line with voltammetric studies (Figure S6B, Supporting Information). Thus, a loading concentration of 5.0 mg mL^{-1} was chosen for subsequent experiments. Furthermore, Figure 6D shows the chronoamperometric response of GTA/Hb/ WS_2 /GC with different applied potentials for the electrocatalytic reduction of H_2O_2 . We observed that the reduction currents increased with increasingly negative potentials applied from -0.1 to -0.5 V. However, the increase in reduction currents is less significant from -0.4 to -0.5 V at lower concentrations of H_2O_2 . We hence selected -0.4 V as a robust value for the applied potential for further chronoamperometric studies where effects of interference species may also be minimized.

Following the optimization of experimental conditions, we investigated the analytical parameters of the GTA/Hb/1T- WS_2 /GC biosensor. In Figure 7A, chronoamperometry was used to probe the reduction currents with successive additions of H_2O_2 into stirred N_2 -saturated 10×10^{-3} M PBS at -0.4 V. An increase in reduction currents is observed with increasing concentrations of H_2O_2 and the concentration dependence of this chronoamperometric signal was evaluated in Figure 7B. The GTA/Hb/1T- WS_2 /GC biosensor demonstrated a wide linear range over the concentration of H_2O_2 from 2×10^{-6} to 38×10^{-6} M ($r^2 = 0.98$) and from 48×10^{-6} to 1728×10^{-6} M ($r^2 = 0.99$) with good linearity. A low limit of detection of 36×10^{-9} M was attained, and the high reproducibility of the system was reflected in the low relative standard deviation (RSD) of 3.9%. Furthermore, repeatability of the constructed biosensor was tested as shown in Figure S7 (Supporting Information). The average RSD obtained from five different H_2O_2 concentrations (20×10^{-6} , 40×10^{-6} , 60×10^{-6} , 80×10^{-6} , 100×10^{-6} M), where three measurements were taken with the same biosensor, over a period of 5 d, is 6.2%. Moreover, we observed from this data that the GTA/Hb/1T- WS_2 /GC biosensor displayed good stability, as it retained 81.5% of its initial current response after 5 d (Figure S8, Supporting Information).

The high analytical performance GTA/Hb/1T- WS_2 /GC biosensor makes it a promising platform for the detection of H_2O_2 in real samples. For this reason, we investigated the interference effect of the GTA/Hb/1T- WS_2 /GC biosensor toward ascorbic acid (AA), dopamine (DA), uric acid (UA), and glucose, which are present in large quantities in the physiological environment.^[42,43] Figure S9A (Supporting Information) shows the current responses of the biosensor with the successive additions of 50×10^{-6} M H_2O_2 , 500×10^{-6} M AA, 500×10^{-6} M DA, 500×10^{-6} M UA, 500×10^{-6} M glucose, and 50×10^{-6} M H_2O_2 into stirred, N_2 -saturated PBS. While no significant change in the reduction currents is observed with high concentrations of the interference species, a significant increase is observed with the additions of H_2O_2 . This can be attributed to the preferential reduction of H_2O_2 , as the negative applied potential allows the detection to be free from interferences by biomolecules.^[44] Moreover, Figure S9B (Supporting Information) shows the measurement upon successive additions of 20×10^{-6} M H_2O_2 in PBS (black line) and 50% v/v human serum (red line); similar responses were observed in both cases. Hence, we conclude that the GTA/Hb/1T- WS_2 /GC

Figure 7. A) Chronoamperometric responses of the GTA/Hb/1T- WS_2 /GC upon successive additions of 2×10^{-6} , 10×10^{-6} , 20×10^{-6} , 100×10^{-6} , 200×10^{-6} , 400×10^{-6} M H_2O_2 (in order). Four successive additions of H_2O_2 were performed for each concentration from 2×10^{-6} to 200×10^{-6} M, followed by one final addition of 400×10^{-6} M H_2O_2 . B) Logarithmic relationship between the concentration of H_2O_2 and the catalytic current was obtained. Error bars represent RSD ($n = 3$; 95% confidence interval). Conditions: stirred, N_2 -saturated, 10×10^{-3} M PBS at pH 7.4; applied potential: -400 mV.

biosensor has wide linear range, low detection limit, high reproducibility, repeatability, and stability with good selectivity, and is a suitable platform for the detection of H_2O_2 in real samples.

Moreover, the constructed GTA/Hb/1T- WS_2 /GC biosensor in this work exhibited one of the best analytical ranges and detection limits when contrasted with previous reports of H_2O_2 detection with TMD-based sensors (Table 1). The use of exfoliated 1T-phase WS_2 sheets in our work has resulted in a H_2O_2 biosensor that significantly outperformed previous works focused on the use of MoS_2 sheets.^[31–33] On the other hand, the biosensor based on ultrasmall MoS_2 NPs/GC^[34] performed better than the GTA/Hb/1T- WS_2 /GC for H_2O_2 detection. This can be attributed to the smaller MoS_2 nanoparticle sizes (< 2 nm) compared to the larger 1T- WS_2 sheets with micrometer lateral

Table 1. Performance parameters of comparable biosensors for the detection of H_2O_2 .

Sensor/transduction method	Analytical ranges [$\times 10^{-6}$ M]	Limit of Detection [$\times 10^{-6}$ M]	Reference
MoS_2 NPs/GC chronoamperometry	0.005–0.1; 0.1–100	0.0025	[34]
GTA/Hb/1T- WS_2 /GC chronoamperometry	2–38; 48–1728	0.036	This work
MoS_2 /Graphene-carbon nanotubes/GC chronoamperometry	5–145	0.83	[31]
Hb-AuNPs@ MoS_2 /GC Cyclic voltammetry	10–300	4	[32]
Nafion/Hb/ MoS_2 /GC chronoamperometry	20–180	6.7	[33]

dimensions, which is in good agreement with size-dependent electrocatalytic performance studies reported previously.^[34,45]

3. Conclusion

We demonstrated the advantageous role of t-BuLi exfoliated, 1T-phase WS_2 for the construction of a heme-based H_2O_2 biosensor compared to 1T-phase MoS_2 , MoSe_2 , and WSe_2 . The GTA/Hb/1T- WS_2 /GC biosensor was fabricated based on a layer-by-layer deposition of 1T- WS_2 and Hb, and entrapment by GTA. Synergistic interactions between 1T- WS_2 and Hb resulted in a highly sensitive and selective H_2O_2 biosensor exhibiting a wide range and low detection limit. Consequently, we showed the viability of this biosensor for H_2O_2 detection in human serum. These findings have strong implications in the development of highly sensitive and low-cost H_2O_2 biosensing systems for applications in the fields of bioanalysis, food safety, and environmental control.

4. Experimental Section

Materials: Molybdenum disulfide MoS_2 (99.9%), molybdenum diselenide MoSe_2 (99.9%), tungsten disulfide WS_2 (99.8%), and tungsten diselenide WSe_2 (99.8%) were acquired from Alfa Aesar, Germany. Tert-butyllithium (1.7 M in pentane) was purchased from Sigma-Aldrich, Czech Republic. Hexane was acquired from Lach-Ner, Czech Republic. Argon (99.9999% purity) was obtained from SIAD, Czech Republic. Phosphate buffered saline tablet, potassium chloride (max 0.0001% Al), potassium hexacyanoferrate(II) trihydrate ($\geq 98.5\%$), potassium hexacyanoferrate(III) ($\approx 99\%$), glutaraldehyde solution ($\approx 50\%$ in water), hemoglobin human (lyophilized powder), and human serum were obtained from Sigma-Aldrich, Singapore. Hydrogen peroxide (35% w/w aqueous solution) was purchased from Alfa Aesar, Singapore. GC, Ag/AgCl reference, and Pt counter electrodes were obtained from CH Instruments, Inc., USA.

Instrumentation: SEM images were acquired by using a Jeol 7600F SEM instrument (Jeol, Japan) operating at 5 kV. Energy-dispersive X-ray spectroscopy (EDS) data were recorded on a Jeol 7600F instrument (Jeol, Japan) at 15 kV. XPS measurements were performed using a Phoibos 100 spectrometer and a monochromatic Mg X-ray radiation source (SPECS, Germany). Survey (wide-scan) and high resolution spectra were recorded for each sample. For SEM, EDS, and XPS measurements, sample preparation was performed by coating a uniform layer of the materials under study onto carbon conductive tape. Raman spectra

were acquired using a confocal micro-Raman LabRam HR instrument (HORIBA Scientific) in backscattering geometry with a CCD detector, a 514.5 nm Ar laser and a 100 $\times$ objective attached to an Olympus optical microscope. Prior to measurements, calibration was achieved using an internal silicon reference at 520 cm^{-1} with a peak position resolution of less than 1 cm^{-1} . The spectra ranged from 100 to 1000 cm^{-1} . HR-TEM was performed with an EFTEM Jeol 2200 FS microscope (Jeol, Japan). An acceleration voltage of 200 keV was used for measurement. The sample was prepared by drop casting the suspension (1 mg mL^{-1} in water) on a TEM grid (Cu; 200 mesh; Formvar/carbon) and drying at 60 $^\circ\text{C}$ for 12 h. XRD data were collected at room temperature on a Bruker D8 Discoverer powder diffractometer with parafocusing Bragg–Brentano geometry using Cu $K\alpha$ radiation ($\lambda = 0.15418$ nm, $U = 40$ kV, $I = 40$ mA). The data were scanned over the angular range of 10–80 $^\circ$ (2θ) with a step size of 0.02 $^\circ$ (2θ). Data evaluation was performed in the software package EVA. All voltammetric and chronoamperometric experiments were carried out using a μ Autolab type III electrochemical analyser (Eco Chemie, The Netherlands) connected to a computer and controlled by the NOVA, Version 1.8.17, software. Electrochemical experiments were conducted in an electrochemical cell (10 mL) at room temperature. A three-electrode setup was employed. A platinum electrode served as the auxiliary electrode, an Ag/AgCl electrode employed as the reference electrode and a GC (3 mm diameter) electrode was functioned as the working electrode.

Synthesis of Tert-Butyllithium Exfoliated Transition Metal Dichalcogenides: Exfoliation of bulk MoS_2 , MoSe_2 , WS_2 materials was performed according to the previously established protocol.^[8] Suspensions were prepared by adding a particular TMD powder (3 g) to t-BuLi (20 mL) in pentane (1.7 M). Subsequently, the suspensions were stirred at 25 $^\circ\text{C}$ under argon atmosphere for 72 h. The resulting Li-intercalated materials were separated by suction filtration under argon atmosphere and washed three times with hexane (dried over sodium). After this, the exfoliated material was dispersed in water (100 mL) and subjected to ultrasonication for 15 min. Centrifugation at 18 000 g and redispersion in water was carried out in order to achieve purification of the samples. This process was repeated until the conductivity fell below 20 μS . Finally, the exfoliated TMD materials were dried in a vacuum oven at 25 $^\circ\text{C}$ for 48 h prior to further use.

Electrode Preparation: Prior to use, the GC electrodes were first renewed using a 5 μm followed by a 0.05 μm alumina particle slurry on a polishing pad. Subsequently, the electrode was thoroughly rinsed with ultrapure water and dried under a flow of nitrogen gas.

Suspensions of t-BuLi exfoliated 1T-phase WS_2 (5 mg mL^{-1}) were prepared in ultrapure water and ultrasonicated for a period of 5 min before each electrochemical measurement to ensure homogeneity of the material under study. An aliquot (4 μL) was then deposited on the electrode surface and left to dry at room temperature. This yields a uniformly distributed film of 1T-phase WS_2 (20 μg ; 1T- WS_2) on the GC electrode surface. The resultant electrode is denoted as 1T- WS_2 /GC. Following this, hemoglobin (0.5 mg mL^{-1} ; Hb) was prepared in ultrapure water. The Hb solution (4 μL) was pipetted onto the surface of the electrode and left to dry at room temperature, resulting in Hb/1T- WS_2 /GC. Subsequently, a solution of glutaraldehyde (0.25% v/v; GTA) in ultrapure water was prepared. Cross-linking of the Hb protein was achieved by spreading a volume of GTA solution (4 μL) over the electrode surface and left to dry in an oven at 35 $^\circ\text{C}$. The final modified electrode is termed here GTA/Hb/1T- WS_2 /GC. The electrode was stored at 4 $^\circ\text{C}$ when not in use.

A similar procedure was used in the fabrication of different types of modified electrodes: GTA/Hb/1T- MoS_2 /GC, GTA/Hb/1T- MoSe_2 /GC, GTA/Hb/1T- WSe_2 /GC, GTA/Hb/B- WS_2 /GC, GTA/Hb/1T- WS_2 /GC, Hb/1T- WS_2 /GC, GTA/Hb/GC, and GTA/GC. Pretreatment of GTA/Hb/1T- MoS_2 /GC, GTA/Hb/1T- MoSe_2 /GC, GTA/Hb/1T- WS_2 /GC, GTA/Hb/1T- WSe_2 /GC, Hb/1T- WS_2 /GC, and GTA/Hb/B- WS_2 /GC electrodes was performed with CV at a scan rate of 50 mV s^{-1} between 0.2 and -0.6 V in PBS electrolyte (10×10^{-3} M) for 2 scans. This served to ensure that the inherent electrochemistry of the TMD materials^[7] would not interfere with subsequent measurements.

Detection of Hydrogen Peroxide: CV was performed at a scan rate of 50 mV s⁻¹ between 0.2 and -0.6 V in a N₂ saturated, PBS electrolyte (10 × 10⁻³ M, pH 7.4). Chronoamperometry was performed at a constant potential of -0.4 V in a N₂-saturated, PBS electrolyte (10 × 10⁻³ M, pH 7.4) under stirring conditions. The resulting chronoamperograms were subjected to baseline correction for further analysis.

HET Measurement: CV was performed at a scan rate of 100 mV s⁻¹ between -0.3 and 0.6 V with ferri/ferrocyanide (5 × 10⁻³ M) in KCl electrolyte (0.1 M).

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Group 6 Layered Transition-Metal Dichalcogenides in Lab-on-a-Chip Devices: 1T-Phase WS₂ for Microfluidics Non-Enzymatic Detection of Hydrogen Peroxide

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S Supporting Information

ABSTRACT: Two-dimensional (2D) layered transition-metal dichalcogenides (TMDs) have been placed in the spotlight for their advantageous properties for catalytic and sensing applications. However, little work is done to explore and exploit them in enhancing the performance of analytical lab-on-a-chip (LOC) devices. In this work, we demonstrate a simple, sensitive, and low-cost fabrication of electrochemical LOC microfluidic devices to be used for enzymatic detection. We integrated four *t*-BuLi exfoliated, group 6 TMD materials (MoS₂, MoSe₂, WS₂, and WSe₂) within the LOC devices by the drop-casting method and compared their performance for H₂O₂ detection. The 1T-phase WS₂-based LOC device outperformed the rest of the TMD materials and exhibited a wide range of linear response (20 nM to 20 μM and 100 μM to 2 mM), low detection limit (2.0 nM), and good selectivity for applications in real sample analysis. This work may facilitate the expanded use of electrochemical LOC microfluidics, with its easier integrability, for applications in the field of biondiagnostics and sensing.

Lab-on-a-chip (LOC) devices have been developed extensively to improve the sensing speed and efficiency, by miniaturizing chemical and biological analytical instruments. They bring benefits such as portability, field deployability, low sample consumption, and low costs.¹ However, it remains a challenge in the field of analytical LOC devices to provide a downsized, yet highly sensitive detector for the minute sample amounts often encountered in real-world applications. In this aspect, electrochemical detection is advantageous, compared with more conventional optical detection methods, due to its ease of miniaturization, low energy consumption, and compatibility with advanced microfabrication schemes.²

For the purpose of improving the sensitivity and detection limits of LOC devices, various nanomaterial-based detectors,^{3–7} have been integrated within LOC systems.^{8,9} While carbon nanotubes are the most extensively studied due to their excellent mechanical properties, high thermal and electrical conductivity, and electrochemical properties,^{10–18} the one-dimensional (1D) nature of the material poses a huge challenge in fabrication.^{19,20} On the other hand, increasing attention is being placed on two-dimensional (2D) nanomaterials²¹ with advantageous electrochemical properties which can offer easy

patternability and device fabrication due to their 2D atomically layered structures.²²

Graphene and graphene oxide-modified transducing platforms have been reported widely to provide highly sensitive detection in LOC systems.^{23–28} Moreover, transition-metal dichalcogenides (TMDs), possessing 2D layered structures, similar to that of graphene, and desirable properties such as high surface-to-volume ratio, excellent conductivity, and capacitive behavior,^{29–31} present as promising candidates for enhancing the performance of electrochemical LOC devices. Sarkar et al. demonstrated the high performance of a MoS₂-based field effect transistor (FET) biosensor, surpassing the sensitivity of that based on graphene by more than 74-fold, and its ability to be scaled down for integration into LOC systems without compromising its sensitivity.¹⁷ Highlighting the promising potential of TMDs for sensing applications, it is surprising that they remain underexplored in the field of analytical LOC devices with only one significant work published at the current time to the best of our knowledge.

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As a major reactive oxygen species (ROS) in living organisms and an oxidative stress marker,^{32–34} highly sensitive and inexpensive point-of-care testing of H_2O_2 is of paramount importance. Considering batch measurements, Wang et al. reported the high electrocatalytic performance of MoS_2 nanoparticles toward H_2O_2 reduction.³⁵ In this work, we integrate 2D layered TMDs (MoS_2 , MoSe_2 , WS_2 , WSe_2) as detector surfaces for hydrogen peroxide (H_2O_2) in LOC devices. Moreover, a simple and reproducible method of preparing TMDs-based LOC microfluidic devices is presented. This method allows investigators without access to state-of-the-art fabrication equipment to explore electrochemical LOC microfluidics and diagnostics with ease and at a low cost. Herein, the TMD materials were prepared by *tert*-butyllithium exfoliation and consequently, we demonstrate the high performance of 2D layered, 1T-phase WS_2 -based LOC device for the detection of H_2O_2 (Figure 1) compared to that based on MoS_2 , MoSe_2 , and WSe_2 . The robust 1T-phase WS_2 -based LOC device is furthermore practical for H_2O_2 determination in human serum, indicating its feasibility for applications in real biosample analysis.

EXPERIMENTAL SECTION

Materials. Carbon conductive paste (BQ242) and polyimide tape (0.5 mil) were purchased from DuPont, U.S.A. and CAPLINQ, Canada, respectively. Conductive silver epoxy (CW2400) was acquired from Chemtronics, U.S.A. SU-8 2035 photoresist and hexamethyldisilane were obtained from MicroChem, U.S.A. and Sigma-Aldrich, St. Louis, MO respectively. Harris Uni-Core cutting tip (2 mm) was obtained from Ted Pella, Inc., U.S.A. Polydimethylsiloxane (Sylgard 184) was acquired from Dow Corning Inc., U.S.A. Liquid chlorine bleach was purchased from GB Chemicals Pte. Ltd., Singapore. Clear silicone sealant was obtained from Sellys, Australia. MoS_2 , MoSe_2 , WS_2 , WSe_2 (<2 μm) bulk powders were obtained from Alfa Aesar, Germany, and *tert*-butyllithium (1.7 M in pentane) was purchased from Sigma-Aldrich, Czech Republic. Hexane was acquired from LACH-NER, Czech Republic. Argon (99.9999% purity) was obtained from SIAD, Czech Republic. Phosphate-buffered saline tablets and human serum were purchased from Sigma-Aldrich, Singapore. Hydrogen peroxide (35% w/w aq soln) and silver conductive ink (S-020) were acquired from Alfa Aesar, Singapore. Ag/AgCl electrode was acquired from CH Instruments, Inc., Singapore.

Instrumentation. Electrode stencils were created by a laser cutter (Universal Laser Systems, Inc., U.S.A.). Plasma bonding of PDMS channels to the glass substrates was achieved using a Covance-MPR plasma system (FemtoScience, Korea). Scanning electron microscopy (SEM) images were acquired by using a Jeol 7600F SEM instrument (Jeol, Japan) operating at 5 kV. Energy-dispersive X-ray spectroscopy (EDS) data were recorded on a Jeol 7600F instrument (Jeol, Japan) at 15 kV. X-ray photoelectron spectroscopy (XPS) measurements were performed using a Phoibos 100 spectrometer and a monochromatic Mg X-ray radiation source (SPECS, Germany). Survey (wide-scan) and high-resolution spectra were recorded for each sample. For SEM, EDS, and XPS measurements, sample preparation was performed by coating a uniform layer of the materials under study onto carbon conductive tape. Contact-angle measurements were performed using an Attension Theta system (Biolin Scientific, Sweden). Injection of phosphate-buffered saline (PBS) and hydrogen peroxide into the microfluidic device was achieved using a Fusion 200 syringe

pump (Chemxy Inc., U.S.A.) and a Pump 11 Elite syringe pump, respectively. The Fusion 200 syringe pump has a flow performance with accuracy and smooth flow ranging from 100 $\mu\text{L min}^{-1}$ to 128 mL min^{-1} while the Pump 11 Elite syringe pump performs in the range of 1.28 $\mu\text{L min}^{-1}$ to 88.28 mL min^{-1} . Flow rates were monitored with a SLI-0430 liquid flow meter (Sensirion AG, Switzerland) which works in a flow range of $-80 \mu\text{L min}^{-1}$ to $80 \mu\text{L min}^{-1}$. Chronoamperometric experiments were carried out using a $\mu\text{Autolab}$ type III electrochemical analyzer (Eco Chemie, The Netherlands) connected to a computer and controlled by the NOVA, Version 1.8.17, software.

Synthesis of 2D Layered Transition-Metal Dichalcogenides. Exfoliation of bulk MoS_2 , MoSe_2 , WS_2 , and WSe_2 materials was performed by *tert*-butyllithium exfoliation. Suspensions were prepared by adding a particular TMD powder (3 g) to *tert*-butyllithium (20 mL; *t*-BuLi) in pentane (1.7 M). Subsequently, the suspensions were stirred at 25 $^\circ\text{C}$ under argon atmosphere for 72 h. The resulting Li-intercalated materials were separated by suction filtration under argon atmosphere and washed three times with hexane (dried over sodium). After which, the exfoliated material was dispersed in water (100 mL) and subjected to ultrasonication for 15 min. Centrifugation at 18 000g, and redispersion in water was carried out in order to achieve purification of the samples. This process was repeated until the conductivity falls below 20 μS . Finally, the exfoliated TMD materials were dried in a vacuum oven at 25 $^\circ\text{C}$ for 48 h prior to further use.

Electrode Preparation. The electrodes were designed using AutoCAD (Autodesk, U.S.A.) and patterned on a glass substrate by laser cutting a polyimide tape mask. Next, carbon conductive paste was spread evenly over the patterned stencil and allowed to cure for 1 h at 100 $^\circ\text{C}$. A three-electrode setup was employed. The carbon electrode serves as the counter and working electrode, as well as a base material for obtaining the pseudo Ag/AgCl reference electrode. The pseudo Ag/AgCl reference electrode was obtained by depositing a thin layer of Ag conductive ink over the carbon material. The Ag conductive ink was allowed to cure for 30 min at 100 $^\circ\text{C}$. Subsequently, the Ag electrode was treated with bleach for 1 min to produce the pseudo Ag/AgCl electrode. Modification of the working electrode was performed by drop-casting a suspension of the exfoliated TMD materials (5 μL) onto the carbon electrode and allowing it to dry for 30 min at room temperature.

Chip Fabrication. All experiments were carried out in microfluidic chips made of polydimethylsiloxane (PDMS). The fabrication process can be broken down into three major steps: (1) fabrication of the SU-8 master, (2) casting of PDMS, and (3) plasma bonding of PDMS over the prepared electrodes on the glass substrate. The master for the PDMS device was fabricated using standard photolithography techniques with SU-8 2035 photoresist. The positive master mold for the device contained channels that were 49 μm deep. In order to prevent adhesion with PDMS, the master mold was treated with hexamethyldisilane for 1 h. After the silane treatment, PDMS was poured onto the master mold, which was degassed in a desiccator with a ~ 5 psi vacuum for 30 min before pouring. After curing in an oven at 80 $^\circ\text{C}$ for 3 h, the PDMS layer was peeled from the silicon master. Inlet and outlet ports were created with a 2 mm cutting tip. It was treated with oxygen plasma in a plasma cleaner for 40 s before it was bonded to the patterned glass substrate. To ensure strong bonding between the PDMS channel and the glass substrate, curing is performed

in an oven at 80 °C for 1 h, and silicone sealant is applied. Subsequently, wires were attached for connection with silver conductive epoxy to the three-electrode system accordingly.

Microfluidic Setup. Two syringe pumps were connected to a Y-connector at the inlet of the microfluidic device. PBS (10 mM, pH 7.4) was injected into the microfluidic chips at a flow rate of 40 $\mu\text{L min}^{-1}$. At ~ 100 s intervals, the flow of PBS was stopped, and simultaneously, hydrogen peroxide (5 μL) was injected into the channel at 50 $\mu\text{L min}^{-1}$. Waste liquid is collected at the outlet of the microfluidic device and via a Teflon tubing to a flow meter to measure the flow rates and monitor any possible leakages in the system.

Potential Determination of Pseudo Ag/AgCl Reference Electrode. Open-circuit potential (OCP) determination of the prepared pseudo Ag/AgCl reference electrode was done with reference to a commercially obtained Ag/AgCl electrode. The relative potential was measured in KCl (3.5 M) over a period of 120 s, and the average OCP was determined for each electrode.

Detection of Hydrogen Peroxide. Chronoamperometry was performed at a constant applied potential of -0.5 V, and the resulting chronoamperograms were subjected to baseline correction before further analysis.

RESULTS AND DISCUSSION

Lab-on-a-Chip Device Characterization. A major step in the development of electrochemical lab-on-a-chip (LOC) devices is the preparation of miniaturized electrodes. In the context of LOC microfluidics, it is crucial for the electrode thickness to be sufficiently low for the efficient attachment of microfluidic channels. Though conventional photolithography techniques can produce electrodes of nanometer thickness, sophisticated facilities and a clean room are required.³⁶ Screen printing has been explored as a cheaper and simpler alternative for the mass printing of conductive inks as electrodes.³⁷ However, high initial cost investment and a well-equipped facility are still necessary. Herein, we introduce a simple and reproducible manual screen printing procedure for the fabrication of low-cost, electrochemical lab-on-a-chip (LOC) devices, and we characterized the LOC device using energy-dispersive spectroscopy (EDS), scanning electron microscopy (SEM), open circuit potential (OCP) determination, and contact-angle measurements.

The optimized LOC device design is illustrated in Figure 1. Linear electrode geometries were chosen for ease of fabrication and channel alignment. A laser cutter is employed to create the electrode stencil on a masked glass substrate. For this reason, an ultrathin polyimide mask with silicone adhesive is used as the stencil for a three-electrode system. The heat-resistant and flame-retardant properties of polyimide³⁸ make it a suitable material for laser cutting while the silicone adhesive ensures that the mask can be removed from the glass substrate without leaving residues.

In order to produce the electrodes, carbon ink is used not only because it is relatively inexpensive but also because of its attractive electrochemical properties for sensing applications such as low background currents and broad potential windows.^{39,40} With carbon ink as a base material, a pseudo reference electrode of silver/silver chloride (Ag/AgCl) is fabricated by printing a layer of silver conductive ink over the carbon material followed by treatment with bleach.⁴¹ The formation of AgCl upon bleach treatment is illustrated in the EDS elemental mapping (Figure S1). OCP determination of

Figure 1. (A,B) Schematic representation (not to scale) and photograph of lab-on-a-chip setup for the electrocatalytic reduction of H_2O_2 with S\$1 coin for scale (C).

three separate pseudo reference electrodes revealed a high reproducibility of the fabrication method with a relative potential of 31.7 ± 0.2 mV versus commercially obtained Ag/AgCl electrode (Figure S2). Moreover, modification of the working electrode with the desired transition-metal dichalcogenides (TMDs) was achieved by the drop-casting method. It is postulated that the layered TMD materials are stably attached to the carbon material by strong hydrophobic interactions.⁴² 1T-phase WS_2 -based LOC device as representative, successful modification of the carbon working electrode is observed through EDS elemental mapping (Figure S3), and contact-angle measurements (Figure S4) showed that attachment of WS_2 to the carbon working electrode is realized as the hydrophobicity of WS_2 increased after deposition onto the highly hydrophobic carbon material. This is further verified by EDS elemental mapping of the WS_2 -modified carbon working electrode where the presence of WS_2 continued to be observed after calibration experiments were performed on the LOC device (Figure S5).

Moreover, further observations under a scanning electron microscopy were made to characterize the electrode widths, separation, and thicknesses. From Figure S6A, it is shown that all three electrodes displayed similar widths of ~ 1.2 mm. The distance between the Ag/AgCl reference electrode (RE) and carbon counter electrode (CE) is ~ 0.2 mm while that between the carbon CE and WS_2 modified (C/ WS_2) working electrode (WE) is ~ 1.7 mm (Figure S6B). Furthermore, the thin polyimide stencil (total thickness of 25 μm) ensures that the resultant electrode has a sufficiently low thickness for efficient channel bonding. The thicknesses of three electrodes were measured and determined to be 4.1 ± 0.6 μm , 4.0 ± 0.5 μm , and 4.0 ± 0.8 μm for the RE, CE, and WE, respectively (Figure 2). The resultant electrode thicknesses were lower than the typical thickness obtained through automated screen-printing on 240 mesh screen (12.7 μm).⁴³ This allowed strong plasma bonding of the polydimethylsiloxane (PDMS) channels over the prepared electrodes. Channel bonding was further enforced using a silicone sealant and appropriate wire connections were made using silver conductive epoxy. Hence, the low-cost (<USD 0.50 per device), simple, and reproducible production of electrochemical LOC devices is realized and used for subsequent investigations of the performance of different layered TMDs for the detection of H_2O_2 .

Figure 2. Measurements of the thickness of the prepared Ag/AgCl, C, and C/WS₂ electrodes under scanning electron microscopy (SEM). Error bars represent RSD ($n = 7$; 95% confidence interval).

Optimization of TMDs-Based Lab-on-a-Chip Devices.

Considering the high availability, low cost, and highly reported catalytic and sensing activity of group 6 transition-metal dichalcogenides (TMDs) (MoS₂, MoSe₂, WS₂, WSe₂), we first synthesized the 2D layered materials by *tert*-butyllithium (*t*-BuLi) exfoliation of their bulk forms. Characterization of the obtained 2D layered materials was performed with scanning electron microscopy (SEM), X-ray photoelectron spectroscopy (XPS), high-resolution transmission electron microscopy (HR-TEM), and Raman spectroscopy. Following that, the respective LOC devices were constructed and their electrocatalytic activities toward the reduction of H₂O₂ were studied using chronoamperometric techniques.

Exfoliation with *t*-BuLi produces single- to few-layer sheets of TMDs composed of the conductive, metallic 1T-phase.⁴⁴ The successful formation of these 2D layered, 1T-phase TMD materials were verified using XPS. The XPS wide scan confirmed the elemental compositions of the individual TMD

materials (Figure S7). In addition, high-resolution XPS spectra of the metals and chalcogens were measured. Figure 3 illustrates the deconvolution analyses of Mo 3d bonding modes of MoS₂ and MoSe₂ and W 4f bonding modes of WS₂ and WSe₂ where the 1T-phase is present. Similar conclusions can be drawn from the deconvolution analyses of their corresponding S 2p and Se 3d bonding modes (Figure S8). Likewise, SEM of the 2D layered TMD materials showed extensive wrinkling of edges with significant spacing between the layers (Figure S9), typical of the exfoliated materials. Subsequently, the prepared 2D layered TMD materials were integrated into LOC devices, and their electrocatalytic performances toward the reduction of H₂O₂ were compared in Figure 4A. The reduction currents obtained with the 1T-phase WS₂-based LOC device significantly surpassed that based on MoS₂, MoSe₂, and WSe₂. This observation aligns well with a previous work which demonstrated the superior electrocatalytic activity of 1T-phase WS₂ compared to the other group 6 TMDs toward the reduction of H₂O₂.⁴⁵ For this reason, an extensive characterization of WS₂ was performed using HR-TEM (Figure 5) and Raman spectroscopy (Figure 6) which further displayed its metallic 1T-phase and 2D layered nature. The high activity of the 1T-phase material can be attributed to the high electrical conductivity as well as increased density of catalytic active sites, on both the basal plane and edges.^{46–49}

Moreover, the 1T-phase WS₂-based LOC device, serving as a suitable H₂O₂ sensor, was optimized for further electrochemical characterizations. The effect of WS₂ loading concentration (Figure 4B) was investigated where bare screen printed (SP) electrode with zero WS₂ loading served as a control. Reduction currents were negligible at the bare SP electrode and started to increase as the WS₂ loading is increased to 2.5 mg mL^{−1} and subsequently 10 mg mL^{−1}. However, the reduction currents remained stagnant as WS₂ loading is further increased to 15 mg mL^{−1}. This is likely due to the saturation of the sensor surface at 10 mg mL^{−1}, which was chosen to be the optimal WS₂

Figure 3. High-resolution X-ray photoelectron spectroscopy (XPS) spectra of Mo 3d (top) of MoS₂ and MoSe₂, and W 4f (bottom) of WS₂ and WSe₂.

Figure 4. Chronoamperometric responses upon injection of $200\ \mu\text{M}$ H_2O_2 : (A) Comparison of *t*-BuLi exfoliated TMDs for the electrocatalytic reduction of H_2O_2 in a lab-on-a-chip device. Optimization of experimental conditions on a 1T-phase WS_2 -based lab-on-a-chip device: (B) different WS_2 loading concentrations at an applied potential of $-0.5\ \text{V}$ and (C) different applied potentials at a WS_2 loading of $10\ \text{mg mL}^{-1}$. Arrows indicate time points for the injection of H_2O_2 .

Figure 5. Transmission electron microscopy (TEM) image of WS_2 sheets with corresponding selective area electron diffraction pattern. High-resolution TEM image of WS_2 shows presence of 1T-phase. Scale bars represent 200 and 2 nm, respectively.

Figure 6. Raman spectrum of 1T-phase WS_2 .

loading concentration for subsequent experiments. Furthermore, different applied potentials were investigated for the electrocatalytic reduction of H_2O_2 . While a low reduction potential is preferred in order to minimize the effects of interference species,⁵⁰ sensitivity of the LOC device cannot be compromised as well. Figure 4C illustrates the increase in reduction currents as the applied potential is increased from $-0.4\ \text{V}$ to $-0.5\ \text{V}$. However, this is not the case as the

reduction potential is increased from $-0.5\ \text{V}$ to $-0.6\ \text{V}$. Therefore, a robust value of $-0.5\ \text{V}$ was chosen as the applied potential for electrocatalytic reduction of H_2O_2 in the WS_2 -based LOC device.

Performance of WS_2 -Based Lab-on-a-Chip Device.

Consequently, analytical parameters of the WS_2 -based LOC device were examined for the detection of H_2O_2 . Figure 7A shows the chronoamperometric responses obtained upon injection of different concentrations of H_2O_2 . An increase in H_2O_2 concentrations resulted in a corresponding increase in reduction currents. The H_2O_2 concentration dependence of this chronoamperometric signal illustrated is typical of sensors, where the tendency toward a sigmoidal relationship with concentration is exhibited on a logarithmic scale (Figure 7B). The calibration curve is divided into two parts where wide linear range from $20\ \text{nM}$ to $20\ \mu\text{M}$ ($r = 0.92$) and from $100\ \mu\text{M}$ to $2\ \text{mM}$ ($r = 0.98$) is observed. Moreover, the limit of detection (LOD) was calculated by following the 3 times s/m criteria established, where s is the standard deviation of the peak current of the lowest analyte concentration and m is the slope of the corresponding calibration graph.^{51–53} The LOC device

Figure 7. Calibration of 1T-phase WS_2 -based lab-on-a-chip device. Current spike obtained upon second of the three H_2O_2 injections is chosen for analysis. (A) Chronoamperometric responses at 1T-phase WS_2 upon injection of different H_2O_2 concentrations. Applied potential: -0.5 V. (B) Logarithmic relationship between the concentration of H_2O_2 (20 nM to 2 mM) and the obtained catalytic current. Error bars represent RSD ($n = 3$; 95% confidence interval). Insets: Magnified views for concentrations of H_2O_2 from 20 nM to 20 μM .

attained a low LOD at 2.0 nM. Next, the relative standard deviation (RSD) value obtained for the system is 17.6%. The RSD is reported as the average of 10 different H_2O_2 concentrations, where triplicates were performed for each concentration on different LOC devices. According to the Bioanalytical Method Validation from Guidance for Industry of the Food and Drug Administration (FDA) federal agency of the United States, the RSD value obtained reflected good reproducibility of the system.^{54,55} In addition, the repeatability of the system was determined by taking the average RSD (3.3%) of 10 different H_2O_2 concentrations where three measurements of each concentration were taken using the same LOC device.

Impelled by the high analytical performance of the WS_2 -based LOC device, it was further investigated as a system for H_2O_2 detection in real samples. Interference study of the system was carried out using biomolecules which are present in the physiological environment: ascorbic acid (AA), dopamine (DA), uric acid (UA), and glucose (Glu).^{56,57} Figure S10A shows a comparison of the chronoamperometric responses upon injection of the interference species into the LOC device at the same concentration of 2 mM. The high selectivity ratios of AA (35.2%), DA (61.8%), and UA (37.5%) showed that interference from these species may be significant because of the highly sensitive system. Taking this into consideration for applications with real samples, we then evaluated the interference effect according to physiological concentrations (Figure 8A).^{58,59} Due to the much lower concentrations of AA

Figure 8. (A) Interference studies of WS_2 -based lab-on-a-chip device. Arrows indicate time points for the injection of H_2O_2 and corresponding interferences. (B) Chronoamperometric responses upon injection of H_2O_2 in PBS and 10% human serum in PBS. Arrows indicate time points for the injection of 200 μM H_2O_2 . Applied potential: -0.5 V.

and DA present at normal physiological conditions, insignificant reduction currents are detected. Although some interference can still be observed with UA, the reduction current is significantly lower than that with H_2O_2 when comparison is made at the same concentration of 200 μM . These observations account for the preferential reduction of H_2O_2 at the applied negative reduction potential. Moreover, an 88.5% signal recovery is observed with 10% human serum in PBS (Figure 8B). Taking into account that the cytotoxic levels of H_2O_2 are usually high at ≥ 50 μM ,^{60–62} our WS_2 -based LOC device proves to be sensitive enough (LOD = 2.0 nM) for the detection of critical H_2O_2 concentrations even after dilution of the real samples.

While the simple design and low sample volumes (<20 μL) of the WS_2 -based LOC device may limit the possibility of additional assay routes for the confirmation of H_2O_2 determination, it must be emphasized that with H_2O_2 being an electroactive molecule, electrochemical techniques have been established as a simple, fast, and effective method for H_2O_2 determination^{63,64} in contrast to other techniques such as chemiluminescence,⁶⁵ fluorescence,⁶⁶ and spectrophotometry.⁶⁷ In addition, a comparison with another work highlighted the advantages of our device. Rusling and co-workers demonstrated a detection limit of 5 nM for H_2O_2 detection with a heme-protein-based microfluidic electrochemical device using glutathione-decorated gold nanoparticles.⁶⁸ While the sensitivity of the heme-based device is similar to that of our WS_2 -based LOC device, the nonenzymatic approach employed in this work confers advantages in experimental conditions and sample preparation. The use of a protein would require specific

experimental and storage conditions, such as temperature and pH, to ensure the structural and conformational stability of the protein.⁶⁹ Moreover, deoxygenation is a necessary step for the function of heme-based biosensors.^{70,71} This is an important consideration for the detection of H₂O₂ in a LOC device where the gas permeability of PDMS leading to oxygen leakage into the flow chamber may decrease the signal-to-noise ratio of heme-based biosensors.⁷² Thinking ahead to applications in mass production, the use of gold nanoparticles can incur high costs in contrast to TMDs, which are abundant and low-cost. Hence, we present a reproducible and highly sensitive WS₂-based LOC device for H₂O₂ detection with wide linear range, low detection limit, and good selectivity for applications in real sample analysis.

CONCLUSION

We demonstrate a low-cost, simple, and reproducible preparation method for electrochemical LOC microfluidic devices and modified the transducing platform with four *t*-BuLi exfoliated group 6 TMD materials by the drop-casting method. The TMDs-based LOC devices were investigated for the detection of H₂O₂. The 1T-phase WS₂-based LOC device exhibited a wide linear range, low detection limit, and feasibility for H₂O₂ determination in human serum. This work not only has strong implications for investigators looking to explore the field of electrochemical LOC microfluidics without sophisticated facilities but also represents one of the first studies exploring and exploiting the desirable electrochemical properties of TMDs for sensing applications in LOC devices. We believe that these findings will serve to spur the development of high-performance point-of-care H₂O₂ sensing systems for bioanalysis, food safety, and environmental applications. In addition, this work demonstrates a low-cost fabrication of electrochemically superior electrodes for other BioMEMS and microfluidic systems.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: [10.1021/acs.analchem.7b00302](https://doi.org/10.1021/acs.analchem.7b00302).

Energy-dispersive X-ray spectroscopy (EDS) and scanning electron microscopy (SEM) analyses for screen-printed electrodes; open-circuit potential (OCP) measurements of pseudo Ag/AgCl reference electrodes; contact-angle measurements of C and 1T phase WS₂; X-ray photoelectron spectroscopy (XPS) and SEM analyses of *t*-BuLi exfoliated TMD materials (PDF)

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Notes

The authors declare no competing financial interest.

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