Top-down approach towards graphene : synthesis and electrochemistry

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**NANYANG
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SYNTHESIS AND ELECTROCHEMISTRY

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SCHOOL OF PHYSICAL & MATHEMATICAL SCIENCES
NANYANG TECHNOLOGICAL UNIVERSITY

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Top-Down Approach Towards Graphene: Synthesis and Electrochemistry

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A thesis submitted to the Nanyang Technological University
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When one door closes, another opens. For the past few years, several doors have closed but many more have opened with brighter prospects. The door for this journey may soon close, but the pursuit of knowledge never ceases. The bonds and friendships that are forged during this journey have provided immense inspirations and motivations along the way. “Though the road has been rocky, it sure feels good to me.” This is not one which can be travelled alone. And it is to these bonds and friendships that I would like to express my heartfelt gratitude.

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Summary

Graphene is widely recognized for its unique structural, physical and electronic properties that can be exploited for electrochemical applications. However, its potential application as an electrochemical material is currently impeded by the lack of methods to synthesize graphene in relatively high quality and large quantities. Consequently, this project has focused on investigating methods for graphene synthesis *via* the top-down approach i.e. the oxidation of graphite to graphite oxide followed by reduction to graphene. Analysis of the various oxidation methods revealed permanganate oxidants to be the most efficient. Five established reduction approaches were demonstrated for the first time to be useful for the conversion of graphene oxide to graphene. These reduction methods offer control over the extent of reduction, production of commercially viable graphene, and restoration of the sp^2 carbon network of reduced graphene. In addition, graphene oxide was successfully introduced as an electroactive label for the detection of single nucleotide polymorphism, demonstrating its potential application for biosensing. Furthermore, graphene materials were found to enhance the electrochemical detection resolutions for catechol and dopamine compounds in a lab-on-a-chip detection platform. The improved methods and demonstrated applications would serve as a useful platform for future studies on the synthesis and usage of graphene materials.

List of Publications

[1] Review article

1. **Chun Kiang Chua**, Martin Pumera. Covalent chemistry on graphene. *Chem. Soc. Rev.* **2013**, 42, 3222-3233.

[2-8] Research articles: Fundamental study on top-down approach of graphene synthesis

2. **Chun Kiang Chua**, Martin Pumera. Selective removal of hydroxyl group from graphene oxide. *Chem. Eur. J.* **2013**, 19, 2005-2011.

Highlighted as “Very Important Paper” – Selected as back cover.

3. **Chun Kiang Chua**, Martin Pumera. Reduction of graphene oxide with substituted borohydrides. *J. Mater. Chem. A* **2013**, 1, 1892-1898.
4. Adriano Ambrosi, **Chun Kiang Chua**, Alessandra Bonanni, Martin Pumera. Aluminium hydride as reducing agent for chemically reduced graphene oxides. *Chem. Mater.* **2012**, 24, 2292-2298.
5. **Chun Kiang Chua**, Martin Pumera. Renewal of sp^2 bonds in graphene oxides via dehydrobromination. *J. Mater. Chem.* **2012**, 22, 23227-23231.
6. **Chun Kiang Chua**, Adriano Ambrosi, Martin Pumera. Graphene oxide reduction by standard industrial reducing agent: Thiourea dioxide. *J. Mater. Chem.* **2012**, 22, 11054-11061.
7. **Chun Kiang Chua**, Sofer Zdeněk, Martin Pumera. Graphite oxides: effects of permanganate and chlorate oxidants on the oxygen composition. *Chem. Eur. J.* **2012**, 18, 13453 -13459.

8. **Chun Kiang Chua**, Sofer Zdeněk, Martin Pumera. Graphene sheet orientation of parent material exhibit dramatic influence on graphene properties. *Chem. Asian. J.* **2012**, 7, 2367-2372.

[9-15] Research articles: Electrochemical applications of graphene materials

9. **Chun Kiang Chua**, Martin Pumera. Chemically modified graphenes as detectors on lab-on-chip devices. *Electroanalysis* **2013**, 25, 945-950.
10. Alessandra Bonanni, **Chun Kiang Chua**, Guanjia Zhao, Sofer Zdeněk, Martin Pumera. Inherently electroactive graphene oxide nanoplatelets as labels for single nucleotide polymorphism detection. *ACS Nano* **2012**, 6, 8546-8551.
11. **Chun Kiang Chua**, Adriano Ambrosi, Martin Pumera. Graphene based nanomaterials as electrochemical detectors in lab-on-a-chip devices. *Electrochem. Commun.* **2011**, 13, 517-519.
12. Shu Min Tan, **Chun Kiang Chua**, Martin Pumera. Graphenes prepared from multi-walled carbon nanotube and stacked graphene nanofiber for detection of 2,4,6-trinitrotoluene (TNT) in seawater. *Analyst* **2013**, 138, 1700-1704.

Highlighted as "Hot Paper".

13. Sze Yin Chee, Hwee Ling Poh, **Chun Kiang Chua**, Filip Sanek, Sofer Zdeněk, Martin Pumera. Influence of parent graphite size on the electrochemistry of thermally reduced graphene oxide. *Phys. Chem. Chem. Phys.* **2012**, 14, 12794-12799.
14. Beng Khong Ong, Hwee Ling Poh, **Chun Kiang Chua**, Martin Pumera. Graphenes prepared by Hummers, Staudenmaier and Hofmann methods for analysis of TNT-based nitroaromatic explosives in seawater. *Electroanalysis* **2012**, 24, 2085-2093.

15. Her Shuang Toh, Adriano Ambrosi, **Chun Kiang Chua**, Martin Pumera. Graphene oxides exhibit limited cathodic potential window due to their inherent electroactivity. *J. Phys. Chem. C*. **2011**, 115, 17647-17650.

[16-17] Research articles: Chemical functionalizations on graphene

16. **Chun Kiang Chua**, Adriano Ambrosi, Martin Pumera. Introduction of carbene on graphene. *Chem. Commun.* **2012**, 48, 5376-5378.
17. **Chun Kiang Chua**, Martin Pumera. Friedel-Crafts acylation on graphene. *Chem. Asian. J.* **2012**, 7, 1009-1012.

[18-20] Research articles: Side projects on fundamental electrochemical study

18. **Chun Kiang Chua**, Martin Pumera, Lubomir Rulišek. The reduction pathway of 2,4,6-trinitrotoluene: An electrochemical and theoretical study. *J. Phys. Chem. C*. **2012**, 116, 4243-4251.
19. **Chun Kiang Chua**, Martin Pumera. Influence of methyl substituent position on redox properties of nitroaromatics related to 2,4,6-trinitrotoluene. *Electroanalysis*, **2011**, 23, 2350-2356.
20. **Chun Kiang Chua**, Martin Pumera. Detection of silver nanoparticles on a lab-on-chip platform. *Electrophoresis* **2013**, DOI: 10.1002/elps.201200426.

Scientific Conference Posters:

1. **Chun Kiang Chua**, Martin Pumera (2013). Organic modifications of graphene: Removal of hydroxyl groups from graphene oxide. In: International Winterschool on Electronic Properties of Novel Materials (IWEPNM), Austria.
2. **Chun Kiang Chua**, Martin Pumera (2012). Organic modifications of graphene: Reduction of graphene oxide. In: 7th Singapore International Chemical Conference (SICC), Singapore.

Thesis Organisation

This thesis is organized into **twelve (12) Chapters**. The reader is presented with the motivations and ideas towards the birth of this thesis in **Chapter 1**. The following **Chapter 2** will provide the reader with a general introduction to graphene materials. In depth discussion on previous work with relation to the structural features of graphite oxide, methods for chemical reduction of graphene oxide and the electrochemistry of graphene 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 4–11**, which consist of a collection of research towards the **aims** established in **Chapter 1**.

In detail, **Chapter 4** will discuss the fundamental study on the preparation methods of graphite oxide. **Chapters 5, 6, 7, 8** and **9** will cover five independent reduction methods of graphene oxide to graphene. **Chapters 10** and **11** will emphasize on the applications of graphene for biosensing 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 12**.

The published version of the results in **Chapters 4-11** are shown in the **Appendices**.

Index of Abbreviations

°C	Degree centigrade
AFM	Atomic Force Microscopy
ATR	Attenuated Total Reflectance
Au-IDE	Gold Inter-Digitated Electrode
cm ⁻¹	Inverse Centimeter
CMG	Chemically Modified Graphene
CRGO	Chemically Reduced Graphene Oxide
CVD	Chemical Vapour Deposition
DMAc	Dimethylacetamide
DMF	<i>N,N</i> -Dimethylformamide
DMSO	Dimethyl Sulfoxide
DRIFTS	Diffuse Reflectance Infrared Fourier Transform Spectroscopy
EA	Elemental Analysis
EIS	Electrochemical Impedance Spectroscopy
ERGO	Electrochemically Reduced Graphene Oxide
FTIR	Fourier Transform Infrared spectroscopy
GC	Glassy Carbon
GeO	Graphene Oxide
GO	Graphite Oxide
HOPG	Highly Ordered Pyrolytic Graphite
hr	Hour (time)
min	Minute (time)
mM	Millimolar
nm	Nanometer
NMP	<i>N</i> -Methyl-2-pyrrolidone
NMR	Nuclear Magnetic Resonance
PBS	Phosphate Buffered Saline
SEM	Scanning Electron Microscopy
TEM	Transmission Electron Microscopy
THF	Tetrahydrofuran
TRGO	Thermally Reduced Graphene Oxide
XPS	X-ray Photoelectron Spectroscopy
wt%	Weight Percentage

Usage of Terminologies

The graphene materials utilized in this project are entirely based on the top-down chemical synthesis approach. These materials, which include **“graphite oxide (GO)”**, **“graphene oxide (GeO)”**, **“chemically reduced graphene oxide (CRGO)”**, **“thermally reduced graphene oxide (TRGO)”**, and **“electrochemically reduced graphene oxide (ERGO)”**, are referred collectively as **“chemically modified graphene (CMG)”**

The term **“reduced graphene oxide (RGO)”** or **“reduced graphene”** would be used as a general term to refer to any or all of these materials – **“chemically reduced graphene oxide”**, **“thermally reduced graphene oxide”**, or **“electrochemically reduced graphene oxide”**

While based on the IUPAC definition of graphene, *“Graphene is a single carbon layer of the graphitic structure, describing its nature by analogy to a polycyclic aromatic hydrocarbon of quasi infinite size”*.¹ On the other hand, the reduced graphene oxide materials obtained from the top-down chemical synthesis methods *via* graphene oxide consists mainly of 99 % few-layer graphene and only 1 % of mono-layer graphene.²

Despite this fact, in order to simplify the discussion in this thesis, the term **“graphene”** will be used as the replacement term or used interchangeably for the **“reduced graphene oxide”** or **“reduced graphene”** materials discussed in the project.

- (1) McNaught, A. D.; Wilkinson, A. *Compendium of Chemical Terminology: IUPAC Recommendations*; International Union of Pure Applied Chemistry, Blackwell Science, 1997.
- (2) Ang, P. K.; Wang, S.; Bao, Q. L.; Thong, J. T. L.; Loh, K. P. *ACS Nano* **2009**, 3, 3587.

Chapter 1

Objectives of the project

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Graphene is the latest carbon nanomaterial that has caught the attention and interest of researchers from all branches of physical sciences, both experimental and theoretical chemists, physicists, and biologists. This is brought about by the exceptional properties and potentials of graphene. Detailed discussion would be provided in **Chapter 2**.

Although many research groups are investigating on the production methods of graphene, the large-scale (kilograms) production of high quality graphene remains a challenge to the graphene community. At the moment, a chemical treatment strategy based on the top-down synthetic approach is the most promising method to achieve that. These production steps involve the oxidation of graphite to graphite oxide and the subsequent reduction of graphene oxide to graphene. As such, fundamental studies on these two chemical steps are important to ensure the production of high quality graphene in large quantities.

Furthermore, graphene is widely applied as electrode materials for electrochemical applications. Since numerous electrochemical sensing and biosensing applications of graphene remain largely untapped, research in this direction could potentially deliver novel applications to improve current technologies.

Hence, **two (2)** general aims, as illustrated in Figure 1.1, were drawn for this project to address and improve these areas of graphene research. Background knowledge leading to these aims would be highlighted in **Chapter 3**.

Aim 1: Investigation on the fundamental preparation procedures of graphene *via* the top-down synthetic approach.

The oxidation methods of graphite towards graphite oxide have been applied interchangeably among four well-known methods (i.e. Staudenmaier, Hofmann, Hummers and Tour) in the graphene community. Graphite oxide is both an important intermediate towards the synthesis of graphene and a material with numerous prospective applications. Therefore, the effects of the types of chemical oxidants on the oxygen contents of graphite oxide materials are important to dictate the suitability of the produced materials for further chemical treatment or specific applications.

Apart from that, while the reduction of graphene oxide towards graphene has been achieved with several chemical reducing agents, most of these reducing agents are, *theoretically*, only capable of reducing oxygen groups to remain as hydroxyl groups. More importantly, numerous more reducing agents are operating on unestablished mechanisms according to the knowledge of synthetic chemistry. As such, rational reduction methods of graphene oxide, based on methods with supported mechanisms, would be investigated. These reduction methods would provide opportunities to fine-tune the extent of reduction and to produce commercially viable graphene. Moreover, with a vision beyond the removal of oxygen containing groups, the restoration of the sp^2 carbon system on reduced graphene, which was previously populated by sp^3 carbon atoms bonded to oxygen functionalities, would be examined.

The research direction of this aim would be elaborated in **Chapters 4, 5, 6, 7, 8 and 9.**

Aim 2: Integration of graphene for applications in biosensing and lab-on-a-chip detection platforms.

Graphene has excellent electrical and physical properties that are highly beneficial for electrochemical sensing and biosensing purposes. However, applications of graphene for such purposes remain largely unexplored. The possibilities of exploiting these unique properties of graphene as an effective detection platform for biosensing purposes, with focus on the detection of single nucleotide polymorphism, would be explored.

Apart from that, the implementation of electrochemical detectors in a lab-on-a-chip platform provides versatility and portability to the system. As materials for electrochemical detectors are often susceptible to fouling and low detection sensitivities, the properties of graphene materials could potentially provide an edge as electrode materials for electrochemical detection.

The applications of graphene materials for electrochemical biosensing and as electrochemical detectors in a lab-on-a-chip platform would be demonstrated in **Chapters 10 and 11.**

Figure 1.1 Summary of the challenges and aims of this project.

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Chapter 2

Introduction

2.1 *Discovery of graphene*

2.2 *General properties of graphene*

2.3 *Production methods of graphene*

 2.3.1 *The bottom-up approach*

 2.3.2 *The top-down approach*

2.4 *The quality of graphene determines its applications*

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2.1 Discovery of graphene

Graphene – a term first coined by Boehm *et. al.* in 1986 for a single carbon layer of a graphitic structure of infinite size – is a fused polycyclic aromatic hydrocarbons analogous to a honeycomb lattice structure.¹ The term '**graphene**' has often been used interchangeably with descriptions such as graphite layers, carbon layers or carbon sheets in the graphene community. This has, however, led to much confusion in the community. It was only 11 years later in 1997, that the IUPAC formalised the recommendation of the term graphene as *"a single carbon layer of the graphitic structure, describing its nature by analogy to a polycyclic aromatic hydrocarbon of quasi infinite size"*.² In terms of the quasi infinite size of graphene, it can be regarded as a basic building block for major graphitic carbon materials of all other dimensionalities (Figure 2.1).³ While graphene could certainly be stacked into three-dimensional graphite materials, it could also be rolled-up into one-dimensional carbon nanotubes or wrapped-up to form zero-dimensional fullerenes.

Although the term graphene has only been acknowledged in recent decades, the concept of graphene has been around since the 1940s. Graphene was initially thought to be thermodynamically unstable to exist under ambient conditions.^{4,5} It was postulated that graphene could not exist in its free state, but would collapse into structures such as fullerenes, carbon nanotubes or amorphous materials instead. As such, graphene was only regarded as a theoretical material for academic purposes. Although graphene was first observed on a platinum (100) surface in 1968⁶ and later probed with scanning tunnelling microscopy in 1992⁷, these discoveries did not spark off any major graphene research boom in the scientific community. The recent explosion of interests in graphene actually refers to the 2004 paper by Geim and Novoselov, which highlighted

the exceptional properties of **free-standing** monolayer sheet of graphene obtained from the mechanical exfoliation of highly oriented pyrolytic graphite.^{8,9}

Figure 2.1 Graphene as a raw building block for carbon nanomaterials of other dimensionalities. Graphene can be balled up into 0D fullerenes, rolled up into 1D carbon nanotubes or stacked into 3D graphite. Reproduced from ref. 3.

2.2 General properties of graphene

This discovery of free-standing graphene sheets opened up a new direction of research in the scientific community. Graphene has been applied in almost all facets of physical sciences, encompassing chemistry, physics and biology. These developments are rapidly driven by the initial experiments that confirmed the charge carriers on graphene as massless Dirac fermions.^{9,10}

Several properties of graphene measured experimentally have currently surpassed those observed in other types of materials. In general, the properties of graphene range from enhanced electrical conductivity, optical transparency, high mechanical strength and thermal conductivity.^{3,11} The ambipolar electric field effect where the charge carriers could be electrons or holes gave rise to its high charge carrier concentrations and mobilities.⁸ The point overlaps between the valence and conduction bands at the Dirac points gave rise to a zero-bandgap graphene. High optical transparency is observed on graphene, with optical absorption of approximately 2.3 % towards visible lights.¹² In terms of thermal conductivity, graphene measured up to above 3000 W mK⁻¹ under ambient conditions. This is approximately seven times higher than the thermal conductivity of pure copper, which is one of the best metallic heat conductor, at ~400 W mK⁻¹.¹³ A defect-free graphene has a breaking strength of 42 Nm⁻¹, corresponding to a Young's modulus of 1.0 TPa, which is one of the strongest materials ever measured.¹⁴ Moreover, graphene is completely impermeable to any gases¹⁵ and is readily functionalized¹⁶ to further fine-tune its properties. However, most of these properties are only observed on graphene samples of the highest quality obtained *via* a mechanical exfoliation method.

2.3 Production methods of graphene

Graphene is essentially a single sheet of polycyclic aromatic carbon displaced from a three-dimensional graphite structure. The effort to obtain this independent sheet of carbon has led to two general approaches i.e. the bottom-up and top-down approaches (Figure 2.2). The bottom-up approach attempts to build graphene from small carbon sources and this is usually carried out through chemical vapour/ solid deposition or epitaxial growth on silicon carbide. The top-down approach works its way through by ‘peeling’ off individual graphene sheets from a graphite material. These include techniques such as mechanical and liquid-phase exfoliation of graphite, oxidation of graphite to graphite oxide with consequent chemical/ electrochemical/ thermal reduction, or unzipping of carbon nanotubes.^{17,18}

Figure 2.2 Production routes towards the synthesis of graphene.

2.3.1 The bottom-up approach

Chemical vapour deposition (CVD) technique is one of the most preferred bottom-up approaches for the growth of large-area uniform graphene films. The growth process involves feeding a feedstock of carbon source (i.e. methane) and hydrogen into a furnace and through a hot zone. A metal substrate placed in the hot zone results in the catalytic reaction between the carbon source and hydrogen to provide layers of graphene upon subsequent cooling. Precise control of the furnace pressure, temperature, growth time, cooling rate, and type of metal substrates determine the quality of the graphene produced. The quality of the “**CVD-graphene**” could vary to different extents in terms of the presence of defects, ripples, domain size, grain boundaries, or thickness ranging from mono-, bi-, to few-layer graphene.^{19,20}

In recent years, CVD-graphene has been produced from metal catalytic surfaces such as platinum, iridium, ruthenium, cobalt, iron, nickel and copper metal.²¹⁻²⁴ The quality of the CVD-graphene produced on these metal surfaces is typically governed by the ability of the metals to dissolve carbon atoms. For instance, cobalt and nickel show higher tendencies to dissolve more carbon atoms than copper, which would result in the latter producing a higher amount of mono-layer graphene. Apart from using vapour carbon source, solid carbon sources have been successfully utilized to grow graphene under similar experimental conditions. Solid carbon sources such as food, insects and waste have been demonstrated to produce mono-layer graphene.²⁵ Despite a good understanding on the growth process, one of the major challenges that lies ahead of CVD-graphene is the transfer process, which is equally complicated as the growth process. The transfer step is necessary to remove the catalytic metal substrates prior to the usage of graphene in further applications. Current transfer techniques rely on carrier

materials such as poly(methyl methacrylate) (PMMA) to transfer graphene sheets onto arbitrary substrates.^{26,27} While the graphene sheets are sandwiched between the carrier material and metal substrate, the latter is subsequently etched. Upon transfer onto an arbitrary substrate, the carrier is then dissolved to complete the transfer process. However, improper transfer techniques could potentially damage the graphene films or introduce unwanted impurities/ dopants onto the films.²⁸

On another hand, “**epitaxial-graphene**” could be grown on the Si- and C-face of hexagonal silicon carbide (SiC) by annealing at a temperature of more than 1000 °C. At that temperature, Si atoms are sublimed while leaving a graphitized surface to provide a layer of epitaxial-graphene. The surface of the SiC highly dictates the properties of the grown graphene.²⁹ Current advancement in this technique has permitted good control over the number of graphene layers grown.³⁰ Growth of graphene on SiC is capable of producing single-crystal high quality graphene films in the size of hundreds of micrometres.³¹

2.3.2 The top-down approach

The most direct top-down approach towards free-standing graphene is *via* the mechanical exfoliation of highly ordered pyrolytic graphite (HOPG). This was, in fact, first demonstrated by Geim and Novoselov in their 2004 paper that set forth the rapid research in graphene. An adhesive tape was used to gently split layers of graphite crystal from the HOPG, until mono-, bi- or few-layer graphene were obtained. Graphene obtained from this method is often labelled as “**pristine graphene**”, as this technique is usually free from dopants, impurities, defects and irregularities in the graphene films.

The exfoliation of graphite could be achieved *via* liquid-phase exfoliation technique as well. In this case, graphite is usually exfoliated in aqueous solutions with surfactants or non-aqueous solvents such as *N*-methylpyrrolidone³², sodium dodecylbenzene sulfonate³³, and ionic liquids³⁴. The exposure of the graphite suspension to a probe ultrasonicator subsequently produces a mixture of mono-, bi- and few-layer graphene, which could be further segregated by centrifugation.³²

A more common method of graphene production is *via* the graphite oxide route. Graphite is usually oxidised with a mixture of strong acid and oxidant to provide “**graphite oxide**”. Graphite oxide refers to a graphite material with a very high content of oxygen groups covalently bonded to its interlayer surfaces while still retains its lamellar structure. The oxidative treatment helps to increase the interlayer distance between graphene sheets in graphite from $\sim 3.33\text{--}3.35^{35}$ Å to $\sim 6\text{--}12^{36}$ Å (varied with humidity upon water intercalation) in graphite oxide for an easy exfoliation, since the sheets are usually held by strong van der Waals forces.³⁷ Several methods available for the oxidation treatment include the Staudenmaier, Hofmann, Hummers and Tour methods. Further ultrasonication treatments result in the collapse of the lamellar structure to give mono-, bi- or few-layer “**graphene oxide**”. Successive chemical or electrochemical reductions would remove most of the oxygen groups to give the corresponding mono-, bi- or few-layer graphene. Various chemical reductants have been utilized, for example, sodium borohydride, hydrazine, hydrohalic acids, and sulphites (reviewed in **Chapter 3**). The electrochemical reduction process is usually achieved by applying a negative potential on an electrode coated with graphene oxide for a fixed period of time. Apart from that, a thermal heat shock treatment at more than 1000 °C on graphite oxide could be applied to simultaneously exfoliate and reduce graphite oxide to give graphene.

Graphene obtained from the reduction of graphite or graphene oxide are often termed as “**chemically/ electrochemically/ thermally reduced graphene oxide**”. These graphene materials and its intermediates obtained *via* initial chemical treatments are collectively labelled as “**chemically modified graphene**” materials since they contain traces of functional groups covalently bonded to their surfaces.

Apart from that, carbon nanotubes could be utilized as a carbon source towards graphene. The treatment of carbon nanotubes with potassium permanganate and sulphuric acid provides unzipped carbon nanotubes that could be reduced to give “**graphene nanoribbons**”.³⁸ In general, graphene nanoribbons possess different morphological and physical properties in comparison to the common graphene sheets. The nanoribbons also exhibit unique electronic properties due to quantum confinement and edge effects.³⁹ More importantly, the bandgap of graphene nanoribbons could be easily engineered and dictated by the length and width of the structures. Numerous attempts to produce well-defined graphene nanoribbons have been accomplished with a bottom-up approach based on polymerization reactions of small chemical monomers.^{40,41}

This project would mainly focus on graphene prepared *via* the top-down approach i.e. conversion of graphite to graphite oxide and subsequent reduction of graphene oxide to graphene. Fundamental studies on the preparation methods of reduced graphene materials, as highlighted in **Chapter 1**, would be investigated in **Chapters 4–9**.

2.4 The quality of graphene determines its applications

The bottom-up and top-down approaches towards graphene provided an array of graphene materials of varying qualities. In fact, the suitability of the graphene materials for specific applications is highly dictated by the sheet quality i.e. defects, thickness, grain size and purity.

The bottom-up approach of CVD growth method provides high quality graphene that is suitable for applications in transparent conductive layers, photonics, nanoelectronics, sensors and bioapplications.¹⁸ The excellent electrical (low sheet resistance) and optical (high optical transparency) properties of graphene grant its usage as transparent conductive layers that are usually applied in electronic products such as organic light-emitting diodes (OLEDs),⁴² e-paper (electronic paper) and touch screen displays⁴³. Given the unique high mechanical flexibility and chemical durability of graphene, it could easily replace the traditional inflexible indium tin oxide (ITO) provided that the cost of production is manageable. The mechanical flexibility and uniform absorption of CVD-graphene across the visible spectrum also ensure its suitability as a rollable e-paper. Furthermore, CVD-graphene has been fabricated for electrochemical sensing applications, such as gas detector⁴⁴ and DNA sequencing⁴⁵. Despite the bright prospect of CVD-graphene, improvements in the growth and transfer processes have to be further attained to ensure the consistent quality of CVD-graphene i.e. types of defects, number of layer, grain boundaries. Currently, the production of CVD-graphene has been achieved in the dimensions of square metres.⁴³

Epitaxial-graphene from SiC based on the bottom-up approach provides high quality graphene as well. Epitaxial-graphene is attractive to applications in the area of

high-power electronics such as high-frequency transistors. The applicability of graphene in this area is still in the development stage as the performance lags greatly behind that of current compound semiconductors (III-V materials). Moreover, the high substrate cost, high-temperature growth, and small-diameter wafers limit any further applications of epitaxial graphene in other areas.¹⁸

While the mechanical exfoliation technique of highly ordered pyrolytic graphite provides pristine graphene, the production volume is often too small. As such, it is only suitable for fundamental research on the properties of graphene. Similarly, the liquid-phase exfoliation could potentially provide layers of mono-, bi- and few-layer graphene but only in very small quantities. However, the produced graphene is less pristine as compared to the graphene obtained *via* mechanical exfoliation technique. The usage of chemical or surfactants might introduce unwanted dopants or impurities while the ultrasonication treatment might cause defects to the graphene sheets.

On the other hand, the top-down approach *via* the route of graphite oxide offers an opportunity for the large-scale production of graphene (reduced graphene). Since reduced graphene comprises of major structural defects and fragments of oxygen containing groups, its applications are limited to the areas of coatings, paint/ink, composites, energy storage, transparent conductive layers and bioapplications.¹⁸ As graphene is relatively inert, it can be used as a transparent protective layer and corrosion barrier against water and oxygen diffusion on the upper layer of metal surfaces. Moreover, the high aspect ratio, electronic, chemical, mechanical and barrier properties of reduced graphene make it a suitable candidate as an additive in composite matrix polymers. Reduced graphene might also grant properties such as gas and moisture barrier, electrical and thermal conductivity, gas and moisture barrier, antistatic

behaviour, and improved composite compressive strength to composite polymers.^{18,46} Apart from that, reduced graphene has been applied in dye solar cells⁴⁷, lithium-ion batteries⁴⁸ and supercapacitors⁴⁹. More importantly, the large-scale production capability of reduced graphene offers opportunities as electrode materials for electrochemical sensing purposes.^{50,51}

Last but not least, graphene is currently being considered for bioapplications such as drug delivery, bioimaging, regenerative medicine and tissue-engineering applications. Despite the huge potential of graphene, in depth understanding on the biocompatibility and toxicity of graphene should be pursued prior to its wide usage in the biomedical area. On top of these, the usage of graphene as detection materials for biosensing systems is prevalent in the recent years.⁵² However, careful examinations on the batch-to-batch reproducibility and reliability of the produced graphene have to be established prior to commercial use.

Specifically in this project, the applications of graphene for electrochemical biosensing and sensing purposes were extended. Graphene was applied for the indirect detection of single nucleotide polymorphism and as electrode materials in a lab-on-a-chip platform in **Chapters 10 and 11**.

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Chapter 3

Literature review

3.1 *Discovery and synthesis of graphite oxide*

3.2 *The elusive structure of graphite oxide*

3.3 *Conversion of graphite oxide to graphene*

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3.1 Discovery and synthesis of graphite oxide

The idea of graphite oxide came around much earlier as compared to graphene. In 1859, when an abundance of graphite from Ceylon (now known as Sri Lanka) arrived in Britain, Professor Brodie from the University of Oxford attempted to measure the molecular weight of the graphite.¹ A mixture of graphite with fuming nitric acid in the presence of potassium chlorate was heated at 60 °C for three to four days. The material was then washed, and re-oxidised under the same condition, up to four repetitions, to finally give a light yellow solid upon drying at 100 °C. The C : H : O ratio of the material was determined as 61.04 : 1.85 : 37.11. The material was found to disperse in pure or basic water, but not in acidic media. As such, Brodie termed the materials as “**graphic acid**”. That was the very first sample of graphite oxide prepared experimentally.

In 1898, Staudenmaier improved the oxidation method by adding the potassium chlorate in small portions and further acidifying the mixture with concentrated sulphuric acid.² The material obtained by Staudenmaier gave almost similar C/O ratio as that prepared by Brodie. However, this method was practically more convenient since it does not require four repetitions of oxidation as that of Brodie’s.

A few decades later in 1937, Hofmann replaced the usage of fuming nitric acid with non-fuming nitric acid in his oxidation method.³ In 1958, Hummers and Offeman presented a safer approach by utilizing potassium permanganate as oxidant in a mixture of concentrated sulphuric acid and sodium nitrate.⁴ This method was safer since it produced nitric acid *in situ*, thus the usage of highly corrosive fuming nitric acid could be avoided. Despite the absence of fuming nitric acid, the combination of potassium permanganate and sodium nitrates resulted in a more heavily oxygenated form of

graphite oxide. As such, the Hummers method has been very well received and adopted by many materials scientists. On the other hand, all these procedures resulted in the production of toxic gases, mainly NO_2 , N_2O_4 and explosive ClO_2 (Staudenmaier/Hofmann).

Timeline of Graphite Oxide Synthesis Methods

Figure 3.1 The discovery of graphite oxide and evolution of the synthesis methods.

An effort to improve the oxidation method introduced by Tour in 2010 has seen a replacement of the *in situ* production of nitric acid with the addition of a less corrosive phosphoric acid.⁵ The method was reported to result in a more oxidised form of graphite oxide and highlighted a more intact graphitic basal-plane. The safer method also suggested the possibility for large-scale production of graphite oxide since the process does not involve a large exotherm or release of toxic gas.

Over the span of 150 years, improvements over the synthesis methods of graphite oxide have been constantly pursued to provide a safer and more effective method (Figure 3.1). The extent of the oxidation process, which was often gauged by the magnitude of carbon/oxygen ratios, was however insufficient to truly understand the structural properties of graphite oxide. Further investigations were thus required to probe in this area of subject.

3.2 The elusive structure of graphite oxide

Graphite oxide is primarily composed of carbon, oxygen and hydrogen atoms. It is common for graphite oxide to retain an atomic carbon/oxygen ratio of approximately 1.5 to 2.5. Although the synthesis method of graphite oxide has been known for nearly 150 years, the exact chemical structure of graphite oxide remains elusive to the graphene community. Several structures have been proposed, such as the Hofmann, Ruess, Scholz-Boehm, Nakajima-Matsuo, Lerf-Klinowski, and Dekany models (Figure 3.2).

The very first model of graphite oxide was proposed by Hofmann and Holst.⁶ The structure was proposed to consist of repeating units of 1,2-epoxides on the entire basal-planes of graphene. In 1946, Ruess proposed a new model made up of sp^3 hybridised basal-planes as opposed to the sp^2 hybridised system of Hofmann and Holst.⁷ The model consists of 1,3-epoxides and hydroxyls to account for the presence of hydrogen atoms found in graphite oxide. Twenty years later in 1969, Scholz and Boehm proposed a new model which was made up of only hydroxyl and ketone groups.⁸ Subsequent model proposed by Nakajima and Matsuo gave rise to a model which was very similar to a graphite intercalation compound.⁹ The models mentioned above were mostly deduced based on the elemental composition, reactivity and X-ray diffraction studies of graphite oxide at that time.

A recent model was proposed by Lerf and Klinowski.¹⁰ This model was derived from the analysis of solid state nuclear magnetic resonance spectroscopy and proposed a distribution of 1,2-epoxides and hydroxyls spread across the basal-planes of graphite. Following subsequent refinement, the edge-plane of the graphite was found to contain small quantities of carboxylic acid groups and other keto groups as well.

However, analysis on the data from FTIR and DRIFTS spectroscopies by Dékány suggested that ketones or quinones are more likely to exist as compared to carboxylic acid. On the other hand, the results from potentiometric acid-base titrations highlighted the presence of acidic sites on the graphite oxide plane. In order to fit the presence of acidic sites on the graphite oxide, the Lerf-Klinowski model is postulated to be populated with α,β -unsaturated ketones that undergo keto-enol isomerizations. The enol form was, as such, the source of acidic protons. On the other hand, the keto form was more thermodynamically stable as compared to the enol form. A recent report by Tour attempted to justify the acidic properties of graphite oxide as due to the presence of incompletely hydrolysed covalent sulphates.¹¹

Despite that, by building on the Ruess and Scholz-Boehm models, Dékány proposed a refined model composing of two distinct domains.¹² The proposed model was composed of tertiary hydroxyls and 1,3-epoxides as well as corrugated regions of cyclic ketones and quinones formed as a result of cleaved C-C bonds. Although the actual identity of graphite oxide remains debatable, the Lerf-Klinowski model is one of the most accepted models to date, by the graphene community. Numerous reports have emerged in recent years that build on the chemistry of the carboxylic groups postulated to exist in graphite oxide. In this project, the Lerf-Klinowski model was closely adhered to in order to explain and visualise work on graphite oxide and graphene derivatives.

Hofmann **Nakajima-Matsuo** **Ruess** **Lerf-Klinowski** **Scholz-Boehm** **Dékány****Figure 3.2** The proposed models for graphite oxide.

3.3 Conversion of graphite oxide to graphene

The amount of scientific discoveries revolving around graphite oxide has spanned over a long period of time. It should not be forgotten that, above all, graphite oxide is a very important intermediate material towards the production of graphene. The oxidative treatment on graphite helps to increase the interlayer distance between graphene sheets in graphite for an easy exfoliation, since the sheets are usually held by strong van der Waals forces. This is referring to the top-down approach, which is the most direct routes, and it is also one which provides the opportunity for the large-scale production of graphene (**reduced graphene oxide**). While the disparities between reduced graphene oxide and pristine graphene are largely noticeable, the former is nevertheless important for a big portion of the graphene community that endeavours to use graphene in applications such as composites, coatings, paint/ink, transparent conductive layers, bioapplications, and energy storage.

Graphite oxide is seen as a highly oxidised form of graphite, which retains the lamellar structure of its precursor albeit with a higher interlayer spacing due to the presence of oxygen functionalities. Based on the Lerf-Klinowski model of graphite oxide, the oxygen functionalities encompass moieties such as hydroxyl, epoxide, carbonyl and carboxylic groups. The basal-plane of the graphite oxide is highly populated with hydroxyls and epoxides while the edge-plane contains mainly of carboxylic and carbonyl groups. These groups account for the structural defects that deviate graphite oxide from the state of pristine graphene.

Its exfoliated form, graphene oxide, is seen as an important intermediate between graphite oxide and graphene. Graphene oxide is structurally different as

compared to graphite oxide. Graphene oxide still retains most of the oxygen functionalities of its precursor, but largely exists as mono-, bi- or few-layer graphene sheets. Graphene oxide is usually achieved *via* mechanical stirring or ultrasonication methods in a polar organic solvent or aqueous media. Although the ultrasonication method ensures a more efficient and faster exfoliation of the stacked graphite oxide sheets, it often entails with structural damages and results in the breaking of graphene oxide sheets into smaller fragments.¹³

In order to achieve the conversion of graphene oxide to graphene, thermal, electrochemical and chemical reduction methods are often applied (Figure 3.3). The reduction methods are envisaged to facilitate the removal of the oxygen functionalities on graphite/ graphene oxide. These three different methods would result in graphene materials of varying performances in terms of their electronic, structural, physical and surface morphological properties. Although these reduced graphene oxide materials consist of defective sheets of sp^2 carbon network, they share close resemblance to pristine graphene and are highly applicable for applications that require a large amount of graphene materials.

In this project, with a bulk emphasis on the chemical reduction of graphene oxide towards graphene materials based on the top-down approach, the current state of the available methods for the chemical reduction of graphene oxide would, as such, be reviewed in the following sub-chapter.

Figure 3.3 Conversion routes from graphite to graphene. Graphite is oxidised to graphite oxide. A thermal treatment on graphite oxide provides thermally reduced graphene oxide (TRGO). An ultrasonication treatment on graphite oxide gives graphene oxide. Graphene oxide can be reduced electrochemically or chemically to result in electrochemically reduced graphene oxide (ERGO) and chemically reduced graphene oxide (CRGO), respectively.

3.4 Chemical reduction of graphene oxide

While the preparation of graphite oxide was first carried out 150 years ago, the attempt to remove the oxygen functionalities on the surface of graphite oxide *via* chemical means did not occur until another 100 years later in 1948. During that time, graphite oxide was treated with hydrazine, hydroxylamine, hydroiodic acid, iron(II) and tin(II) ions to result in a reduction process.¹⁴ Fast forward to the year 2000s, this knowledge has been transferred onto graphene oxide. Based on the chemical compositions, graphite oxide does not differ much from graphene oxide at all. They are only structurally different in terms of the size of the stacked graphene layers.

The transformation of graphene oxide to graphene is, by experimental observations, often indicated by a colour change of the reaction mixture from brown (of graphene oxide) to black (of graphene), as well as an increase of hydrophobicity/ aggregation of the material as a result of the removal of oxygen containing groups. Based on more detailed analyses, the decrement of the elemental oxygen contents (often represented by a change of C/O ratio) and increment in the current conductivity of the graphene materials often signify the efficiency of a particular reduction method.

Despite the wide arrays of chemical reduction strategies, most of these reducing agents are known to reduce graphite/ graphene oxide without any proper support of mechanisms based on the knowledge of synthetic chemistry. Moreover, numerous methods of reduction have surfaced or *resurfaced* in recent years, which include the reduction of graphene oxide with hydrazine monohydrate, dimethylhydrazine, hydroquinone, sodium borohydride, hydroxylamine, phenylenediamine, sulphite, hydrohalic acid, iron/ aluminium/ zinc metals in acidic media, L-ascorbic acid,

saccharides, wild carrot root, alcohols, tea extract, and others. An overview of these reduction methods would be covered in the following text. For discussion purposes, these reductants would be categorized into two different categories, mainly reduction methods with ‘well-supported’ mechanisms and reduction methods with ‘proposed’ mechanisms (Table 3.1). Reductants under the category of ‘well-supported’ mechanisms consist of reductants which have been traditionally applied in synthetic chemistry and have demonstrated proven/ definite modes of reaction towards specific functional groups (i.e. sodium borohydride is reactive towards carbonyl functional groups). On the other hand, reductants under the category of ‘proposed’ mechanisms include reductants that are uncommonly applied in synthetic chemistry as reducing agents and do not have definite modes of reaction towards specific functional groups (i.e. alcohol as a reducing agent). The conductivities and C/O ratios of the resulting graphene materials are also summarised in Table 3.1.

It should be noted that all the mechanisms presented in this sub-chapter (in 3.4.1 and 3.4.2 with exception of Scheme 3.1) are reproduced from the corresponding articles which had introduced and discussed about the reductants of interest.

Table 3.1 List of reductants and their efficiencies in terms of conductivity and C/O ratio.

Reductants	Conductivities (S/m)	C/O ratios (analysis)	References
Reduction methods with ‘well-supported’ mechanisms			
Sodium Borohydride	-	-	15
	17	-	16
	82	4.8 (XPS)	17
	45	8.6 (XPS)	18
Hydrohalic Acid <i>HI/ AcOH</i> <i>HBr</i> <i>HI</i> <i>HI/ TFA</i>			
	30400	15.3 (EA)	19
	-	12.5 (EA)	20
	29800	12.0 (XPS)	21
	2.3×10^{-2}	3.9 (XPS)	22
Reduction methods with ‘proposed’ mechanisms			
Hydrazine	200	10.3 (EA)	23
Hydroxylamine	1122	9.7 (XPS)	27
Pyrrole	-	7.7 (XPS)	28
<i>p</i>-Phenylene diamine	15000	7.4 (XPS)	29
Polyelectrolyte	-	-	30
Metal/ acid <i>Al/ HCl</i> <i>Fe/ HCl</i> <i>Zn/ Ammonia</i> <i>Zn/ HCl</i> <i>Zn/ H₂SO₄</i>			
	2100	-	31
	2300	7.9 (XPS)	32
	-	8.6 (XPS)	33
	15000	33.5 (XPS)	34
	3416	21.2 (XPS)	35
Sulphur-containing compounds <i>NaHSO₃</i>			
	6500	7.9 (EA)	36
Sodium Hydrosulphite	1377	-	37
Amino acid <i>L-Cysteine</i> <i>Glycine</i>			
	1.2×10^{-1}	-	38
	-	11.2 (XPS)	39
L-Glutathione	-	-	40

Alcohol			
<i>Methanol</i>	3.2×10^{-5}	4.0 (EA)	41
<i>Ethanol</i>	1.8×10^{-4}	6.0 (EA)	41
<i>Isopropyl Alcohol</i>	1019	6.9 (EA)	41
<i>Benzyl Alcohol</i>	4600	30.0 (EA)	41
Hydroquinone	-	-	42
L-Ascorbic acid			
<i>with L-tryptophan</i>	14	-	43
<i>L-Ascorbic acid</i>	800	-	44
<i>with alkaline media</i>	7700	12.5 (XPS)	45
Saccharides			
<i>Glucose</i>	-	-	46
<i>Dextran</i>	1.1 (10000: anneal at 500 °C)	-	47
Gallic acid	36	5.3 (XPS)	48
Plant extracts			
<i>Green tea</i>	53	-	49
<i>C. esculenta leaf</i>	4006	7.1 (EA)	50
<i>M. ferrea Linn. leaf</i>	3185	6.1 (EA)	50
<i>C. sinensis peel</i>	3033	6.0 (EA)	50
Microorganisms			
<i>Shewanella</i>	-	-	51
<i>E. coli culture</i>	-	-	52
<i>Baker's yeast/ NADPH</i>	43	5.9 (XPS)	53
<i>Wild carrot roots</i>	-	11.9 (XPS)	54
Bovine serum albumin	-	-	55

3.4.1 Reduction methods with ‘well-supported’ mechanisms

Although the chemical reagents currently available for the reduction of graphene oxide are in abundance, most of these reductants are not supported by well-established mechanisms. The only few reductants which could be included in this category of reductants with ‘well-supported’ mechanisms are sodium borohydride and hydrohalic acids. These reductants are known to possess definite modes of reaction towards specific functional groups. These reductants would be discussed in the following text.

3.4.1.1 Sodium borohydride

Sodium borohydride, NaBH_4 , is a common reducing agent widely known to synthetic chemists. It is a salt containing a tetrahedral BH_4^- anion, which readily solubilize in aqueous and alcoholic media. In the presence of an electrophile such as carbonyl groups, the borohydride anion readily perform a ‘hydride transfer’ reaction (Scheme 3.1) to result in an oxyanion and electron-deficient BH_3 molecule. Subsequent stabilization of the BH_3 molecule with the oxyanion reinstates the borohydride as a ‘hydride transfer’ agent. This is ideally the case until all the B-H bonds are exhausted. However, the reaction is rarely as efficient in practice. The ability of BH_4^- to reduce a carbonyl group is also limited by the types of carbonyl groups. Simple carbonyl compounds such as aldehyde and ketone are reducible by NaBH_4 to alcohol groups, while less reactive carbonyl compounds like ester or amide are not possible.

The usage of NaBH_4 as a reducing agent for graphene oxide was first reported by Kamat and co-workers to achieve physisorption of gold nanoparticles on a graphene-octadecylamine material.¹⁵ Samulski and co-workers next integrated the reduction of

Scheme 3.1 Mechanism of reduction of a carbonyl group with sodium borohydride.

graphene oxide with NaBH₄ as a first of three-step synthesis towards a total reduction effort to obtain a sulphonated graphene which disperses well in water and organic solvents.¹⁶ Based on their method, graphene oxide dispersed in basic water was stirred with NaBH₄ for 1 hr at 80 °C. The pre-reduced sulphonated graphene measured a conductivity of 17 S/m. A similar reduction method with NaBH₄ was also carried out by Ajayan and co-workers on their investigation towards the total reduction of graphene oxide.¹⁷ Following that, Lee and co-workers conducted a study on the effect of NaBH₄ concentration on the electrical properties of the resulting graphene materials.¹⁸ A conductivity of 45 S/m was measured on the graphene prepared by dipping a strip of graphene oxide into a 150 mM solution of NaBH₄. The presence of boron oxide complexes on graphene strips were discovered when the graphene oxide materials were subjected to a low concentration of 15 mM solution of NaBH₄. Apart from that, the electrical resistances of the graphene materials were also measured to be lower than graphene obtained from hydrazine reduction treatment, due to the absence of heteroatoms.

3.4.1.2 Hydrohalic acid

Hydrohalic acids such as HI, HBr, and HCl are often utilized in synthetic chemistry for the transformation of ethers to alcohol and alkyl halides. The halides are nucleophilic in nature, with increasing nucleophilicity from $\text{Cl}^- < \text{Br}^- < \text{I}^-$, and reacts *via* both $\text{S}_{\text{N}}1$ or $\text{S}_{\text{N}}2$ pathways depending on the reacting substrates. The hydrohalic acids are capable of opening epoxides as well.

Hydrohalic acid has been previously applied for the reduction of graphite oxide and has *resurfaced* for the reduction of graphene oxide in recent years. The usage of hydroiodic acid/ acetic acid mixture for the reduction of graphene oxide was achieved by Lee and co-workers.¹⁹ The group indicated that this work was inspired by a report on the conversion of polycyclic quinones and phenols into their corresponding arenes. In their work, the reduction was carried out in two different phases, mainly the liquid and vapour phases. The liquid phase reaction performed much efficiently than the vapour phase reaction. In the liquid phase reaction, graphite oxide was dispersed in acetic acid, ultrasonicated, added with hydroiodic acid and stirred at 40 °C for 40 hr. The resulting graphene provided a measured conductivity of 30400 S/m. The group pointed out that, based on the analysis with combustion ion chromatography, residual I^- or I_2 were absent in the graphene. On top of that, a series of plausible mechanisms were suggested by the group as shown in Scheme 3.2. These included the iodination of alcohols, cleavage of ether, reduction of aromatic iodides and partial reduction of carbonyl group.

While the usage of hydroiodic acid/acetic acid involved heating of the reaction mixture, the group suggested a new reduction method at $-10\text{ }^{\circ}\text{C}$ in a recent study.²⁰ The new method utilized a mixture of hydroiodic acid and trifluoroacetic acid to produce a highly graphitized graphene at a low energy cost. The trifluoroacetic acid was expected to perform much better than acetic acid used in the previous study, since it is a 100000-fold more acidic and had a lower melting point of $-15.4\text{ }^{\circ}\text{C}$.

In another similar study by Cheng and co-workers, graphene oxide was subjected to reduction in hydroiodic acid at $100\text{ }^{\circ}\text{C}$ for 1 hr to result in graphene with a measured conductivity of 29800 S/m .²¹ The group reported that traces of residual I^{-} or I_2 were found by X-ray photoelectron spectroscopy analysis even after thermal annealing at $400\text{ }^{\circ}\text{C}$ for 2 hr. The group also suggested a mechanism based on the epoxide ring opening of iodide atoms and subsequent elimination of the halogen atoms due to the weak binding energy of C-I bond, which were similar to that suggested by Lee and co-workers.

Apart from that, Ma and co-workers achieved a reduction of graphene oxide *via* treatment with hydrobromic acid at $110\text{ }^{\circ}\text{C}$ for 24 hr.²² The measured conductivity of the graphene was 0.023 S/m . The group postulated that the low conductivity could be resulted from the presence of residual bromides.

Scheme 3.2 Suggested mechanisms for the reduction of epoxide, hydroxyl and diketone groups with hydroiodic acid/acetic acid. Reproduced from ref. 19.

3.4.2 Reduction methods with ‘proposed’ mechanisms

Despite the fact chemical reagents should only be applied if it is supported by reasonable mechanisms, it is surprising to realise the availability of more than 20 reduction methods of graphene oxide, which are not supported by reasonable mechanisms based on the knowledge of synthetic chemistry. The reductants range from some simple organic molecules to complex biological compounds. Moreover, most of these reductants remained adhered to the surface of the graphene upon reduction. Although such physisorption provided good dispersion stability to graphene, the respective methods failed to produce clean graphene sheets as the removal of the by-products could be unfeasible. The list of such reduction methods are summarised in Table 3.1.

It should be highlighted again that the proposed mechanisms included in the following text are reproduced from the corresponding articles which had introduced and discussed about the reductants of interest.

3.4.2.1 Hydrazine

While hydrazine has been previously utilized as a reductant on graphite oxide, the effort to obtain individual sheets of graphene from graphite oxide has thus led to its usage on graphene oxide. This was demonstrated by Ruoff and co-workers in their work in 2007.²³ An aqueous suspension of graphene oxide was treated with hydrazine monohydrate at 100 °C for 24 hr to result in graphene with a measured conductivity of 2420 S/m (graphite: 2500 S/m). The obtained graphene was highly hydrophobic and consisted of aggregated layers of graphene sheets, as a result from the loss of oxygen

containing groups. Although the graphene showed a low amount of oxygen content, at a C/O ratio of 10.3, traces of atomic nitrogen were discovered in the sample.

Since graphite oxide consists mostly of hydroxyls and epoxides based on the Lerf-Klinowski model, the group proposed a reduction pathway for the epoxide moiety. The initial mechanism of reduction was postulated to proceed *via* a direct nucleophilic attack of hydrazine on an epoxide group to result in a hydrazine alcohol moiety, which released a water molecule towards the formation of an aminoaziridine and finally underwent a thermal elimination of diimide to form a double bond as shown in Scheme 3.3. The presence of residual nitrogen atoms was explained by the possible formations of hydrazides and hydrazones, owing to the reaction between hydrazine with lactones, anhydrides or quinones found on graphite oxide.

In order to probe the structure of graphite oxide and to verify their initial claims, the group reported a detailed study based on solid state NMR and XPS analyses of ^{13}C - and ^{15}N -labelled graphene obtained *via* hydrazine monohydrate treatment.²⁴ Although the mechanisms of the reduction remained questionable, the study revealed the formation of five-membered pyrazole or pyrazoline rings at the edges of the graphene sheet (Scheme 3.4). The chemical substitution occurred at the edges of the graphene sheets where the presences of diketone groups were most abundant. Further efforts to elucidate the mechanisms of reduction was further pursued by Nagase and co-workers based on density functional theory method.²⁵ The proposed mechanisms are shown in Scheme 3.5.

Apart from that, anhydrous hydrazine treatment on graphene oxide films towards a large-scale formation of graphene was carried out by Kaner and co-workers.²⁶ The graphene showed excellent dispersion stabilities in hydrazine, DMSO and DMF, due to the charge stabilization of N_2H_4^+ counter-ion pairs. The group also postulated that the extent of reduction on carboxylic groups was greater when pure hydrazine was utilized instead of aqueous hydrazine.

Scheme 3.3 Proposed mechanism for the reduction of epoxide group with hydrazine. Reproduced from ref. 23.

Scheme 3.4 Formation of pyrazole ring upon usage of hydrazine as a reductant. Reproduced from ref. 24.

Scheme 3.5 Proposed mechanisms for the reduction of epoxides with hydrazine *via* four different routes. Reproduced from ref. 25.

3.4.2.2 Hydroxylamine

Guo and co-workers introduced a reduction method of graphene oxide *via* hydroxylamine, NH_2OH .²⁷ Based on the method, the hydroxylamine was generated *in situ* from a mixture of hydroxylamine hydrochloride and ammonia. This work was built on prior knowledge whereby hydroxylamine and its chloride and sulphate salts were usually used for reducing metal ions such as Cu^{2+} , Pd^{2+} , Ag^+ and Au^{3+} . Reactions such as the removal of monochloroamine and oxygen could be achieved with hydroxylamine as well. Moreover, the weak reactivity of hydroxylamine with water and its high reducing strength prompted its usage as a reductant for graphene oxide in an aqueous medium.

As such, the reaction mixture which consisted of aqueous dispersion of graphene oxide, hydroxylamine, and ammonia was heated at 90 °C for 1 hr to result in graphene with a measured conductivity of 1122 S/m. The prepared graphene was able to form a stable suspension in aqueous solution possibly due to the repulsive negatively charged graphene surfaces, as a result of the basic reaction condition.

The group proposed a series of mechanisms for the reduction of graphene oxide as shown in Scheme 3.6. For the reduction of epoxide group, the hydroxylamine resulted in a ring opening followed by a proton transfer and elimination of a water molecule to arrive at a *N*-hydroxylaziridine intermediate. Similar intermediate was derived for the removal of hydroxyl groups. Finally, each *N*-hydroxylaziridine intermediate was then converted into a conjugated vinyl while accompanied by the release of unstable species of nitrogen oxide and water molecule.

Scheme 3.6 Proposed mechanisms for the reduction of epoxide and hydroxyl groups with hydroxylamine. Reproduced from ref. 27.

3.4.2.3 Pyrrole

The usage of pyrrole as a reducing agent and stabilizer was demonstrated by Lee and co-workers.²⁸ The group was initially inspired by the work of Sakura and Nagasaki which utilized pyrrole derivatives as reductants for the preparation of gold colloids. As such, an aqueous suspension of graphene oxide was reacted with pyrrole at 95 °C for 12 hr to obtain graphene that displayed good dispersion stability in several organic solvents such as ethanol, isopropanol, DMF, DMSO, NMP, THF and acetone. The stabilities of the dispersions were supported by the adsorbed oxidation product of pyrrole on the graphene surfaces. The group postulated that a molecule of pyrrole donated an electron to graphene oxide while the pyrrole was oxidised to form oligomers (Scheme 3.7).

Scheme 3.7 Proposed steps for the reduction of graphene oxide with pyrrole. Reproduced from ref. 28.

3.4.2.4 *p*-Phenylene diamine

Ma and co-workers introduced the reduction protocol using *p*-phenyl diamine (PPD), which also acted as a reducing agent and stabilizer.²⁹ An aqueous dispersion of graphene oxide was reduced with a solution of PPD in DMF and refluxed at 90 °C for 24 hr. The resulting graphene has a measured conductivity of 15000 S/m in the plane of the graphene film. Moreover, the graphene was readily dispersed in ethanol, glycol and NMP, while only DMF showed short-term dispersion stability. The good dispersion stability was postulated to be a result of the positively charged $-N^+$ residuals of the oxidised PPDs that were adsorbed on the graphene surfaces. This prevented π - π stacking interactions between the graphene sheets. The group did not provide any possible mechanisms for the reduction process.

3.4.2.5 Polyelectrolyte

Apart from that, Lin and co-workers demonstrated the usage of an electrolyte, poly(diallyldimethylammonium chloride) (PDPA), for the reduction of graphene oxide.³⁰ PDPA was initially utilized to aid the dispersion of graphene in aqueous solution. PDPA was previously observed to adsorb on the surface of carbon nanotubes through π - π interaction and electrostatic interaction to result in electrostatic repulsion between the nanotubes in order to prevent aggregations in aqueous suspensions. However, an aqueous solution of graphene oxide which was added with PDPA and heated at 90 °C for 5 hr highlighted a colour change from brown to black, which prompted the usage of PDPA as both a reductant and stabilizer. The resulting graphene was found to form homogeneous colloidal suspensions in water, ethanol, glycol and DMF. The group postulated that the positively charged PDPA, which contained inherent N^+ groups, would attack the oxygen atom of epoxide in graphene oxide to induce a ring-opening reaction (Scheme 3.8). This resulted in a single electron on the α -carbon followed by a subsequent elimination of the nitroso group to provide an olefin.

Scheme 3.8 Proposed mechanism for the reduction of epoxide group with polyelectrolyte. Reproduced from ref. 30.

3.4.2.6 Metal-acid

The utilization of metal ions as a reductant for graphite oxide has *resurfaced* as well, but with a current emphasis on graphene oxide. Fan and co-workers recently reported the reduction of graphene oxide with aluminium powder in an acidic solution.³¹ An aqueous suspension of graphene oxide was reacted with aluminium powder and hydrochloric acid for 30 min to afford graphene with a measured conductivity of 2100 S/m (graphite: 3200 S/m). Aluminium powder was applied as a reductant since the standard reduction potential of aluminium is considerably more negative than those of sodium borohydride and hydrazine, which stood at -1.68 , -1.24 and -1.16 V respectively. The mechanism of the reduction process remained unclear to the group. Although the treatment of aluminium powder with acid would result in the production of hydrogen gas, the group denounced the involvement of hydrogen gas as a reductant. This was based on their control experiment which observed no traces of reduction on a dispersion of graphene oxide bubbled with pure hydrogen gas.

In a follow-up work by the group, iron powder was utilized in the presence of hydrochloric acid for 6 hr to reduce a graphene oxide dispersion.³² The graphene sample showed a measured conductivity of 2300 S/m. In this work, the group postulated the reduction of graphene oxide to occur based on a fast electron transport from Fe/Fe^{2+} to the negatively charged graphene oxide sheets, based on Equation 1. In a control experiment, the reduction of graphene oxide was not observed in the absence of hydrochloric acid.

Subsequently in the work of Wang and co-workers, zinc powder in the presence of ammonia solution was observed to reduce graphene oxide after a 10 min treatment at room temperature.³³ The reduction mechanism was postulated to proceed *via* a “Zn-graphene oxide primary battery” system whereby the zinc acted as an anode, the graphene oxide as a cathode and the dissolved ammonia as an electrolyte. The Zn particle was initially oxidised (Equation 2) to provide free electrons that led to the conversion of NH_4^+ to NH_3 and $\text{H}^\bullet$ (Equation 3). The formation of zinc ammonia complex (Equation 4) drove Equation 2 forward to provide more Zn^{2+} species. The reduction of graphene oxide was finally achieved when $\text{H}^\bullet$ reacted with graphene oxide (Equation 5), resulting in the removal of hydroxyl and epoxide groups.

Apart from that, Ouyang and co-workers showed that zinc powder in hydrochloric acid solution mixture was able to reduce graphene oxide within 1 min under ultrasonication at room temperature.³⁴ At a weight ratio of 2 for Zn/graphene oxide, the obtained graphene exhibited a measured conductivity of 15000 S/m. The group postulated that the spontaneous reduction process was driven by a wide gap between the standard reduction potentials of Zn^{2+}/Zn and graphene oxide. The standard reduction potential of Zn^{2+}/Zn was -0.76 V while the standard reduction potential of graphene oxide at pH 4 was -0.40 V, thus promoted the effective reduction process.

Scheme 3.9 Proposed mechanisms for the reduction of epoxide, carbonyl and carboxyl groups with zinc and sulphuric acid. Reproduced from ref. 35.

In a more recent report by Panigrahi and co-workers, the group achieved the reduction of graphene oxide with solid zinc filings and sulphuric acid after a reaction time of 2 hr at room temperature.³⁵ The graphene showed a measured conductivity of 3416 S/m (graphite oxide: <3 S/m). The group highlighted that the reduction of graphene oxide failed to reach completion within 2 hr if sulphuric acid was used in replaced of hydrochloric acid. The group also proposed a series of mechanisms that facilitated the reduction processes, as shown in Scheme 3.9. In short, the reduction of carbonyl and epoxide groups followed through a series of steps towards the formation of hydroxyl groups, which ultimately underwent dehydrations to afford olefins (Routes 1 and 2). The dehydration process was believed to be catalysed by the presence of $ZnSO_4$.

As for the carboxyl group, the C=C bond conjugated to the C=O was protonated at the α -position, with subsequent decarboxylation and regeneration of the C=C bond (Route 3).

3.4.2.7 Sulphur-containing compounds

Sulphur-containing compounds were found to confer reduction capabilities on graphene oxide as well. The usage of sulphur-containing compounds, mainly NaHSO₃, Na₂SO₃, Na₂S₂O₃, Na₂S•9H₂O, SOCl₂ and SO₂, were demonstrated by Bangal and co-workers.³⁶ The reduction process was carried out by adding portions of the sulphur-containing compounds into a mixture of graphene oxide in DMAc/H₂O (6:1) medium and left to stir at 95 °C for 3 hr. Among the sulphur-containing compounds, SOCl₂ provided the best extent of reduction, with a C/O ratio of 8.48. However, due to the exothermic nature of SOCl₂ upon contact with water, the graphene obtained from NaHSO₃ as reductant, which has a C/O ratio of 7.89, was taken as a benchmark. In comparison to graphene reduced with hydrazine, the latter provided a C/O ratio of 11.4. While the disparities of C/O ratios were rather wide, the measured conductivity varied by a small extent at which the NaHSO₃-graphene gave a measured conductivity of 5100 S/m and the hydrazine-graphene was determined at 6500 S/m. Similar to the reduction with hydrazine, traces of reactants, mainly sulphur atoms were detected from the elemental analyses of the graphene materials.

On top of that, the group proposed a possible mechanism for NaHSO₃ as a reducing agent based on a two-step S_N2 nucleophilic reaction followed by a thermal elimination (Scheme 3.10). In short, the HSO₃⁻ was oxidised to SO₄²⁻. This conjecture was supported by a positive qualitative test whereby Ba²⁺ cation formed a precipitate of

BaSO₄ in an aqueous solution of the graphene. As for SOCl₂, the HSO₃⁻ anion was readily produced upon contact with water based on Equations 6, 7 and 8. Moreover, the group also suggested that the Na₂S, Na₂SO₃ and Na₂S₂O₃ reductants proceeded through the HSO₃⁻ anion route somewhere along their reaction pathway.

Scheme 3.10 Proposed mechanisms for the reduction of epoxide groups with HSO₃⁻ ion. Reproduced from ref. 36.

3.4.2.8 Sodium hydrosulphite

Sodium hydrosulphite, Na₂S₂O₄ was reported by Fu and co-workers as an efficient reductant for graphene oxide.³⁷ The group subjected an aqueous suspension of graphene oxide to a mixture of Na₂S₂O₄ and sodium hydroxide at 60 °C for 15 min, as inspired by the usage of sodium hydrosulphite as an ancient reducing agent in the textile industry. Although the carboxylic groups on graphene oxide were barely reduced, the resulting graphene exhibited a measured conductivity of 1377 S/m (graphite oxide: 3 × 10⁻⁴ S/m). The group postulated that due to the low electrode potential of Na₂S₂O₄, (E₀

$\text{SO}_3^{2-}/\text{S}_2\text{O}_4^{2-} = -1.12 \text{ V}$) in alkaline solution, graphene oxide was more likely to be reduced. Based on their postulation, the $\text{S}_2\text{O}_4^{2-}$ nucleophilic attacked the epoxide or hydroxyl groups on graphene oxide, followed by consequent release of a H_2O molecule to result in the formation of an “intermediate”. A subsequent thermal elimination of the “intermediate” led to the formation of graphene, while the $\text{S}_2\text{O}_4^{2-}$ was oxidised to SO_3^{2-} .

3.4.2.9 Amino acid

The very first amino acid which was introduced as a reducing agent for graphene oxide was L-cysteine. Guo and co-workers selected L-cysteine as a reductant since the presence of the thiol group of L-cysteine was susceptible to oxidation towards the formation of a disulphide derivative cysteine.³⁸ As such, its ability to undergo a redox activity rendered it as a potential antioxidant. The group subjected an aqueous suspension of graphene oxide to L-cysteine at room temperature for 72 hr. The obtained graphene provided a measured conductivity of 0.124 S/m (graphite oxide: 7.97×10^{-7} S/m). This low conductivity was deduced to be a result of an ineffective and incomplete reduction of carboxyl groups on graphene oxide, which on the hindsight granted the ability to form a homogenous aqueous dispersion of graphene at a pH of 10. In addition, the reduction mechanism was proposed to go through a two-step $\text{S}_\text{N}2$ nucleophilic reaction on epoxide and hydroxyl groups, followed by thermal eliminations as shown in Scheme 3.11. The initial nucleophilic attack was driven by the release of a proton from the thiol group to provide a nucleophilic thiol. The free proton formed a high affinity with oxygen atoms on the hydroxyl groups and departed as water molecules. Finally, the L-cysteine was oxidised into L-cysteine while graphene oxide was reduced to graphene.

Scheme 3.11 Proposed mechanisms for the reduction of epoxide and di-hydroxyl groups with L-cysteine. Reproduced from ref. 38.

Thereafter, glycine was utilized as a reducing agent by Lee and co-workers.³⁹ Based on the method developed by the group, glycine was added into an aqueous suspension of graphene oxide and the mixture was allowed to stir for 15 min, ultrasonicated for 30 min, reacted for 12 hr and finally refluxed at 95 °C for 24 hr. Based on their study, a higher amount of glycine used in the reduction would help to prevent aggregations of the graphene sheets. The resulting graphene achieved a C/O ratio of 11.24. Moreover, the group postulated a two-step reduction process whereby the graphene oxide was initially functionalized prior to being reduced. In the first part, the amine functionality of glycine acted as a nucleophile and readily attacked the epoxide groups and β -carbon atom of the hydroxyl groups on graphene oxide. As for the reduction step, the first postulation by the group was based on the fact that the attached glycine could act as a catalyst at a high temperature to facilitate the reduction

process. The second postulation depicted the formation of intermediate structures upon heat treatment which would ultimately be released as an unwanted species while the π -conjugated network of graphene oxide was replenished. The group also stated that other factors that could potentially affect the reduction process could not be overruled.

3.4.2.10 L-Glutathione

Jeong and co-workers demonstrated the usage of L-glutathione reduced (GSH) as a reducing agent for graphene oxide.⁴⁰ GSH was selected by the group as it is a natural antioxidant in cells and is capable of reducing many reactive oxygen species. Apart from that, the group envisaged to use GSH as a capping agent to stabilize and prevent the agglomeration of graphene sheets after the reduction process. As such, an aqueous suspension of graphene oxide was treated with GSH at 50 °C for 6 hr to provide graphene with high dispersion stabilities in aqueous and polar aprotic solvents such as THF, DMF, and DMSO. The group postulated that the stability of the graphene dispersion could originate from the formation of hydrogen bonding between the oxidised products of GSH and the residual oxygen functionalities on the graphene surface. This stabilization effect could have been further enhanced by the presence of residual carboxylic groups as well. The combination of these effects thus prevented the occurrence of π - π stacking and agglomeration of the graphene sheets. Based on the proposed mechanism, GSH was able to release a proton and undergo intermolecular reactions with another GSH to form a glutathione disulphide (GSSG) (Scheme 3.12). The released protons would then bind to the oxygen groups present on the surfaces of graphene oxide, and subsequently released as water molecules.

Scheme 3.12 Proposed mechanisms for formation of glutathione disulphide with simultaneous release of electrons for the reduction of graphene oxide. Reproduced from ref. 40.

3.4.2.11 Alcohol

The reduction of graphene oxide with alcohol was highlighted by Bielawski and co-workers.⁴¹ The group reduced graphene oxide in excess alcoholic solutions of MeOH, EtOH, iPrOH and BnOH at 100 °C for 5 days. Graphene obtained using BnOH as a reducing agent exhibited the best performance, with a C/O ratio of 30 and a conductivity of 4600 S/m. The group postulated that the reduction process could have proceeded *via* thermally mediated processes. In addition, the presence of benzaldehyde and benzoic acid as detected by nuclear magnetic resonance spectroscopy indicated the involvement of BnOH in the reduction process.

3.4.2.12 Hydroquinone

Yao and co-workers introduced the reduction of graphene oxide with hydroquinone.⁴² The reduction process was carried out by refluxing an ethanol/water suspension of graphene oxide in the presence of hydroquinone for 20 hr. The group postulated that the hydroquinone acted as a reducing agent by losing either one H^+ to form a monophenolate ion or two H^+ to form a diphenolate ion (quinone) as shown in Scheme 3.13.

Scheme 3.13 Proposed mechanisms for the transformation of hydroquinone to quinone with simultaneous release of electrons for the reduction of graphene oxide. Reproduced from ref. 42.

3.4.2.13 L-Ascorbic acid

Zhang and co-workers demonstrated the reduction of graphene oxide with L-ascorbic acid (Vitamin C) in the presence of L-tryptophan.⁴³ The usage of L-ascorbic acid was inspired by its nature as an antioxidant in the cells. In this work, the L-ascorbic acid acted as a reductant while L-tryptophan functioned to stabilize the graphene *via* physisorption onto the graphene sheet based on π - π interactions. The stabilization effect of L-tryptophan acted synergistically with the remaining negatively charged carboxylic acid residuals to provide electrostatic repulsions between individual graphene sheets. Based on the method developed, an aqueous suspension of graphene oxide was

subjected to treatment with L-(+)-ascorbic acid, L-tryptophan and NaOH at 80 °C for 24 hr to provide graphene with a measured conductivity of 14.1 S/m (graphite oxide: 5.72×10^{-6} S/m).

The mechanism of reduction was postulated to proceed via a two-step S_N2 nucleophilic reaction followed by another one-step thermal elimination as shown in Scheme 3.14. In the first step, the electron withdrawing side chain of the five-membered ring of L-ascorbic acid increased the acidities of the β - and γ -hydroxyl groups, resulting in the dissociation of the two protons to form an oxygen anion of L-ascorbic acid (H₂AO⁻). In the second step, the anion underwent a back-side S_N2 nucleophilic attack, twice on the epoxide (Route 1) and diol (Route 2) groups of graphene oxide, to arrive at an intermediate. Finally in the third step, the intermediate was subsequently eliminated thermally while the ascorbic acid was oxidised to dehydroascorbic acid. The graphene oxide was then successfully converted to graphene.

At about the same time, Guo and co-workers reported the reduction of graphene oxide using L-ascorbic acid as well.⁴⁴ Based on the method developed by the group, an aqueous dispersion of graphene oxide was subjected to reaction with L-ascorbic acid at room temperature for up to 48 hr to provide graphene with a measured conductivity of 800 S/m. The reduction achieved completion in a shorter time frame upon increasing the loading of L-ascorbic acid. The resulting graphene displayed good dispersion stability in an aqueous dispersion. The group attributed the stability of the aqueous dispersion to the presence of oxalic and guluronic acids, where were obtained from the breakdown of dehydroascorbic acid. These acids could subsequently form hydrogen bonds with the residual oxygen functionalities found on the graphene surfaces to prevent agglomeration and π - π stacking of the graphene sheets.

Scheme 3.14 Proposed mechanisms for the reduction of epoxide and di-hydroxyl groups with L-ascorbic acid. Reproduced from ref. 43.

In a comparative study by Tascón and co-workers, graphene oxide reduced by L-ascorbic acid was shown to disperse well in organic solvents such as DMF and NMP.⁴⁵ On top of that, the conductivity measurements of graphene obtained from L-ascorbic acid (2 mM) reductant was 7700 S/m as compared to graphene obtained from hydrazine (21 mM) treatment at 9960 S/m.

3.4.2.14 Saccharides

Saccharides, mainly sucrose, fructose and glucose, have been utilized as reducing agents for the reduction of graphene oxide by Dong and co-workers.⁴⁶ The reduction step was performed by stirring an aqueous dispersion of graphene oxide with saccharides and ammonia solution at 95 °C for 1 hr. The resulting graphene displayed excellent dispersion stabilities in aqueous solutions. The synthetic mechanism of the reduction process was examined by the group to show that glucose, an aldohexose was first oxidised to aldonic acid by graphene oxide in the presence of ammonia solution. The aldonic acid was then further converted to a lactone. The mixture of aldonic acid and lactone, which were rich in hydroxyl and carboxyl groups, could form hydrogen bonds with the residual oxygen functionalities on the graphene surfaces to prevent aggregations by π - π stacking. On the other hand, fructose also displayed reducing capability with graphene oxide since it was capable of undergoing a keto-enol tautomerism under basic conditions. As for sucrose, since it could readily break down to fructose and glucose upon hydrolysis, it highlighted a similar reduction capability on graphene oxide as well.

In another work, the usage of a dextran (a polysaccharide) as a reductant for graphene oxide was later demonstrated by Min and co-workers.⁴⁷ The graphene oxide was treated with dextran and ammonia solution at 95 °C for 3 days. The mechanism of the reduction was postulated to go through similar mechanisms as that of glucose.

3.4.2.15 Gallic acid

The reduction of graphene oxide with gallic acid was performed by Xiao and co-workers.⁴⁸ Gallic acid was utilized as a reductant as it has been widely adopted as an effective antioxidant in the area of food packaging, food processing, and toiletries for the purpose of preventing rotting induced by lipid peroxidation and spoilage. The three hydroxyl groups on gallic acid were envisaged to induce the reduction process of graphene oxide. In their work, an aqueous dispersion of graphene oxide was subjected to treatment with gallic acid and ammonia solution under two different conditions. The first set of condition was carried out by stirring the mixture at room temperature for 24 hr, while the second set of mixture was stirred at 95 °C for 6 hr. The graphene materials showed different values of measured conductivities, where the former exhibited 0.96 S/m while the latter showed 36 S/m. Both graphene materials were readily dispersed in deionized water, NMP, DMSO, DMF and methanol. The group did not provide any plausible mechanism for the reduction process.

3.4.2.16 Plant extracts

Apart from the usage of chemical reductants, the utilization of biological materials as reducing agents for graphene oxide has gained much attention as well. These reducing agents were claimed to be more environmentally friendly as compared to chemical reduction methods, as the biological reductants are obtained from nature.

The reduction of graphene oxide with green tea was first demonstrated by Yin and co-workers.⁴⁹ The multiple pyrogallol and catechol groups on the polyphenolic compounds found in green tea were foreseen to act as excellent antioxidant agents and

react actively with oxygen species. The phenol groups on the compounds were expected to convert to the corresponding quinone forms upon reaction. Moreover, the reductive capabilities of the polyphenols were previously observed in the synthesis and stabilization of Au, Ag, Pd and Fe microparticles. The reduction process was performed by ultrasonication of graphene oxide in a tea solution for 30 min followed by a subsequent reflux treatment at 90 °C in nitrogen atmosphere for 2 hr. The resulting graphene provided a conductivity of 53 S/m. Moreover, the graphene showed good dispersion stability in polar solvents such as ethanol, methanol, acetone, DMF, NMP, DMAc, and DMSO upon ultrasonication treatments.

The application of phytochemicals towards the reduction of graphene oxide was reported by Karak and co-worker.⁵⁰ Phytochemicals obtained from *Colocasia esculenta* leaf, *Mesua ferrea* Linn. leaf, and *Citrus sinensis* leaf were selected for this study. The phytochemicals such as pectins, flavonoids, ascorbic acid, apigenin, luteolin and various flavones could be potentially oxidised to their respective quinone moieties during the process of graphene oxide reduction. The graphene obtained from *C. esculenta*, *M. ferrea* Linn., and *C. sinensis* extracts provided measured conductivities of 4006, 3185, and 3033 S/m, respectively. In terms of the oxygen contents, the graphene gave C/O ratios of 7.11, 6.09, and 5.97 in the corresponding order. In comparison, the precursor graphene oxide highlighted a C/O ratio of 2.68 and a measured conductivity of 10^4 lower. A series of mechanisms was suggested by the group, which was highly similar to that proposed for L-ascorbic acid as shown in the previous paragraphs.

3.4.2.17 Microorganisms

A group of heterotrophic and facultative anaerobes, *Shewanella*, usually found in the environment, has the ability to utilize inorganic or organic compounds in their respiratory pathway as terminal electron acceptors. These organisms are able to utilize solids as terminal electron acceptors as well. In the work of Tour and co-workers, they highlighted the usage of this bacterium towards the reduction of graphene oxide.⁵¹ The group investigated the reducing capabilities of five different strains of *Shewanella* from different places to highlight the extents of reduction among the five strains. One of the strains highlighted an increase of conductance by 10^3 - 10^4 S/m over the starting graphene oxide materials after 72 hr of incubation period. Although the group obtained some initial hint on the mechanism of extracellular electron transfer occurring on *Shewanella*, further investigations are required as suggested.

Apart from that, the usage of *Escherichia coli* (*E. coli*) bacteria living in a mixed-acid fermentation under anaerobic conditions for the reduction of graphene oxide was highlighted by Akhavan and co-workers.⁵² Graphene oxide sample and *E. coli* were incubated for 48 hr at 37 °C in the dark to result in a reduction process to graphene. The sheet resistance of the produced graphene was measured at 3.4×10^9 Ω /sq as compared to graphene oxide at 2.9×10^{11} Ω /sq. The metabolically-generated electrons and hydrogen from the glycolysis process were postulated to be involved in the mechanism of reduction. Moreover, graphene oxide was found to provide biocompatible sites for the adsorption and proliferation of *E. coli* while the resulting graphene inhibited further proliferation of *E. coli*.

Similar reduction with microorganisms based on baker's yeast containing nicotinamide adenine dinucleotide phosphate (NADPH) was described by Lee and co-workers.⁵³ The group was motivated by previous reports on the ability of baker's yeast in reducing prochiral ketones and α,β -epoxide ketones as well as the reducing capability of NADPH towards organic ketones. The reduction was carried out by stirring an aqueous dispersion of graphene oxide with baker's yeast at 35-40 °C for 72 hr. The resulting graphene highlighted a C/O ratio of 5.9 and a conductivity of 43 S/m (graphite oxide: 0.002 S/m). Based on this method, the graphene was simultaneously bio-functionalized since NADP/NADPH remained attached to the surfaces of the graphene.

Moreover, Lee and co-workers highlighted the application of wild carrot root for the reduction of graphene oxide.⁵⁴ This was pursued based on a previous report on the reduction capability of wild carrot root on aliphatic and aromatic ketones, cyclic ketones, β -keto esters, and azidoketones. The reduction process was attributed to the presence of endophytic microorganisms. By treating aqueous dispersion of graphene oxide with slices of wild carrot roots for 48 hr and another 24 hr under reflux condition provided graphene with a C/O ratio of 11.9.

3.4.2.18 Bovine serum albumin

The conjugation of bovine serum albumin (BSA) with graphene oxide was demonstrated by Deng and co-workers.⁵⁵ A change in the conjugation conditions (temperature and pH) could provide two different types of complexes, mainly BSA-graphene oxide (25 °C at pH 8) and BSA-graphene (55-90 °C, pH 12) conjugates. The group claimed that BSA was expected to function as a reductant since it contains the amino acid group, tyrosine. It is interesting to note that while the group claimed that tyrosine was the source of reductant and that reduction occurred only at a pH of 12 upon the addition of 1 M NaOH, a previous report⁵⁶ has shown that the addition of NaOH into a graphene oxide dispersion would result in a deoxygenation process. The presence of BSA was also claimed to act as a stabilizer for the BSA-graphene oxide/graphene conjugates. More importantly, the BSA-graphene oxide/graphene hybrids provided well-controlled and well-dispersed assembly of Au, Pt, Pd, Ag and latex nanoparticles.

3.5 Graphene for electrochemical applications

The top-down approach of graphene has provided the possibility for large-scale production. However, the produced graphene is mainly defective in nature – the sp^2 carbon networks are filled with holes and defects. Such graphene materials are highly unsuitable for applications such as high-power electronics since the defects would interrupt the ballistic transport and lead to scattering of electrons on graphene. Nevertheless, these graphene materials have found potential usage in less demanding applications such as coatings, paint/ink, composites, energy storage, transparent conductive layers and bioapplications. More importantly, the incorporation of graphene materials for electrochemical sensing has gained much attention in recent years. This was, in fact, brought about by the excellent conductivity, ease for functionalization and large specific surface area of graphene. Brief electrochemical characteristics of graphene and its subsequent applications in electrochemical and biological sensing would be discussed below.

3.5.1 Electrochemical properties of graphene

Electrochemical processes are fundamentally governed by electron transfer. In the case of graphene, an understanding on the basis of *heterogeneous electron transfer* (HET) phenomenon, where electrons are exchanged between a chemical species and a solid-state electrode, is important to probe the electrochemical properties of graphene.

The structural feature of graphene is essentially similar to that of graphite where both are made up entirely of *basal*- and *edge*-plane sites. As such, the electrochemical performances of graphite could be easily related to that of graphene. In order to

establish the specific sites of heterogeneous electron transfer on graphene, highly defect-free samples such as highly ordered pyrolytic graphite (HOPG) are usually the preferred experimental substrates. Compton and co-workers established that the edge-plane sites exhibited a HET rate constant in the order of ~ 0.01 cm/s while the basal plane sites gave $\sim 10^{-9}$ cm/s.⁵⁷ Further investigations carried out by the same group highlighted that the basal-plane sites of graphite was electrochemically inert and that the electrochemical response from the basal-plane was solely contributed by edge-plane defects.⁵⁸ This study was also supported by the work of Loh and co-workers, where they highlighted that the basal-plane of an epitaxial-graphene provided low HET rate.⁵⁹ Upon electrochemical anodization to create defects on the epitaxial-graphene, the HET rate improved significantly. A direct comparison between folded and opened edge sites of graphene supported the argument that fast HET rate occurred at the edge plane sites as well.⁶⁰ Moreover, the electrochemical activities of graphene sheets are closer to the performance of edge-plane pyrolytic graphite compared to basal-plane pyrolytic graphite electrodes.⁶¹ A recent report based on Scanning Electrochemical Cell Microscopy (SECM) indicated that the basal-plane of freshly cleaved HOPG was electroactive, albeit time dependent whereby, the electron transfer rate decreased to negligible value upon prolonged exposure to air within an hour.^{62,63}

In light of these studies, investigations on the electrochemical properties of graphene often involve the comparison of the graphene materials of interest against two standard electrodes, which are the edge and basal plane pyrolytic graphite (BPPG and EPPG) electrodes. On another note, the electrochemistry of graphene is not determined by the number of graphene layers. Based on the electrochemical oxidation of dopamine and ascorbic acid, mono-, few- and multi-layer graphene materials showed

negligible differences in the electrochemical performances.⁶⁴ However, the capacitance of mono-layer graphene was observed to be lower than that of few- and multi-layer graphene.⁶⁵

3.5.2 Graphene contains metallic impurities

The usage of graphene as a material in electrochemical systems is growing exponentially due to the enhanced performances demonstrated over that of carbon nanotubes. It is however important to mention that the presence of impurities, in particular those of metallic nature, alter and in some cases dominate the electrochemistry of the parent carbon material.⁶⁶ Numerous reports have demonstrated that the residual metallic impurities from chemical vapour deposition synthesis were, in fact, responsible for the electrocatalytic properties observed with carbon nanotubes.⁶⁷⁻⁷⁰ As such, reports regarding the excellent electrochemical properties of graphene electrodes towards the redox reactions of numerous compounds have thus surfaced. These encompassed compounds such as sulphides,⁷¹ hydrazine,⁷² dopamine, ascorbic acid,⁶⁴ nitroaromatic explosives⁷³ and DNA bases⁷⁴. Despite the growth of graphene in the area of electrochemical sensing applications, the *absolute* purity of graphene utilized in this direction of research remains questionable. Afterall, the bottom-up chemical vapour deposition method is analogous to the production method of carbon nanotubes, where metal catalyst surfaces (Fe, Ni) are employed to grow graphene. Similarly, the top-down approach cannot guarantee the production of an impurity-free graphene since the graphite starting material (natural or synthetic) contains a variable amount of

Table 3.2: Metallic impurities content (ppm) in graphite, graphite oxide (GO), and thermally reduced graphene (TRGO) determined by ICP-MS analysis. Reproduced from ref. 76.

Sample	Co	Cu	Fe	Mo	Ni
Graphite	0.37	7.2	240	4.7	190
GO	0.31	7.2	560	3.0	220
TRGO	7.8	59	160	5.2	200

metallic impurities.⁷⁵ Moreover, the usage of oxidising and reducing agents could further contaminate the graphene samples.

Based on the top-down approach *via* thermal heat shock treatment to obtain graphene, Pumera and co-workers have shown that the resulting graphene materials contained residual metallic impurities at levels of hundreds of ppm for Fe and Ni and tens of ppm for Co, Cu and Mo as shown in Table 3.2. The presence of these metallic impurities are directly related to the electrocatalytic reduction of cumene hydroperoxide, and the oxidation of sulphides and L-glutathione.⁷⁶ Moreover, the presences of carbonaceous impurities in graphene samples were shown to affect the electrochemical behaviours as well.⁷⁷

The presence of such impurities not only alters the electrochemical properties but also the electronic and physical properties of the graphene materials, thus demonstrating the global importance of this area of research.^{78,79} In such a scenario, it is therefore advisable that prior to any electrochemical applications of graphene, detailed characterizations of the graphene samples should be performed in order to ensure the accuracy of the electrochemical properties.

3.5.3 Graphene for electrochemical sensing

Graphene is an excellent candidate for electrochemical sensing applications given its large specific surface area, excellent conductivity and ease for surface functionalizations. Moreover, as graphene consists of a higher amount of edge-plane sites as compared to carbon nanotubes, graphene naturally provided superior performance for electrochemical sensing. Graphene has been demonstrated for the electrochemical detection of simple molecules such as ascorbic acid, dopamine, uric acid, hydrogen peroxide, and DNA bases.⁸⁰ The electrochemical detections are usually performed with cyclic voltammetry or amperometric measurements.

Papakonstantinou and co-workers were one of the earliest groups that demonstrated the usage of graphene for electrochemical sensing.⁸¹ A multi-layer graphene synthesized *via* microwave plasma enhanced chemical vapour deposition method was able to simultaneously detect dopamine, ascorbic acid, and uric acid with well resolved peaks. Although the performance was superior to that of glassy carbon electrode, it was still comparable to edge plane pyrolytic graphite electrode. In another work, Loh and co-workers demonstrated the usage of anodized epitaxial-graphene as an excellent electrochemical platform that is able to resolve the anodic peaks of four different nucleic acid bases in single and double stranded DNAs.⁵⁹ These reports also highlighted the importance of edge-plane sites for the enhanced electron transfer kinetics.

Most electrochemical sensing analyses were typically performed with graphene obtained *via* the top-down approach (graphite oxide, graphene oxide, chemically/electrochemically/ thermally reduced graphene oxide). The thermally reduced graphene

oxide was found to exhibit the most favourable electrochemical performances among these materials.⁸² It highlighted the highest structural defects and the lowest density of oxygen containing groups. A higher amount of oxygen containing groups also tends to reduce the HET rates of the graphene materials towards the detection of ferro/ferricyanide electrochemical probe.

The performances of the chemically modified graphene varied significantly for the detection of DNA bases. In comparison to bare glassy carbon electrode, Pumera and co-workers highlighted that graphite oxide, graphene oxide, thermally and electrochemically reduced graphene oxide exhibited enhanced sensitivities towards the detection of adenine and cytosine. However, only thermally and electrochemically reduced graphene oxide were efficient for the detection of guanine and thymine.⁸³ In a similar work by Dong and co-workers, they compared the performances of chemically reduced graphene oxide with graphite towards the detection of DNA bases as well.⁸⁴ The chemically reduced graphene oxide was able to detect the presence of four DNA bases with a good resolution while graphite and glassy carbon electrode were unable to. The work also highlighted the ability of graphene to detect single nucleotide polymorphism for short oligomers, which provided opportunities towards a label-free electrochemical detection system.

Apart from that, chemically reduced graphene was also reported to exhibit a higher level of sensitivity towards the detection of cadmium and lead as demonstrated by Wang and co-workers.^{85,86} Furthermore, graphite oxide showed a good detection sensitivity towards paracetamol and provided well resolved peaks in a mixture containing dopamine and ascorbic acid.⁸⁷

3.5.4 Graphene for electrochemical biosensing

Electrochemical biosensors are capable of detecting biological interactions on the electrode surfaces with high sensitivity and selectivity. An electrochemical biosensor consists of two essential elements – a receptor layer (biomolecules such as enzyme, DNA or protein) and an electrode transducer (enhanced with carbon nanomaterials or metal nanoparticles). The receptor layer is typically sensitive and selective to only a specific interaction. The electrode transducer, on the other hand, functions to convert chemical information into an electrical signal. Graphene has been applied in recent years as an effective electrode material due to the large specific surface area and significant density of defects of the basal-plane. These properties facilitated fast heterogeneous electron transfer kinetics and increased the loading of receptors for an improved detection sensitivity.⁸⁸

Enzyme biosensor is one of the most extensively studied models of biosensors. Enzymes such as glucose oxidase, horseradish peroxidase, alcohol dehydrogenase, catalase and urease, have been used as specific bioreceptors in well demonstrated electrochemical biosensing devices.⁸⁹ Glucose sensor, for example, is one of the most successful and longest standing type of enzyme biosensors given its wide commercial availability for diabetic patients. A typical glucose sensor consists of a glucose oxidase enzyme layer immobilized over an electrode surface which oxidise glucose to gluconic acid and subsequently lead to the production of hydrogen peroxide after shuffling electrons *via* oxygen molecules. The presence of hydrogen peroxide is then detected electrochemically. Despite that, a direct detection method based on the direct electron transfer from glucose oxidase to the electrode surface has garnered much interest as well. Glucose biosensors that function based on such direct electron transfer of glucose

oxidase have been demonstrated with chemically⁹⁰ and electrochemically reduced graphene oxide⁹¹. Chemically reduced graphene oxide provided a detection range, detection limit and sensitivity of 0.1-10 mM, 10 μ M and 110 μ A mM⁻¹ cm⁻², respectively.⁹⁰ On the other hand, electrochemically reduced graphene oxide showed the corresponding performances of 2-18 mM, 20 μ M and 7 μ A mM⁻¹ cm⁻², respectively.⁹¹ Apart from that, the application of N-doped graphene has provided significantly enhanced oxidation currents for the detection of glucose as compared to graphene.⁹² Other hybrid structures based on metal nanoparticles or composites with graphene materials have been developed to a large extent as well.⁸⁹

Immunosensor is another class of biosensors that utilises antibodies as biorecognition receptors to specifically bind and detect certain antigens. The high affinity and specific binding between antigens to particular regions of antibodies provided high selectivity and sensitivity of detection. Hou and co-workers have reported the immobilization of anti-sulphate reducing bacteria (anti-SRB) on chemically reduced graphene oxide/doped chitosan film towards the detection of sulphate reducing bacteria. The impedimetric analysis provided a linear relationship between charge transfer resistance and SRB concentration in the range of 1.8×10^1 to 1.8×10^7 cfu/mL.⁹³ Subsequent work based on the interaction between Rabbit IgG-AntiRabbit IgG on chemically reduced graphene platform provided a detection limit of 0.67 nM.⁹⁴ A linear relationship between charge transfer resistance and increasing concentration of IgG was observed over a concentration of 3.3 nM to 683 nM. Pumera and co-workers further evaluated the application of various graphene materials towards the label-free impedimetric detection of IgG.⁹⁵

The application of graphene in biosensors has been extrapolated also to the detections of DNA and single nucleotide polymorphism in recent years. Detection of single nucleotide polymorphism has been largely carried out using electrochemical impedance spectroscopy (EIS) since it allows for a sensitive detection of electrode surface changes without the need of electroactive labels.⁹⁶ Linear and hairpin-shaped DNA probes, immobilized *via* covalent bonding^{97,98} and physical adsorption^{99,100}, have been adopted as receptor layers on graphene surfaces. A study on the influences of mono-, few- and multi-layer graphene towards single nucleotide polymorphism by Pumera and co-workers has concluded that few-layer graphene provided the best sensitivity.¹⁰⁰ The detection limits exhibited by mono-, few- and multi-layer graphene were 50 nM, 6.6 pM, and 66 μ A, respectively. Similar study based on graphene oxide, electrochemically and thermally reduced graphene oxide highlighted a better detection sensitivity with graphene oxide.⁹⁹ On another study by Loh and co-workers, covalently bonded DNA probes on anodized epitaxial graphene resulted in a higher sensitivity and a larger detection range than π - π stacked DNA probes.⁹⁷

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Chapter 4

Effects of oxidant types on the oxygen contents of graphite oxide

4.1 *Introduction*

4.2 *Results*

4.2.1 *Structural characterisation of the graphite oxide materials*

4.2.2 *Electrochemical characterisation of the graphite oxide materials*

4.3 *Discussion*

4.4 *Experimental*

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

1. **Chun Kiang Chua**, Sofer Zdeněk, Martin Pumera. Graphite oxides: effects of permanganate and chlorate oxidants on the oxygen composition. *Chem. Eur. J.* **2012**, 18, 13453-13459.

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Contributions:

C. K. Chua conducted most of the experiments. S. Zdeněk conducted the elemental analysis. M. Pumera conceptualized and directed the project. C. K. Chua and M. Pumera contributed to discussions and wrote the manuscript.

In this chapter, the **first step** of chemical treatment based on the top-down synthesis approach to obtain graphene was evaluated. The first synthetic step, based on Scheme 4.1, refers to the **chemical oxidation** of graphite to graphite oxide. This study focused on **how** the selection of chemical oxidants affected the overall structural and electrochemical properties of the resulting graphite oxide materials. This chapter formed part of a collective work towards addressing Aim 1.

Scheme 4.1 The top-down synthetic approach towards graphene. The chemical oxidation step of graphite to graphite oxide is highlighted.

4.1 Introduction

The preparation of graphite oxide was first introduced 150 years ago. Over the years, four distinctively different oxidation techniques were established to achieve the oxidation process of graphite (Figure 4.1). These include methods known as the Staudenmaier, Hofmann, Hummers and Tour methods.¹⁻⁴ These methods differ in the usage of either the types of chemical oxidants or the types of strong acid. Table 4.1 shows a short summary of chemical reagents used in the following four methods.

Given the varying oxidation conditions available, the proportions of oxygen functionalities on the prepared graphite oxide materials could differ drastically. To note, these oxygen functionalities include hydroxyl, epoxide, carbonyl, and carboxylic acids. The distribution of the types of oxygen functionalities could subsequently affect the performance of graphite oxide (or its exfoliated form, graphene oxide) in different applications. For example, graphite oxide (or graphene oxide) is commonly used in electrochemical immunosensor⁵ and glucose sensor,⁶ as starting material for chemical functionalization,⁷⁻⁹ or could potentially confer passivation of NADH¹⁰ due to the presence of carboxylic groups. As such, it is important to obtain detailed analyses on the relative compositions of oxygen containing groups on graphite oxide produced *via* the different methods.

Although these oxidation methods have been performed interchangeably by the graphene community, there remain several pressing issues which should be addressed in such a practice. **Firstly**, does it make any difference at all to use different oxidants and acid mixtures towards the production of graphite oxides and in which aspects would the products differ in? **Secondly**, specifically between the Staudenmaier and Hofmann

methods, is there any difference between the usage of fuming and concentrated nitric acid? **Thirdly**, based on the different qualities of graphite oxide materials, which method would then be advisable for usage in graphene research?

To address the questions raised above, a further understanding on the abilities of the different oxidation methods in tuning the compositions of oxygen functionalities has to be developed. This was achieved by carrying out structural and electrochemical characterizations on the graphite oxide materials prepared using the Staudenmaier (GO-ST), Hofmann (GO-HO), modified Hummers (GO-HU) and Tour (GO-TO) methods. To necessitate for the convenience of the discussion, the oxidation methods were further grouped into two categories – the chlorate (GO-ST and GO-HO) and permanganate (GO-HU and GO-TO) oxidation methods. Characterisations of the graphite oxide materials were carried out using various spectroscopic methods, specifically, high resolution X-ray photoelectron spectroscopy (XPS), electrochemical impedance spectroscopy (EIS), nuclear magnetic resonance (NMR), Fourier transform infrared (FTIR), Raman spectroscopy and electrochemical cyclic voltammetry (CV).

Figure 4.1 Schematic for the oxidation of graphite to graphite oxide using the Staudenmaier, Hofmann, Hummers and Tour methods.

Table 4.1 Chemical reagents of the four oxidation methods of graphite.

Year	Method	Oxidants	Acid 1	Acid 2/ Reagent
1898	Staudenmaier	Chlorate	H ₂ SO ₄	Fuming HNO ₃
1937	Hofmann	Chlorate	H ₂ SO ₄	HNO ₃
1958	Hummers	Permanganate	H ₂ SO ₄	Sodium nitrate
2010	Tour	Permanganate	H ₂ SO ₄	H ₃ PO ₄

4.2 Results

4.2.1 Structural characterisation of the graphite oxide materials

The initial structural characterisations of the graphite oxide materials prepared with the Staudenmaier, Hofmann, modified Hummers and Tour methods were conducted with XPS analysis. This method provides sensitive detections of surface elemental compositions on the materials of interest. A typical XPS survey scan provides general information about the types of elements present on the materials, whereas a high resolution scan reveals information on the chemical environment of the particular element. Based on Figure 4.2A, the XPS survey spectra indicated obvious peaks of carbon and oxygen elements on graphite, GO-ST, GO-HO, GO-HU and GO-TO which gave atomic C/O ratios of 21.6, 2.47, 2.71, 2.05 and 1.95, respectively. The low atomic C/O ratios observed on the graphite oxide materials indicated the success of the oxidative treatments. Overall, the permanganate (GO-HU and GO-TO) oxidation method showed a higher extent of oxidation compared to the chlorate (GO-ST and GO-HO) oxidation method.

Subsequently, high resolution XPS scans were conducted as shown in Figure 4.2B to investigate the types of oxygenated carbon bonds found in the graphite oxide materials. The C1s core level spectra were deconvoluted into Gaussian peaks centred at 284.5, 286.5, 288.5 and 290.5 eV which corresponded to C=C, C-O, C=O and O-C=O bond types respectively. The C1s core level spectrum of graphite provided a sharp peak attributed to the sole presence of C=C bond type. The deconvoluted C1s peak of chlorate (GO-ST and GO-HO) oxidation method gave prominent peaks for C=C and C-O bond types and only a small contribution of C=O bond. On the other hand, the permanganate

Figure 4.2 X-ray photoelectron spectroscopy spectra of graphite, GO-ST, GO-HO, GO-HU and GO-TO. (A) Survey and (B) C1s core level spectra.

(GO-HU and GO-TO) oxidation method indicated a higher proportion of C=O bond type. The occurrence of O-C=O bond type in GO-TO indicated a significant presence of carboxyl group. In short, the higher proportions of C=O and O-C=O bond types in GO-HU and GO-TO highlighted the higher extent of oxidation by the permanganate method as compared to the chlorate oxidation method.

In order to support the XPS study, elemental analyses were carried out to determine the elemental compositions of the graphite oxide materials as summarized in Table 4.2. The materials consisted mainly of carbon, hydrogen and oxygen elements,

whereas their atomic compositions varied according to the methods of oxidation. Graphite exhibited a low presence of oxygen, while GO-ST, GO-HO, GO-HU and GO-TO showed a greater amount of oxygen denoted by the low atomic C/O ratios of 1.17, 1.15, 0.84 and 0.74 respectively. The trend observed on the atomic C/O ratios, between the chlorate and permanganate methods, was consistent with that obtained using the XPS technique.

The specific chemical environments of the carbon atoms present on the graphite oxide materials were subsequently probed using solid state ^{13}C NMR analyses. The analyses were carried out using a ^1H - ^{13}C cross polarization (CP) mode at 8 kHz MAS (contact time 1 ms) and direct ^{13}C pulse mode at 12 kHz MAS (90° ^{13}C pulse) with average scans of 16000, as shown in Figure 4.3A and 4.3B. The NMR spectra in Figure 4.3 highlighted typical peaks due to the presence of C=O, O-C=O, graphitic sp^2 , C-OH and C-O-C bond types at chemical shifts of 190, 165, 129, 69 and 59 ppm respectively.¹¹ The CP mode provided a lesser amount of information on the compositions of graphite oxide materials as compared to the direct ^{13}C pulse technique, as observed similarly in previous reports.^{11,12} Nevertheless, the graphite oxide materials showed obvious presence of graphitic sp^2 , C-OH and C-O-C bond types on both NMR techniques. In agreement with the XPS analyses, the prominent carbonyl and carboxyl peaks that were only present on the permanganate (GO-HU and GO-TO) oxidation method marked a clear distinction between the two different oxidative treatments.

Table 4.2 Elemental analyses of graphite, GO-ST, GO-HO, GO-HU and GO-TO showing the respective atomic percentage of carbon, hydrogen and oxygen atoms.

Materials	% C	% H	% O
Graphite	99.88	—	0.11
GO-ST	52.68	2.42	44.89
GO-HO	52.09	2.39	45.50
GO-HU	44.18	2.92	52.89
GO-TO	41.22	2.92	55.86

Figure 4.3 Solid state 1D ^{13}C magic-angle spinning (MAS) NMR spectra of graphite, GO-ST, GO-HO, GO-HU and GO-TO. (A) ^1H - ^{13}C cross polarization (CP) and (B) Direct ^{13}C pulse spectra.

Figure 4.4 ATR-FTIR spectra of graphite, GO-ST, GO-HO, GO-HU and GO-TO.

Successively, FTIR analyses were carried out to investigate the presence of specific bond types in the graphite oxide materials. As seen in Figure 4.4, prominent peaks corresponding to O-H, C=O, and C-OH stretching were observed at approximately 3200, 1718 and 1050 cm^{-1} . The large peak at about 1600 cm^{-1} corresponded to the unoxidised sp^2 regions of the graphite material. A closer examination into the FTIR spectra revealed a larger peak of C=O stretching, which could account for both carbonyl and carboxyl functional groups, for the permanganate (GO-HU and GO-TO) oxidation method. This was consistent with the observations obtained from both the XPS and NMR studies.

Figure 4.5 Raman spectra of graphite, GO-ST, GO-HO, GO-HU and GO-TO collected using a 514.5 nm laser.

Apart from that, Raman spectroscopic analyses were carried out to determine the extent of defects introduced by the oxidation processes as shown in Figure 4.5. A pristine graphene sheet typically exhibits a single sharp signal at $\sim 1560\text{ cm}^{-1}$ (G band). Presence of an additional signal at $\sim 1350\text{ cm}^{-1}$ (D band) is usually a result of sp^3 defects in the sp^2 carbon lattice. As such, the intensity ratio of the D and G bands (I_D/I_G) provides vital information on the degree of disorder in the sp^2 carbon lattice. The Raman spectra of graphite, GO-ST, GO-HO, GO-HU and GO-TO showed average I_D/I_G ratios of 0.09, 0.89, 0.87, 0.87 and 0.85, respectively. The graphite oxide materials were equally defective in comparison. Moreover, the broaden D and G bands highlighted greater disorder on the oxidised materials, as compared to graphite, due to extensive oxidation treatments.

4.2.2 Electrochemical characterisation of the graphite oxide materials

Electrochemical study of graphite and graphite oxide materials were accomplished using three different electrochemical approaches, mainly electrochemical impedance spectroscopy, cyclic voltammetry and solid state reduction techniques.

Firstly, the ability of the graphite oxide materials to exchange and transfer charges with their surrounding molecules in the environment was examined by electrochemical impedance spectroscopy technique. This method measures the heterogeneous charge transfer resistance, which is highly influenced by the surface morphology of the material under study. The EIS measurements were carried out on graphite, GO-ST, GO-HO, GO-HU and GO-TO modified electrodes in a solution containing ferro/ferricyanide electrochemical probe. The data obtained from the EIS study were analyzed and fitted based on the Randles equivalent circuit as shown in Figure 4.6 inset.

Based on the Nyquist plot shown in Figure 4.6, the diameter of the semicircle corresponds to the charge transfer resistance (R_{ct}) for the material of interest. The GC electrode gave a R_{ct} of 1.13 k Ω while graphite was 1.18 k Ω . The graphite oxide materials gave higher R_{ct} compared to graphite with GO-HO indicating the lowest R_{ct} at 1.68 k Ω , followed by GO-ST at 1.74 k Ω . The GO-HU and GO-TO resulted in higher R_{ct} at 1.98 and 2.15 k Ω , respectively. The R_{ct} values were higher for materials with lower atomic C/O ratios. This indicated that a percentage of the oxygen containing groups in the graphite oxides were negatively charged at the working pH, which resulted in electrostatic repulsions with the negatively charged ferro/ferricyanide redox complexes. Since GO-TO exhibited the lowest atomic C/O ratio (richest in oxygen containing groups), it thus gave a higher R_{ct} value.

Figure 4.6 Nyquist diagrams for EIS measurements of bare GC, graphite, GO-ST, GO-HO, GO-HU and GO-TO. Conditions: PBS 50 mM, pH 7.2 background electrolyte, 10 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$. Randles equivalent circuit used for data fitting is shown as inset.

Figure 4.7 Cyclic voltammograms on ferro/ferricyanide of bare GC, graphite, GO-ST, GO-HO, GO-HU and GO-TO. Conditions: PBS 50 mM, pH 7.2 background electrolyte, 10 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$. Scan rate: 100 mV/s.

Apart from that, cyclic voltammetry analyses were carried out to determine the heterogeneous electron transfer (HET) rate of the graphite oxide materials with ferro/ferricyanide probe, as shown in Figure 4.7. The lowest peak-to-peak separation (ΔE) was observed on GC electrode at 0.30 V. Graphite resulted in a higher ΔE at 0.33 V. The GO-ST and GO-HO materials gave ΔE of 0.35 and 0.34 V, respectively. Higher ΔE were observed for GO-HU and GO-TO at 0.36 and 0.38 V, correspondingly. The respective heterogeneous electron transfer (HET) rates were calculated according to the Nicholson method which showed an increasing HET in the order of: GO-TO (k_{obs}^0 : $1.1 \pm 0.4 \times 10^{-4} \text{ cm s}^{-1}$) < GO-HU (k_{obs}^0 : $1.3 \pm 0.5 \times 10^{-4} \text{ cm s}^{-1}$) < GO-ST (k_{obs}^0 : $1.4 \pm 0.1 \times 10^{-4} \text{ cm s}^{-1}$) < GO-HO (k_{obs}^0 : $1.8 \pm 0.3 \times 10^{-4} \text{ cm s}^{-1}$) < graphite (k_{obs}^0 : $2.0 \pm 0.1 \times 10^{-4} \text{ cm s}^{-1}$) < GC (k_{obs}^0 : $2.8 \pm 0.4 \times 10^{-4} \text{ cm s}^{-1}$).¹³ Based on the observation, the HET rates were also affected by the electrostatic repulsions between the negatively charged oxygen containing groups on graphite oxide materials and the ferro/ferricyanide redox probes. This resulted in GO-TO showing the lowest HET rate.

A solid state electrochemical reduction based on the cyclic voltammetry technique was also carried out to determine the presence of electrochemically reducible oxygen containing groups. The presence of any electrochemically reducible oxygen functionalities (mainly epoxide, carbonyl, and peroxide) would usually give rise to intense reduction waves at peak potentials lower than -1.0 V (vs. Ag/AgCl reference electrode). The electrochemical reduction was carried out as shown in Figure 4.8. Reduction peak for graphite modified electrodes could not be observed since electrochemically reducible oxygen groups were not present on the surface. However, intense reduction peaks were observed for the graphite oxide materials.

Figure 4.8 Cyclic voltammetric profiles of electrochemical reduction of oxygen containing groups on graphite, GO-ST, GO-HO, GO-HU and GO-TO. Conditions: PBS 50 mM, pH 7.2 background electrolyte. Scan rate: 100 mV/s.

The graphite oxide materials obtained using chlorate (GO-ST and GO-HO) oxidation method resulted in a sharp intense peak at approximately -1.2 to -1.3 V. On the other hand, the graphite oxide materials from permanganate (GO-HU and GO-TO) oxidation method gave reduction bands at higher potentials of approximately -1.4 to -1.8 V. The higher reduction potentials were postulated to be due to the presence of a higher amount of carbonyl and carboxyl functional groups, which usually required higher reduction energies. It should be noted that the common reduction potentials of peroxide group is ~ -0.7 V; aldehyde ~ -1.0 V; epoxide ~ -1.5 V and carboxyl group ~ -2.0 V (vs. Ag/AgCl).

4.3 Discussion

The structural, morphological and electrochemical characterizations of the graphite oxide materials have allowed us to draw conclusions for the questions posed earlier. **Firstly**, graphite oxide materials obtained from the four different oxidation methods exhibited varying characteristics. The structural characterizations highlighted similar extents of oxidation indicated by the atomic C/O ratios (based on XPS and elemental analyses) although the permanganate (GO-HU and GO-TO) oxidation method introduced a higher amount of oxygen groups. On top of that, the permanganate oxidation method also presented a higher proportion of carbonyl and carboxylic functional groups (based on XPS, NMR, and FTIR analyses) as compared to the chlorate (GO-ST and GO-HO) oxidation method. The electrochemical performances, however, highlighted vast differences where the permanganate oxidation method resulted in a higher heterogeneous charge transfer resistance by EIS study and a lower HET rate as compared to the chlorate oxidation method. Moreover, the solid state electrochemical reduction technique, with respect to the reduction peaks observed in the cyclic voltammetry scans, indicated drastic differences in the structural features on the two categories of materials. This was resulted by the differences in the compositions of electrochemical reducible oxygen groups on the graphite oxide surfaces.

Secondly, the strength of the concentrated nitric acid was typically assumed to affect the oxidation process as observed in the availability of both the Staudenmaier and Hofmann methods. By comparing these two methods side-by-side, our results have highlighted that there are negligible differences on the two graphite oxide materials. The GO-ST and GO-HU gave almost identical structural properties and electrochemical performances. As such, with respect to the Staudenmaier and Hofmann oxidation

methods, the graphite oxide materials showed almost similar properties despite the variation of nitric acid concentrations.

Thirdly, prior to the selection of a preferred oxidation method, the intended applications at which graphite oxide materials would be utilized should be established. The usage of graphite oxide materials as electrode materials in electrochemical applications should be careful with the possible occurrence of electrode surface passivation. For example, graphite oxide produced from the Hummers and Tour oxidation methods might not be suitable the detection of NADH since the oxidation product of the NADH could interact strongly with the carboxylic groups on graphite oxide to result in subsequent passivation of the electrode surface. Otherwise, our detailed characterisations have suggested that the Tour oxidation method is the most efficient since it provides a higher degree of oxidation and is safer as it avoids the usage or *in situ* production of concentrated nitric acid.

In summary, the structural and electrochemical properties of the different graphite oxides prepared using the Staudenmaier, Hofmann, Hummers and Tour oxidation methods have been highlighted. The four oxidative treatments provided graphite oxide materials of dissimilar properties due to the compositions of oxygen containing groups (hydroxyl, epoxide, carbonyl, and carboxyl). The graphite oxide obtained using permanganate (Hummers and Tour) as oxidant introduced a higher amount of carbonyl and carboxyl functional groups as compared to the chlorate (Staudenmaier and Hofmann) oxidant according to the structural characterizations. The electrochemical characterizations also highlighted a higher heterogeneous electron transfer rate for graphite oxide obtained using chlorate oxidant. This study would be vital towards providing fundamental knowledge on selecting a production method of

graphite oxide for applications such as electrochemical sensing, biosensing, or optoelectronics in the future.

As graphite oxide is the first intermediate material based on the top-down approach towards the production of graphene, this part of the study has provided a basic understanding on the quality of the produced graphite oxide materials. Subsequent rational reduction methods could be applied to remove the oxygen containing groups on graphene oxide. The following **Chapters 5, 6, 7, 8** and **9** focused on the introduction of five established reduction approaches that are useful for the conversion of graphene oxide to reduced graphene materials.

4.4 Experimental

4.4.1 Materials

Graphite (< 20 μm), sulphuric acid (95–98 %), sodium nitrate, hydrochloric acid (37 %), phosphoric acid (85 %), potassium chlorate (98 %), *N,N*-dimethylformamide (DMF), potassium phosphate dibasic, sodium phosphate monobasic, potassium ferricyanide, and potassium ferrocyanide were purchased from Sigma-Aldrich, Singapore. Potassium permanganate and fuming nitric acid (> 90 %) were obtained from J.T. Baker. Glassy carbon (GC) electrode with a diameter of 3 mm was obtained from Autolab, the Netherlands. Milli-Q water (resistivity: 18.2 $\text{M}\Omega\text{ cm}$) was used throughout the experiments.

4.4.2 Apparatus

X-ray photoelectron spectroscopy (XPS) was performed with a Phoibos 100 spectrometer and a monochromatic Mg X-ray radiation source (SPECS, Germany). Both wide survey and high-resolution spectra for C1s were collected. Relative sensitivity factors were used for evaluation of atomic C/O ratios from XPS wide survey spectra measurements. XPS samples were prepared by coating a carbon tape with a uniform layer of the materials under study. The elemental analysis was performed with Vario EL III from Elemental. Solid state ^{13}C magic-angle spinning (MAS) nuclear magnetic resonance (NMR) spectra were obtained on a JEOL-ECA400 (100 MHz ^{13}C , 400 MHz ^1H) under ^1H - ^{13}C cross polarization and direct ^{13}C pulse mode using standard Delta software. Attenuated total reflectance (ATR) Fourier transform infrared (FTIR) measurements were carried out on a PerkinElmer Spectrum 100 system coupled with a universal ATR

accessory. A diamond/ZnSe was used as the ATR crystal. Raman spectroscopy analysis was performed using a confocal micro-Raman LabRam HR instrument from Horiba Scientific in backscattering geometry with a CCD detector, a 514.5 nm Ar laser and a 100× objective mounted on a Olympus optical microscope. The calibration is initially made using an internal silicon reference at 520 cm^{-1} and gives a peak position resolution of less than 1 cm^{-1} . The spectra are measured from 1000 to 3000 cm^{-1} . All voltammetric experiments were performed on a μ Autolab type III electrochemical analyser (Eco Chemie, The Netherlands) connected to a personal computer and controlled by General Purpose Electrochemical Systems Version 4.9 software (Eco Chemie).

4.4.3 Procedures

Graphite source was used as-received without additional treatment prior to oxidations.

Precautions should be taken when handling corrosive acids and strong oxidants.

Graphite oxide preparation with Staudenmaier method (GO-ST). Sulphuric acid (17.5 mL, 95–98 %) and nitric acid (9 mL, > 90 %) were added to a reaction flask containing a magnetic stir bar. The mixture was cooled at $0\text{ }^{\circ}\text{C}$ for 15 min. Graphite (1 g) was then added to the mixture under vigorous stirring to avoid agglomeration and obtain a homogeneous dispersion. Potassium chlorate (11 g) was slowly added to the mixture (over 15 min) at $0\text{ }^{\circ}\text{C}$ in order to avoid sudden increases in temperature and the formation of chlorine dioxide gas, which is explosive at high concentrations. After the complete dissolution of potassium chlorate, the reaction flask was loosely capped to allow evolution of gas and the mixture was stirred vigorously for 96 hr at room temperature. On completion of the reaction, the mixture was poured into 1 L of

ultrapure water and filtered. GO was then redispersed and washed repeatedly in 5 % HCl solutions to remove sulphate ions. GO was finally washed with ultrapure water until a neutral pH of the filtrate was obtained. The GO slurry was then dried in a vacuum oven at 50 °C for 5 days before use.

Graphite oxide preparation with Hofmann method (GO-HO). Sulphuric acid (17.5 mL, 95–98 %) and nitric acid (9 mL, 63 %) were added to a reaction flask containing a magnetic stir bar. The mixture was cooled at 0 °C for 15 min. Graphite (1 g) was then added to the mixture under vigorous stirring to avoid agglomeration and obtain a homogeneous dispersion. Potassium chlorate (11 g) was slowly added to the mixture (over 15 min) at 0 °C in order to avoid sudden increases in temperature and the formation of chlorine dioxide gas, which is explosive at high concentrations. After the complete dissolution of potassium chlorate, the reaction flask was loosely capped to allow evolution of gas and the mixture was stirred vigorously for 96 hr at room temperature. On completion of the reaction, the mixture was poured into 1 L of ultrapure water and filtered. GO was then redispersed and washed repeatedly in 5 % HCl solutions to remove sulphate ions. GO was finally washed with ultrapure water until a neutral pH of the filtrate was obtained. The GO slurry was then dried in a vacuum oven at 50 °C for 5 days before use.

Graphite oxide preparation with modified Hummers method (GO-HU). Graphite (0.5 g) was stirred with sulphuric acid (23.0 mL, 95–98 %) for 20 min at 0 °C prior to the addition of NaNO₃ (0.5 g) in portions. The mixture was left to stir for 1 hr. KMnO₄ (3 g) was then added in portions at 0 °C. The mixture was subsequently heated to 35 °C for 1 hr. Water (40 mL) was then added into the mixture and resulted in the temperature of the mixture to rise up to 90 °C. The temperature was maintained at 90 °C for 30 min. Additional

water (100 mL) was added into the mixture. This was followed by a slow addition of 30 % H_2O_2 (~10 mL). The warm solution was filtered (RC membrane, 0.22 μm) and washed with warm water (100 mL). The solid was subsequently washed with a copious amount of water until a neutral pH was obtained. The materials were kept in the oven at 50 °C for 5 days prior to usage.

Graphite oxide preparation with Tour method (GO-TO). Graphite (0.3 g) was added into a mixture of concentrated $\text{H}_2\text{SO}_4\text{--H}_3\text{PO}_4$ (36:4 mL), followed by addition of KMnO_4 (1.8 g). The reaction mixture was then heated to 50 °C and stirred for 12 hr. The reaction was then cooled to room temperature and poured in ice water (~40 mL) with addition of 30 % H_2O_2 (0.5 mL). The mixture was then filtered and washed with water, 30 % HCl, ethanol and ether. The obtained solid materials were kept in the oven at 50 °C for 5 days prior to usage.

Electrochemical characterization of materials. The electrochemical experiments were carried out in a 5 mL voltammetric cell at room temperature (25 °C) using a three-electrode configuration. A platinum electrode served as an auxiliary electrode and a Ag/AgCl electrode as a reference electrode; a glassy carbon electrode was utilized as a working electrode. Prior to a measurement, the glassy carbon electrode was polished with 0.05 μm alumina on a polishing cloth. The working electrode was then modified by applying 1 μL of DMF containing 1 mg/mL carbon material (GO-ST, GO-HO, GO-HU or GO-TO) and allowed to dry.

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Chapter 5

Novel methods for the reduction of graphene oxide: Method I

Lithium aluminium hydride as a reducing agent

5.1 *Introduction*

5.2 *Results*

5.2.1 *Structural characterisation of the graphene materials*

5.2.2 *Electrochemical characterisation of the graphene materials*

5.3 *Discussion*

5.4 *Experimental*

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

1. Adriano Ambrosi, **Chun Kiang Chua**, Alessandra Bonanni, Martin Pumera. Aluminium hydride as reducing agent for chemically reduced graphene oxides. *Chem. Mater.* **2012**, 24, 2292-2298.

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Contributions:

A. Ambrosi conducted most of the analyses. C. K. Chua synthesized the graphene materials. A. Bonanni conducted the EIS study. M. Pumera conceptualized and directed the project. A. Ambrosi, A. Bonanni and M. Pumera wrote the manuscript. All authors contributed to discussions.

The **chemical reduction step** for the conversion of graphene oxide to graphene was then evaluated, as highlighted in Scheme 5.1. Five different types of reduction methods to reduce oxygen containing groups on graphene oxide were examined for the first time in the subsequent chapters i.e. **Chapters 5, 6, 7, 8 and 9**.

In **Chapters 5, 6 and 7**, three different types of reducing agents that are supported by well-established mechanisms based on the knowledge of synthetic chemistry were introduced. These reducing agents provided the opportunities to fine-tune the amount of oxygen containing groups on the reduced graphene and to produce commercially viable graphene. Specifically, the reducing agents introduced in these chapters were lithium aluminium hydride (**Chapter 5**), derivatives of borohydrides (**Chapter 6**), and thiourea dioxide (**Chapter 7**).

In **Chapters 8 and 9**, two different methods to remove residual hydroxyl groups on graphene oxide were explored since most reducing agents could theoretically, *at best*, only reduce the oxygen containing groups to hydroxyl groups. Attention was placed specifically on methods that could not only remove the hydroxyl groups but also regenerate the sp^2 carbon framework of the resulting graphene. Specifically, the two methods introduced for these purposes were based on a dehydrobromination process (**Chapter 8**) and the usage of ethanethiol-aluminium chloride complex (**Chapter 9**).

A summary of the methods introduced in the following chapters is shown in Scheme 5.2. **Chapters 5, 6, 7, 8 and 9** formed parts of the collective work in addressing Aim 1.

Scheme 5.1 The top-down synthetic approach towards graphene. Highlighted portion shows the transformation of graphite oxide to graphene oxide *via* ultrasonication treatment followed by the reduction of graphene oxide to reduced graphene with chemical reductants.

Scheme 5.2 Summary of the reducing agents introduced in Chapters 5, 6, 7, 8, and 9 for the conversion of graphene oxide to chemically reduced graphene oxide.

5.1 Introduction

The reduction of graphene oxide to graphene has been previously conducted with various reducing agents. Most of these reducing agents were summarised in **Chapter 3**. It is important to notice that, except for sodium borohydride¹ and hydrohalic acids², *none* of the reducing agents adopted so far is commonly used in synthetic chemistry for the conversion or elimination of oxygen functionalities. In the case of the widely used reducing agent for graphene oxide, hydrazine,³ when utilized under the conditions of Wolff-Kishner reduction, the carbonyl groups are converted to methylene groups.⁴ Moreover, under the conditions of Wharton reaction, α,β -epoxy-ketones could be reduced to allylic alcohols.⁵ It is also known that, in the presence of α,β -unsaturated ketones, hydrazine could not reduce the carbonyl groups to methylenes, but pyrazolines are resulted instead.⁶ Such eventuality is highly probable considering the sp^2 hybridised network of graphene oxide and would also explain the presence of nitrogen atoms typically found in the graphene framework after reduction with hydrazine.⁷ Nitrogen doping causes the loss of important electronic properties of the graphene materials and certainly should be taken into consideration when selecting a reduction procedure.

It is surprising that the reducing agent “toolbox” of graphene oxide is lacking in powerful well-known and widely-used reducing agents typically used in synthetic chemistry, specifically lithium aluminium hydride.⁶ In view of that, the performance of lithium aluminium hydride as a reducing agent of graphite oxide to graphene was investigated. Lithium aluminium hydride is a very powerful and unselective reagent capable of reducing carbonyl, epoxide, ester, and carboxylic groups to hydroxyl groups. It should be emphasized here that the reactivity of lithium aluminium hydride is very different from that of hydrazine and sodium borohydride. Hydrazine should theoretically

target carbonyl groups specifically while sodium borohydride has a lower reactivity as compared to lithium aluminium hydride.

In this part of the study, lithium aluminium hydride, alongside popular reducing agents such as sodium borohydride and hydrazine hydrate were utilized for the chemical reduction of graphene oxide (Scheme 5.3). The properties of the chemically reduced graphene materials were subsequently investigated.

Scheme 5.3 Schematic of the procedure followed for the preparation of the chemically reduced graphene materials. a) An oxidative treatment was initially performed to generate graphene oxide from graphite as starting material. Then graphene oxide is reduced by means of b) NaBH_4 ; c) N_2H_4 ; d) LiAlH_4 .

5.2 Results

Three reducing agents for the reduction of graphene oxide, mainly sodium borohydride (NaBH_4), hydrazine hydrate (N_2H_4) and LAH (LiAlH_4) were compared. The reduction procedures employed in this work followed an initial oxidation of graphite to graphite oxide and consequent exfoliation to graphene oxide. This oxidation step was performed in accordance to the Staudenmaier method which involved the use of concentrated nitric and sulphuric acid together with potassium chlorate as the main oxidizing agent. Graphite oxide (GO) produced in this way was exfoliated to graphene oxide by ultrasonication followed then by a chemical reduction with three different reducing agents: NaBH_4 (CRGO1), N_2H_4 (CRGO2) and LiAlH_4 (CRGO3). Scheme 5.3 summarizes the procedural steps involved. GO and the chemically reduced graphene have been comprehensively characterized by X-ray photoelectron spectroscopy (XPS), Raman and Fourier transform infrared (FTIR) spectroscopies, electrochemical cyclic voltammetry and electrochemical impedance spectroscopy (EIS). By using these methods, the structural and chemical information related to the reduced graphene materials were obtained and thus allowed for the evaluation of the reduction reactions. It is also possible to relate the structures of the materials to the corresponding performances in electrochemical applications.

5.2.1 Structural characterisation of the graphene materials

XPS represents an invaluable characterization tool which could reveal superficial atomic composition of a solid sample and more importantly the type of chemical groups bonded to it. In our study, XPS was adopted to gain two pieces of important information: i) the atomic C/O ratio composing the sample surface which could be used to evaluate the extent of the oxidation first and then the reduction method proposed; ii) the type of oxygenated carbon groups. Figure 5.1A shows the XPS survey spectra of the reduced graphene materials as well as the starting GO. It could be seen that GO presented an intense oxygen signal due to the introduction of oxygen containing groups into the graphene backbone by the oxidative treatment. According to the XPS measurement, the atomic C/O ratio for GO was 3.4, which corresponded to an atomic oxygen content of about 22.5 % (mass oxygen content of 28.2 %). The elemental analysis of GO resulted with an atomic C/O ratio of 1.2. It was clear that the oxidative treatment introduced a significant amount of oxygen species. The three reduction procedures were able to eliminate most of the oxygen functionalities from the GO material indicated by a much less intense oxygen signal. However, a precise evaluation revealed different efficiencies in the removal of oxygen groups by the three reducing agents. The atomic C/O ratios resulted to 9.5 for CRGO1, 11.5 for CRGO2 and 12.0 for CRGO3. These values corresponded to an atomic oxygen content of 9.5, 8.0 and 7.6 % (mass oxygen content of 12.3, 10.4 and 10.0 %) for CRGO1, 2 and 3, respectively.

It should be noted that the use of hydrazine as reducing agent introduces nitrogen atoms into the sp^2 carbon network resulting in the presence of nitrogen content in CRGO2 of about 1 % according to our XPS analysis. Again, the presence of nitrogen moieties into the graphene network might be resulted from the incomplete reduction of

Figure 5.1 XPS survey scan (A) and high-resolution XPS C1s core spectra (B) of GO, CRGO1, CRGO2 and CRGO3 materials

α,β -unsaturated carbonyl groups using hydrazine, providing pyrazole as a final product.⁷ The reduction with LAH was the most efficient method to eliminate the oxygen containing groups on graphene oxide. Consequently, high resolution XPS of C1s core levels (Figure 5.1B) were carried out in order to obtain insights into the amount and type of oxygen containing species available. GO gave four main peaks centred at 284.5 eV (C=C: graphitic sp^2 C), 286.0 eV (C-O: epoxide and hydroxyl), 287.0 eV (C=O: carbonyl) and 288.7 eV (O-C=O: carboxyl). The signal at 291 eV could be assigned to the π - π^* shake-up satellite of the sp^2 band centred at 284.5 eV. The C1s core level envelope of the chemically reduced graphene oxides were fitted to five components centred at 284.5 eV (C=C: graphitic sp^2 C), 285.5 eV (C-C: sp^3 C), 286.5 eV (C-O: epoxide and hydroxyl), 287.6

eV (C=O: carbonyl) and 289.7 eV (O-C=O: carboxyl). It could be seen that upon chemical reduction, the graphitic sp^2 C band became narrower than that of GO, indicating a more homogeneous chemical environment and a more ordered structure. The amount of graphitic sp^2 C increased from a value of 36 % in GO to a value of 68, 69 and 73 % in CRGO1, 2 and 3 respectively. The main difference among the three methods is the remaining amount of carboxylic groups. By using LAH as a reducing agent, only about 2 % of carboxylic groups remained on the graphene material, while with N_2H_4 and $NaBH_4$, the remaining amount were 7 and 6 %, respectively. LAH is known to be among the most powerful reducing agents used in synthetic chemistry reactions and it is clearly demonstrated here as having a more effective ability to reduce carboxylic groups especially when compared to $NaBH_4$ and N_2H_4 .

Raman spectroscopy could be used to gain structural information on the materials. The first order spectra ($1100-1800\text{ cm}^{-1}$) show the characteristic D and G bands of carbon materials. The G band at about 1590 cm^{-1} involves in-plane bond stretching of graphitic sp^2 carbon pairs and thus is indicative of pristine graphene sheets. The D band located at about 1350 cm^{-1} is related to disordered conformations and defects of the sp^2 carbon lattice of graphene. The ratio between the intensities of the D and G bands (I_D/I_G) is thus used as an indicator of the relative disorder present in graphene structures.⁸ Figure 5.2 shows the Raman spectroscopy spectra of GO, CRGO1, CRGO2 and CRGO3. The obtained I_D/I_G ratio of GO was 1.10. This indicated a quite extensive disorder in the graphitic structure due to the presence of oxygen functionalities. Upon reduction, the I_D/I_G ratios decreased to 0.61, 0.97 and 0.63 for CRGO1, CRGO2 and CRGO3, respectively. Although structural changes introduced by chemical reductions are still not well understood, a reduced I_D/I_G ratio has been

Figure 5.2 Raman spectra of GO and the reduced materials CRGO1, CRGO2 and CRGO3. The spectra have been normalized according to the G band for a better comparison.

explained by the elimination of oxygen species with the reintroduction of large aromatic domains.⁹ NaBH₄ and LAH are clearly the most effective in reducing structural disorders as compared to hydrazine, according to our measurements.

In order to gain more information on the functionalities present on the material surface, solid-state FTIR measurements on the materials of investigation were carried out (Figure 5.3). The FTIR spectrum of graphite showed almost undetectable signals with no significant bands. GO presented intense bands at 1060 and 1370 cm⁻¹ corresponding to C-OH vibrations, signals at 870 and 1250 cm⁻¹ were generated by bending and stretching vibrations of epoxide groups, signals at 1620 and 1720 cm⁻¹ generated by C=O and COOH

Figure 5.3 FT-IR spectra of Graphite, GO and the reduced materials CRGO1, CRGO2 and CRGO3. Attenuated Total Reflectance (ATR) mode is used with all samples in solid state.

vibrations and finally an intense and wide band at $2500\text{--}3700\text{ cm}^{-1}$ was due to OH groups and adsorbed water. Upon reduction, the elimination of oxygen functionalities was clearly apparent for the three CRGO materials, thus resulting in signals which were barely detectable. An interesting exception was given by the appearance of a clear band at $2800\text{--}3000\text{ cm}^{-1}$ for CRGO3, produced using LAH as reducing agent. These peaks correspond to the C-H stretching modes.¹⁰ This band, which was not visible for the other reducing agents, might further show how LAH is the most effective in reducing oxygen containing groups. In particular, the reduction of carboxylic groups, possible only with LAH, generates $\text{CH}_2\text{-OH}$ functionalities. The stretching modes of C-H appearing at $2800\text{--}3000\text{ cm}^{-1}$, could be assigned to such resulting functionalities. C-H stretching modes were

not observed after using hydrazine as reducing agent. This further confirms that in the absence of a strong base (normally used in the Wolff-Kishner reaction, but not in the present work), pyrazole moieties are the final product at the carbonyl group locations due to the extensive conjugation. It is also known that C=C double bonds are usually not affected by metallic hydrides. However, when the double bond is conjugated to a C=O group, a possible reduction of the double bond might also occur with powerful reducing agents such as LAH. Graphene oxide is by nature a highly conjugated sp^2 carbon structure and therefore a partial reduction of C=C bonds to CH_2-CH_2 might occur,¹¹ giving rise to the C-H stretching mode band. A significantly increased amount of hydrogen for CRGO3 was also confirmed by the elemental analysis. CRGO3 resulted, in fact, with 11.4 % of atomic hydrogen versus the 2.5 % of the starting GO material. From a physical point of view, CRGO3 resulted with an increased surface area of about $31 \text{ m}^2 \text{ g}^{-1}$ compared to the starting GO material at about $9.4 \text{ m}^2 \text{ g}^{-1}$.

In an attempt to understand the reductive action of LAH, it was important to take into consideration that the starting GO material contained, in its nature, a significant amount of adsorbed water intercalated within the graphene oxide layers. Such a possibility with the GO material adopted in this work (Figure 5.4) was analysed using TGA. From the analysis, GO gave mass losses at three different temperatures: i) about 10-15 % of mass was lost between 20 and 100 °C, attributable to the elimination of the adsorbed water; ii) about 25 % of mass loss at about 250 °C due to the decomposition of oxygenated groups or to the decomposition of oxygenated debris;¹² and iii) complete mass loss at 600 °C due to the oxidation of the damaged graphitic structure. As expected a significant amount of water was present within the GO material.

Figure 5.4 Thermogravimetric analysis (black) and derivative thermogravimetric analysis (red) in air of the starting material GO.

Figure 5.5 Digital photographs of GO and the reduced materials CRGO1, CRGO2 and CRGO3 dispersed in A) ultrapure water and B) DMF, immediately after 30 min sonication. All dispersions are prepared at 1 mg/mL concentration.

This might have important consequences during the reaction with LAH. The reduction reaction was performed in a “dry” THF solution to avoid side reactions of LAH with water molecules, but the intrinsic water present within the GO might react with LAH to generate a strong base locally, along with the evolution of H₂ gas. In such conditions, as described by Rourke and co-workers,¹² detachment of the oxygenated debris might occur, resulting in a primary deoxygenation of GO. In the second step of the experimental, an excess amount of LAH was added to react with the remaining covalently bonded oxygen groups on the GO material. Such double-effect would most likely explain the excellent reducing capability of LAH. However on the down side, the detachment of oxygenated debris caused the restacking of the GO sheets due to the reduced repulsive forces.¹² This effect was clearly observed from our AFM measurements (data not shown). The thickness of GO sheets resulted, in fact, of about 2 nm, while after the reduction, CRGO3 material had a sheet thickness of 3-5 nm. Despite this fact, aggregation of the graphene sheets is commonly experienced with most of the reducing agents.

The formation of methylene moieties should have a tremendous effect on the solubility properties of the material. The solubility of the four materials in pure water and in DMF, both after 30 min of ultrasonication treatment were tested. It could be seen in Figure 5.5 that CRGO3, produced with LAH, was practically insoluble in water, while the other materials showed good dispersion stabilities in this solvent. Using DMF as solvent, all the materials showed good dispersion stabilities. This could be explained by the fact that GO, CRGO1 and CRGO2 structures comprised of both hydrophobic and hydrophilic moieties. CRGO3, on the contrary, resulted with predominantly hydrophobic functionalities (C-H) which caused a low dispersion stability in water.¹³

In more detail, the good solubility of GO, CRGO1 and CRGO2 in water could mostly be attributed to the presence of carboxylic groups, which are the only groups dissociated at a neutral pH. Such functional groups are certainly present on CRGO1 and CRGO2 since NaBH_4 and hydrazine are not able to reduce carboxylic groups. LAH is the only reducing agent that is able to reduce carboxylic groups and therefore their absence on the reduced material accounts for a significantly reduced solubility in water.

5.2.1 Electrochemical characterisation of the graphene materials

To further characterize the materials, electrochemical measurements were performed with the aim to evaluate the electron transfer rate and the charge transfer resistance. Cyclic voltammetry, which is a large amplitude electrochemical technique, was used to measure the electron transfer rate between the materials under investigation and the ferro/ferricyanide redox probe in solution. Figure 5.6A shows representative cyclic voltammograms of GO, CRGO1, CRGO2 and CRGO3-modified GC electrodes in the presence of 5 mM ferro/ferricyanide in comparison to a bare GC electrode. According to the Nicholson method, the peak separation between the oxidation and reduction signals could be correlated to the heterogeneous electron transfer (HET) rate.¹⁴ For a more precise evaluation, Figure 5.6B shows the averaged ($n=5$) peak separations for all materials and the corresponding HET rates. GO resulted with the highest ΔE of 415 mV. CRGO1 and CRGO2 resulted in almost similar peak separations of 326 and 320 mV, respectively. CRGO3 generated using LAH as reducing agent resulted with a higher peak separation of 387 mV. Such increment resulted in about a three-fold decrease of HET rate as compared, for example, to CRGO2. The HET rate constants are 0.6×10^{-4} , 1.9×10^{-4} , 2.1×10^{-4} and $0.8 \times 10^{-4} \text{ cm s}^{-1}$ for GO, CRGO1,

Figure 5.6 Electrochemical characterization of GO and the reduced materials CRGO1, CRGO2 and CRGO3. A) Representative cyclic voltammograms of 5 mM $[\text{Fe}(\text{CN})_6]^{4-/3-}$ using bare GC and GO, CRGO1, CRGO2, CRGO3 modified GC electrodes. Conditions: KCl 0.1 M background electrolyte, scan rate 0.1 V/s. B) Peak-to-peak separation average values ($n=5$) between the oxidation and reduction of $[\text{Fe}(\text{CN})_6]^{4-/3-}$ (left axis) and corresponding value of k^0_{obs} (right axis). C) Nyquist plots for EIS measurements of bare GC and GO, CRGO1, CRGO2, CRGO3 modified electrodes. Conditions: KCl 0.1 M background electrolyte, 5 mM $[\text{Fe}(\text{CN})_6]^{4-/3-}$. Inset: Randles equivalent circuit used for data fitting.

CRGO2 and CRGO3, respectively. This meant that electrons were transferred the fastest at N_2H_4 reduced graphene, slower at NaBH_4 reduced graphene and even slower at LAH reduced graphene. The reasons for such behaviour would be discussed below after describing the results of the independent electrochemical method, impedance spectroscopy.

EIS is a low amplitude sensitive technique that is able to evaluate the resistance of HET between material, in our case graphene, and a molecular probe in the solution.

Such electron-exchange ability is strongly influenced by structural conformation and surface chemistry of the material and therefore EIS could provide further insights into the structures. The charge transfer resistance (R_{ct}) corresponds to the diameter of the semicircle in Nyquist plots obtained from the measurements. As it could be seen in Figure 5.6C, GO presented the highest R_{ct} value of 7.3 k Ω which was due to the low conductivity of the material and the presence of negatively charged oxygen functionalities that repelled negatively charged ferro/ferricyanide complexes. The reduced materials CRGO1, CRGO2 and CRGO3 presented R_{ct} of 4.0, 3.3 and 5.9 k Ω , respectively. This meant that N_2H_4 reduced graphene showed the lowest resistance to HET while the graphene reduced with LAH showed the highest resistance to HET. It is clear that the EIS data are consistent with those obtained by cyclic voltammetry. This might be explained by considering the structures of these materials. It is well-known that the HET of graphene structures towards ferro/ferricyanide is very fast at the edges of the graphene sheets (where defects are also considered as “edge sites” for HET) and very slow (by means of 10^7 difference) at the basal-plane of graphene.¹⁵⁻¹⁷

The fastest HET at N_2H_4 reduced graphene was possibly due to its large density of defects, as determined by Raman spectroscopy, while the slowest HET on LAH reduced graphenes was due to low defect density and additional presence of C-H moieties.

For a qualitative comparison on the conductivities of the materials, I-V curves were obtained by means of interdigitated gold electrodes (Au-IDE).¹⁸ Materials with “ohmic” behaviours would present linear I-V curves with slopes directly proportional to their conductivities. Using this method, an immediate qualitative comparison of GO, CRGO1, CRGO2 and CRGO3 was obtained as depicted in Figure 5.7. It could be seen that

Figure 5.7 Current-voltage curves of GO, CRGO1, CRGO2 and CRGO3 measured after deposition of the materials onto an Au-IDE.

GO presented an extremely high resistance, giving almost zero current signal over the scanned voltage range. Such result confirmed the insulator-semiconducting nature of GO.³ The three chemically reduced materials showed ohmic behaviours with linear I-V curves. By comparing each I-V curve, it could be seen that the steepest slope was observed on CRGO1 (62.4 mA/V), indicating the highest conductivity. CRGO2, which has a slope of 46.6 mA/V, presented a slightly lower value of conductivity. CRGO3 on the contrary, has the lowest conductivity with a slope of 2.9 mA/V, which was one order of magnitude lower than the other materials. These findings confirmed the results obtained using cyclic voltammetry and EIS. The presence of C-H moieties was most likely responsible for the low conductivity of CRGO3.

5.3 Discussion

The reducing ability of lithium aluminium hydride has proven to be consistent with theoretical expectations even though it was applied on graphene oxide as a starting material. Lithium aluminium hydride, similar to most conventional reducing agents, should only reduce oxygen containing groups on graphene oxide to hydroxyl groups. It was surprising to observe that lithium aluminium hydride was able to induce further reduction of oxygen containing groups on graphene oxide compared to typical reducing agents, specifically hydrazine or sodium borohydride. In comparison, the oxygen containing groups on graphene oxide were reduced to a larger extent, resulting in a higher C/O ratio when using lithium aluminium hydride. Moreover, the presence of $-CH$ band from the FTIR spectrum and the hydrophobicity of the LAH-reduced graphene indicated its better reducing strength. However, the LAH-reduced graphene showed a lower performance on its electrical properties as compared to hydrazine- and $NaBH_4$ -reduced graphene due to the presence of the saturated carbons.

From a wider point of view, a well-established and conventional reducing agent that is commonly applied in synthetic chemistry could be beneficial for the reduction of graphene oxide. This study has served as a platform for the usage of conventional reducing agents and has driven subsequent research towards the removal of oxygen containing groups in **Chapters 6, 7, 8 and 9**.

5.4 Experimental

5.4.1 Materials

Graphite (< 20 μm), sulphuric acid (95–98 %), potassium chlorate (98 %), hydrochloric acid (37 %), *N,N*-dimethylformamide (DMF), tetrahydrofuran (THF), methanol, ethanol, sodium hydroxide, potassium phosphate dibasic, sodium carbonate, sodium phosphate monobasic, sodium sulphate, potassium ferrocyanide, potassium chloride, sodium borohydride and lithium aluminium hydride were obtained from Sigma-Aldrich, Singapore. Fuming nitric acid (> 90 %) was obtained from J.T. Baker. Hydrazine hydrate was obtained from Alfa Aesar, Singapore. Glassy carbon (GC) electrode with a diameter of 3 mm was obtained from Autolab, the Netherlands.

5.4.2 Apparatus

X-ray photoelectron spectroscopy was performed with a Phoibos 100 spectrometer and a monochromatic Mg X-ray radiation source (SPECS, Germany). Both survey and high-resolution spectra for C1s were collected. Relative sensitivity factors were used for evaluation of C/O ratios from survey spectra XPS measurements. XPS samples were prepared by coating carbon tape with a uniform layer of the materials under study. Raman spectroscopy analysis was performed using a confocal micro-Raman LabRam HR instrument from Horiba Scientific in backscattering geometry with a CCD detector, a 514.5 nm Ar laser and a 100 $\times$ objective mounted on a Olympus optical microscope. The calibration was initially made using a silicon reference at 520 cm^{-1} and gave a peak position resolution of less than 1 cm^{-1} . The spectra were measured from 1000 to 3000 cm^{-1} . Attenuated total reflectance (ATR) Fourier transform infrared measurements were

carried out on a PerkinElmer Spectrum 100 system coupled with a universal ATR accessory. TGA analysis was performed heating the material from room temperature to 900 °C with ramp of 6 °C/min and in the presence of air at 60 mL/min flow. All voltammetric experiments were performed on a μ Autolab type III electrochemical analyzer (Eco Chemie, The Netherlands) connected to a personal computer and controlled by General Purpose Electrochemical Systems Version 4.9 software.

5.4.3 Procedures

Graphene oxide. Prepared according to the Staudenmaier method whereby 17.5 mL of sulphuric acid (95–98 %) and 9 mL of nitric acid (fuming) were added to a reaction flask containing a magnetic stir bar. The mixture was cooled at 0 °C for 15 min. 1 g of graphite was then added to the mixture under vigorous stirring to avoid agglomeration and obtain a homogeneous dispersion. 11 g of potassium chlorate was added slowly to the mixture (over 15 min) at 0 °C in order to avoid a sudden increase in temperature and the formation of chlorine dioxide gas, which is explosive at a high concentration. After the complete dissolution of potassium chlorate, the reaction flask was loosely capped to allow evolution of gas and the mixture was stirred vigorously for 96 hr at room temperature. On completion of the reaction, the mixture was poured into 1 L of ultrapure water and filtered. Graphite oxide so obtained was then redispersed and washed repeatedly with HCl (5 %) solutions to remove sulphate ions and subsequently with ultrapure water until a neutral pH of the filtrate was obtained. Graphite oxide slurry was then dried in a vacuum oven at 60 °C for 48 hr before use. Exfoliation of graphite oxide to obtain graphene oxide was performed by ultrasonication of a 1 mg/mL dispersion of graphite oxide for 2 hr.

Graphene oxide reduction by sodium borohydride (CRGO1). Dry graphite oxide powder (80.5 mg) was dispersed in ultrapure water to give a 1.0 mg/mL colloidal solution. The mixture was ultrasonicated (150 W) for 3 hr prior to the addition of 5 wt % sodium carbonate to obtain a dispersion of pH 9-10. Sodium borohydride (800 mg, 21.1 mmol) was added over 20 min into the dispersion under vigorous stirring. The reaction mixture was heated up to 80 °C for 1 hr. The mixture was cooled to room temperature and washed repeatedly with ultrapure water using centrifugation (8000 rpm, 10 min). The sample pellet was dried at 60 °C for 5 days to yield CRGO1.

Graphene oxide reduction by hydrazine (CRGO2). Dry graphite oxide powder (49.8 mg) was dispersed in ultrapure water to give a 1.0 mg/mL colloidal solution and ultrasonicated (150 W) for 3 hr. Hydrazine hydrate (2 mL, 32.1 mmol) was added drop wise at 47 °C and then the reaction mixture was heated up to 80 °C for 24 hr. After cooling to room temperature the mixture was washed repeatedly with methanol and ultrapure water using centrifugation (8000 rpm, 10 min). The sample pellet was dried at 60 °C for 5 days to yield CRGO2.

Graphene oxide reduction by lithium aluminium hydride (CRGO3). Dry graphite oxide powder (50 mg) was dispersed in dry THF (30 mL) and ultrasonicated (150 W) for 3 hr. LAH (190 mg, 5 mmol) dispersed in dry THF (10 mL) was added drop wise at 0 °C until bubbling was no longer observed. The mixture was then transferred into a solution of LAH (760 mg, 20 mmol) in dry THF (20 mL) drop wise at 0 °C. The reaction mixture was left to stir under reflux for 24 hr. The mixture was quenched with saturated solution of sodium sulphate followed by 1 M hydrochloric acid at 0 °C until a clear solution was obtained. The mixture was filtered (RC membrane filter, 0.2 µm) and washed repeatedly with ultrapure water to give CRGO3 upon drying at 60 °C for 5 days.

Electrochemical characterization of materials. Immobilization of the carbon materials onto the glassy carbon working electrode was performed firstly by preparing a suspension of the desired material with a concentration of 1 mg/mL in DMF. After 30 min ultrasonication, 1 μ L aliquot from the appropriate suspension was deposited onto the electrode surface. The solvent was then allowed to evaporate at room temperature, leaving a randomly distributed film onto the GC electrode surface.

Cyclic voltammetry experiments were performed at a scan rate of 100 mV/s in 0.1 M KCl as supporting electrolyte and using 5 mM ferro/ferricyanide as redox probe at room temperature (25 °C). Electrochemical impedance spectroscopy measurements were performed in 0.1 M KCl as supporting electrolyte and using 5 mM ferro/ferricyanide as redox probe. The GC electrode surfaces were renewed by polishing with 0.05 mm alumina particles on a cloth (DP-Plus, Streurs) before each voltammetry measurement.

The k_{obs}^0 values were determined using the method developed by Nicholson that relates ΔE_p to a dimensionless parameter ψ and consequently to k_{obs}^0 .⁴⁰ The roughness factor was not taken into account. The diffusion coefficient $D=7.26 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ for $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 M KCl was used for calculations.

Current-voltage measurements (I-V curve) were conducted by depositing 1 μ L of the material suspension (prepared at 1 mg/mL concentration in ethanol) onto the interdigitated electrode having 10 μ m spacing. The electrode was then dried under a lamp for 30 min leaving a randomly deposited material film on the interdigitated area bridging the two Au electrode bands. I-V curves were obtained by linear sweep voltammetric measurements at 20 mV/s scan rate. The displayed data corresponds to the average from five measurements.

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Chapter 6

Novel methods for the reduction of graphene oxide: Method II

Substituted borohydrides as reducing agents

6.1 *Introduction*

6.2 *Results*

6.2.1 *Structural characterisation of the graphene materials*

6.2.2 *Electrochemical characterisation of the graphene materials*

6.3 *Discussion*

6.4 *Experimental*

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Contributions:

C. K. Chua conducted the experiments. M. Pumera conceptualized and directed the project. All authors contributed to discussions and wrote the manuscript.

6.1 Introduction

The study on the reduction of graphene oxide with standard synthetic reagents has motivated subsequent explorations into substituted borohydrides. Sodium borohydride is the most common reducing agent in general synthetic chemistry. Moreover, the usage of sodium borohydride has been demonstrated for the reduction of graphene oxide to graphene. Lee and co-workers have reported the usage of sodium borohydride to produce graphene with superior conductivity as compared to graphene obtained *via* the hydrazine reduction method.¹ Furthermore, sodium borohydride has also been applied on graphene oxide to selectively reduce common oxygen containing groups except carboxylic groups for further applications.²

In theory, sodium borohydride has a well-established mechanism that involves the reduction of carbonyls and acyl chloride functionalities to hydroxyl groups, but it is rarely useful for epoxide and carboxylic acid groups. Derivatives of sodium borohydride such as sodium cyanoborohydride and sodium triacetoxyborohydride usually display relatively lower reducing strength (for chemical structures, see Figure 6.1).³ Since the reduction of graphene oxide with substituted borohydrides has not been demonstrated before, it is of high importance to examine the performances of these substituted borohydrides. A thorough investigation on the three different borohydrides, mainly sodium borohydride (NaBH_4), sodium cyanoborohydride ($\text{NaBH}_3(\text{CN})$) and sodium triacetoxyborohydride ($\text{NaBH}(\text{OAc})_3$) on the graphene oxide platform is crucial as these derivatives could provide opportunities to fine-tune the oxygen composition of the resulting graphene materials. The possible differences in the reducing strength demonstrated by these substituted borohydrides could provide an opportunity to

prepare various quality of reduced graphene for specific applications i.e. electrochemical sensing and electronic devices.^{4,5}

In this part of the study, the reducing strength of $\text{NaBH}_3(\text{CN})$ and $\text{NaBH}(\text{OAc})_3$ were examined in comparison to NaBH_4 on graphene oxide based on the analyses of the structural and electrochemical properties of the resulting graphene materials.

Figure 6.1 Chemical structures of sodium borohydride, sodium cyanoborohydride and sodium triacetoxyborohydride.

6.2 Results

The reducing strength of various substituted borohydrides on graphene oxide were investigated. Graphite oxide was first produced by the modified Hummers' oxidation method from graphite. The graphite oxide was ultrasonicated to give graphene oxide and subsequently treated with sodium borohydride (BH-CRGO), sodium cyanoborohydride (CN-CRGO) and sodium triacetoxyborohydride (OAc-CRGO) in methanol under reflux for 2 hr. A control experiment (Control) was carried out concurrently without the addition of any reductants, to examine the effects of solvent and heat on the properties of the resulting graphene. Methanol was selected as reaction solvent instead of water, since standard reductions with borohydrides in synthetic chemistry are usually carried out in an alcohol solution. More importantly, sodium triacetoxyborohydride decomposes readily upon contact with water. These borohydrides are most likely converted to borates, such as boric acid, trimethoxy borate and triacetoxy borate.^{6,7} While the possibilities of such borates being trapped in the interlayer spacing of graphene sheets are highly probable, subsequent washing should remove most of them.

The graphene oxide treated with the substituted borohydrides were then analysed using high resolution X-ray photoelectron spectroscopy (XPS), Fourier transform infrared spectroscopy (FTIR), Raman spectroscopy, electrochemical impedance spectroscopy (EIS) and electrochemical cyclic voltammetry (CV).

6.2.1 Structural characterisation of the graphene materials

The first structural analysis was carried out on the reduced graphene materials with X-ray photoelectron spectroscopy. XPS is a very sensitive and semi-quantitative analysis method which probes the surface of a material to provide its surface elemental compositions. Survey scans of XPS, from a binding energy of 1200 eV to 0 eV, were conducted to determine an overall composition of available elements as shown in Figure 6.2A. The survey scans highlighted prominent presence of carbon and oxygen elements for all the materials. A peak corresponding to sodium element was observed for the reduced graphene as well. This could correspond to the remaining sodium ions which bound ionically with the oxygen atoms on the reduced graphene materials. The survey scans provided C/O ratios of 1.93, 2.14, 2.51, 2.24 and 2.52 for GO, Control, BH-CRGO, OAc-CRGO and CN-CRGO respectively. A lower C/O ratio, as presented by GO, indicated the presence of a higher amount of oxygen containing groups. As such, the borohydride reductants resulted in a lower amount of oxygen containing groups based on the C/O ratios, possibly *via* hydrogen insertion into carbonyl groups. While possible decomposition of sodium triacetoxymborohydride in the non-anhydrous methanol solvent could not be ruled out, traces of acetate could contribute to the low C/O ratio of OAc-CRGO. Boron element was not detected on the survey scan, which assured that the samples were washed thoroughly and there was no interference from residual reagents.

High resolution XPS on C1s core levels were carried out on the graphene materials as well, as shown in Figure 6.2B. A high resolution C1s core level spectrum provides information on the chemical environment or types of bondings present in the material of interest. With respect to a C1s core level analysis, peaks centred at 284.5

Figure 6.2 X-ray photoelectron spectroscopy spectra of GO, Control, BH-CRGO, OAc-CRGO and CN-CRGO (A) Survey and (B) C1s core level spectra.

(C=C: graphitic sp^2 C), 286.5 (C-O: epoxide and hydroxyl), 288.5 (C=O: carbonyl), and 289.5 eV (O-C=O: carboxyl) are usually observed. For the GO material, peaks corresponding to all the four carbon bond types were observed. Upon ultrasonication and treatment with heat, the amount of C=O reduced drastically and the presence of O-C=O were not detected. This could be due to the exposure of less oxidised basal-planes of previously stacked graphene sheets upon ultrasonication⁸ and the thermal deoxygenation/ decarboxylation under heating conditions.⁹ Subsequent treatment with borohydride reductants provided varying degree of changes on the ratios of carbon

Figure 6.3 ATR-FTIR spectra on GO, Control, BH-CRGO, OAc-CRGO and CN-CRGO.

bond types. The BH-CRGO showed 58.5, 32.8 and 8.7 % of C=C, C-O and C=O bond types, respectively. The OAc-CRGO provided 46.5, 43.2, and 10.3 % while CN-CRGO resulted in 53.9, 36.1 and 10.0 % of C=C, C-O and C=O bond types correspondingly. These highlighted a higher reducing strength of sodium borohydride as compared to the other two reductants; this is in line with the expectations based on general synthetic chemistry knowledge whereby sodium triacetoxymborohydride should provided the lowest reducing strength among the three borohydride reductants, while the extent of reduction resulted from sodium cyanoborohydride lied in between the two borohyrides. This clearly showed that the variation of substituents in the borohydrides reductants allowed

for fine tuning of the reducing strength in the organo-boron family, and subsequently the quality of the reduced graphene.

Subsequent structural analysis was performed using attenuated total reflectance Fourier transform infrared spectroscopy as shown in Figure 6.3. GO gave peaks corresponding to O-H stretching mode ($\sim 3200\text{ cm}^{-1}$), C=O vibrational mode ($\sim 1718\text{ cm}^{-1}$), C-OH vibrational mode (~ 1380 and 1050 cm^{-1}), and C-O-C from epoxide groups ($\sim 870\text{ cm}^{-1}$). The peak at $\sim 1600\text{ cm}^{-1}$ was attributed to unoxidised graphitic regions. Heat treatment on GO provided the Control material, which showed almost similar peak patterns to that of GO. Upon reductive treatment with the borohydrides, persistent peaks from O-H, C=O, C-OH and C-O-C moieties were observed. However, specifically for the C=O bond peak, BH-CRGO gave almost negligible traces while OAc-CRGO and CN-CRGO still displayed prominent peaks for it. This observation was consistent with earlier XPS analyses which indicated that sodium borohydride provided a stronger reducing strength as compared to sodium cyanoborohydride and sodium triacetoxyborohydride.

Raman spectroscopy analysis was carried out as well to evaluate the structural properties of the graphene-based materials as shown in Figure 6.4. Raman spectroscopy is able to identify presence of defects on the graphene sp^2 carbon network by measuring the D (defect, breathing mode of sp^2 carbon) and G (graphitic, sp^2 bonded carbon) bands at ~ 1346 and $\sim 1567\text{ cm}^{-1}$, respectively. The ratio of the peak intensities of D and G bands (I_D/I_G) provides information on the extent of defects for the graphene-based materials. Based on the analyses, GO, Control, BH-CRGO, OAc-CRGO and CN-CRGO resulted in I_D/I_G ratios of 0.89, 0.82, 0.84, 0.87 and 0.88, respectively. The Raman analyses highlighted similar extents of defects on the reduced graphene, possibly due to the already defective GO precursor.

Figure 6.4 Raman spectra on GO, Control, BH-CRGO, OAc-CRGO and CN-CRGO.

6.2.2 Electrochemical characterisation of the graphene materials

Following the structural characterizations of the graphene-based materials, the electrochemical properties were examined *via* electrochemical impedance spectroscopy technique and cyclic voltammetry. Both techniques provide sensitive analyses for the resistance of the graphene-based materials towards heterogeneous charge transfer. EIS is a technique which applies small alternating-current voltage perturbation to the system (in the order of millivolts) at different frequencies while cyclic voltammetry is a complementary technique which applies a large perturbation to the system (in the order of hundreds of mV). The ability of the graphene-based materials to transfer and exchange charges with the surrounding molecules is highly influenced by their surface

morphologies. The EIS analyses were performed in a ferro/ferricyanide electrochemical probe and analysed based on experimental fitting with a Randles equivalent circuit (shown inset in Figure 6.5) on the Nyquist plots shown in Figure 6.5.

The diameter of the semicircle on a typical Nyquist plot corresponds to the charge transfer resistance (R_{ct}) of the material. The bare GC electrode showed a R_{ct} of 0.6 k Ω while the oxygen-rich GO gave a high R_{ct} of 5.30 k Ω . The heat treatment on GO resulted in a decrease of R_{ct} value at 2.27 k Ω . The BH-rGO and CN-rGO exhibited a comparative R_{ct} of 1.64 and 1.76 k Ω , respectively. R_{ct} for OAc-rGO was much higher than BH-rGO and CN-rGO, which was at 4.92 k Ω . Materials with inherently higher contents of oxygen-containing groups would usually result in higher R_{ct} values.⁸ This is possibly due to the electrostatic repulsion between the negatively charged ferro/ferricyanide complexes (electrochemical probe) and a portion of the oxygen containing groups on the graphene-based materials, which are dissociated and negatively charged at the working pH. The EIS data concluded that $\text{NaBH}(\text{OAc})_3$ was a mild reductant towards graphene oxide, while a stronger reducing strength was observed with $\text{NaBH}_3(\text{CN})$. The strongest reduction of the oxygen containing groups (lowest R_{ct}) could be achieved with NaBH_4 . These observations were consistent with the earlier results obtained from XPS and FTIR measurements, as discussed above.

Figure 6.5 Nyquist diagrams for EIS measurements for GO, Control, BH-CRGO, OAc-CRGO and CN-CRGO. Conditions: PBS 50 mM, pH 7.2 background electrolyte, and 10 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$. The Randles equivalent circuit used for data fitting is shown as inset.

Figure 6.6 Cyclic voltammograms on ferro/ferricyanide for GO, Control, BH-CRGO, OAc-CRGO and CN-CRGO. Conditions: PBS 50 mM, pH 7.2 background electrolyte, 10 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$. Scan rate: 100 mV/s.

Apart from that, cyclic voltammetry was carried out as an independent large-scale perturbation method to verify the findings of EIS analyses. In fact, CV scans were carried out prior to each impedimetric scans to examine the heterogeneous electron transfer (HET) rate of the graphene-based materials. A similar ferro/ferricyanide electrochemical probe was used for this purpose. As ferro/ferricyanide probe is sensitive to electrode surfaces, the resulting HET rate could be correlated to the amount of oxygen containing groups on the graphene surfaces. It was demonstrated by various groups that upon an increasing amount of oxygen containing groups on the graphene-based materials, the HET decreased (this is indicated by an increase of the peak-to-peak separation, ΔE).^{8,10} The representative cyclic voltammograms of GO, Control, BH-CRGO, OAc-CRGO and CN-CRGO in comparison to bare GC are shown in Figure 6.6. It could be observed that GO presented the highest peak separation, ΔE of 0.59 V while the bare GC electrode gave the lowest ΔE at 0.20 V. The Control, BH-CRGO, OAc-CRGO and CN-CRGO resulted in peak separations of 0.37, 0.34, 0.54 and 0.36 V, respectively. The heterogeneous electron transfer rate were subsequently calculated based on the Nicholson method¹¹ which showed an increasing HET for the graphene-based materials in the order of: GO (k_{obs}^0 : $5.8 \pm 1.3 \times 10^{-6} \text{ cm s}^{-1}$) < OAc-CRGO (k_{obs}^0 : $1.1 \pm 0.2 \times 10^{-5} \text{ cm s}^{-1}$) < Control (k_{obs}^0 : $1.2 \pm 0.6 \times 10^{-4} \text{ cm s}^{-1}$) < CN-CRGO (k_{obs}^0 : $1.2 \pm 0.2 \times 10^{-4} \text{ cm s}^{-1}$) < BH-CRGO (k_{obs}^0 : $1.6 \pm 0.6 \times 10^{-4} \text{ cm s}^{-1}$). It should be noted that the HET rates determined by CV analyses were highly affected by the interactions between the negatively charged ferro/ferricyanide probe and oxygen containing groups on the graphene material. This resulted in GO having the lowest HET rate while the reduced graphene provided HET rates of about two orders of magnitude higher. These results were consistent with the observations from EIS analyses

Figure 6.7 Cyclic voltammetric profiles of electrochemical reduction of oxygen containing groups for GO, Control, BH-CRGO, OAc-CRGO and CN-CRGO. Conditions: PBS 50 mM, pH 7.2 background electrolyte. Scan rate: 100 mV/s.

and have the same implications towards the efficiency of the borohydrides on the reduction of graphene oxide.

Moreover, the power of electrochemical technique consists of the fact that in an exhaustive mode (coulometric mode), one could relate the number of exchanged electrons to the absolute number of electroactive species. In the case of graphene-based materials, it is possible to *absolutely*, by using solid state voltammetry, determine the amount of electrochemically reducible groups (epoxide, peroxide, aldehyde and carbonyl) on graphene sheets.¹² As such, a solid state electrochemical reduction method using cyclic voltammetry technique to verify the presence of electrochemically reducible oxygen containing groups was carried out. This was achieved by a reductive scan from +0 to -2.0 V as shown in Figure 6.7. The presence of oxygen functionalities such as epoxide, carbonyl, and peroxide would result in intense reduction peaks appearing at

potentials lower than -1.0 V (vs. Ag/AgCl reference electrode). The graphene-based materials were tested against a bare GC electrode. It could be observed that the bare GC electrode did not provide any voltammetric reduction peak. However, the GO and Control graphene materials gave almost identical reductive peak centred at -1.4 V. Upon treatment with sodium borohydride, the area of the reductive peak decreased drastically. The CN-CRGO and OAc-CRGO gave larger reductive peak areas than BH-CRGO, indicating the presence of a higher amount of electrochemically reducible oxygen groups on the graphene surfaces. Based on the integrated reduction peak areas and the amount of deposited graphene materials on the electrode surface, on average, there was one electrochemically reducible oxygen-containing group (epoxide, peroxide and aldehyde) per every 6.26, 6.89, 12.43, 27.23 and 154.90 of carbon atoms for GO, Control, OAc-CRGO, CN-CRGO, and BH-CRGO.¹² This highlighted that the extent of reduction increased in the order of OAc-CRGO < CN-CRGO < BH-CRGO, which is in agreement with the observed electrochemical measurements of R_{ct} and HET rate, as well as with the XPS and FTIR analyses.

6.3 Discussion

While sodium borohydride, sodium cyanoborohydride, and sodium triacetoxyborohydride were expected to show a decreasing reducing strength in this order when applied in synthetic chemistry, a similar trend was observed on the reduction of graphene oxide materials. Sodium borohydride provided the highest extent of reduction based on the high resolution XPS C1s core level analysis. This was also evident from the FTIR analyses, whereby the carbonyl band of the sodium borohydride-reduced graphene was not as apparent as that of sodium cyanoborohydride- and sodium triacetoxyborohydride-reduced graphene materials. In fact, the extents of reduction were correlated to the electrochemical properties. The sodium borohydride-reduced graphene exhibited the highest heterogeneous electron transfer rate and the lowest heterogeneous charge transfer resistance. Moreover, the sodium borohydride-reduced graphene exhibited an average of one electrochemically reducible oxygen containing group per 6.26 of carbon atoms, which was the lowest among the three borohydride derivatives. On the other hand, sodium cyanoborohydride provided a higher extent of reduction than sodium triacetoxyborohydride.

In comparison to using lithium aluminium hydride as a reducing agent in **Chapter 5**, these substituted borohydrides provided a milder approach for reduction purposes. The borohydrides were less likely to reduce carbonyl and carboxylic groups. Furthermore, the reducing strengths of the substituted borohydrides were strongly influenced by their substituent groups. This has provided an opportunity to fine-tune and tailor the structural and electrochemical properties of reduced graphene materials for use in future practical applications.

6.4 Experimental

6.4.1 Materials

Graphite was obtained from Asbury Carbons. Sulphuric acid (95–98 %), sodium nitrate, *N,N*-dimethylformamide (DMF), sodium borohydride, sodium cyanoborohydride, sodium triacetoxymborohydride, potassium phosphate dibasic, sodium phosphate monobasic, potassium ferrocyanide, sodium chloride and potassium chloride were purchased from Sigma-Aldrich, Singapore. Potassium permanganate was obtained from J.T. Baker. Hydrogen peroxide was obtained from Alfa Aesar, Singapore. Methanol was obtained from Tedia, Singapore. Glassy carbon electrode with a diameter of 3 mm was obtained from Autolab, the Netherlands. Milli-Q water (resistivity: 18.2 M Ω cm) was used throughout the experiments.

6.4.2 Apparatus

X-ray photoelectron spectroscopy was performed with a Phoibos 100 spectrometer and a monochromatic Mg X-ray radiation source (SPECS, Germany). Both survey and high-resolution spectra for C1s were collected. Relative sensitivity factors were used for evaluation of C/O ratios from survey spectra XPS measurements. XPS samples were prepared by coating carbon tape with a uniform layer of the materials under study. Raman spectroscopy analyses were performed using a confocal micro-Raman LabRam HR instrument from Horiba Scientific in backscattering geometry with a CCD detector, a 514.5 nm Ar laser and a 100 $\times$ objective mounted on a Olympus optical microscope. The calibration was initially made using an internal silicon reference at 520 cm⁻¹ and gave a peak position resolution of less than 1 cm⁻¹. The spectra were measured from 1000 to

3000 cm^{-1} . Attenuated total reflectance Fourier transform infrared measurements were carried out on a PerkinElmer Spectrum 100 system coupled with a universal ATR accessory. A diamond/ZnSe was used as the ATR crystal. All voltammetric experiments were performed on a μ Autolab type III electrochemical analyzer (Eco Chemie, The Netherlands) connected to a personal computer and controlled by General Purpose Electrochemical Systems Version 4.9 software (Eco Chemie).

6.4.3 Procedures

Graphite oxide (GO). Graphite oxide was prepared using graphite according to the Hummers method whereby graphite (0.5 g) was stirred with sulphuric acid (23.0 mL, 95–98 %) for 20 min at 0 °C prior to the addition of NaNO_3 (0.5 g) in portions. The mixture was left to stir for 1 hr. KMnO_4 (3 g) was then added in portions at 0 °C. The mixture was subsequently heated to 35 °C for 1 hr. Water (40 mL) was then added into the mixture and resulted in the temperature of the mixture to rise up to 90 °C. The temperature was maintained at 90 °C for 30 min. Additional water (100 mL) was added into the mixture. This was followed by a slow addition of 30 % H_2O_2 (~10 mL). The warm solution was filtered (RC membrane, 0.22 μm) and washed with warm water (100 mL). The solid was subsequently washed with a copious amount of water until a neutral pH was obtained. The materials were kept in a vacuum oven at 30 °C for 5 days prior to usage. Precautions should be taken when handling corrosive acids and strong oxidants. Graphene oxide was obtained by subjecting GO through ultrasonication [37 kHz] for 2 hr in the methanol to give a 1 mg/mL dispersion.

Sodium borohydride-reduced graphene oxide (BH-CRGO). Dispersion of graphene oxide in methanol (1 mg/mL) was added Na_2CO_3 (5 %) to adjust the pH to ~8-9. Sodium borohydride (284 mg, 6.57 mmol) was added into the graphene oxide dispersion and stirred at 70 °C (reflux) for 2 hr. Upon completion, the mixture was filtered under vacuum and washed repeatedly with methanol and water until a neutral pH of the filtrate was obtained. The solid samples were dried in a vacuum oven at 30 °C for 5 days before usage.

Sodium cyanoborohydride-reduced graphene oxide (CN-CRGO). Sodium borohydride (412 mg, 6.57 mmol) was added into the graphene oxide dispersion and stirred at 70 °C (reflux) for 2 hr. Upon completion, the mixture was filtered under vacuum and washed repeatedly with methanol and water until a neutral pH of the filtrate was obtained. The solid samples were dried in a vacuum oven at 30 °C for 5 days before usage. Precaution should be taken for possible evolution of hydrogen cyanide vapour.

Sodium triacetoxyborohydride-reduced graphene oxide (OAc-CRGO). Sodium triacetoxyborohydride (1.39 g, 6.57 mmol) was added into the graphene oxide dispersion and stirred at 70 °C (reflux) for 2 hr. The pH of the solution was examined to be ~3-4. Upon completion, the mixture was filtered under vacuum and washed repeatedly with methanol and water until a neutral pH of the filtrate was obtained. The solid samples were dried in a vacuum oven at 30 °C for 5 days before usage.

Control experiment (Control). A control experiment carried out for 2 hr in the same set of conditions but in the absence of borohydride reductants was carried out simultaneously to test the effect of heat treatment on the reduction process. Upon completion, the mixture was filtered under vacuum and washed repeatedly with

methanol and water until a neutral pH of the filtrate was obtained. The solid samples were dried in a vacuum oven at 30 °C for 5 days before usage.

Electrochemical characterization of materials. Immobilization of the carbon materials onto the glassy carbon working electrode was performed firstly by preparing a suspension of the desired material with a concentration of 1 mg/mL in DMF. The CRGO suspensions were ultrasonicated for 5 min prior to depositing 1 μ L of aliquot of the appropriate suspension onto the electrode surface. The DMF solvent was then allowed to evaporate at room temperature, leaving a randomly distributed film onto the GC electrode surface. Electrochemical experiments were performed by using 50 mM phosphate buffer (pH 7.2) as supporting electrolyte for ferro/ferricyanide (10 mM) redox probe at room temperature (25 °C). The GC electrode surfaces were renewed by polishing with 0.05 mm alumina particles on a cloth (DP-Plus, Streurs) before each voltammetry/impedimetric measurement.

The k_{obs}^o values were determined using the method developed by Nicholson that relates ΔE_p to a dimensionless parameter ψ and consequently to k_{obs}^o . The roughness factor was not taken into account. The diffusion coefficient $D=7.26 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ for $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 M KCl was used for calculations.

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Chapter 7

Novel methods for the reduction of graphene oxide:

Method III

Thiourea dioxide as a reducing agent

7.1 *Introduction*

7.2 *Results*

7.2.1 *Morphological characterisation of the graphene materials*

7.2.2 *Structural characterisation of the graphene materials*

7.2.3 *Electrochemical characterisation of the graphene materials*

7.3 *Discussion*

7.4 *Experimental*

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Contributions:

C. K. Chua conducted the experiments. A. Ambrosi and M. Pumera directed the project.

All authors contributed to discussions and wrote the manuscript.

7.1 Introduction

While the introduction of new reducing agents with well-established mechanisms is important, the suitability of the reducing agents towards a commercially viable large-scale production is equally critical. An ideal reducing agent should be able to provide an excellent reducing capability, cheap, safe and produce only wastes that are manageable with current technologies. Current reducing agents, for example, hydrazine and sodium borohydride, might not be ideal for the large-scale preparation of graphene. Hydrazine is highly unstable and carcinogenic in nature while sodium borohydride is reactive and harmful to health.

Efforts to provide alternate reducing agents that could fit into the criteria mentioned above have led us to select thiourea dioxide as a potential candidate. Thiourea dioxide, formamidine sulfinic acid (Figure 7.1), has been utilized as a strong reducing agent in textile printing, paper, photographic and leather processing industries for a long time.¹ It has also been applied in academia research towards the deoxygenation of α,β -epoxy ketones and the reduction of ketones, aromatic nitro-, azoxy-, azo-, hydrazo- and organosulphur compounds.²⁻⁵ The reactivity of thiourea dioxide on oxygen containing groups is, as such, deemed highly relevant for the reduction of graphene oxide. Moreover, the reduction process generates sodium sulphite and urea,⁴ which are easy to manage since these are common commercial wastes. On top of that, thiourea dioxide is easily available in bulk quantities, safer and less expensive than other reducing agents such as hydrazine. The reduction of graphene oxide using thiourea dioxide is thus expected to be scalable and robust since it is backed by proven methods. In this part of the study, the effectiveness of thiourea dioxide as a reducing agent for graphene oxide was established.

Figure 7.1 Chemical structure of thiourea dioxide/ formamidine sulfinic acid.

7.2 Results

A systematic investigation is necessary to determine the reducing strength of thiourea dioxide. To this aim, a variation of reaction time was performed, specifically at a reaction time of 1, 2, 5, 10 or 20 hr (consequently labelled as T1-, T2-, T5-, T10- and T20-CRGO). The reduction procedure was elaborated in the experimental section. A control experiment, which was carried out for 2 hr in a similar reaction condition but in the absence of thiourea dioxide, was performed simultaneously to test the effect of NaOH and heat on the reduction process (labelled as NaOH-Control).

7.2.1 Morphological characterisation of the graphene materials

The morphological properties of the graphene materials were investigated using scanning electron microscopy (SEM) and transmission electron microscopy (TEM) techniques. Figure 7.2 shows the representative TEM and SEM images of GO, NaOH-Control, and T1-CRGO. TEM images of GO showed highly orientated sheets with precise edges. The areas of low contrast indicated locations of multi-layer stacked graphene configurations (Figure 7.2A). When GO was subjected to ultrasonication and NaOH treatment, thinner layers of graphene sheets which apparently have similar smooth and “clean” appearances as GO were observed (Figure 7.2C). Subsequent reduction using thiourea dioxide (1 hr reaction time) gave highly pleated and wrinkled graphene sheets

Figure 7.2 TEM (A, C and E) and SEM (B, D, and F) images of GO (A and B), NaOH-Control (C and D) and T1-CRGO (E and F).

(Figure 7.2E). Such conformation is typical for highly exfoliated materials with structural defects most probably located along the wrinkle lines.

The SEM analysis of GO showed presence of large aggregated and heavily stacked sheets (Figure 7.2B). Similar characteristics were observed on NaOH-Control (Figure 7.2D). On the other hand, the SEM image of T1-CRGO (Figure 7.2F) indicated graphene sheets which were less aggregated, randomly folded and wave-like.

Figure 7.3 Photographs of dispersions in (A) water (0.5 mg/mL) and (B) DMF (1 mg/mL) after ultrasonication (10 min) of (a) GO, (b) NaOH-Control, (c) T1-CRGO, (d) T2-CRGO, (e) T5-CRGO, (f) T10-CRGO, and (g) T20-CRGO.

Figure 7.4 Photographs of reaction mixture upon completion of (A) 2 hr (B) 10 hr (C) 20 hr reaction time. (D) solid samples of 2 and 20 hr of reaction time.

Further evaluations on the dispersion stabilities of the graphene materials in water (Figure 7.3A) and DMF (Figure 7.3B) revealed the physical properties of the materials. The GO and NaOH-Control materials were readily dispersed in water while the reduced graphene materials were highly hydrophobic. The hydrophobic nature of the reduced graphene materials was initially observed during the chemical reactions, whereby the materials formed black aggregations. The extent of aggregation increased with reaction time (Figure 7.4, A-C). Apart from that, the T20-CRGO appeared powder-like and shiny as compared to the rest of the reduced graphene materials which were chunky in appearances (Figure 7.4, D). Interestingly, the T20-CRGO suspended in water has a tendency to remain at the glass interface above the water level, indicating a higher hydrophobic property compared to the materials obtained with shorter reaction times. On the contrary, stable dispersions were formed upon ultrasonication treatments in DMF solvent. This demonstrates that the GO and NaOH-control materials possess amphiphilic features, while the reduced graphene materials have predominantly hydrophobic characteristics.

7.2.2 Structural characterisation of the graphene materials

The extent of reduction with thiourea dioxide was monitored using X-ray photoelectron spectroscopy (XPS), which is a sensitive analytical technique in determining the surface elemental compositions and types of functional groups available on a material of interest. Initial XPS survey scan (Figure 7.5A) of GO showed a C/O ratio of ~2.5, which is typical for graphite materials that have undergone strong oxidation processes.⁶ Treatment with NaOH in the absence of thiourea dioxide (NaOH-

Figure 7.5 XPS spectra of GO, NaOH-Control and T1-CRGO materials: (A) Survey scan and (B) C1s core level spectra. (C) Variation in the C/O ratio of CRGO materials obtained using different reaction time.

Control) produced a material that has undergone a partial deoxygenation, in fact, with a C/O ratio of only ~ 3.0 . Despite a certain deoxygenation ability of NaOH which was already demonstrated in the past, specifically on the ability to remove highly oxygenated debris from graphene oxide materials, such an effect was not observed with the reaction conditions used in this work.⁷ The obvious peak from the Na1s core level was due to the presence of sodium salt, which also explained the high dispersion stability of NaOH-Control in water (Figure 7.5B).⁸ Upon ultrasonication and treatment of GO with thiourea dioxide for 1 hr, T1-CRGO exhibited a higher C/O ratio of ~ 14.5 . Subsequent reduction attempts at extended reaction time saw an increment of C/O ratio up to ~ 16.0 between 2 and 5 hr of reaction time. A slight decrease of C/O ratio for longer reaction times was observed which could be a result of possible chemical degradation (Figure 7.5C). The low C/O ratio measured from the NaOH-Control demonstrated the ability of thiourea dioxide as the main reducing agent which acted synergistically with NaOH towards the production of few-layer reduced graphene sheets. The success of the reduction was also shown by the hydrophobic nature of the reduced graphene materials in water (Figure 7.3A), indicating that a significant removal of oxygen functionalities was achieved.

Moreover, the C/O ratios of CRGOs obtained here using thiourea dioxide were significantly higher than those obtained using other reducing agents such as sodium borohydride (C/O: 5.8),⁹ hydrazine anhydrous (C/O: 12.0),¹⁰ and hydrazine monohydrate (C/O: 10.3).¹¹ Careful analyses of the C1s core level peaks of the XPS spectra facilitate the identification of oxygen containing groups present on the surfaces of the graphene materials. Figure 7.5B presents the C1s core level regions for GO, NaOH-Control and T1-CRGO materials. GO gave intense peaks at about 284.5 and 286.5 eV, corresponding to the contribution of graphitic sp^2 C and C-O groups, respectively. A minor contribution at

288.3 eV was also observed and could be assigned to the C=O functionality. These indicated that the GO material was highly oxidised prior to the reduction step. The subsequent treatment with NaOH as a control experiment resulted in only a slight decrement of the C-O peak and thus reiterated the secondary role of NaOH in the reduction process. Upon reductive treatment with thiourea dioxide, a less broad and well-defined peak with small tailing towards higher binding energies was observed in T1-CRGO. The reduction step resulted in drastic decrement of intensities for the C–O, C=O and O–C=O peaks. An additional band at 285.3 eV for sp^3 carbon bonding (C–C) was observed as well, which could be due to some structural defects generated after the removal of the oxygen functionalities. A well-defined and sharper signal at 284.5 eV along with the π - π^* shake up satellite band at ~290.0 eV demonstrated an improved characteristic of aromatic/conjugated systems. Reduced graphene materials obtained using longer reaction times presented similar XPS spectra to T1-CRGO (data not shown).

Subsequent analyses with attenuated total reflectance Fourier transform infrared (FTIR) spectroscopy were carried out as shown in Figure 7.6. The FTIR spectrum of GO indicated the presence of O–H stretching mode ($\sim 3200\text{ cm}^{-1}$), C=O vibrational mode ($\sim 1718\text{ cm}^{-1}$), C–OH vibrational mode (~ 1380 and 1050 cm^{-1}) and C–O–C in epoxide groups (~ 1220 and 820 cm^{-1}). The peak at 1600 cm^{-1} could result from the unoxidised graphitic region.¹² NaOH-Control showed obvious and identical peaks as that of GO, indicating that most of the oxygen containing groups was not removed by the hot alkali treatment. After the reduction processes, the FTIR spectra of the reduced graphene materials were featureless, without any presence of peaks at ~ 3200 , 1718 , 1380 and 1050 cm^{-1} . This indicated that most of the oxygen containing groups in the reduced graphene materials had been eliminated.

Figure 7.6 ATR-FTIR spectra of GO, NaOH-Control and CRGO materials.

In order to further understand the structural properties of the reduced graphene materials, Raman spectroscopy analyses were carried out using a green laser at 514.5 nm to identify any defects on the surfaces of the carbon materials, which were directly related to the extent of functionalization. Figure 7.7A shows the Raman spectra for GO, NaOH-Control and T1-CRGO. The D (defect, breathing mode of sp^2 carbon)¹³ and G (graphitic, sp^2 bonded carbon)¹⁴ bands at ~ 1346 and ~ 1567 cm^{-1} , respectively, appeared as sharp peaks for T1- CRGO whereas broadened bands were observed for both GO and NaOH-Control. An intense and broad D band indicates a significant presence of structural disorder with short range sp^2 carbon features and/or the presence of sp^3 hybridized carbons. This indicated that the reduction process created structural defects

Figure 7.7 (A) Raman spectra for GO, NaOH-Control and T1-CRGO materials. **(B)** Variation in the I_D/I_G ratio of CRGO materials obtained using different reaction times.

resulting in a higher D band. However, other features were also observed in the Raman spectra. In particular, the 2D band (second order two phonon process) at $\sim 2700\text{ cm}^{-1}$ is usually analysed to approximate the number of graphene layers, where a single sharp and symmetrical 2D band exceeding the G band usually represents a single layer graphene. T1-CRGO (and the remaining reduced graphene materials; data not shown) resulted in a 2D band which was well-defined and of higher intensity than those of GO and NaOH-Control materials, showing that the reduced graphene materials consisted of few-layer graphene sheets.¹⁵ The ratio for the intensities of D and G bands (I_D/I_G) is usually applied to show the extent of chemical modification or defects in a carbon sample.¹⁶ Based on the analyses, GO provided a I_D/I_G ratio of 0.83 while that of the reduced graphene materials produced using different reaction times resulted in I_D/I_G ratios higher than 1.00 as shown in Figure 7.7B. This indicated a higher extent of disorder in the reduced graphene materials when compared to the starting GO and was in good agreement with other reduction methods that used hydrazine anhydrous/monohydrate and sodium borohydride.⁹⁻¹¹ The high defect densities were also reflected as wrinkle- and line-like areas in the TEM image (Figure 7.2E).

7.2.3 Electrochemical characterisation of the graphene materials

Following the structural characterisations of the graphene materials, electrochemical study was carried out to verify the reduction of oxygen containing groups as well. A solid-state electrochemical reduction using the cyclic voltammetry technique as shown in Figure 7.8 was performed to verify the presence of electrochemically reducible oxygen containing groups. The presence of oxygen functionalities (mainly epoxide, carbonyl, and peroxide) on the carbon materials could

Figure 7.8 Cyclic voltammetric profiles of electrochemical reduction of oxygen containing groups. Conditions: PBS 50 mM, pH 7.2 background electrolyte. Scan rate: 100 mV/s.

be confirmed by an intense reduction peak appearing at potentials lower than -1.0 V (vs. Ag/AgCl reference electrode).¹⁷ The electrochemical analyses of the reduced graphene materials were tested against bare glassy carbon (GC), GO and NaOH-Control modified electrodes. It could be observed that GO was the only material which showed a clear and intense reduction peak at a potential of -1.3 V. The NaOH-Control gave a broad reduction peak signifying the presence of electrochemically reducible oxygen functionalities. On the other hand, the T1-CRGO did not display any reduction peaks within the potential range scanned. This further confirmed the absence of electrochemically reducible groups on T1-CRGO (and similarly with the other reduced graphene materials; data not shown), thus indicating the effectiveness of thiourea dioxide as a reductant.

The electrochemical properties were then examined with electrochemical impedance spectroscopy technique (EIS). EIS is useful to investigate the resistance of graphene materials towards heterogeneous charge transfer, thus indicating the ability of the graphene materials to transfer and exchange charges with the surrounding molecules (redox probe). However, such ability is strongly influenced by the surface morphology of the graphene materials. As such, the EIS measurements of bare GC, GO, NaOH-Control and the CRGOs modified electrodes using a ferro-ferricyanide electrochemical probe were conducted. In a typical Nyquist plot, the charge transfer resistance (R_{ct}) of the material of interest corresponds to the diameter of the semicircle. The Nyquist plots were analysed by fitting with a Randles equivalent circuit. It could be observed in Figure 7.9A that the bare GC electrode showed a R_{ct} of 0.76 k Ω . The oxygen-rich GO showed a higher R_{ct} at 5.43 k Ω while NaOH-Control showed a slightly lower R_{ct} of 1.63 k Ω as a result of the mild deoxygenation process. The T1-CRGO exhibited the lowest R_{ct} of 0.11 k Ω compared to the other materials. Interestingly, the R_{ct} increased upon longer reaction time (Figure 7.9B); however, the values obtained remained consistently lower than that of GO and NaOH-Control materials. The reduced graphene materials also exhibited lower R_{ct} as compared to NaBH₄-reduced graphene, which resulted with a R_{ct} of 5.78 k Ω , comparatively to the inherent R_{ct} of its bare GC electrode.¹⁸ It is also interesting to note that materials with inherently higher C/O ratios were observed to provide higher R_{ct} values. This suggested a possible electrostatic repulsion between the negatively charged ferro/ferricyanide complexes (redox probe) and a portion of the oxygen containing groups on the graphene materials, which were negatively charged at the working pH.

Figure 7.9 (A) Nyquist diagrams for EIS measurements. Conditions: PBS 50 mM pH 7.2 background electrolyte, 10 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$. Randles equivalent circuit used for data fitting is shown in the inset. **(B)** Average R_{ct} values for all materials and their corresponding standard deviations.

Cyclic voltammetry was also utilized to examine the heterogeneous electron transfer (HET) rate and interaction of the graphene materials with the ferro/ferricyanide redox probe. Figure 7.10A showcased representative cyclic voltammograms of bare GC, GO, NaOH-Control and T1-CRGO. It could be observed that GO showed the highest peak separation, ΔE of 0.47 V. Subsequently, the ΔE observed for NaOH-Control, bare GC, and T1-CRGO were 0.28, 0.23 and 0.14 V respectively. The heterogeneous electron transfer rates for these materials were calculated according to the Nicholson method¹⁹ which showed a decreasing HET in the order: T1-CRGO (k_{obs}^0 : $2.6 \pm 0.6 \times 10^{-3} \text{ cm s}^{-1}$) > bare GC (k_{obs}^0 : $7.5 \pm 4.7 \times 10^{-4} \text{ cm s}^{-1}$) > NaOH-Control (k_{obs}^0 : $2.9 \pm 2.3 \times 10^{-4} \text{ cm s}^{-1}$) > GO (k_{obs}^0 : $4.4 \pm 5.2 \times 10^{-5} \text{ cm s}^{-1}$) [$n = 3$]. The HET rate was also affected by the electrostatic repulsions between the negatively charged redox probe and the oxygen containing groups on the graphene materials, thus resulting in GO having the lowest HET rate. It could be seen that T1-CRGO material resulted with an HET rate about two orders of magnitude higher than the parent GO. The variations in ΔE of all the graphene materials are shown in Figure 7.10B.

The performances of the reduced graphene materials were then probed on their abilities to enhance electron transfer kinetics, a property which plays an important role to facilitate oxidation/reduction of molecules which require high over-potentials for oxidation/reduction. As such, cyclic voltammetry analyses were carried out with an important biomarker, ascorbic acid (AA), using bare GC, GO, NaOH-Control and T1-CRGO modified electrodes as shown in Figure 7.11. The GO showed the highest oxidation potential, E_{AA} at 0.53 V, followed by bare GC at 0.48 V. The NaOH-Control material resulted in a slightly lower E_{AA} at 0.41 V as a result of its deoxygenated nature. On the

Figure 7.10 (A) Cyclic voltammograms on ferro/ferricyanide. Conditions: PBS 50 mM pH 7.2 background electrolyte, 10 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$. Scan rate: 100 mV/s. **(B)** Average peak-to-peak separation (ΔE) values for all materials and corresponding standard deviations.

Figure 7.11 Cyclic voltammograms on ascorbic acid. Conditions: PBS 50 mM pH 7.2 background electrolyte, 5 mM ascorbic acid. Scan rate: 100 mV/s.

other hand, T1-CRGO showed the lowest E_{AA} at 0.31 V, coupled with an oxidation wave starting potential at -0.13 V. It was clear that the reduced graphene material obtained using thiourea dioxide showed the lowest over-potential for the oxidation of ascorbic acid. This indicated that the reduced graphene materials produced with thiourea dioxide were potentially suitable for electrochemical sensing or biosensing applications with improved performances.

7.3 Discussion

The reduction of graphene oxide to graphene with thiourea dioxide as a reducing agent showed promising results. Thiourea dioxide has a long history of application as a reducing agent in the paper, textile and printing industries. As a common industrial chemical, thiourea dioxide is generally cheap and easy to handle. Chemical reactions with thiourea dioxide produce wastes such as sodium sulphite and urea, which are common commercial wastes with standard waste management protocols already in place. As such, this method is foreseen to be a reliable, safe, and low cost approach towards commercially viable large-scale production of graphene.

More importantly, this method was initially attempted as it was backed by previous studies based on synthetic chemistry. It was unexpected that this method allowed for the production of high quality reduced graphene with a high C/O ratio. Most of the oxygen containing groups were reduced based on the structural characterisations by XPS and FTIR spectroscopy. Moreover, the graphene materials provided excellent electrochemical performances, showing low charge transfer resistance and catalytic properties towards the oxidation of ascorbic acid, an important biomarker.

While working towards the removal of oxygen containing groups on graphene oxide based on methods supported by the knowledge of synthetic chemistry, there is also a need to investigate methods that are more robust and could target the specific removal of hydroxyl groups to achieve a total removal of oxygen containing groups. Hydroxyl groups were targeted since most reducing agents reduced oxygen containing groups, *theoretically at best*, only to hydroxyl moieties. The following **Chapters 8** and **9** attempted to address this.

7.4 Experimental

7.4.1 Materials

Graphite was obtained from Asbury Carbons. Fuming nitric acid (> 90 %) was obtained from J.T. Baker. Sulphuric acid (95–98 %), potassium chlorate (98 %), hydrochloric acid (37 %), *N,N*-dimethylformamide (DMF), ethanol, sodium hydroxide, thiourea dioxide, potassium phosphate dibasic, sodium phosphate monobasic, potassium ferrocyanide, potassium chloride, ascorbic acid were purchased from Sigma-Aldrich, Singapore. Glassy carbon electrodes with diameters of 3 mm were obtained from Autolab, the Netherlands.

7.4.2 Apparatus

A JEM-1400 field-emission transmission electron microscope (JEOL, Japan) operating at 100 kV was used to acquire TEM images. TEM samples solutions (in DMF, 1 mg/mL, ultrasonicated for 5 min) were loaded on the TEM grid directly by dropping 5 μ L of the solution onto the carbon coated side of the grid. A filter paper was used to wick off the excess solution on the TEM grid and dried overnight. A JEOL-7600F semi-in-lens FE-SEM, operating in gentle-beam mode at 2 kV was used to acquire SEM images. The solid samples were transferred to a carbon tape held onto a SEM sample holder for analyses. The analyses of the samples were carried out at an average working distance of 8 mm. X-ray photoelectron spectroscopy was performed with a Phoibos 100 spectrometer and a monochromatic Mg X-ray radiation source (SPECS, Germany). Both survey and high-resolution spectra for C1s were collected. Relative sensitivity factors were used for evaluation of C/O ratios from survey spectra XPS measurements. XPS samples were

prepared by coating carbon tape with a uniform layer of the materials under study. Raman spectroscopy analyses were performed using a confocal micro-Raman LabRam HR instrument from Horiba Scientific in backscattering geometry with a CCD detector, a 514.5 nm Ar laser and a 100× objective mounted on a Olympus optical microscope. The calibration was initially made using an internal silicon reference at 520 cm^{-1} and gave a peak position resolution of less than 1 cm^{-1} . The spectra were measured from 1000 to 3000 cm^{-1} . Attenuated total reflectance Fourier transform infrared measurements were carried out on a PerkinElmer Spectrum 100 system coupled with a universal ATR accessory. A diamond/ZnSe was used as the ATR crystal. All voltammetric experiments were performed on a μ Autolab type III electrochemical analyzer (Eco Chemie, The Netherlands) connected to a personal computer and controlled by General Purpose Electrochemical Systems Version 4.9 software (Eco Chemie).

7.4.3 Procedures

Graphite oxide. Prepared according to the Staudenmaier 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 $0\text{ }^\circ\text{C}$ for 15 min. 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 min 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 hr at room temperature. On completion, the mixture was then 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 hr before use.

Chemically reduced graphene oxide (CRGO) was obtained from the reaction of thiourea dioxide with graphene oxide (subjecting graphite oxide through ultrasonication [37 kHz] for 3 hr in the water). To 50 mg of graphene oxide in 31.2 mL of water was added 18.7 mL of ethanol and 1.04 g of NaOH in portions. 1.4 g of thiourea dioxide was added in portion wise over 15 min. The mixture was refluxed at 90 °C for 1, 2, 5, 10 or 20 hr (consequently labelled as T1-, T2-, T5-, T10- and T20-CRGO). A control experiment carried out for 2 hr in the same set of conditions but in the absence of thiourea dioxide was done simultaneously to test the effect of NaOH on the reduction process (labelled as NaOH-Control). Upon completion, the mixture was filtered under vacuum and washed repeatedly with ethanol followed with water until a neutral pH of the filtrate was obtained. The solid samples were dried in an oven at 50 °C for 48 hr before usage.

Electrochemical characterization of materials. Immobilization of the carbon materials onto the glassy carbon working electrode was performed firstly by preparing a suspension of the desired material with a concentration of 1 mg/mL in DMF. The CRGO suspensions were ultrasonicated for 5 min prior to depositing 1 μ L of aliquot of the appropriate suspension onto the electrode surface. The DMF solvent was then allowed to evaporate at room temperature, leaving a randomly distributed film onto the GC electrode surface. Electrochemical experiments were performed by using 50 mM phosphate buffer (pH 7.2) as supporting electrolyte for ascorbic acid and ferro/ferricyanide (10 mM) redox probe at room temperature (25 °C). The GC electrode surfaces were renewed by polishing with 0.05 mm alumina particles on a cloth (DP-Plus, Streurs) before each voltammetry measurement.

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Chapter 8

Novel methods for the reduction of graphene oxide:

Method IV

Regeneration of sp^2 carbon network *via* dehydrobromination

8.1 *Introduction*

8.2 *Results*

8.2.1 *Structural characterisation of the graphene materials*

8.2.2 *Electrochemical characterisation of the graphene materials*

8.2.3 *Validation of the dehydrobromination process*

8.3 *Discussion*

8.4 *Experimental*

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

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Contributions:

C. K. Chua conducted the experiments. M. Pumera directed the project. All authors contributed to discussions and wrote the manuscript.

8.1 Introduction

The removal of oxygen containing groups *via* chemical reducing agents discussed in **Chapters 5, 6 and 7**, mainly lithium aluminium hydride, substituted borohydrides and thiourea dioxide (and typical reducing agents such as hydrazine), do not, *theoretically*, remove the oxygen containing groups completely. Instead, these reducing agents (with a rare exception for thiourea dioxide reactivity towards α,β -epoxy ketones) are according to standard and decades of accumulated and proven knowledge to reduced, *at best*, the oxygen containing groups to hydroxyl groups. Even powerful reducing agents, such as lithium aluminium hydride, are bound to this limitation. On the other hand, the usage of hydrazine as a reducing agent often introduced the formation of pyrazole groups onto the edges of graphene lattices.¹ These reduction methods would, as such, not lead to the re-establishment of sp^2 hybridization between carbon atoms (Scheme 8.1).

In this part of the study, a standard synthetic chemistry method for the removal of oxygen containing groups from the graphene oxide lattices along with the regeneration of sp^2 bonds onto the graphene sheets were introduced. This approach has a strong synthetic chemistry foundation and was based on the substitution of hydroxyl groups by a bromine moiety with consequent dehydrohalogenation, as shown in Scheme 8.2. The dehydrohalogenation reaction led to the regeneration of sp^2 bonds, which were previously sp^3 bonds instead. (Scheme 8.1D).

Scheme 8.1 Fragments of targeted reduction sites on graphene oxide using (a) NaBH₄, (b) LiAlH₄, (c) N₂H₄, and (d) HBr–KOTu.

Scheme 8.2 Synthetic route towards dehydrobrominated graphene.

8.2 Results

The dehydrobromination reaction leading to the restoration of sp^2 network on graphene surfaces in this work proceeded after the oxidation of graphite to graphene oxide. The first step of this route was the bromine substitution of hydroxyl groups on the graphene oxide surface. Incorporation of bromine atoms into the graphene sp^2 carbon network was achieved through a bromination step with hydrobromic acid. The treatment of hydrobromic acid resulted in brominated graphene (CRGO-Br), likely through ring openings of epoxide moieties and/or substitution reactions with hydroxyl groups. The subsequent treatment with potassium *tert*-butoxide, a strong and non-nucleophilic base frequently applied in synthetic chemistry to achieve a dehydrohalogenation effect, resulted in a dehydrobrominated graphene (CRGO). The efficiencies and performance of the chemical treatments were then evaluated with X-ray photoelectron spectroscopy (XPS), Raman spectroscopy and conductivity measurement techniques.

8.2.1 Structural characterisation of the graphene materials

X-ray photoelectron spectroscopy is a crucial surface analysis technique that is capable of providing surface elemental composition (survey scan) and the type of chemical bonds (core level scan) present on a material. XPS survey scans of GO, CRGO-Br and CRGO are shown in Figure 8.1A. The semi-quantitative analysis on the GO survey scan gave an atomic C/O ratio of 2.4. The low atomic C/O ratio indicated a well oxygenated GO material, which usually consisted of hydroxyl, epoxide, carbonyl and carboxyl moieties. On the other hand, CRGO-Br and CRGO displayed higher atomic C/O ratios of 6.3 and 7.1, respectively. The increasing atomic C/O ratios after the step-wise chemical treatments indicated successive removals of oxygen-containing groups from the graphene backbone. Treatment with a strong base could result in a partial deoxygenation process as well.² As expected from the treatment with hydrobromic acid, the survey scan of CRGO-Br revealed the presence of Br3s, Br3p and Br3d peaks, which accounted for about 6.8 % collectively. The Br peaks disappeared upon further treatment with potassium *tert*-butoxide, which supported the occurrence of a dehydrobromination step.

Consequently, the removal of the oxygen-containing groups as indicated by the increasing atomic C/O ratios from GO to CRGO was investigated using high resolution C1s core level scans. High resolution XPS scans enable detailed evaluation of the changes on the type of oxygen containing groups present on the graphene materials. A typical C1s core level spectrum could be deconvoluted into several peaks centred at 284.5 eV (C=C, sp² graphitic carbon), 285.5 eV (C-C, sp³ carbon), 286.5 eV (C-O, epoxide and hydroxyl), 288.5 eV (C=O, carbonyl) and 289.5 eV (O-C=O, carboxyl). A peak centred at 291 eV is attributed to the π - π^* shake-up satellite of the sp² graphitic carbon peak at

Figure 8.1 XPS survey (A) and high-resolution XPS C1s core spectra (B) of GO, CRGO-Br, and CRGO materials.

Figure 8.2 High-resolution XPS spectra of (A) Br3d and (B) Br3p of the CRGO-Br material.

284.5 eV, which is a characteristic of conjugated system. As shown in Figure 8.1B, GO consisted of a high percentage of C–O moieties which contributed up to 52.1 % as compared to a 44.1 % of C=C graphitic carbon. Treatment with hydrobromic acid resulted in CRGO-Br, with a higher amount of C=C graphitic carbon at 61.4 % and a drastically lower amount of C–O bond at only 14.7 %. The lower amount of C–O bond was due to the substitution of hydroxyl groups with bromine and the partial reductive effect of hydrobromic acid. On top of that, the C=C graphitic carbon peak was much more defined as compared to that of GO, which signified a more ordered and homogeneous chemical environment. Upon addition of potassium *tert*-butoxide, the amount of C=C graphitic carbon increased to 68.6 % while the amount of epoxide and hydroxyl groups reduced to 9.6 %. The amount of oxygenated groups on CRGO was lower than that of CRGO-Br due to the partial deoxygenation effect of the strong base. Moreover, the dehydrobromination effect resulted in a higher amount of C=C graphitic carbon. In comparison to a reduced graphite oxide obtained *via* a hydrazine reduction method, it showed a 72.8 % of C=C graphitic carbon and 5.2 % of C–O bonds (data not shown). The CRGO produced in this work has a comparable but lower amount of C=C graphitic carbon. This could be due to the remaining hydroxyl groups from the opening of epoxide moieties with the bromine anion. Despite the higher content of C–O bonds, our initial theoretical prediction on the regeneration of the sp^2 carbon network is supported by the changes observed in the XPS C1s carbon spectra in Figure 8.1B.

High-resolution XPS scans were also carried out on the Br3d and Br3p core level peaks (previously observed in the wide scan of CRGO-Br, Figure 8.1A) of CRGO-Br as shown in Figure 8.2. In Figure 8.2A, the prominent peaks at 70.8 eV ($3d_{5/2}$) and 72.0 eV ($3d_{3/2}$) accounted for the presence of C–Br bonds. The peak area ratio of C–Br in Br3d

was 65.4 %, which invariably suggested the formation of covalently bonded bromine on the graphene network.^{3,4} This was also supported by the high-resolution Br3p core level spectrum in Figure 8.2B which present C–Br peaks at 190.3 and 183.6 eV, accounting up to 66.5 % of the peak area ratio. The presence of physically adsorbed Br[−] in Br3d (68.6 and 67.8 eV) and Br3p (187.9 and 181.2 eV) spectra was noted as well. While the peak area ratio of C–Br was >65 %, it unequivocally supported the successful bromination on the graphene carbon network.

Consequently, the graphene materials were further investigated by Raman spectroscopy, using a 514.5 nm laser excitation. A typically Raman spectrum shows two prominent networks of graphene. The D band ($\sim 1357\text{ cm}^{-1}$, symmetry A_{1g} mode) usually results from the presence of sp^3 carbons or defects on the graphene sheets while the G band ($\sim 1580\text{ cm}^{-1}$, E_{2g} mode of sp^2 carbon atom) represents the presence of pristine sp^2 carbon. The calculated ratio of D and G bands peak intensity (I_D/I_G) is a common indication of the extent of defects on graphene materials. In Figure 8.3, the D and G bands on GO were broad and this was highly indicative of a more disordered chemical environment. The I_D/I_G ratios for GO, CRGO-Br and CRGO were 0.75, 1.32 and 1.31, respectively. An increment of the I_D/I_G ratio upon treatment with hydrobromic acid indicated formation of large edges and defects on the graphene carbon network. This could also indicate a smaller size of graphitic domains. Further treatment with potassium *tert*-butoxide did not change the I_D/I_G ratio drastically. Although the dehydrobromination step should result in a lower I_D/I_G ratio, the amount of bromine present on the graphene network was possibly insignificant to cause a drastic change in the I_D/I_G ratio. Apart from that, the evolution of 2D ($\sim 2700\text{ cm}^{-1}$, two-phonon double

Figure 8.3 Raman spectra of GO, CRGO-Br and CRGO materials.

resonance process) peaks on CRGO-Br and CRGO signalled the presence of few-layer graphene in the materials. As compared to the reduced graphene material obtained from hydrazine treatment, a higher density of defect with a I_D/I_G ratio of 1.42 was observed on the hydrazine-reduced graphene material (data not shown).

8.2.2 Electrochemical characterisation of the graphene materials

The electrical performances of the graphene materials were primarily evaluated using an interdigitated gold electrode platform. The platform allows for a qualitative comparison of the conductivities for the graphene materials based on the concept of 'ohmic' behaviour, whereby a typical I–V curve would present a slope directly proportional to its conductivity.⁵ As shown in Figure 8.4, the conductivities of GO, CRGO-Br and CRGO were directly compared against a common chemically reduced graphene material obtained via hydrazine reductant (CRGO-N). The GO exhibited a very high resistance which resulted in almost negligible current signal over the scanned potentials. This could most likely attribute to the insulating feature of GO. On the other hand, the remaining three materials gave ohmic behaviours with linear I–V curves. The CRGO material gave the steepest slope among the three materials at 51 mA/V thus indicating the highest conductivity. A brominated CRGO-Br showed the second highest slope at 39 mA/V. Interestingly, the CRGO-N had the lowest conductivity with a slope of 34 mA/V. These measurements indicated that an enhanced chemically reduced graphene with a high conductivity could be achieved *via* a dehydrobromination treatment, which regenerates the disrupted sp^2 carbon network of graphene oxide.

Figure 8.4 Current–voltage curves of GO, CRGO-Br and CRGO materials.

8.2.3. Validation of the dehydrobromination process

In order to validate the dehydrobromination process on graphene oxide, a control experiment was carried out simultaneously. The GO was treated with potassium *tert*-butoxide (produced G-A) prior to treatment with hydrobromic acid (produced G-B) to exclude the dehydrobromination step. The XPS survey and C1s core level spectra (Figure 8.5) showed an increasing atomic C/O ratio upon chemical treatments, which was in tune with the deoxygenative and reductive effects of the procedures. More importantly, the final graphene product obtained exhibited a lower conductivity as compared to CRGO based on the Au-IDE platform (Figure 8.6). The Raman spectra (data not shown) highlighted the changes in the I_D/I_G ratio of G-A to G-B at 0.92 and 1.13, respectively. This has invariably ascertained that the dehydrobromination effect played a crucial role in enhancing the electronic properties of the chemically reduced graphene material by regenerating the broken sp^2 carbon network.

Figure 8.5 XPS survey (A) and high-resolution XPS C1s core spectra (B) of GO, CRGO-Br, and CRGO materials.

Figure 8.6 Current-voltage curves of GO, CRGO-Br, CRGO, G-A, G-B and CRGO-N materials.

8.3 Discussion

While working towards the removal of hydroxyl groups and the enrichment of sp^2 carbon moieties on reduced graphene material, the potential of a pre-reduction methods using hydrobromic acid and followed by a dehydrobromination step was demonstrated. This method allowed for the conversion of hydroxyl groups to bromine groups and subsequent elimination of the halide *via* dehydrobromination, which resulted in a simultaneous renewal of the sp^2 carbon network of reduced graphene material. In fact, the graphene material showed a higher electronic conductivity as opposed to graphene produced using a conventional hydrazine reductant.

This method could potentially be applied on graphene oxide which has undergone a prior reduction step with a common reducing agent, such as sodium borohydride, to achieve a near pristine chemically reduced graphene. Precise manipulations of the structural composition of reduced graphene materials based on the knowledge of synthetic chemistry provide an opportunity to fine-tune the structural entity of graphene materials. This is, however, preferably achieved in a one-step reaction. In our continuous effort towards the effective removal of oxygen containing groups and the renewal of sp^2 carbon network of graphene oxide, alternate methods which allow for the selective removal of specific oxygen moieties in a single step was examined as well, as highlighted in **Chapter 9**.

8.4 Experimental

8.4.1 Materials

Graphite (natural, 45 μm) was obtained from Asbury Carbons, NJ, USA. Sulphuric acid (95–98 %) and hydrochloric acid (37 %) were obtained from Sigma-Aldrich, Singapore. Fuming nitric acid (>90 %) was obtained from J.T. Baker. Hydrazine monohydrate, potassium *tert*-butoxide, hydrobromic acid (48 %) and potassium chlorate (98 %) were obtained from Alfa Aesar, Singapore.

8.4.2 Apparatus

X-ray photoelectron spectroscopy was performed with a Phoibos 100 spectrometer and a monochromatic Mg X-ray radiation source (SPECS, Germany). Both survey and high-resolution spectra for C1s, Br3d and Br3p were collected. Relative sensitivity factors were used for evaluation of C/O ratios from XPS survey spectra. XPS samples were prepared by adhering the surface of a carbon tape to a uniform layer of the materials under study. Raman spectroscopy analysis was performed using a confocal micro-Raman LabRam HR instrument from Horiba Scientific in backscattering geometry with a CCD detector, a 514.5 nm Ar laser and a 100 $\times$ objective mounted on an Olympus optical microscope. The calibration is initially made using an internal silicon reference at 520 cm^{-1} and gives a peak position resolution of less than 1 cm^{-1} . The spectra are measured from 1000 to 3000 cm^{-1} . All voltammetric experiments were performed on a $\mu\text{Autolab}$ type III electrochemical analyzer (Eco Chemie, The Netherlands) connected to a personal computer and controlled by General Purpose Electrochemical Systems Version 4.9 software (Eco Chemie).

8.4.3 Procedures

Bromination of graphene oxide. Graphite oxide was produced using the Staudenmaier method. Graphite oxide (21 mg) was ultrasonicated (150 W) in ultrapure water (21 mL) for 3 hr to give graphene oxide. Hydrobromic acid (5 mL) was added drop-wise into the graphene oxide suspension and left under vigorous stirring at 110 °C for 24 hr. The suspension was cooled to room temperature and filtered/washed over a PTFE membrane (0.2 µm) with ultrapure water and acetone to give CRGO-Br upon drying at 50 °C for 5 days. Precautions should be taken when handling corrosive acids.

Dehydrohalogenation of brominated graphene. Brominated graphene (9 mg) was dissolved in THF (9 mL) and ultrasonicated (150 W) for 30 min. Potassium *tert*-butoxide (120 mg, 1.07 mmol) was then added to the suspension and refluxed for 30 min. Precaution should be taken when handling potassium *tert*-butoxide as it is highly corrosive and can cause serious chemical burns. The suspension was cooled to room temperature and filtered/washed over a PTFE membrane (0.2 µm) with ultrapure water and acetone. It was then washed with 1 M HCl (20 mL) followed by ultrapure water until a neutral pH was obtained. Finally, the solid was washed with diethyl ether to give CRGO upon drying at 50 °C for 5 days.

Reduction of graphene oxide using hydrazine. Graphite oxide (50 mg) was dispersed in ultrapure water to give a 1.0 mg/mL colloidal solution and ultrasonicated (150 W) for 3 hr. Hydrazine monohydrate (2 mL, 32.1 mmol) was added drop-wise and the reaction mixture was left to stir under reflux for 24 hr. 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-N upon drying at 50 °C for 5 days. Precautions should be taken when handling carcinogenic hydrazine monohydrate.

Conductivity measurement of materials. Current–voltage measurements (I–V curve) were conducted with an interdigitated gold electrode platform by depositing 2 μm of the material suspension (prepared at 1 mg/mL concentration in water) onto an electrode surface with 10 μm spacing. The electrode was then dried under a lamp for 20 min, leaving a randomly deposited material film on the interdigitated area bridging the two Au electrode bands. The I–V curves were obtained by linear sweep voltammetric measurements at 20 mV/s scan rate. The displayed data corresponds to the average from three measurements.

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Chapter 9

Novel methods for the reduction of graphene oxide: Method V

Selective removal of hydroxyl groups *via* ethanethiol-aluminium chloride complex

9.1 *Introduction*

9.2 *Results*

9.2.1 *Morphological characterisation of the graphene materials*

9.2.2 *Structural characterisation of the graphene materials*

9.2.3 *Electrochemical characterisation of the graphene materials*

9.3 *Discussion*

9.4 *Experimental*

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

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Contributions:

C. K. Chua conducted the experiments. M. Pumera directed the project. All authors contributed to discussions and wrote the manuscript.

9.1 Introduction

The success of the dehydrohalogenation method has prompted us to look for more alternatives towards the removal of hydroxyl groups from graphene oxide sheets. On top of being effective in removing hydroxyl groups, potential methods should be able to restore the sp^2 carbon backbone of graphene oxide in a simple chemical step.

In this part of the study, a standard synthetic method for the selective removal of hydroxyl groups from the graphene oxide lattice to re-expose the sp^2 carbon bonds on the graphene sheets was investigated. This method was based on the selective defunctionalisation by radical intermediates, with ethanethiol-aluminium chloride complexes to selectively remove hydroxyl groups from the graphene oxide sp^2 carbon lattice.¹ A previous work by Fujita and co-workers has indicated the effective removal of alkoxy, peroxy, hydroxyl, alkylthio and halo groups from the parent aromatic moieties.¹ Thus, this approach was expected to be useful for the removal of hydroxyl and regeneration of sp^2 carbon bonds, as well as to provide a selective defunctionalisation method that could be utilized to tailor the types of oxygen functionalities preferred on the graphene sheets (Scheme 9.1). Moreover, the method was foreseen to work in conjunction with other reducing agents in a *two-step* process to obtain graphene materials with minimal oxygen functionalities.

Scheme 9.1 Fragments of small graphene oxide sheets treated with LiAlH_4 , NaBH_4 and EtSH-AlCl_3 .

9.2 Results

The investigation on the selective defunctionalisation of hydroxyl groups was carried out in the presence of an ethanethiol-aluminium chloride (EtSH-AlCl₃) complex in dry THF solvent (see experimental section for detailed procedure) to provide a reduced graphene material. In conjunction with the process for the selective removal of hydroxyl groups, three other control experiments were carried out at a similar temperature, 70 °C, and which included graphene oxide stirred in the absence of ethanethiol and aluminium chloride (GO-C), graphene oxide stirred in the presence of only ethanethiol (ES-C) or aluminium chloride (AC-C). Aluminium chloride functioned as a Lewis acid while ethanethiol acted as a nucleophile to result in a reductive displacement of hydroxyl group. The reaction underwent the formation of an intermediate sulphide group with a consequent one electron transfer process.¹ Ethanethiol was converted to diethyl disulphide thereafter while aluminium chloride was regenerated; both could be removed in the final washing steps.

Subsequent structural, morphological and electrochemical properties of the obtained graphene materials were carefully characterized and analysed based on high resolution X-ray photoelectron spectroscopy (XPS), Raman spectroscopy, Fourier transform infrared (FTIR), scanning electron microscopy (SEM), electrochemical impedance spectroscopy (EIS), electrochemical cyclic voltammetry (CV) and conductivity analysis.

9.2.1 Morphological characterisation of the graphene materials

The morphological properties of the graphene materials were investigated using scanning electron microscopy technique as shown in Figure 9.1. The SEM analysis of GO showed the presence of heavily stacked and aggregated graphene sheets. On the other hand, the graphene materials obtained from the control experiments (GO-C, ES-C, and AC-C) showed wrinkled, wave-like and less-aggregated graphene sheets. Upon treatment for selective defunctionalization, the graphene sheets of CRGO appeared clean and smooth, with fewer wrinkles and folded edges. The success of the selective defunctionalisation of hydroxyl groups also implied that oxygen containing groups, such as carboxyl, are left untouched. As such, the resulting graphene material displayed good dispersion stability in aqueous solution, as shown in Figure 9.2.

Figure 9.1 SEM images for GO, GO-C, ES-C, AC-C and CRGO.

Figure 9.2 GO and CRGO dispersed in aqueous solution: dispersion of 1 mg/mL of material in ultrapure water after ultrasonication for 10 min.

9.2.2 Structural characterisation of the graphene materials

X-ray photoelectron spectroscopy is a highly sensitive and semi-quantitative analysis method that is valuable for probing the surface elemental compositions of materials. This could be accomplished by an XPS wide scan survey, which translates the kinetic energies of electrons into specific binding energies that correspond to distinctive elements found up to a depth of 10 nm from the surface of the materials. On top of that, high resolution XPS core level scans are able to provide information on the chemical environment for the elements of interest. The XPS survey scans for the graphene materials are shown in Figure 9.3A. The survey scans highlighted the prominent presence of the O1s and C1s peaks; these atoms formed the main composition of graphene oxide based materials. The atomic ratio of carbon to oxygen elements (C/O ratio) obtained from the survey scans provided a good sense on the extent of oxidation/reduction of the graphene materials. Based on the analysis, GO, GO-C, ES-C, AC-C and CRGO provided C/O ratios of 2.16, 2.22, 2.33, 2.56, and 4.71, respectively. The control experiments (GO-C, ES-C and AC-C) showed negligible changes in the C/O ratios, which indicated that the presence of either the ethanethiol or aluminium chloride alone would not result in the reduction/selective defunctionalization of hydroxyl groups on graphene oxide. However, upon the formation of EtSH-AlCl₃ complexes, the selective removal of hydroxyl groups from the graphene oxide surfaces was achieved, as shown by the higher C/O ratio provided by the XPS survey scan of CRGO.

Figure 9.3 X-ray photoelectron spectroscopy spectra for GO, GO-C, ES-C, AC-C and CRGO. (A) Survey and (B) C1s core-level spectra.

In an attempt to gain more insight into the specific defunctionalisation of graphene oxide, high resolution XPS scans were carried out on the C1s core level as shown in Figure 9.3B. A typical C1s core level spectrum of graphene materials often provides characterized Gaussian peaks fitted to the presence of C=C (graphitic sp^2 C, 284.5 eV), C-C (sp^3 C, 285.7 eV), C-O (hydroxyl or epoxide, 286.8 eV), C=O (carbonyl, 288.1 eV) and O-C=O (carboxylic, 289.1 eV) bond types. The presence of $\pi-\pi^*$ (290.6 eV) shake up satellite peak is often observed in graphene materials that displayed an improved characteristic of aromatic/conjugated systems and a more orderly chemical environment. The C1s core level spectrum of graphite oxide indicated significant presence of C-O and C=O bond types. The ultrasonication treatment towards graphene

oxide and thermal treatment at 70 °C did not result in significant changes in the C1s core level spectrum for GO-C. The control experiment with only the addition of ethanethiol showed a similar observation. It is interesting to note that treatment of aluminium chloride alone resulted in a small reduction of the C-O peak, which was lower in intensity than the C=C peak. However, upon treatment of graphene oxide with the EtSH-AlCl₃ complex, an obvious reduction in the intensity of the C-O peak was observed. Despite that, the presence of C=O and O-C=O bond types were still rather prevalent in the CRGO C1s core level spectrum. This highlighted the specific defunctionalisation of hydroxyl groups in graphene oxide; the the remaining C-O bond could most likely be attributed to the presence of small amounts of remaining epoxide groups or inaccessible hydroxyl groups.

Apart from that, Raman spectroscopy analyses were carried out on the graphene materials, as shown in Figure 9.4. Raman spectroscopy is often utilized to probe the quality of the graphene materials. More specifically, a typically Raman spectrum of a graphene material consists of D (~1200 cm⁻¹, defect, breathing mode of sp² carbon) and G (~ 1580 cm⁻¹, graphitic, sp² bonded carbon) bands. The intensity ratio of the D and G bands (I_D/I_G ratio) is often used as an indicator for the extent of defects that occurs in a graphene material. As observed from the analysis, GO, GO-C, ES-C, AC-C and CRGO showed I_D/I_G ratios of 0.93, 0.91, 0.89, 0.96 and 1.07, respectively. The I_D/I_G ratios for the graphene materials obtained from the control experiments (GO-C, ES-C and AC-C) indicated a similar extent of defects due to the presence of oxygen containing groups. Moreover, the broad D band indicated significant structural disorders with the presence of sp³ hybridised carbons and short ranged sp² carbon moieties.

Figure 9.4 Raman spectra for GO, GO-C, ES-C, AC-C and CRGO.

Upon the selective removal of hydroxyl groups, CRGO registered a higher I_D/I_G ratio at 1.07. The increment of I_D/I_G ratio is prevalent and common for reduced graphene materials, as previously observed in several other studies on reduction of graphene oxide.²

To further understand the structural properties for the resulting graphene materials, analysis with attenuated total reflectance Fourier transform infrared spectroscopy was carried as shown in Figure 9.5. Graphite oxide provided peaks corresponding to the O-H stretching mode ($\sim 3200\text{ cm}^{-1}$), C-OH vibrational mode (~ 1380 and $\sim 1050\text{ cm}^{-1}$), C=O vibrational mode ($\sim 1718\text{ cm}^{-1}$) and C-O-C from epoxide groups ($\sim 870\text{ cm}^{-1}$). The unoxidised graphitic region of the graphene materials typically provided

Figure 9.5 ATR-FTIR spectra for GO, GO-C, ES-C, AC-C and CRGO.

a peak at $\sim 1600\text{ cm}^{-1}$. The control experiments (GO-C, ES-C, and AC-C) gave similar FTIR spectra as that of graphite oxide, albeit with a lower peak intensity of C=O vibrational mode. This was well correlated with the XPS C1s core level spectra, which showed smaller presences of the C=O bond type in the control experiments. Upon selective defunctionalisation treatment, the O-H stretching mode on CRGO disappeared and the remaining characteristic peaks were greatly diminished or non-existent. This observation was highly consistent with earlier high-resolution XPS analyses too.

9.2.3 Electrochemical characterisation of the graphene materials

The electrochemical properties of the graphene materials were examined based on electrochemical impedance spectroscopy and cyclic voltammetry techniques. Although these techniques have different methods of measurement, they ultimately measure the resistance of the graphene materials towards heterogeneous electron transfer. The EIS technique applies a small alternating-current voltage perturbation to the system at various frequencies, on the order of 1–10 millivolts, whereas the cyclic voltammetry technique applies a large perturbation to the system on the order of hundreds of millivolts. The ability of graphene materials to transfer and exchange electrons with their surrounding molecules in the environment is highly influenced by their surface morphologies. Both the EIS and cyclic voltammetry analyses were carried out in a ferro/ferricyanide electrochemical probe.

The Nyquist plots obtained from the EIS study are shown in Figure 9.6. In a typical Nyquist plot, the diameter of the semi-circle corresponds to the charge transfer resistance (R_{ct}) of the material. Materials that exhibit higher resistances towards charge transfer would result in higher R_{ct} values. Since EIS is highly sensitive to the surface morphologies of the materials, slight changes of the surfaces would result in significant changes in their R_{ct} values. The bare glassy carbon electrode provided a R_{ct} of 0.55 k Ω , whereas GO gave a R_{ct} of 4.56 k Ω . The control experiments resulted in GO-C, ES-C and AC-C with R_{ct} values of 1.53, 1.10 and 1.16 k Ω , respectively. Upon the selective defunctionalisation treatment of GO, the CRGO material provided the lowest R_{ct} value at 0.09 k Ω . Based on this observation, the graphene materials that contained the largest amount of oxygen containing groups (i.e., lower C/O ratio from XPS analyses) provided higher R_{ct} values. This is probably due to the electrostatic repulsion between both the

Figure 9.6 Nyquist diagrams for EIS measurements for GO, GO-C, ES-C, AC-C, CRGO and bare GC. Conditions: PBS (50 mM), pH 7.2 background electrolyte, $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (10 mM). Inset: Randles equivalent circuit used for data fitting.

Figure 9.7 Cyclic voltammograms on ferro/ferricyanide for GO, GO-C, ES-C, AC-C, CRGO and bare GC. Conditions: PBS (50 mM), pH 7.2 background electrolyte, $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (10 mM). Scan rate: 100 mV/s.

negatively charged oxygen containing groups on the graphene materials and the ferro/ferricyanide probe at the working pH of the electrochemical analyses. This is

generally consistent with previous findings that show C/O ratios correlated with R_{ct} .^{3,4} More importantly, the EIS analyses highlighted the improved heterogeneous charge transfer ability of the CRGO material as a result of the selective removal of hydroxyl groups from the GO material.

Following that, cyclic voltammetry analyses were carried out to reaffirm the results of the electrochemical impedance spectroscopy, as shown in Figure 9.7. In fact, CV scans were carried out prior to each impedimetric scans to probe the heterogeneous electron transfer (HET) rate of the graphene materials. By using a similar electrochemical probe as in the impedimetric analyses, the ferro/ferricyanide system provided a reversible CV scan in which the peak-to-peak separation (ΔE) could be utilized for the measurement of the HET based on the Nicholson method.⁵ The ferro/ferricyanide electrochemical probe is surface sensitive, thus the number of oxygen containing groups on the graphene surfaces could be easily correlated to the HET rate. Graphene materials that exhibited smaller peak-to-peak separations account for higher HET rates. The cyclic voltammograms of bare GC, GO, GO-C, ES-C, AC-C and CRGO provided ΔE values of 0.19, 0.52, 0.26, 0.21, 0.28, and 0.14 V, respectively. The calculated heterogeneous electron transfer rate subsequently indicated an increasing value for the graphene materials in the order of: GO (k_{obs}^o : $1.5 \pm 0.3 \times 10^{-5} \text{ cm s}^{-1}$) < AC-C (k_{obs}^o : $3.8 \pm 2.0 \times 10^{-4} \text{ cm s}^{-1}$) < GO-C (k_{obs}^o : $4.7 \pm 1.0 \times 10^{-4} \text{ cm s}^{-1}$) < ES-C (k_{obs}^o : $9.7 \pm 0.2 \times 10^{-4} \text{ cm s}^{-1}$) < bare GC (k_{obs}^o : $1.2 \pm 0.1 \times 10^{-3} \text{ cm s}^{-1}$) < CRGO (k_{obs}^o : $2.4 \pm 0.4 \times 10^{-3} \text{ cm s}^{-1}$). The GO material gave the lowest HET rate and the CRGO material provided the highest HET rate, at about two magnitudes higher. The results were in tune with the EIS analyses carried out earlier, which showed similar implications for the successful selective removal of the hydroxyl groups.

Figure 9.8 Current–voltage curves of GO, GO-C, ES-C, AC-C and CRGO.

Subsequently, the conductivities of the graphene materials were investigated using an interdigitated gold electrode (Au-IDE) system. The analyses on the Au-IDE platform provided I-V curves for each graphene materials in which the slopes of the curves would be directly proportional to their conductivities. This provided a qualitative comparison of the conductivities based on the concept of ‘ohmic’ behaviour. The I-V curves of the graphene materials are shown in Figure 9.8. The GO provided negligible current signal across the scanned potentials as a result of high sheet resistances. The control experiments provided current signals with ohmic behaviours, as indicated by the linear I-V curves. The slopes of the curves were measured in the range of 2-3 mA/V. Upon treatment with the EtSH- AlCl_3 complex, resulting in the selective removal of hydroxyl groups, the CRGO provided an I-V curve with a slope of 75 mA/V. This indicated that the selective removal of hydroxyl groups from the parent graphene oxide greatly improved the electronic properties of the resulting graphene material.

9.3 Discussion

The selective defunctionalisation of hydroxyl moieties from the graphene oxide surfaces, based on a well-established synthetic chemistry method, has been demonstrated. The ethanethiol-aluminium chloride complex has provided a robust method to selectively remove hydroxyl groups. The structural characterisations (X-ray photoelectron spectroscopy and Fourier transform infrared spectroscopy) highlighted the presence of carbonyl and carboxylic groups while most hydroxyl groups were removed. Moreover, the resulting graphene materials exhibited enhanced heterogeneous electron transfer rate, a lower charge transfer resistance and improved conductivity as compared to the parent graphene oxide. The ability to tailor the composition of oxygen containing groups on graphene materials would be important for certain applications (i.e. electrochemical sensing, chemical functionalizations) which may not require the presence of hydroxyl groups. Furthermore, this method could potentially be applied as a stand-alone method or used synergistically with other reducing agents to provide highly reduced graphene materials.

Chapters 5, 6, 7, 8 and 9 have introduced five different approaches that convert graphene oxide to graphene. These reduction methods were expected to provide sensible approaches towards the reduction of graphene oxide. The reducing agents could specifically fine-tune the extent of reduction, provide commercially viable graphene, regenerate the sp^2 carbon network on reduced graphene. A good understanding on the specific chemistry that is occurring on the surface of the graphene oxide materials would allow for a precise control over the properties of the resulting graphene materials with a high level of confidence and certainty. In fact, these reducing agents are capable of providing reduced graphene of different qualities. These tailor

made graphene would potentially find their niche of applications in the near future. Further work would be carried out to utilize these materials in specific applications, while efforts to provide improved conversion techniques would be continuously pursued in our group..

A related research area of these graphene materials is in the area of electrochemical sensing. Since chemical or biological sensors usually require bulk quantities of electrode materials, the top-down production approach towards graphene is favourable for this aim. Moreover, most of the graphene materials provided favourable electrochemical properties for electrochemical sensing purposes. As such, subsequent **Chapters 10** and **11** shifted the focus of this project towards the application of graphene materials for biosensing and as electrochemical sensors in a lab-on-a-chip platform.

9.4 Experimental

9.4.1 Materials

Graphite (< 20 μm), sulphuric acid (95–98 %), sodium nitrate, aluminium chloride, *N,N*-dimethylformamide, potassium phosphate dibasic, sodium phosphate monobasic, potassium ferricyanide, and potassium ferrocyanide were purchased from Sigma-Aldrich, Singapore. Ethanethiol and hydrogen peroxide was obtained from Alfa Aesar, Singapore. Potassium permanganate was obtained from J.T. Baker. Glassy carbon electrode with a diameter of 3 mm was obtained from Autolab, the Netherlands. Milli-Q water (resistivity: 18.2 $\text{M}\Omega\text{ cm}$) was used throughout the experiments.

9.4.2 Apparatus

X-ray photoelectron spectroscopy was performed with a Phoibos 100 spectrometer and a monochromatic Mg X-ray radiation source (SPECS, Germany). Both wide survey and high-resolution spectra for C1s were collected. Relative sensitivity factors were used for evaluation of atomic C/O ratios from XPS wide survey spectra measurements. XPS samples were prepared by coating a carbon tape with a uniform layer of the materials under study. Attenuated total reflectance Fourier transform infrared measurements were carried out on a PerkinElmer Spectrum 100 system coupled with a universal ATR accessory. Raman spectroscopy analysis was performed using a confocal micro-Raman LabRam HR instrument from Horiba Scientific in backscattering geometry with a CCD detector, a 514.5 nm Ar laser and a 100 $\times$ objective mounted on a Olympus optical microscope. The calibration is initially made using an internal silicon reference at 520 cm^{-1} and gives a peak position resolution of less than 1 cm^{-1} . The spectra are measured

from 1000 to 3000 cm^{-1} . A JEOL-7600F semi-in-lens FE-SEM, operating in gentle-beam mode at 2 kV was used to acquire the SEM images. The solid samples were transferred to a carbon tape held onto a SEM holder for analyses. The analyses of the samples were carried out at an average working distance of 6 mm. All voltammetric experiments were performed on a μ Autolab type III electrochemical analyzer (Eco Chemie, The Netherlands) connected to a personal computer and controlled by General Purpose Electrochemical Systems Version 4.9 software (Eco Chemie).

9.4.3 Procedures

Graphite oxide (GO). Graphite oxide preparation with modified Hummers method. Graphite (0.5 g) was stirred with 23.0 mL of sulphuric acid (95–98 %) for 20 min at 0 °C prior to the addition of NaNO_3 (0.5 g) in portions. The mixture was left to stir for 1 hr. KMnO_4 (3 g) was then added in portions at 0 °C. The mixture was subsequently heated to 35 °C for 1 hr. Water (40 mL) was then added into the mixture and resulted in the temperature of the mixture to rise up to 90 °C. The temperature was maintained at 90 °C for 30 min. Additional water (100 mL) was added into the mixture. This was followed by a slow addition of 30 % H_2O_2 (~10 mL). The warm solution was filtered (RC membrane, 0.22 μm) and washed with warm water (100 mL). The solid was subsequently washed with a copious amount of water until a neutral pH was obtained. The materials were kept in the oven at 50 °C for 5 days prior to usage. Precautions should be taken when handling corrosive acids and strong oxidants.

Chemically reduced/ defunctionalised graphene oxide (CRGO). Ethanethiol (2.88 mL, 27.7 mmol) was added into aluminium chloride (2.66 g, 20.9 mmol) in an ice bath and

stirred for 15 min. A dispersion of graphene oxide (1 mg/mL) in dry THF (25 mL) was added into the mixture at 0 °C and stirred for 30 min. Following that, the reaction mixture was heat to reflux (70 °C) for 5 hr. The reaction mixture was subsequently cooled and filtered (PTFE membrane, 0.22 µm), washed with 1 M HCl solution (25 mL) and with ultrapure water until a pH 7 of filtrate was obtained. It was subsequently washed with THF and diethyl ether. The materials were kept in a vacuum oven at 30 °C for 5 days prior to usage. It should be noted that ethanethiol has a strong disagreeable odor and should only be handled in a fumehood.

Control experiments. GO Control (GO-C) was obtained in a similar method as reduced graphene, but in the absence of ethanethiol and aluminium chloride. EtSH Control (ES-C) was obtained in a similar method as reduced graphene, but in the absence of aluminium chloride. AlCl₃ Control (AC-C) was obtained in a similar method as reduced graphene, but in the absence of ethanethiol.

Electrochemical characterisation of materials. The electrochemical experiments were carried out in a 5 ml voltammetric cell at room temperature (25 °C) using a three-electrode configuration. A platinum electrode served as an auxiliary electrode and a Ag/AgCl electrode as a reference electrode; a glassy carbon electrode was utilized as a working electrode. Prior to a measurement, the glassy carbon electrode was polished with 0.05 µm alumina on a polishing cloth. The working electrode was then modified by applying 1 µL of DMF containing 1 mg/mL carbon material and allowed to dry.

Current–voltage measurements (I–V curve) were conducted with an interdigitated gold electrode platform by depositing 2 µL of the material suspension (prepared at 1 mg/mL concentration in water) onto electrode surface with 10 µm spacing. The electrode was

then dried under a lamp for 20 min, leaving a randomly deposited material film on the interdigitated area bridging the two Au electrode bands. The I–V curves were obtained by linear sweep voltammetric measurements at 20 mV/s scan rate. The displayed data corresponds to the average from three measurements.

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Chapter 10

Electrochemical application of graphene I

Electrochemical biosensing – Indirect detection of single nucleotide polymorphism with graphene oxide

10.1 *Introduction*

10.2 *Results*

10.2.1 Structural characterisation of the graphene materials

10.2.2 Electrochemical characterisation of the graphene materials

10.2.3 Graphene materials as electrochemical-active label for genosensing

10.3 *Discussion*

10.4 *Experimental*

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

1. Alessandra Bonanni, **Chun Kiang Chua**, Guanjia Zhao, Sofer Zdeněk, Martin Pumera.

Inherently electroactive graphene oxide nanoplatelets as labels for single nucleotide polymorphism detection. *ACS Nano* **2012**, 6, 8546-8551.

Article can be retrieved from <http://dx.doi.org/10.1021/nn301359y>.

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Contributions:

A. Bonanni conducted most of the experiments and wrote the manuscript. C. K. Chua synthesized the graphene material. G. Zhao contributed to the AFM study. S. Zdeněk contributed to the dynamic light scattering analysis. M. Pumera directed the project.

In **Chapters 10** and **11**, two general electrochemical applications of graphene materials were demonstrated. The unique physical and electrical properties of the graphene materials were exploited for electrochemical biosensing and sensing purposes. On top of that, both of these applications were envisaged to advance the development of point-of-care detection system.

In **Chapter 10**, focus was placed on the biosensing aspect of graphene materials. This chapter highlighted the indirect detection of single nucleotide polymorphism with graphene oxide nanoplatelets. The graphene oxide nanoplatelets would function as an electroactive label, instead of an electrode transducer in conventional biosensors. This novel detection technique provided clear discrimination between single- and double-stranded DNA, which enabled differentiation among complementary, non-complementary, and one-mismatch DNA sequences correlated with Alzheimer's disease.

In the next **Chapter 11**, graphene materials were incorporated as electrode materials for the electrochemical detection system in a lab-on-a-chip platform. The performances of the graphene materials were probed based on the electrophoretic separation of dopamine neurotransmitter and catechol, as well as with nitroaromatic explosives.

Chapters 10 and **11** formed the collective work in addressing Aim 2.

10.1 Introduction

Graphene is an excellent surface for electrochemistry,¹⁻³ and it is often used as an electrode material in sensing⁴⁻⁶ and energy storage⁷⁻⁹ applications. Graphene is inherently redox inactive at typically used potentials, which means that graphene does not show any electrochemically driven redox (oxidation/reduction) behaviours. On the contrary, graphene oxide displays an inherent redox activity that allows for its electrochemical reduction toward reduced graphene materials (refer to **Chapter 5**, Scheme 5.1).¹⁰ The electrochemical reduction method of graphene oxide is used for the preparation of electrode surfaces^{11,12} or for the quantification of reducible oxygen-containing groups.^{13,14} However, there have not been any attempts shown that utilized the inherent electrochemical redox activity of graphene oxide as a label for DNA detection.

Here, the unique inherent electroactivity of graphene oxide nanoplatelets (GeONPs) for the detection of DNA and single-base polymorphism was exploited. The use of graphene oxide nanoplatelets with a high density of oxygen functionalities is beneficial over standard probes, as the signal amplification is very large, whereby a 50 × 50 nm sized graphene oxide platelet corresponds to almost 22000 withdrawn electrons (given the density of reducible groups of 4.3 nm⁻¹ and two-electron reduction per functional group).¹⁴ The unique interaction between graphene oxide nanoplatelets and single-stranded DNA nitrogenous bases, which differs significantly from its interaction with double-stranded DNA due to the shielding of the bases inside the double helix structure were explored.¹⁵ To the best of our knowledge, this was the first time the reduction peak of graphene oxide was used as an analytical signal in general.

In particular for this work, complementary (wild-type), non-complementary (nc), and one-mismatch (mutant) DNA sequences were employed for the hybridization of the electrode surface modified with DNA probes. After the hybridization steps, the electrode surfaces were incubated with a solution containing a certain amount of GeONPs. The different conjugation ability of graphene with single- and double-stranded DNA (dsDNA) sequences was exploited here. It is well known that single-stranded DNA (ssDNA) sequences lie on the surfaces of graphene sheets via π - π stacking interactions.^{16,17} It was also shown that ssDNA and dsDNA possessed different binding affinities for graphene, with the former being more suitable to this aim.^{18,19} In fact, while the nitrogenous bases in ssDNA are free to form π - π stacking with graphene aromatic rings, the dsDNA nitrogenous bases are less accessible for interactions since they are shielded inside the double-helix structure. The different binding ability of GeONPs to DNA hybrids formed with complementary, non-complementary, and mismatch-containing sequences would result in a different amount of GeONPs conjugated to the electrode surfaces upon incubation. As such, this could provide different electrochemical signals to discriminate among wild-type, mutant, and non-complementary DNA in a simple and fast protocol.

10.2 Results

The unique interactions between graphene oxide nanoplatelets and DNA strands for single-nucleotide polymorphism detection were explored in the following text. The contrasting interactions between single-stranded and double-stranded DNAs with graphene oxide nanoplatelets were demonstrated. Consequently, the unique properties of graphene oxide as an inherently electroactive label were exploited.

10.2.1 Structural characterisations of the graphene materials

GeONPs were obtained by ultrasonication²⁰ of an aqueous solution of graphite oxide nanoplatelets obtained from the oxidation of commercially available graphite nanofibers by the modified Hummers' method. In order to gain more insights into the material used for DNA labelling, the GeONPs used in these experiments were characterized by scanning electron microscopy (SEM), high-resolution transmission electron microscopy (HR-TEM), and atomic force microscopy (AFM).

The SEM image shows the filamentous structure of the nanofibers with uniform size distribution (Figure 10.1a). The HR-TEM image shows that the graphene sheets are perpendicularly aligned along the nanofiber axis (Figure 10.1b). The observed average diameter was around 35 nm. After the exfoliation by ultrasonication, the fibrous structure disappeared and gave separate individual graphene oxide nanoplatelets instead. The AFM image and height profile confirmed the presence of individual nanoplatelets with an average thickness around 1.2 nm (Figure 10.1c). Figure 10.1d shows the colloidal suspension of graphite nanofibers (GNFs) and GeONPs after

Figure 10.1 Microscopy characterizations of GNFs and GeONPs. (a and b) SEM and HR-TEM images of the graphite nanofibers as received. (c) AFM image and height profile of exfoliated GeONPs. (d) Colloidal suspension of graphite nanofibers (GNFs) as received and GeONPs after oxidation/exfoliation.

Figure 10.2 Size distributions of GeONPs measured by dynamic light scattering.

ultrasonicated for 30 min. As seen in the picture, GNFs were deposited on the bottom of the bottle, while GeONPs form a very stable suspension²¹ (picture taken on the third day after preparation). The good dispersion stability of the GeONPs was due to their zeta potential of -41.5 mV ($n = 3$). ASTM defines colloids with a zeta (ζ) potential higher than 40 mV (in absolute value) as having good stability.²² GNFs showed a ζ potential of $+30.9$ mV ($n = 3$), indicating that the low ζ potential is the reason for the poor stability of its colloids. The size distribution of GeONPs was measured by dynamic light scattering (Figure 10.2) and gave an average size value of 37 nm, which closely corresponds to the average diameter of starting graphite nanofibers as well as to the diameter of GeONPs measured by AFM. The full characterizations of GNFs as received and GeONPs confirmed that the fibers were digested, and no nanofibers structure was present after the oxidation/ exfoliation treatment.

10.2.2 Electrochemical characterisation of the graphene materials

The electrochemical behaviour of GeONPs was then investigated by performing cyclic voltammetry analysis between -0.2 and -2.0 V. As illustrated in Figure 10.3, two cyclic voltammetric waves between -1.0 and -1.5 V appeared in the first scan, corresponding to the reduction of the different oxygen-containing groups on the GeONPs surfaces, such as epoxide, aldehyde, and peroxy groups.¹³ This reduction signal was used as the working signal for biosensing purposes. The differential pulse voltammetry (DPV) technique was then employed, as it showed a better sensitivity when compared to cyclic voltammetry.²³ A calibration curve for different GeONPs concentrations was obtained by DPV. The obtained signal increased linearly with increasing concentrations of GeONPs (as shown in Figure 10.4).

Figure 10.3 Graphene oxide nanoplatelets show an inherent electrochemical reduction signal. Cyclic voltammogram for the electrochemical reduction of GeONPs in 10 mM phosphate buffer solution, pH 7.0. Scan rate: 20 mV/s. Reference electrode: Ag/AgCl.

Figure 10.4. Calibration curve for DPV reduction peak given by GeONPs at different concentrations. Error bars correspond to three replicates.

10.2.3 Graphene materials as electrochemical-active label for genosensing

Following the electrochemical characterizations, GeONPs were used as an electrochemical-active label for genosensing. Scheme 10.1 shows the detection procedure steps. First, DNA probes were immobilized on the electrode surface by physical adsorption.²⁴ Then, three different hybridization experiments were performed. The probe-modified electrode was incubated in three different solutions containing (A) complementary target (wild-type); (B) one-mismatch-containing sequence (mutant); and (C) non-complementary target (nc). After hybridization was achieved, the three different electrode surfaces were incubated in a solution containing a specified amount of GeONPs. As illustrated in Scheme 10.1, GeONPs conjugate to the DNA-modified electrode surfaces to varying extents depending on the specific targets used in the hybridization step. For this reason, a different amount of GeONPs was immobilized on the DNA-modified electrode surfaces, thus providing different voltammetric signals.

More specifically, when the hybridization step was performed with the complementary target, a low amount of GeONPs was conjugated to the DNA-modified electrode surface and gave rise to a small reduction signal, as shown by the voltammetric peak (wild-type, red line). For hybridization with the one-mismatch sequence, the amount of GeONPs bound to the DNA-modified electrode surface increased, and this resulted in a higher voltammetric peak (mutant, blue line). Finally, the highest electrochemical signal was given by the electrode surface that was incubated in the non-complementary target solution (nc, black line).

Scheme 10.1 Schematic of the experimental protocol. The hybridization step was performed with complementary target (A); one-mismatch target (B); and non-complementary target (C).

These different behaviours could be attributed to the different binding affinity of graphene nanoplatelets toward single- and double-stranded DNA. Upon hybridization with the complementary target, the electrode surface was modified with a significant amount of dsDNA sequences, while in the case of the non-complementary target the electrode surface consisted mainly of non-hybridized ssDNA probes. Since as already explained, graphene possesses a stronger ability to conjugate to ssDNA, a higher amount of GeONPs will be detected on the electrode surface that was incubated with the non-complementary target. In the case of the one-mismatch sequence the voltammetric signal was higher than the complementary target one, due to the partial hybridization occurring on the electrode surface.

Chronocoulometry experiments were performed to establish the DNA density on the electrode surface after the hybridization step. The results confirmed our hypothesis, the DNA density on the electrode modified with the DNA complementary target being

Figure 10.5 Voltammetric response toward the DNA target concentration in the case of hybridization with wild-type (red diamonds), mutant (blue diamonds), and non-complementary (black diamonds) targets.

2.53×10^{13} molecules/cm², much higher than on the mutant (2.40×10^{13} molecules/cm²) and on the non-complementary target (2.28×10^{13} molecules/cm²).

In order to optimize the amount of DNA to be used in the hybridization steps and to evaluate the limit of detection of the proposed biosensor, the variation of the GeONP voltammetric signal *versus* DNA target concentration was studied. As illustrated in Figure 10.5, for the experiments with the complementary (red diamonds) and the mutant (blue diamonds) sequences, the voltammetric peak heights decreased with increasing concentration of DNA target until a plateau was reached at around 30 nM. At that point, further increment of DNA target concentration did not correspond to any signal decrement. The estimated limit of detection, calculated with consideration of the noise to be 3 times as high as the standard deviation of the value obtained in negative control experiments, was 500 pM. The differentiation between the wild-type and mutant was

detectable to 10 nM. It is expected that with further development of this proof-of-concept principle the limit of detection could be significantly improved.

To establish the proper amount of GeONPs for the labelling step, a calibration with different concentrations of GeONPs was performed. The DNA target concentration was kept constant at a value of 30 nM. The histograms shown in Figure 10.6 represent the voltammetric peak height of GeONP reduction *versus* the different GeONP concentrations for hybridization with the wild-type (red colour), mutant (blue colour), and nc (black colour) sequences. As illustrated in Figure 10.6, the peak height increased when increasing the concentration of GeONPs until a plateau was obtained at 0.2 mg/mL. This point corresponded to the maximum discrimination between the wild-type, mutant, and nc sequences. For higher concentrations of GeONPs, there was no corresponding increase in the voltammetric peak. This indicated that the DNA-modified electrode surface was saturated with GeONPs at a concentration of 0.2 mg/mL.

The optimized values of DNA target and GeONPs were employed for the detection of DNA hybridization and polymorphism. In Figure 10.7, histograms representing the peak height of GeONPs reduction *versus* the different hybridization experiments are shown. As seen in the figure, the discrimination between wild-type, mutant, and nc sequences was achieved with good reproducibility (RSD < 10 %). Moreover, a 26 % increase in the signal for mutant and 45 % for non-complementary sequence as compared to the complementary one was obtained. In addition, Student's t test was applied to compare the results obtained in the three hybridization experiments. The difference between the mean values was significant for all cases analysed, being $t_{\text{calc}} > 6.41$ for all examined cases, larger than $t_{\text{tab}} = 2.13$, at the 95 % confidence level,

Figure 10.6 Voltammetric signal obtained after conjugation with different amounts of GeONPs. Hybridization with wild-type (red histograms), mutant (blue histograms), and nc (black histograms) sequences. Error bars represent triplicate experiments.

Figure 10.7 Histograms representing the peak height values obtained for the conjugation of GeONPs with the different DNA target-modified electrode surfaces. Measurements were performed and are shown in triplicate. Concentration of DNA targets used in the experiments: 30 nM. Concentration of GeONPs: 0.2 mg/mL.

4 degrees of freedom. This confirms that there is a very significant difference among the signals obtained for the three hybridization experiments.

The stability of the biosensor modified with the biorecognition element was studied for a time range of 4 weeks. On day 1 the electrode was modified with the optimized concentration of DNA probes. Hybridization experiments were realized on the same day (day no. 1) and after 2, 5, 7, 10, 14, 21, and 28 days. Results indicate a good stability of the electrode surface within two-weeks' time (variation of the signal in the range of 10 % standard deviation with respect to the first-day experiment). After 3 weeks a 10 % decrease in the signal was observed. After 4 weeks the recorded signal decrease was 12 % and also an increase in the RSD % value was observed.

The response of GeONPs as electroactive labels was also studied for different ultrasonication times employed during the preparation of the nanoplatelets. It is in fact well known that a prolonged ultrasonication can influence the resulting material shape and size.^{25,26} For this reason GeONPs obtained after 2, 8, 16, and 24 hr ultrasonication were employed for the biosensing. The results, illustrated in Figure 10.8, show that when increasing the ultrasonication time from 2 hr up to 24 hr, both the electrochemical signal and the discrimination ability for complementary, non-complementary, and mutant sequences worsen, with the 2 hr ultrasonication time being the condition that provides the best results.

Figure 10.8 Voltammetric signal obtained after the conjugation with GeONPs produced at different ultrasonication times. Hybridization with: wild-type (red histograms); mutant (blue histograms); nc (black histograms) sequences. Concentration of DNA targets used in the experiments: 30 nM. Concentration of GeONPs: 0.2 mg/mL. Error bars represent triplicate experiments.

10.3 Discussion

A proof-of-principle for the use of graphene nanoplatelets as electroactive labels for biosensors has been demonstrated. The ssDNA was shown to interact with graphene oxide nanoplatelets in a different manner than dsDNA. This led to the sensitive discrimination of the single-base mutation in the DNA sequence related to Alzheimer's disease. The reduction signal of graphene oxide nanoplatelets was exploited for the detection of DNA hybridization and polymorphism in a very simple and rapid protocol, by using disposable screen-printed electrodes.

The use of nanoplatelets, which were much smaller than regular sized graphene sheets, allowed for a better conjugation to DNA-modified electrode surfaces and improved the discrimination between single- and double-stranded DNA, which enabled the differentiation among complementary, non-complementary, and one-mismatch DNA sequences correlated with Alzheimer's disease.

A completely new approach in the use of graphene was proposed in this work, showing that the material could now be employed not only as a platform for biomolecule immobilization and analysis but also as a label either for its direct detection or for signal enhancement. Our findings were expected to have a profound impact on point-of-care personalized healthcare.

10.4 Experimental

10.4.1 Materials

Stacked graphite nanofibers were purchased from Strem Chemicals (Newburyport, MA, USA). Potassium ferricyanide ($K_3[Fe(CN)_6]$), potassium ferrocyanide ($K_4[Fe(CN)_6]$), tris(hydroxymethyl)aminomethane (Tris), hexaammineruthenium(III) chloride, sulphuric acid (95–98 %), potassium permanganate, sodium nitrate, hydrogen peroxide, and DNA sequences correlated with Alzheimer's disease were purchased from Sigma-Aldrich (Singapore). Buffer solutions used in the study are the following: 0.1 M PBS (0.1 M NaCl, 10 mM sodium phosphate buffer, pH 7.0), TSC1 (0.75 M NaCl, 75 mM trisodium citrate, pH 7.0), TSC2 (0.30 M NaCl, 30 mM trisodium citrate, pH 7.0), and Tris-HCl (10 mM Tris, pH 7.4). All solutions were made up using Milli-Q water (18.2 M Ω .cm resistivity). The following oligonucleotide sequences were employed: DNA probe 5'-ACCAGGCGGCCGCACACGTCCTCCAT-3'; complementary target 5'-ATGGAGGACGTGTGCGGCCGCCTGGT-3' (wild-type); 1-mismatch target 5'-ATGGAGGACGTGCGCGGCCGCCTGGT-3 (mutant); non-complementary target 5'-AAAAAAAAAAAAAAAAAAAAAAAAA-3' (nc). Oligonucleotides were diluted from stock solutions with sterilized Milli-Q water, separated into fractions, and stored at -20 °C. A single fraction was defrosted when required. Disposable electrical-printed carbon electrodes (DEP-chips, EE-PP model) were purchased from Biodevice Technology (Nomi, Japan). The three-electrode system included a carbon-based working electrode (2.64 mm² area), a Ag/AgCl reference electrode, and a carbon-based counter electrode.

10.4.2 Apparatus

A JEOL-7600F semi-in-lens FE-SEM, operating in gentle-beam mode at 2 kV, was used to acquire the SEM images. AFM analysis was performed using a scanning probe microscope (model Multimode V by Veeco, Plainview, NY, USA). HR-TEM images were taken using a JEM 2100F field emission and Hitachi H-800 transmission electron microscope (JEOL, Japan). Size distribution and zeta potential were measured by a Zetasizer Nano ZS (Malvern) using dynamic light scattering and by laser Doppler microelectrophoresis, respectively. All electrochemical experiments were performed by using an Autolab PGSTAT302 potentiostat (Eco Chemie, Utrecht, The Netherlands) driven by GPES software, version 4.9. DPV measurements were performed in PBS buffer solution. Impedance measurements were recorded between 0.1 MHz and 0.1 Hz at a sinusoidal voltage perturbation of 10 mV amplitude. The experiments were carried out at an applied potential of 0.18 V (vs a Ag/AgCl reference electrode) in a 0.1 M PBS buffer solution containing 10 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1 molar ratio) as a redox probe.

10.4.3 Procedures

Graphene oxide nanoplatelets were produced in bulk quantities by following conventional protocols: (a) a modified Hummers' method was employed to obtain graphite oxide nanoplatelets from graphite nanofibers. A mixture of $NaNO_3$, H_2SO_4 , and $KMnO_4$ was used for the oxidation of graphite nanofibers. Precautions should be taken when handling corrosive acids and strong oxidants. (b) The obtained graphite oxide powder was dispersed in ultrapure water and then ultrasonicated for 3 hr in order to obtain graphene oxide nanoplatelets. For calibration curve purposes 3 μ L of GeONP

suspension in milli-Q water at different concentrations was drop-casted onto each DEP-chip surface, and the solvent was allowed to dry at room temperature (25 °C).

DNA probes were immobilized onto the electrode surface by dry physical adsorption. A 3 μL volume of DNA probe solution in PBS buffer solution at the optimized concentration of 10 μM (as resulted from impedance spectroscopy experiments) was deposited onto the electrode surface for 10 min at 60 °C. The electrode was washed twice in PBS buffer with gentle stirring at room temperature (25 °C) to remove excess, nonadsorbed material. DEP-chips modified with DNA probes were incubated in an Eppendorf tube with the hybridization solution (TSC1 buffer) containing the desired concentration of DNA target (total volume 100 μL). The incubation was performed at 42 °C for 30 min, with gentle stirring. Two brief washing steps were then performed in TSC2 buffer at 42 °C. Incubation with GeONPs was performed in an Eppendorf tube containing 100 μL of GeONPs at the desired concentration in PBS solution. The incubation was performed at room temperature (25 °C) for 20 min, with gentle stirring. This was followed by two brief washing steps in PBS buffer.

DNA oligonucleotide surface density was determined by chronocoulometry according to the method reported by Steel *et. al.* The hexaammineruthenium(III) dilution in Tris-HCl buffer used in the experiments was optimized at 100 μM (as determined by measuring the binding isotherm of the DNA probe-modified platform). The DNA density on the electrode surface was calculated using the Cottrell equation and by considering the extrapolation of the intercept at time zero.

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Chapter 11

Electrochemical application of graphene II

Electrochemical sensing – Graphene as detection materials on a lab-on-a-chip platform

11.1 *Introduction*

11.2 *Results*

11.2.1 The separation of dopamine neurotransmitter and catechol

11.2.2 The separation of nitroaromatic explosives

11.3 *Discussion*

11.4 *Experimental*

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

1. **Chun Kiang Chua**, Adriano Ambrosi, Martin Pumera. Graphene based nanomaterials as electrochemical detectors in lab-on-a-chip devices. *Electrochem. Commun.* **2011**, 13, 517-519.

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Contributions:

C. K. Chua conducted the experiments. A. Ambrosi synthesized the graphene materials. M. Pumera conceptualized, directed the project and wrote the manuscript. All authors contributed to discussions.

2. **Chun Kiang Chua**, Martin Pumera. Chemically modified graphenes as detectors on lab-on-chip devices. *Electroanalysis* **2013**, 25, 945-950.

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Contributions:

C. K. Chua conducted the experiments. M. Pumera directed the project. All authors contributed to discussions and wrote the manuscript.

11.1 Introduction

High-throughput analysis is a fast developing field which offers possibilities for rapid and sensitive chemical or biological screenings. Lab-on-a-chip devices, in particular, are microfabricated microfluidics systems that can considerably improve the speed and scale at which chemical and biological analyses are performed. Such a microfluidics system exhibits the ability to reduce conventional analytical systems and bring advantages of high-throughput, low cost, low sample consumption and portability/ field deployability.¹ Despite that, the major challenge in the field of analytical lab-on-a-chip devices is to provide detectors that are resistant to fouling, sensitive for minute amount of samples (typically less than few nanolitres) and miniaturizable in order not to compromise the portability of the devices. To this aim, electrochemical detection brings great benefits to microfluidics devices as it is inherently miniaturizable, has low energy consumption and it is compatible with advanced microfabrication schemes.²

Several groups, including ours, utilized carbon nanomaterials modified electrochemical electrodes to improve the electrochemical detection limits of lab-on-a-chip devices.³ Several articles were published on high sensitive detections using carbon nanotubes based detectors in microfluidics.⁴⁻¹² Currently, graphene is regarded as the electrochemical materials of the future with reported advantageous electrochemical properties for sensing and energy storage.¹³⁻¹⁷ To the best of our knowledge, there is no report on the use of graphene based nanomaterials as detector surfaces for lab-on-a-chip devices. Only very recently, Chen and co-workers published an article on conventional capillary electrophoresis with graphene based composite detectors.¹⁸

This study would provide a systematic and comprehensive analysis on the integration of chemically modified graphene materials (which include graphite oxide, graphene oxide, chemically/ thermally/ electrochemically reduced graphene) as electrochemical detectors on a lab-on-a-chip device. The performances of the graphene materials were examined based on the electrophoretic separation of important dopamine neurotransmitter and catechol. In the subsequent part, the performances of graphite and graphene oxide were investigated based on the separation of nitroaromatic explosive compounds (2,4,6-trinitrotoluene, 1,3-dinitrobenzene, and 2,4-dinitrotoluene).

11.2 Results

11.2.1 The separation of dopamine neurotransmitter and catechol

It is known that chemically modified graphene materials exhibit different heterogeneous electron transfer rates, depending on the density of the oxygen containing groups and defects, in a bulk solution based measurements.¹⁹ It is important to underline that, in most cases, the bulk-based electrochemical performance of a detection material is not linked to its performance on a lab-on-a-chip platform due to various factors which come into play, such as minute size of the sample and active electrode area, flow conditions, and presence of high-voltage electric field during electrophoretic separation. The performances of various chemically modified graphene materials in a microfluidic system are compared based on the electrophoretic separation of important dopamine neurotransmitter and catechol with the graphene-based electrochemical detection. The electrophoretic separation in this part of the study was conducted with a Topas polymer microchip.

Prior to performing the microfluidic separation/ detection of dopamine neurotransmitter and catechol, the optimal detection potential has to be established. Hydrodynamic voltammograms for the oxidation of dopamine and catechol were carried out using a glassy carbon electrode as shown in Figure 11.1. The voltammograms were recorded at a step potential of +0.1 V starting from +0.0 to +0.8 V. Both dopamine and catechol displayed similar sigmoidal voltammetric profiles on the bare glassy carbon electrode. The initial potential intervals from +0.0 to +0.1 V did not provide any response. The peak currents increased at higher operation potentials and peaked at +0.5 V for both dopamine and catechol. When an insufficiently high detection potential was applied, the sample plug which entered the detector volume would not be oxidised

Figure 11.1. Hydrodynamic voltammograms of 200 μm dopamine and 200 μm catechol on a bare glassy carbon electrode. Conditions: 25 mM MES buffer, pH 6.5; injection and separation voltage, +2000 V; injection time, 3 s. EOF (catechol as a neutral marker): $4.11 \pm 0.04 \times 10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ ($n = 4$).

immediately. The samples would oxidise over a longer period and thus resulted in band broadening.²⁰ At a higher potential, the peak currents decreased with increasing detection potential as the signal-to-noise ratio decreases. As such, the amperometric detection of dopamine and catechol with the chemically modified graphene materials employed a detection potential of +0.5 V since it provided the most favourable signal-to-noise characteristic. Increase in the baseline current and noise were observed at higher potential. Moreover, the selection of the separation voltage would affect the selection of an optimal detection potential as the voltammetric profiles would shift.²¹ The variation in peak current of dopamine and catechol could be a result of variation in injection volume and other variables such as the capillary-to-electrode distance.²²

Figure 11.2. Electropherograms of a mixture of 250 μM dopamine (a) and 150 μM catechol (b) at (A) bare GC, (B) graphite, (C) graphite oxide, (D) graphene oxide, (E) CRGO, (F) ERGO and (G) TRGO modified electrodes. Conditions: 25 mM MES buffer, pH 6.5; injection and separation voltage, +2000 V; injection time, 3 s; detection potential, +0.5 V.

Figure 11.2 shows the typical microchip electropherograms of a mixture of dopamine (a) and catechol (b) at different electrode materials, specifically (A) bare glassy carbon, (B) graphite, (C) graphite oxide, (D) graphene oxide, (E) chemically reduced graphene oxide, (F) electrochemically reduced graphene oxide, and (G) thermally reduced graphene oxide. The detailed analysis of the obtained data showed that the peak currents obtained for dopamine and catechol, respectively, were as follow: bare GC (26.4 and 13.2 nA), graphite (23.5 and 11.7 nA), graphite oxide (24.3 and 10.6 nA), graphene oxide (18.3 and 8.8 nA), CRGO (19.2 and 10.4 nA), ERGO (20.3 and 6.9 nA) and TRGO (2.3 and 2.1 nA). Among the chemically modified graphene materials, graphite oxide modified electrode achieved the highest peak current for the detection of

dopamine while graphite modified electrode gave the highest peak current for catechol detection. The lowest peak currents for both the dopamine and catechol were observed on the TRGO modified electrode. However, the peak currents obtained from these graphene-based materials were lower in comparison to the bare GC electrode. The reason for TRGO materials to exhibit poor performance at lab-on-a-chip platform likely originates from the fact that it shows the largest capacitive behaviour of all graphene materials²³ which leads to high background currents in microfluidic CE/EC systems.³

The resolution of the lab-on-a-chip devices with electrochemical detectors depends on the performances of electron transfer at the detection surfaces.²⁴ The resolution for the separation of dopamine and catechol were 1.49, 1.29, 1.69, 1.35, 1.13, 2.77 and 0.87 for the bare GC, graphite, graphite oxide, graphene oxide, CRGO, ERGO and TRGO detectors used in the microfluidic setup, respectively. The electrochemically reduced graphene oxide gave the highest resolution, with almost a two-fold increment as compared to the bare glassy carbon. On the other hand, the thermally reduced graphene oxide provided the lowest resolution among all the modified electrodes. The separation column efficiency, described as the number of theoretical plates per meter were 2871, 2143, 3045, 1999, 1386, 5191, and 257 for the bare GC, graphite, graphite oxide, graphene oxide, CRGO, ERGO and TRGO, respectively.

In light of these findings, the performances of the chemically-modified graphene materials on a lab-on-a-chip device differed greatly as compared to a three-electrode electrochemical-cell setup analyses which were well studied.¹⁹ Therefore, it must be emphasized that the performances of graphene materials prepared from various synthetic routes must be tested on lab-on-a-chip devices prior to arriving at conclusive remarks with regards to the performances of these materials on a microfluidic setup.

11.2.2 The separation of nitroaromatic explosives

Consequently, we turned our attention to the investigation of microfluidic separation with end-column amperometric detection of nitroaromatic explosives.^{25,26} Namely, we selected 2,4,6- trinitrotoluene (TNT), 2,4-dinitrotoluene (DNT) and 1,3-dinitrobenzene (DNB) as these are important compounds that can be found in the list by the Environmental Protection Agency (U.S. EPA) as TNT related environmental contaminants.²⁷ In this part of the study, the investigation was focused only on graphite and graphene oxide materials. The electrophoretic separation in this part of the study was conducted with a glass microchip.

Figure 11.3 shows the representative micellar electrokinetic electrophoregrams of separation of TNT, DNT and DNB at (A) bare GC, (B) graphite and (C) graphene oxide modified electrodes using amperometric detection in reduction mode. In terms of separating nitroaromatic compounds, it was clear that graphene oxide and graphite detectors provided similar amperometric peak currents of 15.3, 7.5 and 2.8 nA and 15.6, 5.6 and 2.5 nA for DNB, TNT and DNT at graphene oxide and graphite electrodes, respectively. Lower reduction currents originated from bare GC electrode, with 10.0, 7.2 and 2.2 nA for DNB, TNT and DNT. The effect of the graphene oxide based detector on separation efficiency was also significantly higher than that of bare GC electrode; however, graphite resulted in an even higher number of theoretical plates per meter than graphene oxide; see on the example of DNB, where N/m is 9854, 11429 and 4662 per meter for graphene oxide, graphite and bare GC electrode, respectively. The findings were in line with our previous research on mono-, few- and multi-layer graphene in batch measurements where we found that the difference between the redox currents at the mono-layer and multi-layer graphene are similar to those of graphite.²⁸⁻³⁰

Figure 11.3 Electrophoregrams of a mixture of (a) DNB, (b) TNT and (c) DNT (20 ppm for all) at bare GC (A), graphite (B), and graphene oxide (C) modified electrodes. Conditions: 15 mM borate buffer (pH 9.2) with 20 mM SDS; separation voltage, +2000 V; injection voltage, +2000 V; injection time, 3 s; detection potential, -0.5 V.

11.3 Discussion

The potential of graphene materials as electrochemical detectors in a lab-on-a-chip device was demonstrated in this study. Electrochemically reduced graphene oxide highlighted the best performance for the electrophoretic separation of dopamine neurotransmitter and catechol, with a resolution of 2.77 and theoretical plates of 5191 per meter. Subsequent study on the electrophoretic separation of nitroaromatic explosives has shown that graphene oxide provided no advantages in terms of sensitivity or selectivity over that of graphite. The analyses on the nitroaromatic explosives could be extended further with other chemically modified graphene materials.

More importantly, there is a need to emphasize on the requirement for testing of various graphene electrodes in lab-on-a-chip devices in order to provide well-justified, advantageous detection system since the performances of these materials could differ in a bulk solution based environment.

11.4 Experimental

11.4.1 Materials

Graphite (<20 μm), sulfuric acid (95–98%), potassium chlorate (98%), hydrochloric acid (37%), N,N-dimethylformamide (DMF), 4-morpholineethanesulfonic acid sodium salt (MES), acetic acid, catechol, dopamine, trinitrotoluene, 1,3-dinitrobenzene, 2,4-dinitrotoluene, sodium dodecyl sulphate (SDS), acetonitrile and sodium hydroxide, potassium phosphate dibasic, sodium phosphate monobasic, and sodium borohydride were obtained from Sigma-Aldrich, Singapore. Fuming nitric acid (>90%) was obtained from J.T. Baker. Glassy carbon (GC) electrodes with a diameter of 3 mm were obtained from Autolab, The Netherlands. Stock solutions of catecholamines were prepared daily in deionized water. Stock solutions of explosives were prepared in acetonitrile.

11.4.2 Apparatus

The glass microchip was obtained from Micrux Technologies (Model MCE-Glass-001T). The 44 mm $\times$ 16 mm chip consisted of a four-way injection cross, with a 38 mm long separation channel and three side arms of 5 mm each in length (see Figure 11.4). The original detection reservoir was cut off, resulting in an exposed microchannel at the end of the chip, to facilitate end-column amperometric detection. The channels were 50 μm wide and 20 μm deep.

The cross-shaped microchip made from Topas polymer was obtained from Microfluidic ChipShop, Germany. The 85 mm $\times$ 16 mm chip consisted of a four-way injection cross, with a 75 mm separation channel and three side arms of 5 mm each in length (see Figure 11.4). The original detection reservoir was cut off to expose the end of the microchannel

in order to facilitate end-column amperometric detection. The channels were 50 μm wide and 50 μm deep.

A plexiglass holder was specially fabricated to hold the microchip and to accommodate the detectors and detection reservoir.³¹ The detection reservoir consisted of a platinum wire counter (0.25 mm diameter, Aldrich), an Ag/AgCl wire reference (0.5 mm diameter, home-made) and a bare glassy carbon (GC) or carbon material film-modified disk working GC electrode (CH Instruments, Austin, TX, USA) with diameter of 3 mm. The working electrode, housed in the plastic screw was placed at the end of the exposed microchannel.

Figure 11.4 The microchip setup: (a) Topas chip, (b) separation channel, (c) run buffer reservoir, (d) run buffer reservoirs, (e) sample reservoirs, (f) platinum separation electrode, (g) detection electrode, (h) Ag/AgCl wire reference electrode, and (i) platinum cathode for separation.

11.4.3 Procedures

Graphite. The powder was used without further treatment.

Graphite oxide. Sulphuric acid (17.5 mL, 95–98 %) and nitric acid (fuming, 9 mL) were added into a round bottom flask and stirred under 0 °C for 15 min. Graphite (1 g) was then added into the mixture under vigorous stirring to avoid agglomeration and to obtain a homogeneous dispersion. Potassium chlorate (11 g) was slowly added into the mixture (over 15 min) at 0 °C. The reaction mixture was left to stir for 96 h at room temperature. On completion of the reaction, the mixture was poured into deionized water (1 L) and filtered. Graphite oxide was then dispersed and washed repeatedly in HCl (5 %) solutions to remove sulphate ions and finally washed with deionized water until a neutral pH of the filtrate was obtained. The graphite oxide slurry was then dried in a vacuum oven at 40 °C for 48 h before use. Precautions should be taken when handling corrosive acids and strong oxidants.

Graphene oxide. Graphene oxide was obtained by ultrasonication (Fischerbrand, FB 11203 ultrasonic unit at a frequency of 37 kHz, 180 W) of a graphite oxide/DMF dispersion (1 mg/mL) for 1 h. The graphene oxide prepared by this method is typically single layer.

Thermally reduced graphene oxide (TRGO). Thermally reduced graphene oxide was obtained *via* thermal exfoliation/ reduction of graphite oxide at 1050 °C. Graphite oxide (0.2 g) was placed in a porous quartz glass capsule connected to a magnetic manipulator inside a vacuum-tight tube furnace under a controlled atmosphere. A magnetic manipulator created a temperature gradient of over 1000 °C/min. The sample was flushed with nitrogen by repeated evacuation of the tube furnace to remove any traces

of oxygen and then quickly inserted into the furnace and was held in the furnace for 3 min. The flow of nitrogen during the exfoliation procedure was 1000 mL/min, which removed the by-products.

Chemically reduced graphene oxide (CRGO). Chemically reduced graphene was obtained by mixing graphite-oxide (10 mg) with an aqueous solution of NaBH_4 (50 mM, 20 mL). The mixture was ultrasonicated (37 kHz) for 1 h. The reduction reaction was indicated by a colour change from brown (graphene-oxide) to black (CRG) during the sonication process. The CRG dispersion was then washed with deionized water to remove NaBH_4 residues and finally dried in a vacuum oven at 60 °C for 48 h.

Electrochemically reduced graphene oxide (ERGO). 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 7.2).

The graphene materials have been fully characterised as previously reported.¹⁹

Electrode preparation. A 1 mg of the carbon materials were dissolved in 1 mL of DMF. The working electrode was modified by applying a 1 μL of DMF containing 1 mg/mL carbon material (graphite or graphene oxide powder) and allowing to dry overnight.

Electrophoretic procedure. The microchannels of the glass chip were rinsed with 0.1 M NaOH and deionized water for 10 min each, prior to use. MES buffer (25 mM, pH 6.5) was chosen as the electrophoretic buffer for the analysis of catecholamines whereas borate buffer (15 mM, pH 9.2) with addition of 20 mM SDS was used as the electrophoretic buffer for analysis of nitroaromatic explosive materials. Prior to the amperometric measurements, the sample injection channel was initially filled with the sample solution electrokinetically. A separation voltage of +1500 V was subsequently

applied on the buffer reservoir (the detection reservoir grounded) until a flat amperometric baseline was observed. The microchip would then be ready for amperometric measurements. The amperometric detection was performed using HVSTAT2010 (Micrux Technologies) connected to a desktop. The electropherograms were recorded with a time resolution of 30 ms. Sample injection was carried out only after the stabilization of baseline was achieved. All the experiments were carried out in room temperature (25 °C).

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Chapter 12

Conclusion and Epilogue

12.1 Conclusion

12.1.1 Preparation procedures of graphene via the top-down chemical approach

12.1.2 Graphene in biosensing and lab-on-a-chip devices

12.2 Epilogue

12.1 Conclusion

The birth of graphene has brought about major excitement to the scientific community in recent years. Graphene has been exploited for numerous electrochemical applications due to its unique structural, physical, and electronic properties. As a result of the lack of available methods to obtain graphene in relatively high quality and large quantities, the progresses of electrochemical applications has been hindered. The top-down approach of graphene synthesis is highly promising in providing large quantities of the material. However, the quality of the resulting graphene often suffers from high irregularities and defects.

Therefore, this project focused on investigating the methods for graphene synthesis *via* the top-down approach. The oxidation methods of graphite to graphite oxide were investigated and five different established reduction methods for the conversion of graphene oxide to graphene were demonstrated for the first time. In addition, the graphene materials were utilized in electrochemical biosensing towards the detection of single nucleotide polymorphism. Furthermore, the potential application of graphene materials for electrochemical sensing was demonstrated on a lab-on-a-chip platform.

Hence, this project has highlighted two areas of research (Figure 12.1). The first area focused on improving current methods for the top-down synthesis of graphene. The second area focused on the application aspects of graphene in electrochemical biosensing and sensing.

Figure 12.1 Graphical summary of this thesis project. In Chapter 4, the variants of oxidation methods were examined. In Chapters 5, 6, 7, 8 and 9, five reduction methods of graphene oxide were introduced for the first time. In Chapters 11 and 12, the applications of graphene materials in biosensing and as electrode materials in a lab-on-a-chip platform were demonstrated.

12.1.1 Preparation procedures of graphene *via* the top-down chemical approach

The top-down approach for graphene synthesis allows for the production of graphene in large quantities. Even though the resulting graphene materials are defective in nature, these materials have nonetheless found their niche of applications. However, it is still important to expand and improve the current methods to obtain graphene of high quality for future practical applications. Moreover, it is also imperative to understand and apply only well-established chemical modifications in the top-down approach towards graphene.

The oxidation methods of graphite towards graphite oxide based on four known methods, mainly Staudenmaier, Hofmann, Hummers and Tour, were investigated in **Chapter 4**. The study highlighted the influences of oxidant types towards the formation of oxygen containing groups (hydroxyl, epoxy, carbonyl and carboxyl) on the surface of graphene. The graphite oxide obtained using permanganate oxidant provided the highest extent of oxidation and resulted in the highest amount of carbonyl and carboxyl moieties. However, this has led to a lower heterogeneous electron transfer rate based on electrochemical measurements. This comparison study has provided insights into selecting suitable graphite oxide materials for specific applications in the future.

Subsequently, the reduction of graphene oxide towards graphene was explored in **Chapters 5, 6, 7, 8 and 9**. Five rational reduction approaches, which are supported by well-established mechanisms based on the knowledge of synthetic chemistry, were examined in detail to achieve the production of high quality graphene. The reduction methods with lithium aluminium hydride, substituted borohydrides and thiourea dioxide

were highlighted in **Chapters 5, 6** and **7**. The usage of reducing agents with well-established mechanisms is essential to provide confidence on the exact structural modifications that occur on graphene oxide. In fact, the amount of oxygen containing groups could be tweaked to a certain desired composition by applying the appropriate types of reducing agents. Apart from that, the usage of thiourea dioxide provided a prospect for the production of commercially viable graphene.

More importantly, despite the usage of strong reducing agents, they are often only capable of reducing the oxygen containing groups to hydroxyl groups. As such, in **Chapter 8** and **9**, the specific removal of hydroxyl groups coupled with the simultaneous regeneration of the sp^2 carbon network on the resulting graphene were examined. This was achieved by subjecting graphene oxide to a dehydrobromination process or a reaction with ethanethiol-aluminium chloride complexes. Both of these approaches were successful in the removal of the targeted oxygen containing groups and provided reduced graphene materials of superior conductivities based on our structural and electrochemical characterisations.

12.1.2 Graphene in biosensing and lab-on-a-chip devices

The enhanced electronic and biocompatibility properties of graphene has made it a suitable candidate for bioapplications. The highlighted work in **Chapter 10** demonstrated a proof-of-principle for the usage of graphene oxide nanoplatelets as an electroactive label for electrochemical biosensors. The reduction signal of graphene oxide nanoplatelets was exploited to provide a sensitive discrimination of single-base mutation in the DNA sequence related to Alzheimer's disease. This protocol was able to provide a simple and rapid method based on the contrasting interactions between single- and double-strand DNAs with the graphene oxide nanoplatelets. More importantly, this work has redefined the conventional role of graphene materials as platforms for biomolecule immobilization and analysis to showcase the ability of graphene to act as a label for direct detection or for signal enhancement.

Apart from that, graphene materials have been successfully adapted as electrode materials in a lab-on-a-chip electrochemical detection platform as discussed in **Chapter 11**. This investigation was vital since the performances of graphene electrode materials in bulk solution-based electrochemical measurements were not direct reflections of their performances on a lab-on-a-chip platform. The performances of the graphene materials were examined based on the electrophoretic separation of dopamine neurotransmitter and catechol as well as nitroaromatic explosives. Electrochemically reduced graphene oxide provided the best resolution for the separation of dopamine and catechol. On the other hand, graphene oxide did not provide any advantage in terms of selectivity and sensitivity for the detection of nitroaromatic explosives over that of graphite. These fundamental studies could potentially provide profound impacts for point-of-care diagnostics in personalized healthcare and environmental applications.

12.2 Epilogue

Perspective

As we moved into the 21st century, research in carbon nanomaterials has shifted its attention from classical fullerene and carbon nanotubes to graphene. It was barely nine years since graphene was first isolated and only three years since the 2010 Nobel Prize in Physics was awarded to Andre Geim and Konstantin Novoselov for their groundbreaking experiments on the two-dimensional graphene material. Nevertheless, graphene has unveiled an entirely new branch of nanomaterials science. The expansion of graphene research has caught the attention of numerous scientific groups and resulted in an increasing number of graphene publications at a staggering amount of 8655 manuscripts designated for graphene research in 2012 (Thomson Reuters Web of Knowledge)

The enhanced electrical conductivity, optical transparency, high mechanical strength and thermal conductivity properties of graphene have promised to tackle the technological limitations of current materials (i.e. III-V semiconductor) and to introduce new applications (i.e. flexible OLED, e-paper) into the consumer market. However, the suitability of graphene for a specific application is highly dictated by its sheet quality. If graphene were to function in high-technology devices, fundamental research into improving the synthesis methods is important to obtain defect-free graphene in large quantities.

At the moment, the top-down synthetic approach towards the production of graphene is the most promising method towards large-scale production of graphene,

albeit of a lower quality due to sheet defects. Nevertheless, these graphene materials have been applied in potential applications ranging from transparent conductive layers, photonics, nanoelectronics, sensors, bioapplications, high-frequency transistors, coatings, paint/ink, composites and energy storage. It is thus envisaged that a cheaper cost of production and the ability to fine-tune the quality of graphene could result in a faster development of these technologies and subsequent introduction into the consumer market. Apart from that, rational synthetic modifications on the graphene materials could potentially provide high quality graphene sheets or even novel graphene-based materials for future practical applications.

In all likelihood, research on graphene is still in its early stage. Collective efforts from the graphene community is utmost important towards the realization of graphene as the 'wonder-material'.

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Appendices

Graphite Oxides: Effects of Permanganate and Chlorate Oxidants on the Oxygen Composition

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Abstract: Research on graphene materials has refocused on graphite oxides (GOs) in recent years. The fabrication of GO is commonly accomplished by using concentrated sulfuric acid in conjunction with: a) fuming nitric acid and KClO₃ oxidant (Staudenmaier); b) concentrated nitric acid and KClO₃ oxidant (Hofmann); c) sodium nitrate for in situ production of nitric acid in the presence of KMnO₄ (Hummers); or d) concentrated phosphoric acid with KMnO₄ (Tour). These methods have been used interchangeably in the graphene community, since the properties of GOs produced by these different methods were assumed as almost similar. In light of the wide applicability of

GOs in nanotechnology applications, in which presence of certain oxygen functional groups are specifically important, the qualities and functionalities of the GOs produced by using these four different methods, side-by-side, was investigated. The structural characterizations of the GOs would be probed by using high resolution X-ray photoelectron spectroscopy, nuclear magnetic resonance, Fourier transform infrared spectroscopy, and Raman spectroscopy.

Keywords: electrochemistry • graphite oxides • materials science • oxidation • oxygen-containing groups

Further electrochemical applicability would be evaluated by using electrochemical impedance spectroscopy and cyclic voltammetry techniques. Our analyses highlighted that the oxidation methods based on permanganate oxidant (Hummers and Tour methods) gave GOs with lower heterogeneous electron-transfer rates and a higher amount of carbonyl and carboxyl functionalities compared with when using chlorate oxidant (Staudenmaier and Hofmann methods). These observations indicated large disparities between the GOs obtained from different oxidation methods. Such insights would provide fundamental knowledge for fine tuning GO for future applications.

Introduction

The research on graphite oxide (GO) and its exfoliated form has regained much attention from the graphene community in recent years.^[1] GO is a highly oxidized form of graphite, which retains the lamellar structure of its precursor albeit with a higher interlayer spacing due to the presence of oxygen-containing groups.^[2] GO has been utilized for the microfabrication of single particle and carbonaceous electronic circuits,^[3] conductive composites,^[4] ultrathin carbon films,^[5] and molecular-recognition hosts.^[6] On top of that, GO is an important intermediate in the widely adopted top-down approach towards chemically converted graphene.^[7] An in-depth understanding of its properties and production methods would be important in fine tuning GO for specific applications.^[8]

GO was first synthesized by Brodie in 1859 through the oxidation of graphite with fuming nitric acid and potassium chlorate as an oxidant.^[9] In 1898, Staudenmaier improved the oxidation method by adding potassium chlorate in small portions and further acidifying the mixture with concentrated sulfuric acid.^[10] A few decades later in 1937, Hofmann utilized nonfuming nitric acid in his production of GO.^[11] In 1958, Hummers and Offeman introduced a safer alternate method, which used potassium permanganate as an oxidant in a mixture of concentrated sulfuric acid and sodium nitrate that produced nitric acid in situ.^[12] This method has been widely adopted to date and has gone through numerous modifications to reach a higher extent of oxidation.^[13] Most recently, Tour's group introduced a safer alternative, which omitted the usage or in situ formation of nitric acid through an oxidation mixture of potassium permanganate, concentrated sulfuric acid, and phosphoric acid.^[14]

The chemical structure of GO remains debatable, although several structural models have been proposed, such as the Hofmann,^[15] Ruess,^[16] Scholz-Boehm,^[17] Nakajima-Matsuo,^[18] Lerf-Klinowski,^[19] and Dékány^[20] models. The Lerf-Klinowski model is currently the most known and accepted model in the graphene community. The model highlighted two different regions, mainly a region with purely sp² carbons, whereas another filled with oxygen functional groups. The functional groups on the basal planes contain mostly of hydroxyl and epoxyl functionalities, whereas the

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edge planes are filled with carbonyl and carboxylic acid moieties. Given the varying oxidation conditions available, the proportions of the oxygen functionalities could differ drastically. The distribution of the types of oxygen-containing groups available could affect the performance of the GO (or its exfoliated form, graphene oxide) materials in certain applications. For example, GO (or graphene oxide) was commonly used in electrochemical immunosensor^[21] and glucose sensor,^[22] as starting material for chemical functionalization,^[23] or could potentially conferred passivation of NADH^[24] due to the presence of carboxylic groups. As such, it is important to obtain detailed analyses on the relative compositions of the oxygen containing groups on GO produced by these different methods.

Although these different oxidation methods have been freely used by the graphene community, a few pressing issues, which should be addressed in such a practice, remain. First, does it make any difference at all to use different oxidants and acid mixtures towards the production of GOs and in which aspects would the products differ in. Second, specifically between the Staudenmaier and Hofmann methods, is there any difference between the usage of fuming and concentrated nitric acid. Third, based on the possibly different GO materials, which method would then be advisable for application in graphene research.

To address the questions above, we would like to develop a further understanding on the ability of different oxidation methods in tuning the composition of oxygen-containing groups. This was achieved by carrying out structural and electrochemical characterizations on GO materials prepared by using the Staudenmaier (GO-ST), Hofmann (GO-HO), modified Hummers (GO-HU), and Tour (GO-TO) methods (Scheme 1). We have further grouped the oxidation methods into two categories for an easier comparison in the text—the chlorate (GO-ST and GO-HO) and permanganate (GO-HU and GO-TO) oxidation methods.

Characterizations of the GO materials would be carried out by using various spectroscopic methods, specifically, high resolution X-ray photoelectron spectroscopy (XPS), electrochemical impedance spectroscopy (EIS), NMR, Fourier transform IR (FTIR), Raman spectroscopy, and electrochemical cyclic voltammetry.

Results and Discussion

Structural characterizations of the GO materials: The GO materials prepared by using the Staudenmaier, Hofmann, modified Hummers and Tour methods (for details, see the Experimental Section) were initially characterized by using XPS. This method allows the sensitive detections of surface elemental compositions on the materials. A typical XPS survey scan provides information about the presence of any element on the materials, whereas a C1s high-resolution scan reveals more information on the chemical environment of the carbon atoms. Based on Figure 1A, the XPS survey

Scheme 1. Schematic for the oxidation procedures of graphite to GOs by using the Staudenmaier (GO-ST), Hofmann (GO-HO), Hummers (GO-HU), and Tour (GO-TO) methods.

Figure 1. X-ray photoelectron spectroscopy spectra on graphite, GO-ST, GO-HO, GO-HU, and GO-TO. A) Survey and B) C1s core-level spectra.

spectra indicated obvious peaks of carbon and oxygen elements on graphite, GO-ST, GO-HO, GO-HU, and GO-TO, which gave atomic C/O ratios of 21.6, 2.47, 2.71, 2.05, and 1.95, respectively. The low atomic C/O ratios observed for all the GO materials indicated successful and extensive oxidative treatments.^[12] Overall, the permanganate (GO-HU and GO-TO) oxidation methods showed a higher extent of

oxidation compared with the chlorate (GO-ST and GO-HO) oxidation method.

High-resolution XPS scans were also carried out as shown in Figure 1B to investigate the types of oxygenated carbon bonds found in the GO materials. The C1s core-level spectra were deconvoluted into Gaussian peaks centered at 284.5, 286.5, 288.5, and 290.5 eV, which corresponded to the C=C, C–O, C=O, and O–C=O bond types, respectively. The C1s core-level spectrum of graphite gave a sharp peak attributed to the sole presence of C=C bond type. The deconvoluted C1s peak of chlorate (GO-ST and GO-HO) oxidation method gave prominent peaks for C=C and C–O bond types and only a small contribution of C=O bond. On the other hand, the permanganate (GO-HU and GO-TO) oxidation method indicated a higher proportion of C=O bond type. The occurrence of O–C=O bond type in GO-TO indicated a significant presence of carboxyl group. In short, the higher proportions of C=O and O–C=O bond types in GO-HU and GO-TO highlighted the higher extent of oxidation by the permanganate method compared with the chlorate oxidation method.

Apart from that, the elemental compositions of the GO materials were further analyzed with elemental analyses as summarized in Table 1. The materials consisted mainly of

Table 1. Elemental analyses of graphite, GO-ST, GO-HO, GO-HU, and GO-TO showing the respective atomic percentage of carbon, hydrogen, and oxygen atoms.

Materials	C [%]	H [%]	O [%]
Graphite	99.88	–	0.11
GO-ST	52.68	2.42	44.89
GO-HO	52.09	2.39	45.50
GO-HU	44.18	2.92	52.89
GO-TO	41.22	2.92	55.86

carbon, hydrogen, and oxygen elements, whereas their atomic compositions varied according to the methods of oxidation. Graphite exhibited a low presence of oxygen, whereas GO-ST, GO-HO, GO-HU, and GO-TO showed a greater amount of oxygen denoted by the low atomic C/O ratios of 1.17, 1.15, 0.84, and 0.74 respectively. The trend observed on the atomic C/O ratios, between the chlorate and permanganate methods, was consistent with that obtained by using the XPS technique.

Furthermore, solid-state ^{13}C NMR analyses were carried out to ascertain the chemical environment of the carbon atoms present on the GO materials. The analyses were performed by using a ^1H – ^{13}C cross polarization (CP) mode at 8 kHz MAS (contact time 1 ms) and direct ^{13}C pulse mode at 12 kHz MAS (90° ^{13}C pulse) with average scans of 16000, as shown in Figure 2A and B. The NMR spectra in Figure 2 highlights typical peaks due to the presence of C=O, O–C=O, graphitic sp^2 , C–OH and C–O–C bond types at chemical shifts of δ =190, 165, 129, 69, and 59 ppm, respectively.^[25] The CP mode gave a lesser amount of information on the compositions of GO materials compared with the direct ^{13}C

Figure 2. Solid-state 1D ^{13}C magic-angle spinning (MAS) NMR spectra of graphite, GO-ST, GO-HO, GO-HU, and GO-TO. A) ^1H – ^{13}C cross-polarization (CP) and B) direct ^{13}C pulse spectra.

pulse technique, as was similarly observed in previous reports.^[25–26] The GO materials highlighted obvious presence of graphitic sp^2 , C–OH, and C–O–C bond types on both NMR techniques. In agreement with the XPS analyses, the prominent carbonyl and carboxyl peaks that were only present on the permanganate (GO-HU and GO-TO) oxidation method marked a clear distinction between the two different oxidative treatments.

Subsequently, FTIR analyses were carried out to investigate the presence of specific bond types in the GO materials. As can be seen in Figure 3, prominent peaks corresponding to O–H, C=O, and C–O–C stretching were observed at approximately $\tilde{\nu}$ =3200, 1718, and 1050 cm^{-1} .^[27] The large peak at about $\tilde{\nu}$ =1600 cm^{-1} corresponds to the unoxidized sp^2 regions of the graphite material.^[28] A closer examination of the FTIR spectra revealed a larger peak of C=O stretching, which could account for both carbonyl and carboxyl

Figure 3. ATR-FTIR spectra of graphite, GO-ST, GO-HO, GO-HU, and GO-TO.

functional groups, for the permanganate (GO-HU and GO-TO) oxidation method. This is consistent with the observations obtained from both XPS and NMR studies.

Apart from that, Raman spectroscopic analyses were performed to determine the extent of defects introduced by the oxidation processes as shown in Figure 4. A pristine graphene sheet typically exhibits a single sharp signal at ap-

Figure 4. Raman spectra of graphite, GO-ST, GO-HO, GO-HU, and GO-TO collected by using a 514 nm laser.

proximately 1560 cm^{-1} (G band). Presence of an additional signal at about 1350 cm^{-1} (D band) is usually a result of sp^3 defects in the sp^2 lattice. As such, the intensity ratio of the D and G bands (I_D/I_G) provides vital information on the degree of disorder in the sp^2 lattice. Based on Figure 4, the Raman spectra of graphite, GO-ST, GO-HO, GO-HU, and GO-TO showed average I_D/I_G ratios of 0.09, 0.89, 0.87, 0.87, and 0.85, respectively. The GO materials were equally defective in comparison. Moreover, the broadened D and G bands highlighted greater disorder on the material due to extensive oxidation treatments.

Electrochemical characterization of the GO materials: Electrochemical study of graphite and GO materials were accomplished by using three different electrochemical approaches, mainly electrochemical impedance spectroscopy, cyclic voltammetry, and solid-state reduction techniques.

First, the ability of the GO materials to exchange and transfer charges with their surrounding molecules in the environment was examined by EIS technique. This method allows the determination of heterogeneous charge-transfer resistance (R_{ct}), which is highly influenced by the surface morphology of the material under study. The EIS measurements were carried out on graphite, GO-ST, GO-HO, GO-HU, and GO-TO modified electrodes in a solution containing ferro/ferricyanide electrochemical probe. The data obtained from the EIS study were then analyzed and fitted

Figure 5. Nyquist diagrams for EIS measurements. Conditions: PBS (50 mM), background electrolyte (pH 7.2), $\text{K}_3[\text{Fe}(\text{CN})_6]/\text{K}_4[\text{Fe}(\text{CN})_6]$ (10 mM). Randles equivalent circuit used for data fitting is shown as inset.

based on the Randles equivalent circuit shown in Figure 5 inset.

Based on the Nyquist plot shown in Figure 5, the diameter of the semicircle corresponds to the R_{ct} for the material of interest. The GC electrode gave an R_{ct} of $1.13\text{ k}\Omega$, whereas graphite gave $1.18\text{ k}\Omega$. The GO materials gave higher R_{ct} compared with graphite with GO-HO indicating the lowest R_{ct} at $1.68\text{ k}\Omega$, followed by GO-ST at $1.74\text{ k}\Omega$. The GO-HU and GO-TO resulted in higher R_{ct} at 1.98 and $2.15\text{ k}\Omega$, respectively. The R_{ct} values were higher for materials with lower atomic C/O ratios. This indicates that a percentage of the oxygen-containing groups in the GOs were negatively charged at the working pH, which resulted in electrostatic repulsions with the negatively charged ferro/ferricyanide redox complexes. Because GO-TO exhibited the lowest atomic C/O ratio (richest in oxygen-containing groups), it thus gave a higher R_{ct} value.

Apart from that, cyclic voltammetry analyses were carried out to determine the heterogeneous electron transfer (HET) rate of the GO materials in ferro/ferricyanide probe, as shown in Figure 6. The lowest peak-to-peak separation (ΔE) was observed on GC electrode at 0.30 V . Graphite resulted in a higher ΔE at 0.33 V . The GO-ST and GO-HO materials gave ΔE of 0.35 and 0.34 V , respectively. Higher ΔE were

Figure 6. Cyclic voltammograms on ferro/ferricyanide. Conditions: PBS (50 mM), background electrolyte (pH 7.2), $\text{K}_3[\text{Fe}(\text{CN})_6]/\text{K}_4[\text{Fe}(\text{CN})_6]$ (10 mM). Scan rate: 100 mV s^{-1} .

observed for GO-HU and GO-TO at 0.36 and 0.38 V, respectively. The respective HET rates were calculated according to the Nicholson method, which showed an increasing HET in the order of: GO-TO (k_{obs}^0 : $1.02 \times 10^{-4} \text{ cm s}^{-1}$) < GO-HU (k_{obs}^0 : $1.20 \times 10^{-4} \text{ cm s}^{-1}$) < GO-ST (k_{obs}^0 : $1.40 \times 10^{-4} \text{ cm s}^{-1}$) < GO-HO (k_{obs}^0 : $1.76 \times 10^{-4} \text{ cm s}^{-1}$) < graphite (k_{obs}^0 : $1.94 \times 10^{-4} \text{ cm s}^{-1}$) < GC (k_{obs}^0 : $2.79 \times 10^{-4} \text{ cm s}^{-1}$).^[29] Based on the observation, the HET rates were also affected by the electrostatic repulsions between the negatively charged oxygen-containing groups on GO materials and the ferro/ferricyanide redox probes. This resulted in GO-TO showing the lowest HET rate.

A solid-state electrochemical reduction based on the cyclic voltammetry technique was also performed to determine the presence of electrochemically reducible oxygen-containing groups.^[30] The presence of any electrochemically reducible oxygen functionalities (mainly epoxyl, carbonyl, and peroxy) would usually give intense reduction waves at peak potentials lower than -1.0 V (vs. Ag/AgCl reference electrode). The electrochemical reduction was carried out (Figure 7). Reduction peaks for both the GC and graphite

Figure 7. Cyclic voltammetric profiles of electrochemical reduction of oxygen-containing groups. Conditions: PBS (50 mM), background electrolyte (pH 7.2). Scan rate: 100 mV s^{-1} .

modified electrodes could not be observed, because electrochemically reducible oxygen groups were not present on these surfaces. However, intense reduction peaks were observed for the GO materials. The GO materials obtained by using chlorate (GO-ST and GO-HO) oxidation method resulted in a sharp intense peak at approximately -1.2 to -1.3 V . On the other hand, the GO materials from permanganate (GO-HU and GO-TO) oxidation method gave reduction bands at higher potentials of approximately -1.4 to -1.8 V . We postulated that the higher reduction potentials could be due to the presence of a higher amount of carbonyl and carboxyl functional groups, which usually required higher reduction energies. It should be noted that the common reduction potentials of peroxy group is about -0.7 V ; aldehyde about -1.0 V ; epoxyl about -1.5 V ; and carboxyl group about -2.0 V (vs. Ag/AgCl).

Based on the structural, morphological, and electrochemical characterizations, several conclusions for the questions raised earlier could be drawn. First, GO from the four dif-

ferent oxidation methods exhibited varying characteristics. The structural characterizations highlighted similar extents of oxidation indicated by the atomic C/O ratios (based on XPS and elemental analyses), although the permanganate (GO-HU and GO-TO) oxidation method introduced a higher amount of oxygen groups. On top of that, the permanganate oxidation method also presented a higher proportion of carbonyl and carboxylic functional groups (based on XPS, NMR, and FTIR analyses) compared with the chlorate (GO-ST and GO-HO) oxidation method. However, the electrochemical performances highlighted vast differences, in which the permanganate oxidation method resulted in a higher heterogeneous electron-charge transfer resistance by EIS study and a lower HET rate compared with the chlorate oxidation method. Moreover, the solid-state electrochemical reduction technique indicated the very different structural features on the two categories of materials with respect to the reduction peaks observed in the cyclic voltammetry.

Second, the strength of concentrated acid has typically been assumed to affect the oxidation process as can be seen in the Staudenmaier and Hofmann methods. By comparing these two methods side-by-side, our results have highlighted that there are negligible differences on the GO materials produced. The GO-ST and GO-HU gave almost identical structural properties and electrochemical performances. As such, with respect to the Staudenmaier and Hofmann oxidation methods, they are almost similar, despite the strength of nitric acid utilized.

Third, with regard to the selection of oxidation method towards GO, we should first reflect on the purposes of the applications. Applications, which most likely would result in the passivation of the analytes of interest, such as NADH,^[24] should probably avoid the Hummers and Tours oxidation methods, because the carboxylic groups are mainly responsible for the passivation effect. Otherwise, our observations suggested that the Tour oxidation method is best utilized, because it provided a higher degree of oxidation and is safer since it avoids the usage of concentrated nitric acid.

Conclusion

In summary, the structural and electrochemical properties of the different GOs prepared by using the Staudenmaier, Hofmann, Hummers, and Tour oxidation methods were highlighted. The different oxidative treatments displayed dissimilar properties due to the varying proportions on types of oxygen-containing groups (hydroxyl, epoxyl, carbonyl, and carboxyl) present. The GO obtained by using permanganate (Hummers and Tour) as oxidant introduced a higher amount of carbonyl and carboxyl functional groups compared with the chlorate (Staudenmaier and Hofmann) oxidant according to the structural characterizations. The electrochemical characterizations also highlighted a higher heterogeneous electron-transfer rate for GO obtained by using chlorate oxidant. This study would be vital towards providing fundamental knowledge on selecting a production

method of GO for applications, such as electrochemical sensing, biosensing, or optoelectronics in the future.

Experimental Section

Materials: Graphite (<20 µm), sulfuric acid (95–98%), sodium nitrate, hydrochloric acid (37%), phosphoric acid (85%), potassium chlorate (98%), *N,N*-dimethylformamide (DMF), potassium phosphate dibasic, sodium phosphate monobasic, potassium ferricyanide, and potassium ferrocyanide were purchased from Sigma–Aldrich (Singapore). Potassium permanganate and fuming nitric acid (>90%) were obtained from J.T. Baker. Glassy carbon (GC) electrode with a diameter of 3 mm was obtained from Autolab (The Netherlands). Milli-Q water (resistivity: 18.2 MΩ cm) was used throughout the experiments.

Instruments: XPS was performed with a Phoibos 100 spectrometer and a monochromatic Mg X-ray radiation source (SPECS, Germany). Both wide survey and high-resolution spectra for C1s were collected. Relative sensitivity factors were used for evaluation of atomic C/O ratios from XPS wide survey spectra measurements. XPS samples were prepared by coating a carbon tape with a uniform layer of the materials under study. The elemental analysis was performed with Vario EL III from Elemental. Solid-state ¹³C MAS NMR spectra were recorded on a JEOL-ECA400 (100 MHz ¹³C, 400 MHz ¹H) under ¹H–¹³C cross polarization and direct ¹³C pulse mode by using standard Delta software. Attenuated total reflectance (ATR) FTIR measurements were carried out on a PerkinElmer Spectrum 100 system coupled with a universal ATR accessory. A diamond/ZnSe was used as the ATR crystal. Raman spectroscopy analysis was performed by using a confocal micro Raman LabRam HR instrument from Horiba Scientific in backscattering geometry with a CCD detector, a 514.5 nm Ar laser and a 100× objective mounted on a Olympus optical microscope. The calibration was initially made by using an internal silicon reference at 520 cm^{−1} and gave a peak position resolution of less than 1 cm^{−1}. The spectra were measured from 1000 to 3000 cm^{−1}. All voltammetric experiments were performed on a µAutolab type III electrochemical analyzer (Eco Chemie, The Netherlands) connected to a personal computer and controlled by General Purpose Electrochemical Systems, Version 4.9, software (Eco Chemie).

Procedure (Graphite source was used as-received without additional treatment prior to oxidations): *GO preparation with Staudenmaier method*.^[10] Sulfuric acid (17.5 mL, 95–98%) and nitric acid (9 mL, >90%) were added to a reaction flask containing a magnetic stir bar. The mixture was cooled at 0°C for 15 min. Graphite (1 g) was then added to the mixture under vigorous stirring to avoid agglomeration and obtain a homogeneous dispersion. Potassium chlorate (11 g) was slowly added to the mixture (over 15 min) at 0°C to avoid sudden increases in temperature and the formation of chlorine dioxide gas, which is explosive at high concentrations. After the complete dissolution of potassium chlorate, the reaction flask was loosely capped to allow evolution of gas, and the mixture was stirred vigorously for 96 h at RT. Upon completion of the reaction, the mixture was poured into ultrapure water (1 L) and filtered. GO was then redispersed and washed repeatedly in HCl solutions (5%) to remove sulfate ions. GO was finally washed with ultrapure water until a neutral pH of the filtrate was obtained. The GO slurry was then dried in a vacuum oven at 50°C for five days before use.

GO preparation with Hofmann method.^[11] Sulfuric acid (17.5 mL, 95–98%) and nitric acid (9 mL, 63%) were added to a reaction flask containing a magnetic stir bar. The mixture was cooled at 0°C for 15 min. Graphite (1 g) was then added to the mixture under vigorous stirring to avoid agglomeration and obtain a homogeneous dispersion. Potassium chlorate (11 g) was slowly added to the mixture (over 15 min) at 0°C to avoid sudden increases in temperature and the formation of chlorine dioxide gas, which is explosive at high concentrations. After the complete dissolution of potassium chlorate, the reaction flask was loosely capped to allow evolution of gas, and the mixture was stirred vigorously for 96 h at RT. Upon completion of the reaction, the mixture was poured into ultrapure water (1 L) and filtered. GO was then redispersed and washed

repeatedly in HCl solutions (5%) to remove sulfate ions. GO was finally washed with ultrapure water until a neutral pH of the filtrate was obtained. The GO slurry was then dried in a vacuum oven at 50°C for five days before use.

GO preparation with modified Hummers method.^[12] Graphite (0.5 g) was stirred with sulfuric acid (23.0 mL, 95–98%) for 20 min at 0°C prior to the addition of NaNO₃ (0.5 g) in portions. The mixture was left to stir for 1 h. KMnO₄ (3 g) was then added in portions at 0°C. The mixture was subsequently heated to 35°C for 1 h. Water (40 mL) was then added into the mixture, which resulted in the temperature of the mixture to rise up to 90°C. The temperature was maintained at 90°C for 30 min. Additional water (100 mL) was added into the mixture. This was followed by a slow addition of H₂O₂ (30%, ca. 10 mL). The warm solution was filtered (RC membrane, 0.22 µm) and washed with warm water (100 mL). The solid was subsequently washed with a copious amount of water until a neutral pH was obtained. The materials were kept in the oven at 50°C for five days prior to usage.

GO preparation with Tour method.^[14] Graphite (0.3 g) was added into a mixture of concentrated H₂SO₄/H₃PO₄ (36:4), followed by addition of KMnO₄ (1.8 g). The reaction mixture was then heated to 50°C and stirred for 12 h. The reaction was then cooled to RT and poured in ice water (ca. 40 mL) with addition of H₂O₂ (30%, 0.5 mL). The mixture was then filtered and washed with water, HCl (30%), ethanol, and diethyl ether. The obtained solid materials were kept in the oven at 50°C for five days prior to usage.

The electrochemical experiments were carried out in a voltammetric cell (5 mL) at RT by using a three-electrode configuration. A platinum electrode served as an auxiliary electrode and a Ag/AgCl electrode served as a reference electrode; a glassy carbon electrode was utilized as a working electrode. Prior to a measurement, the glassy carbon electrode was polished with 0.05 µm alumina on a polishing cloth. The working electrode was then modified by applying DMF (1 µL) containing carbon material (1 mg mL^{−1}; GO-ST, GO-HO, GO-HU, or GO-TO) and allowed to dry.

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Lithium Aluminum Hydride as Reducing Agent for Chemically Reduced Graphene Oxides

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S Supporting Information

ABSTRACT: Chemical reduction of graphene oxide is one of the main routes of preparation for large quantities of graphenes. A wide range of reducing agents was described for this task, such as hydroquinone, ascorbic acid, saccharides, proteins, hydrazine, or sodium borohydride. With exception of sodium borohydride and hydrazine, no “standard” organic chemistry agents have been described for reduction of graphene oxides. Lithium aluminum hydride (LAH) is a very powerful reducing agent frequently used in organic synthetic methodologies to convert several types of oxygen containing carbon moieties with a well-known reduction mechanism. Here, we describe, for the first time, the use of LAH toward the reduction of graphene oxide and compare its reduction strength to that of hydrazine and sodium borohydride, which are generally adopted in such application. We show that LAH is far more efficient in reducing oxygen functionalities present on graphene oxide. This is a step forward toward applicability of “standard” organic chemistry reducing agents for reduction of graphene oxides.

KEYWORDS: chemically reduced graphene oxide, lithium aluminum hydride, electrochemistry

1. INTRODUCTION

Graphene and graphene-related materials have attracted tremendous attention from researchers in recent years, due to their unique physical, chemical, and mechanical properties.^{1,2} The range of potential applications of graphene is very wide, and in addition to the most claimed possibility of replacing silicon substrates in electronic devices,³ graphene can also be adopted with promising performance in composite materials,^{4,5} solar cells,⁶ sensing and energy-storage systems,^{7–9} or drug delivery.¹⁰ Graphene can be prepared either by bottom-up method, such as (i) chemical vapor deposition (CVD),^{11,12} or top-down methods, such as (ii) micromechanical exfoliation of graphite,¹³ (iii) exfoliation/unzipping of carbon nanotubes,^{14,15} or (iv) exfoliation of graphite by chemical means, with a preliminary oxidation to graphite oxide, followed by the exfoliation to graphene oxide before the final chemical reduction to graphene.^{16,17}

It should be emphasized that methods i–iii are not suitable for large scale production due to challenging fabrication procedures or high costs. On the contrary, the top-down solution-based graphene preparation can be easily scaled-up. This route consists of a preliminary chemical oxidation and exfoliation of graphite to obtain graphene oxide, followed by a reduction process that could be carried out using irradiation,¹⁸ chemical agents, high temperature, or electrochemical methods.¹⁹ Chemical reduction is the focus of considerable research studies, and several reducing agents have been tested with different procedures to eliminate oxygen groups from the graphene oxide material in order to restore the sp^2 carbon network together with the electronic properties.

Chemical reducing agents used on graphene oxide include hydrazine,^{20,21} hydroquinone,²² sodium borohydride,^{23–25} ascorbic acid,^{26,27} saccharides,²⁸ proteins,²⁹ carbon monoxide,³⁰ norepinephrine,³¹ or even KOH.³² It is important to notice that, except for sodium borohydride and hydrazine, none of the reducing agents adopted so far is commonly used in organic synthesis when conversion or elimination of oxygen functional groups is required. Mechanisms of the reductions are not clear for most of the reducing agents (including N_2H_4).³³ KOH and NaOH at high concentration have proven the ability to solubilize the highly oxidized debris strongly adhered to the graphitic material. Such debris can then be easily removed, with the result of a net deoxygenation.^{32,34} Surprisingly, the reducing agents “toolbox” is lacking in powerful well-known and widely used agents typically used in organic synthesis for reduction of oxygen containing groups, such as lithium aluminum hydride (LAH).³⁵ It should be emphasized here that the reactivity of LAH is very different from that of N_2H_4 and $NaBH_4$. LAH is a very powerful and unselective reagent capable of reducing carbonyl, epoxy, ester, and carboxylic groups to hydroxyl. $NaBH_4$ can reduce only carbonyl groups (aldehydes and ketones) to hydroxyl. N_2H_4 , used with the conditions of the Wolff–Kishner reduction, specifically reduce carbonyl groups to methylene.³⁶ However, it is known that, in the presence of α,β -unsaturated ketones, N_2H_4 cannot reduce the $C=O$ group to methylene, and pyrazolines are the resulting products instead.³⁷ Such eventuality is highly probable considering the

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sp^2 hybridized network of graphene oxide (GO) and would also explain the presence of nitrogen atoms typically found in the graphene framework after reduction with hydrazine.³⁸ Nitrogen doping causes the loss of important electronic properties of the graphene material and certainly should be taken into consideration when selecting the reduction procedure. Based on such differences, it is clear that LAH should be more efficient in eliminating most of the oxygen functionalities present on graphene oxide, resulting in a reduced material with different properties.

Our long-term aim is to evaluate a toolbox of standard organic reagents for oxygen-containing group reduction, as these reagents possess the great advantage (in contrary to “new” agents, such as ascorbic acid or epinephrine) of well developed methodology and understood mechanisms. In this work, we demonstrate for the first time the use of the strong reducing agent, lithium aluminum hydride, for the chemical reduction of graphene oxide and investigate the properties of the reduced material by means of Raman spectroscopy, X-ray photoelectron spectroscopy (XPS), attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR), cyclic voltammetry (CV), and electrochemical impedance spectroscopy (EIS). We also compare such properties on materials obtained using common reducing agents such as hydrazine and sodium borohydride.

2. EXPERIMENTAL SECTION

2.1. Materials. Graphite (<20 μm), sulfuric acid (95–98%), potassium chlorate (98%), hydrochloric acid (37%), *N,N*-dimethylformamide (DMF), tetrahydrofuran (THF), methanol, ethanol, sodium hydroxide, potassium phosphate dibasic, sodium carbonate, sodium phosphate monobasic, sodium sulfate, potassium ferrocyanide, potassium chloride, sodium borohydride, and lithium aluminum hydride were obtained from Sigma-Aldrich, Singapore. Fuming nitric acid (>90%) was obtained from J.T. Baker. Hydrazine hydrate was obtained from Alfa Aesar, Singapore. Glassy carbon (GC) electrodes with a diameter of 3 mm were obtained from Autolab, The Netherlands.

2.2. Apparatus. X-ray photoelectron spectroscopy (XPS) was performed with a Phoibos 100 spectrometer and a monochromatic Mg X-ray radiation source (SPECS, Germany). Both survey and high-resolution spectra for C1s were collected. Relative sensitivity factors were used for evaluation of C/O ratios from survey spectra XPS measurements. XPS samples were prepared by coating carbon tape with a uniform layer of the materials under study. Raman spectroscopy analysis was performed using a confocal micro-Raman LabRam HR instrument from Horiba Scientific in backscattering geometry with a CCD detector, a 514.5 nm Ar laser and a 100 $\times$ objective mounted on a Olympus optical microscope. The calibration is initially made using a silicon reference at 520 cm^{-1} and gives a peak position resolution of less than 1 cm^{-1} . The spectra are measured from 1000 to 3000 cm^{-1} . Attenuated total reflectance Fourier transform infrared (ATR-FTIR) measurements were carried out on a PerkinElmer Spectrum 100 system coupled with a universal ATR accessory. A diamond/ZnSe was used as the ATR crystal. Thermogravimetric analysis (TGA) was performed heating the material from room temperature to 900 $^{\circ}\text{C}$ with ramp of 6 $^{\circ}\text{C}/\text{min}$ and in the presence of air at 60 mL/min flow. All voltammetric experiments were performed on a μ Autolab type III electrochemical analyzer (Eco Chemie, The Netherlands) connected to a personal computer and controlled by General Purpose Electrochemical Systems Version 4.9 software (Eco Chemie).

2.3. Procedures. Graphene Oxide (GO). GO was prepared using graphite as starting material and according to the Staudenmaier method.³⁹ Sulfuric acid (17.5 mL; 95–98%) and 9 mL of nitric acid (fuming) were added to a reaction flask containing a magnetic stir bar. The mixture was cooled at 0 $^{\circ}\text{C}$ for 15 min. Graphite (1 g) was then added to the mixture under vigorous stirring to avoid agglomeration

and obtain a homogeneous dispersion. Potassium chlorate (11 g) was slowly added to the mixture (over 15 min) at 0 $^{\circ}\text{C}$ to avoid sudden increases in temperature and the formation of chlorine dioxide gas, which is explosive at high concentrations. After the complete dissolution of potassium chlorate, the reaction flask was loosely capped to allow evolution of gas, and the mixture was stirred vigorously for 96 h at room temperature. On completion of the reaction, the mixture was poured into 1 L of ultrapure water and filtered. Graphite oxide so obtained was then redispersed and washed repeatedly in HCl (5%) solutions, to remove sulfate ions, and ultrapure water, until a neutral pH of the filtrate was obtained. The graphite oxide slurry was then dried in a vacuum oven at 60 $^{\circ}\text{C}$ for 48 h before use. Exfoliation of graphite oxide to obtain graphene oxide (GO) is performed by ultrasonication for 2 h of 1 mg/mL dispersion of graphite oxide.

GO Reduction by Sodium Borohydride. Dry graphite oxide powder (80.5 mg) was dispersed in ultrapure water to give a 1.0 mg/mL colloidal solution. The mixture was ultrasonicated (150 W) for 3 h prior to the addition of 5 wt % sodium carbonate to obtain a dispersion of pH 9–10. Sodium borohydride (800 mg, 21.1 mmol) was added over 20 min into the dispersion under vigorous stirring. The reaction mixture was heated to 80 $^{\circ}\text{C}$ for 1 h. The mixture was cooled to room temperature and washed repeatedly with ultrapure water using centrifugation (8000 rpm, 10 min). The sample pellet was dried at 60 $^{\circ}\text{C}$ for 5 days to yield CRGO1.

GO Reduction by Hydrazine. Dry graphite oxide powder (49.8 mg) was dispersed in ultrapure water to give a 1.0 mg/mL colloidal solution and ultrasonicated (150 W) for 3 h. Hydrazine hydrate (2 mL, 32.1 mmol) was added dropwise at 47 $^{\circ}\text{C}$, and then, the reaction mixture was heated to 80 $^{\circ}\text{C}$ for 24 h. After cooling to room temperature, the mixture was washed repeatedly with methanol and ultrapure water, using centrifugation (8000 rpm, 10 min). The sample pellet was dried at 60 $^{\circ}\text{C}$ for 5 days to yield CRGO2.

GO Reduction by Lithium Aluminum Hydride (LAH). Dry graphite oxide powder (50 mg) was dispersed in dry THF (30 mL) and ultrasonicated (150 W) for 3 h. LAH (190 mg, 5 mmol) dispersed in dry THF (10 mL) was added dropwise at 0 $^{\circ}\text{C}$ until bubbling was no longer observed. The mixture was then transferred into a solution of LAH (760 mg, 20 mmol) in dry THF (20 mL) dropwise at 0 $^{\circ}\text{C}$. The reaction mixture was left to stir under reflux for 24 h. The mixture was quenched with a saturated solution of sodium sulfate, followed by 1 M hydrochloric acid at 0 $^{\circ}\text{C}$ until a clear solution was obtained. The mixture was filtered (RC membrane filter, 0.2 μm) and washed repeatedly with ultrapure water to give CRGO3 upon drying at 60 $^{\circ}\text{C}$ for 5 days.

Immobilization of the carbon materials onto the working electrode (GC) was performed first by preparing a suspension of the desired material with a concentration of 1 mg/mL in DMF. After 30 min ultrasonication, 1 μL aliquot from the appropriate suspension was deposited onto the electrode surface. The solvent was then allowed to evaporate at room temperature, leaving a randomly distributed film onto the GC electrode surface.

Cyclic voltammetry experiments were performed at a scan rate of 100 mV s^{-1} in 0.1 M KCl as supporting electrolyte and using 5 mM ferro/ferricyanide as redox probe. Electrochemical impedance spectroscopy (EIS) measurements were performed in 0.1 M KCl as supporting electrolyte and using 5 mM ferro/ferricyanide as redox probe. The GC electrode surfaces were renewed by polishing with 0.05 μm alumina particles on a cloth (DP-Plus, Streuers) before each voltammetry measurement.

The k_{obs}^0 values were determined using the method developed by Nicholson that relates ΔE_p to a dimensionless parameter ψ and consequently to k_{obs}^0 .⁴⁰ The roughness factor was not taken into account. The diffusion coefficient $D = 7.26 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ for $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 M KCl was used for calculations.

Current–voltage measurements (*I*–*V* curve) were conducted by depositing 1 μL of the material suspension (prepared at 1 mg/mL concentration in ethanol) onto the interdigitated electrode (Au-IDE) having 10 μm spacing. The electrode was then dried under a lamp for 30 min, leaving a randomly deposited material film on the

interdigitated area bridging the two Au electrode bands. I – V curves were obtained by linear sweep voltammetric measurements at 20 mV s^{-1} scan rate. The displayed data corresponds to the average from five measurements.

3. RESULTS AND DISCUSSION

We compare three reduction reagents for reduction of graphene oxide, mainly sodium borohydride ($NaBH_4$), hydrazine hydrate (N_2H_4), and LAH ($LiAlH_4$). The reduction procedures employed in this work followed an initial oxidation of graphite to graphite oxide and consequent exfoliation of this byproduct to graphene oxide (GO). This oxidation step was performed in accordance to the Staudenmaier method,³⁸ which involves the use of concentrated nitric and sulfuric acid together with potassium chlorate as the main oxidizing agent. Graphite oxide produced in this way was exfoliated to graphene oxide (GO) by ultrasonication, followed by a chemical reduction with three different reducing agents: $NaBH_4$, N_2H_4 , and $LiAlH_4$. Figure 1 summarizes the procedural steps involved. GO and the

Figure 1. Schematic of the procedure followed for the preparation of the chemically reduced graphene materials. (a) An oxidative treatment was initially performed to generate graphene oxide from graphite as starting material. Then graphene oxide is reduced by means of (b) $NaBH_4$; (c) N_2H_4 ; (d) $LiAlH_4$.

chemically reduced products have been comprehensively characterized by XPS, Raman and FTIR spectroscopies, cyclic voltammetry, and electrochemical impedance spectroscopy. By using these methods, we are able to obtain structural and chemical information related to the reduced graphene materials, and thus, we are able to evaluate the efficiencies of the reduction reactions. Also, it is possible to relate the structure of the materials to the performance in electrochemical applications.

XPS represents an invaluable characterization tool that can reveal superficial atomic composition of a solid sample and,

more importantly, the type of chemical groups bonded to it. In our study, XPS was adopted to gain two pieces of important information: (i) the atomic C/O ratio composing the sample surface, which could be used to evaluate the extent of the oxidation first and then the reduction methodology proposed; (ii) the type of oxygenated carbon groups. Figure 2a shows the

Figure 2. XPS survey scan (a) and high-resolution XPS C 1s core spectra (b) of GO, CRGO1, CRGO2, and CRGO3 materials.

wide scan XPS survey spectra of the reduced graphene materials as well as the starting GO. It can be seen that GO presents an intense oxygen signal due to the introduction of oxygen containing groups into the graphene backbone by the oxidative treatment. According to the XPS measurement, the atomic C/O ratio for GO was 3.4, which corresponded to an atomic oxygen content of about 22.5% (mass oxygen content of 28.2%). The elemental analysis of GO resulted in an atomic C/O ratio of 1.2 (see Figure S3, Supporting Information). Clearly, the oxidative treatment introduced a significant amount of oxygen species. The three reduction procedures were able to eliminate most of the oxygen functionalities from the GO material indicated by a much less intense oxygen signal. However, a precise evaluation revealed different efficiencies in removal of oxygen groups by the three reducing agents. The atomic C/O ratios resulted 9.5 for CRGO1, 11.5 for CRGO2, and 12 for CRGO3. These values correspond to an atomic oxygen content of 9.5, 8.0, and 7.6% (mass oxygen content of 12.3, 10.4, and 10.0%) for CRGO1, CRGO2, and CRGO3, respectively.

It should be noted that the use of hydrazine as reducing agent introduces nitrogen atoms into the sp^2 carbon network resulting in nitrogen content in CRGO2 of about 1% according to our XPS analysis. Again, the presence of nitrogen moieties into the graphene network may result from the incomplete reduction of α,β -unsaturated carbonyl groups using hydrazine, giving pyrazole as a final product.³⁸ Reduction with LAH was the most efficient method to eliminate the oxygen containing

groups. Consequently, we carried out high resolution XPS of C1s core spectra (Figure 2b) in order to obtain insights into the amount and type of the oxygen containing species present. GO gave four main peaks centered at 284.5 eV (graphitic sp^2 C), 286.0 eV (sp^3 C, epoxy and hydroxyl), 287.0 eV (carbonyl), and 288.7 eV (carboxyl). The signal at 291 eV can be assigned to the π - π^* shakeup satellite of the sp^2 band centered at 284.5 eV. The C1s core level envelope of the chemically reduced graphene oxides were fitted to five components centered at 284.5 eV (graphitic sp^2 C), 285.5 eV (sp^3 C), 286.5 eV (epoxy, hydroxyl), 287.6 eV (carbonyl), and 289.7 eV (carboxyl). It can be seen that, upon chemical reduction, the graphitic sp^2 C band becomes narrower than that of GO, indicating a more homogeneous chemical environment and a more ordered structure. The amount of sp^2 C increased from a value of 36% in GO to a value of 68, 69, and 73% in CRGO1, CRGO2, and CRGO3, respectively. The main difference among the three methods is the remaining amount of carboxylic groups. By using LAH as a reducing agent, only about 2% of carboxylic groups remained on the graphene material, while with N_2H_4 and NaBH_4 , the amounts remaining were 7 and 6%, respectively. LAH is known to be among the most powerful reducing agents used in organic chemistry reactions, and it is clearly demonstrated here as having a more effective ability to reduce the carboxylic groups especially, compared to NaBH_4 and N_2H_4 . Raman spectroscopy can be used to gain structural information on the materials. The first-order spectra (1100–1800 cm^{-1}) show the characteristic D and G bands of carbon materials. The G band at about 1590 cm^{-1} involves in-plane bond stretching of sp^2 carbon pairs and thus is indicative of pristine graphene sheet. The D band located at about 1350 cm^{-1} is related to disordered conformations and defects of the sp^2 carbon lattice of graphene. The ratio between the intensities of the D and G bands (I_D/I_G) is thus used as an indicator of the relative disorder present in graphitic/graphenic structures.⁴¹ Figure 3 shows the Raman spectroscopy spectra of GO, CRGO1, CRGO2, and CRGO3. The obtained I_D/I_G ratio of GO was 1.10. This indicates quite an extensive disorder in the

Figure 3. Raman spectra of GO and the reduced materials CRGO1, CRGO2, and CRGO3. The spectra have been normalized according to the G band for a better comparison.

graphitic structure due to the presence of oxygen functionalities. Upon reduction, the I_D/I_G ratios decreased to 0.61, 0.97, and 0.63 for CRGO1, CRGO2 and CRGO3, respectively. Although structural changes introduced by chemical reduction are still not well understood, a reduced I_D/I_G ratio has been explained by the elimination of oxygen species with the reintroduction of large aromatic domains.⁴² NaBH_4 and LAH are clearly the most effective in reducing structural disorders compared to hydrazine, according to our measurements.

To gain more information on the functionalities present on the material surface, we also performed solid-state FTIR measurements on the materials in investigation (Figure 4). The

Figure 4. FTIR spectra of graphite, GO, and the reduced materials CRGO1, CRGO2, and CRGO3. Attenuated total reflectance (ATR) mode is used with all samples in solid state.

FTIR spectrum of graphite shows almost undetectable signals with no significant bands. GO presented intense bands at 1060 and 1370 cm^{-1} corresponding to C–OH vibrations, signals at 870 and 1250 cm^{-1} were generated by bending and stretching vibrations of epoxy groups, signals at 1620 and 1720 cm^{-1} were generated by C=O and COOH vibrations, and finally, an intense and wide band at 2500–3700 cm^{-1} was due to OH groups and adsorbed water. Upon reduction, the elimination of oxygen functionalities was clearly apparent for the three CRGO materials, thus resulting in signals that were barely detectable. An interesting exception was given by the appearance of a clear band at 2800–3000 cm^{-1} for CRGO3, produced using LAH as reducing agent. These peaks correspond to the C–H stretching modes.⁴³ This band, not visible for the other reducing agents may further show how LAH is the most effective in reducing oxygen containing groups. In particular, the reduction of carboxylic groups, possible only with LAH, generates CH_2 –OH functionalities. The stretching modes of C–H appearing at 2800–3000 cm^{-1} , can be assigned to such resulting functionalities. C–H stretching modes were not observed after using hydrazine as reducing agent. This further confirms that in the absence of a strong base (normally used in the Wolff–Kishner reaction, but not in the present work), pyrazole moieties are the final product at the carbonyl group locations due to the extensive conjugation. It is also known that $\text{C}=\text{C}$

double bonds are usually not affected by metallic hydrides. However, when the double bond is conjugated with a C=O group, a possible reduction of the double bond may also occur with powerful reducing agents such as LAH. Graphene oxide is, by nature, a highly conjugated sp^2 structure, and therefore, a partial reduction of C=C bonds to CH_2-CH_2 may occur,³⁵ giving rise to the C–H stretching mode band. A significantly increased amount of hydrogen for CRGO3 was also confirmed by the elemental analysis. CRGO3 resulted, in fact, with atomic 11.4% of hydrogen versus the 2.5% of the starting GO material (Figure S1 of Supporting Information). From a physical point of view, CRGO3 resulted in an increased surface area of about $31\text{ m}^2\text{ g}^{-1}$, compared to the starting GO material ($9.4\text{ m}^2\text{ g}^{-1}$).

In an attempt to better understand the reductive action of LAH, it is important to take into consideration that the starting GO material contains for its nature, a significant amount of adsorbed water, intercalated within the graphene oxide layers. We used TGA to confirm such possibility with the GO material adopted in this work (Figure 5). From the analysis, GO

Figure 5. TGA (black) and DTG (red) in air of the starting material GO.

presents mass losses at three different temperatures: (i) about 10–15% of mass was lost between 20 and 100 °C, attributable to the elimination of the adsorbed water; (ii) about 25% of mass loss at about 250 °C due to the decomposition of oxygenated groups or to the decomposition of oxygenated debris;³⁴ and (iii) complete mass loss at 600 °C due to the oxidation of the damaged graphitic structure. As expected, a significant amount of water is present within the GO material.

This may have important consequences during the reaction with LAH. The reduction reaction is performed in “dry” THF solution to avoid reaction with water, but the intrinsic water present within the GO may react with LAH generating locally a strong base along with the evolution of H_2 gas. In such conditions, as described by Rourke et al.,³⁴ detachment of the oxygenated debris may occur resulting in a primary deoxygenation of GO. In a second step, the excess of LAH added reacts with the remaining covalently bonded oxygen groups on the GO material. Such double effect most likely explains the excellent reducing capability of LAH. As a side effect, the oxygenated debris detachment causes the restacking of the GO sheets due to the reduced repulsive forces.³⁴ We noticed this effect from our atomic force microscopy (AFM) measurements (Figure S2, Supporting Information). The thickness of the GO sheets was about 2 nm, while after the reduction, CRGO3 material had 3–5 nm sheet thickness. However, aggregation of the graphene sheets is commonly experienced with most of the reducing agents.

The formation of methylene moieties should have a tremendous effect on the solubility properties of the material. We tested the solubility of the four materials in pure water and in DMF after a 30 min ultrasonication treatment. It can be seen in Figure 6 that CRGO3, produced with LAH, is practically

Figure 6. Digital photographs of GO and the reduced materials CRGO1, CRGO2, and CRGO3 dispersed in (a) ultrapure water and (b) DMF, immediately after 30 min sonication. All dispersions are prepared at 1 mg/mL concentration.

insoluble in water, while the other materials show a good dispersibility in this solvent. Using DMF as solvent, all the materials showed good dispersibility. This could be explained by the fact that GO, CRGO1, and CRGO2 structures are comprised of both hydrophobic and hydrophilic moieties. CRGO3, on the contrary, resulted in predominant hydrophobic functionalities (C–H), which caused low dispersion and stability in water.⁴⁴

In more detail, the good solubilities of GO, CRGO1, and CRGO2 in water are mostly attributable to carboxylic groups, which are the only groups dissociated at neutral pH. Such functional groups are certainly present on CRGO1 and CRGO2, since $NaBH_4$ and hydrazine are not able to reduce carboxylic groups.

LAH is the only reducing agent able to reduce carboxylic groups, and therefore, their absence on the reduced material accounts for a significantly reduced solubility in water.

To further characterize the materials, we performed some electrochemical measurements with the aim to evaluate the electron transfer rate and the charge transfer resistance. Cyclic voltammetry, which is a large amplitude electrochemical technique, was used to measure the electron transfer rate between the materials under investigation and the redox probe ferro/ferricyanide in solution. Figure 7a shows representative cyclic voltammograms of GO-, CRGO1-, CRGO2-, and CRGO3-modified GC electrodes in the presence of 5 mM ferro/ferricyanide and compared to a bare GC electrode. According to Nicholson method, the peak separation between oxidation and reduction signal can be correlated to the heterogeneous electron transfer (HET) rate.⁴⁰ For a more precise evaluation, Figure 7b shows the averaged ($n = 5$) peak separations for all materials and the corresponding HET rates. GO resulted with the highest ΔE of 415 mV. CRGO1 and CRGO2 resulted in a similar value of peak separation of 326 and 320 mV, respectively. CRGO3, generated using LAH as reducing agent, resulted in higher peak separation of 387 mV. Such increment results in about 3-fold decrease of HET rate compared, for example, to CRGO2. The HET rate constants were 0.6×10^{-4} , 1.9×10^{-4} , 2.1×10^{-4} , and $0.8 \times 10^{-4}\text{ cm s}^{-1}$

Figure 7. Electrochemical characterization of GO and the reduced materials CRGO1, CRGO2, and CRGO3. (a) Representative cyclic voltammograms of 5 mM [Fe(CN)₆]^{4-/3-} using bare GC and GO-, CRGO1-, CRGO2-, CRGO3-modified GC electrodes. Conditions: KCl 0.1 M background electrolyte, scan rate 0.1 V/s. (b) Peak-to-peak separation average values ($n = 5$) between the oxidation and reduction of [Fe(CN)₆]^{4-/3-} (left axis) and corresponding value of k_0^0 (right axis). (c) Nyquist plots for EIS measurements of bare GC and GO-, CRGO1-, CRGO2-, and CRGO3-modified electrodes. Conditions: KCl 0.1 M background electrolyte, 5 mM [Fe(CN)₆]^{4-/3-}. Inset: Randles equivalent circuit used for data fitting.

for GO, CRGO1, CRGO2, and CRGO3, respectively. This means that electrons are transferred the fastest at N₂H₄ reduced graphene, slower at NaBH₄ reduced graphene, and even slower at LAH reduced graphene. We will discuss the reasons for such behavior after describing the results of independent electrochemical method, impedance spectroscopy (EIS). EIS is a low amplitude sensitive technique able to evaluate the resistance of HET between material, in our case graphene, and molecular probe in the solution.

Such electron-exchange ability is strongly influenced by structural conformation and surface chemistry of the material, and therefore, EIS can provide further insights into the structures. The charge transfer resistance (R_{ct}) corresponds to the diameter of the semicircle in Nyquist plots obtained from the measurements. As it can be seen in Figure 7c, GO presents the highest R_{ct} value of 7.3 kΩ, which is due to the low conductivity of the material and the presence of negatively charged oxygen functionalities repelling negatively charged ferro/ferricyanide complex. The reduced materials CRGO1, CRGO2, and CRGO3 present R_{ct} of 4.0, 3.3, and 5.9 kΩ, respectively. This means that N₂H₄ reduced graphene showed the lowest resistance to HET, and while the graphene reduced with LiAlH₄ showed the highest resistance to HET. It is clear that the EIS data are consistent with those obtained by cyclic voltammetry. This might be explained considering the structure of these materials. It is well-known that HET of graphenic structures toward ferro/ferricyanide is very fast at the edges of graphene sheets (where defects are also considered as “edge sites” for HET) and very slow (by means of 10⁷ difference) at the basal plane of graphene.^{45–47}

The fastest HET at N₂H₄ reduced graphene is due to its large density of defects, as determined by Raman spectroscopy, while the slowest HET on LAH reduced graphenes is due to low defect density and additional presence of C–H moieties.

For a qualitative comparison of the conductivities of the materials, we measured I – V curves by means of interdigitated gold electrodes (Au-IDE).⁴⁸ Materials with “ohmic” behaviors would present linear I – V curves with slopes directly proportional to their conductivities. Using this method, an immediate qualitative comparison of GO, CRGO1, CRGO2, and CRGO3 was obtained, as depicted in Figure 8. It can be seen that GO

Figure 8. Current–voltage curves of GO, CRGO1, CRGO2, and CRGO3 measured after deposition of the materials onto an Au-IDE. Upper left inset: zoomed I – V curve from GO. Lower right inset: optical image of the bare Au-IDE.

presented an extremely high resistance giving almost zero current signal over the scanned voltage. Such a result confirms the insulator-semiconducting nature of GO.²⁰ The three chemically reduced materials showed all ohmic behaviors with linear I – V curves. By comparing each I – V curve, it can be seen that the highest slope resulted for the CRGO1 (62.4 mA/V), indicating the highest conductivity. CRGO2 with a slope of 46.6 mA/V presented a slightly lower conductivity. CRGO3, on the contrary, has the lowest conductivity, with a slope of 2.9 mA/V, which was 1 order of magnitude lower than the other materials. These findings confirm the results obtained using cyclic voltammetry and EIS. The presence of C–H moieties is most likely responsible for the low conductivity of CRGO3.

4. CONCLUSIONS

We have demonstrated that lithium aluminum hydride is a powerful reducing agent for graphene oxide and is capable of inducing further reduction of oxygen containing groups than the typically used reagents for this task, specifically hydrazine or NaBH₄. Oxygen containing groups are reduced in a larger extent, resulting in a higher C/O ratio when using LAH when compared to N₂H₄ and NaBH₄. From a wider point of view, we have demonstrated that a well-established reducing agent from “conventional” organic chemistry can be beneficial for the reduction of graphene oxide materials.

■ ASSOCIATED CONTENT

Supporting Information

AFM, Brunauer–Emmett–Teller, and elemental analysis of GO and CRGO3 (LAH). This information is available free of charge via the Internet at <http://pubs.acs.org/>.

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Notes

The authors declare no competing financial interest.

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Reduction of graphene oxide with substituted borohydrides

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The emergence of graphene as a next-generation material promises enhanced improvement in various fields of materials science. So far, the oxidation of graphite to graphite oxide and consequent reduction to chemically reduced graphene oxide is the most relevant method towards large scale production of graphene materials. Our long-standing aim in the investigation of reductive chemical reactions on graphene surfaces is to evaluate standard, well documented organic synthetic reactions with well-known mechanisms. Sodium borohydride is one of the most common reductants to reduce graphene oxide to chemically reduced graphene oxide, and is only one of the very few with a well-known mechanism. It is well-known in synthetic chemistry that the reducing strength of borohydrides can be fine-tuned by alternating their substituents. This knowledge has not yet been applied to the reduction of graphene oxides. Herein, we expand on the scope for reduction of graphene oxides using various derivatives of borohydrides, specifically sodium cyanoborohydride and sodium triacetoxymborohydride, and investigate the extents of reduction conferred by these variations with comparison to sodium borohydride. The reduced graphenes were characterized by high resolution X-ray photoelectron spectroscopy, Fourier transform infrared spectroscopy, Raman spectroscopy, electrochemical impedance spectroscopy and cyclic voltammetry analyses. Our findings highlighted that sodium triacetoxymborohydride is inclined to react unfavourably with graphene oxide, thus resulting in a graphene material with an almost similar electrochemical characteristic to its precursor. On the other hand, sodium cyanoborohydride conferred obvious reductive effects but was still less effective when compared to sodium borohydride. Our findings bring a deeper understanding of the effects of organo-boron substituents on the extent of graphene oxide reduction. This would allow for potential tailoring of the graphene properties for various applications.

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1 Introduction

Graphene, a 2-dimensional sheet of sp^2 carbon network which resembles a honeycomb structure, has attracted enormous attention from the scientific community.¹ Although previously thought to be thermodynamically unstable under ambient conditions,² graphene was first isolated *via* a 'scotch tape' method in 2004 by Geim *et al.*³ The unique mechanical flexibility,⁴ optical transparency,⁵ thermal and electrical conductivity⁶ of graphene provide opportunities to develop graphene as a material for energy storage, electronic and electrical devices.⁷ Two approaches are commonly used towards the production of graphene, a bottom-up approach such as chemical vapor deposition,⁸ or a top-down approach such as micromechanical exfoliation of graphite,³ unzipping of carbon nanotubes,⁹ and exfoliation of graphite *via* oxidative and subsequently reductive/thermal treatments.¹⁰ These methods generally do not allow for

the bulk production of graphene except the chemical treatment option *via* oxidative and reductive/thermal steps. The graphene materials produced through such chemical treatment are termed as chemically reduced graphene oxide, rGOs. Even though rGOs display a considerable amount of sheet defects and lower conductivities compared to a pristine graphene sheet, the performances are by and large acceptable given the fact that it is the only method towards the large scale production of graphene.

The rGOs are usually obtained in large quantities by a two-step method. Firstly, graphite is oxidized to graphite oxide by oxidation methods such as the Staudenmaier or Hummers methods.^{11,12} Subsequently, graphite oxide is exfoliated to graphene oxide (graphene sheets covalently bonded with various oxygen functionalities such as hydroxyl, epoxy, carbonyl and carboxyl) through ultrasonication and then reduced using various reducing agents. The reductive treatments carried out at high temperature aim to remove most of the oxygen-containing groups, although concrete and viable mechanisms have not been experimentally proven yet.^{13,14} Moreover, although most reductants claimed to restore the sp^2 carbon network of

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graphene oxide, their mechanisms of reduction did not provide any hints for the sp^2 restoration to take place. To date, various reducing agents have been applied for the reduction of graphene oxide, including hydrazine,¹⁴ sodium borohydride,¹⁵ lithium aluminium hydride,¹⁶ hydroiodic acid,¹⁷ thiourea dioxide,¹⁸ ascorbic acid,¹⁹ hydroquinone,²⁰ hydroxylamine,²¹ cytochromes,²² and a protein-directed approach.²³ While these reducing agents have been used extensively towards obtaining rGOs, they have varying reducing strength and some of these reducing agents are not very convenient for large scale usage. For example, lithium aluminium hydride requires an inert atmosphere and hydrazine is highly carcinogenic.²⁴ Moreover, for many of them, there are no plausible mechanisms or explanations presented.^{19–23}

Among the reductants mentioned above, sodium borohydride is commonly used as a reductant of graphene oxide towards rGOs. Shin *et al.* have reported on sodium borohydride-based rGO films to display superior conductivity compared to hydrazine-based rGO films.¹⁵ Moreover, sodium borohydride has also been utilized to remove common oxygen-containing groups while preserving the carboxylic groups of graphene oxide for further modifications *via* pH variations.²⁵ We are interested in the effect of borohydride variations on the properties of the produced rGOs. Theoretically, sodium borohydride is an effective reductant for aldehyde, ketone and acyl chloride functionalities, while it is rarely useful for epoxide and carboxylic acid groups. Derivatives of sodium borohydride such as sodium cyanoborohydride and sodium triacetoxymborohydride usually display a relatively lower reducing strength.²⁶ We are interested in investigating the three different borohydrides, mainly sodium borohydride ($NaBH_4$), sodium cyanoborohydride ($NaBH_3(CN)$) and sodium triacetoxymborohydride ($NaBH(OAc)_3$) on the graphene oxide platform (for chemical structures, see Scheme 1) as their use could result in fine-tuning of the oxygen composition of graphene oxides. The difference in the reducing strength achieved by the different borohydrides have strong potential for the preparation of graphene for various specific applications in sensing and electronic devices.^{27,28}

2 Experimental

2.1 Materials

Graphite was obtained from Asbury Carbons. Sulphuric acid (95–98%), sodium nitrate, *N,N*-dimethylformamide (DMF), potassium phosphate dibasic, sodium phosphate monobasic, potassium ferrocyanide, sodium chloride and potassium chloride were purchased from Sigma-Aldrich, Singapore. Potassium

permanganate was obtained from J.T. Baker. Hydrogen peroxide was obtained from Alfa Aesar, Singapore. Methanol was obtained from Tedia, Singapore. A glassy carbon (GC) electrode with a diameter of 3 mm was obtained from Autolab, the Netherlands. Milli-Q water (resistivity: 18.2 $M\Omega\text{ cm}$) was used throughout the experiments.

2.2 Apparatus

X-ray photoelectron spectroscopy (XPS) was performed with a Phoibos 100 spectrometer and a monochromatic Mg X-ray radiation source (SPECS, Germany). Both survey and high-resolution spectra for C1s were collected. Relative sensitivity factors were used for evaluation of C/O ratios from survey spectra XPS measurements. XPS samples were prepared by coating carbon tape with a uniform layer of the materials under study. Raman spectroscopy analyses were performed using a confocal micro-Raman LabRam HR instrument from Horiba Scientific in backscattering geometry with a CCD detector, a 514.5 nm Ar laser and a 100 $\times$ objective mounted on an Olympus optical microscope. The calibration was initially made using an internal silicon reference at 520 cm^{-1} and gave a peak position resolution of less than 1 cm^{-1} . The spectra were measured from 1000 to 3000 cm^{-1} . Attenuated total reflectance (ATR) Fourier transform infrared (FTIR) measurements were carried out on a PerkinElmer Spectrum 100 system coupled with a universal ATR accessory. A diamond/ZnSe was used as the ATR crystal. All voltammetric experiments were performed on a μ Autolab type III electrochemical analyzer (Eco Chemie, The Netherlands) connected to a personal computer and controlled by General Purpose Electrochemical Systems Version 4.9 software (Eco Chemie).

2.3 Procedures

2.3.1 GRAPHITE OXIDE (GRAPHITE-OX). Graphite-Ox was prepared using graphite according to Hummers¹² method whereby graphite (0.5 g) was stirred with sulphuric acid (23.0 mL, 95–98%) for 20 min at 0 $^{\circ}C$ prior to the addition of $NaNO_3$ (0.5 g) in portions. The mixture was left to stir for 1 h. $KMnO_4$ (3 g) was then added in portions at 0 $^{\circ}C$. The mixture was subsequently heated to 35 $^{\circ}C$ for 1 h. Water (40 mL) was then added into the mixture and resulted in the temperature of the mixture rising up to 90 $^{\circ}C$. The temperature was maintained at 90 $^{\circ}C$ for 30 min. Additional water (100 mL) was added into the mixture. This was followed by a slow addition of 30% H_2O_2 (~10 mL). The warm solution was filtered (RC membrane, 0.22 μm) and washed with warm water (100 mL). The solid was

Scheme 1 Chemical structures of sodium borohydride, sodium cyanoborohydride and sodium triacetoxymborohydride.

subsequently washed with a copious amount of water until a neutral pH was obtained. The materials were kept in a vacuum oven at 30 °C for 5 days prior to usage. Graphene oxide was obtained by subjecting Graphite-Ox through ultrasonication [37 kHz] for 2 h in methanol to give a 1 mg mL⁻¹ dispersion.

2.3.2 SODIUM BOROHYDRIDE-REDUCED GRAPHENE OXIDE (BH-rGO). To a dispersion of graphene oxide in methanol (25 mL, 1 mg mL⁻¹) was added Na₂CO₃ (5%) to adjust the pH to ~8–9. Sodium borohydride (284 mg, 6.57 mmol) was added into the dispersion and stirred at 70 °C (reflux) for 2 h. Upon completion, the mixture was filtered under vacuum and washed repeatedly with methanol and water until a neutral pH of the filtrate was obtained. The solid samples were dried in a vacuum oven at 30 °C for 5 days before usage.

2.3.3 SODIUM CYANOBOROHYDRIDE-REDUCED GRAPHENE OXIDE (CN-rGO). Sodium borohydride (412 mg, 6.57 mmol) was added into the graphene oxide dispersion and stirred at 70 °C (reflux) for 2 h. Upon completion, the mixture was filtered under vacuum and washed repeatedly with methanol and water until a neutral pH of the filtrate was obtained. The solid samples were dried in a vacuum oven at 30 °C for 5 days before usage.

2.3.4 SODIUM TRIACETOXYBOROHYDRIDE-REDUCED GRAPHENE OXIDE (OAc-rGO). Sodium triacetoxyborohydride (1.39 g, 6.57 mmol) was added into the graphene oxide dispersion and stirred at 70 °C (reflux) for 2 h. The pH of the solution was examined to be ~3–4. Upon completion, the mixture was filtered under vacuum and washed repeatedly with methanol and water until a neutral pH of the filtrate was obtained. The solid samples were dried in a vacuum oven at 30 °C for 5 days before usage.

2.3.5 CONTROL EXPERIMENT (CONTROL). A control experiment for 2 h under the same set of conditions but in the absence of borohydride reductants was carried out simultaneously to test the effect of heat treatment on the reduction process. Upon completion, the mixture was filtered under vacuum and washed repeatedly with methanol and water until a neutral pH of the filtrate was obtained. The solid samples were dried in a vacuum oven at 30 °C for 5 days before usage.

2.3.6 ELECTROCHEMICAL CHARACTERIZATION OF MATERIALS. Immobilization of the carbon materials onto the working electrode (GC) was performed first by preparing a suspension of the desired material with a concentration of 1 mg mL⁻¹ in DMF. The rGO suspensions were ultrasonicated for 5 min prior to depositing 1 µL of aliquot of the appropriate suspension onto the electrode surface. The DMF solvent was then allowed to evaporate at room temperature, leaving a randomly distributed film on the GC electrode surface. Electrochemical experiments were performed by using 50 mM phosphate buffer (pH 7.2) as a supporting electrolyte for the ferro/ferricyanide (10 mM) redox probe. The GC electrode surfaces were renewed by polishing with 0.05 mm alumina particles on a cloth (DP-Plus, Streurs) before each voltammetry/impedimetric measurement.

The k_{obs}^0 values were determined using the method developed by Nicholson that relates ΔE_p to a dimensionless parameter ψ and consequently to k_{obs}^0 . The roughness factor was not taken into account. The diffusion coefficient $D = 7.26 \times 10^{-6}$ cm² s⁻¹ for [Fe(CN)₆]^{3-/4-} in 0.1 M KCl was used for calculations.

3 Results and discussion

We investigated the reducing power of various substituted borohydrides on graphene oxide (GO). Graphite oxide (Graphite-Ox) was first produced by the Hummers' oxidation method from graphite. The graphite oxide was ultrasonicated to give graphene oxide and was subsequently treated with sodium borohydride (BH-rGO), sodium cyanoborohydride (CN-rGO) and sodium triacetoxyborohydride (OAc-rGO) in methanol under reflux for 2 hours. A control experiment was carried out concurrently without addition of any reductants. Methanol was chosen as the solvent instead of water, since standard reductions with borohydrides in synthetic chemistry are usually carried out in alcohol solution. More importantly, sodium triacetoxyborohydride decomposes readily when in contact with water. Upon treatment with borohydrides, the rGOs were analysed using high resolution X-ray photoelectron spectroscopy (HR-XPS), Fourier transform infrared (FTIR), Raman spectroscopy, electrochemical impedance spectroscopy (EIS) and cyclic voltammetry.

X-ray photoelectron spectroscopy analyses were carried out on the materials as shown in Fig. 1. XPS is a very sensitive and semi-quantitative analysis method which probes the surface of a material to provide its surface elemental compositions. Survey scans of XPS, from a binding energy of 1200 eV to 0 eV, were carried out to provide an overall composition of available elements as shown in Fig. 1A. The survey scans highlighted the prominent presence of carbon and oxygen elements for all the materials. A peak corresponding to sodium element was observed for the reduced graphene. This could correspond to the remaining sodium ions which bound ionically with the oxygen atoms on the graphene materials. The survey scans provided C/O ratios of 1.93, 2.14, 2.51, 2.24 and 2.52 for Graphite-Ox, control, BH-rGO, OAc-rGO and CN-rGO

Fig. 1 X-ray photoelectron spectroscopy spectra on Graphite-Ox, control, BH-rGO, OAc-rGO and CN-rGO (A) Survey and (B) C1s core level spectra.

respectively. A lower C/O ratio, as presented by Graphite-Ox, indicates the presence of a higher amount of oxygen containing groups. As such, we could observe that the borohydride reductants have resulted in a lower amount of oxygen containing groups based on the C/O ratios, possibly *via* hydrogen insertion into carbonyl groups. While we do not rule out the possibility of the decomposition of sodium triacetoxymethylborohydride in the non-anhydrous methanol solvent, traces of acetate could contribute to the low C/O ratio of OAc-rGO as well. Boron element was not detected on the survey scan, which assured that the samples were thoroughly washed and there is no interference from residual reagents.

High resolution XPS on the C1s core level was carried out on the graphene materials as well, as shown in Fig. 1B. A typical C1s core level spectrum provides information on the types of bonding present in the material of interest. With respect to a C1s core level analysis, peaks centred at 284.5 (C=C), 286.5 (C-O), 288.5 (C=O), and 289.5 eV (O-C=O) are usually observed. For the Graphite-Ox material, peaks corresponding to all four carbon bond types were observed. Upon ultrasonication and treatment with heat, the amount of C=O reduced drastically and the presence of O-C=O was not detected. This could be due to the exposure of less oxidised basal planes of previously stacked graphene sheets upon ultrasonication²⁹ and the thermal deoxygenation/decarboxylation under heating conditions.³⁰ Subsequent treatment with borohydride reductants provided varying degrees of change in the ratios of carbon bond types. The BH-rGO gave 58.5, 32.8 and 8.7% of C=C, C-O and C=O bond types, respectively. The OAc-rGO provided 46.5, 43.2, and 10.3% while CN-rGO resulted in 53.9, 36.1 and 10.0% of C=C, C-O and C=O bond types respectively. These highlighted the higher reducing strength of sodium borohydride when compared to the other two reductants; this is in line with the expectations based on general synthetic chemistry knowledge. Sodium triacetoxymethylborohydride provided the lowest reducing strength among the three borohydride reductants, while the extent of reduction that resulted from sodium cyanoborohydride lay in between the two borohydrides. This clearly shows that the variation of substituents in the borohydride reductants allows for fine tuning of the reducing strength in the organo-boron family.

Subsequent analysis was performed using attenuated total reflectance (ATR) Fourier transform infrared spectroscopy (FTIR) as shown in Fig. 2. Graphite-Ox gave peaks corresponding to the O-H stretching mode ($\sim 3200\text{ cm}^{-1}$), C=O vibrational mode ($\sim 1718\text{ cm}^{-1}$), C-OH vibrational mode (~ 1380 and 1050 cm^{-1}), and C-O-C from epoxy groups ($\sim 870\text{ cm}^{-1}$). The peak at $\sim 1600\text{ cm}^{-1}$ resulted from an unoxidised graphitic region. The heat treatment on Graphite-Ox provided the control material, which highlighted almost similar peak patterns to that with Graphite-Ox. Upon reductive treatment with the borohydrides, persistent peaks from O-H, C=O, C-OH and C-O-C moieties were observed. However, specifically for the C=O bond peak, BH-rGO gave almost negligible traces while OAc-rGO and CN-rGO still displayed prominent peaks for it. This observation was consistent with earlier XPS analyses which indicated that sodium borohydride provided a stronger

Fig. 2 ATR-FTIR spectra on Graphite-Ox, control, BH-rGO, OAc-rGO and CN-rGO.

reducing strength when compared to sodium cyanoborohydride and sodium triacetoxymethylborohydride.

Raman spectroscopy analysis was carried out as well to evaluate the structural properties of the graphene-based materials as shown in Fig. 3. Raman spectroscopy is able to identify the presence of defects on the graphene carbon network by measuring the D (breathing mode of sp^2 carbon) and G (graphitic, sp^2 bonded carbon) bands at ~ 1346 and $\sim 1567\text{ cm}^{-1}$, respectively. The ratio of the peak intensities of the D and G bands (I_D/I_G) provides information on the extent of defects on the graphene-based materials. Based on the analyses,

Fig. 3 Raman spectra on Graphite-Ox, control, BH-rGO, OAc-rGO and CN-rGO.

Graphite-Ox, control, BH-rGO, OAc-rGO and CN-rGO resulted in I_D/I_G ratios of 0.89, 0.82, 0.84, 0.87 and 0.88, respectively. The rGOs held similar extent of defects since the Raman analyses did not provide big disparities among the rGOs obtained from the different borohydride reductants.

Following the structural characterizations of the graphene-based materials, we proceeded to examine the electrochemical properties by the electrochemical impedance spectroscopy (EIS) technique as well as by cyclic voltammetry. Both techniques are sensitive and probe the resistance of the graphene-based materials towards heterogeneous charge transfer. EIS is a technique which applies small alternating-current voltage perturbation to the system (on the order of millivolts) at different frequencies while cyclic voltammetry is a complementary technique which applies a large perturbation to the system (on the order of hundreds of mV). The ability of the graphene-based materials to transfer and exchange charges with surrounding molecules is highly influenced by their surface morphologies. The EIS analyses were performed in a ferro/ferricyanide electrochemical probe and the EIS analyses were completed through the fitting of Nyquist plots as shown in Fig. 4, based on the Randles equivalent circuit (shown as inset in Fig. 4).

Based on the Nyquist plot, the diameter of the semicircle corresponds to the charge transfer resistance (R_{ct}) of the material. The GC electrode showed a R_{ct} of 0.6 k Ω while the oxygen-rich Graphite-Ox gave a high R_{ct} of 5.30 k Ω . The heat treatment on Graphite-Ox resulted in a decrease of the R_{ct} value of 2.27 k Ω . BH-rGO and CN-rGO exhibited a comparative R_{ct} of 1.64 and 1.76 k Ω , respectively. R_{ct} for OAc-rGO was much higher than BH-rGO and CN-rGO, which was at 4.92 k Ω . Materials with an inherently higher content of oxygen-containing groups would usually result in higher R_{ct} values.^{18,29} This is due to the electrostatic repulsion between the negatively charged ferro/ferricyanide complexes (electrochemical probes) and a portion of the oxygen containing groups on the graphene-based

materials, which are dissociated and negatively charged at the used pH. The conclusion drawn from EIS data is that OAc-BH reduces graphene oxide very mildly while stronger reduction is observed at CN-BH₃ and the strongest reduction of the oxygen containing groups (lowest R_{ct}) is achieved using NaBH₄. This is consistent with results received from XPS and FTIR measurements, discussed above.

Cyclic voltammetry (CV) was carried out as an independent large-scale perturbation method to verify the findings of electrochemical impedance spectroscopy. CV scans were carried out before each impedimetric scan to examine the heterogeneous electron transfer (HET) rate of the graphene-based materials. Similar to the EIS study, a ferro/ferricyanide electrochemical probe was used for this purpose. In cyclic voltammetry of the ferro/ferricyanide probe, which is surface sensitive, one can correlate the HET to the amount of oxygen containing groups on graphene surfaces. It was demonstrated by various groups that with increasing the amount of oxygen containing group on graphite and graphene, the HET decreases (this is indicated by increase of peak-to-peak separation, ΔE).^{29,31,32} The representative cyclic voltammograms of Graphite-Ox, control, BH-rGO, OAc-rGO and CN-rGO are shown in Fig. 5. It could be observed that Graphite-Ox presented the highest peak separation, ΔE of 0.59 V while the bare GC electrode gave the lowest ΔE at 0.20 V. The control, BH-rGO, OAc-rGO and CN-rGO resulted in peak separations of 0.37, 0.34, 0.54 and 0.36 V, respectively. The heterogeneous electron transfer rate was subsequently calculated based on the Nicholson³³ method which showed an increasing HET for the graphene-based materials in this order: Graphite-Ox (k_{obs}^o : $5.75 \times 10^{-6} \text{ cm s}^{-1}$) < OAc-rGO (k_{obs}^o : $1.09 \times 10^{-5} \text{ cm s}^{-1}$) < Control (k_{obs}^o : $1.06 \times 10^{-4} \text{ cm s}^{-1}$) < CN-rGO (k_{obs}^o : $1.16 \times 10^{-4} \text{ cm s}^{-1}$) < BH-rGO (k_{obs}^o : $1.49 \times 10^{-4} \text{ cm s}^{-1}$). The HET rate determined by CV was highly affected by the negatively charged redox probe and oxygen containing groups on the graphene material. This resulted in Graphite-Ox having the lowest HET rate while the rGOs provided HET rates of about two orders of magnitude higher. These results are consistent with

Fig. 4 Nyquist diagrams for EIS measurements for Graphite-Ox, control, BH-rGO, OAc-rGO and CN-rGO. Conditions: PBS 50 mM, pH 7.2 background electrolyte, and 10 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$. The Randles equivalent circuit used for data fitting is shown as inset.

Fig. 5 Cyclic voltammograms on ferro/ferricyanide for Graphite-Ox, control, BH-rGO, OAc-rGO and CN-rGO. Conditions: PBS 50 mM, pH 7.2 background electrolyte, 10 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$. Scan rate: 100 mV s⁻¹.

results of EIS and have the same implications towards the efficiency of the different borohydrides on the reduction of graphene oxide.

The power of electrochemical technique consists of the fact that in an exhaustive mode (coulometric mode), one can relate the number of exchanged electrons to the absolute number of electroactive species. In the case of graphene oxides and reduced graphene oxides, it is possible to *absolutely*, by using solid state voltammetry, determine the amount of electrochemically reducible groups (epoxy, peroxy, aldehyde and carbonyl) on a graphene sheet.^{18,34} Therefore, a solid-state electrochemical reduction method using the voltammetry technique to verify the presence of oxygen-containing groups, which are electrochemically reducible on graphene materials, was carried out as well.³⁵ This was achieved by a reductive scan from +0 to −2.0 V as shown in Fig. 6. The presence of oxygen functionalities such as epoxy, carboxyl, hydroxyl and peroxides would result in intense reduction peaks appearing at potentials lower than −1.0 V (vs. Ag/AgCl reference electrode). The graphene materials were tested against a bare GC electrode. It can be observed that the bare GC electrode did not provide any voltammetric reduction peak. However, the Graphite-Ox and control graphene materials gave almost an identical reductive peak centered at −1.4 V. Upon treatment with sodium borohydride, the area of the reductive peak decreased drastically. CN-rGO and OAc-rGO gave larger reductive peak areas than BH-rGO, indicating the presence of a higher amount of electrochemically reducible oxygen groups on the graphene surfaces. Based on the integrated reduction peak areas and the amount of deposited graphene materials on the electrode surface, we have determined that on average there is one electrochemically reducible oxygen-containing group (epoxy, peroxy and aldehyde) per every 6.26, 6.89, 12.43, 27.23 and 154.90 of carbon atoms for Graphite-Ox, control, OAc-rGO, CN-rGO, and BH-rGO.³⁴ This highlighted that the extent of reduction increases in the order of OAc-rGO < CN-rGO < BH-rGO, which is in agreement with the observed electrochemical measurements of R_{ct} and HET rate, as well as with the XPS and FTIR measurements.

Fig. 6 Cyclic voltammetric profiles of electrochemical reduction of oxygen containing groups for Graphite-Ox, control, BH-rGO, OAc-rGO and CN-rGO. Conditions: PBS 50 mM, pH 7.2 background electrolyte. Scan rate: 100 mV s^{−1}.

4 Conclusion

In summary, we have compared substituted borohydrides with different reducing strengths. We used these compounds since the reduction mechanism is well known to tailor the quality and quantity of oxygen containing groups on graphene oxides. We observed striking differences in the amount of oxygen containing groups as well as in the electrochemical behavior of the reduced graphene oxides obtained using the different substituted borohydrides. This opens the doors to a well-defined and understood tailoring of the properties of graphene oxides for electrochemical and other applications.

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Graphene oxide reduction by standard industrial reducing agent: thiourea dioxide†

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The current fabrication methods of pristine graphene are not feasible for bulk production. The closest approach, which is through chemical reduction of graphene oxide to chemically reduced graphene oxide that resembles pristine graphene, has been widely adopted instead. Herein, we report a new methodology for the reduction of graphene oxide to chemically reduced graphene oxide using a common industrial reductant, thiourea dioxide. The final product has been fully characterized by X-ray photoelectron spectroscopy (XPS), transmission electron microscopy (TEM), scanning electron microscopy (SEM), Raman spectroscopy, electrochemical impedance spectroscopy (EIS), attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) and cyclic voltammetry. The microscopy techniques revealed reduced graphene of few-layered sheets. Based on the XPS analyses, a C/O ratio as high as 16.0 was achieved. The reduced graphene oxide product demonstrated a good electrochemical performance with a charge transfer resistance as low as 0.11 k Ω based on EIS measurements and a low overpotential for the oxidation of ascorbic acid. Since thiourea is a common industrial reductant, standard protocols are already in place for the waste produced from this methodology. As such, we foresee that this methodology holds the potential for industrial scale reduction of graphene oxide.

1. Introduction

Studies on graphene nanomaterials have been a research highlight in recent years. Graphene, as a one atom thick material consisting of sp²-bonded carbon featuring a honeycomb structure, exhibits high mechanical strength,¹ high elasticity and thermal conductivity, very high electron mobility at room temperature, tunable optical properties,^{2,3} a tunable bandgap,⁴ and room temperature quantum Hall effect.⁵ In addition, the carbon precursors are available at low costs. Graphene has been postulated as a promising material for energy storage,⁶ electronic platforms for post-complementary metal oxide semiconductor (CMOS) electronics,⁷ and biological and chemical sensors.⁸ Graphene has been produced through different methods,⁹ mainly epitaxial growth from silicon substrates,¹⁰ mechanical exfoliation from graphite through ultrasonication,¹¹ unzipping of carbon nanotubes,¹² pyrolysis,¹³ and photo-assisted reduction from oxidised graphite.¹⁴

However, pristine monolayer graphene, which is free of defects, is difficult to produce in bulk quantities through the methods described above. A chemical method based on the preliminary oxidation of graphite to graphite oxide followed by an exfoliation/reduction (*i.e.* chemical, electrochemical, or thermal) process that produces a carbon material that is similar to graphene has been widely adopted instead for the production of bulk quantities of graphene. These materials are coined as ‘chemically modified graphenes’.¹⁵ Reductants such as sodium borohydride,¹⁶ hydrazine anhydrous,¹⁷ hydrazine monohydrate,¹⁸ ascorbic acid,¹⁹ and hydroquinones²⁰ have been employed to produce gram quantities of chemically reduced graphene oxide (CRGO). However, the current methods mentioned above are difficult to envision at large scale since the adopted reductants are usually not appropriate for large scale productions due to the high costs of chemicals and safety issues. More specifically, the most commonly used hydrazine is highly unstable and carcinogenic²¹ while sodium borohydride is very reactive and harmful to health.²² Thus, we were prompted to look for cheaper and safer new reductants for the reduction of graphene oxide to CRGO that also have high industrial scalability potentials.

Thiourea dioxide has been utilized as a strong reductant in the textile printing, paper, photographic and leather processing industries for a long time.²³ It has also been used in academia research towards the deoxygenation of α,β -epoxy ketones and reduction of ketones, aromatic nitro-, azoxy-, azo-, hydrazo- and

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Scheme 1 Functional groups on graphene oxide reduced by thiourea dioxide.

organosulfur compounds.^{24–27} The reduction process generates sodium sulfite and urea which are easy to manage since these are common commercial wastes. On top of that, thiourea dioxide is easily available in bulk quantities, safer and less expensive than other reducing agents mentioned above. As such, we foresee that the reduction of graphene oxide using thiourea dioxide as a reductant is scalable, and has a potential for commercialization and bulk production of graphene materials.

Herein, we investigate the reducing properties of thiourea dioxide and propose a novel reduction procedure to obtain high quality chemically reduced graphene oxide (Scheme 1). Comprehensive characterizations by means of X-ray photoelectron spectroscopy (XPS), transmission electron microscopy (TEM), scanning electron microscopy (SEM), Raman spectroscopy, electrochemical impedance spectroscopy (EIS), attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) and cyclic voltammetry are presented.

2. Experimental

2.1 Materials

Graphite was obtained from Asbury Carbons. Fuming nitric acid (>90%) was obtained from J.T. Baker. Sulfuric acid (95–98%), potassium chlorate (98%), hydrochloric acid (37%), *N,N*-dimethylformamide (DMF), ethanol, sodium hydroxide, thiourea dioxide, potassium phosphate dibasic, sodium phosphate monobasic, potassium ferrocyanide, potassium chloride, and ascorbic acid (AA) were purchased from Sigma-Aldrich, Singapore. Glassy carbon (GC) electrodes with diameters of 3 mm were obtained from Autolab, The Netherlands.

2.2 Apparatus

A JEM-1400 field-emission transmission electron microscope (JEOL, Japan) operating at 100 kV was used to acquire TEM images. TEM sample solutions (in DMF, 1 mg mL^{−1}, ultrasonicated for 5 min) were loaded on the TEM grid directly by dropping 5 μL of the solution onto the carbon coated side of the grid. A filter paper was used to wick off the excess solution on the TEM grid and dried overnight.

A JEOL-7600F semi-in-lens FE-SEM, operating in gentle-beam mode at 2 kV was used to acquire SEM images. The solid samples were transferred to a carbon tape held onto a SEM sample holder for analyses. The analyses of the samples were carried out at an average working distance of 8 mm.

X-Ray photoelectron spectroscopy (XPS) was performed with a Phoibos 100 spectrometer and a monochromatic Mg X-ray radiation source (SPECS, Germany). Both survey and high-

resolution spectra for C1s were collected. Relative sensitivity factors were used for evaluation of C/O ratios from survey spectra XPS measurements. XPS samples were prepared by coating carbon tape with a uniform layer of the materials under study. Raman spectroscopy analyses were performed using a confocal micro-Raman LabRam HR instrument from Horiba Scientific in backscattering geometry with a CCD detector, a 514.5 nm Ar laser and a 100× objective mounted on an Olympus optical microscope. The calibration was initially made using an internal silicon reference at 520 cm^{−1} and gave a peak position resolution of less than 1 cm^{−1}. The spectra were measured from 1000 to 3000 cm^{−1}.

Attenuated total reflectance (ATR) Fourier transform infrared (FTIR) measurements were carried out on a PerkinElmer Spectrum 100 system coupled with a universal ATR accessory. Diamond/ZnSe was used as the ATR crystal.

All voltammetric experiments were performed on a μAutolab type III electrochemical analyzer (Eco Chemie, The Netherlands) connected to a personal computer and controlled by General Purpose Electrochemical Systems Version 4.9 software (Eco Chemie).

2.3 Procedures

Graphite oxide was prepared using graphite according to the Staudenmaier²⁸ method whereby 17.5 mL of H₂SO₄ (95–98%) and 9 mL of HNO₃ (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 a homogeneous dispersion. 11 g of KClO₃ was added into the mixture over 15 min in order to avoid a temperature spike and formation of ClO₂ gas, which is explosive at high concentrations. The mixture was left to stir vigorously for 96 h at room temperature. On completion, the mixture was then poured into 1 L of deionized water and filtered. Graphite oxide was then redispersed and washed repeatedly with 5% HCl solution to remove sulfate 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.

Reduced graphene oxide was obtained from the reaction of thiourea dioxide with graphene oxide (subjecting graphene oxide to ultrasonication [37 kHz] for 3 h in the water). To 50 mg of graphene oxide in 31.2 mL of water was added 18.7 mL of ethanol and 1.04 g of NaOH in portions. 1.4 g of thiourea dioxide was added portion-wise over 15 min. The mixture was refluxed at 90 °C for 1, 2, 5, 10 or 20 h (consequently labelled as T1-, T2-, T5-, T10- and T20-CRGO). A control experiment was carried out

simultaneously for 2 h under the same set of conditions but in the absence of thiourea dioxide to test the effect of NaOH on the reduction process (labelled as NaOH-Control). Upon completion, the mixture was filtered under vacuum and washed repeatedly with ethanol followed with water until a neutral pH of the filtrate was obtained. The solid samples were dried in an oven at 50 °C for 48 h before usage.

Immobilization of the carbon materials onto the working electrode (GC) was performed firstly by preparing a suspension of the desired material with a concentration of 1 mg mL⁻¹ in DMF. The CRGO suspensions were ultrasonicated for 5 min prior to depositing a 1 µL aliquot of the appropriate suspension onto the electrode surface. The DMF solvent was then allowed to evaporate at room temperature, leaving a randomly distributed film on the GC electrode surface.

Electrochemical experiments were performed by using 50 mM phosphate buffer (pH 7.2) as a supporting electrolyte for ascorbic acid (AA) and a ferro/ferricyanide (10 mM) redox probe. The GC electrode surfaces were renewed by polishing with 0.05 mm alumina particles on a cloth (DP-Plus, Streurs) before each voltammetry measurement.

3. Results and discussion

3.1 The characterization of morphological properties

The morphological properties of the graphene materials were investigated using SEM and TEM techniques. Fig. 1 shows representative TEM and SEM images of Graphite-Ox, NaOH-Control, and T1-CRGO. TEM images of Graphite-Ox showed highly orientated sheets with precise edges. The areas of low contrast indicate locations of multi-layered stacked graphene

Fig. 1 TEM (a, c and e) and SEM (b, d, and f) images of Graphite-Ox (a and b), NaOH-Control (c and d) and T1-CRGO (e and f).

configurations (Fig. 1a). When Graphite-Ox was subjected to ultrasonication and NaOH treatment, we observed thinner layers of graphene sheets which have apparently smooth and “clean” appearances, similar to Graphite-Ox (Fig. 1c). Subsequent reduction using thiourea dioxide (1 h reaction time) gave highly pleated and wrinkled graphene sheets (Fig. 1e). Such a conformation is typical for highly exfoliated materials with structural defects most probably located along the wrinkle lines.

The SEM analysis of Graphite-Ox showed the presence of large aggregated and heavily stacked sheets (Fig. 1b). Similar characteristics were observed on the NaOH-Control (Fig. 1d). On the other hand, the SEM image of T1-CRGO (Fig. 1f) indicated graphene sheets which were less aggregated, randomly folded and wave-like.

Further evaluations on the dispersibility of graphene materials in water (Fig. 2A) and DMF (Fig. 2B) revealed the physical properties of the materials. It was observed that the Graphite-Ox and NaOH-Control materials were readily dispersed in water while the CRGO materials were highly hydrophobic. The hydrophobic natures of the CRGOs were initially observed during the chemical reactions, whereby the CRGOs separated into black aggregations. The extent of aggregation increased with reaction time (Fig. S1, A–C†). Apart from that, the T20-CRGO appeared powder-like and shiny as compared to the rest of the CRGOs which were chunky in appearance (Fig. S1, D†). Interestingly, the T20-CRGO suspended in water has a tendency to remain at the glass interface above the water level, indicating a higher hydrophobic property compared to the materials obtained with shorter reaction times. In contrast, stable dispersions were formed upon ultrasonication treatments in DMF solvent. This demonstrates that the Graphite-Ox and NaOH-Control materials possess amphiphilic features, while the CRGO materials have predominantly hydrophobic characteristics.

3.2 The characterization of structural properties

The extent of reduction with thiourea dioxide was monitored using X-ray photoelectron spectroscopy (XPS), which is

Fig. 2 Photographs of dispersions in (A) water (0.5 mg mL⁻¹) and (B) DMF (1 mg mL⁻¹) after ultrasonication (10 min) of (a) Graphite-Ox, (b) NaOH-Control, (c) T1-CRGO, (d) T2-CRGO, (e) T5-CRGO, (f) T10-CRGO, and (g) T20-CRGO.

a sensitive analytical technique in determining the surface elemental compositions and types of functional groups available on carbon materials.^{29,30} An initial XPS survey scan (Fig. 3a) of Graphite-Ox showed a C/O ratio of ~ 2.5 , which is typical for graphite materials that have undergone strong oxidation processes.³¹ Treatment with NaOH in the absence of thiourea dioxide (NaOH-Control) produced a material with partial deoxygenation, in fact, with a C/O ratio of only ~ 3.0 . Despite a certain deoxygenation ability of NaOH which was already demonstrated in the past, specifically on the ability to remove highly oxygenated debris from graphene oxide materials, we did not observe such an effect with the reaction conditions used in this work.³² The obvious peak from the Na1s core level was due to the presence of sodium salt, which also explained its high dispersibility in water (Fig. 2A).³³ Upon ultrasonication and treatment with thiourea dioxide for 1 hour, T1-CRGO exhibited a higher C/O ratio of ~ 14.5 . Subsequent reduction attempts for prolonged times saw an increment of C/O ratio up to ~ 16.0 between 2 and 5 hours of reaction. A slight decrease of C/O ratio for longer reaction times was observed which could be a result of possible chemical degradation (Fig. 3c). The low C/O ratio measured from the NaOH-Control demonstrated the ability of

thiourea dioxide as the main reducing agent which acted synergistically with NaOH towards the production of few-layered reduced graphene sheets. The success of the reduction was also shown by the hydrophobic natures of the CRGOs in water (Fig. 2A), indicating that a significant removal of oxygen functionalities has occurred.

Moreover, the C/O ratios of CRGOs obtained here using thiourea dioxide were significantly higher than those obtained using other reducing agents such as sodium borohydride (C/O: 5.8),¹⁶ hydrazine anhydrous (C/O: 12.0),¹⁷ and hydrazine monohydrate (C/O: 10.3).¹⁸

Careful analyses of the C1s core level peaks of the XPS spectra facilitate the identification of oxygen containing groups present on the surfaces of the carbon materials. Fig. 3b presents the C1s core level regions for Graphite-Ox, NaOH-Control and T1-CRGO materials. Graphite-Ox gave intense peaks at about 284.5 and 286.5 eV, corresponding to the contribution of the sp^2 C and C–O groups, respectively. A minor contribution at 288.3 eV was also observed and can be assigned to the ketone functionality. These indicated that the Graphite-Ox material was highly oxidised prior to the reduction step. The subsequent treatment with NaOH as a control experiment resulted in a slight decrement of the C–O peak and thus reiterated the secondary role of NaOH in the reduction process. Upon reductive treatment with thiourea dioxide, a less broad and well-defined peak with small tailing towards higher binding energies was observed in T1-CRGO. The reduction step resulted in a drastic decrement of intensities for the C–O, C=O and O–C=O peaks. An additional band at 285.3 eV for sp^3 carbon bonding (C–C) was observed as well, which could be due to some structural defects generated after the removal of the oxygen functionalities. A well defined and sharper signal at 284.5 eV together with the $\pi-\pi^*$ shake up satellite band at ~ 290.0 eV demonstrated an improved characteristic of aromatic/conjugated systems. CRGOs obtained using longer reaction times presented XPS spectra similar to T1-CRGO (Fig. S4 and S5†).

We proceeded to carry out subsequent analyses with attenuated total reflectance (ATR) Fourier transform infrared spectroscopy (FTIR) as shown in Fig. 4. The ATR-FTIR spectrum of Graphite-Ox indicated the presence of the O–H stretching mode (~ 3200 cm^{-1}), C=O vibrational mode (~ 1718 cm^{-1}), C–OH vibrational mode (~ 1380 cm^{-1}) and C–O–C in epoxy groups (~ 1050 cm^{-1}).³⁴ The peak at 1600 cm^{-1} could result from the unoxidised graphitic region.³⁵ NaOH-Control showed obvious and identical peaks with that of Graphite-Ox, indicating that most of the oxygen containing groups were not removed by the hot alkali treatment. After the reduction processes, the ATR-FTIR spectra of the CRGOs were featureless without any presence of peaks at ~ 3200 , 1718 , 1380 and 1050 cm^{-1} , thus indicating that most of the oxygen containing groups in the CRGOs had been eliminated.

To further understand the structural properties of the CRGOs, Raman spectroscopy analyses were carried out using a 514.5 nm laser excitation to identify any defects on the surfaces of the carbon materials, which were directly related to the extent of functionalization. Fig. 5a shows the Raman spectra for Graphite-Ox, NaOH-Control and T1-CRGO (remaining spectra of CRGOs are shown in Fig. S6†). The D (breathing mode of sp^2 carbon)³⁶ and G (graphitic, sp^2 bonded carbon)³⁷ bands at 1346

Fig. 3 XPS spectra of Graphite-Ox, NaOH-Control and T1-CRGO materials: (a) Survey scan and (b) C1s core level spectra. (c) Variation in the C/O ratio of CRGO materials obtained using different reduction times.

Fig. 4 ATR-FTIR spectra of Graphite-Ox, NaOH-Control and CRGO materials.

and 1567 cm^{-1} , respectively, appeared as sharp peaks for T1-CRGO whereas broadened bands were observed for both Graphite-Ox and NaOH-Control. An intense and broad D band indicates a significant presence of structural disorder with short range sp^2 carbon features and/or the presence of sp^3 hybridized carbons. As such, the reduction process created structural defects resulting in a higher D band. However, other features were also observed in the Raman spectra. In particular, the 2D band (second order two phonon process) at $\sim 2700\text{ cm}^{-1}$ is usually used to evaluate approximately the number of graphene layers, where a single layer would result in a sharp and symmetrical 2D band exceeding the G band. T1-CRGO produced here resulted in a 2D band which was well defined and more intense than those of Graphite-Ox and NaOH-Control materials, showing that the CRGO consisted of few-layered graphene sheets.³⁸

The ratio of the intensities of the D and G bands (I_D/I_G) is usually used to show the extent of chemical modification or defects in a carbon sample.³⁹ Based on the analyses, Graphite-Ox gave a I_D/I_G ratio of 0.83 while that of the CRGOs produced using different reaction times resulted in I_D/I_G ratios higher than 1.00 as shown in Fig. 5b. This indicated a higher extent of disorder in the CRGOs when compared to the starting Graphite-Ox and was in good agreement with other reduction methods which used hydrazine anhydrous/monohydrate and sodium borohydride.^{16–18} The high defect densities were also reflected as wrinkle- and line-like areas of the TEM images in Fig. 1e and S2†.

3.3 The characterization of electrochemical properties

An electrochemical study was carried out to verify the reduction of oxygen containing groups as well. A solid-state electrochemical reduction using the cyclic voltammetry technique as shown in Fig. 6 was performed to verify the presence of oxygen

Fig. 5 (a) Raman spectra for Graphite-Ox, NaOH-Control and T1-CRGO materials. (b) Variation in the I_D/I_G ratio of CRGO materials obtained using different reduction times.

containing groups which can be reduced electrochemically. The presence of oxygen functionalities (mainly epoxides, carbonyls, and peroxides) on the carbon materials can be confirmed by an intense reduction peak appearing at potential lower than -1.0 V

Fig. 6 Cyclic voltammetric profiles of electrochemical reduction of oxygen containing groups. Conditions: PBS 50 mM, pH 7.2 background electrolyte. Scan rate: 100 mV s^{-1} .

(vs. Ag/AgCl reference electrode).⁴⁰ The electrochemical analyses of the CRGOs were tested against glassy carbon (GC), Graphite-Ox and NaOH-Control modified electrodes. It can be seen that Graphite-Ox was the only material which showed a clear and intense reduction peak at a potential of -1.3 V. The NaOH-Control gave a broad reduction peak signifying the presence of electrochemically reducible oxygen functionalities. On the other hand, the T1-CRGO did not display any reduction peaks within the potential range scanned. This further confirmed the absence of electrochemically reducible groups (epoxides, carbonyls, and peroxides) on T1-CRGO, and similarly with the other CRGOs shown in Fig. S7a†, thus indicating the effectiveness of thiourea dioxide as a reductant.

Following the characterization of the carbon materials, we continued to probe the electrochemical properties mainly based on the electrochemical impedance spectroscopy (EIS) technique. EIS is useful to investigate the resistance of materials towards heterogeneous charge transfer, thus indicating the ability of the materials to transfer and exchange charges with the surrounding molecules. However, such an ability is influenced by the surface morphology of the graphene materials. As such, we conducted EIS measurements of GC, Graphite-Ox, NaOH-Control and the CRGO materials modified electrodes using a ferro/ferricyanide electrochemical probe. The EIS study was accomplished through fitting of data based on the Randles equivalent circuit.

Based on the Nyquist plot, the charge transfer resistance (R_{ct}) corresponds to the diameter of the semicircle. It could be observed in Fig. 7a that the GC electrode showed a R_{ct} of 0.76 k Ω . The oxygen-rich Graphite-Ox showed a higher R_{ct} at 5.43 k Ω while NaOH-Control showed a slightly lower R_{ct} of 1.63 k Ω as a result of the mild deoxygenation process. T1-CRGO exhibited the lowest R_{ct} of 0.11 k Ω compared to the other materials. Interestingly, the R_{ct} increased upon longer reaction time (Fig. 7b); however, the values obtained were lower than the GC electrode and NaOH-Control material.

The CRGOs also exhibited lower R_{ct} as compared to NaBH_4 reduced CRGO which resulted in a R_{ct} of 5.78 k Ω , in comparison to the inherent R_{ct} of its GC electrode.⁴¹ We could thus observe that materials with inherently higher C/O ratios gave higher R_{ct} values. This suggests a possible electrostatic repulsion between the negatively charged ferro/ferricyanide complexes (redox probe) and a portion of oxygen containing groups on the graphene materials, which are negatively charged at the working pH.

Cyclic voltammetry was also utilized to examine the heterogeneous electron transfer (HET) rate and interaction of the graphene materials with various molecules. A ferro/ferricyanide probe was first used to determine the HET rate of the graphene materials. Fig. 8a showcases representative cyclic voltammograms of GC, Graphite-Ox, NaOH-Control and T1-CRGO (remaining CRGOs are presented in Fig. S7c†). It can be observed that Graphite-Ox showed the highest peak separations, ΔE , of 0.47 V. Subsequently, the ΔE observed for NaOH-Control, GC, and T1-CRGO were 0.28 , 0.23 and 0.14 V, respectively. The heterogeneous electron transfer (HET) rates for these materials were calculated according to the Nicholson⁴² method which showed a decreasing HET in the order: T1-CRGO (k_{obs}^0 : 2.79×10^{-3} cm s $^{-1}$) > GC (k_{obs}^0 : 7.32×10^{-4} cm s $^{-1}$) > NaOH-Control (k_{obs}^0 : 3.71×10^{-4} cm s $^{-1}$) > Graphite-Ox (k_{obs}^0 :

Fig. 7 (a) Nyquist diagrams for EIS measurements. Conditions: PBS 50 mM pH 7.2 background electrolyte, 10 mM $\text{K}_3[\text{Fe}(\text{CN})_6]/\text{K}_4[\text{Fe}(\text{CN})_6]$. Randles equivalent circuit used for data fitting is shown in the inset. (b) Average R_{ct} values for all materials and their corresponding standard deviations.

2.74×10^{-5} cm s $^{-1}$). The HET rate was also affected by the electrostatic repulsions between the negatively charged redox probe and the oxygen containing groups on the graphene materials, thus resulting in Graphite-Ox having the lowest HET rate. It can be seen that the T1-CRGO material resulted in an HET rate about two orders of magnitude higher than the parent Graphite-Ox. The variations in ΔE and k_{obs}^0 of all the graphene materials are shown in Fig. 8b.

We continued to probe the performances of CRGOs on their abilities to enhance electron transfer kinetics, which play an important role in facilitating oxidation/reduction of molecules which require high over-potentials for oxidation/reduction. As such, cyclic voltammetry analyses were carried out on an important biomarker, ascorbic acid (AA) with GC, Graphite-Ox, NaOH-Control and T1-CRGO modified electrodes as shown in Fig. 9 (remaining CRGOs are presented in Fig. S7d†). Graphite-Ox showed the highest oxidation potential, E_{AA} at 0.53 V, followed by GC at 0.48 V. The NaOH-Control material resulted in a slightly lower E_{AA} at 0.41 V as a result of its deoxygenated nature. On the other hand, T1-CRGO showed the lowest E_{AA} at 0.31 V, coupled with an oxidation wave starting potential at -0.13 V. It can be clearly seen that the CRGO obtained using thiourea dioxide showed the lowest overpotential for the oxidation of an important biomarker such as ascorbic acid. This indicates that CRGO produced with our method is suitable for electrochemical biosensing applications with improved performance.

Fig. 8 (a) Cyclic voltammograms on ferro/ferricyanide. Conditions: PBS 50 mM pH 7.2 background electrolyte, 10 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$. Scan rate: 100 mV s⁻¹. (b) Average peak-to-peak separation (ΔE) values for all materials and corresponding standard deviations.

Fig. 9 Cyclic voltammograms on ascorbic acid. Conditions: PBS 50 mM pH 7.2 background electrolyte, 5 mM ascorbic acid. Scan rate: 100 mV s⁻¹.

4. Conclusion

In conclusion, we have demonstrated the reduction of graphene oxide material to chemically reduced graphene oxide by using thiourea dioxide as a reductant. Thiourea dioxide has a long history of being used as a reductant in the paper, textile and printing industries. Thus, thiourea dioxide is generally cheaper and easier to handle than most other proposed reducing agents. The reaction with thiourea dioxide mainly produces wastes such as sodium sulfite and urea which are common commercial wastes with standard protocols already in place for waste management. As such, this new methodology is reliable, safe, and low cost. In

addition, the material obtained with the proposed method produced high quality chemically reduced graphene oxide with a C/O ratio as high as ~16.0, which is higher than that obtained using other reducing agents such as sodium borohydride and hydrazines. The graphene material produced using the proposed method possesses excellent electrochemical performances, showing low charge transfer resistance and catalytic properties towards the oxidation of ascorbic acid, an important biomarker. We envisage that this new methodology could be applied for industrial scale reduction of graphene oxides.

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Renewal of sp^2 bonds in graphene oxides *via* dehydrobromination†

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Graphene-based materials are emerging to become one of the most exciting candidates for various applications. There has been a keen interest in developing methods that will not only result in their bulk production, but could also generate graphene-based materials that possess good electrical and physical properties. The most convenient method towards bulk production of graphene-based materials is often *via* the reduction of graphene oxide. In this context, numerous reduction techniques have been previously applied based on standard organic chemistry reagents. Although most standard reducing agents work well in the removal of oxygen-containing groups from the graphene oxide surfaces, they do not theoretically result in the important restoration of the defective sp^2 carbon network. Particularly in this work, we focused on the restoration of the defective sp^2 carbon network of graphene oxide *via* a dehydrohalogenation method. This was achieved through a prior substitution of hydroxyl to bromine groups, followed by a dehydrobromination step. This strategy offers a great potential for the production of chemically reduced graphene of high conductivity.

1. Introduction

Graphene has been receiving increasing interest over the past decade due to its unique structural, physical and electronic properties.^{1–4} The potential usage of graphene is not only limited to a new platform for ultrafast electronics,⁵ but also extends to areas in energy storage,⁶ solar cells,⁷ and biochemical sensors.⁸ Graphene is mainly produced using two methods, specifically by the top-down approach such as (i) micromechanical exfoliation of graphite,⁹ (ii) unzipping of carbon nanotubes,^{10,11} and (iii) exfoliation of graphite by means of oxidative treatments followed by reductive/thermal treatments¹² or by the bottom-up approach such as (iv) chemical vapour deposition.¹³ Methods (i) and (iv) are often unfavourable due to the high cost and low yield of graphene production. On the other hand, methods (ii) and (iii) are often preferred since they allow for bulk production of graphene, although the integrity of the sp^2 carbon network of the graphene sheets is often compromised due to the harsh chemical/thermal treatments.

The preparation of graphene-based materials in bulk quantities is largely carried out by oxidative treatments of graphite to graphene oxide and subsequent reduction steps. Graphene oxide is broadly defined as a compound with a large amount of oxygen (C/O ratio of ~ 2) in various bonding arrangements, including

epoxy, hydroxyl, carbonyl, and carboxyl. The broad aim of reducing graphite/graphene oxide is to recreate as much as possible a pristine form of graphene. In short, it is to remove the oxygen-containing groups and restore the sp^2 carbon–carbon bonds, which were previously converted to sp^3 C–C bonds during oxidation. To achieve this, chemical reduction is typically carried out by utilizing standard synthetic chemistry reducing agents, such as NaBH_4 , LiAlH_4 and N_2H_4 , or less standard agents, such as hydroquinone, glucose and ascorbic acid.^{14–19} It is commonly claimed that by utilizing these reductants, the oxygen-containing groups are reduced/removed from the graphene surface and that the sp^2 bond arrangements of graphene are restored. Here, it must be highlighted that for *none* of the reducing agents listed above (with exception of N_2H_4 which we will discuss below), is there any known mechanism of synthetic chemistry leading to the removal of oxygen containing groups. All the reducing agents listed above are according to standard and decades of accumulated and proven knowledge to (at the best) be able to reduce, with different reductive strengths, some oxygen-containing groups to hydroxyl groups. However, these agents, even powerful reducing agents such as LiAlH_4 , are not able to remove hydroxyl groups from the graphene lattice. This reduction of oxygen-containing groups also does not lead to the re-establishment of sp^2 hybridization between carbon atoms. We wish to illustrate this on graphene fragments as depicted in Scheme 1. Rourke *et al.* recently provided an explanation of observed “removal” of oxygen-containing groups from graphene oxide by treatment with NaOH washings.²⁰ Graphene oxide is usually reduced with N_2H_4 , which is known to be a reducing agent in the Wolff–Kishner reduction that interacts only with carbonyl groups and leads to the formation of a hydrazone moiety which

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Scheme 1 Fragments of targeted reduction sites on graphene oxide using (a) NaBH_4 , (b) LiAlH_4 , (c) N_2H_4 , and (d) HBr - KOtBu .

is consequently substituted with two hydrogen atoms.²¹ A recent study also highlighted the formation of a small quantity of pyrazole (see Scheme 1) upon treatment with hydrazine and the mechanisms for the removal of hydroxyl, carboxyl and epoxy groups are mostly not well understood.²²

As such, none of the reagents listed above leads to the direct recreation of sp^2 carbon-carbon bonds.

There is as such an urgent need to bring standard synthetic chemistry methods which would allow for the reintroduction of sp^2 hybridization into graphene oxides. Here, we propose a standard organic chemistry method for removal of oxygen containing groups from the graphene oxide lattice together with the reintroduction of sp^2 bonds into the graphene sheets. This method has a strong synthetic chemistry foundation and it is based on the substitution of hydroxyl groups by a bromine moiety with consequent dehydrohalogenation, as shown in Scheme 2. The dehydrohalogenation reaction leads to the recreation of sp^2 bonds (Scheme 1d).

Scheme 2 Synthetic route towards dehydrobrominated graphene.

2. Experimental

2.1 Materials

Graphite (natural, 45 μm) was obtained from Asbury Carbons, NJ, USA. Sulfuric acid (95–98%) and hydrochloric acid (37%) were obtained from Sigma-Aldrich, Singapore. Fuming nitric acid (>90%) was obtained from J.T. Baker. Hydrazine monohydrate, potassium *tert*-butoxide, hydrobromic acid (48%) and potassium chlorate (98%) were obtained from Alfa Aesar, Singapore.

2.2 Apparatus

X-ray photoelectron spectroscopy (XPS) was performed with a Phoibos 100 spectrometer and a monochromatic Mg X-ray radiation source (SPECS, Germany). Both survey and high-resolution spectra for C1s, Br3d and Br3p were collected. Relative sensitivity factors were used for evaluation of C/O ratios from XPS survey spectra. XPS samples were prepared by adhering the surface of a carbon tape to a uniform layer of the materials under study. Raman spectroscopy analysis was performed using a confocal micro-Raman LabRam HR instrument from Horiba Scientific in backscattering geometry with a CCD detector, a 514.5 nm Ar laser and a 100 $\times$ objective mounted on an Olympus optical microscope. The calibration is initially made using an internal silicon reference at 520 cm^{-1} and gives a peak position resolution of less than 1 cm^{-1} . The spectra are measured from 1000 to 3000 cm^{-1} . All voltammetric experiments were performed on a $\mu\text{Autolab}$ type III electrochemical analyzer (Eco Chemie, The Netherlands) connected to a personal computer and controlled by General Purpose Electrochemical Systems Version 4.9 software (Eco Chemie).

2.3 Procedures

2.3.1 Bromination of graphene oxide.²³ Graphite oxide was produced using the Staudenmaier method.²⁴ Graphite oxide (21 mg) was ultrasonicated (150 W) in ultrapure water (21 mL) for 3 h to give graphene oxide. Hydrobromic acid (5 mL) was added dropwise into the graphene oxide suspension and left under vigorous stirring at 110 $^\circ\text{C}$ for 24 h. The suspension was cooled to room temperature and filtered/washed over a PTFE membrane (0.2 μm) with ultrapure water and acetone to give rGO-Br upon drying at 50 $^\circ\text{C}$ for 5 days.

2.3.2 Dehydrohalogenation of brominated graphene. Brominated graphene (9 mg) was dissolved in THF (9 mL) and ultrasonicated (150 W) for 30 min. Potassium *tert*-butoxide (120 mg, 1.07 mmol) was then added to the suspension and refluxed for

30 min. The suspension was cooled to room temperature and filtered/washed over a PTFE membrane (0.2 μm) with ultrapure water and acetone. It was then washed with 1 M HCl (20 mL) followed by ultrapure water until a neutral pH was obtained. Finally, the solid was washed with diethyl ether to give rGO upon drying at 50 $^{\circ}\text{C}$ for 5 days.

2.3.3 Reduction of graphene oxide using hydrazine. 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-N upon drying at 50 $^{\circ}\text{C}$ for 5 days.

2.3.4 Characterization of materials. Current–voltage measurements (I – V curve) were conducted with an interdigitated gold electrode platform (Au-IDE) by depositing 2 μL of the material suspension (prepared at 1 mg mL^{-1} concentration in water) onto an electrode surface with 10 μm spacing. The electrode was then dried under a lamp for 20 min, leaving a randomly deposited material film on the interdigitated area bridging the two Au electrode bands. The I – V curves were obtained by linear sweep voltammetric measurements at 20 mV s^{-1} scan rate. The displayed data corresponds to the average from three measurements.

3. Results and discussions

The dehydrobromination reaction leading towards the restoration of the sp^2 network on graphene surfaces in this work proceeded after the oxidation of graphite to graphene oxide. The first step of this route is the bromine substitution of hydroxyl groups on the graphene oxide surface. Incorporation of bromine atoms into the graphene sp^2 carbon network was achieved through a bromination step with hydrobromic acid.²³ The treatment of hydrobromic acid thus resulted in brominated graphene (rGO-Br), likely through ring openings of epoxy moieties and/or substitution reactions with hydroxyl groups.²⁵ The subsequent treatment with potassium *tert*-butoxide, which is a strong and non-nucleophilic base frequently used in organic chemistry to achieve the dehydrohalogenation effect, resulted in a dehydrobrominated graphene (rGO). The efficiencies and performance of the chemical treatments were then evaluated with X-ray photoelectron spectroscopy (XPS), Raman spectroscopy and conductivity measurement techniques.

3.1 Structural characterization of graphene materials

X-ray photoelectron spectroscopy is a crucial surface analysis technique that is capable of providing surface elemental composition (survey scans) and the type of chemical bonds (core level scans) present on the materials. XPS survey scans of Graphite-Ox, rGO-Br and rGO are shown in Fig. 1a. The semi-quantitative analysis on the Graphite-Ox survey scan gave an atomic C/O ratio of 2.4. The low atomic C/O ratio indicated a well oxygenated Graphite-Ox material, which usually consisted of hydroxyl, epoxy, carbonyl and carboxyl moieties. On the other

Fig. 1 XPS survey scan (a) and high-resolution XPS C1s core spectra (b) of Graphite-Ox, rGO-Br, and rGO materials.

hand, rGO-Br and rGO displayed higher atomic C/O ratios of 6.3 and 7.1, respectively. The increasing atomic C/O ratios after those chemical treatments indicated successive removals of oxygen-containing groups from the graphene backbone. Treatment with a strong base could result in a partial deoxygenation process as well.²⁰ As expected from the treatment with hydrobromic acid, the survey scan of rGO-Br revealed the presence of Br 2s, Br 3p and Br 3d peaks, which accounted for about 6.8% collectively. The Br peaks disappeared upon further treatment with potassium *tert*-butoxide, which supported the conclusion that the dehydrobromination took place.

Consequently, the removal of the oxygen-containing groups as indicated by the increasing atomic C/O ratios from Graphite-Ox to rGO was investigated using high-resolution C1s core level scans. High resolution XPS scans enable detailed evaluation of the changes on the type of oxygen containing groups present on the graphene materials. A typical C1s core level spectrum could be deconvoluted into several peaks centred at 284.5 eV (C=C, sp^2 graphitic carbon), 285.5 eV (C–C, sp^3 carbon), 286.5 eV (C–O, epoxy and hydroxyl), 288.5 eV (C=O, carbonyl) and 289.5 eV (O–C=O, carboxyl). A peak centred at 291 eV is attributed to the π – π^* shake-up satellite of the sp^2 graphitic carbon peak at 284.5 eV, which is a characteristic for a conjugated system. As shown in Fig. 1b, Graphite-Ox consisted of a high percentage of C–O moieties which contributed up to 52.1% as compared to a 44.1% of C=C graphitic carbon. Treatment with hydrobromic acid resulted in rGO-Br, with a higher amount of C=C graphitic carbon at 61.4% and a drastically lower amount of C–O bonds at only 14.7%. The lower amount of C–O bonds was due to the substitution of hydroxyl groups with bromine and the partial reductive effect of hydrobromic acid. On top of that, the C=C graphitic carbon peak was much more defined as compared to that of Graphite-Ox, which signified a more ordered and homogeneous chemical environment. Upon addition of potassium *tert*-butoxide, the amount of C=C

graphitic carbon increased to 68.6% while the amount of epoxy and hydroxyl groups reduced to 9.6%. The amount of oxygenated groups on rGO was lower than that of rGO-Br due to the partial deoxygenation effect of the strong base. Moreover, the dehydrobromination effect resulted in a higher amount of C=C graphitic carbon. In comparison to a reduced graphite oxide obtained *via* a hydrazine reduction method, we observed a 72.8% of C=C graphitic carbon and 5.2% of C–O bonds (data not shown). The rGO produced in this work has a comparable but lower amount of C=C graphitic carbon. This could be due to the remaining hydroxyl groups from the opening of epoxy moieties with the bromine anion. Despite the higher content of C–O bonds, our initial theoretical prediction on the regeneration of the sp^2 carbon network is supported by the changes observed in the XPS C1s carbon spectra in Fig. 1b.

High-resolution XPS scans were also carried out on the Br3d and Br3p core level peaks (previously observed in the wide scan of rGO-Br, Fig. 1a) of rGO-Br as shown in Fig. 2. In Fig. 2a, the prominent peaks at 70.8 eV ($3d_{5/2}$) and 72.0 eV ($3d_{3/2}$) accounted for the presence of C–Br bonds. The peak area ratio of C–Br in Br3d was 65.4%, which invariably suggested the formation of covalently bonded bromine on the graphene network.^{26,27} This was also supported by the high-resolution Br3p core level spectrum in Fig. 2b which present C–Br peaks at 190.3 and 183.6 eV, accounting up to 66.5% of the peak area ratio. The presence of physically adsorbed Br^- in Br3d (68.6 and 67.8 eV) and Br3p (187.9 and 181.2 eV) was noted as well. While the peak area ratio of C–Br was >65%, it unequivocally supported the successful bromination on the graphene carbon network.

Consequently, the graphene materials were further investigated by Raman spectroscopy, using a 514 nm laser excitation. A typically Raman spectrum shows two prominent networks of graphene. The D band ($\sim 1357\text{ cm}^{-1}$, symmetry A_{1g} mode) usually results from the presence of sp^3 carbons or defects on the graphene sheets while the G band ($\sim 1580\text{ cm}^{-1}$, E_{2g} mode of sp^2 carbon atom) represents the pristine carbon. The calculated ratio of D and G bands peak intensities (I_D/I_G) is a common indication of the extent of defects on graphene materials. In Fig. 3, the D and G bands on Graphite-Ox were broad and this is highly indicative of a more disordered chemical environment. The I_D/I_G ratios for Graphite-Ox, rGO-Br and rGO were 0.75, 1.32 and 1.31, respectively. An increment of the I_D/I_G ratio upon treatment with hydrobromic acid indicated formation of large edges and defects on the graphene carbon network. This could also

Fig. 2 High-resolution XPS spectra of (a) Br3d and (b) Br3p of the rGO-Br material.

Fig. 3 Raman spectra of Graphite-Ox, rGO-Br and rGO materials.

indicate a smaller size of graphitic domains. Further treatment with potassium *tert*-butoxide did not change the I_D/I_G ratio drastically. Although the dehydrobromination step should result in a lower I_D/I_G ratio, we suspected that the amount of bromine present on the graphene network was not significant enough to cause a drastic change in the I_D/I_G ratio. Apart from that, the evolution of 2D ($\sim 2700\text{ cm}^{-1}$, two-phonon double resonance process) peaks on rGO-Br and rGO signalled the presence of few-layered graphene in the materials. As compared to the reduced graphite oxide obtained from hydrazine treatment, we observed a higher density of defect with a I_D/I_G ratio of 1.42.

3.2 Electrical characterization of graphene materials

The electrical performances of the graphene materials were primarily evaluated using an interdigitated gold electrode (Au-IDE) platform. The platform allowed for a qualitative comparison of the conductivities for the graphene materials based on the concept of ‘ohmic’ behaviour, whereby a typical I – V curve would present a slope directly proportional to its conductivity.²⁸ As shown in Fig. 4, the conductivities of Graphite-Ox, rGO-Br and rGO were directly compared against a common chemically reduced graphene oxide obtained *via* a hydrazine reductant (CRGO-N). The Graphite-Ox exhibited a very high resistance which resulted in almost negligible current signal over the scanned potentials. This could most likely attribute to the insulating feature of Graphite-Ox. On the other hand, the remaining three materials gave ohmic behaviours with linear I – V curves. The rGO material gave the steepest slope among the three materials at 51 mA V^{-1} thus indicating the highest conductivity.

A brominated rGO-Br showed the second highest slope at 39 mA V^{-1} . Interestingly, the CRGO-N had the lowest conductivity with a slope of 34 mA V^{-1} . These measurements indicated that an enhanced chemically reduced graphene with a high conductivity could be achieved *via* a dehydrobromination treatment,

Fig. 4 Current–voltage curves of Graphite-Ox, rGO-Br and rGO materials.

which regenerates the disrupted sp^2 carbon–carbon network of graphene oxide.

3.3 Validation of the dehydrobromination process

In order to validate the dehydrobromination process on graphene, a control experiment was carried out. The Graphite-Ox was treated with potassium *tert*-butoxide (produced G-A) prior to treatment with hydrobromic acid (produced G-B) to exclude the dehydrobromination step. The XPS survey scans (Fig. S1†) showed an increasing atomic C/O ratio upon chemical treatments, which was in tune with the deoxygenative and reductive effects of the procedures. More importantly, the final graphene product obtained exhibited a lower conductivity as compared to rGO based on the Au-IDE platform (Fig. S2†). The Raman spectra (Fig. S3†) highlighted the changes in the I_D/I_G ratio of G-A to G-B at 0.92 and 1.13, respectively. This has invariably ascertained that the dehydrobromination effect played a crucial role in enhancing the electronic properties of the chemically reduced graphene by regenerating the broken sp^2 carbon network.

4. Conclusions

We have demonstrated the potential of graphene materials obtained based on a pre-reduction method using hydrobromic acid and followed by a dehydrobromination step. The graphene material possessed a better electronic conductivity as opposed to graphene produced using a conventional hydrazine-reductant. This method could potentially be applied on graphene oxide which has undergone a prior reduction step with strong reductants, such as lithium aluminium hydride, to achieve a near pristine chemically reduced graphene. We are also currently working on the feasibility of transferring other standard organic

chemical procedures onto graphene platforms towards the production of graphene with better electronic properties.

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Selective Removal of Hydroxyl Groups from Graphene Oxide

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Abstract: Graphene has a wide range of potential applications, thus tremendous efforts have been put into ensuring that the most direct and effective methods for its large-scale production are developed. The formation of graphene materials from graphene oxide through a chemical reduction method is still one of the most preferred routes. Numerous methods starting from various reducing agents have been developed to obtain near-pristine graphene sheets. However, most of the reducing agents are not mechanistically supported by classical organic chemistry knowledge and of those that are supported, they are only theoretically ca-

pable of, at most, reducing oxygen-containing groups on graphene oxide to hydroxyl groups. Herein, we present a mechanistically proven method for the selective defunctionalisation of hydroxyl groups from graphene oxide that is based on ethanethiol–aluminium chloride complexes and provides a graphene material with improved properties. The structural, morphological and electrochemical properties of the graphene materials have been fully characterised

Keywords: electrochemistry • graphene • reduction • selective defunctionalisation

based on high-resolution X-ray photoelectron spectroscopy, Fourier transform infrared spectroscopy, Raman spectroscopy, scanning electron microscopy, electrochemical impedance spectroscopy and cyclic voltammetry techniques. Our analyses showed that the obtained graphene materials exhibited high heterogeneous electron-transfer rates, low charge-transfer resistance and high conductivity as compared to the parent graphene oxide. Moreover, the selective defunctionalisation of hydroxyl groups could potentially allow for the tailoring of graphene properties for various applications.

Introduction

When graphene was first theorised, it was thought to be too thermodynamically unstable to exist at ambient temperatures.^[1] It was only in 2004 that Geim, Novoselov et al. succeeded in proving otherwise.^[2] Graphene, a two-dimensional carbon allotrope, is a single layer of conjugated carbon atoms arranged in a honeycomb structure. Graphene possess exceptional mechanical, optical, thermal and electrical properties, even though it is only one atom thick.^[3] Its outstanding properties have shown graphene to be a promising material for energy storage^[4] and electronic and electrical devices.^[5] Routes towards the production of graphene encompass both the top-down and bottom-up approaches. The bottom-up approach consists of a chemical vapour deposition technique,^[6] whereas the top-down method involves micromechanical exfoliation of graphite,^[2] unzipping of carbon nanotubes^[7] or the pre-oxidation of graphite to graphite oxide, followed by subsequent thermal/chemical reduction towards graphene. Towards the mass production of graphene, the chemical oxidation of graphite followed by chem-

ical reduction is currently the most cost effective and viable method.

The chemical oxidation of graphite to graphite oxide is normally accomplished through oxidation methods such as the Staudenmaier or Hummers methods.^[8] The graphite oxide materials obtained are usually rich in oxygen-containing groups such as hydroxyl, epoxy, carbonyl and carboxyl moieties. Upon ultrasonication treatments to give graphene oxide and the subsequent removal of oxygen-containing groups or chemical reduction, graphene is obtained in large quantities. These materials are usually referred to as chemically reduced graphene oxide. Numerous types of reducing agents have been applied to graphene oxide materials in recent years, such as hydrazine,^[9] sodium borohydride (NaBH₄),^[10] lithium aluminium hydride (LiAlH₄),^[11] hydroiodic acid,^[12] thiourea dioxide,^[13] ascorbic acid,^[14] hydroquinone,^[15] hydroxylamine,^[16] cytochromes^[17] and the protein-directed approach.^[18] These reducing agents showed varying degrees of performance to give graphene materials with differing qualities. Although some of these reducing agents have been applied in classical organic chemistry reactions for a long time and are known to work based on specific mechanisms (e.g., sodium borohydride and lithium aluminium hydride), plausible reduction mechanisms for the other reducing agents have not been determined. Moreover, even though most of these reducing agents have well-documented reaction mechanisms, the oxygen-containing groups are often only reduced to a lesser degree to end up as hydroxyl groups on the graphene surfaces (see Scheme 1). In short,

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Scheme 1. Fragments of small graphene oxide sheets treated with LiAlH_4 , NaBH_4 and EtSH-AlCl_3 .

the direct re-establishment of the sp^2 carbon-carbon bonds and complete removal of oxygen functionalities are not achievable with the use of these reducing agents.

The selective reduction and complete removal of oxygen-containing groups based on well-established classical organic chemistry reactions with well-defined mechanisms are imperative to improve our understanding and control over the modification of graphene oxide to graphene materials. Moreover, the ability to achieve selective reduction or removal of oxygen functionalities would allow for the precise tailoring of graphene materials for specific applications. Our group has previously investigated the removal of hydroxy groups to regenerate the sp^2 carbon-carbon bonds on graphene.^[19] Herein, we propose a standard organic method for the selective defunctionalisation of hydroxyl groups from the graphene oxide lattice to re-expose the sp^2 carbon-carbon bonds on the graphene sheets (See Scheme 1). This method is based on the selective defunctionalisation by radical intermediates with ethanethiol-aluminium chloride complexes to selectively remove hydroxyl groups from the graphene oxide sp^2 -carbon lattice.^[20] Previous work by Fujita and co-workers has indicated the effective defunctionalisation of alkoxy, peroxy, hydroxyl, alkylthio and halo groups from the parent aromatic moieties.^[20] Thus, we expect that this method would be valuable for providing a selective defunctionalisation method that could be utilized to tailor the types of oxygen functionalities that are preferred on the graphene sheets. Moreover, this method could be applied in conjunction with other reducing agents in a two-step process to obtain graphene materials with minimal oxygen functionalities.

Results and Discussion

The investigation of the selective defunctionalisation of hydroxyl groups was carried out in the presence of an ethanethiol-aluminium chloride (EtSH-AlCl_3) complex in dry

THF (see the Experimental section for the detailed procedure) to give reduced graphene oxide (rGO). In conjunction with the process for the selective removal of hydroxyl groups, three other control experiments, including graphene oxide stirred in the absence of ethanethiol and aluminium chloride (GO-C) and graphene oxide stirred in the presence of only ethanethiol (ES-C) or aluminium chloride (AC-C), were carried out at a similar temperature, 70°C . Subsequent structural, morphological and electrochemical properties of the obtained graphene materials were carefully characterised and analysed based on high resolution X-ray photoelectron spectroscopy, Raman spectroscopy, Fourier transform infrared (FTIR), scanning electron microscopy, electrochemical impedance spectroscopy, cyclic voltammetry and conductivity analysis.

Characterisation of structural properties: X-ray photoelectron spectroscopy (XPS) is a highly sensitive and semi-quantitative analysis method that is valuable for probing the surface element compositions of materials. This can be accomplished by an XPS wide scan survey, which translates the kinetic energies of electrons into specific binding energies that correspond to distinctive elements found up to a depth of 10 nm from the surface of the materials. On top of that, high-resolution XPS core-level scans are able to provide information on the chemical environment for the elements of interest. The XPS survey scans for the graphene materials are shown in Figure 1A. The survey scans highlight the prominent presence of the O1s and C1s peaks; these atoms form the main composition of graphene oxide based materi-

Figure 1. X-ray photoelectron spectroscopy spectra for GO, GO-C, ES-C, AC-C and rGO. A) Survey and B) C1s core-level spectra.

als. The ratio of carbon to oxygen elements (C/O ratio) obtained from the survey scans provided a good sense of the extent of oxidation/reduction of the graphene materials. Based on our analysis, GO, GO-C, ES-C, AC-C and rGO had C/O ratios of 2.16, 2.22, 2.33, 2.56 and 4.71, respectively. The control experiments (GO-C, ES-C and AC-C) showed negligible changes in the C/O ratios, which indicated that the presence of either the ethanethiol or aluminium chloride alone would not result in the reduction/selective defunctionalisation of hydroxyl groups on graphene oxide. However, upon the formation of EtSH-AlCl₃ complexes, the selective removal of hydroxyl groups from the graphene oxide surfaces was achieved, as shown by the higher C/O ratio provided by the XPS survey scan of rGO.

In an attempt to gain more insight into the specific defunctionalisation of graphene oxide, high-resolution XPS scans were carried out on the C1s core level as shown in Figure 1B. A typical C1s core-level spectrum of graphene materials often provides characterised Gaussian peaks fitted to the presence of C=C (sp² carbon, 284.5 eV), C-C (sp³ carbon, 285.7 eV), C-O (hydroxyl or epoxy, 286.8 eV), C=O (carbonyl, 288.1 eV) and O-C=O (carboxylic, 289.1 eV) bond types. The presence of the π - π^* (290.6 eV) shake up satellite peak is often observed in graphene materials that displayed improved characteristics of aromatic/conjugated systems and a more orderly chemical environment. The C1s core level spectrum of graphite oxide indicated the significant presence of C-O and C=O bond types. The ultrasonication treatment towards graphene oxide and thermal treatment at 70 °C did not result in significant changes in the C1s core-level spectrum for GO-C. The control experiment with only the addition of ethanethiol showed a similar result. It is interesting to note that treatment with aluminium chloride alone resulted in a small reduction of the C-O peak, which was lower in intensity than the C=C peak. However, upon treatment of graphene oxide with the EtSH-AlCl₃ complex, we observed an obvious reduction in the intensity of the C-O peak. Despite that, the presence of C=O and O-C=O bond types were still rather prevalent in the rGO C1s core-level spectrum. This highlights the specific defunctionalisation of hydroxyl groups in graphene oxide; the remaining C-O bond could most likely be attributed to the presence of small amounts of remaining epoxy groups or inaccessible hydroxyl groups.

Apart from that, Raman spectroscopy analyses were carried out on the graphene materials, as shown in Figure 2. Raman spectroscopy is often utilised to probe the quality of graphene materials. More specifically, a typical Raman spectrum of a graphene material consists of D (approximately 1200 cm⁻¹, breathing mode of sp² carbon) and G (approximately 1580 cm⁻¹, graphitic sp²-bonded carbon) bands. The intensity ratio of the D and G bands (I_D/I_G ratio) is often used as an indicator for the extent of defects that occur in graphene materials. As observed from the analysis, GO, GO-C, ES-C, AC-C and rGO showed I_D/I_G ratios of 0.93, 0.91, 0.89, 0.96 and 1.07, respectively. The I_D/I_G ratios for the graphene materials obtained from the control experiments

Figure 2. Raman spectra for GO, GO-C, ES-C, AC-C and rGO.

(GO-C, ES-C and AC-C) indicated a similar extent of defects due to the presence of oxygen-containing groups. Moreover, the broad D band indicated significant structural disorders with the presence of sp³-hybridised carbons and short-range sp²-carbon moieties. Upon the selective removal of hydroxyl groups, rGO registered a higher I_D/I_G ratio of 1.07. The increment of I_D/I_G ratio is prevalent and common for reduced graphene materials, as previously observed in several other studies on reduction of graphene oxide.^[9,13]

To further understand the structural properties of the resulting graphene materials, analysis with attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) was carried out (Figure 3). Graphite oxide provided peaks corresponding to the O-H stretching mode (≈ 3200 cm⁻¹), C-OH vibrational mode (≈ 1380 and 1050 cm⁻¹), C=O vibrational mode (≈ 1718 cm⁻¹) and C-O-C from epoxy groups (~ 870 cm⁻¹). The unoxidised graphitic region of the graphene materials typically provided a peak at ≈ 1600 cm⁻¹. The control experiments (GO-C, ES-C and AC-C) gave similar FTIR spectra to that of graphite oxide, albeit with a lower peak intensity for the C=O vibrational mode. This is well correlated with the XPS C1s core-level spectra, which show a smaller presence of the C=O bond type. Upon selective defunctionalisation treatment, the O-H stretching mode on rGO disappeared and the remaining characteristic peaks were greatly diminished or non-existent. This observation was highly consistent with earlier high-resolution XPS analyses too.

Figure 3. ATR-FTIR spectra for GO, GO-C, ES-C, AC-C and rGO.

Characterisation of morphological properties: The morphological properties of the graphene materials were subsequently investigated by using scanning electron microscopy (SEM; see Figure 4). The SEM analysis of GO showed the presence of heavily stacked and aggregated graphene sheets. On the other hand, the graphene materials obtained from the control experiments (GO-C, ES-C and AC-C) showed wrinkled, wave-like and less-aggregated graphene sheets. Upon treatment for selective defunctionalisation, the graphene sheets of rGO appeared rather clean and smooth,

Figure 4. SEM images for GO, GO-C, ES-C, AC-C and rGO.

with wrinkles and folded edges. The success of the selective defunctionalisation of hydroxyl groups also implied that oxygen-containing groups, such as carboxyl, are left untouched. As such, the resulting graphene material displayed good solubility in aqueous solution, as shown in Figure 5.

Figure 5. GO and rGO dispersed in aqueous solution: dispersion of 1 mg mL^{-1} of material in ultrapure water after ultrasonication for 10 min.

Characterisation of electrochemical and electrical properties: The electrochemical properties of the graphene materials were examined based on electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) techniques. Although these techniques have different methods of measurement, they ultimately measure the resistance of the graphene materials towards heterogeneous electron transfer. The EIS technique applies a small alternating-current voltage perturbation to the system at various frequencies, on the order of 1–10 mV, whereas the cyclic voltammetry technique applies a large perturbation to the system on the order of hundreds of millivolts. The ability of graphene materials to transfer and exchange electrons with their surrounding molecules in the environment is highly influenced by their surface morphologies. Both the EIS and cyclic voltammetry analyses were carried out with a ferro/ferricyanide electrochemical probe.

The Nyquist plots obtained from the EIS study are shown in Figure 6. In a typical Nyquist plot, the diameter of the semi-circle corresponds to the charge-transfer resistance (R_{ct}) of the material. Materials that exhibit higher resistances towards charge transfer would result in higher R_{ct} values. Because EIS is highly sensitive to the surface morphologies of the materials, slight changes of the surfaces would result in significant changes in their R_{ct} values. The bare glassy-carbon electrode provided a R_{ct} of 0.55 k Ω , whereas the GO gave a R_{ct} of 4.56 k Ω . The control experiments resulted in GO-C, ES-C and AC-C with R_{ct} values of 1.53, 1.10 and 1.16 k Ω , respectively. Upon the selective defunctionalisation treatment of GO, the rGO material showed the lowest R_{ct} value of only 0.09 k Ω . Based on this observation, the graphene materials that contain the largest amount of oxygen-

Figure 6. Nyquist diagrams for EIS measurements for GO, GO-C, ES-C, AC-C, rGO and bare GC. Conditions: PBS (50 mM), pH 7.2 background electrolyte, $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (10 mM). Inset: Randles equivalent circuit used for data fitting.

containing groups (i.e., lower C/O ratio from XPS analyses) provided higher R_{ct} values. This is probably due to the electrostatic repulsion between both the negatively charged oxygen-containing groups on the graphene materials and the ferro/ferricyanide probes at the working pH of the electrochemical analyses. This is generally consistent with previous findings that show C/O ratios correlated with R_{ct} .^[21] More importantly, the EIS analyses highlighted the improved heterogeneous charge-transfer ability of the rGO material as a result of the selective removal of hydroxyl groups from the GO material.

Following that, cyclic voltammetry analyses were carried out to reaffirm the results of the electrochemical impedance spectroscopy (Figure 7). In fact, CV scans were carried out prior to each impedimetric scan to probe the heterogeneous

Figure 7. Cyclic voltammograms on ferro/ferricyanide for GO, GO-C, ES-C, AC-C, rGO and bare GC. Conditions: PBS (50 mM), pH 7.2 background electrolyte, $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (10 mM). Scan rate: 100 mV s^{-1} .

electron-transfer (HET) rate of the graphene materials. By using a similar electrochemical probe as in the impedimetric analyses, the ferro/ferricyanide system provided a reversible CV scan in which the peak-to-peak separation (ΔE) could be utilised for the measurement of the HET based on the Nicholson method.^[22] The ferro/ferricyanide electrochemical probe is surface-sensitive, thus the number of oxygen-containing groups on the graphene surfaces could be easily correlated to the HET rate. Graphene materials that exhibited smaller peak-to-peak separations account for higher HET rates. The cyclic voltammograms of bare GC, GO, GO-C, ES-C, AC-C and rGO showed ΔE values of 0.19, 0.52, 0.26, 0.21, 0.28 and 0.14 V, respectively. The calculated heterogeneous electron transfer rate subsequently indicated an increasing value for the graphene materials in the order of: GO (k_{obs}^0 : $1.44 \times 10^{-5} \text{ cm s}^{-1}$) < AC-C (k_{obs}^0 : $3.52 \times 10^{-4} \text{ cm s}^{-1}$) < GO-C (k_{obs}^0 : $4.61 \times 10^{-4} \text{ cm s}^{-1}$) < ES-C (k_{obs}^0 : $9.73 \times 10^{-4} \text{ cm s}^{-1}$) < bare GC (k_{obs}^0 : $1.21 \times 10^{-3} \text{ cm s}^{-1}$) < rGO (k_{obs}^0 : $2.39 \times 10^{-3} \text{ cm s}^{-1}$). The GO material gave the lowest HET rate and the rGO material provided the highest HET rate, at about two magnitudes higher. The results were in tune with the EIS analyses carried out earlier, which showed similar implications for the successful selective removal of the hydroxyl groups.

The conductivities of the graphene materials were investigated by using an interdigitated gold electrode (Au-IDE) system. The analyses on the Au-IDE platform provided I - V curves for each graphene material in which the slopes of the curves would be directly proportional to their conductivities. This provided a qualitative comparison of the conductivities based on the concept of ohmic behaviour. The I - V curves of the graphene materials are shown in Figure 8. The GO provided negligible current signal across the scanned potentials as a result of high sheet resistances. The control experiments provided current signals with ohmic behaviours, as indicated by the linear I - V curves. The slopes of the curves were measured in the range of $2\text{--}3 \text{ mA V}^{-1}$. Upon treatment with the EtSH- AlCl_3 complex, resulting in the selective removal of hydroxyl groups, the rGO provided an I - V curve with a

Figure 8. Current-voltage curve of GO, GO-C, ES-C, AC-C and rGO.

slope of 75 mAV^{-1} . This indicated that the selective removal of hydroxyl groups from the parent graphene oxide greatly improved the electronic properties of the resulting graphene material.

Conclusion

We have demonstrated a selective defunctionalisation of hydroxyl moieties on graphene oxide surfaces that is based on a well-established organic chemistry methodology. The use of the ethanethiol–aluminium chloride complex has provided a robust method to selectively remove hydroxyl groups. The ability to tailor the composition of oxygen-containing groups on graphene materials is important for certain applications (for example, biosensing) that do not require the presence of hydroxyl groups. Moreover, the resulting graphene materials exhibited enhanced heterogeneous electron-transfer rates, a lower charge-transfer resistance and improved conductivity as compared to the parent graphene oxide. This method could potentially be applied as a stand-alone method or used synergistically with other reducing agents to provide improved graphene materials.

Experimental Section

Materials: Graphite ($<20 \mu\text{m}$), sulfuric acid (95–98%), sodium nitrate, aluminium chloride, *N,N*-dimethylformamide (DMF), potassium phosphate dibasic, sodium phosphate monobasic, potassium ferricyanide and potassium ferrocyanide were purchased from Sigma–Aldrich, Singapore. Ethanethiol and hydrogen peroxide (H_2O_2) was obtained from Alfa Aesar, Singapore. Potassium permanganate was obtained from J.T. Baker. Glassy-carbon (GC) electrode with a diameter of 3 mm was obtained from Autolab, the Netherlands. Milli-Q water (resistivity: $18.2 \text{ M}\Omega\text{cm}$) was used throughout the experiments.

Apparatus: X-ray photoelectron spectroscopy (XPS) was performed with a Phoibos 100 spectrometer and a monochromatic Mg X-ray radiation source (SPECS, Germany). Both wide-survey and high-resolution spectra for C1s were collected. Relative sensitivity factors were used for the evaluation of atomic C/O ratios from XPS wide-survey spectra measurements. XPS samples were prepared by coating a carbon tape with a uniform layer of the materials under study. Attenuated total reflectance Fourier transform infrared (ATR-FTIR) measurements were carried out on a PerkinElmer Spectrum 100 system coupled with a universal ATR accessory. Raman spectroscopy analysis was performed by 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 mounted on a Olympus optical microscope. The calibration was initially made by using an internal silicon reference at 520 cm^{-1} and gave a peak position resolution of less than 1 cm^{-1} . Diamond/ZnSe was used as the ATR crystal. The spectra were measured from 1000 to 3000 cm^{-1} . A JEOL-7600F semi-in-lens FE-SEM, operating in gentle-beam mode at 2 kV , was used to acquire the SEM images. The solid samples were transferred to a carbon tape held onto a SEM holder for analyses. The analyses of the samples were carried out at an average working distance of 6 mm . All voltammetric experiments were performed on a $\mu\text{Autolab}$ type III electrochemical analyzer (Eco Chemie, The Netherlands) connected to a personal computer and controlled by General Purpose Electrochemical Systems Version 4.9 software (Eco Chemie).

Procedures

Graphite oxide (GO)—Graphite oxide preparation with modified Hummers method:^[8a] Graphite (0.5 g) was stirred with sulphuric acid (23.0 mL , 95–98%) for 20 min at 0°C and then NaNO_3 (0.5 g) was added in portions. The mixture was left to stir for 1 h. KMnO_4 (3 g) was then added in portions at 0°C . The mixture was subsequently heated to 35°C for 1 h. Water (40 mL) was then added to the mixture, which resulted in the temperature of the mixture rising to 90°C . The temperature was maintained at 90°C for 30 min. Additional water (100 mL) was added to the mixture. This was followed by a slow addition of H_2O_2 (30%, $\approx 10 \text{ mL}$). The warm solution was filtered (RC membrane, $0.22 \mu\text{m}$) and washed with warm water (100 mL). The solid was subsequently washed with a copious amount of water until a neutral pH was obtained. The materials were kept in the oven at 50°C for 5 days prior to use.

Reduced/defunctionalised graphene oxide (rGO): Ethanethiol (2.88 mL , 38.8 mmol) was added to aluminium chloride (2.66 g , 19.9 mmol) in an ice bath and stirred for 15 min. A dispersion of graphene oxide (1 mg mL^{-1}) in dry THF (25 mL) was added to the mixture at 0°C and stirred for 30 min. The reaction mixture was then heated at reflux (70°C) for 5 h. The reaction mixture was subsequently cooled and filtered (PTFE membrane, $0.22 \mu\text{m}$), washed with a solution of HCl (1 M, 25 mL) and ultra-pure water until the filtrate was at pH 7. The mixture was subsequently washed with THF and diethyl ether. The materials were kept in a vacuum oven at 30°C for 5 days prior to use.

Control experiments: The GO control (GO-C) was obtained in a similar manner to reduced graphene, but in the absence of ethanethiol and aluminium chloride.

The EtSH control (ES-C) was obtained in a similar manner to reduced graphene, but in the absence of aluminium chloride.

The AlCl_3 control (AC-C) was obtained in a similar manner to reduced graphene, but in the absence of ethanethiol.

Electrochemical measurements: The electrochemical experiments were carried out in a 5 mL voltammetric cell at room temperature by using a three-electrode configuration. A platinum electrode served as an auxiliary electrode and a Ag/AgCl electrode as a reference electrode; a glassy-carbon electrode was used as a working electrode. Prior to each measurement, the glassy-carbon electrode was polished with alumina ($0.05 \mu\text{m}$) on a polishing cloth. The working electrode was then modified by applying a solution of carbon material (1 mg mL^{-1}) in DMF ($1 \mu\text{L}$) and allowing it to dry.

Conductivity measurements: Current–voltage measurements (I – V curve) were conducted with an interdigitated gold electrode platform (Au-IDE) by depositing $2 \mu\text{L}$ of the material suspension (prepared at 1 mg mL^{-1} concentration in water) onto the electrode surface with $10 \mu\text{m}$ spacing.^[23] The electrode was then dried under a lamp for 20 min, leaving a randomly deposited material film on the interdigitated area that bridged the two Au electrode bands. The I – V curves were obtained by linear-sweep voltammetric measurements at a 20 mVs^{-1} scan rate. The displayed data corresponds to the average from three measurements.

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Inherently Electroactive Graphene Oxide Nanoplatelets As Labels for Single Nucleotide Polymorphism Detection

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Graphene and graphene-related materials, such as graphene oxides, have been attracting great interest from the scientific community due to their interesting electronic, mechanical, thermal, optical, and electrochemical properties. Graphene is an excellent surface for electrochemistry,^{1–3} and it is often used as an electrode material in sensing^{4–6} and energy storage^{7–9} applications. Graphene is inherently redox inactive at typically used potentials, which means that graphene does not show any electrochemically driven redox (oxidation/reduction) behaviors. On the contrary, graphene oxide (GO) materials display an inherent redox activity that allows for their electrochemical reduction toward reduced graphenes.¹⁰ Note that the term “graphene oxide” refers to graphene sheets that were oxidized with various oxygen-containing groups, such as hydroxyl, aldehyde, epoxy, carboxyl, carbonyl, and peroxy.¹¹ The electrochemical reduction method of graphene oxide is used for preparation of electrode surfaces^{12,13} or for quantification of reducible oxygen-containing groups.^{14,15} However, there have not been any attempts shown that utilize the inherent electrochemical redox activity of graphene oxide as a label for DNA detection.

Here we exploit the unique inherent electroactivity of graphene oxide nanoplatelets (GONPs) for DNA and single-base polymorphism detection. The use of graphene oxide nanoplatelets with a high density of oxygen functionalities is beneficial over standard probes, as the signal amplification is very large, whereby a 50 × 50 nm sized graphene oxide platelet corresponds to almost 22 000 withdrawn electrons (given the density of reducible groups of 4.3 nm^{−1} and

ABSTRACT Graphene materials are being widely used in electrochemistry due to their versatility and excellent properties as platforms for biosensing. However, no records show the use of inherent redox properties of graphene oxide as a label for detection. Here for the first time

we used graphene oxide nanoplatelets (GONPs) as electroactive labels for DNA analysis. The working signal comes from the reduction of the oxygen-containing groups present on the surface of GONPs. The different ability of the graphene oxide nanoplatelets to conjugate to DNA hybrids obtained with complementary, noncomplementary, and one-mismatch sequences allows the discrimination of single-nucleotide polymorphism correlated with Alzheimer's disease. We believe that our findings are very important to open a new route in the use of graphene oxide in electrochemistry.

KEYWORDS: electroactive graphene oxide · genosensor · single-nucleotide polymorphism · graphene label

two-electron reduction per functional group).¹⁴ We also explore the unique interaction between graphene oxide nanoplatelets and single-stranded DNA nitrogenous bases, which differs significantly from its interaction with double-stranded DNA due to the shielding of the bases inside the double helix.¹⁶ To the best of our knowledge, this is the first time the reduction peak of GO is used as the analytical signal in general.

In particular, in this work complementary (wild-type), noncomplementary (nc), and one-mismatch (mutant) DNA sequences were employed for the hybridization of the electrode surface modified with DNA probes. After the hybridization steps, the electrode surfaces were incubated with a solution

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containing a certain amount of GONPs. The different conjugation ability of graphene with single- and double-stranded DNA (dsDNA) sequences was exploited here. It is well known that single-stranded DNA (ssDNA) sequences lie on the surfaces of graphene sheets *via* π – π stacking interactions.^{17,18} It was also shown that ssDNA and dsDNA possessed different binding affinities for graphene, with the former being more suitable to this aim.^{19,20} In fact, while the nitrogenous bases in ssDNA are free to form π – π stacking with graphene aromatic rings, the dsDNA nitrogenous bases are less accessible for interactions since they are shielded inside the double-helix structure. The different binding ability of GONPs to DNA hybrids formed with complementary, noncomplementary, and mismatch-containing sequences would result in a different amount of GONPs conjugated to the electrode surface upon incubation. As such, this could provide different electrochemical signals to discriminate among wild-type, mutant, and noncomplementary DNA in a simple and fast protocol.

RESULTS AND DISCUSSION

We explore here the unique interactions between graphene oxide nanoplatelets and DNA strands for single-nucleotide polymorphism detection. We demonstrate that single-stranded and double-stranded DNA interact in different fashions with graphene oxide nanoplatelets. Consequently, we exploit the unique properties of graphene oxide as inherently electroactive labels.

GONPs were obtained by ultrasonication²¹ of an aqueous solution of graphite oxide nanoplatelets obtained from the oxidation of commercial graphite nanofibers by the modified Hummers' method.²² In order to gain more insights into the material used for DNA labeling, the GONPs used in these experiments were characterized by scanning electron microscopy (SEM), high-resolution transmission electron microscopy (HR-TEM), atomic force microscopy (AFM), and electrochemistry.

The SEM image shows the filamentous structure of the fiber with uniform size distribution (Figure 1a). The HR-TEM image showed that the graphene sheets were perpendicularly aligned along the fiber axis (Figure 1b). The average observed diameter was around 35 nm. After the exfoliation by ultrasonication, the fibrous structure disappeared and gave separate individual graphene oxide nanoplatelets instead. The AFM image and height profile confirmed the presence of individual nanoplatelets with an average thickness around 1.2 nm (Figure 1c). Figure 1d shows the colloidal suspension of graphite nanofibers (GNFs) and GONPs after being sonicated for 30 min. As seen in the picture, GNFs were deposited on the bottom of the bottle, while GONPs form a very stable suspension²³ (picture taken on the third day after preparation). The good stability of the

Figure 1. Microscopy characterizations of GNFs and GONPs. (a and b) SEM and HR-TEM images of the graphite nanofibers as received. (c) AFM image and height profile of exfoliated GONPs. (d) Colloidal suspension of graphite nanofibers (GNFs) as received and GONPs after oxidation/exfoliation.

GONPs was due to their zeta potential of -41.5 mV ($n = 3$). ASTM defines colloids with a zeta potential higher than 40 mV (in absolute value) as having good stability.²⁴ GNFs showed a ζ potential of $+30.9$ mV ($n = 3$), indicating that the low ζ potential is the reason for poor stability of its colloids. SEM and TEM images of the material before and after oxidation/exfoliation and a more detailed AFM characterization are shown in the Supporting Information (see Figures S1, S2, S3). The size distribution of GONPs was measured by dynamic light scattering (see Figure S4) and gives an average size value of 37 nm, which closely corresponds to the average diameter of starting carbon fibers as well as to the diameter of GONPs measured by AFM. The whole characterization of GNFs as received and GONPs confirmed that the fibers were digested, and no nanofiber structure is shown after the oxidation/exfoliation treatment.

We then investigated the electrochemical behavior of GONPs by performing cyclic voltammetry between -0.2 and -2.0 V. As illustrated in Figure 2, two cyclic voltammetric waves between -1 and -1.5 V appeared in the first scan, corresponding to the reduction of the different oxygen-containing groups on the GONP surface, such as epoxy, aldehyde, and peroxy.¹⁴ This reduction signal was used as the working signal for biosensing purposes. The differential pulse voltammetry (DPV) technique was then employed, as it shows better sensitivity when compared to cyclic voltammetry.²⁵ A calibration curve for different GONP concentrations was obtained by DPV. The obtained signal increased linearly with increasing concentrations of GONPs (as shown in Figure S5, Supporting Information).

Following the electrochemical characterizations, GONPs were used as an electrochemical-active label

for genosensing. Scheme 1 shows the detection procedure steps. First, DNA probes were immobilized on the electrode surface by physical adsorption.²⁶ Then, three different hybridization experiments were performed. The probe-modified electrode was incubated in three different solutions containing (A) complementary target (wild-type); (B) one-mismatch-containing sequence (mutant); and (C) noncomplementary target (nc). After hybridization was achieved, the three different electrode surfaces were incubated in a solution containing a specified amount of GONPs. As illustrated in Scheme 1, GONPs conjugate to the DNA-modified electrode surfaces to varying extents depending on the specific targets used in the hybridization step. For this reason, a different amount of GONPs was immobilized on the DNA-modified electrode surfaces, thus providing different voltammetric signals.

More specifically, when the hybridization step was performed with the complementary target, a low amount of GONPs was conjugated to the DNA-modified electrode

Figure 2. Graphene oxide nanoplatelets show an inherent electrochemical reduction signal. Cyclic voltammogram for the electrochemical reduction of GONPs in 10 mM phosphate buffer solution, pH 7.0. Scan rate: 20 mV s^{-1} . Reference electrode: Ag/AgCl.

surface and gave rise to a small reduction signal, as shown by the voltammetric peak (wild-type, red line). For hybridization with the one-mismatch sequence, the amount of GONPs bound to the DNA-modified electrode surface increased, and this resulted in a higher voltammetric peak (mutant, blue line). Finally, the highest electrochemical signal was given by the electrode surface that was incubated in the noncomplementary target solution (nc, black line).

These different behaviors could be attributed to the different binding affinity of graphene nanoplatelets toward single- and double-stranded DNA. Upon hybridization with the complementary target, the electrode surface was modified with a significant amount of dsDNA sequences, while in the case of the noncomplementary target the electrode surface consisted mainly of nonhybridized ssDNA probes. Since as already explained, graphene possesses a stronger ability to conjugate to ssDNA, a higher amount of GONPs will be detected on the electrode surface that was incubated with the noncomplementary target. In the case of the one-mismatch sequence the voltammetric signal was higher than the complementary target one, due to the partial hybridization occurring on the electrode surface.

Chronocoulometry experiments were performed to establish the DNA density on the electrode surface after the hybridization step. The results confirmed our hypothesis (see Figure S6, Supporting Information), the DNA density on the electrode modified with the DNA complementary target being 2.53×10^{13} molecules/cm², much higher than on the mutant (2.40×10^{13} molecules/cm²) and on the noncomplementary target (2.28×10^{13} molecules/cm²).

In order to optimize the amount of DNA to be used in the hybridization steps and to evaluate the limit of

Scheme 1. Schematic of the experimental protocol. The hybridization step was performed with complementary target (A); one-mismatch target (B); and noncomplementary target (C).

Figure 3. Voltammetric response toward the DNA target concentration in the case of hybridization with wild-type (red diamonds), mutant (blue diamonds), and noncomplementary (black diamonds) targets. Error bars represent triplicate experiments. Individual values are shown in Table S1 (Supporting Information).

Figure 4. Voltammetric signal obtained after conjugation with different amounts of GONPs. Hybridization with wild-type (red histograms), mutant (blue histograms), and nc (black histograms) sequences. Error bars represent triplicate experiments. Individual values are shown in Table S2 (Supporting Information).

detection of the proposed biosensor, the variation of the GONP voltammetric signal *versus* DNA target concentration was studied. As illustrated in Figure 3, for the experiments with the complementary (red diamonds) and the mutant (blue diamonds) sequences, the voltammetric peak heights decreased with increasing concentration of DNA target until a plateau was reached at around 30 nM. At that point, further increment of DNA target concentration did not correspond to any signal decrement. The estimated limit of detection, calculated with consideration of the noise to be 3 times as high as the standard deviation of the value obtained in negative control experiments, was 500 pM. The differentiation between the wild-type and mutant was detectable to 10 nM. It is expected that with further development of this proof-of-concept principle the limit of detection could be significantly improved.

To establish the proper amount of GONPs for the labeling step, a calibration with different concentrations of GONPs was performed. The DNA target concentration was kept constant at a value of 30 nM. The histograms shown in Figure 4 represent the voltammetric peak height of GONP reduction *versus* the different GONP concentrations for hybridization with

Figure 5. Histograms representing the peak height values obtained for the conjugation of GONPs with the different DNA target-modified electrode surfaces. Measurements were performed and are shown in triplicate. Concentration of DNA targets used in the experiments: 30 nM. Concentration of GONPs: 0.2 mg/mL.

the wild-type (red color), mutant (blue color), and nc (black color) sequences. As illustrated in Figure 4, the peak height increased when increasing the concentration of GONPs until a plateau was obtained at 0.2 mg/mL. This point corresponded to the maximum discrimination between the wild-type, mutant, and nc sequences. For higher concentrations of GONPs, there was no corresponding increase in the voltammetric peak. This indicated that the DNA-modified electrode surface was saturated with GONPs at a concentration of 0.2 mg/mL.

The optimized values of DNA target and GONPs were employed for the detection of DNA hybridization and polymorphism. In Figure 5, histograms representing the peak height of GONPs reduction *versus* the different hybridization experiments are shown. As seen in the figure, the discrimination between wild-type, mutant, and nc sequences was achieved with good reproducibility (RSD < 10%). Moreover, a 26% increase in the signal for mutant and 45% for noncomplementary sequence as compared to the complementary one was obtained. In addition, Student's *t* test was applied to compare the results obtained in the three hybridization experiments. The difference between the mean values was significant for all cases analyzed, being $t_{\text{calc}} > 6.41$ for all examined cases, larger than $t_{\text{tab}} = 2.13$, at the 95% confidence level, 4 degrees of freedom. This confirms that there is a very significant difference among the signals obtained for the three hybridization experiments.

The stability of the biosensor modified with the biorecognition element was studied for a time range of 4 weeks. On day 1 the electrode was modified with the optimized concentration of DNA probes. Hybridization experiments were realized on the same day (day no. 1) and after 2, 5, 7, 10, 14, 21, and 28 days. Results indicate a good stability of the electrode surface within two-weeks time (variation of the signal in the range of 10% standard deviation with respect to the first-day experiment). After 3 weeks a 10% decrease in the signal was observed. After 4 weeks the recorded signal decrease was 12% and also an increase in the

RSD% value was observed (see Supporting Information, Figure S7).

The response of GONPs as electroactive labels was also studied for different ultrasonication times employed during the preparation of the nanoplatelets. It is in fact well known that a prolonged ultrasonication can influence the resulting material shape and size.^{27,28} For this reason GONPs obtained after 2, 8, 16, and 24 h ultrasonication were employed for the biosensing. The results, illustrated in Figure S8, show that when increasing the ultrasonication time from 2 h up to 24 h, both the electrochemical signal and the discrimination ability for complementary, noncomplementary, and mutant sequences worsen, with the 2 h ultrasonication time being the condition that provides the best results.

CONCLUSIONS

To conclude, we showed the proof of principle for the use of graphene nanoplatelets as electroactive labels for biosensors. We showed that ssDNA interacts with graphene oxide nanoplatelets in a different manner

than dsDNA. This leads to the sensitive discrimination of the single-base mutation in the DNA sequence related to Alzheimer's disease. The reduction signal of graphene oxide nanoplatelets was exploited for the detection of DNA hybridization and polymorphism in a very simple and rapid protocol, by using disposable screen-printed electrodes.

The use of nanoplatelets, which are much smaller than regular size graphene sheets, allows for a better conjugation to DNA-modified electrode surfaces and improves the discrimination between single- and double-stranded DNA, which enables differentiation among complementary, noncomplementary, and one-mismatch DNA sequences correlated with Alzheimer's disease.

A completely new approach in the use of graphene was proposed in this work, showing that the material can be employed not only as a platform for biomolecule immobilization and analysis but also as a label either for its direct detection or for signal enhancement. Our findings are expected to have a profound impact on point-of-care personalized healthcare.

EXPERIMENTAL SECTION

Materials. Stacked graphite nanofibers were purchased from Strem Chemicals (Newburyport, MA, USA). Potassium ferricyanide ($K_3[Fe(CN)_6]$), potassium ferrocyanide ($K_4[Fe(CN)_6]$), tris-(hydroxymethyl)aminomethane (Tris), hexaammineruthenium(III) chloride, sulfuric acid (95–98%), potassium permanganate, sodium nitrate, hydrogen peroxide, and DNA sequences correlated with Alzheimer's disease were purchased from Sigma-Aldrich (Singapore). Buffer solutions used in the study are the following: 0.1 M PBS (0.1 M NaCl, 10 mM sodium phosphate buffer, pH 7.0), TSC1 (0.75 M NaCl, 75 mM trisodium citrate, pH 7.0), TSC2 (0.30 M NaCl, 30 mM trisodium citrate, pH 7.0), and Tris-HCl (10 mM Tris, pH 7.4). All solutions were made up using Milli-Q water (18.2 M Ω cm resistivity). The following oligonucleotide sequences were employed: DNA probe 5'-AC-CAGGCGGCCGCACACGTCTCCAT-3'; complementary target 5'-ATGGAGGACGTGTGCGGCCGCCTGGT-3' (wild-type); 1-mismatch target 5'-ATGGAGGACGTGCGGCCGCCTGGT-3 (mutant); noncomplementary target 5'-AAAAAAAAAAAAAAAAAAAAAA-3' (nc). Oligonucleotides were diluted from stock solutions with sterilized Milli-Q water, separated into fractions, and stored at –20 °C. A single fraction was defrosted when required. Disposable electrical-printed carbon electrodes (DEP-chips, EE-PP model) were purchased from Biodevice Technology (Nomi, Japan). The three-electrode system included a carbon-based working electrode (2.64 mm² area), a Ag/AgCl reference electrode, and a carbon-based counter electrode.

Equipment. A JEOL-7600F semi-in-lens FE-SEM, operating in gentle-beam mode at 2 kV, was used to acquire the SEM images. AFM analysis was performed using a scanning probe microscope (model Multimode V by Veeco, Plainview, NY, USA). HR-TEM images were taken using a JEM 2100F field emission and Hitachi H-800 transmission electron microscope (JEOL, Japan). Size distribution and zeta potential were measured by a Zetasizer Nano ZS (Malvern) using dynamic light scattering and by laser Doppler microelectrophoresis, respectively. All electrochemical experiments were performed by using an Autolab PGSTAT302 potentiostat (Eco Chemie, Utrecht, The Netherlands) driven by GPES software, version 4.9. DPV measurements were performed in PBS buffer solution. Impedance measurements were recorded between 0.1 MHz and 0.1 Hz at a sinusoidal voltage perturbation of 10 mV amplitude. The experiments were carried out at an applied potential of 0.18 V (vs a Ag/AgCl reference electrode) in

a 0.1 M PBS buffer solution containing 10 mM $K_3[Fe(CN)_6]$ / $K_4[Fe(CN)_6]$ (1:1 molar ratio) as a redox probe.

Procedures. Graphene oxide nanoplatelets were produced in bulk quantities by following conventional protocols:²⁹ (a) a modified Hummers' method was employed to obtain graphite oxide nanoplatelets from graphite nanofibers.^{22,30} A mixture of $NaNO_3$, H_2SO_4 , and $KMnO_4$ was used for the oxidation of graphite nanofibers. (b) The obtained graphite oxide powder was dispersed in ultrapure water and then ultrasonicated for 3 h in order to obtain graphene oxide nanoplatelets.²¹ More details on the procedures are available in the Supporting Information. For calibration curve purposes 3 μ L of GONP suspension in milli-Q water at different concentrations was drop-casted onto each DEP-chip surface, and the solvent was allowed to dry at room temperature.

DNA probes were immobilized onto the electrode surface by dry physical adsorption. A 3 μ L volume of DNA probe solution in PBS buffer solution at the optimized concentration of 10 μ M (as resulted from impedance spectroscopy experiments,^{31,32} see Figure S9 in the Supporting Information) was deposited onto the electrode surface for 10 min at 60 °C. The electrode was washed twice in PBS buffer with gentle stirring at room temperature to remove excess, nonadsorbed material. DEP-chips modified with DNA probes were incubated in an Eppendorf tube with the hybridization solution (TSC1 buffer) containing the desired concentration of DNA target (total volume 100 μ L). The incubation was performed at 42 °C for 30 min, with gentle stirring. Two brief washing steps were then performed in TSC2 buffer at 42 °C. Incubation with GONPs was performed in an Eppendorf tube containing 100 μ L of GONPs at the desired concentration in PBS solution. The incubation was performed at room temperature for 20 min, with gentle stirring. This was followed by two brief washing steps in PBS buffer.

DNA oligonucleotide surface density was determined by chronocoulometry according to the method reported by Steel *et al.*³³ The hexaammineruthenium(III) dilution in Tris-HCl buffer used in the experiments was optimized at 100 μ M (as determined by measuring the binding isotherm of the DNA probe-modified platform). The DNA density on the electrode surface was calculated using the Cottrell equation and by considering the extrapolation of the intercept at time zero.

Conflict of Interest: The authors declare no competing financial interest.

Supporting Information Available: AFM, SEM, TEM, dynamic light scattering, chronocoulometry, and stability study. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Chemically Modified Graphenes as Detectors in Lab-on-Chip Device

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Abstract

Graphene materials hold immense potentials for electrochemical detectors in lab-on-chip devices. The electronic and electrochemical properties of graphene based materials are significantly affected by the fabrication routes and by the structural features, such as density of defects and amount of oxygen containing groups. Therefore it is paramount to evaluate various graphene-based materials prior to their integration onto the lab-on-chip devices. The performance of various reduced graphenes (so called chemically modified graphene materials), namely, thermally, chemically and electrochemically reduced graphenes as well as graphene-oxide and graphite-oxide as detectors in a microfluidics system was examined and linked to the materials properties of the various graphenes. This work shows that not all graphene materials are beneficial for the detection at lab-on-chip devices. In addition, the findings show that materials exhibiting excellent properties in batch measurements demonstrate poor performance in flow-setup. These findings could provide valuable insights into the future applicability of graphene materials towards practical applications.

Keywords: Lab-on-chip, Microfluidics, Electrochemistry, Graphene

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1 Introduction

High-throughput analysis is a fast developing field which offers possibilities for rapid and sensitive chemical or biological screenings. In general, microfabricated microfluidics systems known as “Lab-on-chip” devices are able to improve the speed and scale at which such analyses are performed [1]. These devices provide advantages in terms of portability, low cost, and low amount of sample consumptions [2]. These benefits are achieved via incorporation of a miniature and sensitive detector for analyses of samples in small volume (typically less than few nanoliters). To this aim, electrochemical detection technique offers great advantages since it is highly miniaturizable, consumes low amount of energy and very much compatible with advanced microfabrication methods [3].

The sensitivity or detection limits of Lab-on-chip devices continue to be the main concern for such platform that is coupled with an electrochemical detection system. The utilization of various electrode surfaces such as carbon-based and boron-doped diamond electrodes have improved the sensitivity and reproducibility [4,5]. Chen et al. and Escarpa et al. have introduced carbon nanotubes and graphene composite materials as detectors to achieve a higher sensitivity [6–13].

Graphene is a one-atom thick material consisting of sp^2 -bonded carbon featuring a honeycomb structure [14]. The properties of graphene sheets, such as high mechanical strength, high elasticity and thermal conductivity, very high room-temperature electron mobility, low cost and low environmental impact, have made this an ideal material for sensing and energy storage [15,16]. Graphene could be obtained by a top-down (chemical or mechanical exfoliation of graphite, and unzipping of carbon nanotubes) [17–19] or bottom-up (chemical vapour deposition) [20] methods. In our work, we focus exclusively on top-down methods, which are suitable for large-scale production of graphene related materials. In these methods, graphite is oxidized to graphite-oxide and this material is consequently exfoliated/reduced by various methods, including thermal exfoliation/reduction, exfoliation of graphite-oxide to graphene-oxide by ultrasonication and consequent chemical or electrochemical reduction. The resulting products, reduced graphenes as well as their precursors, graphene and graphite oxides, are generally termed chemically modified graphene materials. These chemically modified graphene materials differ in terms of thickness and percentage of atomic oxygen contents, which directly affect their electron transfer properties [21]. The routes towards the graphene materials are

Scheme 1. Schematic of the procedure for the production of chemically modified graphene materials using graphite as the starting material.

shown in Scheme 1 [21]. The graphene materials were immobilized on detection electrodes via a thin-film coating technique in the absence of any additives [5].

Herein, we present a systematic comprehensive study of the integration of these chemically modified graphene materials (which include graphite-oxide, graphene-oxide, chemically reduced graphene (CRG), thermally reduced graphene (TRG), and electrochemically reduced graphene (ERG)) as electrochemical detector surfaces on a Lab-on-chip platform.

2 Experimental

2.1 Reagents

Graphite ($<20\ \mu\text{m}$), sulfuric acid (95–98%), potassium chlorate (98%), hydrochloric acid (37%), *N,N*-dimethylformamide (DMF), 4-morpholineethanesulfonic acid sodium salt (MES), acetic acid, catechol, dopamine, potassium phosphate dibasic, sodium phosphate monobasic,

and sodium borohydride were obtained from Sigma-Aldrich, Singapore. Fuming nitric acid ($>90\%$) was obtained from J. T. Baker. Glassy carbon (GC) electrodes with a diameter of 3 mm were obtained from Autolab, The Netherlands.

2.2 Preparation of Chemically Modified Graphene Materials

2.2.1 Graphite-Oxide: Prepared According to the Staudenmaier Method

Sulfuric acid (17.5 mL 95–98%) and nitric acid (fuming, 9 mL) were added into a round bottom flask and stirred under 0°C for 15 min. Graphite (1 g) was then added into the mixture under vigorous stirring to avoid agglomeration and to obtain a homogeneous dispersion. Potassium chlorate (11 g) was slowly added into the mixture (over 15 min) at 0°C . The reaction mixture was left to stir for 96 h at room temperature. On completion of the reaction,

the mixture was poured into deionized water (1 L) and filtered. Graphite oxide was then dispersed and washed repeatedly in HCl (5 %) solutions to remove sulfate ions and finally washed with deionized water until a neutral pH of the filtrate was obtained. The graphite-oxide slurry was then dried in a vacuum oven at 40 °C for 48 h before use.

2.2.2 Graphene-Oxide

Graphene-oxide was obtained by ultrasonication (Fischerbrand, FB 11203 ultrasonic unit at a frequency of 37 kHz, 180 W) of a graphite-oxide/DMF dispersion (1 mg/mL) for 1 h.

2.2.3 Thermally Reduced Graphene (TRG)

Thermally reduced graphene was obtained via thermal exfoliation/reduction of graphite oxide at 1050 °C. Graphite oxide (0.2 g) was placed in a porous quartz glass capsule connected to a magnetic manipulator inside a vacuum-tight tube furnace under a controlled atmosphere. A magnetic manipulator created a temperature gradient of over 1000 °C/min. The sample was flushed with nitrogen by repeated evacuation of the tube furnace to remove any traces of oxygen and then quickly inserted into the furnace and was held in the furnace for 3 min. The flow of nitrogen during the exfoliation procedure was 1000 mL/min, which removed the by-products.

2.2.4 Chemically Reduced Graphene (CRG)

Chemically reduced graphene was obtained by mixing graphite-oxide (10 mg) with an aqueous solution of NaBH₄ (50 mM, 20 mL). The mixture was ultrasonicated (37 kHz) for 1 h. The reduction reaction was indicated by a color change from brown (graphene-oxide) to black (CRG) during the sonication process. The CRG dispersion was then washed with deionized water to remove NaBH₄ residues and finally dried in a vacuum oven at 60 °C for 48 h.

2.2.5 Electrochemically Reduced Graphene (ERG)

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 7.2).

The graphene materials have been fully characterized as previously reported [21].

2.3 Apparatus

The cross-shaped microchip made from Topas polymer was obtained from Microfluidic ChipShop, Germany. The 85 mm × 16 mm chip consisted of a four-way injection cross, with a 75 mm separation channel and three side arms of 5 mm each in length. The original detection reser-

voir was cut off to expose the end of the microchannel in order to facilitate end-column amperometric detection. The channels were 50 μm wide and 50 μm deep. A plexi-glass holder was specially fabricated to hold the microchip and to accommodate the detectors and detection reservoir. The detection reservoir consisted of a platinum wire counter (0.25 mm diameter, Aldrich), an Ag/AgCl wire reference (0.5 mm diameter, home-made) and a bare glassy carbon electrode (CH Instruments, Austin, TX, USA) with a diameter of 3 mm. The working electrode, housed in the plastic screw was placed at the end of the exposed microchannel. The high voltage power supply and amperometric detection were provided and performed on a HVSTAT2010 system (Micrux Technologies, Spain) connected to a desktop.

2.4 Electrophoretic Procedure

The microchannels of the topas chip were rinsed with 0.1 M NaOH and deionized water for 10 min each, prior to use. MES buffer (25 mM, pH 6.5) was used as the electrophoretic buffer for the analysis. The sample injection channel was initially filled with the sample solution electro-kinetically prior to the amperometric measurements. A separation voltage of +2000 V was subsequently applied on the buffer reservoir (the detection reservoir grounded) until a flat amperometric baseline was observed. The microchip would then be ready for amperometric measurements. The electropherograms were recorded with a time resolution of 30 ms. Sample injection was carried out only after the stabilization of baseline was achieved. All the experiments were carried out in room temperature. The scheme of the experimental setup is shown in Scheme 2 [22].

2.5 Immobilization of Graphene Materials

The graphene materials were firstly prepared in suspensions of 1 mg/mL in DMF and ultrasonicated for 10 min. Subsequently, 1 μL of the aliquot was drop casted on the surface of glassy carbon electrodes. The solvent was then allowed to evaporate at room temperature, leaving a randomly distributed film on the glassy carbon electrode surface.

3 Results and Discussion

It is known that chemically modified graphenes exhibit different heterogeneous electron transfer rates (HET), depending on the density of the oxygen containing groups and defects, in a bulk solution based measurements [21]. Here we investigate the performance of various chemically modified graphenes as detectors in microchip electrophoresis devices. It is important to underline that in general, in many cases the bulk-based electrochemical performance of detection material is not linked to its performance on a lab-on-chip platform due to various factors

Scheme 2. The microchip setup: (a) Topas chip, (b) separation channel, (c) run buffer reservoir, (d) run buffer reservoirs, (e) sample reservoirs, (f) platinum separation electrode, (g) detection electrode, (h) Ag/AgCl wire reference electrode, and (i) platinum cathode for separation.

which come into play, such as minute size of the sample and active electrode area, flow conditions, and presence of high-voltage electric field during electrophoretic separation. Here we compare the performance of various chemically modified graphene materials in a microfluidic system by utilizing electrophoretic separation of important dopamine neurotransmitter and catechol with the graphene-based electrochemical detection.

Prior to performing the microfluidic separation/detection of dopamine and catechol, the optimal detection potential has to be established. Hydrodynamic voltammograms for the oxidation of dopamine and catechol were carried out using a glassy carbon electrode as shown in Figure 1. The voltammograms were recorded at a step potential of +0.1 V starting from +0.0 to +0.8 V. Both dopamine and catechol displayed similar sigmoidal voltammetric profiles on the bare glassy carbon electrode. The initial potential intervals from +0.0 to +0.1 V did not provide any response. The peak currents increased at higher operation potentials and peaked at +0.5 V for both dopamine and catechol. Thereafter, the peak currents decreased with increasing detection potential. The amperometric detection of dopamine and catechol with the chemically modified graphene materials employed a detection potential of +0.5 V since it provided the most favorable sound-to-noise characteristic.

Figure 2 shows the typical microchip electropherograms of a mixture of dopamine (a) and catechol (b) at different electrode materials, specifically (A) bare glassy carbon, (B) graphite, (C) graphite-oxide, (D) graphene-oxide, (E)

chemically reduced graphene, (F) electrochemically reduced graphene, and (G) thermally reduced graphene.

The detailed analysis of the obtained data showed that the peak currents obtained for dopamine and catechol, respectively, were as follow: bare GC (26.4 and 13.2 nA),

Fig. 1. Hydrodynamic voltammograms of 200 μ m dopamine and 200 μ m catechol on a bare glassy carbon electrode. Conditions: 25 mM MES buffer, pH 6.5; injection and separation voltage, +2000 V; injection time, 3 s.

Fig. 2. Electropherograms of a mixture of 250 μM dopamine (a) and 150 μM catechol (b) at (A) bare GC, (B) graphite, (C) graphite-oxide, (D) graphene-oxide, (E) CRG, (F) ERG and (G) TRG modified electrodes. Conditions: 25 mM MES buffer, pH 6.5; injection and separation voltage, +2000 V; injection time, 3 s.

graphite (23.5 and 11.7 nA), graphite-oxide (24.3 and 10.6 nA), graphene-oxide (18.3 and 8.8 nA), CRG (19.2 and 10.4 nA), ERG (20.3 and 6.9 nA) and TRG (2.3 and 2.1 nA). Among the chemically modified graphene materials, graphite-oxide modified electrode achieved the highest peak current for the detection of dopamine while graphite modified electrode gave the highest peak current for catechol detection. The lowest peak currents for both the dopamine and catechol were observed on the TRG modified electrode. However, the peak currents obtained from these graphene-based materials were lower in comparison to the bare GC electrode. The reason for TRG materials to exhibit poor performance at lab-on-chip platform likely originates from the fact that it shows the largest capacitive behavior of all graphene materials [23], which leads to high background currents in microfluidics CE/EC systems [24].

The resolution of the lab-on-chip devices with electrochemical detectors depends on the electron transfer at the detection surfaces [25]. The resolution for the separation of dopamine and catechol were 1.49, 1.29, 1.69, 1.35, 1.13, 2.77 and 0.87 for the bare GC, graphite, graphite-oxide, graphene-oxide, CRG, ERG and TRG detectors used in the microfluidics setup, respectively. The electrochemically reduced graphene gave the highest resolution, with almost a two-fold increment as compared to the bare glassy carbon. On the other hand, the thermally reduced graphene provided the lowest resolution among all

the modified electrodes. The separation column efficiency, described as the number of theoretical plates per meter were 2871, 2143, 3045, 1999, 1386, 5191, and 257 for the bare GC, graphite, graphite-oxide, graphene-oxide, CRG, ERG and TRG, respectively.

In light of these findings, the performances of the chemically modified graphene materials on a lab-on-chip device differ greatly as compared to a three-electrode electrochemical-cell setup analyses which were well studied [21], as well as among the reduced graphenes prepared by various routes. Therefore, it must be emphasized that graphene materials prepared from various routes must be tested for detection on lab-on-chip devices before making conclusion of beneficial/detrimental performance of the graphene materials on lab-on-chip devices and their practical applications.

4 Conclusions

We have demonstrated the potential of chemically modified graphene materials as electrochemical detectors in a lab-on-chip device. We found that electrochemically reduced graphene highlighted the best performance with a resolution of 2.77 and theoretical plates of 5191 per meter. These findings could extend the use of electrochemically reduced graphene as a detector for other analytes such as explosives compounds or DNA, towards rapid and sensitive high-throughput analyses. We wish to emphasize here the need for testing of various reduced graphene electrodes in lab-on-chip setups in order to provide well-justified, advantageous detection system.

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Graphene based nanomaterials as electrochemical detectors in Lab-on-a-chip devices

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ABSTRACT

Graphene related materials have received significant attention as new and advantageous materials for electrochemistry. Here we present an evaluation of graphene oxide amperometric detectors in microfluidics devices and we compare the results to those obtained at graphite microparticles and bare glassy carbon electrodes. We evaluate the influence on peak currents (sensitivity) and peak width (resolution and separation efficiency) of neurotransmitters and nitroaromatic explosives. We conclude that there is no major advantage of using graphene oxide as detector in microfluidics devices and graphite microparticles-based amperometric detectors in microfluidics provide an equivalent or better performance.

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1. Introduction

There is an ever-increasing need for sensitive high-throughput analysis. “Lab-on-a-chip” devices, in particular, the microfabricated microfluidics systems can considerably improve the speed and scale at which chemical and biological analyses are performed. Such a microfluidics system exhibits the ability to reduce conventional analytical systems and bring advantages of high-throughput, low cost, low sample consumption and portability/field deployability [1]. The major challenges in the field of analytical Lab-on-a-chip devices is to provide sensitive detector for minute amount of samples (typically less than few nanolitres) which can be miniaturized in order not to compromise the portability of the Lab-on-a-chip device. Electrochemical detection brings great benefits for the microfluidics devices as it is inherently miniaturizable, has low energy consumption and it is compatible with advanced microfabrication schemes [2].

Several groups, including ours, focused on improving detection limits of Lab-on-a-chip devices using nanomaterials modified detectors [see i.e. Review 3]. Several articles were published on high sensitive detection using of carbon nanotubes (CNT) based detectors in microfluidics [4–14]. Currently, graphene is regarded as the electrochemical materials of the future with reported advantageous electrochemical properties for sensing and energy storage [15–19]. Here, it should be mentioned that not all reports are that optimistic [20]. To the best of our knowledge, there is no report on the use of graphene based nanomaterials as detector surfaces for Lab-on-a-chip devices. Only very recently, Chen and coworkers published article on

conventional capillary electrophoresis with graphene based composite detector [21]. We wish to report here the comparative study of graphene oxide and graphite microparticles materials for detection of neurotransmitters and explosives and to investigate for any advantages of graphene oxide to be employed in electrochemical detection in microfluidics.

2. Experimental section

2.1. Reagents

Graphite microparticles powder (particle size <20 µm, Aldrich) was used without further treatment. Graphene oxide was obtained by ultrasonication (37 kHz) of a dispersion of 1 mg/mL graphite oxide in DMF for 2 h [22]. Graphene oxide prepared by this method is typically single layer [23]. The graphite oxide was prepared using graphite nanofibers (Aldrich) according to Staudenmaier method [24] whereby 17.5 mL of H₂SO₄ (95–98%) and 9 mL of HNO₃ (fuming) were stirred in a round bottom flask at 0 °C for 15 min. 1 g of graphite nanofibers was then added to the mixture under vigorous stirring to obtain a homogeneous dispersion. 11 g of KClO₃ was added into the mixture over 15 min in order to avoid temperature spike and the formation of ClO₂ gas, which is explosive at high concentrations. The mixture was left to stir vigorously for 96 h at room temperature. On completion, the mixture was then 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 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. A 1 mg of the carbon materials were dissolved in 1 mL of DMF. Dopamine, catechol, trinitrotoluene, 1,3-dinitrobenzene, 2,4-dinitrotoluene, sodium

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tetraborate decahydrate, 4-morpholineethanesulfonic acid sodium salt (MES), acetic acid, sodium dodecyl sulphate (SDS), acetonitrile and sodium hydroxide were received from Aldrich. Stock solutions of catecholamines were prepared daily in deionized water. Stock solutions of explosives were prepared in acetonitrile. The working electrode was modified by applying 1 μL of DMF containing 1 mg/mL carbon material (graphite or graphene oxide powder) and allowing to dry overnight.

2.2. Apparatus

The glass microchip was obtained from Micrux Technologies (Model MCE-Glass-001T). The 44 mm $\times$ 16 mm chip consisted of a four-way injection cross, with a 38 mm long separation channel and three side arms of 5 mm each in length (see Fig. 1). The original detection reservoir was cut off, resulting in an exposed microchannel at the end of the chip, to facilitate end-column amperometric detection. The channels were 50 μm wide and 20 μm deep. A plexiglass holder was specially fabricated to hold the microchip and to accommodate the detectors and detection reservoir [25]. The detection reservoir consisted of a platinum wire counter (0.25 mm diameter, Aldrich), an Ag/AgCl wire reference (0.5 mm diameter, home-made) and a bare glassy carbon (GC) or carbon material film-modified disk working GC electrode (CH Instruments, Austin, TX, USA) with diameter of 3 mm. The working electrode, housed in the plastic screw was placed at the end of the exposed microchannel.

2.3. Electrophoretic procedure

The microchannels of the glass chip were rinsed with 0.1 M NaOH and deionized water for 10 min each, prior to use. MES buffer (25 mM, pH 6.5) was chosen as the electrophoretic buffer for the analysis of catecholamines [26] whereas borate buffer (15 mM, pH 9.2) with addition of 20 mM SDS was used as the electrophoretic buffer for analysis of nitroaromatic explosive materials [27]. Prior to the amperometric measurements, the sample injection channel was initially filled with the sample solution electrokinetically. A separation voltage of +1500 V was subsequently applied on the buffer reservoir (the detection reservoir grounded) until a flat amperometric baseline was observed [26]. The microchip would then be ready for amperometric measurements. The amperometric detection was performed using HVSTAT2010 (Micrux Technologies) connected to a desktop. The electropherograms were recorded with a time resolution of 30 ms. Sample injection was carried out only after the stabilization of baseline was achieved. All the experiments were carried out in room temperature.

3. Results and discussion

Graphene oxide has been previously used with reported beneficial electrochemical properties for “in batch” electrochemical analysis of a

Fig. 1. Top view of the microchip: (a) the glass chip, (b) separation channel, (c) buffer reservoir, (d) run buffer reservoirs, (e) sample reservoirs, (f) platinum cathode for separation, (g) detection electrode, (h) Ag/AgCl wire reference electrode, and (i) platinum counter electrode.

wide variety of compounds, starting with ferro/ferricyanide through biomolecules and proteins as well as of DNA bases [28,29]. We thus decided to investigate whether there would be any advantages of using graphene oxide (GO) as detectors in microfluidics. We used GO modified GC electrode as electrochemical detector and compared its performance with graphite microparticles modified GC as well as bare GC electrode. We used dopamine, catechol and nitroaromatic explosives related compounds as standard analytes to compare the electrochemical performance of these three materials. Fig. 2 shows typical electropherograms of separation of dopamine and catechol on glass microchip, using amperometric mode for the detection of the neurotransmitters at different electrode materials, namely (A) bare GC, (B) graphite microparticles and (C) graphene oxide. There is no apparent significant difference between the electropherograms of the catecholamines using GC and graphene oxide detection electrodes; however, there is an apparent difference for signal using graphite microparticles. Peak currents of dopamine (DA) and catechol (CA) are higher at graphite microparticles modified electrode (of 14.0 and 5.4 nA for DA and CA, respectively) than on bare GC (of 10.8 and 4.6 nA for DA and CA, respectively) and interestingly, also higher at GO modified electrode (of 10.8 and 3.6 nA for DA and CA, respectively). It is of interest to discuss these results in light of our previous work on detection of DA and CA on CNT, graphite microparticles and bare GC electrodes. There is consistency between the data we present in this manuscript and in Ref. [9] where the bare GC and graphite microparticles modified electrode responses in microfluidics device, with peak currents of DA being about 1.4 times higher on graphite microparticles modified electrode than on bare GC. Oxidative peak currents of DA on CNT based detectors were always lower than that of graphite microparticles.

When quantifying the resolution between DA and CA, the resulting numbers are very similar. The bare GC detector exhibits a resolution of $R_s = 1.94$ while GO detector provides a resolution of $R_s = 2.22$ and graphite microparticles modified electrode gives a resolution of $R_s = 1.99$. Separation column efficiency, described as the number of theoretical plates per meter was 2178, 2781 and 3226 m^{-1} for GC, GO, and graphite microparticles respectively, using DA as example.

Fig. 2. Electropherograms of a mixture of (a) dopamine and (b) catechol at bare GC (A), graphite microparticles (B), and GO (C) modified electrodes. Conditions: Dopamine and catechol at 400 and 100 μm respectively; 25 mM MES (pH 6.5); separation voltage, +1500 V; injection voltage, +1500 V; injection time, 5 s; detection potential, +0.6 V. Carbon material film loading, 1 μg .

Fig. 3. Electrophoregrams of a mixture of (a) DNB, (b) TNT and (c) DNT (20 ppm for all) at bare GC (A), graphite microparticles (B), and GO (C) modified electrodes. Conditions: 15 mM borate buffer (pH 9.2) with 20 mM SDS; separation voltage, +2000 V; injection voltage, +2000 V; injection time, 3 s; detection potential, -0.5 V. Carbon material film loading, $1\text{ }\mu\text{g}$.

These data show that there is only a small difference between graphite microparticles and GO in terms of the detector performance for resolution and separation efficiency, in favor of graphite microparticles-based electrodes. Here we should mention that in this microfluidics/detector design, the detector material can have profound influence upon separation parameters, as it has been demonstrated before [10,30].

Consequently, we turned our attention into the investigation of microfluidic separation with end-column amperometric detection of nitroaromatic explosives [31,32]. Namely, we selected 2,4,6-trinitrotoluene (TNT), 2,4-dinitrotoluene (DNT) and 1,3-dinitrobenzene (DNB) as these compounds are found in the list by the Environmental Protection Agency (U.S. EPA) as TNT related environmental contaminants [33]. Fig. 3 shows representative micellar electrokinetic electrophoregrams of separation of TNT, DNT and DNB at (A) bare GC, (B) graphite microparticles and (C) graphene oxide modified electrodes using amperometric detection in reduction mode. In case of the separation of nitroaromatic compounds, it is clear that GO and graphite microparticles detectors provide similar amperometric peak currents of 15.3, 7.5 and 2.8 nA and 15.6, 5.6 and 2.5 nA for DNB, TNT and DNT at GO and graphite microparticles electrodes, respectively. Lower reduction currents originate from bare GC electrode, with 10.0, 7.2 and 2.2 nA for DNB, TNT and DNT. The effect of the graphene oxide based detector on separation efficiency is also significantly higher than that of bare GC electrode; however, graphite microparticles results in an even higher number of theoretical plates per meter than GO; see on the example of DNB, where Nm^{-1} is 9854, 11429 and 4662 m^{-1} for graphene oxide, graphite microparticles and glassy carbon electrode, respectively. The findings presented in this

article on microfluidics systems with graphene based electrochemical detectors are in line with our previous research on single-, few- and multilayer graphene in batch measurements where we found that the difference between the redox currents at the single layer and multilayer graphene are similar to those of graphite [34–36].

4. Conclusion

Here we demonstrate that the use of graphene oxide as electrochemical detector in microfluidics is of no advantage in terms of sensitivity or selectivity over that of graphite microparticles both in oxidation and reduction mode, as demonstrated for microfluidics separation of neurotransmitters and nitroaromatic explosives.

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