PRODUCTION AND APPLICATIONS OF GRAPHENE AND ITS COMPOSITES

A thesis submitted to
The University of Manchester
for the degree of
Doctor of Philosophy
in the
Faculty of Engineering and Physical Sciences

2015

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SCHOOL OF MATERIALS
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Word count – 62991
ABSTRACT

Production and applications of graphene and its composites

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Doctor of Philosophy
17 September 2015

Graphene, a single layer of graphite, owing to its excellent mechanical, electrical, and thermal properties, has evolved as an exceptional nanomaterial in the past decade. It holds great promise in developing various novel applications from biomedical to structural composites. However, several challenges remain in realising the great potential of this material; one being the bulk scale production of graphene. This thesis has been concerned with production of pristine few-layer graphene (FLG) using liquid phase exfoliation (LPE) of graphite in various solvent media and exploring the applications of graphene-based composite coatings as optical Raman-strain sensors.

LPE of natural graphite using bath sonication was used to produce highly stable pristine FLG in 1-methyl-2-pyrrolidinone (NMP) and N,N-dimethylformamide (DMF). Atomic force microscope (AFM) was used to analyse the exfoliation efficiency and lateral dimensions, while Raman spectroscopy provided an insight about the quality of the graphene flakes. Moreover, the potential for dynamic light scattering (DLS) as an efficient in situ characterisation technique for estimating the lateral dimensions of graphene flakes in dispersions was demonstrated.

LPE was also employed to explore various routes to produce pristine graphene in aqueous media which can be used for toxicity studies. Aqueous dispersions were prepared by a solvent exchange method of graphene originally in organic solvents (NMP and DMF) using dialysis, achieving 0.1 v/v% organic solvent levels. Pristine aqueous graphene dispersions were also prepared by directly exfoliating graphite in biocompatible surfactant (TDOC- Sodium taurodeoxycholate) and biomolecules (Phosphatidylcholine and human serum albumin) solutions. Cell culture studies by collaborators revealed that solvent-exchanged and TDOC-exfoliated pristine FLG displayed minimal toxicity and albumin-exfoliated FLG hardly any cytotoxicity, whereas phosphatidylcholine-exfoliated FLG was cytotoxic.

Raman spectroscopy is a well-established technique used to study the local deformation of carbon-based composites by following the shift rates of the Raman 2D band with strain. Raman active strain coatings were produced from epoxy composites made with the FLG produced by LPE in organic solvents and by electrochemical exfoliation method. The deformation experiments on these coatings revealed little or no strain sensitivity, due to several factors such as length of flakes, processing history, graphene loading, defects in graphene and alignment of flakes within the composites. As an alternative, composite coatings made from chemical vapour deposition (CVD) graphene were investigated. Excellent strain sensitivity was observed upon various cyclic deformational sequences and Raman mapping over 100 × 100 µm area. In comparison to the commercially available wide area strain sensors, CVD graphene composite coatings with a calculated absolute accuracy of ~ ± 0.01 % strain and absolute resolution of ~ 27 microstrains show promise for wide area Raman-based strains sensors.
<table>
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<tr>
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<td>at.%</td>
<td>Atomic percent</td>
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<td>$A$</td>
<td>Absorbance from UV-Vis spectroscopy</td>
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<tr>
<td>$c$</td>
<td>Concentration</td>
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<tr>
<td>$C_G$</td>
<td>Concentration of graphene</td>
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<tr>
<td>$d(H)$</td>
<td>Hydrodynamic diameter</td>
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<tr>
<td>$D$</td>
<td>Translational diffusion coefficient</td>
</tr>
<tr>
<td>$E_{S,G}$</td>
<td>Surface energy graphene</td>
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<tr>
<td>$E_{S,S}$</td>
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<td>$G(\tau)$</td>
<td>Correlation function</td>
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<tr>
<td>Hz</td>
<td>Hertz</td>
</tr>
<tr>
<td>$I$</td>
<td>Raman band intensity</td>
</tr>
<tr>
<td>$I_{D'}$</td>
<td>Intensity of Raman D' band</td>
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<tr>
<td>$I_{2D}$</td>
<td>Intensity of Raman 2D band</td>
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<tr>
<td>$I_D$</td>
<td>Intensity of Raman D band</td>
</tr>
<tr>
<td>$I_G$</td>
<td>Intensity of Raman G band</td>
</tr>
<tr>
<td>$k_B$</td>
<td>Boltzmann’s constant</td>
</tr>
<tr>
<td>$K$</td>
<td>Kelvin</td>
</tr>
<tr>
<td>$l$</td>
<td>Path length of cuvette in UV-Vis measurement</td>
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<tr>
<td>$l_c$</td>
<td>Critical length</td>
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<tr>
<td>$L$</td>
<td>Length of graphene flake</td>
</tr>
<tr>
<td>$L_D$</td>
<td>Inter defect distance</td>
</tr>
<tr>
<td>$\langle L \rangle$</td>
<td>Average length of graphene flake</td>
</tr>
<tr>
<td>$\Delta L/L$</td>
<td>Fractional change in length</td>
</tr>
<tr>
<td>$n$</td>
<td>Refractive index of the dispersant</td>
</tr>
<tr>
<td>$N$</td>
<td>Number of graphene layers in a flake or number of layers/flake</td>
</tr>
<tr>
<td>$\langle N \rangle$</td>
<td>Mean number of layers in a flake</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
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<td>--------</td>
<td>-------------</td>
</tr>
<tr>
<td>$N_1$</td>
<td>Number of monolayer graphene flakes</td>
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<tr>
<td>$N_{1-5}$</td>
<td>Number of graphene flakes containing $&lt; 5$ layers</td>
</tr>
<tr>
<td>$N_T$</td>
<td>Total number of graphene flakes examined</td>
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<td>Pascal</td>
</tr>
<tr>
<td>$q$</td>
<td>Wave vector</td>
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<td>Normalised resistance variation</td>
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<td>Siemens</td>
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<td>$t$</td>
<td>Correlator time delay</td>
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<td>$T$</td>
<td>Absolute temperature</td>
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<tr>
<td>$T_{NS}$</td>
<td>Thickness of graphene nanosheet</td>
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<tr>
<td>$V_{mix}$</td>
<td>Volume of the mixture</td>
</tr>
<tr>
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<td>Volume/volume percent</td>
</tr>
<tr>
<td>vol.%</td>
<td>Volume percent</td>
</tr>
<tr>
<td>w</td>
<td>Width of graphene flake</td>
</tr>
<tr>
<td>w/v%</td>
<td>Weight/volume percent</td>
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<tr>
<td>wt.%</td>
<td>Weight percent</td>
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<tr>
<td>$\langle w \rangle$</td>
<td>Average width of graphene flake</td>
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<tr>
<td>$\varepsilon_m$</td>
<td>Matrix surface strain</td>
</tr>
<tr>
<td>$\phi_G$</td>
<td>Volume fraction of dispersed graphene</td>
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<tr>
<td>$\Delta H_{mix}$</td>
<td>Enthalpy of mixing</td>
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<tr>
<td>$\mu\varepsilon$</td>
<td>Microstrain</td>
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<td>Degree Celcius</td>
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<td>Gruneisen parameter</td>
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<td>$\zeta$</td>
<td>Zeta potential</td>
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<tr>
<td>Symbol</td>
<td>Description</td>
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<td>--------</td>
<td>------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>η</td>
<td>Viscosity</td>
</tr>
<tr>
<td>θ</td>
<td>Scattering angle</td>
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<tr>
<td>λ</td>
<td>Laser wavelength</td>
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<td>µ</td>
<td>Carrier mobility</td>
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<tr>
<td>ν</td>
<td>Poisson ratio</td>
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<tr>
<td>ω</td>
<td>Raman wave number (frequency)</td>
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<tr>
<td>ω_{2D}</td>
<td>Initial position (frequency) of Raman 2D band before straining</td>
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<tr>
<td>ω_G</td>
<td>Initial position (frequency) of Raman G band before straining</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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<td>-------------</td>
</tr>
<tr>
<td>0d</td>
<td>Zero-dimension</td>
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<td>One-dimension</td>
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<td>Two-dimension</td>
</tr>
<tr>
<td>3d</td>
<td>Three-dimension</td>
</tr>
<tr>
<td>AFM</td>
<td>Atomic force microscopy</td>
</tr>
<tr>
<td>aGO</td>
<td>As-produced GO</td>
</tr>
<tr>
<td>b.p.</td>
<td>Boiling point</td>
</tr>
<tr>
<td>BA</td>
<td>Benzyl amine</td>
</tr>
<tr>
<td>BLG</td>
<td>Bilayer graphene</td>
</tr>
<tr>
<td>BSA</td>
<td>Bovine serum albumin</td>
</tr>
<tr>
<td>bwGO</td>
<td>Base-washed GO</td>
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<tr>
<td>CMC</td>
<td>Critical micelle concentrations</td>
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<td>CNC</td>
<td>Cellulose nanocrystals</td>
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<td>CNT</td>
<td>Carbon nanotubes</td>
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<td>CTAB</td>
<td>Cetyltrimethylammonium bromide</td>
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<tr>
<td>CVD</td>
<td>Chemical vapour deposition</td>
</tr>
<tr>
<td>DLS</td>
<td>Dynamic light scattering</td>
</tr>
<tr>
<td>DMA</td>
<td>N,N-dimethylacetamide</td>
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<td>DMF</td>
<td>N,N-dimethylformamide</td>
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<td>DMPC</td>
<td>1,2-dimyristol-sn-glycero-3-phosphocholine</td>
</tr>
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<td>DMPG</td>
<td>1,2-dimyristoyl-sn-glycero-3-phospho-(1-rac-glycerol) sodium salt</td>
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<tr>
<td>DMSO</td>
<td>Dimethyl sulfoxide</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<td>DOPC</td>
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<td>DR</td>
<td>Double resonance</td>
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<td>Egg yolk phosphatidylcholine</td>
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<td>Few-layer graphene</td>
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<td>FRET</td>
<td>Förster resonance energy transfer</td>
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<td>Full width half maximum</td>
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<td>Grain boundaries</td>
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<tr>
<td>GNR</td>
<td>Graphene nanoribbon</td>
</tr>
<tr>
<td>GO</td>
<td>Graphene oxide</td>
</tr>
<tr>
<td>HOPG</td>
<td>Highly oriented pyrolytic graphite</td>
</tr>
<tr>
<td>HRTEM</td>
<td>High resolution transmission electron microscope</td>
</tr>
<tr>
<td>HSA</td>
<td>Human serum albumin</td>
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<tr>
<td>HSP</td>
<td>Hansen solubility parameters</td>
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<tr>
<td>IEP</td>
<td>Isoelectric point</td>
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<td>IL</td>
<td>Ionic liquids</td>
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<td>IPA</td>
<td>Isopropanol</td>
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<tr>
<td>IPSD</td>
<td>Intensity Particle Size Distribution</td>
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<td>IR</td>
<td>Infrared spectroscopy</td>
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<td>ISS</td>
<td>Interfacial shear stress</td>
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<td>LO</td>
<td>Longitudinal optical phonon</td>
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<tr>
<td>LPE</td>
<td>Liquid phase exfoliation/exfoliated</td>
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<tr>
<td>MC</td>
<td>Micromechanical cleavage/cleaved</td>
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<tr>
<td>MLG</td>
<td>Multi-layer graphene</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>MWCO</td>
<td>Molecular weight-cut off</td>
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<tr>
<td>MWNT</td>
<td>Multi-walled carbon nanotubes</td>
</tr>
<tr>
<td>NMP</td>
<td>1-methyl-2-pyrrolidinone</td>
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<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
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<tr>
<td>NP</td>
<td>Nanoparticles</td>
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<td>OD</td>
<td>Oxidative debris</td>
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<tr>
<td>o-DCB</td>
<td><em>ortho</em>-dichlorobenzene</td>
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<tr>
<td>PBS</td>
<td>Phosphate buffer saline</td>
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<tr>
<td>PC</td>
<td>Phosphatidylcholine</td>
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<td>PDI</td>
<td>Poly dispersity index</td>
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<td>PDMS</td>
<td>Polydimethylsiloxane</td>
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<td>Polyethylene glycol</td>
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<tr>
<td>POPC</td>
<td>1-palmitoyl-2-oleoyl-<em>sn</em>-glycero-3-phosphocholine</td>
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<td>PP</td>
<td>Polypropylene</td>
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<tr>
<td>PTFE</td>
<td>Polytetrafluoroethylene</td>
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<td>PVA</td>
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<td>Poly vinyl chloride</td>
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<td>PVP</td>
<td>Polyvinyl pyrrolidone</td>
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<td>SC</td>
<td>Sodium cholate</td>
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<tr>
<td>SDBS</td>
<td>Sodium dodecylbenzenesulfonate</td>
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<td>SDS</td>
<td>Sodium dodecyl sulphate</td>
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<tr>
<td>SEM</td>
<td>Scanning electron microscopy</td>
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<tr>
<td>SERS</td>
<td>Surface enhanced Raman scattering</td>
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<td>SLG</td>
<td>Single-layer graphene</td>
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<tr>
<td>STM</td>
<td>Scanning tunnelling microscope</td>
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List of abbreviations

SU-8 Photoresist
SWNT Single-walled carbon nanotubes
TDOC Sodium taurodeoxycholate
TEM Transmission electron microscope
TLG Tri-layer graphene
TO Transverse optical phonon
TRGO Thermally-reduced graphene oxide
Trp Tryptophan
Tyr Tyrosine
UV-Vis Ultra violet – Visible spectroscopy
vdW van der Walls force
XPS X-ray photoelectron spectroscopy
XRD X-ray diffraction
DECLARATION

No portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other institute of learning.

Any part of the thesis that has already been published in a scientific journal is indicated in the footnote at the start of the relevant chapter.
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PREFACE

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Thesis: Graphene functional composites
Pass, University of Manchester Intellectual Property Company funding

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University of Manchester, UK
Dean’s Award Studentship

Publications from this thesis

(1) A. P. A. Raju, A. Lewis, B. Derby, R. J. Young, I. A. Kinloch, R. Zan, K. S. Novoselov,
Wide-Area Strain Sensors based upon Graphene-Polymer Composite Coatings Probed

(2) A. P. A. Raju, S. C. Offerman, P. Gorgojo, C. Valles, E. V. Bichenkova, H. S. Aojula, A.
Vijayaraghavan, R. J. Young, K. S. Novoselov, I. A. Kinloch, D. J. Clarke, Biologically-
friendly pristine graphene and graphene oxide without lytic activity, Manuscript
submitted.
Contributions to other work


(2) Z. Li, R. J. Young, I. A. Kinloch, N. R. Wilson, A. J. Marsden, A. P. A. Raju, Quantitative determination of the spatial orientation of graphene by polarised Raman spectroscopy, *Carbon*, 88 (2015), 215-224

Conferences

Oral Presentations

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<td>Aug 2013</td>
<td>NanoteC13, University of Surrey, Guildford, UK</td>
<td>- Wide-area Strain Sensors based upon Graphene-Polymer Coatings probed by Raman Spectroscopy</td>
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<td>Sep 2013</td>
<td>CNPComp2013, Leibniz-Institut für Polymerforschung, Dresden, Germany</td>
<td>- Wide-area Strain Sensors based upon Graphene-Polymer Coatings probed by Raman Spectroscopy</td>
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<tr>
<td>Aug 2014</td>
<td>NanoteC14, Hotel Le Plaza, Brussels, Belgium</td>
<td>- Production of pristine few layer graphene in aqueous media for biological studies</td>
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Poster Presentations

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<tr>
<td>Aug 2012</td>
<td>NanoteC12, University of Sussex, Brighton, UK</td>
<td>- Multifunctional Graphene Composites: Reinforcement and Strain Sensor</td>
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Won *Skinner Prize* for Best Poster Presentation
ACKNOWLEDGEMENTS

First and foremost I would like to express my sincere gratitude and appreciation to Prof. Ian Kinloch for the supervision, invaluable counsel and encouragement throughout the course of my PhD. I would also like to thank Prof. Robert Young for all his help and thought-provoking suggestions during the progression of my project.

I would like to extend my thanks to Prof. David Clarke and Dr Elena Bichenkova for their co-operation, support and guidance during my time at the School of Pharmacy, University of Manchester. I would also like to thank Prof Kostya Novoselov from the School of Physics and Astronomy for providing mechanically exfoliated graphene samples.

My heartfelt thanks to all the Material Science Centre staff especially Polly and Andy for their assistance in the labs. I extend my gratitude to Amanda, Cristina, Patricia, Recep, and Amor for their help at several stages during my experiments and the valuable suggestions they provided.

My thanks also go out to my colleagues in the Nanomaterials group – Libo, Lei, Gaoxiang, Bennie, Yanning, George, Alex for their great help, friendship and laughter. Special thanks to Rahul Nair for his continued support, both in my personal and professional life.

I would also like to extend my thanks to the friends I have who over the years have made my student life in Manchester a very special time – Sujji, Venkat, Vas, Viji, Ali, Niki, Mez, Utkarsh, Rajiv, Cris, Ghangesh, Jannanie, Chao, Toto, Ripu, Rani, Baby, Raam, Sashi, Chetty, Kavi, Anju, Meenz, Ash, Kamath, Ramesh, Maddy, Karthik, Alphonse, and anyone else I may have missed unintentionally. Thanks a lot guys.

I would also like to acknowledge and thank the University of Manchester for awarding the Dean’s Award studentship for my PhD, thus providing me with this opportunity.

Last but not the least, I would like to thank my family for their endless love, understanding and constant support.
DEDICATION

To my Family and Friends
Chapter 1 - Introduction
1. **INTRODUCTION**

The famous talk entitled “There is Plenty of Room at the Bottom” by Professor Richard Feynman in 1959 sparked the curiosity in the field of nanotechnology. Nanotechnology is the science, engineering and technology conducted on materials which are 1 – 100 nm in size. The interest in nanomaterials lies in the ability to control a material’s structure on the atomic scale and hence dictate its properties. Over last the 30 years, carbon nanomaterials in particular have been widely studied due to the range of allotropes that can be derived from the three bonds that carbon can make. The well-known bulk allotropes of carbon are: graphite (soft and brittle) purely consisting of sp$^2$ hybridised bonds, diamond (hard and strong) purely consisting of sp$^3$ hybridised bonds, and amorphous carbon (non-crystalline) consisting of both sp$^2$ and sp$^3$ hybridised bonds. Technological improvements in the field of microscopes and other precision instruments have increased the level of detail in which nanomaterials can be studied. These developments in technology enabled the discovery of several other carbon nanomaterials such as fullerenes (0d, *Bucky balls*), carbon nanotubes (1d), and graphene (2d).

Graphene is a 2d allotrope of carbon, composed of a single atomic sheet of sp$^2$ hybridised carbon atoms. It was first isolated in 2004 by Andre Geim and Konstantin Novoselov by the repeated mechanical exfoliation of highly oriented pyrolytic graphite (HOPG). The scientists were awarded the Nobel Prize in Physics in 2010 “for groundbreaking experiments regarding the two-dimensional material graphene”. This paper and the subsequent Nobel Prize sparked a surge of research and launched the graphene field; a topic with regards to which tens of thousands of papers are now published per year.

1.1. **Motivations and Aims**

There are several challenges in realising and exploiting the potential of this material. One of the key issues remains producing graphene on the bulk scale. Liquid phase exfoliation (LPE) of graphite in a solvent media, was relatively new at the commencement of this thesis, but is now one of the most widely used routes for producing pristine graphene in various solvents. The characterisation of graphene in these dispersions, especially its lateral dimensions which is crucial for certain applications
(e.g. composites\textsuperscript{4}), are normally carried out \textit{ex situ} using atomic force microscope (AFM), scanning electron microscope (SEM), and transmission electron microscope (TEM). These techniques, however reliable, can be time consuming and expensive. Hence there is a lack of quick and easy techniques for measuring lateral dimensions of graphene flakes exist.

With the increase in the number of people producing and using graphene every day, the potential toxicity of these materials are a matter of concern, especially so for pristine graphene. Most of the existing toxicity studies\textsuperscript{5} are conducted on Graphene oxide (GO); a derivative of graphene, due to its aqueous media stability. In order to conduct toxicity studies it is required to produce pristine graphene in aqueous media. Moreover, moving the LPE from organic to aqueous media might favour the use of graphene in various applications.

With regards to applications of graphene, one application of interest is using composites made from graphene and epoxy resin to be used as strain sensor ‘paint’ over a wide area. Most of the studies carried out depend on the piezoresistivity of the graphene or its composites for strain sensor applications.\textsuperscript{6} On the other hand, Raman spectroscopy is a well-established technique which measures the energy of the phonons. The energy of phonons depend on the strain on the material, meaning that Raman spectroscopy can be used to study local deformations. This technique is widely used in the field of carbon-based composites to study the deformation patterns and interfacial stress transfer between the matrix and reinforcement.\textsuperscript{4, 7, 8} Moreover, graphene has a well-defined Raman spectrum which is highly strain sensitive (2D band) due to its high stiffness.\textsuperscript{9} These factors allow Raman spectroscopy to be used to measure the strain over wide area by following the 2D band shift of graphene composites.

This thesis aims to address all the above discussed issues and possibilities. Hence the three main research objectives of this thesis are:

1) The production of pristine few-layer graphene (FLG) in different organic solvents and exploration of a quick and simple method that allows the characterisation of lateral dimensions of graphene \textit{in situ} in dispersions.
2) Investigation of various techniques for producing pristine FLG in aqueous media and the use of different biocompatible molecules, to facilitate toxicological examinations.

3) Exploring the use of graphene-based polymer-composites coatings, probed by Raman spectroscopy to have the potential to be an effective, precise and non-contact technique for wide-area strain measurements.

1.2. Thesis Outline

This thesis is organised as follows: Chapter 2 reviews the literature concerning the properties and production techniques of graphene. Chapter 3 gives the current state of the art on the Raman spectroscopy of graphene and graphene-based strain sensors. Chapter 4 describes the experimental methodology used in this thesis, including the working principles of some of the techniques. The results are then split across the next three chapters, with Chapter 5 discussing the production of pristine FLG in organic solvents and the use of dynamic light scattering (DLS) in estimating the lateral dimensions of graphene. Chapter 6 develops new methodologies for the production of “pristine” FLG in aqueous media using biocompatible molecules. These materials were used by collaborators in toxicity studies, with these results presented in Appendix 9.6. Chapter 7 investigates the use of graphene-based composite coatings as Raman-based wide-area strain sensors. Finally, Chapter 8 concludes the thesis and suggests directions for future work.
Chapter 2 – Graphene: Properties and Production
2. GRAPHENE: PROPERTIES AND PRODUCTION

Peierls\textsuperscript{10} and Landau \textit{et al.}\textsuperscript{11} argued more than 75 years ago that strictly 2d crystals were theoretically impossible since the thermal fluctuations would destroy the long range crystalline order. Subsequent experimental observations showed that as the thickness of films decreased (to dozens of atomic layers), the melting point of the films also decreased, making them unstable due to island segregation or decomposition.\textsuperscript{12} Thus whilst atomic monolayers have always been known to be an integral part of larger 3d structures, it was believed that without such a 3d base, 2d materials could not exist. This assumption was dispelled with the isolation of 2d graphene in 2004\textsuperscript{1} and other materials\textsuperscript{13} by Prof. Andre Geim and Prof. Kostya Novoselov at The University of Manchester, UK.\textsuperscript{14} However, these new 2d materials were found not to violate Peierls’ calculations by either being a coating on a bulk material or forming a wave when freely suspended.\textsuperscript{15, 16}

2.1. STRUCTURE AND MORPHOLOGY OF GRAPHENE

Graphene is an allotrope of carbon and is a 2d atomic crystal which consists of carbon atoms arranged in a hexagonal lattice structure. It is considered as a basic building block of other carbon based allotropes; it can be wrapped around to form 0d fullerenes, rolled to form 1d carbon nanotubes (CNTs) and stacked to form 3d graphite (Figure 2.1).

![Graphene Diagram](image)

\textit{Figure 2.1: Graphene is a 2d building material for other dimension carbon nanomaterials. It can be wrapped up into 0d fullerenes, rolled into 1d carbon nanotubes or stacked into 3d graphite.}\textsuperscript{14}
The carbon-carbon (sp² hybridised) bond length in graphene is approximately 0.142 nm with an interplanar spacing of 0.335 nm when sheets are stacked upon each other. The unit cell of monolayer graphene (also known as single-layer graphene (SLG)) has two atoms in it, whereas there are four atoms in bilayer’s unit cell. The carbon atoms in graphene are bonded with in-plane, σ bonds (3 bonds/atom), which are extremely strong and form the rigid backbone of the hexagonal lattice (Figure 2.2a). The out-of-plane partially filled pz orbital, π bonds, perpendicular to lattice, are responsible for the electrical conduction and the interactions between graphene layers or substrates. The known stacking arrangements (Figure 2.2b,c and d) of bi- (two atomic layers) and few- (three to ten atomic layers) layer graphene are: hexagonal (AA stacking), Bernal (AB stacking) and rhombohedral (ABC stacking). Among these, the lowest energy and the most abundant form (80 %) in single crystal graphite is Bernal stacking.

Despite showing long-range crystalline order, transmission electron microscopy (TEM) studies on suspended SLG sheets shown that they were not perfectly flat due to the previously discussed Peierls’ instability. They exhibit intrinsic static microscopic roughening or ‘ripples’ appear randomly on suspended SLG sheets with 8-10 nm wavelength and 0.7-1 nm amplitude with these waves also being predicted by Monte Carlo simulations. Scanning tunnelling microscopy (STM) studies revealed smaller intrinsic ripples (< 0.5 nm height) in SLG on SiO₂ had little influence on its electronic properties, whereas larger ripples (> 3 nm height) had little influence on its electronic properties. Apart from intrinsic ripples, graphene can have other disorders or defects such as
as: topological defects (e.g., pentagons, heptagons and their combinations), adatoms, vacancies, extended defects like edge and cracks, adsorbed impurities or charges on top of graphene.  

### 2.2. PROPERTIES OF GRAPHENE

#### 2.2.1. Electronic properties

The initial enthusiasm in graphene research was mainly because of its 2d nature and symmetry meaning that its charge carriers behave as massless, relativistic particles. Graphene is described as a zero bandgap semiconductor as its valance and conduction band meet at the corners of Brillouin zone. It exhibits ambipolar electric field effect with tuneable concentrations between holes and electrons as high as $10^{13}$ cm$^{-2}$, with room temperature charge carrier mobilities ($\mu$) up to $\sim 15000$ cm$^2$/Vs. Upon minimizing the extrinsic scattering in suspended SLG, ballistic transport, $\mu$ in excess of $2 \times 10^5$ cm$^2$/Vs at an electron densities of $\sim 2 \times 10^{11}$ cm$^{-2}$ was observed. Recently record high values of $\mu$ were reported: $2.5 \times 10^5$ cm$^2$/Vs (room temperature, $\mu$m scale mean free path) for graphene sandwiched between two hexagonal boron-nitride crystals and $\sim 6 \times 10^6$ cm$^2$/Vs (4 K) for epitaxially grown graphene on silicon carbide. Such unusual electronic properties (ambipolar electric field and ballistic transport over sub-micron distances at room temperature) make graphene as a promising candidate of future electronic devices. In particular, there is increasing interest in high frequency applications for the communications sector due to graphene’s zero band gap limiting its use in traditional field-effect transistors (FET).

#### 2.2.2. Mechanical properties

The mechanical properties of graphene were first reported in 2007 by Frank et al. who measured the spring constant of few-layer graphene (FLG) (<5 layers) by the force displacement of an atomic force microscope (AFM) tip. The Young’s modulus of graphene was calculated to be 0.5 TPa with the spring constant ranging from 1-5 N/m.

Lee et al. carried out a similar AFM nanoindentation technique on a SLG sheet suspended over 1 – 1.5 $\mu$m diameter hole (Figure 2.3a) in a silicon substrate. The graphene sheet was indented by the AFM tip until it ruptured (Figure 2.3b,c) and the
Young’s modulus then is determined from the force-depth curve. The estimated Young’s modulus and strength of monolayer graphene was ~1 TPa and ~130 GPa respectively.\textsuperscript{29} Such impressive properties can be attributed to the robustness of in-plane covalent $sp^2$ bond which makes it the strongest material known.\textsuperscript{4, 29} Graphene is also shown to elastically stretch up to 20\%.\textsuperscript{29}

![Figure 2.3: a) SEM image of a graphene monolayer covering an array of circular holes (1-1.5 µm), scale bar – 3 µm. b) Schematic representation of the nanoindentation on a suspended monolayer graphene. c) AFM image of the fractured graphene sheet.\textsuperscript{29}](image)

Using density functional theory, Liu et al. have estimated the Young’s modulus and strength of SLG to be ~1.05 TPa and 107-121 GPa, respectively, which are in excellent agreement to the experimentally calculated values.\textsuperscript{30} Similar values for Young’s modulus of graphene was obtained from other methods such as resonators\textsuperscript{31} and Raman spectroscopy.\textsuperscript{4, 32, 33}

Moreover graphene is impermeable to all the gases including helium.\textsuperscript{34} Change in resistivity values, mainly because of the change in Fermi levels, were observed when individual gas molecules adsorb on graphene surface making it a highly sensitive gas detector.\textsuperscript{35}

\section*{2.2.3. Other properties}

Graphene has outstanding thermal properties. The intrinsic thermal conductivity of SLG was measured between 3000-5000 W/(mK),\textsuperscript{36} and decreases as the number of layers increases (1300 W/(mK) for 4 layers) approaching value for bulk graphite.\textsuperscript{37} High thermal conductivity leads to efficiently dissipate heat, making it an excellent choice for thermal management materials.\textsuperscript{38}

SLG absorbs only 2.3 \% of the light with negligible reflectance (< 0.1 \%).\textsuperscript{39} Such impressive combinations of mechanical (strength, flexibility and elasticity), optical and
electronic properties make graphene a perfect material for flexible electronics and touch screen devices.\textsuperscript{40}

Additionally, the theoretically predicted surface area of SLG is around 2600 m\textsuperscript{2}/g, with experimentally derived values for FLG in the range of 270-1550 m\textsuperscript{2}/g.\textsuperscript{41} Such high surface area can be beneficial in several areas including nanocomposites by surface modification, thereby, enhancing the matrix-graphene interfacial interaction.\textsuperscript{4, 42}

\section{2.3. Production of Graphene}

Even before realising the exciting properties that graphene has to offer, several studies had been carried out to with the aim of producing a very thin graphite film or graphene.\textsuperscript{43-46} This research led to the work in 2004 in which Prof. Andre Geim and Prof. Kostya Novoselov successfully isolated monolayer graphene by the micromechanical cleavage of highly oriented pyrolytic graphite (HOPG).\textsuperscript{1} In wider sense, there are two different approaches for producing graphene:

- “Top-down” - Breaking down of graphite into graphene using external forces such as mechanical, electrical etc. (Sections 2.3.1 to 2.3.4)
- “Bottom-up” – Building up graphene from molecular building blocks or from carbon sources. (Sections 2.3.5)

Based on these two approaches, different methods for producing graphene are discussed in the following sections.

\subsection{2.3.1. Mechanically-cleaved graphene}

In this top-down approach, exfoliation of bulk graphite to graphene takes place by peeling the layers upon exerting external forces overcoming the van der Waals (vdW) attraction between the layers. The most commonly known method is the micromechanical cleavage (MC) or “Scotch tape” method.\textsuperscript{1} In which, a piece of bulk graphite (e.g., HOPG) is repeatedly peeled in between the Scotch\textsuperscript{TM} tape, thus overcoming the vdW attraction between the flakes, thereby reducing its thickness each time to achieve optically transparent flakes upon transferring it to a substrate (Figure 2.4a). Optical microscopy\textsuperscript{47} and Raman spectroscopy\textsuperscript{48} are widely used to identify different layers (Figure 2.4b,c). Upon optimisation of MC process, mm-sized SLG flakes
were obtained (Figure 2.4d). Till-date, this method produces the large-area high-quality flakes that are preferred for fundamental research. However the method is not practical for bulk manufacturing as it is very labour intensive with a low-throughput.

Jayasena et al. have demonstrated that FLG can be obtained by cleaving the HOPG using an ultrasonic oscillator-aided ultrasharp single crystal diamond wedge. Such diamond wedges/knives can produce flakes with several hundred \( \mu \text{m} \) lateral dimension and few tens of nm thickness.

Using three-roll mill, a process inspired from “Scotch tape” method, Chen et al. produced SLG and FLG by continuous mechanical exfoliation of graphite using poly vinyl chloride (PVC). The PVC dissolved in dioctyl phthalate (DOP) acts as an adhesive peeling of the graphene layers from graphite during the continuous three-roll milling process. However, harsh conditions (up to 500 °C) were used to remove the residual PVC and DOP, thereby increasing the complexity of the process.

2.3.2. Liquid-phase exfoliation of graphene

Among the top-down approach, ultrasonication-assisted liquid-phase exfoliation (LPE) is the most common method of producing graphene from bulk graphite (Figure 2.5 and Figure 2.6a,b). Exfoliation can be achieved either using suitable solvents or surfactant/stabilizer molecules in aqueous media. Typical LPE process involve: (i)
dispersion of graphite in solvent, (ii) exfoliation and (iii) “purification” (Removing unexfoliated flakes, usually centrifugation). The general exfoliation principle using ultrasonication is explained in section 4.1.2.1.

Figure 2.5: Schematic representation of the LPE process of graphite into graphene using organic solvents and surfactant molecules

The LPE yield can be estimated by measuring the concentration of graphene ($C_G$) in the dispersion by using UV-Vis spectroscopy and exploiting the Beer-Lambert law (see section 4.1.2). The degree of exfoliation can be determined by analysing the number of layers in a flake ($N$) using TEM, AFM and/or Raman spectroscopy. In TEM, $N$ can be obtained by electron diffraction pattern (Figure 2.6g,h) or counting the edges of the flake (Figure 2.6f). Whereas in AFM, $N$ can be calculated by dividing the graphite interlayer distance to the thickness of the deposited flake (Figure 2.6j,k) on a specific substrate, as the thickness of SLG is shown to be substrate dependent. Raman spectroscopy is generally used to determine the degree of exfoliation as it is sensitive to change in number of layers (Figure 2.4c and Figure 2.6l). However, advancements in understanding the Raman spectroscopy of graphene on the effect of doping, edges, defects, and oxidation had resulted in gaining information on the quality of the exfoliated flakes in addition to other techniques such as high-resolution TEM (HRTEM) and X-ray photoelectron spectroscopy (XPS) (Figure 2.6). Since LPE methods are often
performed in room temperature and do not involve any strong oxidants, large number of studies\textsuperscript{59-63} have classified the resultant graphene sheets as pristine graphene with regards to the basal plane and the defects are mainly attributed in the edges or edge effects.\textsuperscript{53,64}

2.3.2.1. Solvent-based LPE

The main prerequisite of successful exfoliation is to overcome the vdW attraction of the adjacent graphene layers. One way of achieving this is by liquid immersion. Upon liquid immersion, interfacial tension between the solid and liquid plays a major role.\textsuperscript{65} Higher the interfacial tensions between solid and liquid, the poorer the dispersibility of solid in the liquid.\textsuperscript{65} In the case of graphite, a high interfacial tension between liquid causes the flakes to adhere with each other as the cohesion between the flakes (\textit{i.e.}, the energy per unit area required to separate two flat surfaces from contact\textsuperscript{65}) are high, resulting in hindering its dispersion in liquid.\textsuperscript{54} Hence, solvents with surface tension (\textit{i.e.}, the property of surface of a liquid that allows it to resist an external force, due to the cohesive nature of its molecules\textsuperscript{65}) can reduce the interfacial tension between graphene and solvent, thereby enhancing graphene’s dispersion.\textsuperscript{47} However, not all the solvents can efficiently disperse graphene. This can be explained from a thermodynamic perspective in terms of enthalpy of mixing as explained by Coleman \textit{et al.}\textsuperscript{3,54} The enthalpy of mixing of graphene dispersed and exfoliated in a solvent is given as,

$$\frac{\Delta H_{mix}}{V_{mix}} \approx \frac{2}{T_{NS}} \left( \sqrt{E_{S,S}} - \sqrt{E_{S,G}} \right)^2 \phi_G$$

\textbf{Equation 2.1}

Where, $\Delta H_{mix}$ is the enthalpy of mixing, $V_{mix}$ is the volume of the mixture, $T_{NS}$ is the thickness of graphene nanosheet, $E_{S,S}$ and $E_{S,G}$ are surface energy of solvent and graphene, respectively, and $\phi_G$ is the volume fraction of dispersed graphene. It is clear from Equation 2.1, that the $\Delta H_{mix}$ will have a lower value when $E_{S,S}$ and $E_{S,G}$ are closer to each other. This implies that the suitable solvents are those which have similar surface energy as of graphene. In those solvents, exfoliation can readily occur due to the minimum energy cost, which could be induced by sonication.\textsuperscript{64}

By measuring various solvents with different surface energy (surface tensions ($\gamma$)) and the concentration of the dispersed graphene after centrifugation (Figure 2.6c), it has
been shown that the best solvents for dispersing graphene have surface energy of 70-80 mJ/m² (corresponding surface tension of 40-50 mJ/m²). Contact angle measurements estimate the surface energy of graphite to be ~55 mJ/m². Some solvents (not limited to) like benzyl benzoate, 1-methyl-2-pyrrolidinone (NMP), N,N-dimethylformamide (DMF) and ortho-dichlorobenzene (o-DCB) with γ values of 45.95 mJ/m², 40.1 mJ/m², 37.1 mJ/m², and 36.6 mJ/m², respectively, are generally classified as best solvents for exfoliating graphene. Whereas, other solvents like water, ethanol and chloroform with γ values of 72.8 mJ/m², 22.1 mJ/m², and 27.5 mJ/m², respectively, are poor solvents for direct exfoliation of graphene.

Figure 2.6: a) SEM image of starting graphite (scale bar 500 µm). b) Graphene dispersions (A – 6 µg/ml) obtained by sonication and centrifugation in NMP. c) Graphene concentration measured after centrifugation for various solvents plotted versus solvent surface tension and surface energy. d,e) Bright field TEM image of monolayer and multilayer, respectively. f, g) HRTEM images of folded edge of single layer. g,h) Electron diffraction pattern of monolayer and multilayer, respectively. i) Histogram of number of layers per flake from TEM measurements. j) AFM image of graphene produced by LPE in NMP and (k) the corresponding height profile. l) Raman spectra of bulk graphite and vacuum filtered graphene films from LPE process. m) C1s XPS spectrum of a thin film of graphene flakes from LPE process. Contributions from residual solvents are highlighted using arrow marks.
Hernandez et al. have also used more sophisticated parameters like Hansen solubility parameters (HSPs), which consider dispersive, polar and hydrogen-bonding interactions, to explore number of suitable solvents for direct exfoliation of graphene.  

2.3.2.1.1. LPE in organic solvents

Blake et al. reported the first successful LPE of graphite to graphene in DMF by sonicating for 3 hr followed by centrifugation at 13000 rpm resulting in stable dispersion containing mostly SLG (~50 %) and FLG as characterised by AFM and TEM. Whereas, the first detailed investigation on the LPE of graphene in organic solvents like NMP was reported by Hernandez et al.\textsuperscript{54} $C_G$ of 0.01 mg/ml were obtained with the conditions: 30 min sonication, centrifugation - 500 rpm for 90 min. Among the analysed flakes, by combining the electron diffraction pattern and careful analysis of edges to analyse number of layers in a flake, they have identified ~25 % SLG and vast majority were < 5 layers (Figure 2.6).\textsuperscript{54} The relatively defect-free nature of the graphene flakes was confirmed by the absence of D band\textsuperscript{48} in Raman spectra on the exfoliated flakes (larger than laser spot) and the dominant C – C peak of XPS (except residual solvent peaks) as seen in Figure 2.6l,m. Such concentrations are very low for many applications; hence upon increasing the sonication time drastically to 460 hr $C_G$ of 1.2 mg/ml was achieved by Khan et al.\textsuperscript{59} However such approach involving long sonication times and high energy may not be a practically preferred method of producing high $C_G$. It was found that graphene films produced from vacuum filtration of graphene dispersions don’t reaggregate into graphite\textsuperscript{59} and it exhibits Raman spectra similar to FLG\textsuperscript{48} suggesting only weak interactions exist between the flakes. Khan et al. utilised such graphene films to redisperse in fresh solvents (NMP) with aid of sonication to achieve very high concentrations of $C_G$ ~63 mg/ml.\textsuperscript{69} The dispersed graphene flakes were on average ~1 µm long and 3-4 layers thick. Despite the observed sedimentation occurred over time, $C_G$ ~25 mg/ml appeared to be indefinitely stable.\textsuperscript{69}  

It has to be noted that direct LPE in organic solvents like NMP normally produces graphene flakes with lateral dimensions typically < 1 µm, which are too small for applications such as mechanical reinforcement in composites (see section 3.2.6 for Shear lag analysis). Khan et al. have used controlled centrifugation for separating graphene flakes based on their sizes.\textsuperscript{62} Firstly, they separated the smaller sized flakes from larger
flakes by centrifuging at higher speeds. The sediment (which containing larger flakes) from the earlier step was redispersed in fresh solvent followed by subsequent centrifugation at lower speeds. These steps were repeated such that graphene flakes were separated based on their sizes as determined by TEM and Raman: ~3.5 µm for 500 rpm to ~1 µm for 4000 rpm. It is worth noting that the increase in thickness of graphene with decrease of centrifugation speed was observed. Such size-selection technique was even followed to establish the use of Dynamic Light Scattering (DLS) technique in measuring the lateral dimensions of 2d materials in liquid-phase.

As mentioned earlier, due to the matching surface tensions, these organic solvents produce stable dispersions of graphene by LPE. However detailed solvent molecule-graphene interactions are not well explored. Liu et al. investigated the origin of surface charge of unfunctionalised graphene in organic solvents by electrophoresis and zeta potential (ζ) measurements. They have proposed that the charge transfer between the graphene and solvent molecules are responsible for the graphene’s surface charge. It was found that depending on the different donor and acceptor numbers of a solvent, graphene can be positive or negatively charged in the dispersion exhibiting different ζ values. The electrostatic repulsion of charged graphene sheets facilitates stable dispersions. Such observations resulted in finding some unreported solvents such as tetramethylurea and benzoyl chloride for direct LPE of graphene.

![Figure 2.7: a) Zeta potential values of graphene dispersed in different organic solvents. b) Zeta potential of graphene dispersion vs. donor and acceptor numbers of the respective solvents.](image)

**2.3.2.1.2. LPE in aromatic organic solvents**

Hamilton et al. used o-DCB – a solvent with γ values of 36.6 mJ/m² and ability to interact with graphene via aromatic π – π interactions, for LPE of graphite producing
stable graphene dispersions using 30 min cup-horn sonication and centrifugation at 4400 rpm for 30 min.\textsuperscript{72} They found graphene produced from microcrystalline graphite (0.03 mg/ml) resulted in higher $C_G$ than thermally expanded graphite and HOPG (0.02 mg/ml). TEM and AFM characterisation revealed graphene sheets were 100-500 nm lateral dimensions and < 5 layers. They have also observed improved conductivity of the graphene film (1500 S/m) after removing the residual o-DCB by annealing at 400 °C for 12 hr.\textsuperscript{72}

Bourlinos \textit{et al.} have used perfluorinated aromatic solvents such as hexafluorobenzene, octafluorotoluene, pentafluorobenzonitrile and pentafluoropyridine, which are electron-deficient molecules, to LPE of graphite to produce graphene.\textsuperscript{73} Relatively short sonication (1 hr) and natural sedimentation resulted in stable dispersions with varying concentrations in the order: pentafluorobenzonitrile (0.1 mg/ml, 2 % yield) > hexafluorobenzene (0.08 mg/ml, 1.5 % yield) > pentafluoropyridine $\approx$ octafluorotoluene (0.05 mg/ml, 1 % yield). The non-oxidation state of graphene sheets were confirmed by Raman and Infrared (IR) spectroscopy. The average thickness of graphene determined by AFM is 0.5-1 nm. The mechanism of stabilisation was ascribed to both the charge transfer between electron rich graphene sheets to electron-deficient solvent molecules through $\pi - \pi$ stacking and the matching surface energies between solvent and graphene. They also reported stable graphene dispersions were produced by sonication (1 hr) in other aromatic organic solvents like pyridine ($C_G$ - 0.3 mg/ml), 2-cyanopyridine and benzyl amine (BA) and non-aromatic solvents like ethyl acetate, vinyl acetate, methyl chloroacetate, 2-methoxyethyl ether, acetylacetone, and $N,N,N',N'$-tetramethylenediamine with $C_G$ ranging betwen 0.2 to 0.3 mg/ml.\textsuperscript{73}

\textbf{2.3.2.1.3. LPE in low boiling solvents}

Due to the high boiling point (b.p.) of the organic solvents like NMP, DMF etc., complete removal of the solvents is often difficult. Hence researchers explored low bp solvents\textsuperscript{61, 67, 74} for LPE production of graphene. Based on the HSPs, Hernandez \textit{et al.} identified range of solvents, including low bp solvents like isopropanol (IPA), acetone and chloroform, suitable for LPE of graphene.\textsuperscript{67} O’Neill \textit{et al.} have studied those solvents in detail and achieved $C_G$ - 0.4 mg/ml and 0.5 mg/ml after 300 hrs sonication for chloroform and IPA, respectively, approximately half the $C_G$ achieved in high bp NMP.\textsuperscript{61} The flakes
were relatively defect free as characterised by Raman spectroscopy with lateral dimensions $\sim 1 \mu m$ and < 10 layers. In another study, Choi et al. have used more volatile solvent like 1-propanol for LPE achieving low $C_G$ - 25 $\mu g/ml$ (bath sonication 20 min, centrifugation 4500 rpm for 10 min), but better than other reported low bp solvents. Due to their high volatility, the room temperature dried graphene films were almost completely free of any residual solvents as confirmed by XPS measurements.

2.3.2.1.4. Sonication induced defects

Coleman group have shown that in general, sonication of graphite to produce graphene is a non-destructive process and it doesn’t introduce any basal plane defects in the graphene lattice. They have assigned the observed D band in the Raman spectra of thin films, prepared from vacuum filtration of sonication-assisted LPE graphene, are mainly due from edge effects. They suggested if this assumption was true, then the average $I_D/I_G$ ratio should scale to the flake edge to area ratio: $\langle I_D/I_G \rangle \propto [\langle L \rangle^{-1} + \langle w \rangle^{-1}]$, where $\langle L \rangle$ and $\langle w \rangle$ are average length and width of the graphene flakes, respectively. This prediction was well agreed by their results, indicating the observed D band was from edge effects and graphene were free from basal plane defects.

However, some studies have shown ultrasonication (cavitation) does introduce defects in the basal plane of graphene. Skaltsas et al. have carried out XPS analysis on NMP- and o-DCB exfoliated graphene produced at different tip sonication power and times, revealing high O content present as carboxylic acid and ether/epoxy functional groups on the graphene lattice as a result of sonication. Recently, Bracamonte et al. have studied effect of sonication time on defect localisation. They have shown that defects observed for short sonication times were mainly from edges, whereas for longer sonication times ($> 2$ hrs) the defects were of basal plane defects. Besides, they also suggested the observed basal plane defects are not sp$^3$-like or vacancies or substitutional impurities, but topological defects (like pentagon-heptagon pairs).

Since sonication-based LPE is one of the widely studied techniques for producing graphene, such different studies signify the importance of defects that could be induced by sonication, and it remains an interesting area of study.
2.3.2.2. Surfactant-based LPE

The use of surfactant molecules, like small organic molecules or polymers, can effectively exfoliate graphite to graphene both in aqueous and in organic solvents. Such surfactants have a high affinity towards the hydrophobic graphene surface and stabilise the graphene sheets against aggregation by means of electrostatic repulsion or steric stabilisation.

2.3.2.2.1. LPE in aqueous media

The use of water is ideal especially for biological studies due to its non-toxic nature. However due to graphene’s hydrophobic nature, LPE of graphite in water is challenging. This can be overcome by use of surfactants to stabilise the graphene in aqueous media. Lotya et al. have shown successful exfoliation of graphite in aqueous ionic surfactants like sodium dodecylbenzenesulfonate (SDBS)\textsuperscript{77} and sodium cholate (SC).\textsuperscript{60} The $C_G$ obtained for SDBS-based dispersion are 0.002-0.05 mg/ml depending on starting graphite concentration (sonication 30 min, centrifugation 500 rpm for 90 min) and by increasing the sonication time to 400 hrs $C_G = 0.3$ mg/ml was achieved for SC-based dispersion. Unlike for NMP exfoliated graphene, the flake sizes of graphene doesn’t reduce significantly on longer sonication time.\textsuperscript{59} Although the monolayer fraction obtained is lower than the solvent exfoliated graphene, the flakes were found to be relatively defect and oxide free.\textsuperscript{60, 77} Hersam et al. have also used SC to exfoliate graphene in water using intense tip sonication with $C_G = 0.09$ mg/ml and the SC-coated graphene sheets were separated based on thickness by density gradient ultracentrifugation.\textsuperscript{78} Up to ~80% monolayer can be separated using this process, and it was shown to improve the electrical and optical performance of transparent conductive films prepared from these flakes. Sun et al. optimised the sonication parameters (including starting surfactant and graphite concentrations, sonication time etc.) and demonstrated by using sodium taurodeoxycholate (TDOC) high $C_G = 7.1$ mg/ml with 8 % monolayer and 82 % < 5 layers (TEM) can be achieved.\textsuperscript{79}

In such ionic surfactant systems, the stability against aggregation is mainly due to the electrostatic repulsion between the surfactant-coated graphene sheets, and can be characterised by the $\zeta$ values. Smith et al. studied wide range of surfactants and found
the $\zeta$ values ranged between ± 25-65 mV.\textsuperscript{80} Interestingly, they found the $C_G$ scaled with square of the $\zeta$ values, meaning $C_G$ is proportional to the magnitude of the electrostatic potential barrier, which stabilises the surfactant-coated graphene against aggregation.\textsuperscript{3, 80} Recent studies have shown that addition of alcohols like ethanol to the aqueous surfactant (sodium dodecyl sulfate-SDS) solutions can enhance the $C_G$ up to 3-10 times.\textsuperscript{81, 82} Addition of alcohol was shown to reduce the surface tension of the solvent system\textsuperscript{82} and also decrease the mixing enthalpy with improved stability of surfactants\textsuperscript{81} thereby enhancing the final $C_G$.

Biodegradable organic molecules like urea\textsuperscript{83} and cellulose nanocrystals (CNC)\textsuperscript{84} were also used as surfactants in producing graphene. High quality FLG with $C_G = 0.15$ mg/ml was obtained for urea-assisted exfoliation which is higher yield than exfoliation in DMF under similar experimental conditions.\textsuperscript{83} It was proposed that the two primary amine groups of urea are responsible for the exfoliation and stability mechanism: an $\text{–NH}_2$ group of the urea readily adsors on the newly created graphene surface during sonication while the other $\text{–NH}_2$ group undergoes reaction with CO$_2$ from air producing $\text{–NHCOOH}$, which ionises in water thereby stabilising the graphene sheets upon electrostatic repulsion.\textsuperscript{83} CNC-assisted LPE of graphene produced $C_G > 1$ mg/ml with 75% of measured flakes are SLG (AFM analysis) and the stability was due to the electrostatic repulsion from adsorbed sulfate groups.\textsuperscript{84} Due to their higher sulfate content, the $C_G$ obtained by CNC-assisted exfoliation was $> 3$ times than SDS-assisted exfoliation under same experimental conditions.\textsuperscript{84}

Aromatic organic molecules like pyrene and its derivatives have been explored for exfoliating graphene in aqueous media. The adsorption of such molecules on graphene surface happens via $\pi - \pi$ interactions by sharing their electrons of the $\pi$- orbitals through a non-covalent bond. Zhang \textit{et al.} have produced FLG in aqueous solutions using 1,3,6,8-pyrenetetrasulfonic acid tertasodium salt (Py-(SO$_3$)$_3$)$_3$ and 1-pyrenemethylamine hydrochloride (Py-NH$_2$) to fabricate transparent conductive films.\textsuperscript{85} Parviz \textit{et al.} have explored use of several pyrene derivatives in exfoliating graphene in water. Among all the derivatives tested, 1-pyrenesulfonic acid sodium salt (Py-SO$_3$) was found to be more effective in exfoliation with $C_G$ of 0.8-1 mg/ml and the HRTEM results reveal 2-4 layers.\textsuperscript{86} The presence of sulfonyl group had resulted in good stability over wide range of pH values.
and was evidenced from the $\zeta$ values. Such pyrene derivatives were shown to outperform other organic surfactant-assisted and polymer-assisted exfoliation under similar conditions.\textsuperscript{86} Yang \textit{et al.} have carried out similar studies on different pyrene derivatives (sonication 80 min, centrifugation 1000 rpm for 20 min, pyrene derivatives were removed under several washing steps) in water and found that Py-SO$_3$ had better exfoliation efficiency than Py-(SO$_3$)$_4$.\textsuperscript{87} Following their work, and on detailed systematic comparative study on pyrene derivatives (with different sulfonic groups) in water, Schlierf \textit{et al.} have revealed 6,8-dihydroxy-1,3-pyrenedisulfonic acid disodium salt (Py-(OH)$_2$(SO$_3$)$_2$) has better exfoliation efficiency than others.\textsuperscript{88} From the experimental and modelling results, they have suggested that the molecular dipole of Py-(OH)$_2$(SO$_3$)$_2$ was not important \textit{per se}, however the asymmetrical polarity facilitate the adsorption of molecule on graphene by promoting the lateral displacement of water molecules confined between the aromatic regions of pyrene and graphene. They have also shown that the mechanism of stabilisation was mainly due to electrostatic repulsion of the adsorbed molecules, and the stability can be altered by altering the pH or by adding salts.\textsuperscript{88}

Zhang \textit{et al.} designed and synthesised two naphthalene diimide (NDI)-based surfactants: N,N'-bis[2-(ethanoic acid sodium)]-1,4,5,8-naphthalene diimide and N,N'-bis[2-(ethanesulfonic acid sodium)]-1,4,5,8-naphthalene diimide containing ionic –COONa and –SO$_3$Na groups, respectively, covalently bonded to NDI unit through ethylene spacers.\textsuperscript{89} The aromatic NDI unit (electron deficient) adsorbs on the graphene surface \textit{via} $\pi-\pi$ interaction, hydrophobic force, and Coulomb attraction with the hydrophobic electron-rich graphene surface. The ionic group helps in dispersing the graphene in water. Such specifically designed surfactants resulted in high quality FLG with $C_G$ of 1.2 – 5 mg/ml, which are much higher, compared to other surfactants.\textsuperscript{89}

In addition, successful LPE of graphite to graphene in weakly basic solutions containing sodium hydroxide (NaOH, pH=11) was reported.\textsuperscript{90} Despite the low yield (0.4%), TEM analysis revealed 200 nm to 2 $\mu$m flakes and the multi-layer graphene flakes (MLG) (< 10 nm) was observed from AFM analysis. The maximum concentration was obtained when the pH was 10 to 11 indicating the –OH charge build up on exfoliated graphene sheets and the stability was due to electrostatic repulsion ($\zeta$ ~ -47 mV). Upon investigating different types of graphite, it was suggested that the presence of small
amount of thermally liable functional groups (–OH and –COOH groups) in starting graphite could contribute to graphene’s stability under weak basic conditions.\textsuperscript{90}

2.3.2.2.2.  LPE in organic solvents

Surfactants were also shown to improve exfoliation efficiency of graphite to graphene in organic solvents. Vadukumpully \textit{et al.} have used cationic surfactant like cetyltrimethylammonium bromide (CTAB) as a surfactant for direct LPE of graphite to graphene in DMF.\textsuperscript{91} The AFM analysis shows FLG with average thickness of \(\sim 1.18\) nm. The stability of graphene sheets was expected from the repulsion between the adsorbed long alkyl chains of CTAB molecules.\textsuperscript{91} Common organic salts like edetate disodium, sodium tartrate and potassium sodium tartrate have shown to be excellent surfactants for direct exfoliation of graphene in organic solvents including NMP, DMF and dimethyl sulfoxide (DMSO).\textsuperscript{92} In presence of such surfactants, significant enhancement in \(C_G\) (in some cases > 100 times) was observed compared to the pure solvent under similar experimental conditions (sonication 2 hr). The obtained \(C_G \sim 1\) mg/ml had FLG, determined by AFM, Raman, TEM, and FTIR were defect- and oxide-free.\textsuperscript{92}

Ciesielski \textit{et al.} have used simple aliphatic compounds like 1-phenyloctane and arachidic acid to enhance the exfoliation efficiency in NMP.\textsuperscript{93} The adsorption energies of these molecules are higher than NMP and it is miscible with organic solvents. In presence of these compounds in NMP, bath sonication of 6 hrs showed 25-50 \% enhancement of \(C_G\) compared to pure NMP. Raman analysis revealed that these compounds did not affect the quality and the structure of graphene.\textsuperscript{93}

2.3.2.2.3.  Polymer-assisted LPE

Polymers were also used as surfactants for exfoliating graphite to graphene both in organic solvents and in water. Smith \textit{et al.} have shown that exfoliation of graphite to graphene using non-ionic surfactants including IGEPAL CO-890, Triton X-100, Tween 20 and Tween 80 in water using tip sonication for 30 min.\textsuperscript{80} They found that the \(C_G\) scaled linearly with the magnitude of the steric potential barrier which stabilises the flakes against aggregation. Similarly, Guardia \textit{et al.} have shown in general the non-ionic surfactants (including Tween 80, Brij 700, and Pluronic P-123) outperformed other ionic surfactants (SDBS, TDOC etc.) in exfoliation efficiency of graphene under similar
conditions. The main stability mechanism in non-ionic surfactants was due to the steric stabilisation of the long hydrophilic parts of the molecules. The best $C_G$ obtained was around 1 mg/ml for P-123 (0.5 w/v%) under 2 hr of mild sonication (starting graphite concentration – 100 mg/ml). The flakes produced were defect free FLG with lateral dimensions in hundreds of nm with < 5 layers (10-15 % SLG). Notley have significantly improved the $C_G$ up to 15 mg/ml by slight modification in the exfoliation process. Continuous addition of surfactants (Pluronic F108 and F127) to the graphite/water solution during sonication, rather than adding at once, shown to maintain the surface tension at optimum levels (~41 mJ/m²) such that the newly formed graphene surfaces readily adsorb the surfactants forming highly stable and concentrated dispersions.

Bourlinos et al. have used polyvinylpyrrolidone (PVP) to produce non-oxidised graphene flakes in aqueous media (sonication for 9 hr) and the FLG with average thickness < 1 nm as characterised by AFM and TEM analysis. Wajid et al. have used PVP as a surfactant to exfoliate expanded graphite into graphene in various organic solvents including DMF, NMP, DMSO etc. with $C_G$ 0.4-0.72 mg/ml (tip sonication 1 hr, centrifugation 5000 rpm for 4 hr). Without PVP, the solvents were inefficient in dispersing the expanded graphite signifying the surfactants role. The freeze dried powder obtained from these dispersions shown to readily disperse in water without any sonication, indicating steric stabilisation. Additionally, aqueous PVP-stabilised graphene dispersions were found to be stable at higher temperatures (~100 °C) and at low pH (~2). Polymer composites produced from these graphene (lateral dimensions assumed: 2.5 × 1.5 µm) showed both mechanical (37 % increase in Young’s modulus with 0.03 vol.% graphene) and electrical property (Electrical conductivity increase from < $10^{-2}$ S/m to 2.6 × $10^{-6}$ S/m with 0.27 vol.% graphene) enhancements.

2.3.2.3. Co-solvent-based LPE

In general, for direct exfoliation either high b.p. solvents or surfactant-based solutions were used. However, it is difficult to eliminate the solvents and surfactants from the final material which can prove detrimental for application. Hence, other solvent systems have been explored, including co-solvent systems. Khan et al. have demonstrated that NMP-exfoliated graphene can be diluted in water up to 99:1 (vol.%, water:NMP) to produce stable dispersion with slight degree of aggregation and sedimentation.
Following this, Zhu et al. have shown successful exfoliation of expanded graphite in optimal DMF-water (90:10 vol.%) mixtures with no structural defects.\(^98\)

Liu et al. have shown direct exfoliation of graphene is possible in ethanol-water mixtures.\(^99\) Considering the HSPs, they have tested range of ethanol-water mixing ratios, achieving maximum exfoliation (\(C_G - 0.05\) mg/ml, sonication bath 90 min) at 70:30 (vol.%, ethanol:water), with no observed defects (TEM) and dispersion stability spanning for years.\(^99\) Similarly Fedi et al. selected mixtures of alcohol: 1-butanol: IPA (3:2), whose HSPs match with graphene, to produce exfoliated FLG with few defects.\(^100\) The chemiresistors made from those graphene had similar NO\(_2\) sensitivity that of NMP-exfoliated graphene.

Halim et al. have carried out systematic investigations on different cosolvent systems of alcohol (methanol, ethanol, IPA and t-butyl alcohol)-water mixtures in exfoliating graphite and MoS\(_2\).\(^101\) It was found that the \(C_G\) peaked (~80, 50, 30 and 10 wt.% for methanol, ethanol, IPA and t-butyl alcohol, respectively) when the surface tensions of the cosolvent mixtures were 20-25 \(\text{mJ/m}^2\) (Change from predicted optimal surface tensions of 30-35 \(\text{mJ/m}^2\) was attributed to alcohol evaporation during sonication). Additionally, the \(C_G\) was found to be higher for cosolvent with more \(-\text{CH}_3\) groups, following the trend of t-butyl alcohol > IPA > ethanol > methanol. They explained the observed minimum interfacial energy at optimal cosolvent concentrations by proposing that alcohol behaves in analogous to surfactants, such that at critical micelle concentrations (CMC, for alcohols > 10 wt.%) the non-polar \(-\text{CH}_3\) groups adsorb on graphene surface and \(-\text{OH}\) groups interact with water. However, at higher alcohol concentrations (> CMC) the alcohol aggregates microscopically, similar to highly hydrophobic surfactants, thereby reducing the graphene dispersion concentrations.\(^101\)

Very high \(C_G\) up to 50 mg/ml was also reported in cosolvent systems including 1:1 mixture of benzene and hexafluorobenzene by Oyer et al.\(^102\) TEM, AFM and Raman analysis confirms the FLG nature of the flakes. The advantage of this method is due to the cosolvent mixtures’ high vapour pressure and high freezing point, graphene produced could be readily transformed into powder \textit{via} evaporative cooling, suitable for other applications.\(^102\)
2.3.2.4. Solvent exchange methods

In order to facilitate broader applications of graphene, presently limited due to high bp solvents, several solvent exchange methods have also been realised by researchers. Zhang et al. have followed simple technique to solvent exchange from NMP to ethanol\textsuperscript{103}: The NMP-exfoliated graphene was vacuum filtered in poly tetrafluoroethylene (PTFE) membrane to form a filter cake, which was dispersed in ethanol and filtered again. Repeating the washing step five times, followed by a final dispersion (centrifugation 1000 rpm 30 min) in ethanol produced homogenous graphene dispersion (\(C_G\) - 0.04 mg/ml) in ethanol with \(~0.3\) vol.% NMP. Good conductivity (1130 S/m) was shown by films prepared from such dispersions.\textsuperscript{103} Similar method was followed by Yi et al. to exchange solvents from DMF to water.\textsuperscript{104} Extensive washing steps (10 times) and drying conditions (200 °C for 72 hr) of the filter cake have resulted in negligible amount of DMF in final dispersion. The main reason for stability in water was found to be the enhanced edge effects (O-containing, 7.8 at.% by XPS, groups attached at edges, whereas basal plane is defect free) of small-sized graphene sheets. Graphene films prepared from such water-dispersed graphene showed \(\sim1052\) S/m conductivity,\textsuperscript{104} comparable to earlier method.\textsuperscript{103}

Liang et al. have followed a phase separation method to enhance the \(C_G\).\textsuperscript{105} Firstly, the graphene was produced (sonication 3 hr, centrifugation 7500 rpm for 4.5 hr) in 1 w/v% ethyl cellulose (EC)/ethanol mixture (\(C_G\) - 0.12 mg/ml). To this, 1:5 volume ratio of terpineol was added. Finally, water (4 times the initial solution) was added to the solution. Owing to the strong hydrophobicity of EC-stabilised graphene, they migrate to terpineol phase resulting in \(C_G\) – 1.02 mg/ml. Raman analysis evidenced less defects with negligible oxidation.\textsuperscript{105} Following this work, realising the excellent stability of graphene in terpineol, and knowing terpineol’s compatibility in inkjet printing technology,\textsuperscript{106} Li et al. have carried out different approach to exchange DMF-exfoliated graphene into terpineol.\textsuperscript{107} Firstly, stable graphene dispersions in DMF were obtained by sonication (20 hr) and centrifugation (10000 rpm 15 min), to which terpineol was added. Low pressure-vacuum distillation process (80 °C water bath) was employed to evaporate the DMF at low pressure (~30 mbar) leaving behind graphene in terpineol (it is stable until pressure reaches 10 mbar) with \(C_G\) – 0.39 mg/ml.\textsuperscript{107} In order to achieve, higher \(C_G\) in terpineol
suitable for inkjet printing, Li et al.\textsuperscript{108} took advantage of EC's affinity for graphene as demonstrated by Liang et al.\textsuperscript{105} Addition of EC before the distillation process prevented the aggregation of graphene flakes, thereby increasing $C_G$ to 1 mg/ml. Additional process of annealing at 400 °C (maximum of few hours) was involved for removing EC in the inkjet printing process. Transparent conductive films (90 % transmittance) with 200 kΩ □⁻¹ sheet resistance, were attained from these solvent exchanged dispersions.\textsuperscript{108}

2.3.2.5. Graphite intercalation compound-based LPE

Increasing the interlayer spacing of the graphite (0.34 nm) weakens the vdW force between the layers, normally achieved by high temperature treatments or using intercalants, producing graphite intercalation compounds (GIC).\textsuperscript{109-111} GIC compounds are widely studied as the starting material for LPE.

Liu et al. have reported by adding NaOH improved exfoliation efficiency in organic solvents like BA, NMP, $N,N$-dimethylacetamide (DMA) and cyclohexanone, due to its intercalation.\textsuperscript{110} Addition of NaOH to graphite and sonicating for 1.5 hrs in NMP resulted in ~3 times higher $C_G$ than the pure solvent. X-ray diffraction (XRD) measurements showed increased interlayer spacing in the graphite sediment treated with NaOH compared to the non-treated graphite, suggesting successful NaOH intercalation. Niu et al. have used a different approach by using inorganic salts like sodium chloride (NaCl) and copper(II) chloride (CuCl$_2$) to intercalate the graphite under aqueous conditions using stirring and eventually boiling away the water.\textsuperscript{111} Mild sonication for 2-3 hr of the GIC followed by centrifugation at 3000 rpm for 5 min produced stable dispersions in different organic solvents including NMP, DMF, toluene and ethanol with $C_G$ between 0.05-0.12 mg/ml. Advantage of this method is that the exfoliation doesn’t breakdown the starting graphite significantly thereby producing large area flakes typically 5-100 µm$^2$ with 86 % of flakes between 1-5 layers. In another study, Zheng et al. have shown large graphene sheets up to 300 µm$^2$ can be produced in oleyl amine from GIC (intercalated using sulphuric and nitric acids) under solvothermal conditions with $C_G$ – 0.15 mg/ml (60 % SLG).\textsuperscript{109} The advantage of this method is due to their relatively mild acid treatment for shorter period of time (40 min), large graphene sheets with low levels of O (< 3-4 at.%),\textsuperscript{109, 111} as confirmed by XPS analysis and Raman spectroscopy, were obtained.\textsuperscript{32}
2.3.2.6. Ionic liquid-based LPE

Another top-down approach to produce graphene have been investigated using ionic liquids (IL) - which are defined as special salts that remain liquid below 100 °C. Due to their unique physicochemical characters: chemical and thermal stability, high ionic conductivity, wide electrochemical window, negligible vapour pressure, nonvolatility, IL are proposed as “green” organic solvents.

2.3.2.6.1. Sonication-assisted IL-based LPE

Wang et al. directly exfoliated graphite in 1-butyl-3-methylimidazolium bis(trifluoro-methane-sulfonylimide ([Bmim][Tf$_2$N]) using tip sonication (60 min) and centrifugation at 10000 rpm for 20 min to produce stable graphene dispersions with $C_G$ - 0.95 mg/ml. TEM analysis showed the flakes are in µm-dimensions and having < 5 layers. The stabilisation mechanisms in ionic liquids were expected to be from the $\pi - \pi$ interactions between aromatic cations and the electrostatic interactions between the $\pi$ electrons of the graphitic planes and the charged ionic species. The XPS analysis of the [Bmim]-[Tf$_2$N] exfoliated graphene showed ~10 at.% of oxygen (O) suggesting some level of oxidation, which are probably due to strong non-covalent interactions ($\pi - \pi$) and/or covalent functionalisation with [Bmim]-[Tf$_2$N] itself.

2.3.2.6.2. Grinding-assisted IL-based LPE

Grinding of the staring graphite with IL has been used in several studies for exfoliating graphene. Nuvoli et al. have used of 1-hexyl-3-methylimidazolium hexafluorophosphate ([HMIM][PF$_6$]) to exfoliate graphite to graphene. Firstly, they grind the graphite flakes with [HMIM][PF$_6$] in a mortar followed by bath sonication for 24 hr and centrifugation at 4000 rpm for 30 min, resulting in stable dispersion with $C_G$ – 5.33 mg/ml. AFM analysis of the exfoliated flakes show lateral dimensions of 3-4 µm with average apparent height of 2 nm and Raman spectra confirming the FLG nature. Chloroform was used to wash the IL for producing powdered graphene.

Shang et al. have developed a method to produce graphene dispersions by using mechanical grinding of small quantities of IL (0.1-0.5 ml) such as 1-butyl-3-methylimidazolium hexafluorophosphate ([BMIM][PF$_6$]) with graphite. The graphite-IL mixture was grinded in a mortar for 0.5-4 hr turns into a gel due to the $\pi - \pi$ interactions.
of graphite and IL. During the grinding process, due to the shear forces, the IL helps shearing the graphite layers into graphene by acting as a lubricant.\textsuperscript{114,115} The obtained gel was further washed extensively using DMF and acetone mixture removing the IL. Dispersion of the sediment in DMF results in a stable graphene dispersion with typical dimensions between 0.006-0.36 µm\(^2\) and 2-5 layers as determined by TEM. Most importantly, XPS analysis showed \(\sim 3.6\) at.\% of O in the final graphene which was very close to the starting graphite (\(\sim 3.4\) at.\%), suggesting graphene sheets are free from any impurities.\textsuperscript{114} Benson \textit{et al.} followed the same process but produced graphene flakes with different dimensions using sequential centrifugation steps (400-1 µm with 2-6 nm thick for 3000 rpm and few hundred nm with < 10 layers for 10000 rpm).\textsuperscript{115} The O content determined by XPS analysis was \(\sim 2.6\) at.\%, slightly higher than the starting graphite (\(\sim 1.1\) at.\%), attributed to the oxygen from environment attacking the large amount of edges.\textsuperscript{115} The advantage of the latter two methods is that it not only avoids any sonication steps, thereby preventing functionalisation of IL to graphene, but also complete removal of IL in final graphene was achieved.

\subsection*{2.3.3. Electrochemical exfoliation of graphene}

Electrochemical methods to prepare graphene materials typically utilises the application of electric current to encourage structural expansion (either via cathodic reduction or anodic oxidation) of a graphite working electrode (normally in form of rod, flake or HOPG), in a liquid electrolyte (aqueous: acidic or surfactant, or non-aqueous: organic or ILs).\textsuperscript{117,118} In general, during the electrochemical exfoliation approach, upon application of potential to the electrodes, intercalation of anions or cations from the electrolyte results in the production of GICs, which are then further exfoliate into graphene layers that disperse in the electrolyte (Figure 2.8).\textsuperscript{117} A common method is to intercalate anions into graphite anodes to exfoliated graphene sheets with surface oxidation or chemical functionalisation. Whereas, utilising cations to intercalate graphite cathodes have shown to produce graphene sheets with a lower degree of surface oxidation or chemical functionalisation, thereby retaining the pristine nature of graphene.\textsuperscript{118}
2.3.3.1. Anodic exfoliation

In anodic exfoliation, application of positive potentials to the graphite working electrode results in intercalation of anions such as: sulphate-based ions, nitrate ions, etc., and subsequent exfoliation to produce graphene flakes. Parvez et al. have reported exfoliation in different aqueous inorganic salts, including sodium sulphate, ammonium sulphate (showed highest exfoliation efficiency at 0.1 M concentration in water, +10 V for 3-5 min) and potassium sulphate, producing graphene sheets with low defect density (C/O ~ 17.2).\textsuperscript{119} The produced graphene sheets contained ~80 % > 5 µm lateral dimensions and ~85 % flakes between 1-3 layers. Lu et al. have reported IL containing nitrate ions (ethylammonium nitrate) can be used for electrochemical exfoliation of graphite in aqueous media.\textsuperscript{120} Unlike the failure of exfoliation in pure IL, addition of water showed successful exfoliation. Under applied potential (+2.2 V), the water oxidises generating oxygen-reactive species which opens the edges and grain boundaries of graphite sheets, that are utilised by the nitrate ions to intercalate and exfoliate, producing nitrogen doped graphene sheets.\textsuperscript{120}

![Figure 2.8](image)

*Figure 2.8: a,b) Schematic representation of electrochemical approaches using anions or cations for intercalation and exfoliation of graphene flakes\textsuperscript{117}. c-g) Electrochemical exfoliation of HOPG using cations\textsuperscript{121}: (c) Picture of HOPG. d) HOPG expansion after 1000 sec tetraethylammonium cation intercalation, e, f) HOPG expansion after 1000 sec and 10000 sec tetrabutylammonium cation (TBA\textsuperscript{+}) intercalation, respectively. g) SEM image of HOPG expansion of 6000 sec TBA\textsuperscript{+} intercalation.*
2.3.3.2. Cathodic exfoliation

Since the anodic exfoliation involves oxidation at the graphite anode, it results in producing graphene either partially oxidised or chemically functionalised. Hence, non-oxidative routes of electrochemical exfoliation at graphite cathode have been realised. Wang et al. have demonstrated the use of Li in propylene carbonate to successfully intercalate the graphite at the cathode (-15 V).\textsuperscript{122} Subsequent sonication of the GIC in solvents like DMF produced high quality FLG (< 5 layers) as confirmed by XPS and Raman analysis.\textsuperscript{122} Cooper et al. have used tetraalkylammonium salts to cathodically intercalate (Figure 2.8c-g) HOPG (-2 V) producing FLG (2-5 layers) with no functionalisation and very little O (< 1 at.%, thought to be from exposure to atmosphere).\textsuperscript{121}

Abdelkader et al. have used DMSO-based electrolyte containing Li and triethylammonium ions to electrochemically exfoliate graphene in gram quantities.\textsuperscript{123} They have suggested that the triethylammonium ions which are dissociated between the graphite layers to give triethylamine and hydrogen gases, that helps in spontaneous exfoliation of the flakes from the graphite electrode. This process has produced FLG with large lateral dimensions (up to 20 µm) with very little defects as analysed by Raman spectroscopy.\textsuperscript{123} Such electrochemically exfoliated large graphene sheets (20 µm) have shown increased reinforcing ability (even at 20 wt.%) in poly propylene (PP)\textsuperscript{124} and poly methyl methacrylate (PMMA)\textsuperscript{125} matrices, compared to smaller graphene sheets (5 µm).

2.3.4. Other top-down methods

2.3.4.1. High-shear exfoliation

Recent studies have realised the use of high-shear rotor-stator mixer to produce large-scale exfoliation of graphene dispersions.\textsuperscript{126, 127} Paton et al. reported the graphene produced in NMP (Figure 2.9a,b,c) by high-shear rotor-stator were predominantly FLG with lateral dimensions between 200-800 nm (TEM analysis) and are unoxidised and free from basal plane defects (XPS and Raman analysis).\textsuperscript{127} They have shown regardless of laminar or turbulent flow, successful exfoliation can occur when the shear rate exceeds 10^4 s\textsuperscript{-1},\textsuperscript{127} which is mainly at the vicinity of the rotor-stator.
Whereas, Yi et al.\textsuperscript{128} and Varalla et al.\textsuperscript{129} have exploited the turbulent flow in the kitchen blender, produced by the rotating blade mixer, to produce FLG in DMF and aqueous media ($C_G = 1 \text{ mg/ml}$\textsuperscript{129}), respectively. Unlike rotor-stator, high shear regions are not localised in kitchen blender, and can be maintained if the turbulence is fully developed. The combination of various fluid dynamic events including, viscous shear, turbulence, collisions, were considered as the main exfoliation mechanisms.\textsuperscript{128, 129} Such techniques show a promising way for large-scale graphene production (Figure 2.9d,e,f).

### 2.3.4.2. Sonication free LPE

Pu \textit{et al.} have shown FLG can be produced by intercalation and exfoliation using supercritical $\text{CO}_2$.\textsuperscript{130} The graphite was immersed in supercritical $\text{CO}_2$ for 30 min followed by rapid depressurising the supercritical fluid to expand and exfoliate graphite. The FLG were collected by discharging the expanding $\text{CO}_2$ gas directly into a solution containing SDS to avoid aggregation or restacking. TEM and AFM analysis show the FLG are $\sim$10 layers.\textsuperscript{130}

Behabtu \textit{et al.} have demonstrated spontaneous exfoliation of graphite to graphene using chlorosulphonic acid with isotropic concentrations of $C_G = 2 \text{ mg/ml}$.\textsuperscript{131}
The exfoliation is mainly because the acid protonates the graphene to form a salt and induces repulsion between the layers.\textsuperscript{131}

### 2.3.5. Epitaxial graphene

Several bottom-up methods including, chemical vapour deposition (CVD) growth technique, thermal decomposition of silicon carbide (SiC), etc., were also studied for producing \textit{pristine} high quality graphene.

#### 2.3.5.1. CVD-grown graphene

Among the bottom-up methods for producing graphene, CVD technique is one of the most investigated approaches, especially holding promises in producing large-area graphene on inexpensive transition metals. The principle of CVD is to decompose a carbon feedstock with the help of heat to provide a source of carbon, which can then rearrange to form sp\textsuperscript{2} carbon species, normally achieved over a catalyst.\textsuperscript{132} In case of graphene, generally hydrocarbon gases (\textit{e.g.}, methane gas, CH\textsubscript{4}) are generally utilised as feedstock and successful catalysts by far are transition metal surfaces (\textit{e.g.}, Ni and Cu).\textsuperscript{133} The CVD process mainly depends on the carbon saturation of a transition metal upon exposure to a hydrocarbon gas at a higher temperature.

A wide variety of transition metals have been used for graphene growth including, Ru,\textsuperscript{134} Ir,\textsuperscript{135} Pt and Pd\textsuperscript{136}. However, graphene growth on relatively inexpensive polycrystalline metals like Ni\textsuperscript{137},\textsuperscript{138} and Cu\textsuperscript{139},\textsuperscript{140} have triggered interest in optimising the CVD conditions for producing large-area high quality graphene. It has been found that FLG films tend to be grown on Ni due to growth via carbon segregation-precipitation process.\textsuperscript{137},\textsuperscript{138} In contrast, owing to the low solubility of C atoms in Cu, continuous SLG films were grown on Cu surface via surface adsorption process (Figure 2.10a). Graphene growth on Cu is a self-limiting process;\textsuperscript{139},\textsuperscript{140} once the Cu surface is covered by a monolayer of graphene, the growth terminates due to the unavailability of catalyst (Cu), therefore BLG and FLG are barely formed (darker regions in Figure 2.10c).\textsuperscript{140} Graphene ‘wrinkles’ as seen in Figure 2.10c and Figure 2.12g usually originate due to the different thermal expansion coefficient of graphene and Cu and/or surface reconstruction of the Cu and these wrinkles can be observed spanning across the Cu grain boundaries, a way to identify the continuous graphene film.
2.3.5.1.1. **Large single crystal graphene on Cu**

Despite the continuous growth of SLG on Cu, the as-grown graphene tends to be polycrystalline in nature containing grain boundaries (GBs); formed by the coalescence of adjacent graphene domains during the CVD growth (Figure 2.11a). GBs contain defects like heptagon-pentagon pairs (Figure 2.11b)\(^\text{142}\) or overlapped bilayer regions (Figure 2.11c)\(^\text{143}\). Moreover, the presence of GBs is shown to alter the mechanical and electrical properties. Ruiz-Vargas \textit{et al.} have shown the indentation experiments on poly crystalline CVD graphene had an order of magnitude reduction in both elastic modulus and breaking force compared to MC graphene.\(^\text{144}\) Additionally, AFM analysis have demonstrated that tears often occurs at the grain boundaries during indentation experiments (Figure 2.11d,e).\(^\text{142}\) Yu \textit{et al.} have shown the GBs between the coalescing grains affected graphene’s electronic transport properties.\(^\text{145}\) Conversely, Tsen \textit{et al.} have demonstrated that well-stitched GBs (overlapped bilayer regions, Figure 2.11c) in polycrystalline graphene showed enhanced electrical conductance.\(^\text{143}\) Hence, large numbers of studies have been carried out to produce large single crystal graphene on Cu.

The one key point in achieving large single crystal graphene is preferably to reduce the number of graphene nucleation sites, which could be obtained by surface treatment/smoothening of Cu.\(^\text{146}, \text{147}\) Yan \textit{et al.} have used electrochemical polishing to remove the protection layers that are normally present on commercial Cu foils, followed by annealing them at 2 atm (H\(_2\)) for 7 hr to reduce the surface wrinkles and defects.\(^\text{148}\) Large hexagonal single crystal grains ~2.3 mm (Figure 2.11f) was grown on such pre-treated Cu foils. The carrier (hole) mobility of such single crystal graphene grain was ~11000 cm\(^2\)/Vs, comparable to MC graphene.\(^\text{148}\) Similarly, Chen \textit{et al.} followed
electrochemical polishing of the Cu foil, before rolling it into a tube. They proposed that during the annealing process, the evaporative loss of Cu in the inner surface of Cu tube was suppressed due to re-deposition of Cu in the confined space, resulting in flat inner surface.\textsuperscript{149} This was confirmed by AFM analysis which indicated the inner Cu tube surface were smoother and had fewer defects than the outer surface. As a result, single crystal graphene grains \(~2\) mm were formed on the inner surface of Cu the tube, with observed carrier mobilities of \(5200\ \text{cm}^2/\text{(Vs)}\).\textsuperscript{149} Mohsin \textit{et al.} have demonstrated mm-sized hexagonal single graphene grains could be grown on Cu by following a brief \textit{in situ} treatment of the Cu foil.\textsuperscript{150} By melting and subsequent resolidification of Cu at atmospheric pressure, smoother surface (AFM analysis: resolidified Cu roughness \(~8\) nm, as-received Cu roughness \(~160\) nm) was obtained, resulting in the low nucleation density thereby achieving large grains.\textsuperscript{150}

Another method to produce large single grain graphene is to maintain the catalytic inactivity of copper(I) oxide (\(\text{Cu}_2\text{O}\)) layer thereby reducing the graphene nucleation. Zhou \textit{et al.} followed the idea by annealing the Cu in presence of pure Ar (rather than Ar/H\(_2\) mixture) gas, where the catalytic inactive \(\text{Cu}_2\text{O}\) was formed.\textsuperscript{151} The presence of \(\text{Cu}_2\text{O}\) reduced the graphene nucleation density from \(10^{-6}\) to 4 nuclei \text{cm}^{-2}.\textsuperscript{151} Such conditions resulted in \(~5\) mm (Figure 2.11g) sized hexagonal single graphene grain with straight edges (48 hr growth), with carrier mobilities up to \(16000\ \text{cm}^2/\text{(Vs)}\). Using

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2_11}
\caption{a) Images of many graphene domains among CVD graphene film.\textsuperscript{142} b) CVD GB containing aperiodic heptagon-pentagon pairs.\textsuperscript{142} c) Overlapped bilayer regions at CVD graphene GB.\textsuperscript{143} d, e) AFM image showing the tears occurred along the GBs during indentation.\textsuperscript{142} f) Optical and SEM image of mm-sized graphene single crystals grown on pre-treated Cu foils.\textsuperscript{148} g) mm-sized graphene grains grown on oxygen-rich Cu (48 hr growth).\textsuperscript{151} h) Merged sub-cm single crystal graphene grown on Cu foil.\textsuperscript{152} i) cm-sized single crystal graphene on Cu exposed to \(\text{O}_2\).\textsuperscript{153}}
\end{figure}
similar Ar-gas assisted approach (suppressing graphene nucleation by oxygen-rich Cu) single crystal graphene ~5.9 mm (Figure 2.11h) was grown by Gan et al.\textsuperscript{152} Hao et al. discovered by introducing O onto Cu, the graphene nucleation density was substantially decreased by passivating the surface active sites on Cu.\textsuperscript{153} By controlling the surface oxygen, successful and repeatable growth of cm-sized (Figure 2.11i) single grain graphene was obtained, with room temperature carrier mobility ranging from 15000-30000 cm\(^2/(Vs)\).

Recent studies have shown by aligning the graphene domains, leading to seamless stitching of the grains, large single crystal graphene can be obtained. Lee \textit{et al.} have shown this concept on hydrogen-terminated Ge(110) over layer on Si.\textsuperscript{154} However, uniform growth over large area is difficult due to the impurities and defects on the Ge(110). Whereas, Nguyen \textit{et al.} have used large (6 cm \times 3 cm) polished monocrystalline Cu(111) to grow large area single crystal graphene with no GBs, by merging multiple hexagonal graphene domains which are orientated in same direction.\textsuperscript{155} Babenko \textit{et al.} have recently demonstrated growth of mm-sized single grain graphene on polycrystalline Pt foils coated with Si-containing film.\textsuperscript{156} When heated, the Si film reacts with Pt forming a liquid platinum silicide layer, which screens the Pt lattice and fills the topographic defects. This results in formation of large graphene domains. This growth technique resulted in graphene growth rate of 120 µm/min, an order of magnitude higher than reported literature, was achieved.

2.3.5.1.2. Transfer of CVD graphene on substrates

The CVD grown graphene on the metallic substrates should be transferred on to arbitrary substrates like glass, quartz, SiO\(_2\) etc. for realising its properties and applications. In commonly used wet-transfer process (Figure 2.12a), a thin protective layer of PMMA\textsuperscript{157, 158} or polydimethylsiloxane (PDMS)\textsuperscript{137} is coated on the CVD graphene followed by etching the metallic Ni or Cu foil in an etchant (\textit{e.g.}, Iron(III) chloride, FeCl\(_3\)). After transferring to target substrate, the protective PMMA layer could be removed by dissolving in acetone (Figure 2.12b-d). Nevertheless, complete removal of residual PMMA remains difficult, resulting in p-doped graphene in some samples.\textsuperscript{157, 158} Following such wet-transfer method, roll-to-roll transfer of graphene using a thermal release tape as the support polymer has been demonstrated by Bae \textit{et al.} producing flexible 30-inch
graphene on plastic substrates for flexible electronic applications (Figure 2.12e,f). However, such transfer methods inevitably result in cracks as seen in Figure 2.12g. Kobayashi et al. have demonstrated large scale synthesis (100 m long) of CVD graphene on Cu foil via a roll-to-roll process in a specially designed CVD reactor. A photocurable epoxy resin was used to bond graphene with polyethylene terephthalate (PET, 230 mm wide), followed by spray etching (FeCl$_3$) of Cu in a roll-to-roll process, producing 100 m long transparent conductive film.

Other modified wet-transfer methods have also been reported. Gao et al. have demonstrated H$_2$ gas bubbling transfer of graphene. The PMMA-protected CVD grown graphene on Pt substrate was used as cathode in an electrolysis cell (electrolyte NaOH aqueous solution). Due to water reduction reaction at the cathode, H$_2$ gases (bubbles) are produced at the interface of graphene and Pt substrate resulting in detachment of graphene/PMMA layer by substrate etching. This process facilitates the repeatable use of Pt for graphene growth. Such electrochemical delamination method (for reusing Cu for graphene growth) was also reported for transfer of graphene grown on Cu by Wang et al. However, a thin layer (~40 nm thick) of Cu was found to etch away during the electrochemical delamination transfer process. In a recent study, face-to-face transfer method for transferring wafer-scale graphene films was demonstrated. The clean and
large-area transfer process was facilitated by the nascent gas bubbles and capillary bridges between the graphene film and underlying substrate.\textsuperscript{163}

\subsection*{2.3.5.2. Thermal decomposition of silicon carbide}

Transfer free wafer-scale graphene can be produced by thermal decomposition of silicon carbide (SiC). In this process, the SiC substrates are annealed at higher temperatures (1000-1600 °C) under ultrahigh vacuum conditions, results in Si atom sublimation and graphitisation of remaining C atoms forming epitaxial graphene. In general, by optimising the growth conditions, thickness of graphene can be controlled on Si-terminated face thereby producing FLG\textsuperscript{164} whereas, on C-terminated face MLG are commonly formed.\textsuperscript{165} Unlike the Si-face, the nucleation occurs throughout the C-face and the resulting number of graphene layers is not homogenous. Recently, very high $\mu$ on a 40 nm wide graphene nanoribbon (GNR) grown on the Si-face of (0001) SiC was demonstrated. Ballistic conductance (4 K) of $\sim 6 \times 10^6$ cm\textsuperscript{2}/(Vs) over length scales greater than 10 nm was observed for such GNRs.\textsuperscript{26}

\section*{2.4. \textbf{PRODUCTION, STRUCTURE AND PROPERTIES OF GRAPHENE OXIDE}}

\subsection*{2.4.1. Production of Graphene oxide}

One of the most popular alternatives for producing LPE of graphene-based materials is \textit{via} graphene oxide (GO) route. In this method, the graphite is normally oxidised to form graphite oxide, which with aid of ultrasonication exfoliated into individual GO sheets in various solvents. The most commonly used method for oxidising graphite is Hummers’ method that includes strong oxidising agents like potassium permanganate, nitric acid and sulphuric acid.\textsuperscript{166} The oxidisation results in functionalising the basal plane and edges of graphite with epoxy, hydroxyl and carbonyl groups. These functional groups increases the interlayer distance of graphite oxide, and upon exfoliation, the hydrophilic nature of the functional groups facilitates excellent stability of GO in water.\textsuperscript{167} GO is highly negative charged due to the ionisation of the functional groups, that renders stability in water, alcohols, and certain organic solvents\textsuperscript{168} without any surfactants by electrostatic repulsion.\textsuperscript{167}
2.4.2. Structure of Graphene oxide

GO is a nonstoichiometric compound, hence the determination of its structure has been challenging. However in general, the accepted chemical structure of GO has the epoxy and hydroxyl groups on the basal plane and carboxyl group on the edges (similar to Lerf-Klinowski model\textsuperscript{169}, Figure 2.13a), and confirmed by solid-state nuclear magnetic resonance (NMR) spectroscopy.\textsuperscript{169, 170} Gao \textit{et al.} have also proposed that five- and six-membered lactol rings decorating the edges and esters of tertiary alcohols on the surface (Figure 2.13b).\textsuperscript{171}

![Figure 2.13: Various proposed structures of GO: a) Schematic model of a GO sheet based on Lerf-Klinowski model\textsuperscript{169} b) GO structure proposed by Gao \textit{et al.} containing lactol rings at edges.\textsuperscript{171} c) GO structure proposed by Rourke \textit{et al.} including highly oxidised surface-bound debris.\textsuperscript{172}](image)

Recently, Rourke \textit{et al.} has reported a new structure (Figure 2.13c) for GO produced from Hummers’ method.\textsuperscript{172} They proposed that highly functionalised low molecular weight oxidative debris (OD) non-covalently decorates the as-produced GO (aGO). Upon washing with a base, this OD can be stripped from aGO producing base-washed GO (bwGO). They have also found that bwGO cannot be easily redispersed in water suggesting the OD acts as a surfactant. The bwGO film was also found to be electrically conductive ($10^0$-$10^1$ S/m) compared to aGO, and very similar to reduced GO (rGO).\textsuperscript{172}

2.4.3. Reduction process of Graphene oxide

The oxygen groups in GO can be reduced by reduction process producing rGO. Chemical approach is most common among the reduction process. The commonly used reducing agents are hydrazine (N\textsubscript{2}H\textsubscript{4})\textsuperscript{173}, sodium borohydride (NaBH\textsubscript{4})\textsuperscript{171}, hydrogen iodide (HI)\textsuperscript{174}, \textit{etc}. During the reduction treatment with such chemicals, the brown coloured GO turns black and precipitates in the solution. The obtained rGO is less hydrophilic due to the removal of oxygen containing groups. The reduction process, apart from removing the oxygen groups, destroys the sp\textsuperscript{2} carbon plane irreversibly at many places, leaving sp\textsuperscript{3
carbon, vacancies, etc. Hence, the structure of rGO contains patches of sp$^2$ carbon islands (3-6 nm size) surrounded by holes and defects (Figure 2.14). The C/O ratio varies with different chemicals used for the reduction process: $N_2H_4 - 10.3$, $NaBH_4 - 4.8$, $HI - 12$, $Zn/HCl - 33.5$, etc.

**Figure 2.14**: a) HRTEM image of single-layer rGO. Colour scheme highlighting different features: dark grey – contaminated regions, blue – disordered single-layered carbon networks or extended topological defects, red – individual adatoms or substitutions, green – isolated topological defects, yellow – holes and their edge reconstructions. Scale bar – 1 nm. b) Atomic model illustrating disordered rGO basal plane consisting of holes, topological defects and remnants of oxygen groups.

Thermal reduction is another alternative method to reduce GO, that employs very high temperatures, to remove the oxygen groups. The C/O ratio produced were around 12.5 to 14.1. In general, the rGO show less hydrophilicity and solubility in solvents. The stability of the dispersions can be improved by covalent or non-covalent modifications of the surface of rGO. Yuan et al. used cyclopentadienyl-capped poly (ethylene glycol) monomethyl ether to covalently functionalise rGO, thereby improving the stability of rGO in otherwise not soluble solvents like water, ethanol, acetone, chloroform etc. Stankovich et al. *in situ* reduced GO in presence of poly(sodium-4-styrenesulfonate) producing stable aqueous dispersions of non-covalently adsorbed polymer-coated rGO. Xu et al. have used 1-pyrenebutyrate (PB) functionalise rGO via $\pi - \pi$ interactions. The conductivity ($2 \times 10^2$ S/m) of the flexible films produced from PB-rGO was at least 7 orders of magnitude higher than the GO precursor. The main advantage of the chemical reduction to produce rGO is its scalability due to relatively simple process. However, the main drawbacks of this process are: complete removal of oxygen and sp$^3$ carbon groups in
rGO is not possible.\(^{173}\) Therefore, the chemical reduction process does not completely restore the sp\(^2\) carbon lattice and the electronic properties of rGO are found to be orders of magnitude lower than the pristine graphene.\(^{185, 186}\)

However, recent studies\(^{187-191}\) have demonstrated that by high temperature heat treatments (\(\geq 1500\) °C) \(i.e.,\) graphitisation, efficient conversion of GO or rGO to graphene is possible with dramatic reduction of surface defects and residual oxygen. Such graphitisation studies demonstrate restoration of sp\(^2\) carbon lattice as evidenced by Raman spectroscopy (Figure 2.15a,b), XPS (Figure 2.15c), STM (Figure 2.15d,e), \(\textit{etc.}\) The obtained graphene showed improved electronic properties with election mobility \(~1000\) cm\(^2\)/\((\text{Vs})\) (precursor rGO had a value of \(130\) cm\(^2\)/\((\text{Vs})\))\(^{191}\) and electrical conductivity as high as \(577\,000\) S/m.\(^{190}\)

**Figure 2.15:** a,b) Raman spectra of starting GO (FGS), GS (treated GO:1900 °C, \(10^{-6}\) torr vacuum) and its corresponding Raman map of 2D band intensity, respectively.\(^{187}\) c) XPS C1s and O1s spectra of graphite oxide, CG (rGO) and GG (treated rGO: 2800 °C, argon atmosphere).\(^{188}\) d,e) Atomic-scale STM images of starting rGO and treated (first treatment-1500 °C, second treatment-2700 °C, argon atmosphere), respectively.\(^{190}\)

Rozada \textit{et al.} have used STM and theoretical simulations to understand the restoration process in individual GO sheets upon graphitisation treatments.\(^{192}\) They proposed the healing process is mainly dominated by the generation and migration of atomic vacancies in the carbon lattices at high temperatures (1500-1800 °C) and is dependent on the starting concentration of oxygen groups in GO and also the nature of the substrate.
2.4.4. Mechanical properties of Graphene oxide

The mechanical properties of the GO are inferior compared to pristine graphene due to the presence of severe lattice distortions in form of sp$^3$ carbons, holes, impurities etc. Moreover due to surface functionalisation, thickness of GO (0.7-1 nm) is higher than pristine graphene (0.34 nm). Dikin et al. investigated the mechanical properties of GO paper and found stiffness of 40 GPa and strength of 120 MPa.$^{193}$ Suk et al. reported the Young’s modulus of GO as $\sim$208 GPa (effective thickness of GO $\sim$0.7 nm) from AFM indentation measurements.$^{194}$ Gomez-Navarro et al. carried out AFM indentation technique, similar for pristine graphene$^{29}$, on rGO suspended over a trench in Si/SiO$_2$ wafer, measuring Young’s modulus of $\sim$250 GPa.$^{195}$ Paci et al. carried out simulation studies exploring the mechanical properties of GO and found the modulus $\sim$750 GPa (thickness of GO considered as $\sim$0.34 nm), and found that changes in the chemical bonding and presence of holes limits the mechanical properties of GO.$^{196}$

2.5. Production of Biocompatible Graphene-based materials

Due to graphene’s extraordinary properties, graphene-based materials (GBMs) have gained lot of interest in the biomedical field exploring their uses as biosensors, tissue engineering and drug delivery.$^{197-202}$ These potential applications make environmental health and safety of GBMs an important topic of investigation$^2, 203, 204$ leading to several toxicity studies both in vitro and in vivo.$^2, 5, 205-207$

Since GBMs have variety of types (Figure 2.16) including: pristine SLG, FLG, GO, rGO, GNRs, etc., their biocompatibility is expected to vary with types. Recent reviews have shown indeed the biological response of GBMs vary depending on number of layers, layer dimensions, stiffness, hydrophobicity, surface functionalities, purity and dosage of the material.$^5, 203, 204$ More importantly, Bianco highlighted that most of the available studies were performed on GO or rGO due to their better solubility/stability in aqueous media compared to other GBMs.$^5$
2.5.1. Biocompatible materials as surfactants

In general, due to different production methods for obtaining GBMs for bioanalysis, several issues were faced including; aggregation or poor dispersibility in solvents or in physiological conditions. Covalent and non-covalent functionalisation of GBMs is often adapted to address this issue, thereby producing biocompatible GBMs facilitating further analysis.

Functionalisation (covalent or non-covalent) of GBMs using biocompatible polymers like polyethylene glycol (PEG) (PEGylation) is one of the commonly studied methods for producing biocompatible GBMs. PEGylated GO (covalent functionalised) showed excellent stability in the physiological solutions and have been employed for drug delivery and live cell imaging. Moreover, the PEGylated GO showed modulated effect on cytotoxicity on series of cell lines without affecting the cell viability up to 100 µg/ml. Yang et al. have carried out in vivo studies on fluorescent labelled PEGylated GO in mice for photo thermal therapy of cancer, showing excellent tumour ablation effect. Zhang et al. have shown PEGylated nano-GO (10-30 nm diameter) can be cleared out from the mouse body after intravenous injection, without any noticeable toxicity to the treated animals. Similar to PEG, covalent functionalisation of GO with dextran showed excellent stability in the physiological solutions with excellent in vitro biocompatibility and no noticeable in vivo toxicity (mice).

Pluronic-functionalised GBMs were also studied as biocompatible materials. Li et al. dispersed commercially available pristine graphene in 1 % Pluronic F108, showing dosage dependant in vitro cytotoxic effects on murine macrophages. Duch et al. have shown direct administration of GO into mice lung resulted in severe and persistent lung injury. In contrast, pristine well-dispersed graphene produced from direct LPE of
graphite using 2 w/v.% Pluronic F108NF as surfactant, showed significant reduction in pulmonary toxicity compared with GO.\textsuperscript{215} Biocompatible cationic polymers like polyethyleneimine (PEI) were used to non-covalently functionalise GO (GO-PEI).\textsuperscript{216} Owing to its improved stability in physiological solutions, at higher concentrations (300 µg/ml) GO-PEI displayed higher human epithelial carcinoma cell viability compared to the GO.\textsuperscript{216}

In another study, Wojtoniszak et al. investigated the toxicity of GO and rGO dispersed using different biocompatible surfactants including: PEG, Pluronic P123 and sodium deoxycholate (DOC, bile surfactant), on mice fibroblast cells.\textsuperscript{217} Despite the stability in physiological conditions, they found that toxicity response was surfactant- and concentration of nanomaterial-dependant. Within their experimental conditions, PEGylated GO showed best biocompatibility with lowest toxicity compared to other nanomaterials.\textsuperscript{217}

Recent study by Conroy et al. showed no detectable cellular morphology changes of cultured human lung epithelial cell lines A549 grown on pristine graphene films (CVD-grown and LPE using SC bile surfactant) or when exposed to LPE graphene flakes (0.1 to 5 µg/ml).\textsuperscript{218} Moreover they found ultrafine carbon black is more toxic than graphene.

\subsection*{2.5.2. Functional biomolecules as surfactants}

\subsubsection*{2.5.2.1. DNA-based}

Researchers have explored the use of functional biomaterials such as DNA (deoxyribonucleic acid),\textsuperscript{219-221} lipids\textsuperscript{222-224} and proteins\textsuperscript{225-228} to enhance the biocompatibility of GBMs through non-covalent interactions. Patil et al. have used single-stranded DNA (ssDNA) to successfully prepare stable aqueous solution of rGO with concentrations up to 2.5 mg/ml.\textsuperscript{219} Hydrazine-based reduction of GO in presence of ssDNA resulted in stable aqueous rGO, whereas reduction process in absence of ssDNA followed by its stabilisation in aqueous ssDNA solution was not successful. DNA molecules were principally adsorbed through hydrophobic as well as weak electrostatic/hydrogen bonding interactions between primary amines of the nitrogen bases, and the carboxylic and phenolic groups of the rGO sheets. Due to this, the DNA was oriented with its
charged polar moieties of the sugar-phosphate backbone exposed to solvent, rendering its negative $\zeta$ values and stability.

Following such non-covalent interactions, Liu et al.\textsuperscript{221} used thiol-labelled DNA to stabilise the GO and rGO in aqueous media, whereas, Liu et al.\textsuperscript{220} used pyrene-labelled ssDNA to directly LPE graphite to produce pristine graphene. In both the studies the resulting bio-conjugates were used as a scaffold for self-assembly of gold (Au) nanoparticles (NPs).

### 2.5.2.2. Lipids-based

Lipids are biomolecules, which are like surfactants having a hydrophobic tail group and hydrophilic head group, and could effectively stabilise GBMs in aqueous media. Liu et al. demonstrated that, anionic liposomes (DMPG – 1,2-dimyristoyl-sn-glycero-3-phospho-(1-rac-glycerol) sodium salt) deposited as monolayers around the rGO surface (Figure 2.17a,b), thereby enhancing colloidal stability in water by electrostatic repulsion.\textsuperscript{222} In contrast, removal of excess positively charged liposomes resulted in rGO aggregation, and neutral (zwitterionic) DMPC (1,2-dimyristol-sn-glycero-3-phosphocholine) liposomes failed to stabilise rGO in water in their experimental conditions.\textsuperscript{222} An interesting study conducted by Ip et al.\textsuperscript{223} following cryo-TEM measurements and fluorescence spectroscopy revealed that zwitterionic liposomes like DOPC (1,2-dioleoyl-sn-glycero-3-phosphocholine) adsorbs on GO as intact liposomes mainly at the edges (Figure 2.17c,d), whereas spontaneous liposome rupture (varies depending on level of oxidation) was observed for rGO (Figure 2.17e) and commercial-graphene (Figure 2.17f), suggesting lipid molecule-graphene interactions. It is crucial to understand the GBMs-lipids interactions as lipids (phosphatidylcholine) forms the major part of cell membranes. Molecular dynamic (MD) simulations have shown that SLG sheets covered by phospholipids (POPC – 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine) exhibited strong interaction with the cell membrane such that the graphene sheets gets internalised in the hydrophobic regions of biological membranes (Figure 2.17g-l).\textsuperscript{229} Li et al. have also absorbed the hydrophobic interactions of FLG and the biological cell membranes from both simulation and experimental studies.\textsuperscript{230}
Figure 2.17: a,b) AFM images of rGO and rGO-DMPG on mica, respectively. c-f) Cryo-TEM images of GO-DOPC (c, d), rGO-DOPC (e) and commercial graphene-DOPC (f). Intact liposomes can be clearly seen adsorbed on the edges and surface of GO, while for rGO it can be observed only at edges. Absence of any intact liposomes on the surface of rGO and graphene was correlated to the rupture of adsorbed liposomes. g-i) MD simulation of self-insertion of SLG inside the phospholipid membrane. The graphene micelle merges with the membrane and releases the monolayer, which penetrates the membrane.

Additionally, Pykal et al. have demonstrated that lecithin molecules can be used for direct LPE of graphite producing stable FLG in chloroform. Their MD simulation revealed the formation of reverse micelles on the graphene surface thereby preventing aggregation via steric stabilisation.

2.5.2.3. Proteins-based

Proteins are complex amphiphilic biopolymers, comprising both hydrophobic and hydrophilic regions on their surface. Such protein molecules could be used to efficiently exfoliate or to functionalise the GBMs to enhance their potential applications and biocompatibility. Liu et al. showed that bovine serum albumin (BSA) can functionalise and reduce the GO at the same time to produce BSA-coated rGO. Both BSA-coated GO and rGO showed excellent stability in physiological solutions and exhibited pH dependant stability. They have also demonstrated the use of protein as a ‘universal glue’ molecule on GO and rGO resulted in successful incorporation of other NPs (e.g., Au, latex, etc.) of different sizes, shapes and surface properties (Figure 2.18a-c). Lu et al. have shown β-lactoglobulin added during the hydrazine-based GO reduction process, produced stable protein-coated rGO biocomposite in aqueous solutions. Au NPs assembly on these biocomposite yielded strong surface enhanced Raman scattering (SERS) of Rhodamine 6G.
Similar to lipids, understanding the interactions between the protein molecules and GBMs are crucial as they are concerned with nanomaterial toxicity.\textsuperscript{231} For \textit{e.g.}, Hu \textit{et al.} reported fetal bovine serum (FBS) can mitigate the cytotoxicity of GO by preventing the direct contact of cell membranes with the nanomaterials.\textsuperscript{232} However, the interactions between GBMs and protein molecules could be complex as they both depend strongly on their environment (\textit{e.g.}, pH values, \textit{etc.}). Theoretical studies have predicted that amino acids/proteins could interact with GBMs through vDW interactions (Figure 2.18d)\textsuperscript{233} and strong $\pi - \pi$ interactions (Figure 2.18e).\textsuperscript{234, 235} Experimental evidences have demonstrated electrostatic interactions and hydrogen bonding between functionalised and charged GO with proteins.\textsuperscript{236} In case of rGO, the hydrophobic interactions dominate as shown by Zhang \textit{et al.}\textsuperscript{237} They have found enzyme loadings on rGO increased with increasing levels of rGO reduction, and attributing this behaviour to enhanced hydrophobic interactions between the rGO and the enzyme molecules.

![Figure 2.18: a-c) TEM images of rGO/BSA, rGO/BSA/Au NPs and GO/BSA/latex NPs, respectively. d) MD simulation study showing vDW interactions of L-leucine amino acid molecules on graphene sheet. e) MD simulation study showing typical structure of villain headpiece (HP35) protein adsorbed on the graphene surface, revealing strong $\pi - \pi$ interactions (blue regions). f) Structure of HFBI protein showing the well-defined hydrophobic regions (green patch) and schematics showing HFBI-facilitated exfoliation of graphene sheets. g, h) TEM images of graphene sheets produced from Kish graphite by sonication for 2 min (sonic probe) and 40 min (sonic bath), respectively. i) SEM image of lithographically formed HOPG micro pillars. j) TEM image of thin sheet exfoliated from HOPG pillars.]

Such strong hydrophobic interactions were exploited by other researchers by using proteins such as hydrophobin HFBI\textsuperscript{225} and lysozyme\textsuperscript{228} to directly exfoliate graphite
to graphene in aqueous media. Since these protein molecules have well-defined hydrophilic and hydrophobic regions on their surface, once exfoliated, the hydrophobic regions could readily adsorb on the basal plane of graphene resulting in stable dispersions (Figure 2.18f-j). The stability can be attributed to the reduced surface energy difference between the graphene and hydrophobic region of the protein, and the solvent and hydrophilic regions of the protein.\textsuperscript{225} Other variants of hydrophobins with specific functionalities were also shown to efficiently exfoliate graphene. Successful Au NPs decorations on such protein-exfoliated graphene molecules were also demonstrated.\textsuperscript{225, 228}
Chapter 3 – Raman Spectroscopy of Graphene and Graphene-based Strain Sensors
3. RAMAN SPECTROSCOPY OF GRAPHENE AND GRAPHENE-BASED STRAIN SENSORS

Among all the characterisation techniques used for graphene, Raman spectroscopy plays a major role mainly due to its simplicity and range of information that can be obtained. In this chapter, a short introduction to Raman spectroscopy and the characteristics of Raman bands of graphene are presented. Additionally, application of graphene as strain sensors and a brief introduction to existing strain gauge technology are also presented.

3.1. RAMAN SPECTROSCOPY

Raman spectroscopy is a form of molecular spectroscopy based on the Raman Effect or Raman scattering.\(^{238}\) When light (photons) interacts with any material or matter, some of the photons may be absorbed, reflected, transmitted or scattered. Most of the scattered photons have the same frequency (and thus energy) as that of the incident beam and this elastic process is called Rayleigh scattering. A very small number of the scattered (1 in $10^6$-$10^8$) photons have a different frequency than the incident beam and these are due to Raman scattering (inelastic scattering).\(^{239}\) These inelastic scatterings are characteristic of the chemical bonds present in the molecule allowing identification of the samples.

*Figure 3.1: a) Schematic representation of Raman Effect. b) Schematic representation of the energy level diagram of Rayleigh, Stokes and anti-Stokes Raman scattering.\(^{239}\)*
Typically the interaction of a photon with a molecule excites the molecule to a temporary virtual state. Upon relaxation, the corresponding photon gets emitted to certain vibrational state and can have three different possibilities (Figure 3.1b):

- **Rayleigh (elastic) scattering** - The incident and emitted photon have the same energy
- **Stokes Raman scattering** - The molecule absorbs energy through the creation of a phonon and thus the emitted photon shifts to lower frequency than the incident photon
- **anti-Stokes Raman scattering** - The molecule loses energy through the absorption of a phonon and thus the emitted photon shifts to higher frequency than the incident photon

Raman spectroscopy is a type of vibrational spectroscopy, and these energy differences arise from the molecular vibrations. Moreover, the intensity of Raman bands depends on the number of molecules present in different vibrational states. According to the Boltzmann distribution (which gives number of molecules present at different states of energy level) more molecules will occupy the lowest energy state (at ambient temperature). Thus at ambient temperature, due to decreased population of excited vibrational state, Stokes scattering is more intense than anti-Stokes scattering. Furthermore, with increase in temperature (increased population of excited vibrational state) the intensity of anti-Stokes scattering increases relative to Stokes scattering. The vibrational information is specific to the chemical bonds and symmetry of molecules, which act as a fingerprint of that particular molecule. Hence Raman spectroscopy is a powerful tool to probe the molecular bonds of a material.

The Raman spectrum is typically plotted as the scattered light intensity as a function of the wavenumber difference between incident and scattered light, typically in $\text{cm}^{-1}$ (which corresponds to difference between incident and scattered photon energy, 1 $\text{meV} = 8.0655447 \text{ cm}^{-1}$).\(^9\)
3.2. **Raman Spectroscopy of Graphene**

Raman spectroscopy has proved its integral part in characterising carbon-based materials, especially graphene. The main Raman bands of graphene are D (around 1350 cm\(^{-1}\)), G (around 1580 cm\(^{-1}\)) and 2D band (around 2700 cm\(^{-1}\)), among which the most prominent bands are G and 2D bands (Figure 3.2a).

![Raman spectra of bulk graphite and SLG (2.41 eV) showing the prominent G and 2D bands.](image1)

![Raman spectrum of a graphene edge showing the defect induced D and D' bands (2.41 eV).](image2)

**Figure 3.2:** a) Raman spectra of bulk graphite and SLG (2.41 eV) showing the prominent G and 2D bands. b) Raman spectrum of a graphene edge showing the defect induced D and D' bands (2.41 eV).

### 3.2.1. Raman bands of graphene

The G band is a first-order Raman scattering process, associated with the doubly degenerated E\(_{2g}\) phonon at the Brillouin zone centre, and common to all sp\(^2\) carbon systems. The two phonons responsible for G band are (Figure 3.3a,b): in-plane transverse optical phonon (iTO) and longitudinal optical phonon (LO), having same frequency at the Brillouin zone centre. The G band is a single resonance process as seen in Figure 3.3c.

![Schematic representation of Raman active E\(_{2g}\) mode.](image3)

**Figure 3.3:** Schematic representation of Raman active E\(_{2g}\) mode. a) LO phonon mode, b) iTO phonon mode. c) First order G band process.

The D band is a first-order Raman scattering process, associated with the A\(_{1g}\) radial breathing modes of hexagon rings (Figure 3.4a). The activation mechanism for D band
is a double resonance (DR) process (Figure 3.4b): the excited charge carrier undergo one-elastic scattering by a defect and one-inelastic scattering by an iTO phonon, satisfying the momentum conservation. The second-order 2D band is the overtone of D band, appearing approximately at twice the frequency of the D band, is also activated by DR mechanism. However, with 2D band, the excited charge carrier undergoes two-inelastic scattering process by iTO phonons (Figure 3.4d) before momentum is conserved. Hence, unlike the D band, 2D band doesn’t need a defect to be active, and is always present in graphitic systems with hexagonal carbon symmetry.

Figure 3.4: a) Schematic representation of A1g breathing mode. b, c) Second order DR process involving one phonon for the D band (intervalley process) and D’ band (intravalley process), respectively. d) Second order DR process involving two phonons for the 2D band. e) Two phonon involved triple resonance process in SLG.

The D band also gives rise to another disorder band: D’ band at ~1620 cm⁻¹ (close to G band). This D’ band is analogous to the D band with respect to DR mechanism, in which D’ band corresponds to “intravalley” scattering (K to K) (Figure 3.4c), whereas D band is “intervalley” scattering (K to K’) (Figure 3.4b) process.

The D band and the D’ band are highly sensitive to the disorder or defect, so in highly crystalline SLG these bands do not appear apart from the edges (Figure 3.2b).

Due to the linear electron dispersion near the Fermi level, the D band, D’ band and 2D bands are dispersive with different laser energies. They show a blue shift in their frequency with increasing laser excitation energy: D band – 50 cm⁻¹/eV, D’ band – 10 cm⁻¹/eV, and 2D band – 88-100 cm⁻¹/eV.
3.2.2. Sensitivity of Raman bands of graphene to number of layers

Raman spectra of graphene are sensitive to change in number of layers. Ferrari et al. reported the change in 2D band shape, frequency (position) and width (FWHM) with number of layers.\textsuperscript{48} SLG is characterised by a single Lorentzian peak with an intense and narrow FWHM(2D) \ (~24-30 cm\(^{-1}\)). The intensity of 2D band is \ (~2-4\) times more than G band for SLG,\textsuperscript{48} and can be explained by the triple resonance process (Figure 3.4e).\textsuperscript{240} This characteristic feature is often used to identify SLG using Raman spectroscopy.

As the number of layers increases in Bernal stacked graphene, the evolution of electronic band structure means that different DR process becomes possible, each contributing to different components in the 2D band.\textsuperscript{48, 240} For instance, in Bernal stacked BLG, four DR processes are possible resulting four components of its 2D band: 2D\(_{1B}\), 2D\(_{1A}\), 2D\(_{2A}\) and 2D\(_{2B}\) (of which 2D\(_{1A}\) and 2D\(_{2A}\) have relatively higher intensities than other two) each having FWHM(2D) \ (~24 cm\(^{-1}\)), with higher frequency.\textsuperscript{48} In Bernal stacked tri-layer graphene (TLG), the 2D bands becomes much broader and splits into 15 components as described by Malard et al.,\textsuperscript{240} but are usually difficult to deconvolute, so generally fitted with six components with FWHM(2D) \ (~24 cm\(^{-1}\)). For more than 5 Bernal stacked graphene, only two peaks; 2D\(_1\) and 2D\(_2\), similar to bulk graphite, can be observed.\textsuperscript{48}

![Figure 3.5](image)

*Figure 3.5: a) Evolution of Raman spectra at 514 nm (2.41 eV) and 633 nm (1.96 eV) with the number of layers. b) DR process 2D band for BLG. c) The four components of the 2D band in BLG at 514 nm and 633 nm.*\textsuperscript{48}

In the case of turbostratic graphene, the electronic structures of the individual layers are decoupled, and so the 2D band retains the single Lorentzian form (Figure 3.6a,b).\textsuperscript{240, 245-247} However, broadening of 2D band, with FWHM(2D) \ (~40-60 cm\(^{-1}\)),\textsuperscript{243, 245, 248} of turbostratic graphene is generally observed due to its polycrystalline nature.\textsuperscript{249}
Whereas the 2D band position was shown to blue shift \((10-20 \text{ cm}^{-1})\) with increasing number of turbostratic layers (Figure 3.6a).\(^{247,248}\)

Hence when dealing with epitaxial grown graphene, precise determination of number of layers using 2D band shape can be challenging. Therefore intensity of 2D band to G band, \(I_{2D}/I_G\), is normally considered for turbostratic graphene. In general, \(I_{2D}/I_G > 1\) is considered as SLG, and \(I_{2D}/I_G \sim 1\) is evidenced as turbostratic FLG.\(^{240,245}\)

The G band is also sensitive to number of layers and its intensity has been shown to increase with number of layers.\(^{250,251}\) This method was followed by Ni et al. to determine number of layers in their experiments.\(^{251}\) Graf et al. have shown that the intensities of G band to 2D band, \(I_G/I_{2D}\) (inverse of \(I_{2D}/I_G\)), increases with number of layers (Figure 3.6c).\(^{250}\) They have also showed that the G band position shifts to lower wavenumber as the number of layers increases (Figure 3.6d). Similar results were reported by Das et al.\(^{252}\)

3.2.3. Sensitivity of Raman bands of graphene to disorders

The otherwise forbidden D and D’ bands appear in Raman spectra of graphene when Raman active defects such as edges, vacancies or sp\(^3\)-type are present in graphene lattice.\(^9,57,253\) However, Raman inactive defects such as charged impurities,\(^{55,254}\) intercalants,\(^{255}\) and strain,\(^{256,257}\) do not generate D band, but affects the G and 2D bands characteristics.\(^9\)

The edge of graphene acts as defects, generating the D band, except for perfectly aligned zigzag edge.\(^{56}\) Whereas, the D’ band appears for both zigzag and armchair edges,
as it is independent of edge structure due to its “intravalley” DR process.\textsuperscript{258} Casiraghi et al. showed that when a D band is present and FWHM(G) < 16 cm\textsuperscript{-1}, the D band is due to edges.\textsuperscript{254} If the FWHM(G) > 16 cm\textsuperscript{-1} and the position of G band blue shifts from 1580 cm\textsuperscript{-1}, the D band is due to structural disorder.\textsuperscript{254}

The intensity of D band is not related to the number of graphene layers but only to the amount of disorder.\textsuperscript{56, 259} In general, the $I_D/I_G$ ratio indicates the amount of disorder (bulk\textsuperscript{259, 260} or edges\textsuperscript{258}) present in the carbon materials, and has been studied widely for graphene. The $I_D/I_G$ is related to the defect quantity, represented by $L_D$(inter defect distance), by the relationship: $I_D/I_G \propto 1/L_D^2$ for 25 nm < $L_D$ < 4 nm.\textsuperscript{260, 261} From this relationship, it is clear that higher the amount of disorder present in graphene lattice, higher the $I_D/I_G$ ratio.

It was also found that the FWHM(G) increases with bulk disorder,\textsuperscript{260} but doesn’t increase upon introducing edges.\textsuperscript{56} Therefore, by combining FWHM(G) with $I_D/I_G$ ratio, information regarding bulk disorder in graphene can be obtained,\textsuperscript{9, 260} i.e., the $I_D/I_G$ ratio will positively correlate with FWHM(G) in presence of bulk disorder and no correlation in absence of bulk disorder.

Moreover the $I_D/I_G$ ratio gives information on edges\textsuperscript{56} or bulk disorder,\textsuperscript{260} but it cannot discriminate between them. However, Eckmann et al. recently showed that $I_D/I_{D'}$ ratio is very sensitive to the type of defects.\textsuperscript{57} They reported $I_D/I_{D'} \approx 3.5$ for boundaries (edges), $\approx 7$ for vacancies, $\approx 13$ for sp\textsuperscript{3} and values between vacancies and sp\textsuperscript{3} for substitutional impurities.\textsuperscript{57, 253} This makes Raman spectroscopy a powerful tool in characterising type of defects on graphene.

### 3.2.4. Raman spectroscopy in liquid-phase exfoliated graphene

Raman spectroscopy is widely used for characterising LPE graphene in complementary to AFM/TEM etc. In general, the shape of 2D band for LPE graphene is used to derive information about the number of layers, i.e., efficiency of exfoliation.\textsuperscript{54, 59} However, often the spectra are obtained from drop casted or vacuum filtered samples, where the aggregation of individually exfoliated flakes lead to a 2D band with broad and symmetric peak, similar to turbostratic graphene.\textsuperscript{59, 79, 262}
Moreover, the LPE graphene mostly contains a D band (in bulk measurement), and the $I_D/I_G$ ratio are often related to edges produced due to the LPE process. The Coleman group, who carried out extensive study\textsuperscript{59-63, 77} on sonication-assisted production of graphene, have shown that sonication does not produce any body defects apart from edges. Assuming the flakes are rectangular, they have probed the edge-length-to-flake-area ratio, $\langle w \rangle^{-1} + \langle L \rangle^{-1}$, with the $\langle I_D/I_G \rangle$ ratio and showed that they scale linearly (Figure 3.7a), suggesting the increase in $I_D$ is mainly due to the formation of new edges.\textsuperscript{3}

![Figure 3.7: a) $\langle I_D/I_G \rangle$ ratio vs $\langle w \rangle^{-1} + \langle L \rangle^{-1}$ for NMP-sonicated graphene (36-192 hr sonication, 500-4000 rpm centrifugation). The straight line in this plot represent that the formation of new edges are responsible for increase in $I_D/I_G$ ratio. The straight line also coincides with the starting graphite, $\langle I_D/I_G \rangle_{powder}$.\textsuperscript{59} b) $I_D/I_G$ as a function of FWHM(G) for 120 min NMP-sonicated graphene showing a positive linear correlation. Upon annealing, effectively healing the basal plane defects, the correlation is lost.\textsuperscript{76}

On the contrary, Bracamonte et al. reported that sonication of graphite to produce graphene induce basal plane defects by probing the relationship between the $\langle I_D/I_G \rangle$ ratio to FWHM(G).\textsuperscript{76} As mentioned in the previous section, FWHM(G) doesn’t increase upon introducing edges whereas it increases with bulk disorder. They have found that shorter sonication times doesn’t have any correlation between $I_D/I_G$ ratio to FWHM(G), whereas it positively correlates for longer sonication times. Moreover, upon annealing the sample, thereby healing the body defects, they observed loss of correlation (Figure 3.7b), suggesting perhaps sonication does introduce basal plane defects such as topological defects (like pentagon-heptagon pairs).\textsuperscript{76} These studies on LPE graphene using Raman spectroscopy show its significance in deriving wealth of information.
3.2.5. Sensitivity of Raman bands of graphene to strain

Changes to crystal phonons can be caused by external perturbations such as doping, strain, etc. Strain alters/affects the crystal symmetry and its phonon frequency. In case of graphene, such changes have huge impact on its Raman bands: The tensile strain causes phonon softening (red shift) and the compressive strain causes phonon hardening (blue shift). The rate of this change is given by Gruneisen parameter (γ), and is crucial for quantifying amount of strain in the system. First detailed investigation on Raman bands of graphene upon straining and its Gruneisen parameters was reported by Mohiuddin et al. Clear G band splitting (G+ and G−) was observed (Figure 3.8a,c) at uniaxial strains greater than 0.4%, among which one component was polarised parallel (G+) to the strain and another was polarised perpendicular (G−) to the strain (Figure 3.8d). Both the components shifted to lower wavenumber with shift rates of ~ -10.8 cm⁻¹/% strain (G+) and ~ -31.7 cm⁻¹/% strain (G−). Whereas no splitting of the 2D band was observed with a measured shift rate of ~ -64 cm⁻¹/% strain (Figure 3.8b,e).
In case of uniaxial strain the Gruneisen parameters for G ($\gamma_G^\text{uniaxial}$) and 2D ($\gamma_{2D}^\text{uniaxial}$) band are given by following equations as determined by Mohiuddin et al.\textsuperscript{256}

\[
\gamma_G^\text{uniaxial} = -\frac{\Delta \omega_G^+ + \Delta \omega_G^-}{2\omega_G^0(1-\nu)\varepsilon} \quad \text{Equation 3.1}
\]

\[
\gamma_{2D}^\text{uniaxial} = -\frac{\Delta \omega_{2D}}{\omega_{2D}^0(1-\nu)\varepsilon} \quad \text{Equation 3.2}
\]

Where, $\Delta \omega_G^+/\varepsilon$, $\Delta \omega_G^-/\varepsilon$ and $\Delta \omega_{2D}/\varepsilon$ are the uniaxial strain shift rates of G\textsuperscript{+} mode, G\textsuperscript{-} mode and 2D band, respectively, $\omega_G^0$ and $\omega_{2D}^0$ are initial position (frequency) of G and 2D band before straining, respectively, and $\nu$ is the Poisson’s ratio of the substrate. It is clear from the Equation 3-1 and Equation 3-2 that the Poisson ratio plays an important role in determining the strain in the system. It should be noted when analysing the strain in a system in which the graphene is adhered well to a substrate, the Poisson ratio of the substrate should be used rather than the in-plane Poisson ratio of graphite. Whereas in case of biaxial graphene, the Gruneisen parameter of G and 2D (or D) band is given by,\textsuperscript{256,267}

\[
\gamma_m^\text{biaxial} = -\frac{\Delta \omega_m}{2\omega_m^0\varepsilon} \quad \text{Equation 3.3}
\]

Where $m$ being G or 2D band, $\Delta \omega_m/\varepsilon$ is the biaxial strain shift rate of G or 2D band and $\omega_m^0$ is the initial frequency of G or 2D band before straining. Biaxial strains are more suitable for calculating the $\gamma$ since it does not depend on the Poisson ratio. Several groups have studied the effect of strain on Raman bands of graphene and have calculated various values for $\gamma$, and it is still a matter of debate.

The uniaxial or biaxial strain can be applied to graphene via various ways including; inducing strain by utilising the difference in thermal expansion coefficient of substrate and graphene\textsuperscript{268}, piezoelectric substrates\textsuperscript{266}, and the most common method by stretching or bending the graphene deposited on a flexible substrates such as PMMA\textsuperscript{32,269-274}, PET\textsuperscript{256,263,264} and PDMS.\textsuperscript{265,275-277} The Table 3-1 reports the various literature values of shift rates of Raman bands of graphene (mainly G and 2D band are presented). It also includes calculated or experimentally derived Gruneisen parameter values.
Table 3-1: Shift rates of Raman bands of graphene under strain and its Gruneisen parameters ($\gamma$) values reported in the literature

<table>
<thead>
<tr>
<th>Reference</th>
<th>Laser (nm)</th>
<th>Strain</th>
<th>Graphene type</th>
<th>Shift rates (cm$^{-1}$/% strain)</th>
<th>Gruneisen parameter ($\gamma$)</th>
<th>Maximum Strain induced (%)</th>
<th>Substrate/Strain induction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yu et al.$^{264}$</td>
<td>532</td>
<td>Uni (T)</td>
<td>MC SLG</td>
<td>G: -8</td>
<td></td>
<td>0.6</td>
<td>PET/Bending</td>
</tr>
<tr>
<td>Ni et al.$^{263}$</td>
<td>532</td>
<td>Uni (T)</td>
<td>MC SLG</td>
<td>G-: -14.2, G+: -27.8, 2D: -21.9</td>
<td>1.24 (from CNT)$^{278}$</td>
<td>0.8</td>
<td>PET/Stretching</td>
</tr>
<tr>
<td>Mohiuddin et al.$^{256}$</td>
<td>514</td>
<td>Uni (T)</td>
<td>MC SLG</td>
<td>G: -31.7, G-: -10.8, G+: -64</td>
<td>2D$^g$ = 3.55, G$^g$ = 1.99, 2D$^T$ = 2.7, G$^T$ = 1.8</td>
<td>1.3</td>
<td>PET/2&amp;4 point bending</td>
</tr>
<tr>
<td>Huang et al.$^{265}$</td>
<td>532</td>
<td>Uni (T)</td>
<td>MC SLG</td>
<td>G: -12.5 ± 2.6, G-: -5.6 ±12, G+: -21 ±4.2</td>
<td>G$^g$ = 0.69 ±0.14</td>
<td>3.1</td>
<td>PDMS/3 point bending</td>
</tr>
<tr>
<td>Tsoukleri et al.$^{269}$</td>
<td>514</td>
<td>Uni (T)</td>
<td>MC SLG</td>
<td>G: -59.1 (b), G-: -65.9 (s)</td>
<td></td>
<td>0.9 (b), 1.3 (s)</td>
<td>PMMA/Cantilever beam bending</td>
</tr>
<tr>
<td>Gong et al.$^{32}$</td>
<td>633</td>
<td>Uni (T)</td>
<td>MC SLG</td>
<td>G: ~ -40 (s)</td>
<td></td>
<td>0.7</td>
<td>PMMA/4 point bending</td>
</tr>
<tr>
<td>Frank et al.$^{270}$</td>
<td>785</td>
<td>Uni (T)</td>
<td>MC SLG</td>
<td>G: -31.4 ± 2.8, G-: -9.6 ±1.4</td>
<td></td>
<td>0.62</td>
<td>PMMA/Cantilever beam bending</td>
</tr>
<tr>
<td>Ding et al.$^{266}$</td>
<td>532</td>
<td>Bi</td>
<td>MC SLG</td>
<td>G: -57.3, 2D: -160.3</td>
<td>2D$^g$ = 2.98, G$^g$ = 1.8</td>
<td>0.1 to 0.15</td>
<td>PMMA/Piezoelectric actuator</td>
</tr>
<tr>
<td>Bissett et al.$^{277}$</td>
<td>532</td>
<td>Uni (T)</td>
<td>MC SLG</td>
<td>G: -49, 2D: -72</td>
<td>2D = 2.7 from ref.$^{256}$</td>
<td>0.25</td>
<td>PDMS/Stretching</td>
</tr>
<tr>
<td>Zabel et al.$^{279}$</td>
<td>488</td>
<td>Bi (T)</td>
<td>MC SLG</td>
<td>G: -57, 2D: -140</td>
<td></td>
<td>1</td>
<td>SiO$_2$/Bubbles</td>
</tr>
<tr>
<td>Authors</td>
<td>Reference</td>
<td>Strain Type</td>
<td>Material</td>
<td>Initial Displacement</td>
<td>2D (elastic)</td>
<td>G (elastic)</td>
<td>Notes</td>
</tr>
<tr>
<td>------------------</td>
<td>-----------</td>
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<td>-------------------------------------------</td>
</tr>
<tr>
<td>Raju et al.</td>
<td>280</td>
<td>Uni (T)</td>
<td>MC SLG</td>
<td>~ - 53 (s)</td>
<td>2D = 2.7</td>
<td></td>
<td>PMMA/4 point bending</td>
</tr>
<tr>
<td>Li et al.</td>
<td>281</td>
<td>Uni (T)</td>
<td>CVD SLG</td>
<td>- 12.8</td>
<td>2D = 3.55</td>
<td></td>
<td>PET-PMMMA/4 point bending</td>
</tr>
<tr>
<td>Xu et al.</td>
<td>282</td>
<td>Uni (T)</td>
<td>CVD SLG</td>
<td>- 36</td>
<td></td>
<td></td>
<td>PET/Stretch</td>
</tr>
<tr>
<td>Metzger et al.</td>
<td>283</td>
<td>Bi (C)</td>
<td>MC SLG</td>
<td>203 ± 20</td>
<td>2D&lt;sup&gt;E&lt;/sup&gt; = 3.8, G&lt;sup&gt;E&lt;/sup&gt; = 2.4</td>
<td>- 0.066 ± 0.006</td>
<td>SiO&lt;sub&gt;2&lt;/sub&gt;/Shallow depression</td>
</tr>
<tr>
<td>Tsoukleri et al.</td>
<td>269</td>
<td>Uni (C)</td>
<td>MC SLG</td>
<td>25.8 (b) 59.1 (s)</td>
<td></td>
<td>- 0.9 (b) 1.2 (s)</td>
<td>PMMA/Cantilever beam bending</td>
</tr>
<tr>
<td>Frank et al.</td>
<td>270</td>
<td>Uni (C)</td>
<td>MC SLG</td>
<td>22.3 to 33.1 5.5 to 10.1</td>
<td></td>
<td>- 0.7</td>
<td>PMMA/Cantilever beam bending</td>
</tr>
</tbody>
</table>

Abbreviations (where applicable) related to Table 3-1:


Uni – Uniaxial strain, Bi – Biaxial strain, (T) – Tension, (C) – Compression

(b) – Bare graphene on substrate without any top coat, (s) – Graphene sandwiched between substrate and a top coat (polymer)

Superscripts: <sup>E</sup> – Experimentally calculated, <sup>T</sup> – Theoretical calculation
From Table 3-1 it is evident that the shift rates of Raman bands with strain varies with different groups. The G band shift rate varies from -60 (tension) to 80 (compression) cm\(^{-1}\)/% strain for biaxial strain. Whereas the 2D band has more varied shift rate values and is dependent on the wavelength used due to its dispersive nature with laser energies. One of the main reasons for this variation of shift rates could be variation in interfacial stress transfer between graphene and the substrate.\(^\text{269,277}\)

Most of the studies (for uniaxial strain) had been carried out inducing strain by bending or stretching the MC graphene deposited on polymer substrate (vdW interaction), without any top coat of polymer. During the deformation in order to prevent the slippage of graphene the strain could be induced very slowly or the graphene can be coated with a polymer top coat to ensure efficient stress transfer. For instance, Yu et al.\(^\text{264}\) and Ni et al.\(^\text{263}\) assumed that there is no slippage during the deformation, which might have resulted in the lower shift rates. Huang et al.\(^\text{265}\) deposited narrow strips of titanium were deposited to clamp the graphene, but the measured shift rates were small compared to other studies. On the other hand, Mohuddin et al. applied strain very slowly for loading, unloading and a loading cycle over two different set ups to compare the shift rates, and obtained 2D band shift rate of \(~-64\) cm\(^{-1}\)/% strain for uniaxial strain, in excellent agreement with the Gruneisen parameters.\(^\text{256}\) A top coat of polymer was applied on MC graphene by Tsoukleri et al.\(^\text{269}\), Frank et al.\(^\text{270}\), and Gong et al.\(^\text{32}\) in their studies to prevent slippage of graphene during deformation and to reduce the uncertainty of transfer of applied strain to graphene. Despite the topcoat, slight slippage and compression was observed during unloading cycle by Gong et al.\(^\text{32}\)

Other plausible reasons could be; the type of substrates (PMMA, PET, PDMS etc.) that are used in these studies, as substrates have different Poisson ratio could affect the uniaxial strain applied on to graphene; and the shape of the studied flake itself.

The CVD graphene, however, is a continuous film of SLG with grain boundaries (acting as defects). Bissett et al. reported G band blue shift (41 cm\(^{-1}\)/% strain) for CVD graphene as opposed to red shift (-49 cm\(^{-1}\)/% strain) for MC graphene during uniaxial strain (0.25 % strain) on PDMS substrate.\(^\text{277}\) They attributed this anomalous behaviour to the stress response of 5- or 7- membered rings along the grain boundaries.
Moreover, due to the asymmetry in graphene lattice caused by uniaxial strain, the band structure of graphene gets disrupted, causing splitting of G, G' and 2D bands. However, the G band splitting was not clear for strains below 0.4%, rather band broadening was observed (Figure 3.8a,c).

3.2.6. Stress transfer studies in graphene nanocomposites using Raman Spectroscopy

The sensitivity of Raman bands to strain facilitates the study of the interfacial stress transfer between graphene and the matrix in nanocomposites. This use of micro-Raman spectroscopy builds upon over 30 years of work in systems such as carbon and polymer fibres. Gong et al. carried out the first stress transfer study on graphene using a model experimental composite system comprising of a SLG sandwiched between two polymer layers. In this case, as the graphene flake is discontinuous and reinforcing the composite matrix, stress transfer from the matrix to the flake is assumed to take place through a shear stress at the flake/matrix interface as shown in (Figure 3.9a,b). When the system is subjected to axial stress, $\sigma_1$, parallel to the flake axis, the perpendicular lines drawn to the flake becomes distorted since the Young’s modulus of the matrix is much less than that of the flake. This induces a shear stress at the flake/matrix interface. The axial stress in the flake will build up from zero at the flake ends to a maximum value in the middle of the flake (Shear lag model). The uniform strain assumption means that, if the flake is long enough, in the middle of the flake the strain in the flake equals that in the matrix. Since the flakes have much higher Young’s modulus it means that the flakes carry most of the stress in the composites. Gong et al. produced graphene by MC and subsequent deposition by Scotch tape (Figure 3.9c, d). Raman spectroscopy was then used to map the local strain distribution across the flake and the data fitted to shear-lag theory which had been adapted to a planar filler.
Figure 3.9: a, b) Deformation patterns for a discontinuous flake in a polymer matrix. c) Optical micrograph showing SLG. d) Schematic diagram (not to scale) of composite showing the sandwich arrangement. e and f) Variation of axial strain with position across the SLG in x-direction at 0.4 % and 0.6 % matrix strain, respectively. g) Representation of flake strain with respect to flake size using Shear-lag theory.

Figure 3.9e shows the variation of axial strain (determined from the Raman 2D band shift with strain) with position across the SLG in the x-direction at 0.4 % applied matrix strain. It can be seen that the strain builds up from the edges of the flake and reaches the maximum in the middle and eventually falls off on the other end. This behaviour is analogous to short fibre reinforcement as explained by Shear-lag model. Increasing the applied strain to 0.6 % results in interface failure as evidenced in Figure 3.9f, suggesting poor interfacial adhesion. This is reflected in the measured interfacial shear stress (ISS) at edges of flake which is \( \sim 1 \text{ MPa} \), an order of magnitude lower than carbon fibre composites (\( \sim 20 - 40 \text{ MPa} \)). In case of fibre reinforcement, critical length, \( l_c \), \( 2 \times \) the distance over the strain rises from fibre ends to 90 % of the plateau level, is an important parameter, and is given by,
\[ l_c = \left( \frac{\sigma_f d}{\tau_c} \right) \]

Equation 3.4

Where, \( \sigma_f \) is the tensile strength of the fibre, \( d \) is the diameter of the fibre and \( \tau_c \) is the interfacial shear strength between matrix and fibre. The critical length of graphene to achieve effective reinforcement as calculated by Shear-lay model is \( > 3 \, \mu m \) (more preferably \( > 30 \, \mu m \)). It can be explained from Figure 3.9g when the flakes are larger, the strain in the flake matches the strain in the matrix and therefore resulting in good stress transfer. But if the flakes are smaller, the strain in the flakes cannot build up to the matrix strain and hence leading to poor stress transfer. In other words, the edge effect of graphene dominates in such composites and the graphene effectively acts as a short reinforcement. One way to enhance the stress transfer is by increasing the interfacial adhesion by means of functionalising the graphene surface or the edges.\(^4\), \(^32\) Doing so, reinforcement could be even achieved from smaller flakes (Equation 3.4) as a result of better interface.

Jiang et al. carried out similar interfacial stress transfer studies on MC SLG on PET substrate by applying strains up to 7 \% (stretching).\(^{288}\) They have found that in their system, the failure mechanisms occurs during straining and relaxing (unloading) by shear sliding and buckling, respectively. The calculated ISS by Shear-lag theory was 0.46-0.69 MPa and is comparable to Gong et al.\(^{32}\), again suggesting a weak interface. The critical tensile strain for interfacial sliding was found to be 0.3 \%. The critical compressive strain for onset of buckling is around \(~ -0.7 \%)\(^{288}\), which is similar to the experimentally observed critical buckling strain by Tsoukleri et al.\(^{269}\) and Androulidakis et al.\(^{289}\) for sandwiched graphene systems.

Recently Anagnostopoulos et al. studied the interfacial stress transfer in systems MC SLG deposited on SU-8/PMMA substrate.\(^{290}\) They have studied the local strain distribution across a graphene flake with 100 nm steps. Such small distances, compared to \(~ 1 \mu m \) steps by Gong et al.\(^{32}\), have allowed them to investigate the stress transfer at the edge of the flake in more detail. They have reported that the observed maximum ISS (0.4 MPa) does not occur at the edge of the flake, rather \(~ 2 \mu m \) from the edge, deviating the Shear-lag theory.\(^{290}\) However, they attribute this behaviour mainly to the presence of doping and residual compressive strains (from fabrication step) near the edges.
Chapter 3 – Raman Spectroscopy of Graphene and Graphene-based Strain Sensors

The shear-lag analysis have been extended and adapted for CVD graphene as reported by Li et al.\textsuperscript{281} They have studied the deformation of CVD graphene on PET substrates which had wrinkles in the order of ~1.2 µm domains (graphene islands, Figure 3.10a). The observed 2D band shift rate of such system was ~ -12.8 cm\textsuperscript{-1}/% strain (Figure 3.10b), as opposed to ~ -52.5 cm\textsuperscript{-1}/% strain\textsuperscript{288} for flat MC SLG on PET. This lower shift rate, according to shear-lag theory, is due to the short fibre effect (Figure 3.10c). In this case, despite having a fairly good interface between PET and the CVD graphene, each graphene island acts as a short fibre, smaller than the \( l_c \), such that the stress transfer is non uniform (Figure 3.10d) within the islands leading to lower shift rates.\textsuperscript{281}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig310}
\caption{a) SEM image of CVD graphene showing the graphene islands (wrinkles). b) Shift rate of 2D band under uniaxial strain. c) Schematic diagram explaining the short fibre effect in CVD graphene system with wrinkles. d) Predicted strain distribution within ~1.2 µm diameter graphene island for applied PET strain of 0.4 %.\textsuperscript{281}}
\end{figure}

Such lower shift rates upon straining was not reported by Bissett et al. who investigated Raman band shifts of CVD graphene on PDMS, and reported a 2D band shift rate of ~ -72 cm\textsuperscript{-1}/% strain.\textsuperscript{277} It has to be noted that they have used more deformable PDMS substrate \( (v_{PDMS} = 0.5) \) than Li et al.\textsuperscript{281} (PET, \( v_{PET} = 0.35 \)), that could have induced a biaxial strain. Moreover, they have assumed the MC SLG and CVD SLG would have same
shift rate, hence direct comparison of shift rates/interfacial stress transfer are not possible.

The experimental studies using AFM revealed the Young’s modulus of SLG is 1 TPa, making SLG attractive for polymer reinforcement. Whereas, studies conducted on model graphene composites on various graphene layers, including SLG, BLG, TLG and many-layer graphene by probing Raman 2D band shift rates, suggests this might not be the case. Gong et al. have reported that BLG with a top polymer coat behaves similar to SLG having same shift rate, and for BLG without a top polymer coat has lower shift rate than SLG, suggesting poor stress transfer between the layers of flake, similar to CNTs. The TLG and many-layer graphene had further lower shift rates compared to SLG and BLG suggesting, again confirming the stress transfer between the flakes are poor. Despite this observation, they have showed that more volume fraction of graphene between the minimum separations of polymer chains (> 1 nm) is required for effective reinforcement. It was found that the optimum number of layers to effectively reinforce the polymer matrix is 3-4 layers graphene rather than SLG or BLG.

![Figure 3.11:](image)

Figure 3.11: a) Raman 2D band shift with strain for SLG, BLG, TRG and many layer graphene (fitted using single Lorentzian). b) Schematic diagram of the microstructure of graphene-based nanocomposites with SLG and TLG as reinforcements. c) Effective Young’s modulus and maximum volume fraction for different polymer layer thickness as function of number of layers. d) Maximum nanocomposite modulus predicted for different polymer layer thickness as a function of number of layers showing optimum number of layers around 3-4 layers.
These studies are beneficial in terms for using graphene as reinforcement in bulk composites, because, the bulk scale production of graphene indeed produces few-layer graphene onwards with various thickness and lateral dimensions.\textsuperscript{50, 118, 127} For instance, it has been shown by electrochemical synthesis FLG (with little defects) with different lateral dimensions can be produced in large scale.\textsuperscript{123} Bulk composites produced, in PP\textsuperscript{124} and PMMA\textsuperscript{125}, from the 20 µm (electrochemical synthesis method) diameter flakes showed increased reinforcing ability (even at 20 wt.%) than 5 µm diameter flakes, in agreement with the above argument by Gong \textit{et al.}\textsuperscript{274} Whereas the LPE method produces FLG flakes that are few microns or sub-micron in diameter, which showed very little reinforcement in polyvinyl alcohol (PVA) composite films.\textsuperscript{291}

In some cases, researchers have followed G band shift with strain to study the stress transfer in bulk composites. Srivastava \textit{et al.} have observed G band shift rate of -2.4 cm\textsuperscript{-1}/% strain for thermally-reduced GO (TRGO)/PDMS composites compared to -0.1 cm\textsuperscript{-1}/% strain for single-walled carbon nanotubes (SWNT)/PDMS composite, suggesting enhanced load transfer effectiveness for graphene.\textsuperscript{275} While Xu \textit{et al.} observed slightly higher G band shift rate of -4.2 cm\textsuperscript{-1}/% strain for TRGO/PDMS composites, which was attributed to slight degree of functionalisation and doping during the sample preparation procedure.\textsuperscript{292}

In case of GO, where the 2D band is absent and the G band is overlapped with D’ band, the D band could be followed for stress transfer studies in composites as described by Li \textit{et al.}\textsuperscript{293} With the knowledge of the shift rates and Gruneisen parameter, effective Young’s modulus of GO was derived, and found it decreased with increased loading. Similar results were obtained when using GO as reinforcements in bulk composites\textsuperscript{294}, contrary to unfunctionalised graphene produced by electrochemical methods\textsuperscript{124, 125}, suggesting that increasing the concentration of GO (more than 1 wt.%) resulted in aggregation within the composites with deteriorating properties.

All these studies demonstrate the unambiguous importance of Raman band shifts with strain. Most importantly all the Raman bands of graphene are sensitive to strain. Even though the 2D band exhibits a much broader line width compared to G band, its high Raman sensitivity/shift rate (Table 3-1) allows even small strain/stress variations to
be easily detected, making this band highly sensitive upon application of tensile or compressive strain/stress. This high strain sensitivity of Raman 2D band of graphene makes graphene composite/coatings as a potential candidate for optical Raman-based strain sensors, a concept which is explored in this thesis.

### 3.3. APPLICATIONS OF GRAPHENE

Given the extraordinary properties of graphene, various potential multifunctional applications are possible, ranging from conductive inks to structural composites (Figure 3.12).

![Figure 3.12: Potential applications of graphene in different sectors ranging from conductive inks to chemical sensors, light emitting devices, composites, energy, touch panels and high frequency electronics.](image)

Among the applications, the use of graphene in the area of sensors has been studied widely. The 2d nature of graphene leads to a high surface area, resulting in a high degree of sensitivity to changes in the environment, making it a suitable candidate as sensors; ranging from gas sensors, mass sensors, bio sensors, optical sensors to strain sensors. It has been shown that the absorption of individual gas molecules (e.g., NO₂) can be detected using MC graphene, and a 3d foam containing FLG produced via CVD can detect NO₂ and NH₃ at an order of magnitude lower concentration than commercial
sensors. Micro-engineered vibrating beam sensors have taken advantage of graphene’s high stiffness and low density to act as mass sensors with detection limits up to 2 zeptograms. Graphene has also been explored as photodetectors and has demonstrated photodetection from the visible to mid-IR (higher than 1 ampere/watt) spectrum at room temperature. Graphene have also been employed as biosensors, e.g., pH change detection in aqueous environment, DNA sensing, real-time glucose detection and protein monitoring. Functionalised graphene have also used to detect specific protein molecules with immunoglobulin E aptamer functionalised MC graphene for selective detection of immunoglobulin E proteins and biotin functionalised rGO (using PEG and PEI) for selective detection of avidin.

3.3.1. Graphene-based strain sensors

It has been shown that graphene can be elastically stretched up to 20 % strain, this flexibility combined with its optical transparency has made graphene a strong candidate for flexible electronics. This flexibility also meant that graphene can be used as a strain sensor, by following its piezoresistive behaviour. The sensitivity of such sensors is normally measured by gauge factor (GF), which is the change in resistance over the strain and is generally given by:

\[ GF = \frac{(\Delta R/R)}{(\Delta L/L)} = \frac{(\Delta R/R)}{\varepsilon} \]

Equation 3.5

Where, \( \Delta R/R \) is normalised resistance variation, \( \Delta L/L \) is fractional change in length, and \( \varepsilon \) is the strain. The GF of commercially available resistance based strain sensors are in range of 2 to 5.

Different types of graphene-based strain sensors have been reported and the literature is summarised in the following sections.

3.3.1.1. Strain sensors based on SLG sheets

The strain sensitivity of graphene is studied normally by inducing structural deformation directly on the graphene or graphene supported on a deformable substrate. Huang et al. carried out nanoindentation experiments on suspended MC SLG while
measuring the electrical response in situ during deformation, and found little change in electrical resistance up to 3% strain, with GF ~ 1.9.\textsuperscript{308}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3_13.png}
\caption{a) AFM image of rippled MC graphene ribbons.\textsuperscript{276} b) Resistance response of the graphene ribbons with strain. The inset is the optical images before and after straining (20% strain).\textsuperscript{276} c) Current-Voltage curves of CVD graphene strain sensor devices under different uniaxial strain. Inset show the schematics of strain sensor on PDMS substrate.\textsuperscript{309} d) Relative variation of resistance of the graphene strain sensor devices as a function of uniaxial strain (GF).\textsuperscript{309}}
\end{figure}

Wang et al. demonstrated the use of rippled SLG on PDMS to obtain strain sensors with GF ~ -2.\textsuperscript{276} They fabricated the strain sensors by depositing the MC graphene on a pre-strained PDMS substrate, and the ripples were generated upon relaxing the substrate (Figure 3.13a). The resistance of the device decreased on straining (up to 20% strain) due to flattening of the ripples, suggesting its sensitivity to strain (Figure 3.13b).

The use of CVD SLG as a strain sensor was first demonstrated by Lee et al.\textsuperscript{310} The resistance increased (~492 to ~522 kΩ) upon application of strain (1% strain), giving a GF of 6.1.\textsuperscript{310} Later Fu et al. reported improved strain sensitivity of strain sensor produced from CVD SLG on PDMS substrates with GF ~ 151 (Figure 3.13c,d).\textsuperscript{309} They have also noticed the resistance decreased slightly during the initial straining cycle followed by increase in resistance in the subsequent straining cycles. The decrease in resistance at the
initial cycles was attributed to the relaxation of residual strains (from fabrication steps) in their CVD/PDMS films (Figure 3.13c).\textsuperscript{309} It should be noted that several factors including defects, grain boundaries, residual strains, transfer methods and device fabrication procedure can influence the performance a strain sensor, leading to large variations in the strain sensitivity and hence the GF.

### 3.3.1.2. Strain sensors based on interconnected graphene sheets

Graphene produced by other methods, for instance LPE, mainly produces FLG. These FLG were explored as strain sensors by Hempel et al. by forming percolated networks on plastic substrates by spray coating.\textsuperscript{311} The working principle of the strain gauge was described as, when the strain was applied, number of connections between the overlapped graphene sheets decreases, which reduces the conductive path thereby increasing the resistance (Figure 3.14a). The advantage of this technique is that it allows sensors to be fabricated with tuneable GF (Figure 3.14b,c), by increasing or decreasing the flake number density (by varying the amount of graphene sprayed), thereby altering the initial resistance of the device, which could be used for different applications.\textsuperscript{311}

![Figure 3.14](image)

**Figure 3.14:** a) Modelling of percolation through graphene flake network under strain. Representative of voltage drop at fixed current in a graphene film at various strain levels. b) Curves of normalised change in electrical resistance vs. strain for several strain gauges. c) Extracted GF vs initial resistance for different devices.\textsuperscript{321}

Interconnected 3d graphene sheets can be obtained by other methods such as CVD growth on Ni\textsuperscript{312} or Cu\textsuperscript{312, 313} templates. Interconnected graphene foams or woven fabrics with enhanced surface area were obtained after etching the metal. These foams or woven fabrics was supported on polymer substrate (PDMS)\textsuperscript{314} or impregnated by polymers (PDMS)\textsuperscript{312} to create composites which were demonstrated as strains sensors. Li et al. demonstrated the synthesis of highly sensitive strain sensors based on graphene woven fabrics on PDMS substrate (GF ~ 10\textsuperscript{3} under 2-6 % strain, and 10\textsuperscript{6} for > 7 % strain)
which has polycrystalline structure and high crack density.\textsuperscript{314} When the strain was applied the width of the overlapped regions changed, rather than deforming individual sheets, increasing the resistance. Additionally the resistance also increased due to decreased conduction \textit{via} the cracks present in the networks, leading to the higher strain sensitivity.\textsuperscript{314}

Recently Feng \textit{et al.} demonstrated the synthesis of 3d stretchable and highly conductive micro-wrinkled rGO/PDMS composites.\textsuperscript{315} These composites were shown to have stable electrical conductivity and failure resistance up to 500 fatigue deformation cycles (for 10 \% - 30 \% strain levels). The advantage of this technique is that due to the isotropic nature of rGO shrinkage during fabrication procedure these sensors can be stretched in any direction within the plane of the film.

### 3.3.1.3. Strain sensors based on bulk graphene composites

Graphene bulk composites have been demonstrated as strain sensors with varying GFs. These systems work by the strain deforming the percolated network in the composites, and hence changing its impedance. Kim \textit{et al.} demonstrated 3 wt.\% graphene/epoxy bulk composites as strain sensors with a GF \( \sim 11.4 \).\textsuperscript{316} Eswaraiah \textit{et al.} have observed 2 wt.\% graphene/PVDF based composites out performed CNT/PVDF composites in strain sensing.\textsuperscript{317} The flexibility of 2d graphene induced rapid changes in contact and tunnelling resistance leading to the enhanced strain sensitivity than CNT composites.\textsuperscript{317} In another study by Eswaraiah \textit{et al.}, strain sensors based on rGO/PVDF was produced by \textit{in situ} reduction of GO in PVDF matrix during composite fabrication, and exhibited good strain sensitivity of GF \( \sim 12.1 \) (2.2 wt.\%) due to the availability of sufficient interconnections.\textsuperscript{318} Increasing the rGO concentration decreased the GF, suggesting the physical movements of the conductive fillers were attenuated due to saturation, reducing the changes in electrical resistance.\textsuperscript{318}

### 3.3.1.4. Wearable graphene sensors

The piezoresistivity of graphene has also been explored in creating strain sensors that can sense movement of the body parts. Bae \textit{at al.} demonstrated a transparent CVD graphene rosette-based strain sensor fixed to a wearable glove that could sense the bending movements of fingers.\textsuperscript{319}
Human motion sensors have also been demonstrated based on polymer nanocomposites produced from 3d structures of graphene-based materials. Yan et al. showed the use of stretchable PDMS nanocomposites prepared from a mixture of commercially available crumpled graphene (from rGO) and cellulose nanofibers as an all-direction human motion sensor which can stretch up to 100 % and having GF ~ 7.1.\(^\text{320}\) Jeong et al. have demonstrated PDMS composites produced from fragmented graphene foams (produced by CVD growth of 3d Ni template) showed higher strain sensitivity (GF ~ 15 to 29), as opposed to unfragmented graphene foam/PDMS composites (GF ~ 2.2), due to the large change in contact resistance within fragmented graphene foams during stretching.\(^\text{321}\) These strain sensors shown to function as human health-monitoring devices by monitoring movement of fingers, elbows and even pulse of radial artery.

Boland et al. have produced body motion sensors from graphene-rubber composites. The elastic bands (natural rubber) were coated by LPE graphene (produced in NMP/water), by soaking the elastic bands in suitable solvents, producing nanocomposite which were shown to sense strains by displaying resistance changes even at 800 % strain levels with GF ~ 35.\(^\text{322}\) These sensors were able to sense the body movements such as joint and muscle motion as well as breathing and pulse.

### 3.3.2. Existing strain sensor technology

Strain sensors are devices that measure the strain, tensile or compression, on an object in a particular part or over an area resulting from applied load. It has to be noted that, the measurement of strain using strain sensor always assumes that the strain on the object under investigation is transferred equally to the gauge.\(^\text{307}\) There are different types of strain sensors available that can measure strain over a single point or over an area, are described below.

#### 3.3.2.1. Resistance-based strain sensors

The most common type of strain sensors that are used to obtain single point measurements is resistance-based strain gauge. The working principle of these strain gauges is based on change in resistance of a conductor when it is stretched or compressed within its elasticity limits, such that it doesn’t deform permanently.
Most commonly used resistance-based strain gauges are the metallic foil-based strain gauge. These foil strain gauges are produced normally from a copper-nickel alloy which are photo-etched to a desired pattern, and are encapsulated within a flexible polymer substrate (e.g., polyimide) which acts as a carrier (Figure 3.15a). The gauge is bonded to the specimen using an adhesive, usually a thin layer of epoxy resin or cyanoacrylate adhesive, hence also called bonded resistance strain gauges.

![Figure 3.15: Different types of foil-based bonded resistance strain gauges. a) Common strain gauge showing the grid encapsulated within polymer matrix. Basic rosette types classified by grid orientation: b) tee; c) 45°-rectangular; d) 60°-delta.]

The variations in the electrical resistance of the grid are measured as an indication of strain. The overall resistance of this type ranges from 30 to as much as 5000 Ω, but gauges with 120-350 Ω are normally used. Higher resistance gauges are mainly used for transducer applications and on composite materials. These strain gauges can be used to measure both static and dynamic strains. When the principal direction of strain/deformation/stress is known, the strain gauge can be fixed in specific direction. When the principal direction of strain is unknown or in case of biaxial strains, rosette type of strain gauges as shown in Figure 3.15b,c,d are normally used. Foil strain gauges can be used to measure strains up to 5 % or 50000 microstrain (µε). By changing the grid metal (Constantan P alloy), strains as high as 20 % can be measured, but high cyclic strains at this strain levels tend to induce permanent resistance changes. The GF of the commercially available sensors are in the range of 2 to 5. Moreover these sensors are only moderately affected by the temperature changes, and have service temperatures -269 °C to 230 °C. Other advantages of these sensors are:

- Uniform characteristics within different range of gauges
- Small size, light weight and durable
- Inexpensive
Accuracy of measured strain is better than 0.01 %, and resolutions up to 0.1 µε.

However, the main disadvantage in using these sensors for a wide-area strain measurement is that, large number of these gauges should be fixed on the sample, leading to significant amount of infrastructure: soldering wires, connections, etc. making it unsuitable for wide-area strain sensing applications. Additionally their susceptibility to electromagnetic interference (noise) makes their usage challenging in many applications.

3.3.2.2. Optical strain sensors

3.3.2.2.1. Fibre optic sensors

Fibre optic sensors use the optical fibres as the ‘intrinsic’ sensing element. By modifying the fibre, fibre optic sensors can be used to measure strain, temperature or pressure. The sensitive factors that were followed for measurements in these sensors are; intensity, phase, polarisation, wavelength and transit time of the light in the fibre. Fibre optic sensors have been developed to measure temperature and strain simultaneously with high accuracy by using Fibre Bragg grating (FBG).

A FBG is a distributed Bragg reflector in an optical fibre (Figure 3.16a). In FBG sensor, the refractive index of the core of the optical fibre changes radically along short lengths. When a white light with many wavelengths passes through a FBG, one particular wavelength is in-plane with the grating period (Bragg wavelength), which is reflected back to the input end of the fibre leaving all other wavelengths to transmit to other end (Figure 3.16b).

![Figure 3.16: Schematic representation of (a) An FBG optical fibre and (b) Operation of FBG optical sensor](image)

The reflected Bragg wavelength is sensitive to strain and temperature showing shift in the wavelength. The sensors sensitivity to strain and temperature makes this system complex when proper strain measurements are needed. An independent FBG
temperature sensor should be installed in close thermal contact with the FBG strain sensor, and their responses should be subtracted to compensate the temperature effect, yielding the temperature-compensated proper strain value.\textsuperscript{326} In general, when the FBG is subjected to tensile strain the Bragg wavelength shifts to higher wavelength and to lower wavenumber on compressive strain. Several advantages of these sensors are:

- Can be directly integrated into composites, or can be fixed or as patches on the surface of the test specimen for measuring strains.
- Can measure very high strains (> 10000 µm/m) and can be used in highly stressed composite constructions.
- Long term stability is very high.
- Special grades can be used at > 700 °C and general usage (-30 °C to 60 °C).
- Since the sensors are selected based on measuring within selected wavelength or colour range, several gratings can be located in a row (> 20 FBG, with special interrogator technologies up to 100 FBG), each measuring different parameters such as strain, temperature, pressure and acceleration.

The accuracy and resolution of commercially available FBG sensors is very high:\textsuperscript{326, 327} Accuracy for strain is \(\sim 1.2 \text{ pm/µε} \) and temperature \(\sim 0.1-11 \text{ pm/°C} \) (depending on sensor); Resolution is 0.4-1 µε for strain and 0.05 °C for temperature. However, their cost and need for complex fibre optical lay-up to achieve sufficient point resolution over wide-area are some disadvantages.

### 3.3.2.2.2. Photoelasticity-based sensors

The photoelasticity-based sensors (Figure 3.17) utilise birefringence, which is the splitting of incident light into two components due to different refractive indexes within a transparent materials. When a polarised light beam passes through a birefringent material which is under stress/strain, the incident light splits into two components (due to birefringence, with two refractive indexes) along the principal stress directions (perpendicular to the direction of light propagation). The magnitude of the refractive indexes is directly related to the stress/strain at each point (analogous to resistance change in strain gauge). This causes a change in the polarisation state of the transmitted light resulting interference fringes, which can be analysed by a Polariscope (Figure 3.17c).
A video camera is normally used to image the fringe patterns (Figure 3.17d) that can reveal information related to orientation and principal stress/strain directions.\textsuperscript{328,329}

![Figure 3.17](image)

\textit{Figure 3.17: a) Schematic representation of reflection Polariscope. b) Application of strain sensitive photoelastic coatings on a complex part. c) Reflection Polariscope. d) Images from Polariscope of a product being incrementally loaded revealing the fringe patterns.}\textsuperscript{328}

Commercially available photoelasticity-based sensors like PhotoStress\textsuperscript{®} (Vishay Precision Group)\textsuperscript{328} and Grey-field Polariscope (Stress Photonics)\textsuperscript{330}, supply a photoelastic sensitive polymer coatings (PMMA/epoxy- or PS/epoxy-based) with a Polariscope for strain/stress analysis.

The advantages of this type of sensors are:

- Full field strain/stress maps with stress distributions can be obtained in real time
- Strain/stress levels in complex parts can be imaged (Figure 3.17b)
- Relatively simple to use

The accuracy of these sensors is about 0.1 \% and the resolutions ranging from 10 – 20 \(\mu\varepsilon\). However, one of the disadvantages is in the slightly complex coating application process on the target substrate. Nevertheless, this technique is suitable for wide-area strain sensing applications due to their simplicity and flexibility.
Chapter 4 - Methods
4. METHODS

4.1. SYNTHESIS OF GRAPHENE

The graphene used in this thesis was produced in-house by various methods: (i) micromechanical cleavage (MC) using Scotch™ tape, (ii) CVD, (iii) electrochemical exfoliation and (iv) LPE. Commercially available graphene was also used in certain experiments for comparison. The experimental procedures of these production routes are explained in the following sections.

4.1.1. Micromechanical cleavage

A small piece of natural graphite (Nacional de Grafite) was pressed on the Scotch™ tape and repeatedly cleaved (10-15 times) to obtain thin flakes. This tape was then pressed against a substrate (e.g., Si/SiO₂ beam) to transfer one or more of the layers of the thin flakes. Acetone was used to dissolve the tape, leaving graphene attached to the substrate. The sample was then baked on a hot plate (90 °C for 10 min) to remove any residual solvents. It has to be noted that this procedure produces broad variety of flakes with varying dimensions and thickness throughout the substrate. Optical and Raman spectroscopy was further used to identify SLG for analysis. The MC samples on PMMA beam was kindly provided by Prof. Kostya Novoselov of University of Manchester, School of Physics and Astronomy. This MC graphene was used to prepare composite coating samples and the detailed procedure is in section 4.2.2.

4.1.2. Liquid-phase exfoliated graphene

The liquid-phase exfoliation used in this project mainly includes ultrasonication as the basis of exfoliating graphene from the graphite flakes. A brief insight to the ultrasonication and exfoliation of graphene is explained in the following sections.

4.1.2.1. General principles of ultrasonication

Ultrasound is classified as sound beyond the frequency that can be detected by human ears. Normal range of human hearing is between 16 Hz to 20 kHz. The ultrasound thus ranges from 20 Hz to 10 MHz. Like any sound wave, ultrasound is transmitted via waves which alternately compress (high pressure) and stretch (refraction, low pressure) the molecular structure of the medium through which they pass. During this stretching
phase, provided the negative pressure is strong enough to overcome intermolecular binding process, a fluid medium can be torn apart producing voids or cavities (micro bubbles). In the succeeding cycles, these bubbles grow in size (at least twice their initial size) and become unstable and collapses resulting in intense local heating (hot spots of ~ 5000 °C), extreme pressures (~ 1000 atm) with rapid heating/cooling rates (> 10^6 K/s).331

The cavitation bubbles when collapses asymmetrically near a micro-particle surface, forming microjets with speed in the order of 100 m/s. These microjets subsequently produce an asymmetric shock wave upon implosion of the bubbles, resulting in erosion on a particle’s surface and de-aggregation of particles.332 In case of layered materials, the microjets aid the exfoliation process (Figure 4.1).

![Schematic representation of generation and collapse of cavitation bubbles during ultrasonication process and the process of graphite exfoliation to graphene.](image)

**Figure 4.1:** Schematic representation of generation and collapse of cavitation bubbles during ultrasonication process and the process of graphite exfoliation to graphene.

### 4.1.2.2. Experimental procedure

Hernandez et al. demonstrated the production of pristine FLG from graphite in NMP using sonication and centrifugation.54 Their technique is broadly followed in this
project; however, different solvents were used for graphene exfoliation. The schematic representation of the whole process is shown in Figure 4.2.

The graphite used in this project was natural graphite (grade 2369) from Branwell Graphite Ltd, UK. This particular grade was chosen for this work as the literature reports that it produces a higher concentration of graphene compared to other grades.\textsuperscript{333} The graphite flakes were sieved in 500 mesh to remove large graphite particles which may not be exfoliated completely in the low power sonication. The initial concentration of graphite of 5 mg/ml is kept constant for all the experiments. Graphite was then added to solvent or surfactant solution and sonicated for different time periods (6 hr to 60 hr) in the ultrasonic bath (Elmasonic P, max power 820 W). Low power sonication (32 W, 37 KHz, 30 % power) was carried out for all the samples. During longer sonication times, the bath water tends to heat up to $\sim$ 50 °C leading to considerable evaporation. Therefore, a circulating cooler was used to maintain the temperature in sonication bath around 22-25 °C at all time to prevent any water evaporation, and temperature related aggregation (At elevated temperatures, decrease in viscosity increases the Brownian motion and

\textit{Figure 4.2: Schematic representation of liquid-phase exfoliation of graphene.}
could result in enhanced vdW attraction causing aggregation) of graphene sheets. The sonicated solution was left overnight to settle. The solution was then centrifuged for 20 min at between 1000 rpm (~ 112 ×g) to 10000 rpm (~ 11180 ×g) in a Thermo Scientific Sorvall LEGEND RT+ centrifuge machine to remove unstable and unexfoliated flakes. The supernatant was carefully decanted and used for further characterisation and use.

4.1.2.3. Exfoliation in organic solvents

It is well-known that graphite can be exfoliated into graphene in organic solvents which has similar surface energies that of graphene.\textsuperscript{54} In this work, N, N-Dimethyl formamide (DMF, Sigma D158550, ≥ 99 %) and N-Methyl-2-pyrrolidone (NMP, Sigma M79603, 99 %) were used to exfoliate graphite to graphene. Different sonication times and centrifugation speeds were used to optimise the process. The results are discussed in the Chapter 5.

The concentration of graphene produced can be obtained by knowing the absorption coefficient of graphene sheets. Absorption coefficient can be determined by using UV-Vis spectroscopy using Lambert-Beer law.

\[ A = \alpha l c \] 

\textit{Equation 4.1}

where, \( A \) is absorbance (dimensionless), \( \alpha \) is absorption coefficient (ml mg\(^{-1}\) m\(^{-1}\)), \( l \) is the path length of cuvette and \( c \) is concentration (mg/ml). While straightforward, different absorption coefficient values for graphene (1390 – 6600 mL mg\(^{-1}\) m\(^{-1}\)) were reported in the literature\textsuperscript{54, 59, 60, 77}, see section 5.2.1 for discussion. In order to avoid this uncertainty, in this study, the value of \( \alpha \) was determined independently by producing different concentration of graphene using different conditions (sonication time and centrifugation speed) and solvents (NMP and DMF). To obtain the concentration for this calibration, known volume of graphene dispersions were filtered through porous aluminium oxide filter membranes (Whatman\textsuperscript{®} anodisc, 100 nm pores, 13 mm diameter). The resultant graphene films ("graphene paper") were dried in tube furnace at 400 °C for 4 hr and carefully weighed in a microbalance. Absorption values of dispersions at 660 nm were obtained from UV-Vis spectrometer using quartz cuvettes (10 mm light path) at a range of dilutions prepared from different experimental batches.
Figure 4.3: Measurement of the absorption coefficient of graphene dispersions produced in different conditions and in different organic solvents (Unfilled black circles). Data from example dispersions before (100 v/v%) and after dialysis (0.1 v/v%) were also added. Note: The slight change in the absorbance value before and after dialysis is due to the aggregation of graphene flakes/dilution (hence decrease in concentration) during dialysis.

From Figure 4.3, the $\alpha$ was calculated to be 4632 mL mg\(^{-1}\) m\(^{-1}\) and this value was used for the measuring concentration of graphene in organic solvents and solvent exchanged dispersions by dialysis in this thesis.

4.1.2.3.1. **Graphene paper preparation**

Graphene dispersions produced in NMP and DMF were used to prepare thin films of graphene paper. These graphene papers were prepared by vacuum filtration as explained in the previous section. Graphene dispersions obtained after centrifugation were filtered through porous aluminium oxide filter membranes (Whatman® anodisc, 100 nm pores, 47 mm diameter) followed by drying in tube furnace at 400 °C for 4 hr. It was used for further characterisation using Raman spectroscopy and XRD.

4.1.2.3.2. **Size selection process**

Coleman et al. have demonstrated that by using controlled centrifugation, graphene flakes with different lateral dimensions can be extracted.\(^62\) This technique was broadly adapted in this thesis to produce graphene flakes with different lateral dimensions. Graphene dispersion produced by direct LPE of graphite in NMP (60 hr) was centrifuged at 2000 rpm for 20 minutes to remove all the unexfoliated material. The
supernatant was centrifuged at 6000 rpm for 20 min, the sediment collected had graphene of nominal lateral dimensions ~500 nm (termed FLG-0.5). The supernatant of 6000 rpm sample was further spun at 10000 rpm for 20 min, the sediment collected had graphene of nominal lateral dimensions ~200 nm (termed FLG-0.2).

The sediments collected were washed twice with acetone (to remove residual NMP) before dispersing it in 10 ml acetone for 30 min. These samples were used for preparing solution processed graphene/epoxy composites (section 4.2.1).

4.1.2.4. Exfoliation in aqueous media using surfactants

Biocompatible anionic bile surfactant, TDOC (Sigma T0557), was used to exfoliate graphite to graphene sheets resulting in surfactant coated graphene sheets. The stock solution was prepared by stirring TDOC (5 mg/ml) with deionised (DI) water (ultrapure water, 18.2 MΩ cm⁻¹, Millipore Milli-Q Plus system). Graphite (5 mg/ml) was added to the stock solution and bath sonicated for different time periods (6 - 36 hr). The dispersion was then centrifuged at 10000 rpm for 20 min to produce a final stable dispersion. The value of α was calculated using the method described above and found to be 1825 mL mg⁻¹ m⁻¹ (Figure 4.4), see section 5.2.1 for discussion.

![Figure 4.4: a) Structure of TDOC. b) Measurement of absorption coefficient of graphene dispersion produced in aqueous TDOC solution (Absorbance at 660 nm).](image)
4.1.2.5. Exfoliation using lipids

Phosphatidylcholine, a type of phospholipid with a polar head (hydrophilic) group and a non-polar (hydrophobic) tail group was used to exfoliate graphite to graphene in aqueous media. 2:1 molar ratio of egg yolk phosphatidylcholine (EggPC) (50 mg, Lipid products, Nutfield, UK) and cholesterol (13 mg, Sigma C8667) were dissolved in 1:1 ratio chloroform-methanol mixture. The mixture was then dried at 40 °C in a rotatory evaporator to form a thin lipid film. The lipid film was further dried overnight in a freeze dryer to completely remove the solvents. The resultant lipid film was dispersed in autoclaved phosphate buffer saline (PBS) (140 mM NaCl, pH-7.4) and then sonicated in a low power bath for 15 min to produce vesicles with monodispersed diameter. Graphite was added to the liposome solution and sonicated for 36 hr in an ultrasonic bath to produce lipid-coated graphene dispersion. The dispersion was centrifuged at 2000 rpm (268 ×g) for 20 min to remove any unstable/unexfoliated graphite to yield stable lipid-coated graphene dispersion (“G/EggPC as prepared”). In order to remove any excess lipids, the as prepared G/EggPC was washed with PBS buffer and centrifuged at 10000 rpm (6708 ×g) for 10 min, and resuspended in PBS buffer. This procedure was repeated thrice to obtain G/EggPC washed dispersion.

The concentration of graphene was measured from UV-Vis spectra using the Lambert-Beer law (α was taken as 1825 mL mg⁻¹ m⁻¹, based upon the value measured for the G/TDOC), whereas, concentration of lipids were measured by using the Stewart assay. The dispersion was filtered using 100 nm pore polycarbonate filters (Nucleopore) in high pressure filter (Avestin Liposofast 100) in room temperature. The graphene film was carefully dried in room temperature and weighed. The well-established Stewart assay was carried out on the lipid-coated graphene dispersion to calculate the lipid content. Briefly, 10 µl of dispersion mixed with 1 ml of chloroform and 1 ml of thiocyanate reagent which is a mixture of 27 g ferric chloride hexahydrate (Sigma F2877) and 30 g of ammonium thiocyanate (Sigma 221988) in 1 L water. The mixture was shaken for 1 min followed by centrifugation for 10 min at 1000 rpm. Due to the complex formation between lipids and ferrothiocyanate, the chloroform changes into red colour depending on the concentration of lipids present. The concentration of lipids was calculated by measuring absorbance at 488 nm. All the solutions were stored in the fridge at 4 °C.
when not used. DLS was employed to determine the size of liposomes and lipid-coated graphene and the results are presented in section 6.4.

**4.1.2.6. Exfoliation using proteins**

Direct exfoliation of graphite was carried out in presence of proteins to produce protein-coated graphene sheets in aqueous media. In a typical experiment, human serum albumin (HSA, Sigma A9511) was dissolved in autoclaved PBS buffer (140 mM NaCl, pH 7.4). Graphite was added and the mixture was directly exfoliated in low power sonication bath followed by centrifugation (20 min at 2000 rpm) to remove any unstable/unexfoliated graphite. The procedure yielded a stable dispersion of protein-coated graphene (“as prepared G/HSA”). In order to optimise (to achieve high graphene concentration) the process, different starting concentration of HSA (0.25 – 10 mg/ml) and sonication times (4 – 36 hr) were used. In order to remove the excess HSA, the as prepared G/HSA was washed with PBS buffer and centrifuged (10000 rpm, 10 min), and resuspended in PBS buffer. This procedure was repeated thrice to obtain G/HSA-wash dispersion.

The concentration of graphene was obtained from UV-Vis spectra using Lambert-Beer law ($\alpha$ was taken as that for G/TDOC: 1825 mL mg$^{-1}$ m$^{-1}$), while the protein concentration was estimated by the Ninhydrin method. The Ninhydrin method is widely used to measure the amino acid concentration. Human serum albumin has 585 amino acids in its sequence. The graphene dispersion was carefully filtered via 20 nm pore anodisc filter membranes (Whatman® 13mm disc) and dried at room temperature and weighed. For Ninhydrin method, typically 10 µl of known concentration of HSA is mixed with 100 µl of pyridine (Sigma 270970) and 25 µl of ninhydrin (Sigma, N4876). The mixture was heated in a heating block at ~80 °C for 10 min followed by cooling it in running tap water. As a result of a reaction between amino acids and ninhydrin, the colour of the solution changes into purple and the absorbance value at 570 nm was noted. A calibration curve as shown in Figure 4.5 was obtained by using different known HSA concentrations. Concentration of unknown protein in graphene dispersion was estimated by reading the absorbance value from the Ninhydrin test and following the calibration curve. All the solutions were stored in the fridge at 4 °C when not used.
4.1.2.7. Summary of different preparation methods

Table 4-1: LPE- conditions used

<table>
<thead>
<tr>
<th>Graphite concentration (mg/ml)</th>
<th>Liquid-media</th>
<th>Concentration (mg/ml)</th>
<th>Sonication time (hr)</th>
<th>Centrifuge conditions (rpm/min)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Solvent/surfactant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>DMF</td>
<td>-</td>
<td>24-60</td>
<td>1000-10000/20</td>
</tr>
<tr>
<td>5</td>
<td>NMP</td>
<td>-</td>
<td>6-60</td>
<td>1000-10000/20</td>
</tr>
<tr>
<td>5</td>
<td>TDOC</td>
<td>5</td>
<td>6-36</td>
<td>10000/20</td>
</tr>
<tr>
<td>5</td>
<td>EggPC</td>
<td>1</td>
<td>36</td>
<td>2000/20</td>
</tr>
<tr>
<td>5</td>
<td>HSA</td>
<td>0.25-10</td>
<td>4-36</td>
<td>2000/20</td>
</tr>
</tbody>
</table>

*1000 rpm – 112 x g, 2000 rpm – 268 x g, 10000 rpm – 11180 x g.

4.1.3. Electrochemically exfoliated graphene

Electrochemical methods, such as electrochemical reduction of GO or direct exfoliation of graphite to graphene are known to be able to produce large diameter FLG (~ 20 µm). Recently Abdelkader et al. exfoliated a graphite cathode to produce FLG sheets with relatively few sp³ defects.¹²³

Electrochemically exfoliated FLG was kindly supplied by Dr. Amr Abdelkader (School of Materials, University of Manchester) with two different mean diameters: 5 µm (termed as FLG-5) and 20 µm (termed as FLG-20). The procedure of electrochemical exfoliation process is published elsewhere.¹²³ These FLG powder was dispersed in 10 ml
acetone for 30 min using sonication and were used to produce epoxy-graphene composite coatings as described in section 4.2.1.

### 4.1.4. CVD graphene

Graphene produced by CVD technique was used in this project to evaluate its potential as a Raman based wide-area strain sensor. CVD graphene was prepared in-house (Figure 4.6) and kindly supplied by Dr. Amada Lewis (School of Materials, University of Manchester).

![Figure 4.6: Photograph of the reactor used to produce the CVD graphene.](image)

The CVD graphene used in this project was grown by hot wall CVD process. The copper foils (Alfa Aesar product #13382) were cut into 1 × 1 cm squares and loaded into 1” fused silica furnace tube. The pressure in the tube was lowered by a rotary vane pump (Edwards RV12) in combination with a turbo pump (Leybold Oerlikon Turbvac 50). Hydrogen was introduced in the furnace tube at 10 sccm for 30 min to displace any air in the tube. Then the hydrogen flow was reduced to 1 sccm to achieve a pressure value of 5 × 10^{-3} mbar. Once the desired pressure was achieved, the temperature was increased to 1040 °C at a heating rate of 40 °C/min and the hydrogen flow was continued for 30 min to anneal the copper foil. The precursor methane was introduced at the rate of 2 sccm and the pressure maintained was about 0.01 mbar. After 30 min, the methane flow was terminated and the furnace was allowed to cool down in the room temperature. This process resulted in the formation of graphene on either side of the Cu foil, which was then carefully removed from the furnace tube.
4.1.5. Commercially sourced graphene

4.1.5.1. Elicarb® graphene

Elicarb® graphene powder from Thomas Swan Ltd, UK was used to prepare epoxy-graphene composite coatings as described in section 4.2.1. The average lateral dimension of graphene sheets are around 1 µm with 5-7 layers. Herein, it is termed as FLG–1.337

4.1.5.2. Graphene supermarket CVD graphene

CVD-grown single layer graphene on Cu foil was bought from Graphene Supermarket, USA.338 This CVD graphene was used in this project as a comparison with the in-house CVD graphene for strain sensor applications. The CVD graphene was supplied on a 2” × 2” Cu foil and were cut into small square pieces before following the transfer process as shown in section 4.2.3.

4.1.6. Solvent exchange of graphene by dialysis

It is well-known that organic solvents like NMP and DMF can readily disperse graphene due to their surface energies being close to that of graphene.54, 59 However, these organic solvents also have disadvantages. Their higher b.p. meaning that is very difficult to remove them fully from the surface of the graphene by heating, limiting the use of graphene produced in these solvents in applications such as electronics and biomedical. Herein dialysis is studied as an alternative route to remove the organic solvents via solvent exchange.

4.1.6.1. Dialysis principle

Dialysis is a process which works on the principle of diffusion. Diffusion is a random, thermal movement of molecules in solution (Brownian motion) which leads to movement of molecules from a high concentration to a low concentration region until equilibrium is reached. In dialysis small molecules (solutes) diffuse from a high concentrated region to a low concentrated region across a semipermeable membrane as shown in Figure 4.7.339 The pore size of semipermeable membrane means that large molecules cannot diffuse through it, whilst small molecules can. Hence the small molecules can reach equilibrium across the entire solution volume, thereby changing
their concentration on either side of the membrane. The final concentration of small molecules after equilibrium depends on the volume of solutions used, if the buffer is replaced with fresh buffer, diffusion will further decrease the concentration of small molecules.

![Diagram of dialysis principle](image)

*Figure 4.7: Dialysis principle and schematic representation of dialysis procedure*

Due to the freedom of movement of small molecules across either side of semipermeable membrane, dialysis could be either used to introduce or to remove small molecules in a sample. Dialysis is widely used in life science to clean or purify DNA, protein samples, serum or blood.

The separation characteristics the semipermeable membrane is determined by its pore size range and is often characterised by the molecular weight-cut off (MWCO). Typically a membrane’s MWCO refers to the smallest average molecular mass of a molecule that will not diffuse across the membrane. Ifmiscible liquids are used on either side of the membrane, effectively a complete solvent exchange could be attained, which has been adapted in this thesis.

### 4.1.6.2. Dialysis experimental procedure

Since NMP and DMF are miscible in water, dialysis was employed in this project as an effective solvent exchange mechanism for graphene dispersions to exchange organic solvents with water.

Preparation of dialysis tubing is very important for effective dialysis. The required length of cellulose-based dialysis tubing (Sigma D0405, MWCO 12400 Da) was cut and immersed in aqueous 1 mM ethylene diamine tetra acetic acid (EDTA, Sigma E9884) solution and boiled for 10 min followed by rinsing in DI water. The tube was further
boiled for 10 min in DI water followed by DI water rinsing. These processes remove the contaminants of dialysis tubes (glycerol, sulphur compounds etc.). Since the molecular weight of DMF, NMP, and water are 73.09 Da, 99.13 Da, and 18.01 Da, respectively, these molecules can exchange effectively through the selected dialysis membrane.

For the solvent exchange process, typically 5 ml – 10 ml of graphene dispersion (DMF or NMP) was contained within the dialysis tubing by tying knots at the top and bottom of the tube. A schematic representation of the typical process is shown in Figure 4.7 and example process shown in Figure 6.2. These tubes were immersed in bottle with the desired volume of distilled water to reduce the concentration of organic solvents step-wise (from 100 v/v% --> 10 v/v%, 10 v/v% --> 1 v/v% etc.). Each dialysis step was carried out for 12 hrs with gentle stirring. For e.g. at the start, a dialysis tube containing 5 ml of graphene-DMF (100 v/v%) was immersed in 50 ml of water (ratio 1:10). At the end of 12 hrs dialysis, the concentration inside the tube had changed to 10 v/v% DMF and 90 v/v% water. 5 ml of 10 v/v% DMF sample was used as the starting solution for next dialysis step immersing in 1:10 ratio water resulting in 1 v/v% DMF and 99 v/v% water. Another step of dialysis leads to 0.1 v/v% DMF and 99.9 v/v% water. Dialysed graphene dispersions were used to prepare graphene paper as described in 4.1.2.3.1, and was characterised by Raman spectroscopy and XRD.

It is important to note the change in volume of sample inside the dialysis tube before and after dialysis. Due to the slight expansion of dialysis tubing or absence of tight knots, the volume inside the dialysis tube tends to increase slightly at the end of each step and this result in diluting the starting graphene concentration. In order to avoid, it is important to have a tight knots with no any air gap.

4.2. PREPARATION OF TYPICAL GRAPHENE COMPOSITE COATINGS

4.2.1. Solution processed graphene/epoxy composites

Epoxy composite coatings (5 wt.%) were produced with different lateral sizes of graphene (Table 4-2) in order to investigate the role of graphene flake size on its performance in Raman-based strain sensors.
Table 4-2: Different size of graphene flakes used in the composite preparation

<table>
<thead>
<tr>
<th>Graphene type</th>
<th>Average diameter (as produced/ as supplied) (µm)</th>
<th>Average number of layers or thickness</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLG-0.2</td>
<td>~ 0.2</td>
<td>&lt; 6 layers</td>
<td>Size selection process (see 4.1.2.3.2)</td>
</tr>
<tr>
<td>FLG-0.5</td>
<td>~ 0.5</td>
<td>&lt; 6 layers</td>
<td>Size selection process (see 4.1.2.3.2)</td>
</tr>
<tr>
<td>FLG-1</td>
<td>~ 1</td>
<td>6-7 layers</td>
<td>Elicarb®, Thomas Swan Ltd.</td>
</tr>
<tr>
<td>FLG-5</td>
<td>~ 5</td>
<td>~ 5 nm</td>
<td>Electrochemical exfoliation (see 4.1.3)</td>
</tr>
<tr>
<td>FLG-20</td>
<td>~ 20</td>
<td>~ 5 nm</td>
<td>Electrochemical exfoliation (see 4.1.3)</td>
</tr>
</tbody>
</table>

The matrix system used in this study was a low viscosity epoxy resin from Huntsman Co. Epoxy resin (Araldite LY5052) mixed with an amine hardener (Aradur HY5052 CH) in ratio of 100:38 by weight.

In order to have good dispersion of graphene in epoxy resin, several mixing steps were followed: the graphene powder or sediment was suspended in acetone (10 ml) using low power sonication bath for 30 min. 1 g of epoxy resin (LY5052) was added to the mixture and further sonicated for 1 hr facilitating homogenisation. Solvent was removed by stirring on a hot plate at 70 °C overnight followed by 2 hr in vacuum oven (room temperature). After the mixture had cooled to room temperature, 0.38 g of hardener (HY5052) was added and mechanically stirred for 5 min. Mixture was degassed for 15 min in vacuum oven before drop casting it on PMMA beam (70 × 20 × 2.5 mm). The coating was spread evenly to form a thin layer (~300 µm thick) and then cured following the manufacturer’s recommended conditions of 24 hr at room temperature followed by 4 hr at 100 °C. The coatings were polished using a sand paper before the deformation procedure to compensate the fluorescence from epoxy in Raman spectra of the composites (Figure 4.8).
4.2.2. Mechanically-cleaved graphene composite coatings

These composite specimens comprised of a sandwich layer of PMMA/SU-8/graphene/PMMA. A 5 mm PMMA beam forms the base of the composite sample. A 300 nm layer of SU-8 photoresist was spin coated on top of the PMMA beam and cross-linked. The photoresist was used in order to increase the visibility of graphene through optical interference (Typically in the Nobel Prize Winners’ group a 300 nm SiO\textsubscript{2} layer on a Si wafer is used). The MC graphene was carefully deposited on the surface of SU-8 by the Scotch\textsuperscript{TM} tape. A thin layer (~100 nm) of PMMA was spin coated on top of the beam so that graphene remains visible in between the sandwich layers and also acting as the composite top layer. The schematic representation of the specimen is shown in Figure 4.9.
Some of the prepared samples were kindly provided by Prof. Kostya Novoselov from School of Physics and Astronomy, University of Manchester.

### 4.2.3. CVD graphene composite coatings

Graphene produced by the CVD technique (both in-house preparation and commercially available) were used to produce a similar sandwich composite to that of the mechanically exfoliated graphene which was explained in previous section. The main step in this process was the transfer of CVD graphene from the Cu growth foil to the substrate. A schematic representation of the process and the sample is shown in the Figure 4.9. For the transfer process, a small square piece of Cu foil was chosen. 200 nm of PMMA was spin coated on one side of the Cu foil in the spin coater. The CVD technique produce graphene on both sides of the Cu foil, the graphene on the other side of Cu foil where there is no PMMA was removed by etching in oxygen plasma for 5 min. The Cu was then etched in a 0.1 M ammonium persulphate (etchant) solution, leaving PMMA/graphene in the solution. This PMMA/graphene was carefully transferred with a glass substrate to DI water to remove any residual etchant present. This process was repeated several times to
ensure the complete removal of etchant leaving behind PMMA/graphene floating in the DI water in a beaker.

Meanwhile SU-8 (300 nm) photoresist was spin coated on the PMMA beam (5 mm) and baked in an oven at 70 °C for 10 min. This beam was then used to pick the PMMA/graphene. The sample was rested for 5 min before baking it in oven at 70 °C for 10 min for drying. Dr Recep Zan from School of Materials, University of Manchester did the transfer of CVD graphene on PMMA beams.

4.3. CHARACTERISATION

4.3.1. Optical microscopy

Optical microscopy (Olympus BH-2 microscope) was used to identify the number of layers, position of MC graphene and the quality of CVD graphene coatings on the composite samples. Either 50× or 100× objective lens was used to obtain the images. The optical microscopes on the Reinshaw Raman spectrometers were also used to obtain images.

4.3.2. Scanning electron microscope

Philips XL 30 Field Emission Scanning Electron Microscope operated at 5-10 kV accelerating voltage was used to study the morphology of the graphene. The samples were mounted on aluminium SEM stubs (Agar Scientific AGG301) using adhesive carbon tape (Agar Scientific AGG3939), followed by sputter coating the non-conductive samples with gold. A line of silver paint was applied over the edge of the sample and the carbon tape for efficient discharging of electrons generated by the beam. Secondary electron mode was used to image all the samples except the CVD coatings, where back scattered electron mode was used to image the grain boundaries in the CVD graphene.340

SEM samples were prepared from graphene dispersions by drop casting ~50 µl of dispersion on a SiO$_2$/Si wafer. The samples were dried at 80 °C in vacuum overnight to remove any solvents.
4.3.3. Atomic force microscope

AFM was used to measure the lateral dimensions and thickness of graphene sheets produced by various processing conditions. The measurements were taken in Bruker Dimension FastScan using FastScan-A (Bruker) probe in tapping mode.

Samples for AFM measurements were prepared either by drop casting or spray coating on freshly cleaved mica sheets (Agar Scientific AGG250-2). For graphene produced in organic solvents, samples were prepared by spraying ~0.5 ml diluted dispersions (at 1.5 bar nitrogen pressure and 5-10 cm away) on mica sheet using an Evolution Solo Airbrush (Harder & Steenbeck). The mica sheet were heated up to 100 °C prior to spraying as it helped flash evaporate the solvent during the spraying procedure, reducing the probability of graphene aggregation. The mica sheets were again heated on hot plate at 150 °C to evaporate excess solvents. This procedure was generally repeated three to five times for good coverage. Residual solvents were removed by annealing the substrates under argon atmosphere at 400 °C for 4 hrs prior to AFM measurements.

For graphene produced using biomaterials, AFM samples were prepared by dropping few drops (50 µl) of dilute dispersion on freshly cleaved mica and drying overnight at room temperature. DI water was used to wash the sample surface to remove excess lipid or proteins and gently dried using nitrogen gas. All the images were analysed using Nanoscope Analysis software.

4.3.4. Dynamic Light Scattering

DLS also called as Photon Correlation Spectroscopy or Quasi-Elctron Light Scattering is a non-invasive method for measuring size of the particles or molecules in dispersion typically in the submicron region.

4.3.4.1. DLS Principle

Particles in dispersion undergo Brownian motion due to its collision with the surrounding solvent molecules. In DLS, the speed at which the particles diffuse due to Brownian motion is measured by determining the rate at which the intensity of scattered light fluctuates. The relationship between the velocity of Brownian motion and the particle size is defined by Stokes-Einstein equation;341, 342
\[ d(H) = \frac{k_B T}{3\pi \eta D} \]  

*Equation 4.2*

Where, \( d(H) \) is hydrodynamic diameter, \( D \) is translational diffusion coefficient, \( k_B \) is Boltzmann’s constant, \( T \) is absolute temperature and \( \eta \) is viscosity of solvent. The hydrodynamic diameter is the diameter of a hard sphere that has the same translational diffusion coefficient as the particle.

![Diagram showing hydrodynamic diameter](image)

*Figure 4.10: Hypothetical dynamic light scattering of larger and smaller particles along with its typical intensity fluctuations with time and typical correlogram showing the signal decay.*

When a light hits particles, the light scatters in all the directions. Different size particles move at different speeds resulting in different scattering intensities. In DLS, a digital autocorrelator is used to analyse the intensity fluctuations with time and a correlation function as a function of delay time is derived by fitting the intensity fluctuations. Larger the particle, slower the Brownian motion hence slower intensity fluctuation and longer correlation time as shown in Figure 4.10. The correlation function \( G(\tau) \) of the scattered intensity from the correlator is given by;

\[ G(\tau) = < I(t). I(t + \tau) > \]  

*Equation 4.3*
where, $\tau$ is the time difference of the correlator.

For large number of monodisperse particles in Brownian motion, the correlation function is an exponential decay function of the correlator time delay $\tau$;

$$G(\tau) = A[1 + B \exp(-2\Gamma \tau)] \quad \text{Equation 4.4}$$

where, $A$ is the baseline of the correlation function, $B$ is the intercept of correlation function and $\Gamma$ is decay constant.

$$\Gamma = Dq^2 \quad \text{Equation 4.5}$$

$$q = \frac{4\pi n}{\lambda} \sin \left(\frac{\theta}{2}\right) \quad \text{Equation 4.6}$$

where, $q$ is wave vector, $n$ is refractive index of the dispersant, $\lambda$ is the laser wavelength and $\theta$ is the scattering angle.

For polydisperse sample, the equation can be written as;

$$G(\tau) = A[1 + Bg_1(\tau)^2] \quad \text{Equation 4.7}$$

where, $g_1(\tau)$ is the sum of all the exponential decays contained in the correlation function.

The size from the correlation functions can be obtained by using different algorithms, with the two fundamental approaches being;

1) Cumulants analysis: Use a single exponential to fit the correlation function to obtain the mean size ($z$-average diameter) and the width of the distribution (polydispersity index).

2) Use multiple exponentials to fit the correlation function to obtain the distribution of particle sizes by using CONTIN or Non-negative least squares.

The $z$-average diameter is normally used for analysing monodisperse or narrow distributions. Whereas, the multi exponential fit is more appropriate for broader and multi-modal distributions. The size distributions obtained from the multi exponential fit is a plot of the relative intensity of light scattered by different sized particles and therefore called as Intensity Particle Size Distribution (IPSD). If IPSD shows more than one peak, by
knowing the sample refractive index and absorbance, Mie theory could be employed to convert intensity distribution to volume distribution or number distribution.\textsuperscript{341, 342}

It has to be noted that the hydrodynamic size data obtained from DLS assumes the particles are of spherical geometry (using Stokes-Einstein equation). Clearly the graphene flakes are not spherical. Hence to compare the size data outputted for the graphene flakes by DLS, lateral dimensions of graphene flakes were independently calculated using AFM and Raman spectroscopy.

4.3.4.2. DLS experimental setup and procedure

Zetasizer Nano ZS from Malvern Instruments Limited was used to measure the intensity particle size distribution of graphene dispersions as shown in Figure 4.11.\textsuperscript{343}

![Figure 4.11: Schematic representation of the Dynamic light scattering instrument](image)

In this DLS system, 633 nm laser acts as a light source to illuminate the sample in the cell. A detector at a backscattering angle (173°) measures the intensity of the scattered light. If the intensity of scattered light is too high, the detector gets saturated. The attenuator installed modifies the intensity of the laser source and hence the intensity of scattered light from the sample. The appropriate attenuator position is automatically determined by the software and hence the graphene dispersions did not need diluting. A correlator acts as a digital signal processor analysing the scattered light intensity signals from the detector. The correlator derives the rate of intensity variations by comparing the
scattered light intensity at successive time intervals. The Malvern Zetasizer software v7.03 analyses these information and derives the size measurements.

The advantages of using the backscattering angle detectors are:

1) Because of the detector angle, the laser does not have to travel through the entire sample thereby reducing multiple scattering, where the scattered light from one particle is scattered by other particles. Since the light passes through shorter length of the sample, higher concentrations, without dilution, could be measured.

2) Large particles such as dust in dispersion normally scatter light in the forward direction. Hence the effects of dust are greatly reduced using backscattering detectors.

The software uses information from the correlation functions and the scattered light intensity to automatically modify the focusing lens position to change the measurement position on the cell. Large samples or highly concentrated solutions scatter more light; in this case, measuring closer to the cuvette walls potentially reduces the effect of multiple scattering.

Sample volumes up to ~1.5 ml were filled in fluorescence quartz cuvettes (Fisher Scientific 12731685) without dilution for analysis. Since the process is very sensitive to the viscosity and temperature, the instrument was given the specific properties (viscosity and refractive index) of the solvents used. Measurements were made using the normal resolution mode and the samples were equilibrated for 120 seconds before the measurements were started. All IPSD values represented are average of 3-5 mean values.

4.3.4.3. Zeta potential

Particles in colloidal suspension usually carry an electrical charge. These electrical charges might arise from surface ionisation of functional groups or adsorption of charged species in the solvent system. Based on the surface charge of the particles, ions with opposite charge form layers around them called electrical double layer. Zeta potential ($\zeta$) is the potential at the slipping plane of the electrical double layer (Figure 4.12). Higher values of $\zeta$ (either +ve or –ve) is indicative of greater electrostatic repulsion between
same charged particles leading to stable dispersion. In general, particles with $\zeta$ higher than $\pm 30$ mV in magnitude are considered to be stable.

![Schematic representation of zeta potential](image)

*Figure 4.12: Schematic representation of zeta potential*

When applying electric current across the dispersion, particles with $\zeta$ move towards opposite charged electrode with an equilibrium velocity (electrophoretic mobility). $\zeta$ values can be derived from the electrophoretic mobility, solvent viscosity, dielectric constant and Smoluchowski theory.

Zeta potential values were measured using Zetasizer Nano ZS (Malvern Instruments Limited, UK) by diluting the dispersions up to 10 to 50 fold (typically graphene concentration $<0.05$ mg/ml). Dispersions were carefully filled without any bubbles in disposable capillary cells (Malvern DTS1061) for zeta potential measurements. Due to these capillary cells incompatibility, zeta potential measurements were not carried out on dispersions with higher than 0.1 v/v% organic solvent content.

**4.3.5. Ultraviolet-visible spectroscopy**

Ultraviolet-visible (UV-Vis) spectroscopy was used to measure the absorbance behaviour and concentration of graphene produced by various methods as explained in section 4.1.2. Perkin Elmer Lambda 25 and Varian Cary® 5000 UV-Vis spectrophotometers were used for absorbance measurements. Samples were diluted 10 to 50 fold before measurement.
4.3.6. **Fluorescence spectroscopy**

Fluorescence spectroscopy was mainly used to investigate the interaction of protein with graphene by following tryptophan and tyrosine residue fluorescence at 495 nm excitation wavelength. Samples were diluted 10 to 50 fold and measured using a Varian Cary Eclipse Fluorescence Spectrophotometer.

4.3.7. **Nuclear magnetic resonance spectroscopy**

Nuclear magnetic resonance (NMR) relies on the magnetic field produced by a spinning nucleus containing an odd number of nucleons (protons and neutrons). In the presence of external magnetic field the nucleus can exhibit more than one spin state and can move between these states by absorption of electromagnetic radiation of a specific frequency (radiofrequency).

In this study, proton (\(^1\)H) NMR spectroscopy was used to determine the residual organic solvent content in dialysed graphene dispersions. A Bruker Avance II operating at a frequency of 400 MHz (\(^1\)H) was employed to obtain the spectra. In all cases, spectra were acquired and processed using Bruker software Topspin 2.1. NMR data were collected using 128 transients into 65 k data points over a spectral width of 8 kHz, with a relaxation delay of 2.4 s between scans. Processing involved multiplication by an exponential window function prior to Fourier transform and phase correction. \(^1\)H chemical shifts (δ) were reported in parts per million (ppm).

For the NMR measurements, the graphene produced in organic solvents were dialysed against deuterium oxide (D\(_2\)O) rather than H\(_2\)O to avoid signal interference. Sample preparations were carried out by careful measurement of solutions. 0.99 mM Trimethyl phosphate (TMP) was used as internal reference (doublet; centred at 3.8 ppm). For control samples (without graphene) stock solutions of 10 v/v\%, 1 v/v\% and 0.1 v/v\% organic solvent levels were prepared by mixing 100 µl, 10 µl and 1 µl of organic solvents (NMP or DMF) in 900 µl, 990 µl and 999 µl D\(_2\)O, respectively. 8.2 µl of TMP was added to 691.8 µl of stock solution to make 700 µl of sample solution for NMR study. For dialysed samples (with graphene), the stock solutions were replaced by dispersions obtained after dialysis.
Assuming the TMP concentration was same, integral values (number of $^1$H) of the organic solvent signals (control and dialysed) were calculated against calibrated TMP signal with an experimental error of ± 5 %. All the spectra were analysed and plotted using Plot™ software.

4.3.8. X-ray diffraction

XRD was used to measure the inter-flake separation of the graphene paper prepared from dialysed graphene dispersions. X’Pert MPD, Philips was used and Bragg’s law was used to estimate the interlayer distance from the obtained $2\theta$ values.

4.3.9. Raman spectroscopy

Raman spectroscopy is one of the primary characterisation techniques used in this project. The experimental setup of the Raman spectroscope is explained below.

4.3.9.1. Raman experimental setup

A Renishaw 2000 spectrometer connected to an optical microscope and equipped with 633 nm HeNe laser (1.96 eV) as shown in Figure 4.13 was used for the majority of the studies. Whereas, for the doping studies, Raman 1000 spectrometer equipped with 514 nm Ar (2.41 eV) was used.

The light beam passes through spatial filters, which contained a pair of lenses and a 10 µm pinhole. The light then passes through holographic notch filters which reflect the light towards the microscope. During the deformation tests, the polarisation of the laser beam was always parallel to the tensile axis.
The sample was placed on the adjustable microscope stage and the laser beam was focused on the sample surface with a 50× ultra-long working distance objective lens. The diameter of the laser spot was about ~2 µm and the laser power on the sample was < 1 mW. The scattered light from the sample was collected in backscatter geometry and passed through the holographic notch filters, which filters out the Rayleigh scattered light. The diffraction grating and triangular mirror separate the scattered Raman lines spatially as a function of wavelength. After this process, a charge coupled device (CCD) camera collects the light. Charges were generated inside the CCD chip due to the scattered photons, which were collected with electrodes. The measured current was proportional to the number of photons detected and in turn to the intensity of scattered light. The intensity of the light was collected as a function of its wavelength (its difference with the incident light wavelength gives the Raman wave number in cm⁻¹), processed and stored on the computer. The spectral resolution of the spectrometer was 1 cm⁻¹ and absolute resolution (e.g., peak shift with respect to a spectrum collected on the same machine and fitted using curve fitting software) was 0.1 cm⁻¹. The spectra were acquired using Wire 1.3 software. The peak positions in the Raman spectra were fitted using a Lorentzian curve as described by:

\[ I = \frac{I_0}{1 + 4\left(\frac{\omega - \omega_c}{\Delta \omega}\right)^2} \]

Equation 4.8
where, $\omega$ is the Raman wave number (cm$^{-1}$), $\omega_c$ at the centre, $I_0$ is the maximum intensity and $\Delta \omega$ the full width at half maximum of the peak.\textsuperscript{333,345,346} The Raman system was calibrated using a standard silicon sample which has a characteristic peak at 520 cm$^{-1}$.

### 4.3.9.2. Deformational procedure

For the deformational analysis, a resistance strain gauge (Vishay Precision Group, Inc.) was fixed on all the composite coating samples on the PMMA beam using a cyanoacrylate adhesive. Electrical wires were soldered to the strain gauge and connected to a multimeter to measure the change in resistance during the deformation. The tensile strain on the sample top surface was introduced by using a four-point bending rig as shown in Figure 4.14.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{four_point_bending_rig.png}
\caption{a) Schematic representation and b) Picture of the four-point bending rig with PMMA beam showing tensile loading on the top surface}
\end{figure}

It is to be noted that, the strain on the thin composite sample was assumed to be same as that of beam surface. The resistance of the strain gauge changes corresponding to the strain applied on the beam. Hence, using the following equation value of applied strain on the sample surface could be calculated:

$$\varepsilon_m = \frac{R_{Si} - R_{S0}}{S_{gf} \times R_{S0}} \times 100\%$$ \hspace{1cm} \text{Equation 4.9}

Where, $\varepsilon_m$ is matrix surface strain, $R_{Si}$ is strain gauge resistance with loading, $R_{S0}$ is strain gauge resistance without loading and $S_{gf}$ is gauge factor of strain gauge (2.080 ± 0.5 from Vishay).

In order to investigate the strain sensing and interfacial stress transfer, composite coatings were subjected to cyclic deformation. Different deformational sequences were followed (Figure 4.15):
1) **Sequence I – Uniform Maximum strain**

A total of four to five deformation cycles (loading and unloading) with a maximum strain level of 0.3 % strain.

2) **Sequence II – Increased Maximum strain**

A total of five deformation cycles with 0.1 %, 0.2 %, 0.3 %, 0.4 % and 0.5 % strain as the maximum strain at each cycle.

3) **Sequence III – Strain Mapping**

To demonstrate the longer term stability and spatial mapping, a total of 25 cycles with a maximum strain of 0.3 % was employed. 5 random points were studied at the maximum and minimum of each cycle. Detailed spatial maps were taken on initial, 6th and 25th cycles at step intervals of 10 ± 2 µm.

4) **Sequence IV – Variable Maximum strain**

A total of nine deformation cycles with maximum strain of 0.3 % strain for first 3 cycles, 0.5 % for subsequent three cycles and 0.3 % for last three cycles.

It has to be noted that the sequence I and II were carried out for both mechanically exfoliated and CVD graphene coatings. Whereas, sequence IV was subjected only for mechanically exfoliated graphene coating to explore about the strain hardening behaviour, and sequence III was carried out only for CVD graphene coating to study the wide-area strain sensing behaviour.
Figure 4.15: Different cyclic deformational sequences followed to investigate the strain sensing and interfacial stress transfer in the composite coatings.

The shift of the 2D band with strain was used to follow the deformation. For deformation studies, all the spectra were obtained using 10 % laser power (< 1 mW, He-Ne laser, 633 nm) with an exposure of 10 seconds and 5 accumulations. The deformation was carried out step-wise in intervals of either 0.02 or 0.05 ± 0.005 % strain. A total of three spectra at each strain level were taken and the average value was used for the calculations. The maximum strain used in these deformation cycles were limited to 0.5 % as the debonding of the flakes and polymer matrix cracking was observed at strains greater than 0.5 % in similar model composites. When the maximum strain was achieved in each cycle, the sample was unloaded step-wise to 0 % strain.

The composite coatings prepared from LPE graphene were subjected to a deformational loading cycle up to a maximum strain of 0.5 % with 0.05 % strain intervals.
Chapter 5 – Production of Few Layer Graphene in Organic Solvents
5. PRODUCTION OF FEW LAYER GRAPHENE IN ORGANIC SOLVENTS

5.1. INTRODUCTION

LPE is one of the most common methods for producing graphene in various solvents mediums. This chapter explains the production of FLG by direct exfoliation of graphite flakes in organic solvents like NMP and DMF. Hernandez et al. have shown that NMP and DMF are among the most effective solvents for graphene owing to having a similar surface tension to that of graphene (see section 2.3.2). AFM was used to analyse the exfoliation efficiency and lateral dimensions, while Raman spectroscopy provided an insight about the quality of the graphene flakes. Finally, the potential for DLS as an efficient characterisation technique for estimating the lateral dimensions of the graphene flakes was demonstrated.

5.2. PRODUCTION OF FLG IN ORGANIC SOLVENTS

5.2.1. Effect of sonication time and centrifugation speed on the concentration of graphene

In order to optimise the production process in this project, natural graphite (5 mg/ml) was exfoliated in various organic solvents using bath sonication for different sonication times (6-60 hr) and centrifugation speeds (2000-10000 rpm). One disadvantage of using bath sonication is its reproducibility as the sonic energy input to the sample is sensitive for many factors including the water level in the bath, temperature of water, position in the bath, shape of the vessel, volume of suspension etc. In addition, the actual power output from the bath to the sample varies with the type of bath used. In order to reduce the number of factors that affects the end results, most of the variables were kept constant. All the measurements presented in this work were made from same sonication bath, using a 1 L round-bottomed flask held in the centre of bath in a constant depth of water. The actual power applied to the sample was calculated to be ~ 32 W by measuring the change in temperature of a known mass of water (The sonic bath was run at ~ 240 W at 37 KHz). As mentioned in the experimental section 4.1.2.2, a circulating cooler was used to maintain the bath water temperature between 22 and 25 °C to avoid evaporation.
of bath water at prolonged sonication times; as increased solvent temperature could affect the sonication process.

The graphite/organic solvent mixture was sonicated in the round-bottomed flask for 60 hr. Aliquots were taken at different times, left overnight for sedimentation, followed by centrifugation (20 min) at different speeds. Figure 5.1a shows photographs of the graphene obtained in the NMP. The dispersions produced using a longer sonication times appear darker at a given centrifugation speed indicating higher graphene concentration.

The quantitative information on the yield (or concentration) of graphene can be estimated using UV-Vis spectroscopy and applying the Lambert-Beer law: \( A = \alpha l c \), where \( A \) is the absorbance, \( l \) (m\(^{-1}\)) is the optical path length, \( c \) (mg/ml) is concentration and \( \alpha \) (ml mg\(^{-1}\) m\(^{-1}\)) is absorption coefficient. \( \alpha \) was experimentally determined by vacuum filtering a known volume of dispersions and measuring the resulting film in a microbalance, as explained in section 4.1.2.3. The value of \( \alpha = 4632 \) mL mg\(^{-1}\) m\(^{-1}\) was obtained for solvent exfoliation. Additionally, value of \( \alpha = 1825 \) mL mg\(^{-1}\) m\(^{-1}\) was obtained for TDOC-based exfoliation (see section 4.1.2.4). It should be noted that the different values for \( \alpha \) have been reported in literature\(^ {54, 59, 60, 69, 77} \) ranging from 1390 to 6600 mL mg\(^{-1}\) m\(^{-1}\) in various solvents and surfactant-based dispersions, and this variation is not clearly understood. In general the \( \alpha \) is constant and assumed to be characteristic material property, however, such variation in the observed and the reported values could probably relate to different surface and layer properties of the material.\(^ {347, 348} \) A study conducted by Su et al. on rGO with varying lateral dimensions, mean number of flakes and functional groups, revealed dispersions with smaller flakes (lateral dimension) and less layer per flake had lower \( \alpha \), attributed to shrinkage of the \( \pi \)-conjugated systems.\(^ {349} \) Therefore, to avoid any uncertainty, calculated \( \alpha \) values of 4632 mL mg\(^{-1}\) m\(^{-1}\) and 1825 mL mg\(^{-1}\) m\(^{-1}\) for organic solvent and aqueous surfactant-based dispersions, respectively, were used in this thesis. The UV-Vis spectra of FLG/NMP and FLG/DMF show characteristic featureless absorption as shown in Figure 5.1b. Unknown concentrations were calculated using the \( A \) value at 660 nm.
Figure 5.1: a) Photograph of graphene dispersions in NMP obtained after different sonication times and centrifugation speeds. b) Example UV-Vis absorption spectra obtained from graphene produced in NMP and DMF, with different concentrations, appearing flat and featureless.

The calculated concentrations after centrifugation are shown in Figure 5.2 for both FLG/NMP and FLG/DMF. It can be seen clearly from Figure 5.2a, that the concentration of FLG/NMP increases steadily with the sonication time and reaches maximum value at 60 hr for all the centrifugation speeds. The maximum concentrations obtained at 60 hr for FLG/NMP were $0.28 \pm 0.04$ mg/ml, $0.22 \pm 0.03$ mg/ml, and $0.12 \pm 0.01$ mg/ml at 2000 rpm, 6000 rpm, and 10000 rpm, respectively. It has been shown direct exfoliation in NMP for longer sonication times of about 270 hr results in concentrations up to $1.2$ mg/ml at 500 rpm.\textsuperscript{59} However, a controlled centrifugation study on graphene dispersions in NMP have shown that the mean number of graphene layers per flake ($\langle N \rangle$) increased as the speed of centrifugation decreased ($\langle N \rangle$ - 7.3, 3, and 3.2 for 500, 1000 and 3000 rpm, respectively).\textsuperscript{62} Hence in this study, relatively higher centrifugation speeds were chosen to remove the thicker flakes. It noteworthy that the larger size flakes are removed at higher centrifugation speeds leaving only smaller size flakes dispersed.\textsuperscript{59} This case was also observed in this study and is reported in the following section.
Figure 5.2: Concentration of graphene produced in (a) NMP and (b) DMF after centrifugation as a function of sonication time.

One of the objectives of this study was to obtain high concentrations of graphene dispersions in order to prepare epoxy-based composites for strain sensor applications (Chapter 7). Thus following the results for the FLG/NMP dispersions, only samples longer than 24 hr sonication were studied for FLG/DMF dispersions. Figure 5.2b shows the concentration against sonication time. As expected, the concentration shows a similar trend to that of the FLG/NMP dispersions, with a maximum concentration attained at 60 hr. The maximum concentrations obtained were 0.24 ± 0.02 mg/ml, 0.15 ± 0.01 mg/ml, and 0.1 ± 0.02 mg/ml at 2000 rpm, 6000 rpm, and 10000 rpm, respectively.

5.2.2. AFM characterisation

It is critical to establish the exfoliation efficiency (degree of exfoliation) of the various production conditions followed. TEM is widely used in the literature to determine the flake size and number of layers. In TEM, the number of layers can be determined both by electron diffraction pattern analysis and, less reliably, by the edge counting method, which requires a very high resolution image. AFM can also be used to obtain the size and thickness distribution of the flakes by depositing dispersions on various substrates.

The AFM samples were prepared by spray coating the graphene dispersions on freshly cleaved mica sheets. The degree of aggregation of flakes was reduced by spraying onto pre-heated mica sheets so that the solvent flash evaporated away.
Representative AFM scans and height profiles of the spray deposited flakes observed are shown in Figure 5.3. As seen in Figure 5.3a, large number of flakes were distributed reasonably uniformly over the substrate. To determine the lateral dimensions, the shape of the flakes was approximated to rectangular in shape and the length was taken as the longest side, as illustrated in Figure 5.3b and Figure 5.3f. The example monolayer flake in Figure 5.3d has an apparent height of ~1 nm, as seen from its height profile in Figure 5.3e. It should be noted that, the type of substrates used and the environmental conditions such as the relative humidity affect the estimation of the height of monolayer graphene by AFM.\footnote{For example, it was reported that the apparent height of monolayer graphene on SiO$_2$/Si was ~1 nm,\textsuperscript{13} while on mica it is be ~0.4 nm.\textsuperscript{350} However, it has been shown that the apparent AFM height in tapping mode depends strongly on the scanning parameters and the experimental environment.\textsuperscript{351}} In order to get more flakes/images for statistical analysis, fast-scan was used (i.e., a low interaction between the tip and substrate) over large area, typically 5 × 5 μm area as shown in Figure

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**Figure 5.3:** a) A representative 5 × 5 μm AFM scan of FLG/NMP flakes spray deposited on mica sheet. The bright flake is > 30 nm thick graphite. b) Zoomed region of the highlighted area in (a) showing the method used to measure the lateral dimensions. c) Height profile of the flake in (b). d) 1 × 1 μm AFM scan from FLG/DMF dispersions showing a monolayer with height profile in (e) of ~ 1 nm apparent height. Arrow indicates aggregated and folded flakes. f) Amplitude image of (d) shows the folds/aggregations clearly.
5.3 and Figure 5.4 under ambient environment. By analysing the height changes in fold and incompletely exfoliated graphene, the step height was determined always as a multiple of ~0.5 nm.

![AFM images of graphene flakes](image.png)

**Figure 5.4:** a, d, and i) AFM height scans showing monolayer graphene flakes in each image. The respective height profiles of monolayer graphene in (b, e, and j) have apparent height between 0.9-1.1 nm. Corresponding amplitude images in (c, f, and k) were also used to measure the lateral dimensions. Samples were prepared from FLG/NMP (a and d) and FLG/DMF (i and j).

Coleman *et al.* have also reported that the measured height of monolayer from such dispersions ranges from 1-2 nm (even up to 2.6 nm), which was characterised by its well-defined edges similar to TEM observations. In this study, the thinnest flakes observed amongst all the analysed AFM images had a height between 0.9-1.1 nm (5-15 %
of analysed flakes) as shown in Figure 5.4, which are assumed to be monolayer graphene. The increase in the thickness might be due to combination of scanning parameters and presence of residual NMP between the graphene and substrate and moisture content. With the knowledge of monolayer height and step height (~0.5 nm), the AFM height data can be converted into number of layers per flake (N).

By analysing the AFM images, statistical data on the exfoliation state could be derived. Statistical data was derived from at least 200 flakes for each condition. It should be noted that, despite pre-heating the mica substrates, the aggregation of flakes was unavoidable (Figure 5.3d and Figure 5.4a). However, through careful analysis of the AFM height image and corresponding amplitude image, the length can be measured (Figure 5.3 and Figure 5.4) reliably. Figure 5.5 shows the histogram of length of graphene flakes ($L_{AFM}$) observed at different sonication times and centrifugation speeds used. In general, as the sonication time increased from 24 hr to 60 hr the $L_{AFM}$ decreased. The decrease is clearer at higher centrifugation speeds; At 10000 rpm, $L_{AFM}$ decreases from 150 ± 80 nm for 24 hr to 80 ± 40 nm for 60 hr. Lower centrifugation speeds retain larger flakes (> 500 nm) as seen in the histogram. Whereas, higher centrifugation speed removes the larger flakes and retains only the smaller flakes leading to relatively small length distribution. For e.g., at 24 hr, $L_{AFM}$ decreases from 290 ± 170 at 2000 rpm to 150 ± 80 nm at 10000 rpm, which is ~50 % reduction in mean size. This behaviour was generally observed for graphene and other 2d materials.60, 62, 352

As explained earlier, the AFM height data was converted to the number of layers, $N$, by determining the apparent monolayer height (~1 nm) and step height (~0.5 nm). Histogram data of $N$ as a function of different sonication time and centrifugation speed is shown in Figure 5.6.
Figure 5.5: Histograms of the length of graphene flakes ($L_{\text{AFM}}$) observed by AFM as a function of sonication time and centrifuge speed.

Figure 5.6: Histograms of number of layers per flake ($N$) observed by AFM as a function of sonication time and centrifuge speed.
In general, the histogram data reveals that as sonication time increases, \( N \) decreases slightly. The mean number of flakes per layer, \( \langle N \rangle \), remains around 3-5 layers, except for 24 hr 2000 rpm sample which may be due to the fact that at low centrifugation speed the thicker flakes are not removed effectively (see Figure 5.3a). It can also be noted that, as the centrifugation speed increases, the number of thicker flakes \( (N > 7) \) reduces considerably suggesting that higher centrifugation speed removes heavier flakes and larger flakes (Figure 5.5) resulting in very stable dispersions. As a result, dispersions obtained at higher speeds (6000-10000 rpm) remain stable over six months without any visible aggregation or sedimentation unlike the dispersions at lower speed (2000 rpm) showing visible aggregation and sedimentation at the bottom of bottle within one month. Example AFM images (FLG/NMP, 60 hr) in Figure 5.7 show the mean length of the flake \( \langle L_{\text{AFM}} \rangle \) and \( \langle N \rangle \) changes with increase in centrifugation speed.

![Figure 5.7: Example AFM images of FLG/NMP at 60 hr at different centrifugation speeds](image)

From the statistical histogram data, additional statistics can be derived; Monolayer percentage \( (N_1/N_T \%) \), where \( N_1 \) is number of monolayers, \( N_T \) is total number of flakes examined) and few-layer percentage \( (N_{1-5}/N_T \%) \), where \( N_{1-5} \) is number of flakes < 5 layers, \( N_T \) is total number of flakes examined). The plots are shown in Figure 5.8.
Figure 5.8: Statistical data derived from the histograms plotted in all cases as a function of sonication time, including different centrifugation speeds. a) Length of flake ($L_{AFM}$), b) Mean number of layers per flake ($\langle N \rangle$), c) Monolayer percentage ($N_1/N_T\%$), d) Few layer percentage ($N_{1-5}/N_T\%$). Highlighted regions show the effect of centrifuge speeds at one sonication time (60 hr). All error bars represent the standard deviation from histogram data.

Figure 5.8a shows the variation of $L_{AFM}$ with different sonication time and centrifugation speed. As seen in histogram data, $L_{AFM}$ decreases as sonication time increases. Despite the reduction in flake length, relatively large standard deviations were observed for 2000 rpm samples. This is due to the presence of large distribution of flake lengths, i.e., the presence of larger flakes (> 500 nm) and also smaller flakes (< 500 nm). As the centrifugation speed increases, the larger flakes are removed, retaining only smaller flakes and hence smaller standard deviation (e.g., at 60 hr sonication, see highlighted region of Figure 5.8a).

It has to be noted that, AFM was used in this study for the lateral size analysis, as opposed to the more widely used TEM technique. However, one disadvantage while using TEM during statistical analysis is that, during the sample preparation technique smaller
flakes might escape through the holes in the TEM grid. This might result in over estimating the flake size.\textsuperscript{59, 61, 77} As observed in this study, even at low sonication times (24 hr), flakes as small as $<100$ nm and as large as $>1300$ nm are present and contribute to the calculated mean size (Figure 5.5).\textsuperscript{77} In addition to this, the present study is not a size selection study\textsuperscript{53} by using controlled centrifugation steps where relatively similar size of flakes can be extracted at a particular centrifugation speed. So, the presence of a large distribution of flake lengths in the samples is not surprising, thus justifying the standard deviation.

With regards to $N$, samples with lower sonication time (24 hr) and a centrifugation speed (2000 rpm) had $\langle N \rangle - 8 \pm 4$ (Figure 5.8b). As the sonication time increased up to 60 hr, even when using a low centrifuge speed of 2000 rpm, the $\langle N \rangle$ decreased considerably to $4.2 \pm 1.6$, suggesting that exfoliation efficiency increases with sonication time. At higher sonication times (48 and 60 hr), as centrifugation speed increased, $\langle N \rangle$ remained between 3-5 suggesting that higher centrifugation speed remove heavier $\langle N \rangle > 7$ and larger flakes $\langle L \rangle > 500$ nm, resulting in a stable dispersion with less sedimentation.

Derived $N_1/N_T \%$ and $N_{1-5}/N_T \%$ from the histogram data were and plotted in Figure 5.8c,d. As the sonication time increases $N_1/N_T \%$ increases steadily (\textit{e.g.}, at 6000 rpm, $\sim 4 \%$ at 24 hr to $\sim 8 \%$ at 60 hr). Most striking observation is that, $N_1/N_T \%$ increases drastically as centrifugation speed increases (\textit{e.g.}, at 60 hr, $\sim 5 \%$ at 2000 rpm to $\sim 16 \%$ at 10000 rpm). This suggests that the smaller sized flakes those remain at dispersed at higher centrifuge speeds must constitute to the higher $N_1/N_T \%$ than the large sized flakes. With regards to $N_{1-5}/N_T$, significant increase was observed as the sonication time increased (\textit{e.g.}, at 2000 rpm, $\sim 50 \%$ at 24 hr to $\sim 80 \%$ at 60 hr). At higher centrifugation speeds, $N_{1-5}/N_T \%$ was roughly constant between 80 \% and 90 \%. These observations were similar to literature.\textsuperscript{59, 350}

Similar trend in $\langle L_{AFM} \rangle$ and $\langle N \rangle$ was observed for FLG/DMF sample. As mentioned in 5.2.1, only higher concentration samples were analysed for DMF based dispersions. At 60 hr sonication time, the $\langle L_{AFM} \rangle$ and $\langle N \rangle$ changed from $\sim 170$ nm and 5.8 at 2000 rpm to $\sim 100$ nm and 3.3 at 10000 rpm, respectively (see Appendix 9.1). Noteworthy, for DMF-based dispersions, the $\langle N \rangle$ are slightly higher and $N_1/N_T \%$ and $N_{1-5}/N_T \%$ are slightly
lower than the NMP based dispersions, suggesting NMP is better solvent than DMF in terms of exfoliation efficiency.\textsuperscript{67}

5.2.3. Raman spectroscopy characterisation

5.2.3.1. Raman spectroscopy on graphene paper

Raman spectroscopy is a fast and non-destructive method for probing the physical and chemical phenomena of graphene. Therefore, it can be used to understand the nature of the graphene flakes produced by sonicated assisted exfoliation. Raman characterisation was carried out on graphene paper prepared by the vacuum filtration procedure described in section 4.1.2.3.1. A 633 nm (He-Ne) laser was used to characterise all the films. For each condition, typically 20 or more spectra were recorded and averaged. Raman spectra of FLG/NMP produced at different conditions are shown in Figure 5.9. A spectrum of starting graphite is shown for comparison.

The Raman spectrum of the starting graphite powder contains a single sharp G band at \( \sim 1580 \text{ cm}^{-1} \), D band at \( \sim 1330 \text{ cm}^{-1} \) and a 2D band at \( \sim 2686 \text{ cm}^{-1} \). The presence of D band suggests that the graphite does have small amount of defects. The 2D band is composed of two components; \( 2D_1 \) at \( \sim 2636 \text{ cm}^{-1} \) and \( 2D_2 \) at \( \sim 2689 \text{ cm}^{-1} \) and was fitted by two Lorentzian curves.

Figure 5.9 shows representative Raman spectra of the FLG/NMP samples prepared under various conditions. All the solvent exfoliated samples had in common a sharp G band (\( \sim 1580 \text{ cm}^{-1} \)), a considerably pronounced D band at \( \sim 1330 \text{ cm}^{-1} \), a broad and symmetric 2D band at \( \sim 2660 \text{ cm}^{-1} \) and a shoulder peak \( D' \) at \( \sim 1617 \text{ cm}^{-1} \). The D and \( D' \) band are defect related bands which can be probed to understand the nature of defects in the graphene.\textsuperscript{67}
Figure 5.9: Representative Raman spectra of FLG/NMP. a) Effect of sonication time (at constant centrifugation speed). b) Effect of centrifugation speed (at constant sonication time). Spectra for starting graphite shown ($I_D/I_G$ graphite ratio ~0.08) is also shown for comparison. All spectra were taken using 633 nm (He-Ne) laser. Dashed line indicates intensity variations between G and D band for various conditions.

In general, all the spectra are similar to those reported in the literature for liquid-phase exfoliated graphene.\textsuperscript{54, 60, 62, 67, 69, 77} One significant difference between the samples (at different conditions) is the intensity variations of the D and G band, as highlighted by dashed lines in Figure 5.9. The intensity of the D band increases slightly as the sonication time increases (Figure 5.9a), but the intensity increases drastically as the centrifugation speed increases (Figure 5.9b). As will be explained later in section 5.2.3.2, the intensity increases are caused mainly because of the edge effects rather than other defects types. The Raman spectra obtained on FLG/DMF graphene films were also very similar as of FLG/NMP and is shown in Appendix 9.1.
Figure 5.10: a) Individual Raman spectra obtained on FLG/NMP (48 hr, 6000 rpm) graphene paper at 20 different positions demonstrating the homogeneity of the film. b) Accuracy of the curve fit on 2D band using single Lorentzian function. All spectra were taken using 633 nm (He-Ne) laser.

Figure 5.10a shows an example of ~15 individual Raman spectra obtained on a graphene film (FLG/NMP, 60 hr, 6000 rpm) produced. As can be seen only very little variations were observed in the spectra. This suggests the film produced is very homogenous and the values obtained were averaged and considered for analysis. The 2D band obtained contains a broad but symmetrical band, which can be fitted using single Lorentzian peak as shown in Figure 5.10b. It has to be noted that, single Lorentzian fit was not accurately fitted for all the observed samples. Some samples obtained at lower centrifugation speed (2000 rpm) had to be forced fitted to single Lorentzian. This may be because the presence of thicker flakes \( \langle N \rangle > 7 \) as observed in the AFM.
The shape of the 2D band can be used to estimate the number of layers in the sample. However, it remains challenging to establish the precise number of layers for LPE samples which have a broad and symmetric 2D band as observed in Figure 5.10. This broad 2D band is similar to that observed from turbostratically stacked graphene/graphite, probably due to the rotational disorder between the flakes breaking the electronic coupling between the individual layers.

Figure 5.11a shows the change in 2D band position with sonication time (e.g., at 6000 rpm, 2D band position is $2662 \pm 0.5$ cm$^{-1}$ at 24 hr to $2659 \pm 0.6$ cm$^{-1}$ at 60 hr). It has been shown for MC flakes, that when a 2D band is fitted with single Lorentzian peak, as the number of layers increase, the position of the 2D band shifts to higher wavenumbers and reaches the maximum position which corresponds to the position of HOPG. Following this observation, empirically, it can be said that as the sonication time increases the number of layers decreases. Hence the 2D band position has a lower wavenumber for longer sonication time samples (Figure 5.11a). The difference in the 2D band position is clearer with different centrifugation speeds. The highlighted region in Figure 5.11a shows the variation of 2D band position at 60 hr with different centrifugation speed; $2665 \pm 1.1$ cm$^{-1}$ at 2000 rpm to $2655 \pm 0.8$ cm$^{-1}$ at 10000 rpm. This observation suggests that at higher centrifugation speeds, only the thinner graphene flakes remain dispersed, confirming AFM observations. The FWHM(2D) fall into
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the range of 83-86 cm$^{-1}$ (Figure 5.11b). The FWHM(2D) is found to hardly to vary with different sonication time and centrifugation speed.

The G band position, D band position and FWHM(D), were invariant with the various sonication time and centrifugation speeds as shown in Figure 5.11c,e,f. Whereas a slight increase in FWHM(G) is observed (Figure 5.11d). However, the lack of significant broadening of the G band and D band confirms that there are no major disruptions to the basal plane as in the case of GO (for GO, FWHM(G) and FWHM(D) > 100 cm$^{-1}$). The position of the G band remained at 1580 ± 1 cm$^{-1}$ for all conditions suggesting that the graphene produced is not heavily doped. The position of the D band remained fairly unchanged at 1331 ± 1 cm$^{-1}$, for all conditions.

![Figure 5.12: a) Mean $I_{2D}/I_G$ ratio, b) Mean $I_D/I_G$ ratio as a function of sonication time with different centrifugation speeds. Highlighted regions show effect of centrifugation speeds at one sonication time (60 hr).](image)

Figure 5.12 shows the mean $I_{2D}/I_G$ and $I_D/I_G$ ratio as a function of different sonication time. It is well-known that $I_{2D}/I_G > 2$ is one of the characteristic Raman features of a monolayer graphene. Ni et al. shown the intensity of G band increases as the number of graphene layers increases. It has also been suggested that number of graphene layers is sensitive to $I_{2D}/I_G$ ratio. It can been seen from Figure 5.12a that as sonication time increases, the $I_{2D}/I_G$ increases slightly at a given centrifugation speed (e.g., at 6000 rpm, $I_{2D}/I_G$ ratio changes from 0.47 ± 0.1 at 24 hr to 0.53 ± 0.1 at 60 hr). Similarly, as the centrifugation speed increases (highlighted region), the $I_{2D}/I_G$ ratio increases (e.g., at 60 hr, $I_{2D}/I_G$ ratio changes from 0.48 ± 0.1 at 2000 rpm to 0.55 ± 0.1 at...
10000 rpm). This observation suggests that at a higher sonication time and centrifugation speed there are more few layer flakes ($\langle N \rangle$ – 1 to 5) than many layer (> 7 layers) flakes, which is indeed what is observed in AFM. However, $I_{2D}/I_G$ ratio cannot be relied on in estimating the number of layers effectively as it has been shown that the ratio varies up to four times for a suspended monolayer to a supported monolayer because of spatially varying doping levels arising from the interaction with the substrate.\textsuperscript{356} Nevertheless, the observation of $I_{2D}/I_G$ ratio increase could be attributed to few layer flakes domination.

The presence of defects in the graphene gives rise to D and D’ band, which are initially are forbidden in non-defective graphene.\textsuperscript{57} The $I_D/I_G$ ratio is widely studied to gain insight on the nature of defects that are present or introduced in the graphene.\textsuperscript{9, 55, 57, 252, 253, 260, 357-359} Figure 5.12b reveals how the $I_D/I_G$ ratio varies with different sonication times and centrifugation speeds. The ratio increases steadily with sonication time at all centrifugation speeds (e.g., at 6000 rpm, $I_D/I_G$ ratio changes from 0.95 ± 0.3 at 24 hr to 1.27 ± 0.5 at 60 hr). The increase in ratio is significant as a function of centrifugation speed (e.g., at 60 hr, $I_D/I_G$ ratio changes from 0.76 ± 0.6 at 2000 rpm to 1.64 ± 0.5 at 10000 rpm).

The quantity of defects has been shown to relate with $I_D/I_G$ ratio, larger the ratio, larger the defect density ($I_D/I_G$ ratio inversely related to the crystal grain size).\textsuperscript{259, 260, 360} In other words, the intensity of D band increases as the defect concentration increases. It is well-known that in graphene the edges act as defects.\textsuperscript{48, 56} So, in this case the defects can be attributed to the edges rather than basal plane defects in graphene (as explained in section 5.2.3.2). The diameter of the laser spot is ~2 µm. The average size of graphene produced by sonication as observed by AFM are at least a magnitude smaller, leading to observation of significant number of edges under the laser spot. This result give rise to the intense D band as observed in the Raman spectra (Figure 5.9).

In addition to that, in a defect-free graphene, any changes in the FWHM(G) are due to doping.\textsuperscript{55, 242} Whereas, FWHM(G) increases considerably for the defected sample.\textsuperscript{242, 259} However, here the broadening of G band in FLG is not as significant as what is observed for GO and perhaps the small increment of FWHM(G) (Figure 5.11d) are due
to edge defects. Unlike the GO or rGO, the FWHM(D) band doesn’t show significant broadening confirming the defects are not of sp$^3$ type.$^{173}$

Similar results were observed for FLG/DMF as shown in Appendix 9.1. The position of 2D band shifts to a lower wavenumber as a function of sonication time when fitted with single Lorentzian suggesting thinner flakes remain dispersed at high centrifugation speeds. The centrifugation speed did not have any significant effect on the position of G and D band and their respective FWHM (not shown). $I_{2D}/I_G$ and $I_D/I_G$ ratio had similar results as seen for FLG/NMP suggesting the increase in $I_D/I_G$ ratio is mainly because of the edges.

### 5.2.3.2. Raman analysis to estimate flake length and access defect type

Coleman et al. have shown that the sonication of graphite to produce graphene is relatively a non-destructive process, in terms of introducing any new basal plane defects, and the observed defects are predominantly due to the edges.$^{59-63}$ They have probed the correlation between the $L$ of the flakes to $I_D/I_G$ ratio to confirm that sonication doesn’t introduce any basal plane defects. This procedure was followed in this present study to prove that the defects are mainly due to the edges produced by sonication process and not that of basal plane defects.

As explained in the previous section, as the size of flakes decreases due to sonication process and centrifugation speeds, the density of edges under the laser spot increases resulting in an intense D band and hence $I_D/I_G$ ratio. If the variation in $I_D/I_G$ ratio with sonication time and centrifugation speed is only because of the flake size, then the $I_D/I_G$ ratio must relate to the mean length of the flake ($\langle L \rangle$) such that,$^{59, 61-63}$

$$\frac{I_D}{I_G} = \left( \frac{I_D}{I_G} \right)_{\text{graphite}} + \frac{k}{\langle L \rangle}$$  \hspace{1cm} \text{Equation 5.1}

Where, $k$ is a constant and $(I_D/I_G)_{\text{graphite}}$ is the $I_D/I_G$ ratio of starting graphite. The starting graphite powder had a $(I_D/I_G)_{\text{graphite}} \sim 0.08$ (Figure 5.9). Since the graphite has relatively large grain size ($\sim 200$ µm), the ratio could not have come from edges, indicating that some basal plane defects are present in the starting graphite powder.
Figure 5.13: $I_D/I_G$ ratio as a function of $1/\langle L \rangle$ for all the samples produced. The linear fit does coincide with the measured value of $(I_D/I_G)_{\text{graphite}}$ which is also shown.

The $\langle L \rangle$ was estimated from AFM analysis for all the different samples produced under different conditions. The plot between $I_D/I_G$ vs $1/\langle L \rangle$ is shown in Figure 5.13. It has to be noted that all data from FLG/NMP and FLG/DMF were used for the plots. The linear relationship can be clearly seen between $I_D/I_G$ and $1/\langle L \rangle$ suggesting the observed increase in D band intensity is only due to the edges. Interestingly, the intercept of the straight line (~0.1) coincides approximately with the measured value of $(I_D/I_G)_{\text{graphite}}$ (~0.08) suggesting that the sonication does not introduce any new basal plane defects in the graphene apart from pre-existing defects in the starting graphite powder. The slope of the graph is $k = 0.14$, which is comparable to literature values on the solvent exfoliated graphene by sonication ($k = 0.17$ and $k = 0.26$) and shear exfoliation ($k = 0.17$). These values can be used in Equation 5-1 such that $L$ can be estimated via Raman measurements.

$$L_{\text{Raman}} = \frac{0.14}{I_D/I_G - 0.08}$$  

Equation 5.2

However, care should be taken when it comes to the $(I_D/I_G)_{\text{graphite}}$ (~0.08) and the constant $k$ (0.14) as it is shown to vary on the type of graphite used. Figure 5.14 shows the length data derived from the Equation 5-2 ($L_{\text{Raman}}$) and its correlation with $L_{AFM}$ for different sonication and centrifugation speeds used. It can be seen that the
$L_{\text{Raman}}$ and $L_{\text{AFM}}$ match each other. Hence this method could be used to estimate of mean lateral size of graphene flakes with a relative error of $\sim 17\%$.

![Image of graph showing Raman and AFM measurements against sonication time.](image)

**Figure 5.14:** Plot between the calculated $L_{\text{Raman}}$ and the measured $L_{\text{AFM}}$ vs sonication time.

Recently Eckmann et al. studied the nature of defects in graphene by probing the $I_D/I_{D'}$ ratio. This ratio depends on the nature of the defects, $I_D/I_{D'} \sim 3.5$ for boundary or edge type defects, $I_D/I_{D'} \sim 7$ for vacancy defects (basal plane defects) and $I_D/I_{D'} \sim 13$ for sp$^3$ type defects.\(^{57}\) Again, this ratio could be analysed for the samples to confirm the type of defects.

![Image of graph showing $I_D/I_G$ and $I'_{D}/I_G$ ratios for FLG/NMP and FLG/DMF samples.](image)

**Figure 5.15:** a) $I_D/I_G$ ratio vs $I'_{D}/I_G$ ratio for all FLG/NMP and FLG/DMF samples. The data point from starting graphite powder is added for reference. b) $I_D/I_{D'}$ ratio as a function of sonication time and centrifugation speed. The superimposed lines in (a) and (b) represent the type of defects from literature.\(^{57}\)
Figure 5.15a is a plot between $I_D/I_G$ ratio and $I_D'/I_G$ ratio for all the samples including FLG/NMP and FLG/DMF. The superimposed lines represent the type of defects as reported by Eckmann et al.\textsuperscript{57} It can be clearly seen that the data exhibits a linear behaviour with a slope, $I_D/I_D' \sim 4.61$. This value lies in between the edge type and vacancy type defects and compares to the measured value of $(I_D/I_D')_{\text{graphite}} \sim 5$ (graphite spectra in Figure 5.9). This again is consistent with the argument of no new basal plane defects been introduced during the sonication procedure.

In addition, if basal plane defects were being introduced during sonication then the $I_D/I_D'$ ratio should increase when sonication time increases. Figure 5.15b shows the variation of $I_D/I_D'$ ratio as a function of sonication time with different centrifuge speeds for both FLG/NMP and FLG/DMF. It can be seen that, invariant of any conditions, the $I_D/I_D'$ ratio stays within the error with a value of $I_D/I_D' \sim 4.5$. This strongly suggests that the sonication of graphite to produce graphene does not introduce any new defects in the basal plane. These observations confirm that the suggestion by Coleman et al. on the $I_D/I_D'$ ratio of boundary defects (edge defects) should perhaps be $3.5 \leq I_D/I_D' \geq 4.5$.\textsuperscript{127}

5.2.4. Lateral size determination using Dynamic Light Scattering

It is well-known that the liquid-phase exfoliation method can be used to produce a large number of 2d materials in dispersion.\textsuperscript{54, 68, 127, 361} Once these dispersions are produced it is important to know/determine the dimensions and the thickness of the nanosheets as they vary in thickness and size. Based on the processing conditions the dimensions of the nanosheets vary, especially the lateral dimensions which become critical for certain applications (e.g., composites).\textsuperscript{32, 291} In order to characterise it, several methods been followed which are indirect, e.g., TEM or AFM (as used in this study). Especially when it comes to scaling up the production, these indirect ways of determining the lateral dimensions become tedious, time consuming and expensive in some cases.

Other existing methods for measuring the in situ size in dispersion include; laser diffraction, and X-ray scanning sedimentation, which require special equipment and are still time consuming.\textsuperscript{362} It has been shown that size exclusion chromatography\textsuperscript{63} and via controlled centrifugation\textsuperscript{62} graphene dispersions could be separated based on their lateral dimensions. Other centrifugation-based techniques have been reported to separate a
range of nanomaterials based on their sizes.\textsuperscript{363-367} Ultracentrifugation was shown to separate nanomaterials based on their size and thickness effectively.\textsuperscript{78, 368} However in all the cases, separate measurements had to be done to extract the size of the flakes. Very recently it has been shown that for other 2d materials like MoS\textsubscript{2}, WS\textsubscript{2}, MoSe\textsubscript{2} and WSe\textsubscript{2}, the size and thickness data could be obtained by studying the extinction spectra.\textsuperscript{368}

DLS is a technique which is a commonly used to measure the size of particles in colloidal dispersions. It works on the principle of measuring the diffusion coefficient of the particle and converting it into hydrodynamic size (assuming spherical geometry) by using Stokes-Einstein’s equation as explained in section 4.3.4. This method works extremely well for the spherical particles, for non-spherical properties like rod or sheets, the relationship between diffusion coefficient and size becomes complicated.\textsuperscript{369}

In this present work, size of graphene flakes produced under different sonication time and centrifugation speed were measured using DLS and the results were compared to the statistically measured size data from AFM (section 5.2.2) and calculated size data from Raman spectroscopy (section 5.2.3).

### 5.2.4.1. DLS on graphene dispersions

The DLS works on principle, of measuring the scattered light fluctuations of particles undergoing Brownian motion as a function of time. The principle and the process is explained in section 4.3.4.1.

In this study, Zetasizer Nano ZS, a commercially available DLS instrument from Malvern Instruments Limited was used. This instrument was equipped with a backscattering angle detector (173\textdegree) thereby reducing multiple scattering of the scattered light. The instrument optimises the position of measurement and attenuation of the incident beam prior to the measurement. Hence, dispersions with high concentrations could be analysed without dilution. The DLS software analyses the scattered light, correlates the fluctuation using a set of algorithms and outputs a plot with relative intensity vs size of the particles. This distribution is referred to as Intensity Particle Size Distribution (IPSD). The primary position of this peak is considered as $L_{DLS}$.\textsuperscript{369}
Same set of dispersions which were analysed by AFM and Raman spectroscopy were examined by DLS. Figure 5.16 show representative IPSD of FLG/NMP dispersions. This type of curve is typical of IPSD and it is characterised by a bell-shaped curve on a relative intensity vs size (nm, logarithmic scale). The distributions are normally characterised by a single peak, however for some samples multi-peak characteristics were also observed.

![Intensity particle size distribution (IPSD) for FLG/NMP. a) At various sonication time, b) At different centrifugation speeds](image)

From Figure 5.16, it can be clearly seen that DLS is certainly sensitive to the change in size of graphene flakes despite its non-spherical geometry. As the sonication time increases the $L_{DLS}$ decreases (e.g., at 2000 rpm, $\sim350$ nm at 24 hrs to $\sim200$ nm at 60 hr), as observed in AFM. Similarly, the $L_{DLS}$ decreased as the centrifugation decreased (e.g., for 48 hr, $\sim300$ nm at 2000 rpm to $\sim100$ nm at 10000 rpm). These values are similar to the length measured by AFM and Raman spectroscopy. As mentioned earlier, some of the IPSDs were characterised by multi-peak. In the case of 48 hr 2000 rpm sample, a small peak appears at $\sim50$ nm. This might be from smaller flakes that are present in the dispersions as seen in AFM images. When TEM is used for statistical analysis these smaller flakes might escape through the grid during the sample preparation leading to underestimating the size of the flakes. One cannot rule out the possible aggregation in AFM when using high boiling solvents as seen in Figure 5.3. A smaller peak appeared at $>3$ µm in some samples. The origin of this peak is unclear, as no such sized graphene sheets
were observed under AFM. These peaks were also reported by others and suggested it might be from air bubbles or dust particles.\textsuperscript{70}

![Graph showing IPD of a non-centrifuged sample (PDI-1) and 10000 rpm centrifuged sample (PDI-0.23)](#)

\textit{Figure 5.17: Example IPD of a non-centrifuged sample (PDI-1) and 10000 rpm centrifuged sample (PDI-0.23)}

In some cases, when the sample is very poly disperse, the built-in quality report of the software notifies the user and suggests that the sample is unsuitable for measurement. This is because the software struggles to fit the correlogram function effectively due to presence of various sizes, hence affecting the derivation of the diffusion coefficient and the hydrodynamic size. Specifically some poorly centrifuged samples which had sedimenting particles (usually larger) were flagged with such notifications by the software. Figure 5.17 show an example of such case, where a non-centrifuged (only sonication) sample was measured. This sample had a population of particles which were sedimenting under gravity. The software stated that the samples poly dispersity index (PDI) measured by the software was PDI-1. The reason could be that the presence of large sedimenting particles constantly affects the observed size population within a certain time period, resulting in drastic change in observed intensity fluctuations, affecting the correlation function. Such samples are characterised by a high intense peak (Figure 5.17) and poor correlation function. However, sample centrifuged at 10000 rpm showed a PDI-0.23. The measured PDI of all the FLG/NMP and FLG/DMF samples was between 0.2-0.42. The value of PDI for a monodisperse sample is $< 0.05$, and such values are not possible for graphene dispersions as there is always a range of sizes present. However, empirically, it can be suggested that smaller the PDI lesser the poly dispersity.

The DLS instrument can assess the sample’s size distribution using a number of measurements; \textit{z}-Average diameter, IPSD, volume particle size distribution (VPSD) and
number particle size distribution (NPSD). The presence of such uncharacteristic peaks (> 3 µm) will have an effect on the value of z-Average diameter as it considers all the peaks in the IPSD. Such peaks would affect the VPSD and NPSD as the software uses Mie theory to convert the peaks of IPSD. Hence, IPSD is the only value which could be considered, as only the solvent temperature and viscosity values are required to derive the size information.  

5.2.4.2. Correlation of lateral sizes determined by AFM, Raman and DLS

In this section, the length data derived from the primary peak position of the DLS IPSD \( (L_{DLS}) \) is compared with \( L_{AFM} \) and \( L_{Raman} \) to see its relevance with the data.

Figure 5.18: Plot between the primary peak position derived from DLS data \( (L_{DLS}) \) vs \( L_{AFM} \) and \( L_{Raman} \) showing its linear dependence. Error data are from the standard deviations.

Figure 5.18 show the dependence between \( L_{DLS}, L_{AFM} \) and \( L_{Raman} \). It has to be noted that all data from FLG/NMP and FLG/DMF has been used in this plot. It is clear that the \( L_{DLS} \) data correlates and scale linearly with both \( L_{AFM} \) and \( L_{Raman} \), suggesting that the data obtained by DLS is indeed the size data of graphene sheets. This in turn confirms
that the IPSD of DLS measures the hydrodynamic diameter, which is the diameter of a sphere whose volume is equal to that of the mean graphene flakes volume, having similar translational diffusion coefficient. One can also note the large error bars which correspond to the AFM standard deviation, and as explained (section 5.2.2) it is because of the presence of large length distributions in samples with lower centrifugation speed. Despite the error bar, the mean length derived from AFM ($L_{AFM}$) and Raman ($L_{Raman}$) does scale with DLS data ($L_{DLS}$).

Hence, the derived $L_{DLS}$ is a reliable estimate of the mean size of the nanosheets in the dispersion and not the distribution. DLS cannot determine the distribution of length due to the limitations of the instrument (data point of x-axis in Figure 5.16). Another limitation is that DLS becomes less sensitive when the size of the particles is > 1-2 µm. However, most of the LPE nanosheets do fall below majorly below 1 µm.

Recently Lotya et al. have measured the size of various nanosheets types (graphene, MoS$_2$ and WS$_2$ in various solvents) using DLS. The lateral size statistical data were derived from TEM images on centrifugation-based size-selected flakes.

![Figure 5.19: Primary IPSD peak position, $a_{DLS}$, vs mean nanosheets size, $〈L〉$, measured from TEM image analysis. Dashed line: fitted power law dependence of $a_{DLS}$ with $〈L〉$. Image used from ref.](image)

They have found that the primary IPSD peak position, $a_{DLS}$, and $〈L〉_{TEM}$ scale linearly and follows a power law dependence (Figure 5.19). The fact that data for different nanosheets types fall on the same line (Figure 5.19), similar to the current study (Figure 5.18), supports this analysis as it shows that only nanosheets lateral dimension
and not material type affects the value outputted by the DLS instrument. Moreover, by fitting the data, they have derived a semi-empirical correlation (Equation 5.3) between the size outputted by DLS and \( \langle L \rangle \), that can be used to estimate the mean size of the dispersed nanosheets.

\[
\langle L \rangle = (0.07 \pm 0.03)a_{DLS}^{(1.5\pm0.15)} \tag{Equation 5.3}
\]

In the current study, by comparing the mean size data of DLS with AFM and Raman it can be said that \( L_{DLS} \) can estimate the size of nanosheets with a relative error of 25\%, agreeing with Lotya \textit{et al.}\textsuperscript{70} This \textit{in situ} length measurement technique, despite its error, could play a crucial role when preliminary ideas of the flake sizes are required, comparing samples in a quality control environment such as production or understanding the \textit{in situ} aggregation of graphene sheets. The latter will be demonstrated in Chapter 6 when considering bio surfactant-graphene dispersions.

\section*{5.3. \textbf{SUMMARY}}

In this Chapter, the production of stable dispersions of FLG in NMP and DMF by sonication of natural graphite was discussed. The concentration of the dispersion was found to increase with sonication time and decrease with centrifugation speed. Moreover, dispersions produced at higher centrifugations speeds were found to stable over six months without any visible sedimentation.

The exfoliation efficiency was characterised using AFM. Longer sonication times and higher centrifugation speeds resulted in dispersions with smaller \( L_{AFM} \) (80 to 120 nm) and thinner \( N \) (3 to 5) flakes and \textit{vice versa} for dispersions produced at shorter sonication times and lower centrifugation speeds. Despite flakes having relatively small \( \langle L \rangle \) \( \sim 130 \) nm, samples produced at 60 hr sonication and 6000 rpm centrifugation speeds were the best of all preparations with \( N_{1-5}/N_T \% \sim 80 \% \) whilst maintaining stable dispersion. FLG/NMP and FLG/DMF prepared at the conditions were used as the starting material for dialysis in Chapter 6.

Raman spectroscopy was used to understand the quality of the flakes. The Raman spectra of all the samples exhibited a symmetric 2D band and its position, FWHM(2D) along with \( I_{2D}/I_G \) ratio indicates that the graphene is few-layer \( \langle N \rangle < 5 \) in nature. By
probing the $I_D/I_G$ ratio along with $I_D/I_D'$, it was shown that the intense D bands are from the edges that are formed during the sonication process, and not because of any new basal plane defects. This shows that the sonication is a non-destructive process with regards to the introduction of any new basal plane defects. Moreover, the $L_{\text{Raman}}$ was also derived from the $I_D/I_G$ which compares to $L_{\text{AFM}}$ with a relative error of $\sim 17\%$.

Finally, the potential for DLS as an efficient characterisation technique for estimating the lateral dimensions of the graphene flakes was explored. The $L_{\text{DLS}}$ derived from the primary peak position of IPSD of DLS were compared to the calculated $L_{\text{Raman}}$ and $L_{\text{AFM}}$. It was found that the $L_{\text{DLS}}$ indeed exhibited a linear relationship with $L_{\text{Raman}}$ and $L_{\text{AFM}}$, with a relative error of $\sim 25\%$ in measuring the mean size of nanosheets (in submicron region). This is consistent with the IPSD obtained from DLS is a measure of the hydrodynamic diameter, which is the diameter of a sphere whose volume is equal to that of the mean graphene flakes volume, having similar translational diffusion coefficient. This \textit{in situ} length measurement technique, despite its error, could play a crucial role when preliminary ideas of the flake sizes are required, comparing samples in a quality control environment such as production or understanding the \textit{in situ} aggregation of graphene sheets.

It has to be noted that, the method established in this chapter for measuring the lateral dimensions using AFM and DLS and the quality of graphene flakes using Raman spectroscopy will be used in the Chapter 6 to characterise the graphene flakes produced using bio-molecules as surfactants. Furthermore, the FLG/NMP and FLG/DMF dispersions produced in this chapter will be used as the starting material for further studies (dialysis – section 6.2.2).
Chapter 6 – Production of Few Layer Graphene in Aqueous Media for Biological Studies
6. PRODUCTION OF FEW LAYER GRAPHENE IN AQUEOUS MEDIA FOR BIOLOGICAL STUDIES

6.1. INTRODUCTION

Graphene-based materials (GBMs), due to their exceptional properties, have gained much interest in recent years in the biomedical field with potential applications in biosensors, tissue engineering and drug delivery\textsuperscript{197-202}. These biomedical applications combined with the wide use of graphene in laboratories, resulted in a strong need for the study of the environmental health and safety issues associated with graphene,\textsuperscript{203, 204} leading to several toxicity studies.\textsuperscript{5, 205, 206}

GBMs exist in different forms: SLG, FLG (2-10 layers), GO, rGO, ultrafine graphite (more than 10 layers but less than 100 nm thick), GNRs and graphene dots. The biocompatibility of GBMs is expected to vary with the graphene type.\textsuperscript{5} Recent reviews have shown that the biological responses of GBMs vary depending on the number of layers, layer dimensions, stiffness, hydrophobicity, surface functionalities, purity and dosage of the material.\textsuperscript{5, 203} The environmental health and safety of graphene has been complicated and confused by the bias of the studies to date towards functionalised or GO-based, with few studies considering pristine graphene.\textsuperscript{207} Biomedical investigation with rationally-selected and carefully-characterised graphene materials has been urged to study interactions with cells, tissues and organisms.\textsuperscript{370}

As highlighted by Bianco, the extrinsic properties of graphene, such as surface chemistry, play a major part in determining the \textit{in vivo} and \textit{in vitro} toxicity.\textsuperscript{5} Most available studies concerns GO, rGO or functionalised-GO, mainly due to their stability in aqueous media.\textsuperscript{5, 209, 210, 371, 372} GO cytotoxicity includes biomembrane and inflammatory damage, diminished by some chemical modifications,\textsuperscript{373} and oxidative degradation, resulting in holes as basal plane defects,\textsuperscript{374} which could potentially result in highly toxic polyaromatic hydrocarbon type compounds.

\textsuperscript{1} This work is a part of Graphene Environmental Health & Safety (EHS) Working Group, a collaborative project between School of Materials and Manchester Pharmacy School, funded by Dean of Engineering and Physical Science Faculty, University of Manchester. UK.
The toxicity of pristine graphene (sp² carbon lattice) remains largely unexplored, but increasingly indicates minimal toxicity. A recent study by Conroy et al. has shown no detectable cellular morphology changes in cultured human lung epithelial cell lines A549 grown on pristine graphene films (CVD and LPE-based) or when exposed to LPE graphene flakes (0.1 to 5 µg/ml). The lung has been most considered the most important organ with regard to entry by graphene into the external epithelial surfaces of the body. Aqueous surfactant-dispersed pristine FLG produced minimal inflammation in lung cells compared to GO. Pristine graphene nanoplatelets possessing a sufficiently large aerodynamic diameter to be deposited in lung alveolae were indicated as a possible inflammation risk with the alveolar macrophages up taking the flakes and producing reactive oxygen species. However, on extended medium term studies, minimal oxidation and lung inflammation damage was found by the same group.

Surfactant polymer (Pluronic F108) exfoliated FLG (~170 nm hydrodynamic diameter, 2-3 nm thickness & ζ of -20 mV) decreased the viability of mitochondrial cells in a dose-dependent manner (50% viability loss for 100 µg/ml FLG). This cytotoxicity is believed to have resulted from both the loss of the mitochondrial membrane potential and intracellular reactive oxygen species (ROS), resulting in triggering of apoptosis. Sub-lethal concentrations (20% viability loss for 20 µg/ml) resulted in the secretion of pro-inflammatory cytokines of both Th1 and Th2, but reduced led to phagocytosis and binding to extracellular matrix, preventing over-activation.

Pristine GBMs are often presumed to aggregate in aqueous biological media in the absence of added surfactants and to be unsuitable for biological studies. However, the more likely exposure risk will be from pristine GBMs. Pristine GBMs will associate with organic materials and adsorb biomolecules, which are likely to affect their dispersion-aggregation and biological behaviour. For example, suggest a ‘slice and dice’ action of pristine FLG on biomembranes, however, their experimental studies included mixing with phospholipids to disperse the FLG, which combination was found to be lytic and cytotoxic in this study.

In this chapter, several routes for producing pristine graphene via LPE were explored in order to provide well-characterised aqueous preparations of pristine FLG for
biological and toxicity studies. Aqueous dispersions were first prepared by solvent exchange method of graphene originally in NMP or DMF by dialysis. Aqueous graphene dispersions were then prepared directly, by exfoliating graphite in in biocompatible surfactant and biomolecule (phospholipids and albumin protein) solutions. Cell culture experiments by my collaborators found that the solvent-exchanged and biocompatible surfactant-exfoliated pristine FLG displayed minimal cytotoxicity and albumin-exfoliated FLG hardly any cytotoxicity, whereas GO and phospholipid-exfoliated FLG were particularly cytotoxic (Appendix 9.6.1).

6.2. **Solvent Exchange of Graphene**

6.2.1. **Why solvent exchange is needed?**

The direct LPE of graphite in organic solvents using sonication produces pristine graphene (SLG to FLG) at high concentrations. The hydrophobic nature of graphene makes it unstable in aqueous media without it being stabilized by a surfactant. The organic solvents NMP and DMF are among the most effective solvents for direct exfoliation due to similar surface tension to that of graphite. However, these organic solvents have high boiling points and, due to their incompatibility/toxicity, they are unsuitable for biological studies. Whereas, so far, the aqueous media stability of GO, rGO and functionalised-GO makes them a favourable candidates for the biological investigations.

NMP and DMF however are miscible with water, so it may be possible to dilute the dispersions. As a part of a control experiment, the metabolic activity of a Breast Cancer cells line (MCF-7) were investigated against different concentrations of NMP and DMF in water (see Appendix 9.6.1 and Figure 9.12a). It was found that at least 90 % of cells survived when the organic solvent level was reduced up to 0.1 v/v% (99.9 v/v% water).
Figure 6.1: Photographs showing the stability and concentration of G/DMF and G/NMP on different dilution with water. G/DMF and G/NMP dispersions were produced at 24 hr sonication and 6000 rpm centrifuge speed.

One way of achieving this low level of organic solvents is just by simple dilution. It can be seen clearly from Figure 6.1 that graphene dispersions upon dilution with water, the organic solvent content in the dispersion can certainly be reduced while leading to a stable dispersion even after 12 hr. However, the concentration of graphene also reduces upon dilution (as observed even by the naked eye), making it unsuitable for biological studies, where higher concentrations may be required, certainly for the toxicity studies here. Solvent exchange techniques such as dialysis could potentially be employed to reduce the organic solvent content in these graphene dispersions when dialysed against water.

6.2.2. Dialysis of graphene dispersions against aqueous media

Dialysis works on the principle of the diffusion of molecules across a semipermeable membrane separating regions of high and low concentration. The working principle and experimental details are given in section 4.1.6.

The initial graphene dispersions were produced by the direct exfoliation of graphite in DMF and NMP using a sonication time of 60 hr (see 5.2.1). The resultant dispersions were then centrifuged at 6000 rpm for 20 min, the supernatant was collected and centrifuged at 10000 rpm. The sediment from this step was then collected and redispersed in 10-15 ml of fresh solvent to achieve a high concentration of graphene. The concentrations obtained for graphene in DMF (G/DMF) and graphene in NMP (G/NMP) were ~0.78 mg/ml and ~0.51 mg/ml, respectively.
Figure 6.2: a) Example experimental set up of dialysis procedure. Graphene dispersions were contained within the dialysis bag, secured by knots top and bottom, and dialysed against stepwise 10× dilution volumes of deionised water to achieve the solvent dilutions indicated. b) Photographs of stable dispersions obtained after step-by-step dialysis. Each dialysis step was carried out with gentle stirring for 12 hr.

The step-wise dialysis of the graphene dispersions was carried out as shown in Figure 6.2a; 100 v/v% \(\rightarrow\) 10 v/v% \(\rightarrow\) 1 v/v% \(\rightarrow\) 0.1 v/v%. In a typical procedure, the dialysis bag was filled with the starting graphene dispersion (100 v/v% organic solvent) and secured by knots on both ends followed by immersion of the bag in (1:10 ratio) DI water. The dialysis step was assisted by gentle stirring over 12 hr. During the dialysis procedure, the organic solvents diffused from the inside (high concentration) to the outside (low concentration) of the dialysis bag through the semipermeable membrane, and vice versa for water. This diffusion resulted in an equilibrium concentration of organic solvent and water on either side of the dialysis membrane. These steps were repeated again in a stepwise fashion by replacing the outside liquid with fresh DI water (1:10 ratio). Stable dispersions of G/DMF and G/NMP were obtained at different levels of dialysis (Figure 6.2b). The highlighted stable dispersions contained only 0.1 v/v% organic solvent and 99.9 v/v% water.

6.2.3. Confirmation of dialysis

UV-Visible spectroscopy confirmed the extent of dialysis of the dispersions by monitoring the solvent’s UV cut-off wavelength, the wavelength at which the solvent absorbance in a 1 cm path cell is equal to 1 a.u. (water in reference cell).\(^{379}\) The UV cut-off wavelengths of 100 v/v% DMF and NMP (against 100 v/v% water) are 268 nm and 285 nm, respectively.\(^{380, 381}\)
Figure 6.3: UV-Visible absorbance spectra of control and dialysed dispersions along with solvent cut-off wavelengths. a) G/DMF and b) G/NMP. Cut-off wavelength was measured at \( A=1 \) (reference cell-water). The concentration of the dispersions were calculated from absorbance at 660 nm using Beer-Lambert law \( (\alpha \cdot 4632 \text{ mL mg}^{-1} \text{ m}^{-1}) \). All the dispersions were diluted 50 fold with water.

UV-Visible absorbance spectra of the dispersions were recorded along with control samples without graphene (Figure 6.3). The control samples were carefully prepared by diluting the organic solvents in water at the specified concentrations. The absorbance spectra of the starting (100 v/v%) and the dialysed (10 v/v%, 1 v/v% and 0.1 v/v%) dispersions show flat and featureless absorption. The graphene absorption was observed around \( \sim 269 \) nm (attributed to \( \pi \rightarrow \pi^* \) transition of the aromatic \( C - C \) bonds), indicating that the electronic conjugation within graphene was retained.\(^{167}\) The level of dialysis was confirmed by comparing the cut-off wavelengths of the organic solvents in the control and the dialysed samples, which are in excellent agreement with each other (Table 6-1) indicating successful dialysis. The organic solvent levels in the dialysed samples were further confirmed by NMR spectroscopy in section 6.2.3.2.

**Table 6-1: UV cut-off wavelength and concentrations of control and dialysed graphene dispersions**

<table>
<thead>
<tr>
<th>Sample</th>
<th>DMF Control</th>
<th>G/DMF (dialysed)</th>
<th>NMP Control</th>
<th>G/NMP (dialysed)</th>
<th>Concentration after dialysis (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 v/v%</td>
<td>242 ± 3</td>
<td>238 ± 3</td>
<td>241 ± 3</td>
<td>0.78 ± 0.02</td>
<td>0.51 ± 0.01</td>
</tr>
<tr>
<td>10 v/v%</td>
<td>234 ± 2</td>
<td>237 ± 2</td>
<td>230 ± 3</td>
<td>0.46 ± 0.02</td>
<td>0.21 ± 0.02</td>
</tr>
<tr>
<td>1 v/v%</td>
<td>225 ± 3</td>
<td>228 ± 3</td>
<td>221 ± 2</td>
<td>0.41 ± 0.02</td>
<td>0.19 ± 0.01</td>
</tr>
<tr>
<td>0.1 v/v%</td>
<td>213 ± 3</td>
<td>214 ± 3</td>
<td>207 ± 3</td>
<td>0.33 ± 0.02</td>
<td>0.16 ± 0.01</td>
</tr>
</tbody>
</table>

*Samples were 50 fold diluted in water*
6.2.3.1. Stability of dialysed dispersions

The concentrations of the graphene dispersions were calculated from the absorbance at 660 nm using the Beer-Lambert law ($\alpha = 4632 \text{ mL mg}^{-1} \text{ m}^{-1}$). In order to check the dialysed dispersions follow this $\alpha$ value, separate dispersions of FLG/NMP and FLG/DMF were dialysed to 0.1 v/v% of organic solvents. The data obtained after vacuum filtration, drying and weighing of the graphene films fall on the linear fit observed for all other preparations (see Figure 4.3), suggesting the $\alpha = 4632 \text{ mL mg}^{-1} \text{ m}^{-1}$ holds good for dialysed graphene dispersions. One important observation of Figure 6.3 is the drop in concentration of the dialysed dispersions compared to the starting dispersions (100 v/v%). This drop can further seen in Table 6-1 and in the highlighted regions of Figure 6.4. For the G/DMF dispersions, the concentration dropped by $\sim 40 \%$ during the 100 v/v% $\rightarrow$ 10 v/v% dialysis. In the subsequent steps, the concentration dropped $\sim 11 \%$ and $\sim 20 \%$ for 10 v/v% $\rightarrow$ 1 v/v% and 1 v/v% $\rightarrow$ 0.1 v/v% dialysis steps, respectively. G/NMP dispersions showed similar behaviour (Figure 6.4b and Table 6-1). The drop in concentration could be attributed to a combination of the following:

1) Aggregation and sedimentation of the graphene sheets during the dialysis process.

2) Dilution within the dialysis bag. As mentioned in section 4.1.6.2, during the dialysis procedure, the dialysis bag expands slightly resulting in dilution. Despite the tight knots, the expansion of the bag could not entirely be avoided.

Figure 6.4: Sedimentation behaviour of starting and dialysed graphene dispersions over 38 days. Concentrations were calculated from UV-Vis absorbance at 660 nm. Highlighted region show the drop in concentration during dialysis.
The final concentration obtained for the G/DMF and G/NMP dispersions at 0.1 v/v% organic solvent content (99.9 v/v% water) was $0.33 \pm 0.02 \text{ mg/ml}$ and $0.16 \pm 0.01 \text{ mg/ml}$, respectively.

The stability of the dialysed dispersions was measured over a period of a month by using UV-Vis spectroscopy. The sedimentation curves of the G/DMF and G/NMP dialysed dispersions are shown in Figure 6.4a,b. The dispersions were relatively stable over a month. The 0.1 v/v% G/DMF and 0.1 v/v% showed < 25 % sedimentation over 10 days, however, 55-60 % sedimentation was observed over a month. Khan et al. reported that a graphene dispersion in NMP (0.7 mg/ml) showed faster sedimentation (25 % over 160 hr) upon lesser, 100 fold dilution in water (1 v/v% NMP, 0.007 mg/ml).

6.2.3.2. Organic solvent content by $^1$H NMR spectroscopy

The amount of organic solvent content present in the dialysed dispersions was determined by estimating the $^1$H present using $^1$H NMR spectroscopy. In order to avoid the signal interference from H$_2$O to organic molecules, the graphene dispersions were dialysed against heavy water (D$_2$O) using a similar procedure to that for water dialysis. This allows the quantification of residual organic solvent content by integration of the $^1$H signals.

![Figure 6.5: $^1$H NMR spectra of 10, 1 and 0.1 v/v% D$_2$O-dialysed G/DMF (a) and G/NMP (b) dispersions. Note: black trace – graphene dispersions, red trace – control (without graphene).]
TMP (0.099M) was used as an internal reference for all the dispersions (see 4.3.7 for experimental details). Control (without graphene) samples of the relevant solvent concentrations, along with pure solvent references were carefully prepared. It was assumed that the TMP concentration was the same in all the samples, hence all the signals could be calibrated against the reference TMP signal during analysis. It can be clearly seen from Figure 6.5, that the \(^1\)H NMR spectra of both control (red trace) and dialysed graphene dispersions (black trace) were in excellent agreement (see Appendix 9.2) with each other, suggesting successful dialysis of organic solvents.

By integrating the area under the NMR signals, the information regarding the number of chemically distinct hydrogen states could be obtained. The integral values (number of hydrogens) of all the signals were derived by calibrating against the reference TMP signals (Appendix 9.2, Figure 9.5). These integral values were then converted into the concentration of organic solvents present in the dispersions (Table 6-2).

\[
\begin{array}{c|cc}
\text{Sample} & \text{Level of dialysis quantified by } \text{\(^1\)H NMR signal integration (v/v\%)} \\
& \text{G/DMF} & \text{G/NMP} \\
\hline
10 v/v\% & 9.64 \pm 0.43 & 8.87 \pm 0.44 \\
1 v/v\% & 0.85 \pm 0.04 & 0.77 \pm 0.04 \\
0.1 v/v\% & 0.07 \pm 0.01 & 0.07 \pm 0.01 \\
\end{array}
\]

Table 6-2 shows the levels of dialysis quantified by integrating the NMR signals. Again, during the step-by-step dialysis, the dialysis levels achieved were lower (e.g., \(~0.07\ v/v\%\) than the expected (\(~0.1\ v/v\%\). The slight expansion of the dialysis bags during the dialysis procedure, resulting in dilution of graphene within the bag, would not be expected to dilute the NMP in the bag equilibrated to the whole volume (bag and dialysate). The graphene-adsorbed NMP signal may be quenched by metallic behaviour of the graphene, possibly through eddy currents, though no Knight shift or line broadening was observed in the resolved spectra of NMP or DMF (i.e., the results with and without graphene in Figure 6.5 are almost identical). Alternatively, the experimental errors (~5\%) associated with preparing such low concentrations could influence the NMR results. Nevertheless, NMR spectroscopy confirmed that the dialysis was at least an order of
magnitude lower at each step, suggesting dialysis is a controlled solvent exchange process suitable for preparation of aqueous graphene dispersions.

6.2.4. Characterisation of dialysed graphene

The quality of the 0.1 v/v% dialysed graphene dispersions was characterised using AFM, Raman spectroscopy and XRD. AFM statistical analysis was carried out on graphene flakes spray-deposited onto mica substrates. Figure 6.6 shows representative AFM scans (see Appendix 9.3 for more AFM scans), with histograms of the length of flakes ($L_{AFM}$) and the number of layers per flake ($N$). The mean length of flakes ($\langle L_{AFM} \rangle$) was around 140 ± 70 nm and 170 ± 80 nm for the 0.1 v/v% dialysed G/DMF and G/NMP dispersions, respectively. The $\langle L_{AFM} \rangle$ obtained was similar to that of the starting 100 v/v% dispersions.

Similar to the starting dispersions, the mean number of layers per flake ($\langle N \rangle$) for 0.1 v/v% dialysed G/DMF and G/NMP dispersions remained around 4.5 ± 2.3 and 4.3 ± 1.9, respectively. However, a ~7 % increase in $N_{>8}/N_T$ compared to the starting dispersions was observed, indicating a possible slight increase in the degree of aggregation in the dialysed dispersions. Nevertheless, $N_1/N_T$ and $N_{1-5}/N_T$ remained around ~9 % and ~67 %, respectively.
The presence of a large number of relatively thin and smaller-sized graphene flakes (range of 50-100 nm) was observed (Figure 6.6j and Appendix 9.3). Some of the smaller flakes had no defined edges, compared to the other graphene flakes. This observation, as reported recently\textsuperscript{382}, could be due to the crumpling of thin pristine graphene sheets (from aqueous media) as a result of the AFM spray preparation process.\textsuperscript{382}
Chapter 6 – Production of Few Layer Graphene in Aqueous Media for Biological Studies

Raman spectra and XRD were obtained from the thin films prepared by filtering the dispersions on 100 nm porous aluminium oxide filter membranes. The Raman spectra (Figure 6.7a) of the 0.1 v/v% dialysed dispersions show typical Raman bands: a sharp G band at ~1580 cm\(^{-1}\), a pronounced D band at ~1330 cm\(^{-1}\), a broad and symmetric 2D band at ~2660 cm\(^{-1}\), and shoulder peak, D’ band at ~1617 cm\(^{-1}\). These bands are typical of the solvent-exfoliated graphene as discussed in the previous chapter.\(^{54, 59, 67}\) Most importantly, the Raman spectra of dialysed and starting (non-dialysed) dispersions are similar to each other (Figure 6.7a and Table 6-3).

![Figure 6.7](image_url)

Figure 6.7: a) Raman spectra and b) XRD data of the starting (100 v/v%) and dialysed (0.1 v/v%) graphene dispersions. Data from graphite is shown for reference.

The position, shape (broad) of the 2D band and the \(I_{2D}/I_G\) ~0.5 of the dialysed graphene dispersions suggest that they are few layer (< 5 layers) in nature.\(^{48, 59}\) The relative intensity of the D band (\(I_D/I_G\)) could suggest either presence of defects or be attributed to the edges. Since the laser spot was around 2 µm in diameter, a considerable amount of edge phonons would have contributed to the intense D band observed for both dialysed and starting dispersions. As explained in section 5.2.3.2 and the literature\(^3\).
sonication does not introduce any new basal plane defects in the graphene. Additionally, for the dialysed and starting graphene dispersions, the $I_D/I_D'$ was ~4, suggested that the defects are mainly boundary type defects (edges). Moreover, the absence of obvious FWHM(G) and FWHM(D) broadening (Table 6-3) of the dialysed dispersions suggested negligible structural disorder in the basal plane.

Table 6-3: 2D band position, FWHM(G), FWHM(D), $I_{2D}/I_G$, $I_D/I_G$, $I_D/I_{D'}$ of starting (100 v/v%) and dialysed (0.1 v/v%) graphene dispersions

<table>
<thead>
<tr>
<th>Sample</th>
<th>Raman 2D position (cm$^{-1}$)</th>
<th>FWHM(G) (cm$^{-1}$)</th>
<th>FWHM(D) (cm$^{-1}$)</th>
<th>$I_{2D}/I_G$</th>
<th>$I_D/I_G$</th>
<th>$I_D/I_{D'}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starting G/DMF</td>
<td>2669.7 ± 0.6</td>
<td>22.5 ± 0.6</td>
<td>42.1 ± 0.4</td>
<td>0.51 ± 0.02</td>
<td>0.63 ± 0.06</td>
<td>3.92 ± 0.25</td>
</tr>
<tr>
<td>0.1 v/v% G/DMF</td>
<td>2668.5 ± 0.4</td>
<td>23.1 ± 0.5</td>
<td>42.7 ± 0.5</td>
<td>0.45 ± 0.04</td>
<td>0.74 ± 0.02</td>
<td>3.98 ± 0.31</td>
</tr>
<tr>
<td>Starting G/NMP</td>
<td>2667.2 ± 0.5</td>
<td>21.5 ± 0.5</td>
<td>43.4 ± 0.3</td>
<td>0.47 ± 0.03</td>
<td>0.81 ± 0.05</td>
<td>4.05 ± 0.31</td>
</tr>
<tr>
<td>0.1 v/v% G/NMP</td>
<td>2667.1 ± 0.6</td>
<td>22.9 ± 0.7</td>
<td>43.3 ± 0.2</td>
<td>0.42 ± 0.06</td>
<td>0.86 ± 0.03</td>
<td>4.24 ± 0.08</td>
</tr>
</tbody>
</table>

The XRD analysis (Figure 6.7b) of the starting graphite showed a sharp diffraction peak around 26.5° due to diffraction from the (002) crystalline planes of graphite corresponding to an interlayer distance of ~3.34 Å. Whereas the diffraction peak was slightly broader for the starting and dialysed dispersions with an interlayer distance ranging between ~ 3.35 – 3.36 Å. This implies that during filtration, graphene restacks to form turbostratic graphene rather than graphite. These observations, along with the Raman results, confirm that the basal plane of the dialysed graphene had not suffered any severe in-plane disruptions, as in the case of GO.

### 6.2.4.1. Discussion on stability mechanism

The colloidal stability of dispersions can be indicated by measuring their $\zeta$ values. The $\zeta$ values of as-prepared 0.1 v/v% dialysed dispersions were ~ -48.87 mV (pH ~7.6) and ~ -47.7 mV (pH ~7.7) for G/DMF and G/NMP, respectively. Colloidal solutions with $\zeta > \pm 30$ are normally considered to be stable systems, thus these high values indicate that the dialysed dispersions were stabilised through electrostatic repulsion between the graphene particles.
It is known that solvents with surface tensions values of 40-50 mJ/m² are the best solvents for dispersing graphene, as they reduce the interfacial tension between the solvent and graphene. It has been shown by molecular simulations that the last layer of confined solvent molecules around graphene sheets hinders their aggregation. Liu et al. have shown that unfunctionalised graphene in organic solvents was either positively or negatively charged and that their stability was mainly due to electrostatic repulsion. For NMP and DMF (higher donor number), the charge transfer happens from solvent to graphene, rendering graphene sheets net negatively charged with respect to the solvent, calculated $\zeta \sim -30$ mV.

Meanwhile Yi et al. demonstrated that repeated washing and re-dispersal in water of DMF-exfoliated graphene resulted in stable water-dispersed graphene with negligible residual DMF. It was found that, as the size of the graphene decreases, the $\zeta$ increases, due to graphene’s enhanced edge effects (bonding of edge carbon atom to oxygen in water) resulting in higher stability of smaller-sized graphene ($\zeta > -30$ mV for <0.09 µm²) in water, with no structural disorder in the basal plane.

MD simulation studies have also shown that organic solvents such as NMP, due to their higher affinity, form dense layers around graphene surfaces. Wetting simulations have also shown that NMP could spread around graphene surfaces, whilst water remains at the hydrophobic contact angle.

From these observations, the observed high $\zeta$ values for 0.1 v/v% dialysed dispersions could be attributed mainly to the charge transfer from the residual organic solvents surrounding graphene surfaces (acting like surfactant) and that their colloidal stability was largely due to electrostatic repulsion of the charged graphene sheets. Even though direct evidence of edge functionalisation was not observed in the dialysed samples (no significant broadening in Raman G and D band, no considerable increase in interlayer spacing by XRD), the enhanced edge effects (bonding of edge carbon atom to oxygen in water) of smaller-sized graphene in water, as described by Yi et al., cannot be fully neglected.
6.3. EXFOLIATION OF FEW LAYER GRAPHENE USING BILE SURFACTANT

Surfactant-based (ionic and non-ionic) LPE is another promising method to produce relatively defect-free graphene in aqueous media.\textsuperscript{60, 77-80, 94, 390, 391} Electrostatic repulsion is the main stability mechanism with ionic surfactant-coated graphene. Whereas, steric hindrance play a major role in stabilising non-ionic polymeric surfactant-coated graphene. Among various available surfactants, bile salt surfactants such as SC, SDC, TDOC are some of the few ionic biologically-relevant surfactants. These are similar to bile acids secreted into the gut, which have the advantage of being relatively non-toxic and had already been shown to display good colloidal stability of high concentration of graphene in water.\textsuperscript{60, 79, 80, 391}

Smith \textit{et al.} have shown that the surfactants with high electrostatic potential barrier (ζ values) can stabilises graphene concentration with low degree of aggregation.\textsuperscript{80} Thus, TDOC which is an anionic surfactant with a high ζ value (> -60 mV), as an ideal candidate to produce stable graphene in aqueous media.\textsuperscript{79}

Aqueous graphene dispersions were produced by direct the LPE of graphite in TDOC solution (5 mg/ml) for using a range of different sonication times (see 4.1.2.4 for experimental details). UV-Vis absorbance spectra of 10 fold diluted G/TDOC dispersions were similarly recorded (Figure 6.8a). The strong absorbance at ~269 nm can be attributed to π → π* transition of the aromatic C – C bonds.\textsuperscript{79, 167} The concentration of the graphene was obtained by using the optical absorbance at 660 nm and applying the Lambert-Beer law, taking α as – 1825 mL mg\textsuperscript{-1} m\textsuperscript{-1}. The typical concentration of G/TDOC obtained as a function of sonication time are shown in Figure 6.8b (Note: These dispersions were centrifuged at 10000 rpm for 20 min prior to the UV measurement). The concentration obtained after 36 hr sonication was ~0.1 mg/ml. This concentration is higher than that reported by Smith \textit{et al.}\textsuperscript{80} and Guardia \textit{et al.}\textsuperscript{94} who obtained concentrations of ~0.03 mg/ml and ~0.02 mg/ml, respectively, using TDOC. The difference is believed to be that their starting surfactant concentration was less than the CMC of TDOC (CMC\textsubscript{TDOC}-0.52-2.08 mg/ml), whereas herein the concentration was above the CMC.\textsuperscript{79} (The dispersed graphene concentration critically depends on the CMC of the surfactants,\textsuperscript{391} and hence a starting surfactant concentration of 5 mg/ml was used in this
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study, leading to a very stable dispersion without any visible aggregation over 8 months.)

Sun et al. have achieved very high concentration of G/TDOC up to 7.1 mg/ml (24 hr, 500 rpm) using more intense, probe tip sonication. Lotya et al. observed smaller graphene flakes were produced at higher centrifugation rates (longer sonication time, up to 400 hr), which had basal plane defects. The surfactant used in their study was SC rather than TDOC and hence may not be directly comparable to this study. Nevertheless, as explained below, Raman spectra of G/TDOC did not show the presence of basal plane defects in these TDOC preparations.

![Image of Raman spectra](image)

**Figure 6.8:** a) UV-Vis absorbance spectra of G/TDOC (36 hr, 10 fold diluted). Inset showing a stable G/TDOC dispersion. b) Graphene concentration obtained against sonication time (all samples centrifuged at 10000 rpm for 20 min). c) Raman spectra of thin film prepared G/TDOC (36 hr) dispersions. Spectrum of starting graphite is shown for reference. d) Intensity particle size distribution of G/TDOC (36 hr) dispersion. e) AFM height image of spray coated G/TDOC (36 hr) dispersion. f) Corresponding amplitude image of (e). Note: after spray deposition, the mica substrate was rinsed with DI water to remove the surfactant.

Representative Raman spectra obtained on a thin film prepared by vacuum filtration of G/TDOC (36 hr, 10000 rpm) are shown in Figure 6.8c. Typical Raman bands (G band, 2D band and D band) of graphene are clearly seen and are similar to that of LPE graphene. The 2D band is broad (but still a single Lorentzian) and down shifted (2652 ± 2.1 cm⁻¹) by ~20 cm⁻¹ compared to the starting graphite, and the \( I_{2D}/I_G \) ~0.75 suggesting G/TDOC as few-layer (< 5 layers) in nature. The characteristic
G band appears around ~1580 cm\(^{-1}\) and the presence of an intense D band (~1330 cm\(^{-1}\)) and D’ band (~1617 cm\(^{-1}\)) indicates the presence of defects. These defects could be due to the edges as the flakes produced are smaller as determined by DLS and AFM.

The G/TDOC dispersions were spray deposited on mica followed by rinsing with DI water (to remove excess free surfactant molecules) followed by annealing (400 °C in argon) before AFM measurements. The \(L_{\text{AFM}}\) obtained was ~90 nm and is comparable with the \(L_{\text{DLS}}\) (~100 nm) as seen in Figure 6.8d,e. AFM statistical measurements over 100 flakes indicate that the dispersion contained \(N_1/N_T \approx 17\%\) and \(N_{1-5}/N_T > 92\%\) with \(\langle N\rangle\) as 3.2 ± 1.8.

As mentioned earlier, the observed \(I_D/I_G\) ratio ~1.25 could be attributed to the flake edges rather than basal plane defects based on the following considerations:

- Since the \(L_{\text{AFM}}\) ~ 90 nm and the laser spot was ~2 µm, a considerable amount of edges contributed to the D band intensity.
- No obvious broadening of FWHM(G) ~21.5 ± 1.1 or FWHM(D) ~40.1 ± 1.2 was observed, suggesting the absence of high structural disorder (For highly disordered graphene the G band merges with the D’ band, and G and D band exhibit large FWHM \(^{173, 259}\)).
- The observed \(I_D/I_{D'}\) ~4.15 suggests boundary-type defects rather than basal plane defects.\(^{57, 127}\)

Finally, the as-produced G/TDOC dispersion had a pH value of ~7.6 with \(\zeta\) ~ -63 mV, similar to that reported in the literature.\(^{80}\) This \(\zeta\) value suggests that the colloidal stability against aggregation was mainly due to electrostatic repulsion of TDOC-coated graphene sheets.\(^{79, 80}\)

### 6.4. Exfoliation of Few Layer Graphene Using Lipids

The hydrophobic nature of pristine graphene has led to the assumption that its stability in aqueous media is possible only with the addition of surfactants\(^{60, 77, 79}\) facilitating biological studies.\(^{218}\) Whereas, due to its hydrophilic functional groups, GO remains stable in water, making it by far the most studied (among GBMs) candidate for biological applications and toxicity studies.\(^5\) However, in an electrolyte environment
(physiological conditions), the screening of surface electrostatic charge results in the aggregation of GO.\textsuperscript{392} Functionalisation of the GO or rGO using biocompatible polymers (\textit{e.g.}, PEG) has resulted in stable dispersions in a saline environment.\textsuperscript{209, 393, 394} One possible way of dispersing pristine graphene in a saline environment is to make use of the hydrophobicity of the biomolecules, lipids and proteins (effectively as surfactants), present in the human body. Doing so, could not only it enhance the colloidal stability in a saline environment, but also shed light on the interactions of biomolecules with graphene, and their interaction with cells in the body.

Phosphatidylcholine (PC, Figure 6.9a), a type of phospholipid, is the main component of lecithin. PC has a zwitterionic hydrophilic head group (choline) linked to a pair of hydrophobic fatty acids (mixture both of saturated and unsaturated of various chain lengths in natural PC) esterified to a glycerol moiety. PC is a major component of the biological cell membranes and, due to its chemical inertness and neutral charge, it used as a model for cell membrane studies\textsuperscript{395} and is widely used in drug delivery carriers (\textit{e.g.}, liposomes).\textsuperscript{396–398} The surfactant-like nature of these amphiphilic phospholipids can stabilise graphene in aqueous media as demonstrated by Liu \textit{et al.}, where negatively-charged liposomes deposited as lipid monolayers around the rGO surface, resulted in enhanced colloidal stability in water by electrostatic repulsion.\textsuperscript{222} In contrast, removal of excess positively-charged liposomes resulted in rGO aggregation, and neutral liposomes (1,2-dimyristol-sn-glycero-3-phosphocholine) failed to stabilise rGO in water in their experimental conditions.\textsuperscript{222} However, Pykal \textit{et al.} directly exfoliated graphite in lecithin/chloroform solution producing FLG, stabilised by formation of reverse micelles on the graphene surface in chloroform, as suggested by their MD simulations.\textsuperscript{224}

In this study, natural graphite was added while hydrating the lipid film (2:1 molar ratio of egg PC and cholesterol) in PBS buffer (140 mM NaCl, physiological conditions) as explained in section 4.1.2.5. The low power exfoliation for \textasciitilde 36 hr in sonication bath, followed by 2000 rpm centrifuge for 20 min (removing unexfoliated graphite and large multi-lamellar vesicles) resulted in colloidally-stable, lipid-coated graphene dispersions (G/EggPC) as shown in Figure 6.9b. Aggregation was observed after 7 days, but upon shaking and 5 min sonication, the graphene was redispersed, suggesting a weak interaction exists between graphene and EggPC in aqueous media.
Figure 6.9: a) Structure of the predominant species of phosphatidylcholine in egg yolk and cholesterol. Photographs of G/EggPC as prepared. Aggregation was observed after 7 days, but with shaking and short sonication stable dispersions were regained. c) UV-Vis spectra (diluted in DI water), and d) DLS (10 fold diluted in DI water) of EggPC (control), G/EggPC and G/EggPC-wash dispersions, respectively.

UV-Vis spectra of G/EggPC showed a flat and featureless absorption along with lipid absorption. Whereas, upon eliminating excess liposomes by using a washing step (see 4.1.2.5), the concentration of graphene was reduced and surprisingly no lipid absorption was observed (Figure 6.9c). However the absorption around ~269 nm for the G/EggPC-wash did suggest the electronic conjugation within graphene was retained. The concentration of graphene in G/EggPC (pH ~6.9) was ~0.13 mg/ml (using Lambert-Beer law, $\alpha = 1825$ mL mg$^{-1}$ m$^{-1}$), which is high compared to lecithin-based exfoliation of graphene. The concentration of lipids in G/EggPC was determined as ~0.35 mg/ml by the Stewart assay.

The size of liposomes (EggPC control, without graphene) obtained by sonication as determined by DLS was ~50 nm (PDI = 0.09 ± 0.02), suggesting unusually small unilamellar
DLS on the diluted G/EggPC showed two clear size distributions (PDI = 0.58 ± 0.12): ~60 nm attributed to the excess liposomes present in the dispersion and ~400 nm ($L_{DLS}$) attributed to the lipid-coated graphene, whereas, the $L_{DLS}$ of the washed G/EggPC was ~350 nm (PDI = 0.38 ± 0.02).

The graphene exfoliation efficiency of EggPC was investigated using AFM analysis. Figure 6.10 shows representative AFM height and phase image of G/EggPC on mica. Statistical analysis over 60 flakes indicated the $L_{AFM}$ was 350 ± 150 nm, and the exfoliation yield of $N_1/N_T$ ~17 % and $N_{1-5}/N_T$ > 92 % with $\langle N \rangle$ as 5.3 ± 2.1.

Representative Raman spectra of G/EggPC (Figure 6.10g and Appendix 9.4) showed typical Raman bands: G band, 2D band and D band. The shape of the 2D band and $I_{2D}/I_G$ ~0.45 indicates FLG, agreeing with the AFM analysis. The presence of the D’
band, with $I_D/I_{D'} \sim 3.52$, with no obvious broadening of FWHM(G) $\sim 20.2 \pm 0.6$ and FWHM(D) $\sim 39.2 \pm 1.4$, suggests that the basal plane of graphene was relatively defect free, and the D band with $I_D/I_G \sim 0.31$ was mainly due to edge defects.\textsuperscript{57, 79, 127}

From all the above observations, it is unambiguous that EggPC aided the exfoliation of graphite into graphene by the ultrasonication method. However, it was surprising not to see any clear layer of adsorbed lipid layers on the graphene surface in AFM, except some residual lipid molecules seen on very thin graphene surfaces (Figure 6.10f, phase degree difference).\textsuperscript{400} This suggests that the interaction between the graphene and lipid is weak, and possibly disordered, such that the lipids were successful in exfoliating graphene, but did not form stable structures long term, which were largely removed by AFM preparation, and also eventually resulted in loose aggregates and sedimentation. However, it has to be noted that this aggregation was reversible; shaking and 5 min sonication brought back colloidal stability.

In order to understand better the aggregation effect, a small volume of G/EggPC was filtered through a 400 nm filter under high pressure (2-3 bar N$_2$) yielding a grey hazy suspension, suggesting the presence of graphene ($\sim 0.01$ mg/ml), which would be expected to be coated with lipid molecules. DLS was used to observe any change in the size over a time period. As seen in Figure 6.11, the starting size of $\sim 100$ nm could be attributed to either liposomes or lipid coated graphene or both (Note: graphene sheets $> 100$-200 nm would have been eliminated during high pressure filtration). In due course of time, the IPSD clearly splits into two different sizes: $\sim 50$ nm (attributed to small liposomes) and $\sim 350$ nm (attributed to graphene aggregates). Sonication resulted in one IPSD $\sim 100$ nm, suggesting that the graphene sheets were again redispersed with weak interactions.

This suggests that during the sonication process, the lipid molecules in addition to forming liposomes, exfoliated graphene by forming a lipid layer (lipid monolayer/bilayer) around the graphene and stabilised the dispersion (stable dispersion after centrifugation in Figure 6.9b and DLS of G/EggPC in Figure 6.9d) by electrostatic repulsion or steric stabilisation. However, due to the saline environment used (PBS buffer, 140 mM NaCl, and physiological conditions), the surface negative charge of graphene would be screened.
by the salt (Na + ion)\textsuperscript{394} resulting in possible detachment of lipid molecules from the graphene surface. These lipid molecules would then tend to form more thermodynamically-stable structures like micelles, lipid lamellae or liposomes (as the most stable in excess water) and aggregating the graphene (schematics in Figure 6.11) with residual lipids on its surface (Figure 6.10f). Re-sonicating the suspension could cause the graphene to be dispersed by these excess lipid molecules in the suspension through weak interactions.

\begin{center}
\textbf{Figure 6.11: Change in IPSD with time of high pressure filtered G/EggPC monitored by DLS (left image). Schematics explaining possible mechanisms of lipid detachment and graphene aggregation (right image).}
\end{center}

\section*{6.5. Exfoliation of Few Layer Graphene Using Human Serum Albumin}

Human serum albumin (HSA) is the most abundant protein in blood plasma (40-60\% of total protein content) and is one of the widely-studied model proteins.\textsuperscript{401, 402} HSA contains a single polypeptide chain of 585 amino acid residues (66.5 kDa), including fluorophores: one tryptophan (Trp) and 18 tyrosine (Tyr) residues.\textsuperscript{401} The subdomains IIa and IIIa of HSA are the hydrophobic domains or ‘pockets’ (Figure 6.12a), which act as primary binding sites for water-insoluble fatty acids, drugs etc.\textsuperscript{402, 403} The water solubility, the inherent hydrophobic domains and conformational flexibility of HSA make it a good bio-surfactant for stabilisation of graphene. Researchers have also demonstrated direct
exfoliation (sonication) of graphite in proteins like hydrophobins$^{225}$ and lysozyme$^{228}$ producing protein coated FLG. The hydrophobic regions of the protein adsorb on to the graphene surface, when the resulting stable dispersions are presumably due to the reduced surface energy difference between the graphene and hydrophobic side of the protein, and the solvent and hydrophilic side or regions of the protein.$^{225}$

In this study, natural graphite was directly exfoliated in aqueous HSA solution to produce stable protein-coated FLG dispersion. In a typical experiment, HSA was dissolved in saline PBS buffer (140 mM NaCl, physiological conditions) mixed with natural graphite (5 mg/ml), followed by sonication in water-cooled sonication bath and 2000 rpm centrifugation, to produce protein-coated FLG dispersion (G/HSA) as shown in Figure 6.12. The effect of sonication time and HSA concentration on graphene concentration was first investigated to optimise the process. The concentration of graphene was calculated using Lambert-Beer law by measuring absorbance at 660 nm ($\alpha = 1825 \text{ mL mg}^{-1} \text{ m}^{-1}$).

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Figure 6.12: a) Crystal structure of HSA and the hydrophobic ‘pockets’ (highlighted region) and the structure of tryptophan and tyrosine.$^{404}$ b) Effect of sonication time on the graphene concentration (HSA concentration: 2.5 mg/ml). c) Effect of HSA concentration on the graphene concentration (Sonication time: 24 hrs, bath sonication). d) UV-Vis absorption spectra of HSA control, as produced G/HSA (24 hrs, HSA conc.:
5 mg/ml) and G/HSA-wash (Note: all samples were 25 fold diluted). Inset shows photograph of as-produced G/HSA after 2000 rpm centrifugation.

The concentration of graphene obtained increased with sonication time, as seen in Figure 6.12b at a fixed HSA concentration of 2.5 mg/ml. However, the graphene concentration dropped substantially after 24 hrs, which may be due to HSA denaturation or aggregation during the prolonged sonication. As the starting HSA concentration was varied, the maximum graphene concentration of ~0.21 mg/ml was obtained at a HSA concentration of 5 mg/ml (Figure 6.12c). Increasing the HSA concentration up to 10 mg/ml resulted in protein aggregation during the sonication, resulting in poor graphene exfoliation efficiency. Hence, the samples prepared with a starting HSA concentration of 5 mg/ml and 24 hrs sonication (2000 rpm centrifuge) were used for subsequent analysis.

The UV-Vis spectra of the HSA control showed a typical amino acids absorption peak around 280 nm (Figure 6.12d). The absorption spectra of as-prepared G/HAS showed a featureless absorption in the visible region, and an absorption peak around 280 nm, which is attributed to HSA molecules. Upon eliminating excess HSA by a washing step (see 4.1.2.6), the absorbance peak of the washed G/HSA shifted to ~269 nm, which is attributed to $\pi \rightarrow \pi^*$ transition of the aromatic $C - C$ bonds in graphene, suggesting the electronic conjugation within graphene was retained. Removing excess HSA not only reduced the concentration of the graphene, but reduced the stability of the dispersion (G/HSA-wash) in PBS buffer, suggesting that the presence of excess HSA helped in stabilising the graphene in saline environment for longer periods (stable more than 15 days). Using the Ninhydrin method, the concentration of excess HSA in G/HSA was estimated to be ~2.6 mg/ml (cf. starting HSA concentration - 5 mg/ml).

The graphene sheets produced by HSA exfoliation were characterised using AFM (Figure 6.13 and Appendix 9.5). AFM analysis showed that the graphene flakes obtained by HSA assisted exfoliation were much smaller and thinner structures, when compared to the starting graphite flakes (~250 µm diameter), indicating successful exfoliation. Statistical analysis over 160 flakes indicated the $L_{AFM}$ is 370 ± 120 nm and thickness of 7.6 ± 1.5 nm. Presumably, this higher thickness was due to the HSA adsorption, as evidenced in the amplitude and phase images.
Figure 6.13: Representative AFM images of G/HSA. a), d) and i) are height scans of G/HSA with average observed thickness. b and c) are corresponding amplitude and phase images of (a) showing HSA coverage on graphene surface. e and j) are corresponding phase images of (d) and (i) showing clear HSA adsorption at the edges. f and k) are enlarged phase images of HSA showing several individual ellipsoidal HSA molecules.

Crystallographic studies have shown that HSA has a heart-shaped (Figure 6.12a) structure (approximated to an equilateral triangle of $8 \times 8$ nm dimension and $\sim 3$ nm height). The reported size of individual HSA molecule in PBS buffer (pH 7.4, 140 mM NaCl) determined by DLS is $\sim 3.8$ nm and this is similar to the observed size of as-prepared, unsonicated HSA control (Figure 6.14a). This control, though, also contains some larger protein aggregates ($\sim 50$ nm) due to the high concentration of HSA used (5 mg/ml). DLS on HSA controls that had been sonicated for 24 hr (Figure 6.14b) indicated individual HSA molecules, suggesting that such a long period of sonication did not damage the HSA completely. This sample, also contained large particles ($> 200$ nm), which could be attributed to the formation of aggregates, possibly sonication-induced amyloid like
structures. However, such features were not observed on the graphene surface in the AFM images (Figure 6.13, no adsorbed feature > 200 nm). It should also be noted that for AFM analysis, 50 µl of G/HSA was drop casted on mica by allowing overnight drying, followed by DI water rinsing and N₂ drying. Such extensive washing may have washed away the excess HSA molecules and any aggregates, leaving behind only tightly-bound HSA molecules on the hydrophobic graphene surface.

Recent AFM studies on HSA adsorption on HOPG surfaces have shown individual HSA molecules are ellipsoidal in shape with a length of 12.6 nm and width of 6.5 nm. Upon careful analysis of a selected area of the AFM phase images (Figure 6.13f,k), several molecules with oblong/ellipsoidal shape (∼ 16 × 11 nm) were observed, which could be attributed to individual HSA molecules. The larger dimensions observed were probably due to AFM tip broadening/distortion effect, but HSA conformational change upon adsorption may have been a factor. The average height of HSA measured by AFM on hydrophobic substrates is ∼2 nm. Hence in the present study, assuming HSA is adsorbed on both sides of graphene, the ⟨N⟩ of G/HSA should be calculated as 6.1 ± 3, confirming FLG production by HSA-assisted exfoliation.

Figure 6.14: DLS of as-prepared non-sonicated HSA control (a) and sonicated HSA control (b). Concentration of HSA was 5 mg/ml. Arrow shows the expected size of individual HSA molecules. The highlighted region in (b) corresponds to aggregated/denatured HSA.
Figure 6.15: SEM images of supernatant from G/HSA-wash, showing leaf like arrangements of HSA molecules with the residual graphene as the nucleation point (shown by arrow marks).

The supernatant of the G/HSA-wash was studied to investigate the protein aggregation seen in Figure 6.14b. SEM images (Figure 6.15a,c and e) show self-assembled ‘leaf’-like structures with dendritic distributions and graphene at the nucleation point. During the drying process, the evaporation of water in PBS buffer resulted in rapid aggregation of protein molecules driven by hydrophobic interactions forming such structures. The dendritic architectures could be explained by the diffusion limited-aggregation model, in which graphene nucleation at the tip of the leaf structure is an outcome of a diffusion-limited process. Similar results were observed for DNA-HSA and silk proteins morphology.
Representative Raman spectra of G/HSA are shown in Figure 6.16 and Figure 9.11. The shape of the 2D band and \( I_{2D}/I_G \sim 0.48 \) indicates FLG, agreeing with previous AFM analysis.\(^{48}\) The presence of D' band with \( I_D/I_{D'} \sim 4.3 \), with no obvious broadening of FWHM(G) \( \sim 21.2 \pm 0.5 \) and FWHM(D) \( \sim 41.3 \pm 2.1 \) suggests that the basal plane of graphene is relatively defect free, and the D band with \( I_D/I_G \sim 0.5 \) was mainly due to edge defects.\(^ {57, 79, 127}\) However, the contribution of adsorbed HSA to the D band of graphene cannot be fully neglected.

Fluorescence spectroscopy is widely used to study the interactions of amino acid fluorophores of proteins (Trp and Tyr) with nanoparticles such as DNA\(^ {411}\), graphene quantum dots\(^ {414}\) and GO\(^ {236, 415, 416}\) and also denaturing of HSA\(^ {417}\). It has been demonstrated that graphene-based materials like GO can quench the intrinsic fluorescence of fluorophores like organic dyes\(^ {418-420}\), quantum dots\(^ {421, 422}\), fluorescent labels\(^ {423-427}\), amino acids\(^ {415}\) and proteins\(^ {236, 415, 416}\) through Förster resonance energy transfer (FRET). The fluorescence quenching is usually due to the interaction of aromatic rings of the fluorophores with the graphene-based materials via non covalent interactions such as electrostatic interactions, hydrogen bonding, hydrophobic and \( \pi - \pi \) interactions.\(^ {236, 415, 416, 420, 424-426}\) Hence, the intrinsic fluorescence of Trp and Tyr of HSA was investigated to obtain information regarding its interaction with FLG.
HSA has one Trp residue in subdomain IIA and 18 Tyr residues distributed throughout the protein at distances short enough for mutual Tyr → Tyr or Tyr → Trp FRET but, due to the lack of spectral overlap between Trp emission and Tyr absorption, Trp → Tyr energy transfer is unlikely. Hence, the dominant emission (~340 nm) in the fluorescence spectra excited at 280 nm (which is the absorption maximum of HSA, Figure 6.12c) is from Trp, with some contribution from Tyr (~310 nm). Tyr does not contribute to the emission at longer wavelength excitation (295 nm), when selective Trp interactions could be investigated at this wavelength.

![Figure 6.17: Fluorescence spectra of HSA-Control, G/HSA and G/HSA-wash, excited at 280 nm (a) and 295 nm (b) respectively. Samples were 10 fold diluted.](image)

The fluorescence emission spectra (300 nm - 500 nm region) of the control HSA and as-produced G/HSA excited at 280 nm and 295 nm are shown in Figure 6.17a and Figure 6.17b, respectively. The quenching of Tyr and Trp fluorescence in G/HSA was clearly observed compared to HSA control, due to its interaction with the graphene surface. Complete fluorescence quenching of Tyr and Trp was observed for G/HSA-wash, indicating the observed fluorescence of G/HSA is from the excess HSA present in the dispersion (~2.6 mg/ml excess HSA as estimated by Ninhydrin assay). These observations show that Tyr and Trp, which are the hydrophobic regions of HSA, are indeed interacting with graphene, presumably then responsible for enhancing the stability of the dispersion.

Theoretical studies have shown that Tyr and Trp amino acids can be strongly adsorbed on graphene surfaces via π → π interactions. Unlike GO which has several groups attached to its surface, G/HSA comprised pristine FLG (no significant Raman D
or G band broadening), meaning that the basal plane of graphene contained mostly sp$^2$ carbons facilitating $\pi \rightarrow \pi$ interactions of Tyr and Trp.$^{415,416}$

The as-prepared G-HSA had a pH value of $\sim$6.8 in PBS buffer. The isoelectric point (IEP), the pH value at which a molecule does not have any electrical charge, of HSA is around 4.7.$^{236}$ Consequently, in the G/HSA adsorbate, the anionic charge density would become dominant due to the carboxylate ions, which would induce electrostatic repulsion between the graphene sheets and, with excess HSA, enabling a stable dispersion ($\zeta$ value of G/HSA is -27.87 mV).$^{228}$ The $\zeta$ value of G/HSA-wash and its supernatant was -13.4 mV and -22.87 mV, respectively. One possible explanation for the observed reduced stability of G/HSA-wash upon removing excess HSA could be that the relative effect of surface charge screening by Na$^+$ of PBS buffer was slightly higher than the repulsion between the graphene sheets. However, as the protein is net charged and has positively-charged regions on its surface, the electrostatic attractions cannot be fully excluded.$^{236}$

From the above results, it is clear that direct exfoliation of graphite in HSA resulted in HSA-coated graphene sheets. It has been reported that serum proteins, which are normally present in the media during cell growth has strong adsorption on GO, mitigating cytotoxicity.$^{232}$ In order to understand the interaction of pristine graphene in the presence of neat HSA, 0.1 v/v% dialysed G/DMF dispersion was added slowly to the neat HSA solution (5 mg/ml filtered in 20 nm filter) and characterised by DLS and fluorescence spectroscopy. The HSA control had a size of $\sim$3.8 nm similar to reported.$^{406,407}$ A clear evolution of the size differences could be observed from Figure 6.18a. Gradual addition of graphene reduced the light scattering intensity of HSA and the IPSD spectra became dominated by its hydrodynamic size around 100 nm. However, one cannot neglect the possibility of observing the interaction of HSA and graphene by DLS. Fluorescence spectroscopy confirmed HSA and graphene interaction by means of fluorescence quenching (Figure 6.18b,c). As mentioned earlier, graphene acted as a fluorescence quencher by interacting with the Tyr and Trp residues of HSA.$^{236,415,416}$ Upon gradual addition of graphene to HSA control solutions, the Tyr and Trp fluorescence reduces rapidly ($\sim$ 86 % quenching with 0.36 µg of 0.1 v/v% G/DMF addition) confirming its strong interaction.
Figure 6.18: a) Change in IPSD size profile upon addition of 0.1 v/v% G/DMF. The fluorescence quenching of Tyr and Trp (b) and Trp(c) alone by addition of different concentration of 0.1 v/v% G/DMF excited at 280 nm and 295 nm, respectively. d) Stern-Volmer plot of 0.1 v/v% G/DMF quenching Tyr and Trp residues of HSA.

The Stern-Volmer plot (Figure 6.18d) shows an upward curvature which indicates the quenching mechanism is combined static and dynamic quenching or predominantly static quenching.\cite{415} It has been determined that, for GO, the main quenching mechanism is static quenching with some contribution of dynamic quenching, which is most likely the reason here as both electrostatic and hydrophobic interactions play a role in stabilising the graphene.\cite{415}
Chapter 6 – Production of Few Layer Graphene in Aqueous Media for Biological Studies

6.6. SUMMARY

In summary, in this chapter several methods to produce pristine FLG in aqueous media were discussed.

(a) Dialysis was employed to exchange the organic solvents with water in the organic solvent-exfoliated FLG dispersions. UV-Vis and \(^1\)H NMR spectroscopy confirmed that the organic solvent could be reduced down to levels of 0.1 v/v% (99.9 v/v% water) while maintaining sufficient colloidal stability. Raman spectroscopy and XRD indicated no in-plane disruptions, suggesting the pristine nature was retained in FLG. The higher absolute \(\zeta\) values for the dialysed 0.1 v/v% FLG dispersions meant that the electrostatic repulsion was the main stability mechanism with some contribution from edge effects.

(b) TDOC, a bile surfactant effectively exfoliated graphite to highly-stable FLG dispersions with the best exfoliation conditions of 36 hr sonication and 10000 rpm centrifuge speed producing flakes with \(\langle L_{AFM}\rangle \sim 100\ \text{nm}\) and \(N_{1-5}/N_T > 92\%\).

(c) A one-step process of using neutral phospholipids like PC (the major component of cell membrane) to produce pristine FLG in PBS buffer was demonstrated (Exfoliation conditions: 36 hr sonication and 2000 rpm centrifuge speed). The absence of adsorbed lipid molecules on graphene surface by AFM analysis confirmed their weak interaction. The surface charge screening effect by salt results in poor stability of graphene in PBS buffer. Despite its poor stability, it was shown that PC indeed efficiently exfoliated graphite to FLG (graphene monolayers by AFM) and its pristine nature was confirmed by Raman analysis.

(d) Finally, the intrinsic nature of proteins to adsorb onto the surface of particles via their hydrophobic domains (‘pockets’) was explored in producing FLG, by directly exfoliating graphite in aqueous solutions of HSA, most abundant protein in human plasma. FLG dispersions with concentrations up to \(~0.21\ \text{mg/ml}\) in saline PBS buffer were obtained at the best exfoliation conditions of 24 hrs sonication and 2000 rpm centrifuge speed (starting HSA concentration of 5 mg/ml) and were found to be stable for up to 15 days. AFM analysis revealed strong adsorption of HSA onto graphene surfaces, identifying individual HSA molecules. Raman analysis confirmed the pristine nature of the graphene. Strong fluorescence quenching of
Tyr and Trp suggested that the hydrophobic domains of HSA were indeed interacting ($\pi \rightarrow \pi$ and hydrophobic interactions) with graphene, possibly as a result of conformational changes due to its structural flexibility. Such HSA-coated graphene could act as ‘stealth’ nanomaterials in biological studies.

All the materials produced in this Chapter were examined for their cytotoxicity effects in cell-based biological systems. The results are presented in Appendix 9.6.1 and Figure 9.12.
Chapter 7 – Application of Graphene Composites: Raman Strain Sensors
7. APPLICATION OF GRAPHENE COMPOSITES: RAMAN STRAIN SENSORS

7.1. INTRODUCTION

The monolayered nature of graphene makes it of particular promise for sensor applications as every atom is present at both surfaces, increasing its sensitivity to environmental changes. For example, the Hall Effect has been used to detect the absorption of single NO₂ and NH₃ gas molecules. Graphene has also been used as a photo detector. Micro-engineered vibrating beam sensors have taken advantage of graphene’s high stiffness and low density to act as mass sensors with detection limits reported of up to 2 zeptograms. Graphene has also shown promise as a piezoresistivity based strain sensor, with studies based on MC graphene, CVD-grown graphene, and graphene-composites. CVD graphene on PDMS was found to have a GF of 6.1 whilst MC graphene on PDMS had a GF ~ 2 for applied strains up to 30 %. In a recent study, thin films, produced by spray coating of LPE graphene, on a plastic substrate were used to demonstrate strain sensitive coating with a tuneable gauge factor based on the stretching of a percolated graphene network. These sensors are analogous to the conventional foil strain gauges (GF 2-5) which give high accuracy single-point measurements.

In addition to high accuracy, single-point, deformation measurements, there is a need to be able to measure local strain at multiple points over a structure. Whilst this can be achieved with electronically-based sensors, every point of interest needs to be individually wired, leading to a significant amount of infrastructure. Thus it is preferable to measure the strain using optical measurements based upon the photoelastic properties of either the material itself or an active coating upon it. A classic example of such a system is the birefringence measurements made on PMMA and polystyrene (PS) structures. There are already commercially available photoelasticity-based sensors, such as the Grey-field Polariscope from Stress Photonics and PhotoStress® from Vishay.

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2 Sections of 7.1 and 7.3 of this chapter are based upon a paper of “Wide-Area Strain Sensors based upon Graphene-Polymer Composite Coatings Probed by Raman Spectroscopy” in Adv. Funct. Mater. 2014, 24, 2865-2874. DOI: 10.1002/adfm.201302869.
In these techniques, photoelastic sensitive coatings based on PS/epoxy resin are applied over complex structures and a polariscope measures the strain-induced birefringence to form a full-field strain map with resolutions from 10 to 20 $\mu \varepsilon$ (see manufacturer’s data sheet in reference\textsuperscript{328, 330}). Bragg diffraction gratings are another option but require a complex fibre optical lay-up to achieve sufficient point resolution over a large area.\textsuperscript{435} A promising rival to these optical systems is the development of Raman active coatings, where a strain active material is coated onto a device and the strain is read using a hand-held Raman spectrometer. This technique relies on the sensitivity of the Raman bands to the bond strain; tension causes frequency downshift (phonon softening) while the compression causes frequency upshift (phonon hardening).

The development of the Raman strain sensors has been previously limited due to the lack of hand-held spectrometers and the development of suitable active coatings. Raman systems, however, are now available based upon solid-state lasers for under £10k. Earlier studies on Raman active coatings focused upon using carbon nanotubes. Wagner \textit{et al.} used SWNT as Raman sensors in glass fibre polymer composites to measure the stress concentrations around defects and demonstrated that the nanotubes could be used to produce a two-dimensional mapping of the strain distribution in the composite.\textsuperscript{436, 437} Sureeyatanapas \textit{et al.} demonstrated that SWNTs could be incorporated into the sizing of glass fibres to give a strain sensitive coating.\textsuperscript{437} It was found that functionalized SWNT showed higher strain sensitivity due to a better interface with the sizing. Li \textit{et al.} demonstrated the use of nanotube as macro-scale strain sensors in Bucky papers by both Raman and electrical measurements.\textsuperscript{438} Following this work, Vega \textit{et al.} developed SWNT-epoxy coatings as strain sensors by simultaneously following Raman spectroscopic and electrical impedance measurements.\textsuperscript{439} The impedance and Raman response of these coatings followed each other in single-cycle deformation experiments. Whereas, when subjected to cyclic deformation, the Raman method gave information about the interfacial damage.\textsuperscript{439}

It has been suggested by our group\textsuperscript{4, 32} and others,\textsuperscript{269, 272} that graphene would be the ideal Raman active coating and superior to CNTs due to
Graphene’s two-dimensional nature making it more suitable for coatings.

(2) The higher inherent band shift per unit strain of the 2D Raman band in graphene.\(^{274}\)

(3) The high intensity of graphene’s most strain sensitive band (2D band).

In section 7.2, FLG/epoxy composite coatings prepared from different flakes sizes and production techniques were investigated as a strain sensor by following the Raman 2D band with strain. In section 7.3, the performance of a model single grain system (MC flake) is compared to CVD graphene on a polymer substrate. The latter uses materials that are being readily scaled by a number of companies and groups.\(^{40, 159, 440, 441}\) The shift of the Raman 2D band with strain under cyclic deformation was followed to determine the in-service behaviour of the sensor coating. In particular, the interfacial characteristics of the graphene-polymer interface were studied during the cyclic deformation as these determine the stress transfer to the graphene and hence its strain sensing ability. These measurements also have significance for the wider graphene composite community, particular on the fatigue behaviour of such materials. Finally, the absolute accuracy and resolution of the graphene-based Raman strain sensor as a function of graphene source were calculated.

### 7.2. APPLICATIONS OF FLG/COMPOSITES: RAMAN STRAIN SENSORS

#### 7.2.1. Types of FLG used

Epoxy composites containing graphene flakes with different diameters were produced to investigate the role of graphene’s lateral dimensions on its performance in a Raman-based strain sensor. These flakes were produced by the following techniques:

- Solvent exfoliation was used to produce the samples labelled FLG-0.2 and FLG-0.5.
- Commercially available graphene was used to produce the samples labelled FLG-1 (This sample was produced using a surfactant-based exfoliation method).
- Electrochemical exfoliation was used to produce the samples labelled FLG-5 and FLG-20.

As seen in the Chapter 5, sonication of graphite in organic solvents produces a stable dispersion of FLG. However, the size of those flakes is predominantly in the sub-
micron range. Coleman et al. have shown that controlled centrifugation could be used to produce flakes with different lateral dimensions. This process has been adapted as described in section 4.1.2.3.2 to produce flakes with \( \sim 0.2 \mu m \) (FLG-0.2) and \( \sim 0.5 \mu m \) (FLG-0.5) diameters. Commercially available Elicarb® Graphene powder with average size of \( \sim 1 \mu m \) was also used (FLG-1).  

![Figure 7.1: SEM images of sonication-based exfoliated graphene. a, d) Starting graphite used to produce FLG-0.2 (b and c) and FLG-0.5 (e and f). g) As-supplied Elicarb® graphene powder from Thomas Swan Ltd. h) Magnified image from (g) with the arrow showing non graphene-like features (bright white). c, f and i) Images of FLG-0.2, FLG-0.5 and FLG-1 after 30 min sonication in acetone, respectively.](image)

Figure 7.1 shows the SEM images of FLG-0.2, FLG-0.5 and FLG-1 produced from sonication-based process. As seen clearly, the sizes of flakes have dramatically reduced from starting graphite (\( \sim 200 \mu m \)) to produce graphene flakes with sub-micron diameter. Figure 7.1b,c,e,f are SEM images from drop casting of dispersions in acetone on SiO\(_2\)/Si substrate, showing aggregation of flakes as a result of drying. Despite the aggregation, individual flakes can be observed with defined edges. These images were used to analyse the average flake sizes obtained. SEM image of as-received Elicarb® Graphene powder is
shown in Figure 7.1g. The presence of some species which are not graphene-like could also be observed in the magnified images (arrow marks in Figure 7.1h).

Figure 7.2: SEM images of electrochemically exfoliated graphene. a, d) As produced FLG-5 and FLG-20 from electrochemical process. b, e) Magnified images showing the intercalated graphite flakes (arrow marks) as a result of electrochemical exfoliation. c, f) Images from FLG-5 and FLG-20 after 30 min sonication in acetone, respectively.

Recently it has been shown that electrochemical exfoliation of graphite can produce large diameter FLG flakes. The diameter of the flakes is shown to be controlled by the graphite grain size in the initial electrode. FLG with two different sizes were produced by electrochemical exfoliation: 5 µm (FLG-5) and 5 µm (FLG-20). SEM images of as-supplied material of FLG-5 and FLG-20 is shown in Figure 7.2a and Figure 7.2b, respectively. Magnified images in Figure 7.2b,e show the intercalated graphite along with individual flakes. These samples were dispersed in acetone for 30 min by ultrasonication to homogenise them. Individual flakes can be observed in Figure 7.2c. Whereas for FLG-20, the flakes were still aggregated (Figure 7.2f) and were relatively thick when compared to the other samples. These SEM images were used to analyse the flake size after the solution processing and is shown in Table 7-2.

7.2.2. Raman spectroscopy characterisation of FLG and FLG/epoxy composite coatings

It is well-known that Raman spectroscopy can give information on the degree of exfoliation, nature of defects, and size of the flakes. So, Raman spectroscopy was
employed to characterise the nature of as-produced/as-received FLG and the FLG/epoxy composites.

Figure 7.3: a) Raman spectra of solution processed FLG. A spectrum of graphite is shown for comparison. b) Raman spectra of 5 wt% FLG/epoxy composite coatings. The highlighted regions show the epoxy region of the spectra.

The Raman spectra of the starting FLGs (after solution processed in acetone for 30 min) is shown in Figure 7.3a. The first observation is that all the spectra are different to that of graphite, indicating successful exfoliation. FLG-0.2 has a symmetrical 2D band, similar to the observations in 5.2.3.1, suggesting turbostratic graphene. The presence of an intense D band is mainly due to the edges of the flakes and not of in-plane defects. For FLG-0.5, the shape of the 2D band suggests few-layer nature (< 6 layers) and the D band is due to the edges. It has been shown in 5.2.3.2 that the sonication does not produce any new basal-plane defects and the increase in the D band is due to the edges of graphene. For FLG-1 (Elicarb® Graphene powder), as shown in the manufacturer’s product information, the 2D band shows few-layer nature with average of 5-7 atomic layers and the D band is associated with the edges. The shape of the 2D band for
electrochemically exfoliated graphene (FLG-5 and FLG-20) again shows few-layers in nature (~5 layers), similar to the observations by Abdelkader et al.\textsuperscript{123}

Table 7-1: Average number of layers, thickness, Raman 2D band position, FWHM(2D), $I_{2D}/I_G$ and $I_D/I_G$ of all types of graphene used for epoxy composite coatings

<table>
<thead>
<tr>
<th>Graphene type</th>
<th>$\langle N \rangle$ or thickness (nm)</th>
<th>Raman 2D band position (cm$^{-1}$)</th>
<th>FWHM(2D)</th>
<th>$I_{2D}/I_G$</th>
<th>$I_D/I_G$</th>
<th>$I_D/I_G^{\text{comp}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLG-0.2</td>
<td>&lt; 6 layers</td>
<td>2663.9 ± 1.6</td>
<td>76 ± 0.3</td>
<td>0.43 ± 0.01</td>
<td>1.18 ± 0.22</td>
<td>1.02 ± 0.35</td>
</tr>
<tr>
<td>FLG-0.5</td>
<td>&lt; 6 layers</td>
<td>2672.2 ± 0.4</td>
<td>75 ± 0.7</td>
<td>0.4 ± 0.02</td>
<td>0.42 ± 0.03</td>
<td>0.45 ± 0.08</td>
</tr>
<tr>
<td>FLG-1</td>
<td>6 – 7 layers</td>
<td>2663.2 ± 4.7</td>
<td>83 ± 4.3</td>
<td>0.47 ± 0.05</td>
<td>0.33 ± 0.11</td>
<td>0.38 ± 0.24</td>
</tr>
<tr>
<td>FLG-5</td>
<td>~ 5 nm</td>
<td>2668.4 ± 3.5</td>
<td>80 ± 3.2</td>
<td>0.42 ± 0.03</td>
<td>0.3 ± 0.25</td>
<td>0.42 ± 0.12</td>
</tr>
<tr>
<td>FLG-20</td>
<td>~ 5 nm</td>
<td>2674.5 ± 3.7</td>
<td>74 ± 2.6</td>
<td>0.4 ± 0.04</td>
<td>0.33 ± 0.08</td>
<td>0.4 ± 0.11</td>
</tr>
<tr>
<td>MC monolayer</td>
<td>-</td>
<td>2645 ± 1.5</td>
<td>25 ± 0.9</td>
<td>&gt; 2</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

As mentioned before, the 2D band of the graphene is the most strain sensitive of all the Raman bands. Hence the 2D band was fitted with one Lorentzian function for comparison. Table 7-1 show the 2D band position of all the FLGs used. The 2D band position varied between 2662 – 2675 cm$^{-1}$ with FWHM(2D) ranging between 75-85 cm$^{-1}$, confirming the few-layer nature of the samples. Generally, for the sonication-based exfoliation process, the $I_D/I_G$ ratio is normally due to the edges of the flakes (see section 5.2.3.2) as the flakes are smaller than the laser spot (~2 μm).\textsuperscript{54} However for electrochemically exfoliated flakes, which have large sizes than laser spot the $I_D/I_G$ ratio suggests that there is some in-plane defects present. Abdelkader et al. suggested these defects could be caused due to gas evolution between the layers or fatigue caused by repeated intercalation/deintercalation during the production process.\textsuperscript{123} The 2D band position, FWHM(2D), $I_{2D}/I_G$ ratio and $I_D/I_G$ ratio of a reference monolayer is also given for comparison.

The low intensity of the 2D band for FLG when compared to a monolayer graphene (Table 7-1) and high fluorescence from the polymer at low graphene loadings meant reliable data could not be obtained for Raman strain sensing measurements.
Similar observations were reported by Valles et al. for PMMA/GO nanocomposites.\textsuperscript{294} Reasoning this, only 5 wt.% epoxy/FLG composite coatings were prepared for this study. As can be seen in Figure 7.3b, the solution processed 5 wt.% FLG/epoxy composite coatings show relatively good intensity of 2D band when compared to epoxy. The shapes of the 2D band of graphene in the composite samples were almost similar to the starting material suggesting the exfoliated nature is retained within the sample. It can also be noted a slight increase in the $I_D/I_G$ ratio of the FLG-5 and FLG-20/epoxy composite ($I_D/I_G$)\textsubscript{comp}) coatings. This increase in ($I_D/I_G$)\textsubscript{comp} ratio (Table 7-1) suggests that, during the solution processing of these composites, the sonication does reduce the size of the flakes.

![Figure 7.4: a) Schematic representation of MC Graphene composite coating. b) Schematic representation of the 4-point bending. c) Raman spectra of a MC SLG. The spectrum of the substrate is also shown for comparison. The absence of D band indicates the high quality of graphene.](image)

In order to evaluate the strain response of FLG/epoxy composite, a reference composite coating with MC SLG was prepared as described in section 4.2.2. Figure 7.4a shows the schematic of the reference SLG composite coating. Raman spectra of the SLG composite coating shows an intense 2D band with $I_{2D}/I_G > 3$. The absence of a D band in the composite indicates high crystalline order of the graphene.
7.2.3. **Raman deformational study of FLG/epoxy composite coatings**

The FLG/epoxy composite coatings and the reference monolayer composite coating samples were subjected to strain using 4-point bending as shown in Figure 7.4b. The position of 2D band with strain was followed to study to the strain response of the graphene.

The Raman 2D band shifted significantly with the applied strain for the reference monolayer graphene composite coating indicating stress transfer occurred in the coatings (Figure 7.5a). The shift rate of ~ -48 cm\(^{-1}\)/% strain obtained is comparable to the previous studies on such sandwich composites systems.\(^{32}\)

The 2D band response of FLG/epoxy composites with strain is completely different to that of the reference monolayer composite (Figure 7.5a). The 2D band position of all the FLG/epoxy samples remained unchanged within error. It has to be mentioned that, for the reference MC SLG, the average from three different positions within the same flake are considered for calculations. Whereas for FLG/epoxy composite coatings, the average values from 3-5 random positions at each strain level were considered to obtain an overall response of the coating. Thus, the relatively large error bars are due variation in the Raman response within the sample.

![Image](image.png)

**Figure 7.5:** a) The shift of 2D band with loading strain for all the FLG/epoxy composite coatings along with the reference MC SLG composite coating at random positions within the composites. b) The shift of 2D band with loading strain of FLG-0.5, FLG-1 and FLG-20 at the same spot.

Instead of taking measurements from the different positions within the composite coatings, the 2D band response was monitored at the same spot during deformation for
some of the coatings. The response of the 2D band at the same spot with strain is shown in Figure 7.5b. Shift rates of ~ -1.3, ~ -3.8 and ~ -4.5 cm\(^{-1}\)/% strain where observed for FLG-0.5, FLG-1 and FLG-20 respectively (Table 7-2). The mean diameter as calculated from the SEM images are also given in Table 7-2.

**Table 7-2: SEM mean length of the FLG, shift rate of the FLG/epoxy composites**

<table>
<thead>
<tr>
<th>Composite coating</th>
<th>SEM Mean length* (&lt; L &gt;) (µm)</th>
<th>Shift rate ((cm^{-1}/% strain))</th>
<th>R(^2) value for linear fit</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLG-0.2</td>
<td>0.24 ± 0.2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>FLG-0.5</td>
<td>0.56 ± 0.3</td>
<td>~ - 1.3(^\dagger)</td>
<td>0.55</td>
</tr>
<tr>
<td>FLG-1</td>
<td>1.2 ± 1.6</td>
<td>~ - 3.8(^\dagger)</td>
<td>0.90</td>
</tr>
<tr>
<td>FLG-5</td>
<td>2 ± 1.7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>FLG-20</td>
<td>6.1 ± 5.9</td>
<td>~ - 4.5(^\dagger)</td>
<td>0.40</td>
</tr>
<tr>
<td>MC SLG</td>
<td>&gt; 20(^\dagger)</td>
<td>~ - 48</td>
<td>0.99</td>
</tr>
</tbody>
</table>

*SEM mean length were calculated after 30 min sonication, \(^\dagger\)Length from optical microscope (see Figure 7.8a). \(^\dagger\)Shift rates at the same spot in the respective composite coatings.

These shift rates suggest the interfacial stress transfer does occur within the composite coatings, *i.e.*, the FLG does act as reinforcement in the composite. However the strain sensibility (shift rate) of the FLG/epoxy coatings (*e.g.*, FLG-20 ~ -4.5 cm\(^{-1}\)/% strain) is at least a magnitude worse when compared to the reference exfoliated graphene composite coating (~ -48 cm\(^{-1}\)/% strain). Several possible explanations can explain this behaviour:

1. **Number of layers:**

   It has to be noted that, in the MC SLG, the surface of the graphene on either side is in complete contact with the polymer matrix resulting in large interface. It is known that, the stress transfer in the graphene-polymer composite occurs via interfacial shear stress.\(^4\), \(^32\), \(^273\), \(^274\) Gong *et al.* have reported a shift rate of -8 cm\(^{-1}\)/% strain for their ‘many layer’ graphene flake sandwiched between two polymer films (similar to that shown in Figure 7.4a) under uniaxial tension.\(^274\) This was believed to the flake having good stress transfer at the polymer-graphene interface but poor levels of stress transfer at the internal graphene-graphene interface. However, it was found out that 4-5 layers of graphene is the optimum thickness for reinforcement taking into account the maximum achievable loading volume fraction and the drop in modulus.\(^4\), \(^274\) For the FLG used here,
the solution processing of as-supplied aggregated powder might not have separated the layers well-enough before the addition of epoxy into the mixture. This would result in thicker graphene which would account for and hence the observed reduced strain sensitivity.

2. **Length of the flakes:**

   It has been shown that the length of flakes plays a major role in achieving efficient reinforcement as well as shift rates and hence strain sensing ability.\(^{32, 124}\) The critical length of graphene to achieve effective reinforcement as calculated by Shear-lag theory is > 3 \(\mu\)m (more preferably > 30 \(\mu\)m).\(^{4, 32, 124}\) It can be explained from Figure 7.6 that when the flakes are larger, the strain in the flake matches the strain in the matrix and therefore resulting in good stress transfer. But if the flakes are smaller, the strain in the flakes cannot build up to the matrix strain and hence leasing to poor stress transfer. In other words, the edge effect of graphene dominates in such composites and the graphene effectively acts as a short reinforcement resulting in less or no band shift (strain sensitivity).

![Figure 7.6: Representation of flake strain with respect to flake size using Shear-lag theory](image)

As a result of solution processing (dispersing as-supplied/produced FLGs in acetone), the \(< L >\) of FLG decreases (Table 7-2). Especially for FLG-5 and FLG-20, the size decreased considerably. It is not surprising to see no shift rates for the FLG-0.2, FLG-0.5 and FLG-1/epoxy composites, as their average sizes are smaller than the critical length. The small shift rate of \(-1.3\) and \(-3.8\) cm\(^{-1}\)/\% strain observed for FLG-0.5 and FLG-1, respectively, at the same spot could be attributed to regions where relatively larger flakes might have dominated. It is surprising to see no shift rates for FLG-5 and FLG-20 despite the average length (as-supplied) is higher than the critical length of reinforcement. There are number of possible reasons why this might be the case:
• **Alignment of flakes in the composite**: In bulk composite coatings, the graphene flakes are 3d randomly-oriented during processing resulting in reduced/no shift rate. The observed \( \sim 4.5\ \text{cm}^{-1}/\% \) strain at the same spot for FLG-20/epoxy composite coating could perhaps be from a FLG which is aligned to the deformation axis at the measured region. It is also plausible that the larger SLG or FLG could roll and fold up during the processing, again affecting the shift rates.

• **Homogeneity of graphene dispersed within the composite**: In order to achieve efficient reinforcement throughout the composite, it is important that the dispersion of graphene within the polymer matrix is uniform. Figure 7.7, shows Raman spectra of random positions of a FLG-1/epoxy composite. The variation of the intensities between the 2D band of graphene and the epoxy at various positions indicates that the graphene indeed is not homogeneously dispersed within the composite. This could be a result of:
  
  o The processing technique not being effective in homogeneously dispersing graphene within the composites.
  
  o Re-aggregation of graphene flakes due to the higher loadings used (5 wt.%). Similar problems were encountered with nanotubes\(^{442}\) and GO.\(^{294}\)

These reasons contribute to the observed large standard deviation for 2D band positions for such composites produced (Figure 7.5 and Table 7-1).

![Figure 7.7: Raman spectra of FLG-1/epoxy composite showing the difference in the observed intensities of graphene 2D band and epoxy, suggesting non-homogenous dispersion of graphene within the composite.](image-url)
Defects: The Raman spectra of the as-supplied FLG-5 and FLG-20 does show a strong D band indicating the presence of in-plane defects as a result of production procedure.\textsuperscript{123} It was shown that the presence of defects and holes in the graphene will reduce the Young’s modulus of the reinforcement, which would result in the reduced shift rates.\textsuperscript{4}

Along with all the above mentioned plausible reasons, some other general reasons like force fitting a single Lorentzian to the 2D band of FLG could add certain degree of uncertainty to the data.

All the above observations confirm that, when using graphene bulk composite coatings as a Raman-based strain sensors, several factors influence the response of the 2D band with strain. Even if the length of the flakes is greater than the critical length of reinforcement, factors such as; processing methods, loading percentage, defects in the material and alignment of flakes within the composites influences the shift rate of the 2D band with strain. These reasons lead to better performance of composite coatings based on MC graphene, than the FLG bulk composite coatings as Raman strain sensor. However, MC graphene is not scalable, so a realistic alternative is to explore the use of CVD graphene as a Raman based strain sensor.

7.3. APPLICATIONS OF CVD GRAPHENE COMPOSITE COATINGS: WIDE-AREA RAMAN STRAIN SENSORS

Due to the high strain sensitivity, graphene is a perfect candidate for being wide-area strain sensor. Reserbat-Plantey \textit{et al.} showed that graphene’s suitability for sensing was such that even real time dynamic measurements could be taken from a single point.\textsuperscript{443} However, to date graphene has not been studied as a wide-area strain sensor, partly due to a lack of suitable material;

- MC SLG as demonstrated in this chapter works well but is not scalable.
- Solvent-exfoliated graphene, as explained in the previous section, is shorter than the critical length of graphene required for efficient stress transfer, meaning that not enough strain is transferred from the composite to the flake.\textsuperscript{32} Electrochemical-exfoliated graphene despite being longer than the critical length
of graphene, is affected by several other factors such as the; processing methods, loading percentage, defects in the material and alignment of flakes within the composites. These factors influence the shift rate of the 2D band with strain (section 7.2.3).

- In the last few years, though, large area CVD films have become available and Sony Corporation has demonstrated roll-to-roll production and transfer of a 100 m long graphene films.\textsuperscript{159}

Hence, composites coatings made from CVD graphene could be a potential candidate for Raman-based wide-area strain sensors. In this section, the performance of in-house prepared CVD graphene strains sensor coating is compared to the model mechanically cleaved graphene strain sensor. The performance of commercially available CVD graphene coatings were also studied for comparison.

\textbf{NOTE: In this section, data of MC graphene for uniform maximum strain deformation is being used from author’s previous work (Optical image and Raman 2D band response with loading strain).}\textsuperscript{444} Those results support the discussions of the present work.

\section*{7.3.1. Composite coatings characterisation}

\subsection*{7.3.1.1. Optical microscopy characterisation}

An optical microscope was used initially to identify the MC graphene flakes in the composite coatings by differentiating through contrast. Figure 7.8 shows the optical images of exfoliated graphene flakes on which cyclic deformations were carried out. The bright areas in Figure 7.8b are the bulk graphite. Figure 7.8c shows a large area of transferred PMMA/CVD film. The white streaks observed were due to the drying of the PMMA top coat. Wrinkles and damage were observed in the PMMA/CVD graphene thin film (Figure 7.8d) as a result of transfer process.
Figure 7.8: a, b) Optical image of few SLG in the exfoliated coatings. c, d) Transferred CVD graphene coatings. Wrinkles and damages were observed due to the transfer process.

7.3.1.2. Raman spectroscopy characterisation

Composite coatings were prepared from both MC and in-house CVD-grown graphene. The schematics of the sample preparation were shown in section 4.2.2 and 4.2.3. The in situ Raman measurements were carried out on the graphene monolayer flakes as shown in the optical micrographs (Figure 7.8). Well-defined Raman spectra with a characteristic 2D band (around 2640 cm\(^{-1}\)), G band (around 1580 cm\(^{-1}\)) and D band (1350 cm\(^{-1}\)) were obtained from both the MC and CVD graphene. Figure 7.9a shows the example flakes found in the composite system with increasing number of layers, with the \(I_{2D}/I_G\) ratio greater than 3 for the SLG. The absence of a D band for the MC graphene reveals its high-quality.\(^{48}\) Figure 7.9b shows the Raman spectra of as-grown CVD graphene on Cu foil and in the coatings. \(I_{2D}/I_G\) ratio was greater than 2 and the FWHM(2D) of ~25 cm\(^{-1}\).
Figure 7.9: a) Raman spectra of different layers of graphene in MC composite coatings. The shape of 2D band varies with the number of graphene layers; monolayer could be fit with single Lorentzian, whereas the band splits into four for bilayer. Absence of D band at ~1350 cm$^{-1}$ indicates high crystalline order of the samples. b) Raman spectra of the as-produced CVD graphene on Cu foil and in the composite coating. The spectra from PMMA top coat and SU-8 is shown as a reference in both cases. Spectra offset for clarity.

7.3.1.3. SEM characterisation

SEM was used to calculate the GBs (wrinkles$^{281}$) of CVD graphene. The wrinkles are produced during the cooling phase of CVD growth, due to difference in thermal coefficients for graphene and Cu substrates. CVD graphene used in this study was prepared from hot wall CVD process (section 4.1.4).$^{336}$ Wet transfer (section 4.2.3 and Figure 4.9) of CVD graphene was carried out leaving PMMA/graphene film floating in the DI water. The film was carefully tilted upside down before being picked on a SiO$_2$ (300 nm)/Si wafer and dried. The samples were gold coated by sputtering before imaging. As shown in Figure 7.10a,c &e, the Cu GBs were clearly visible in very low magnifications. However in the higher magnifications and back scattered electron detector with low contrast levels, the graphene GBs were clearly visible. The dashed lines and arrow marks were used for clarity. Statistical analysis was carried out on the graphene grain boundaries resulting in an average value of $\sim 7.3 \pm 4.8 \mu m$ over the area of $\sim 100 \mu m \times \sim 100 \mu m$. Damage from the transfer process can be seen in the form of small holes in the magnified images.
Figure 7.10: SEM images of CVD graphene/PMMA film. a,c &e) Cu GBs were clearly visible (black dotted lines were drawn for better clarity). b,d & f) Graphene GBs were visible in the magnified regions (white dotted lines and arrows were drawn for clarity). Damages from the transfer process were also visible.
7.3.2. Cyclic deformation behaviour

The 2D band of graphene was used to follow the cyclic deformation of the coatings. Upon axial strain, 2D band splitting was not observed for graphene (both MC and CVD) as reported in literature.\textsuperscript{271, 285, 445} But broadening of the 2D band was observed as shown in Figure 7.11a. The accuracy of the fit of the 2D band using the Lorentzian function is shown in Figure 7.11b.

\textbf{Figure 7.11: a) Change in the shape and position of Raman 2D band of MC SLG graphene during and after deformation, b) Accuracy of the curve fit by using the Lorentzian function}

7.3.2.1. Sequence I: Uniform maximum strain

The coatings were subjected to cyclic deformation using a four-point bending rig with the maximum strain of 0.3 % at each cycle. Figure 7.12a,d show the deformation sequence applied and the response of the 2D band position. The Raman 2D band shifts significantly with the applied strain, indicating that stress transfer occurred in the coatings (Figure 7.12b,e). Elastic deformation occurred in the MC graphene coatings, with the loading and unloading curves for the first cycle overlaying each other. The shift rate of $-53 \text{ cm}^{-1}/\% \text{ strain}$ (Figure 7.12c) was obtained for both loading and unloading curve is similar to the previous studies.\textsuperscript{269, 274} As the sample undergoes subsequent deformation cycles, the linearity between the loading and unloading curve is lost, giving rise to a hysteresis loop. This behaviour can be related to the coatings undergoing damage at the graphene-matrix interface. Cyclic deformation of bulk CNT composites revealed similar behaviour due to damage at nanotube-matrix interface. The hysteresis loop increases in area and shifts upwards with the deformation cycle (Figure 7.13). This behaviour suggests that the coating underwent irreversible damage at the graphene-matrix interface. Furthermore at the end of each cycle, the 2D band position at 0 % strain is at
higher wavenumber than in the previous cycle. This is consistent with graphene undergoing slippage during the loading cycle and then in-plane compression during unloading, resulting in the development of residual compression.

Figure 7.12: Sequence I cyclic deformation with uniform maximum strain level. a, d) The sequence of the deformation and the response of 2D band position of both MC and CVD graphene. The highlighted area
shows the relaxation of residual pre-compression of CVD graphene in the initial two cycles. The strain sensing ability was calculated at 0.1 % and 0.3 % strain levels as indicated. b,e) The shift of 2D band vs. loading strain. The hysteresis loop observed in the exfoliated graphene indicates the interfacial damage. Boxes at 0 % strain indicate the behaviour of the residual stresses at the end of the deformation cycles. c,f) Shift rate of 2D band, obtained from slopes of (b) and (e). The dashed horizontal line indicates the theoretical ideal shift rate calculated by using the Gruneisen parameter (\(\gamma = 2.7\)).

The initial 2D band position was significantly higher in the CVD coating (~2660 cm\(^{-1}\)) compared to exfoliated graphene (~2640 cm\(^{-1}\)), indicating that the CVD graphene was under residual compression. This residual compression was found throughout the sample, with the average band position being ~2659.7 ± 2.5 cm\(^{-1}\) over 200 random spots. The residual strain is believed to be developed from the transfer process and drying of the PMMA top coat.\(^{270}\) The initial two deformation cycles had allowed the graphene to relax this compression (Figure 7.12d,e). From the third cycle onwards, approximately elastic deformation occurs, indicating good stress transfer between the graphene and matrix. Unlike in the exfoliated graphene, the 2D band position at 0 % strain is constant at the end of the final three deformation cycles, indicating no further significant relaxation of residual strains.

![Graph of Raman 2D band shift](image)

Figure 7.13: Shift of Raman 2D band of MC graphene composite coating for cyclic deformation with same maximum loading strain. Elastic deformation was observed in the cycle 1 and the development of hysteresis loop and its upshift as a result of interfacial deformation is clearly observed in cycle 4.

The theoretical ideal shift rate for graphene is calculated by using the Gruneisen parameter, \(\gamma\), which is a measure of rate of change of phonon frequency in a crystal with
For the uniaxial strain the Gruneisen parameter of 2D band \((\gamma_{2D})\) could be defined as,

\[
\gamma_{2D} = -\frac{1}{\omega_{2D}^0} \frac{\Delta \omega_{2D}}{\varepsilon (1-v)}
\]

Equation 7.1

where, \(\Delta \omega_{2D}/\varepsilon\) is the shift rate of 2D band, \(\omega_{2D}^0\) is initial position of 2D band before straining and \(v\) is the Poisson’s ratio. In the literature, various values of Gruneisen parameters are reported and it is still a matter of argument.\(^{267, 447}\) The Poisson’s ratio of the polymer coatings was used 0.33 as the graphene exhibited elastic deformation indicating good adhesion between graphene and the substrates once the residual strain had been annealed. This ratio was taken as 0.33, as the Poisson ratio of PMMA is 0.3-0.35 and SU-8 is a transversely isotropic material with an in-plane Poisson ratio of 0.33. The Gruneisen parameter value of 2.7 was used as calculated by the first-principle calculations of Mohiuddin et al.\(^{256}\) near ideal shift rates of -62 cm\(^{-1}\)/% strain (Figure 7.12c,f) were experimentally obtained for both MC and CVD graphene which is similar to the value (\(~\) -60 cm\(^{-1}\)/% strain) reported in the published literature.\(^{256, 269, 273, 274}\)

The shift rates of the loading and unloading curves of MC graphene increases with the deformation cycles and reached the theoretical ideal shift rate (Figure 7.12c). This behaviour could be related to the strain hardening of the graphene, meaning graphene exhibits more resistance to the deformation in the subsequent deformation cycles. The reason for this behaviour is unclear at this point, but the flattening of the ripples present in the graphene during the deformation could be one plausible explanation. However, strain hardening behaviour was not observed for MC graphene with pre-existing residual strains (from fabrication step), undergoing cyclic loadings. As explained in 7.3.2.4, exfoliated graphene was under residual tension before the cyclic deformation. Upon cyclic deformation, the residual strain relaxed and graphene exhibited elastic deformation.

Whereas for the CVD graphene, the shift rate is approximately half of the theoretical ideal shift rate \(\sim\) -30 cm\(^{-1}\)/% strain. This shift rate however is lower than the results of -72 cm\(^{-1}\)/% strain from stretching CVD graphene on PDMS. It should be noted that the work of Bissett et al. used a more deformable substrate (PDMS) and hence the
graphene was under biaxial deformation and thus is not directly comparable.\textsuperscript{277} Furthermore since Bissett \textit{et al.} were not able to accurately measure the strain in their PDMS substrate, they assumed that their exfoliated and CVD graphene would have the same band shift rate.\textsuperscript{277}

The Raman band shift could be used to estimate the effective Young’s modulus of the graphitic based carbon reinforcements. As mentioned before, for uniaxial strain, the common shift rate obtained/calculated is \( \sim -60 \, \text{cm}^{-1}/\% \) for 1 TPa monolayer graphene.\textsuperscript{256, 269, 273, 274} From the shift rates obtained in Figure 7.12c,f the average effective modulus of exfoliated graphene and CVD graphene are in the order of \( \sim 0.93 \pm 0.1 \) TPa and \( \sim 0.52 \pm 0.12 \) TPa, respectively.

\textbf{7.3.2.2. Sequence II: Increased maximum strain}

In this section, the results of subjecting the coatings to deformation with increased maximum strain levels are discussed. Figure 7.14a,d shows the deformation sequence used and the response of the 2D band position of both exfoliated and CVD graphene coatings. The significant shift of 2D band with applied strain as seen in Figure 7.14b,e indicates that the stress transfer occurred in the coatings. For the MC graphene, the 2D band shifts approximately linearly with the strain up to 0.4 \% strain. When the strain level increased to 0.5 \%, the loading and the unloading curve were not superimposable, indicating the onset of the interfacial damage in the coating leading to the hysteresis loop. Also, similar to the previous deformation sequence, the 2D position at the 0 \% strain level at the end of the deformation cycle is at a higher wavenumber than the previous cycle (Figure 7.14b). This indicates that the graphene underwent slippage during the loading cycle leading to the development of residual compression at the end of the cycle. The shift rates calculated from the slope of Figure 7.14b is plotted in Figure 7.14c. Strain hardening was observed with each cycle, as seen in the previous deformation sequence.

As before, the as-made CVD graphene coating had residual compression, leading to a relatively high 2D band position (\( \sim 2660 \, \text{cm}^{-1} \)). The initial deformation cycles (Figure 7.14d,e) allowed the graphene to relax the pre-strain, after which the coating exhibited almost elastic deformation. The 0 \% 2D band position of last three deformation cycles remains almost constant around \( \sim 2650 \, \text{cm}^{-1} \) suggesting no further relaxation of residual
strain. The average shift rate per unit strain was $-31 \pm 7.9 \text{ cm}^{-1}/\%\text{ strain}$, which is $\sim 35\%$ lower than the exfoliated graphene ($-48 \pm 7.5 \text{ cm}^{-1}/\%\text{ strain}$). The reason for the lower shift rate may be the many fine grain structure of the CVD graphene.

Figure 7.14: Sequence II cyclic deformation with increased maximum strain level. a,d) The sequence of the deformation and the response of 2D band position of both MC and CVD graphene. The highlighted area shows the relaxation of residual pre-compression of CVD graphene in the initial three cycles. The strain hardening

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sensing ability was calculated at 0.1 % and 0.3 % strain levels as indicated. b,e) The shift of 2D band vs. loading strain. Boxes at 0 % strain indicate the behaviour of the residual stresses at the end of the deformation cycles. c,f) Shift rate of 2D band, obtained from slopes of (b) and (e). The dashed horizontal line indicates the ideal shift rates calculated by using the Gruneisen parameters (γ=2.7).

### 7.3.2.3. Sequence III: Mapping of strain over a wide-area of CVD graphene

In order to demonstrate both the spatial strain mapping ability and life time of graphene sensors, a CVD graphene coating was deformed for 25 cycles using the sequence shown in Figure 7.15a. The average 2D position within the 100 square micron region of interest is shown in Figure 7.15b. As seen in previous samples, the film was initially in compression, with this compression being relaxed over the first few cycles of deformation. The strain response of the film was then found to be stable for the rest of the experiment where the maximum and minimum peak positions were constant within error. The 2D band position was also manually mapped at the start (0 % strain), mid-point (0.3 % strain), and end (0 % strain) of the initial, 6th, and 25th cycles (Figure 7.15c). The mapping was taken at step intervals of 10 ± 2 µm and confirms that the residual pre-compression relaxes over the first few deformation cycles to give a stable system. In particular, the Raman maps for cycles 6 and 25 are very similar confirming that the strain behaviour of graphene film stabilises. As noted earlier, the grain size (wrinkles) of graphene film is approximately 7 µm (Figure 7.10), hence the mapped region corresponds to approximately 14 randomly orientated grains in each direction. Therefore the relative uniformity of the Raman band position strongly suggests that individual grain orientation relative to the direction of deformation is not significant factor in the strain response.
Figure 7.15: a) The deformation cycle applied to a CVD composite coating over 25 cycles. The highlighted points are the points in the cycle where a full Raman map was taken in (c). The strain sensing ability was calculated at 0.3 % strain as indicated. b) The average Raman position in the 100 µm × 100 µm region of interest, based on five spectra taken from random locations. c) Raman maps of the 2D band position. The black spots represent the place where the spectra were obtained (error ± 2 µm).
7.3.2.4. Sequence IV: Variable maximum strain

Since MC graphene coatings with relatively no residual strains exhibited strain hardening behaviour (see section 7.3.2.1 and 7.3.2.2), investigations were carried on another MC graphene flake with pre-existing residual strains under cyclic loading. This composite was subjected to three sets of deformation with three cycles each with 0.3 %, 0.5 % and 0.3 % as the maximum strain at each set. Figure 7.16a show the deformation sequence followed and the response of 2D band position. On observing the trend of the 2D band position with strain in Figure 7.16a,b it is clear that the graphene flake was under pre-tension. This pre-tension in the graphene might be;

1. Because of in-plane residual strain present in the exfoliated graphene. Recent study has shown the presence of residual strain in the range of - 0.2 % to 0.4 % in exfoliated graphene on the Si/SiO$_2$ substrate regardless of charge-doping effects$^{448}$ and/or

2. Caused during the initial deposition process on the polymeric substrates. The surface of the rough polymeric substrates could also be a reason for the development of residual strains in the graphene layers as suggested in the literature.$^{269,270}$

Upon cyclic deformation, the pre-tension in graphene relaxes in the initial set of deformation cycles from the 5$^{th}$ deformation cycle the graphene exhibited elastic deformation. When analysed detail, during the initial two cycles, the loading and the unloading curve don’t follow each other. The unloading curves shift to a higher wavenumber with higher shift rates than the loading as seen in Figure 7.16b,c. Almost elastic deformation was observed on the 3$^{rd}$ cycle, but, when the maximum strain increased from 0.3 % to 0.5 % in the 4$^{th}$ cycle, the interface was damaged resulting in change of slope in its loading curve (Figure 7.16b) thus the drop in the shift rate (Figure 7.16c). From the 5$^{th}$ deformation cycle, the graphene exhibited elastic deformation with an average shift rate of $\sim -49.3 \pm 0.9$ cm$^{-1}$/% strain which is equivalent to having an effective Young’s modulus of $\sim 0.82 \pm 0.02$ TPa ($\sim -60$ cm$^{-1}$/% strain for 1 TPa SLG). The linearity between the loading and unloading curves from 5$^{th}$ cycle to 9$^{th}$ cycle indicates good stress transfer in the composite. A drop in the shift rates between the 6$^{th}$ and 7$^{th}$ deformation cycle could be related to the reduced stress transfer efficiency through the
damaged interface and hence the reduced shift rates (~\(-40 \text{ cm}^{-1}/\% \text{ strain, effective modulus of } 0.67 \pm 0.01 \text{ TPa}\)).

The position of 2D band at 0 % strain at the 1\textsuperscript{st} cycle (~\(2626 \text{ cm}^{-1}\)) is similar to the 2D band position at 0.5 % strain of the 5\textsuperscript{th} and 6\textsuperscript{th} deformation cycle (highlighted regions of Figure 7.16b) indicating that the graphene flake was under a pre-tension in order of ~0.5 % strain which is in agreement with the recent report on the presence of residual strain in the exfoliated graphene.\(^{448}\)

Unlike the MC flakes studied for sequence I and II, this graphene flake was under residual pre-tension (from fabrication step). Upon cyclic deformation, similar to CVD graphene, the residual stresses relaxed in the initial few deformation cycles and elastic deformation was observed in the subsequent deformation cycles. The interesting result was that, no strain hardening effect was observed here unlike the previously studied exfoliated graphene flakes in sequence I and II, suggesting that presence of residual strains and the initial conditioning steps (few cyclic deformation cycles) are in fact a benefit from the strain sensing point of view, thereby eliminating any unreliability during strain measurements.
Figure 7.16: Sequence IV cyclic deformation with variable maximum strain level for MC graphene composite coatings. a) The sequence of the deformation and the response of 2D band position. The highlighted area shows the relaxation of residual pre-tension of MC graphene in the initial three cycles. The strain sensing ability was calculated at 0.1% and 0.3% strain levels as indicated. b) The shift of 2D band vs. loading strain. Highlighted points indicate the residual pre-tension was ~0.5% strain. c) Shift rate of 2D band, obtained from slopes of (b). The dashed horizontal line indicates the ideal shift rates calculated by using the Gruneisen parameters (γ=2.7).
7.3.2.5. Discussion: Comparison of micromechanics of exfoliated and CVD graphene

In the current study, MC and CVD graphene coatings (in-house produced) were subjected to various cyclic deformation sequences to understand their deformation behaviour. It can be seen from the results that, the residual pre-strains (mainly from the transfer process) present in the graphene flakes play a major role in their mechanical behaviour as identified previously by other research groups. For example, Fu et al. reported a slight decrease in the electrical resistance in initial stretching cycles followed by an increase in resistance in the subsequent cycles which they attributed to the relaxation of residual strains in the transferred CVD/PDMS film. In general, in all the experiments residual pre-compression was observed in CVD graphene which relaxes over the initial few deformation cycles. Whereas, the MC flakes initially has relatively little pre-strain.

However, in general, the shift rates of MC graphene were at least ~ 35 % higher than the CVD graphene. In order to understand this difference, the structure of the two materials needs to be considered. MC graphene is a large single crystal and hence it exhibits higher stiffness and modulus and hence the shift rates. In contrast, the CVD graphene exists as a continuous single-layer film with large number of smaller domains approximately 7 µm in diameter. The domain boundaries act as a defect in the crystal structure potentially meaning that each domains act as an individual flake. Hence shear lag theory would predict that there would be a lower strain at the edges of the domains, based upon similar arguments as that for the solvent exfoliated flakes (as explained in 7.2.3). Furthermore, some domains could be small enough for edge effects to dominate so that full stress transfer does not occur within the flake, lowering the band shift rate. Another plausible reason for incomplete transfer of stress to the graphene CVD may be the poor interface being formed or wrinkling of the PMMA/graphene film taking place during the transfer process.
7.3.2.5.1. **G band shift in model CVD graphene coatings**

Bissett *et al.* showed a G band upshift on tension in CVD graphene compared to a down shift in MC graphene. This behaviour was related to the presence of domain grain boundaries in the polycrystalline CVD graphene. This anomalous behaviour was not observed in the CVD graphene coatings in this study.

Attempts were made to check if the behaviour was followed in the CVD graphene used in this study. Figure 7.17 show the shift of G band with strain after five deformation cycles (uniform maximum strain). It should be noted that, the G band of graphene is overlapped by the substrate peak at similar wavenumber. Since the substrate peak is unaffected by the applied strain, when the curve was fitted with one Lorentzian function the general behaviour (downshift on straining) of the G band on uniaxial tension was observed. A shift rate of $\Delta \omega = -14.72 \text{ cm}^{-1}$/% strain was observed during loading. Relatively large error bars were related to the curve fitting variations. However, careful analysis of Bissett *et al.*’s experimental details show that their upshift was probably due to the fact that strain was not measured directly but rather inferred from the Raman peak shift, assuming the band shift per unit strain for a perfect crystal.277

![Figure 7.17: Shift of G band with strain in model CVD graphene coatings](image)
7.3.2.5.2. **Doping levels in the CVD graphene coatings**

The analysis of the G band in the samples does let the relative effects of doping and strain on the Raman spectra to be calculated, as the G band is more strain sensitive to dopants than the 2D band.

The Raman data was also used to evaluate the degree of doping in the samples. For the doping study, spectra were obtained from low power (< 1 mW) Argon laser (514 nm) in Renishaw 2000 spectrometer. The position and FWHM of the G and 2D band are sensitive to both doping\(^{55, 354}\) and strain\(^{32, 256}\). It has been shown previously that the position of the 2D band shifts < 1 cm\(^{-1}\) for carrier concentrations below 3 \(\times\) 10\(^{13}\) cm\(^{-2}\), whereas the G band shifts up to 30 cm\(^{-1}\). Hence the position of the G band was used to analyse the doping levels. It can be clearly seen from Figure 7.18, that the position of G band before and after cyclic deformation are \(~1592\) cm\(^{-1}\) and \(~1588\) cm\(^{-1}\), respectively. The difference in the position of the G band after deformation (\(~1588\) cm\(^{-1}\)) compared to the literature values (\(~1583\) cm\(^{-1}\))\(^{55, 354}\) for very little or no doping, corresponds to an estimated hole doping level less than 0.5 \(\times\) 10\(^{13}\) cm\(^{-2}\).\(^{55, 451}\) For this hole doping levels, the 2D band shift is < 1 cm\(^{-1}\), which in turn again supports the argument that shift of 2D band position (\(~20\) cm\(^{-1}\) before deformation) was mainly because of the strain effects in this study and not doping.

![Figure 7.18: Position of G band before and after cyclic deformation of CVD graphene coatings](image-url)
7.3.2.6. Cyclic deformation of commercial vs in-house CVD graphene

The performance of commercially produced CVD graphene was also used to explore its use as strain sensor. Raman spectroscopy was used to characterise the as-received commercial CVD graphene on Cu foil from Graphene supermarket Ltd.\textsuperscript{338} and in-house grown graphene (used in sections 7.3.2.1 to 7.3.2.3).\textsuperscript{336} The Raman spectra of both the samples are shown in Figure 7.19.

![Raman spectra comparison](image)

Figure 7.19: a) Raman spectra of as-supplied commercial CVD graphene and in-house grown CVD graphene on Cu (after baseline correction). b) Raman spectra of composite coating using commercial CVD graphene.

The as-supplied commercial graphene has an intense D band around 1320 cm\(^{-1}\) which corresponds to the intrinsic defects of graphene and the \(I_D/I_G \sim 1\) with \(I_{2D}/I_G \sim 1.5\). Whereas with the in-house grown graphene, the absence of D band indicates its high quality, along with \(I_{2D}/I_G\) ratio > 3 confirming the graphene is predominantly monolayer.

Model composite coatings prepared from commercial CVD graphene was subjected to sequence II cyclic deformation (increased maximum strain). The Raman spectrum of the composite sample is shown in in Figure 7.19b and the D band is clearly visible after transfer.
Chapter 7 – Application of Graphene composites: Raman Strain Sensors

Figure 7.20: a) Cyclic deformation sequence II and the response of the 2D band position of commercial CVD graphene. b) The shift of 2D band vs. loading strain with respect to the deformation cycle. c) Shift rate of 2D band, obtained from slopes of (b). The dashed horizontal line indicates the theoretical ideal shift rate calculated by using the Gruneisen parameter ($\gamma = 2.7$). d) Shift rates of different graphene composite coating with 0.5 % maximum strain.

The commercial CVD graphene coating had relatively less residual compression when compared to the in-house grown graphene (~2660 cm$^{-1}$ for in-house CVD and ~2654 cm$^{-1}$ for commercial CVD). Unlike the in-house CVD graphene, the residual compression relaxed slightly in the initial cycle before the response stabilises in the subsequent deformation cycles as observed in Figure 7.20a, b. Some of the plausible reasons are:

- Broad FWHM(2D) band and the $I_{2D}/I_G \approx 1.5$, indicates that the graphene might be bilayer than monolayer and hence the upshift of the observed 2D band position.
- It is reported previously that the exfoliated bilayer graphene with top and bottom polymer coat is almost similar in transferring stress to the polymer layers when compared to monolayer with top and bottom polymer coat.$^{4,274}$ However, the observed shift rate in this case (~ -14 cm$^{-1}$/% strain) is at least 70 % and 60 %
lower than exfoliated and in-house produced graphene, respectively. This corresponds to the intrinsic defects (D band) of CVD graphene, reduced stress transfer due to grain boundaries$^{142,277}$ and/or incomplete transfer of applied stress to the CVD graphene due to poor interface formed during transfer process. Moreover, the presence of smaller wrinkles$^{281}$ or boundaries in the transferred film could be a plausible reason for the observed reduced stress transfer.

These above reasons indicate that the quality of CVD graphene plays a crucial role in optical based wide-area sensor applications where the uniformity of graphene over a large area is required. Presence of few-layer regions and intrinsic defects in the region of interest would result in estimating different strain rather than the actual strain.

### 7.3.3. Sensing ability of the coatings

The resolution and accuracy are the main two parameters that define the sensitivity of any strain sensor. The resolution is the smallest difference in relative strain that can be measured and is typically quoted in microstrain ($\mu \varepsilon$). The accuracy is the repeatability in measure of an absolute strain from sample to sample and is typically quoted in percentage strain ($1 \% \equiv 10^4 \mu \varepsilon$). Commercially-available single-point strain sensors have very high resolutions up to 0.1 $\mu \varepsilon$, whereas large area photo-elasticity based strain sensors have relative resolutions ranging from 10 – 20 $\mu \varepsilon$ and an absolute accuracy about 0.1 $\%$ strain.$^{328,330}$

The accuracy of measuring the strain in the graphene coatings could be determined from the cyclic deformational sequences. Absolute accuracy is given by the standard deviation of the difference of the 2D band position at one particular strain level divided by the ideal graphene shift rate of $\sim -62$ cm$^{-1}$/% strain as determined by using the Gruneisen parameter. The maximum achievable resolution (absolute resolution) of these graphene-based Raman strain sensors could be calculated from the Equation 7.2.

$$\text{Absolute resolution (}$\mu \varepsilon$$) = \frac{\text{Absolute resolution of Raman spectrometer (cm}^{-1}\text{)}}{\text{Maximum band shift obtained(cm}^{-1}$/% strain\text{)}} \times 10000$$

*Equation 7.2*

Example calculations for both absolute accuracy and resolution are given in the following sections. The strain sensing ability of these graphene-based Raman strain
sensors were calculated at two reference strain levels; 0.1 % and 0.3 % as shown in Figure 7.12a,d, Figure 7.14a,d, Figure 7.15a, Figure 7.16a and Figure 7.20a. It should be noted that, only the data where graphene in the composite coatings relaxed from its pre-strains were considered for the calculations (see Figure 7.12a,d, Figure 7.14a,d, Figure 7.15a, and Figure 7.16a). The calculated values of absolute accuracy and resolutions are given in Table 7-4.

7.3.3.1. Absolute accuracy – Example calculations

In this context, the absolute accuracy of the system is defined as the accuracy in determining the strain levels. It could be calculated from the standard deviation of the difference in wavenumbers at one particular strain level to the ideal shift rate of that band. It could be explained with an example; Figure 7.21 is the applied strain and the response of 2D band for CVD graphene with uniform maximum strain levels.

![Figure 7.21: Applied strain and response of 2D band in model CVD coating. The absolute accuracy was calculated at 0.1% and 0.3% strain levels as indicated by the horizontal lines.](image)

It should be noted that for these calculations, only data after the relaxation of pre-strains were considered. The 2D band position at 0.1 % at 3\textsuperscript{rd} deformation cycle was considered as the reference position (2646.36 cm\textsuperscript{-1}). The calculations are shown in Table 7-3.
### Table 7-3: Absolute accuracy calculations

<table>
<thead>
<tr>
<th>Reference position - 2646.36 cm⁻¹ (3rd cycle, loading)</th>
<th>New positions</th>
<th>Difference (reference position – new position)</th>
<th>Absolute accuracy = standard deviation/ ideal shift rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>3rd cycle, unloading 2646.19 cm⁻¹</td>
<td>0.17</td>
<td>± 0.01 %</td>
</tr>
<tr>
<td>4th cycle, loading 2645.4 cm⁻¹</td>
<td>4th cycle, unloading 2644.59 cm⁻¹</td>
<td>0.96</td>
<td>-</td>
</tr>
<tr>
<td>5th cycle, loading 2644.55 cm⁻¹</td>
<td>5th cycle, unloading 2644.52 cm⁻¹</td>
<td>1.81</td>
<td></td>
</tr>
</tbody>
</table>

**7.3.3.2. Absolute resolution – Example calculations**

As explained earlier, absolute resolution could be calculated from the Equation 7.2. The absolute resolution of the spectrometer with the peak fitting software is 0.1 cm⁻¹. The maximum shift obtained is ~ 37 cm⁻¹/% strain for the sequence II (increased strain deformation, after relaxation of pre-strains) which computes to an absolute resolution of ~ ± 27 µε.

This in perspective, if CVD graphene coating was to be used as a large area Raman-based strain sensor, it could measure strains from 0 % up to the interfacial failure with a resolution of 27 µε with a strain sensing absolute accuracy of ~ ± 0.01 % strain.

**7.3.3.3. Discussion: Strain sensing**

The calculated values of absolute accuracy and absolute resolution are given in Table 7-4 and it can be observed that, the absolute accuracy and the resolution values change between different deformation sequences. This is due to the deformation behaviour of the graphene during the cyclic deformation. The variation in the absolute accuracy values of MC graphene in sequences I and II could be attributed to the strain hardening behaviour observed in the coatings. Whereas, once the residual strain in the
graphene is relaxed, the value of accuracy is constant at \( \pm 0.02 \% \), which is better than the commercially-available large-area strain sensors.\(^{328, 330}\) The maximum achievable resolutions of the exfoliated composites are in the range of \( \sim 15–18 \mu \varepsilon \) which is comparable to the commercially-available large-area strain sensors. These high resolution values correspond to a strong interface between the exfoliated graphene and the composite which leads to higher shift rates. As discussed earlier, the in-house CVD graphene has a lower resolution of \( \sim 27 \mu \varepsilon \), but still comparable to the exfoliated graphene, whereas, the commercial CVD graphene had poor resolution of \( \sim 72 \mu \varepsilon \). Nevertheless, the absolute accuracy values of the in-house CVD graphene after the relaxation of residual strains remained at \( \sim \pm 0.01 \% \) strain for sequences I, II and III. It should be noted that, the maximum strain that these sensors could withstand is defined by the strain to failure of the interface.

Table 7-4: Calculated absolute accuracy and resolution of exfoliated and CVD graphene composite coatings

<table>
<thead>
<tr>
<th>Composite coatings</th>
<th>Deformational sequence</th>
<th>Absolute Accuracy (% strain)</th>
<th>Absolute Resolution (( \mu \varepsilon ))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>@ 0.1</td>
<td>@ 0.3</td>
</tr>
<tr>
<td><strong>MC</strong></td>
<td>Sequence I (Same max. strain)</td>
<td>(~ \pm 0.04)</td>
<td>(~ \pm 0.02)</td>
</tr>
<tr>
<td></td>
<td>Sequence II (Increase max. strain)</td>
<td>(~ \pm 0.03)</td>
<td>(~ \pm 0.02)</td>
</tr>
<tr>
<td></td>
<td>Sequence IV (Variable max. strain)</td>
<td>(~ \pm 0.02)</td>
<td>(~ \pm 0.02)</td>
</tr>
<tr>
<td><strong>CVD (in-house)</strong></td>
<td>Sequence I (Same max. strain)</td>
<td>(~ \pm 0.01)</td>
<td>(~ \pm 0.01)</td>
</tr>
<tr>
<td></td>
<td>Sequence II (Increase max. strain)</td>
<td>(~ \pm 0.01)</td>
<td>(~ \pm 0.01)</td>
</tr>
<tr>
<td></td>
<td>Sequence III</td>
<td>-</td>
<td>(~ \pm 0.01)</td>
</tr>
<tr>
<td><strong>CVD (commercial)</strong></td>
<td>Sequence II (Increase max. strain)</td>
<td>(~ \pm 0.03)</td>
<td>(~ \pm 0.03)</td>
</tr>
</tbody>
</table>
7.4. **Summary**

Information on strain over a large structure (e.g. bridge) is crucial both during its design and in service. In addition to high accuracy, single-point, deformation measurements, there is a need to be able to measure local strain at multiple points over a structure. Whilst this can be achieved with electronically-based sensors (e.g. resistance strain gauges made from Cu-Ni alloy), every point of interest needs to be individually wired, leading to significant amount of infrastructure. Thus it is preferable to measure the strain using optical measurements, e.g. by using Raman spectroscopy, especially on carbon-based composites as their Raman bands are strain sensitive. Graphene is an ideal candidate for Raman active coatings because: 1) Its 2d nature suitable for coatings (each atom in graphene is sensible for change in its environment, whereas CNTs are 1d and has less surface area), 2) Higher inherent band shift per unit strain of the Raman 2D band, and 3) The highest intensity of graphene’s most strain sensitive band (2D band).

FLG/epoxy composite coatings were initially investigated as a Raman active strain sensor by following the changes in the 2D band with applied strain. Little or no strain sensitivity was observed for coatings made from flakes having a length shorter than the critical length of graphene (i.e., < 3 µm). Whereas for flakes longer than the critical length, several factors such as the processing history, loading of graphene, defects in the graphene and alignment of flakes within the composites could influence the shift rates of the band.

Graphene coatings were also prepared from both MC (top-down approach) and CVD methods (bottom-up approach) and were successfully demonstrated as Raman-based strain sensor. The CVD based coatings were found to have residual compression stresses which relaxed over the first 2 to 3 deformation cycles. These results suggest that sensors made from transferred graphene should be cycled by an external strain as a conditioning step during manufacture to ensure stable and accurate readings while in use. Moreover, a hysteresis loop (interfacial damage) and its upshift were observed in MC graphene coatings in the absence of residual strains. These MC graphene samples, also exhibited a strain hardening behaviour with a higher shift rates and thus a more effective modulus than CVD graphene. In general, the shift rates of MC graphene are at least ~ 35
% higher than the CVD graphene, making the former more sensitive to strain, which may be due to MC graphene being a large single crystal domain.

The absolute accuracy and resolution of these strain sensor coatings were calculated from the knowledge of 2D band position and absolute resolution of the Raman spectrometer. In comparison to the commercially available wide area strain sensors, CVD graphene films with a calculated absolute accuracy of $\pm 0.01$ % strain and absolute resolution of $\sim 27 \, \mu \varepsilon$ show promise for a wide-area Raman-based strain sensors.
Chapter 8 – Conclusions and Future Works
8. CONCLUSION AND FUTURE WORKS

8.1. CONCLUSIONS

This thesis has explored various LPE production methods for pristine FLG in both organic and in aqueous media. The graphene produced in aqueous media facilitated its use in toxicological examinations. Moreover, epoxy-based composite coatings made from graphene, produced using LPE, MC, and CVD processes, were investigated as wide-area Raman-based strain sensor applications. The main results of this thesis are summarised below:

- Firstly, FLG was successfully produced by sonicating natural graphite in an ultrasonic bath in organic solvents such as NMP and DMF to produce FLG/NMP and FLG/DMF. The concentration of the dispersion was found to increase with sonication time and decrease with centrifugation speed. Dispersions produced with higher centrifugations speeds were found to be stable over six months without any visible sedimentation. AFM was used to characterise the exfoliation efficiency. It was found that the higher the sonication time the lower the $L_{AFM}$ and $N$. Similar behaviour was observed while using different centrifugation speeds. This suggests that smaller and thinner flakes dominate the dispersions produced at longer sonication time and higher centrifugation speed. Statistical analysis by AFM revealed the $L_{AFM}$ ranged from ~300 to 80 nm and $N$ ranged between ~8 – 3 for various conditions used. Despite flakes having relatively small $\langle L \rangle$ ~ 130 nm, samples produced at 60 hr sonication and 6000 rpm centrifugation speeds were the best of all preparations with $N_{1-5}/N_T \%$ ~ 80 % whilst maintaining stable dispersion. Raman spectroscopy was used to understand the quality of the flakes. The Raman spectra of all the samples exhibited a symmetric 2D band and its position, FWHM(2D) along with $I_{2D}/I_G$ ratio indicates that the graphene was few-layer in nature. By probing the $I_D/I_G$ ratio along with $I_D/I_{D'}$, it was shown that the intense D band are from the edges that are formed during the sonication process, and not because of any new basal plane defects. This shows that the sonication is a non-destructive process with regards to the introduction of any new basal plane defects. The $L_{Raman}$ was also derived from the $I_D/I_G$ which compares to $L_{AFM}$ with a relative error of ~17 %. The potential for
DLS as an efficient characterisation technique for estimating the lateral dimensions of the graphene flakes was also explored. The $L_{DLS}$ derived from the primary peak positions of IPSD of DLS were compared to the calculated $L_{Raman}$ and $L_{AFM}$. It was found that the $L_{DLS}$ indeed exhibited a linear relationship with $L_{Raman}$ and $L_{AFM}$, with a relative error of ~25% in measuring the mean size of nanosheets (in submicron region). This is consistent with the IPSD obtained from DLS as a measure of the hydrodynamic diameter, which is the diameter of a sphere whose volume is equal to that of the mean graphene flakes volume, having similar translational diffusion coefficient. This in situ length measurement technique, despite its error, could play a crucial role when preliminary ideas of the flake sizes are required, comparing samples in a quality control environment such as production or understanding the in situ aggregation of graphene sheets.

- Secondly, various techniques for producing pristine FLG in aqueous media were explored. A simple solvent exchange method like dialysis was employed to exchange the organic solvents with water in the organic solvent-exfoliated FLG dispersions. UV-Vis and $^1$H NMR spectroscopy confirmed that the organic solvent could be reduced down to levels of 0.1 v/v% (99.9 v/v% water) while maintaining sufficient colloidal stability. Raman spectroscopy and XRD indicated no in-plane disruptions, suggesting the pristine nature was retained in FLG. The higher absolute $\zeta$ values for the dialysed 0.1 v/v% FLG dispersions meant that the electrostatic repulsion was the main stability mechanism with some contribution from edge effects. TDOC, a bile surfactant effectively exfoliated graphite to highly-stable FLG dispersions with the best exfoliation conditions of 36 hr sonication and 10000 rpm centrifuge speed producing flakes with $\langle L_{AFM} \rangle \sim 100$ nm and $N_{1-5}/N_T > 92\%$. A one-step process of using neutral phospholipids like PC (the major component of cell membrane) to produce pristine FLG in PBS buffer was demonstrated (Exfoliation conditions: 36 hr sonication and 2000 rpm centrifuge speed). The absence of adsorbed lipid molecules on graphene surface by AFM analysis confirmed their weak interaction. The surface charge screening effect by salt results in poor stability of graphene in PBS buffer. Despite its poor stability, it was shown that PC indeed efficiently exfoliated graphite to FLG (graphene monolayers by AFM) and its pristine nature was confirmed by
Raman analysis. Moreover, the intrinsic nature of proteins to adsorb onto the surface of particles via their hydrophobic domains (‘pockets’) was explored in producing FLG, by directly exfoliating graphite in aqueous solutions of HSA, most abundant protein in human plasma. FLG dispersions with concentrations up to ~0.21 mg/ml in saline PBS buffer were obtained at the best exfoliation conditions of 24 hrs sonication and 2000 rpm centrifuge speed (starting HSA concentration of 5 mg/ml) and were found to be stable for up to 15 days. AFM analysis revealed strong adsorption of HSA onto graphene surfaces, where identified individual HSA molecules were visible. Raman analysis confirmed the pristine nature of the graphene. Strong fluorescence quenching of Tyr and Trp suggested that the hydrophobic domains of HSA were indeed interacting (\(\pi \rightarrow \pi\) and hydrophobic interactions) with graphene, possibly as a result of conformational changes due to its structural flexibility. Such HSA-coated graphene could act as ‘stealth’ nanomaterials in biological studies.

- All the aqueous graphene dispersions produced in this thesis were examined for their cytotoxicity effects in cell-based biological systems (Red blood cells, breast cancer cells MCF-7, and macrophages murine J774A.1) by my collaborators. The results are presented in Appendix 9.6.1 and Figure 9.12. Cell culture experiments found that the solvent-exchanged and biocompatible surfactant-exfoliated pristine FLG displayed minimal cytotoxicity and albumin-exfoliated FLG hardly any cytotoxicity, whereas GO and phospholipid-exfoliated FLG were particularly cytotoxic.

- Finally, information on strain over a large structure (e.g. bridge) is crucial both during its design and in service. In addition to high accuracy, single-point, deformation measurements, there is a need to be able to measure local strain at multiple points over a structure. Whilst this can be achieved with electronically-based sensors (e.g. resistance strain gauges made from Cu-Ni alloy), every point of interest needs to be individually wired, leading to significant amount of infrastructure. Thus it is preferable to measure the strain using optical measurements, e.g. by using Raman spectroscopy, especially on carbon-based composites as their Raman bands are strain sensitive. Graphene is an ideal candidate for Raman active coatings because: 1) Its 2d nature suitable for coatings (each atom in graphene is sensible for change in its
environment, whereas CNTs are 1d and have less surface area), 2) Higher inherent band shift per unit strain of the Raman 2D band, and 3) The highest intensity of graphene’s most strain sensitive band (2D band). FLG/epoxy composite coatings were investigated as a Raman active strain sensor by following the changes in the 2D band with applied strain. Little or no strain sensitivity was observed for coatings made from flakes having a length shorter than the critical length of graphene (i.e., < 3 µm). Whereas for flakes longer than the critical length, several factors such as the processing history, loading of graphene, defects in the graphene and alignment of flakes within the composites could influence the shift rates of the band. Graphene coatings were also prepared from both MC (top-down approach) and CVD methods (bottom-up approach) and were successfully demonstrated as Raman-based strain sensor. The CVD based coatings were found to have residual compression stresses which relaxed over the first 2 to 3 deformation cycles. These results suggest that sensors made from transferred graphene should be cycled by an external strain as a conditioning step during manufacture to ensure stable and accurate readings while in use. Moreover, a hysteresis loop (interfacial damage) and its upshift were observed in MC graphene coatings in the absence of residual strains. These MC graphene samples, also exhibited a strain hardening behaviour with a higher shift rates and thus a more effective modulus than CVD graphene. In general, the shift rates of MC graphene are at least ~ 35 % higher than the CVD graphene, making the former more sensitive to strain, which may be due to MC graphene being a large single crystal domain. The absolute accuracy and resolution of these strain sensor coatings were calculated from the knowledge of 2D band position and absolute resolution of the Raman spectrometer. In comparison to the commercially available wide-area strain sensors, CVD graphene films with a calculated absolute accuracy of ~ ± 0.01 % strain and absolute resolution of ~ 27 µε show promise for a wide area Raman-based strain sensors.
8.2. **FUTURE WORKS**

One of the intriguing outcomes of the LPE in organic (NMP and DMF) and TDOD-based aqueous dispersions is the observation of different values for the absorption coefficient (\(\alpha\)) of graphene. The value of \(\alpha\) ranging from 1390 to 6600 mL mg\(^{-1}\) m\(^{-1}\) in various solvents and surfactant-based dispersions was reported in the literature\(^5^4, 5^9, 6^0, 6^9, 7^7\) these variations are not clearly understood, and remains an interesting area to study given that graphene dispersions are widely used. Su et al. recently conducted a study on variations of \(\alpha\) on rGO systems with different lateral dimensions, mean number of layers per flake and functional groups.\(^3^4^9\) They reported that dispersions containing smaller (lateral dimensions) and less layer per flake had lower \(\alpha\), attributed to the shrinkage of the \(\pi\)-conjugated systems. They also reported that the absorption coefficient increases with an increase in the number of functional groups on the flakes due to auxochromic effect. It would be interesting to study the effect of change in lateral dimensions and thickness of flakes of pristine graphene in various solvent media with the value of \(\alpha\). Sequential centrifugation at different speeds and ultracentrifugation can be employed to produce flakes with different lateral dimensions and thickness, respectively.

One of the challenges in achieving higher shift rates (higher strain sensitivity) in epoxy-based graphene composites remains in the homogenous dispersion of FLG in the matrix. Other preparation methods, such as using high speed mixers or three-roll mills can be employed to homogeneously disperse FLG flakes directly into epoxy resin, avoiding the use of a solvent processing step. These methods could also be employed to exfoliate the FLG \textit{in situ} due to the high shear causing minimal damage to the large lateral dimensions (electrochemically exfoliated FLG).

In epoxy composite coatings where pristine FLG is used, the interaction between FLG and epoxy is mainly through vdW interactions, where the interfacial stress transfer is relatively poor. One way to improve the stress transfer is \textit{via} graphene functionalisation (e.g. amine group functionalisation to use in epoxy matrix). Functionalisation could improve the interfacial adhesion between epoxy and FLG by forming covalent bonds, thereby, enhancing the stress transfer and strain sensitivity. Moreover, functionalisation can ensure homogenous dispersion of FLG in the matrix with less aggregation. However,
disruption to the sp² structure could result in reducing the intensity of the Raman 2D band of graphene. In such cases, defect active Raman D band (2D band is overtone of D band) could be used to monitor the strain measurements.²⁹³

With regards to CVD graphene composite coatings, the shift rate (strain sensitivity) obtained in Chapter 7 for CVD graphene is ~ 35 % lower than MC graphene. This can be attributed to MC graphene being large single crystal domain. Recent improvements on CVD growth (as explained in section 2.3.5.1.1), have reported large single crystal domains growth in mm- to cm- sizes. It would be interesting to conduct the strain sensitivity study on large single crystal CVD grain-composite coatings. Furthermore, with the large area of CVD graphene films, effects of external cracks or notches in the film can be studied using automated Raman maps under strain, revealing the strain field.
9. **APPENDIX**

9.1. **FLG/DMF CHARACTERISATION**

9.1.2. **AFM Characterisation**

Figure 9.1: Histogram data on $L_{AFM}$ and $N$ of 60 hr FLG/DMF at different centrifugation speeds.
9.1.3. Raman Characterisation

Figure 9.2: a) Representative Raman spectra of 60 hr FLG/DMF sample at different centrifugation speeds. b) Change in 2D band, G band and D band position as a function of centrifugation speed.

Figure 9.3: $I_{2D}/I_G$ and $I_D/I_G$ ratio of 60 hr FLG/DMF as a function of centrifugation speed.
9.2. **NMR CHARACTERISATION OF DIALYSED GRAPHENE DISPERSIONS**

Figure 9.4: Enlarged section of $^1$H NMR signals from control (red trace, without graphene) and dialysed graphene (black trace) dispersions. 0.099 M TMP was used as internal reference.

Figure 9.5: Example $^1$H NMR spectra of control and dialysed dispersions showing the integral values. These integral values correspond to the number of $^1$H and were used to quantify the organic solvents in dialysed dispersions.
9.3. **AFM CHARACTERISATION OF DIALYSED GRAPHENE DISPERSIONS**

*Figure 9.6: Additional AFM images of dialysed 0.1 v/v% G/DMF dispersions.*

*Figure 9.7: Additional AFM images of dialysed 0.1 v/v% G/NMP dispersions.*
9.4. **AFM AND RAMAN CHARACTERISATION OF LIPID ADSORPTION ON GRAPHENE**

*Figure 9.8: Additional AFM images of G/lipid showing no adsorbed lipid molecules on graphene surface*

*Figure 9.9: Additional Raman spectra of G/lipid showing typical Raman bands of graphene. The shape of 2D band confirms few-layers.*
9.5. **AFM and Raman characterisation of HSA adsorption on graphene**

![AFM images of G/HSA showing strongly adsorbed HSA on graphene surfaces](image1.png)

*Figure 9.10: Additional AFM images of G/HSA showing strongly adsorbed HSA on graphene surfaces (clearly visible in the phase image).*

![Raman spectra of G/HSA showing few-layer nature of graphene](image2.png)

*Figure 9.11: Additional Raman spectra of G/HSA showing few-layer nature of graphene.*
9.6. BIOLOGICAL EXAMINATIONS

9.6.1. Cytolytic and cytotoxic activities of aqueous graphene

9.6.1.1. Materials and methods

Hemolytic assay

Defibrinated horse blood (Oxoid Basingstoke UK SR0050C) was centrifuged (100 ×g for 5 min) and washed in 0.9 w/v% NaCl until the supernatant was colourless, when the harvested red blood cells (RBCs) were counted using a haemocytometer and diluted to 5x10⁶ cells/ml. Diluted RBCs (100 μl) were added to replicates (n=4) of each concentration of each graphene preparation (100 μl) and made up to 1 ml with sterile 0.9 w/v% NaCl. After incubation at 37 °C for 30 min and removal of the RBCs by similar centrifugation, absorbance at 550 nm of aliquots (200 μl) of the supernatants was estimated (Tecan Safire plate reader). Controls of RBCs alone and RBCs treated with surfactant (5 w/v% Triton X-100) were taken as zero and 100 % lysis (respectively).

Cell Culture

Breast cancer cells (MCF-7) were grown at 37 °C under 5 % CO₂ in Roswell Park Memorial Institute (RPMI) medium (Sigma Aldrich, UK. R8758), supplemented with 10 % Foetal Calf Serum (FCS, Sigma Aldrich, UK. F7524) and 1 w/v% penicillin/streptomycin solution (Sigma Aldrich, UK. P4333). Mouse macrophages (J774A.1) were grown in Dulbecco’s Modified Eagle’s Medium (DMEM) media (Sigma Aldrich, UK. D6429) supplemented with 10 v/v% FCS and a 1 w/v% penicillin/streptomycin. When confluent, cells were removed: MCF-7 by Trypsin EDTA treatment and J774A.1 by gentle cell scraping. Cells were harvested and washed in phosphate buffered saline (PBS) by centrifugation at 100 ×g for 5 min at room temperature and counted by haemocytometer.

AlamarBlue Assay

Both MCF-7 and J774A.1 cells were allowed to adhere overnight to the surface of micro-well plates (Costar 96well Cell Culture Cluster, Cat No. 3596) at a density of approximately 5000 cells per well, with replicates (n=6), before treatment with graphene preparations diluted into growth medium. Double strength growth medium was prepared.
from solid medium (Sigma Aldrich, UK. RPMI-R6504, DMEM-D7777) in FCS (100 ml) and 1 w/v% penicillin/streptomycin (10 ml) with sodium bicarbonate (2 g; Sigma Aldrich, UK. S5761) and adjusted to 500 ml with sterile DI water. Sterile graphene preparations were adjusted to double the concentrations tested with DI water and mixed with an equal volume of the double strength medium, when 200 μl was added to the adhered cells in each well. After incubation for 24 h at 37 °C under 5 % CO\(_2\), Resazurin (Sigma Aldrich, UK. 199303) was added (10 μl of 0.1 mg/ml) to each well and the plates further incubated for 6 h, when their fluorescence was read (Ex 570 nm, Em 590 nm, Tecan Safire plate reader). Controls of media only and cells with media were taken as zero and 100 % respectively.

All of the biological studies were performed by Dr. Shaun Offerman at the Manchester Pharmacy School under the supervision of Dr. Harmesh Aojula and Prof. David Clarke. These results were presented in this thesis as supporting information for the materials produced in Chapter 6. The summary of all the aqueous graphene preparations (from Chapter 6) used for toxicity examinations are given in Table 9-1. GO was also used in the toxicity examinations for comparison. GO was kindly supplied by Dr. Cristina Valles from School of Materials, University of Manchester.

Table 9-1: Summary of all the aqueous graphene preparation used for toxicity examinations

<table>
<thead>
<tr>
<th>FLG preparation</th>
<th>Hydrodynamic diameter (DLS) nm</th>
<th>Mean flake length (AFM) nm</th>
<th>Mean number of layers (AFM)</th>
<th>Zeta potential mV</th>
<th>Residual organic solvent ((^1)H-NMR) (v/v%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dialysed 0.1 v/v% G/DMF</td>
<td>~170</td>
<td>140 ± 70</td>
<td>4.5 ± 2.3</td>
<td>-48.9 (pH ~7.6)</td>
<td>0.07 ± 0.01</td>
</tr>
<tr>
<td>Dialysed 0.1 v/v%G/NMP</td>
<td>~200</td>
<td>170 ± 80</td>
<td>4.3 ± 1.9</td>
<td>-47.7 (pH ~7.7)</td>
<td>0.07 ± 0.01</td>
</tr>
<tr>
<td>G/TDOC</td>
<td>~100</td>
<td>90 ± 40</td>
<td>3.2 ± 1.8</td>
<td>-63.0 (pH ~7.6)</td>
<td>NA</td>
</tr>
<tr>
<td>G/Egg PC</td>
<td>~350</td>
<td>350 ± 150</td>
<td>5.3± 2.1</td>
<td>ND</td>
<td>NA</td>
</tr>
<tr>
<td>G/HSA</td>
<td>~390</td>
<td>370 ± 120</td>
<td>6.1 ± 3</td>
<td>-27.9 (pH ~6.8)</td>
<td>NA</td>
</tr>
</tbody>
</table>
Graphene materials have been reported to act on cell membranes and mitochondria, which were considered here by well-established assays for cell lysis and reductive metabolic activity of cells using the alamarBlue assay.

Red blood cells serve as model non-phagocytic cells, where activity of the graphene preparations is likely to involve direct penetration of cell membranes with loss of cytosol, including hemoglobin where membrane activity is cytolytic. Organic solvents may also cause lytic damage to cell membranes. Though cells could withstand higher...
Appendix

levels of DMF, NMP was more toxic with over 70 % loss of metabolic activity at 1 %, and 20 % loss at 0.2 v/v% NMP (Figure 9.12a). Solvents in the G/DMF and G/NMP preparations were reduced by dialysis to <0.1 v/v% to minimise solvent cytotoxicity. Most FLG preparations were then not significantly cytolytic. Whatever association of the surfactant, albumin and trace solvent FLG preparations with cell membranes, they were not cytolytic up to the highest concentrations tested (100 µg/ml). However, G/Egg PC and GO were cytolytic at relatively low concentrations: G/lipid at >10 µg/ml and GO at >20 µg/ml released hemoglobin from red cell cells (Figure 9.12b).

G/Egg PC also inhibited reductive cell metabolic activity of cancer cell lines (breast cancer MCF-7 cells, Figure 9.12c) at relatively low concentrations (>20 µg/ml), whereas GO and G/HSA were not significantly inhibitory at the highest concentration tested (100 µg/ml). G/TDOC was also not significantly inhibitory and slightly stimulatory of metabolism up to the highest concentration tested (50 µg/ml). Dialysed (0.1 v/v%) G/DMF and G/NMP were of intermediate cytotoxicity, reducing cell metabolism by about 50 % at the highest concentration tested (100 µg/ml).

G/HSA was least inhibitory to macrophages (murine J774A.1) with loss of reductive metabolism by only 15-20 %, with no significant dose response effect (Figure 9.12d). GO and G/Egg PC were most cytotoxic to macrophages, with relatively low concentrations (>10 µg/ml) leaving little reductive metabolism, whereas dialysed 0.1 v/v% G/DMF was stimulatory at low concentrations and G/TDOC was not significantly toxic at relatively low concentrations (<20 µg/ml).

9.6.1.3. Discussion

Aqueous exfoliation of graphene from graphite by sonication and adsorption of very different biomolecules (HSA, bile salt & phospholipids) produced pristine graphene with the typical range of physical characteristics of FLG exfoliated in organic solvents (NMP & DMF). Longer flake lengths and 1-2 more graphene layers may reflect the shorter sonication times in aqueous media to avoid basal plane defects. Though adsorption of the biomolecules modified surface charge to some extent, FLG exfoliated in the organic solvents had a zeta potential ($\zeta$ of -48 mV) in the middle of the range ($\zeta$ of -27 to -63 mV). Adsorption of molecules by FLG appears to have a strong contribution to the major
FLG became highly cytolytic by prior association with lipids common in biomembranes (phosphatidyl choline/cholesterol). The association was relatively weak with no lipid clusters on graphene surfaces discerned by AFM after washing. The lipid-FLG association was also unstable, with eventual phase separation and agglomeration, which instability may promote penetration of cell membranes, with localised regions of high curvature and disorder in the lipid bilayer sufficient to cause the observed lysis and release of haemoglobin. Despite the interaction between the hydrocarbon region of lipids and graphene, the entry barrier of polar lipid head groups may repel pristine graphene away from lipid bilayers, which barrier may be lessened in the lytic action of GO by its edge polar regions. The sharp edges of exfoliated pristine graphene are also proposed to slice through the polar entry barrier and allow graphene to penetrate through the hydrocarbon of lipid bilayers without causing lytic disorder in biomembranes and loss of viability. However, the microscopic evidence for sharp edge penetration of cell membranes was not from FLG per se. Though not well characterised, the larger flakes were first dispersed in a similar lipid (dipalmitoyl-phosphatidylcholine), but were only considered at low graphene concentrations (~10 µg/ml). Such graphene levels were only weakly cytolytic for the smaller FLG flakes used here, and may be less so for larger flakes, given lower particle concentrations for the same mass concentration. The pristine FLG here was smaller with similarly sharp edges, when any such interaction with lipid bilayers was not cytolytic, even for high concentrations of FLG with only traces of organic solvent, nor when dispersed in bile salt surfactant to facilitate entry through the polar barrier into the hydrocarbon region.

Only lipid-associated FLG seriously inhibited reductive metabolism at low concentrations when tested on a breast cancer cell line (MCF-7). Though there have been reports of graphene interference with some assays, the alamarBlue assay used here did not suffer from such underestimates of cell viability compared to other assays. However, very low concentrations of GO and FLG, particularly with traces of DMF, though not albumin nor NMP-exfoliated FLG, initially increased alamarBlue reduction in macrophages. Stimulated mitochondrial activity in response to weak uncoupling or repair...
of cell damage has been observed for low levels of GO. Given their lytic activity, GO and lipid-associated FLG inhibited alamarBlue reduction at low concentrations (>10 μg/ml), similar to possible mitochondrial damage with ROS production. Presumably related to the greater uptake and transport in macrophages, higher concentrations were inhibitory for the solvent and surfactant-exfoliated FLG. HSA association with pristine FLG may be expected in cell studies, where culture media is supplemented by blood serum, but this did not mask differences in the short term, where the FLG was produced by prior adsorption of other molecules (e.g., lipids, surfactant). Though albumin would normally be recycled out of macrophages and other cells, to maintain blood circulation, murine macrophages were selected because human serum albumin is not bound by the murine recycling receptor and is catabolised. Even so, albumin-exfoliated FLG was least inhibitory with no dose response suggesting albumin adsorption minimised intracellular effects on reductive metabolism.

### 9.6.2. Graphene oxide characterisation

![Figure 9.13: Representative AFM images and Raman spectrum of GO prepared by Hummer’s method. (a) Height sensor image showing aggregated GO flakes spread on a silicon wafer (with substrate surface contaminants/undried water bubbles). Corresponding (b) line scan showing apparent thickness of ~1 nm for monolayer GO flake. (c) Representative Raman spectra of GO showing D and G band with FWHM(D) ~125 cm⁻¹ and FWHM(G) ~120 cm⁻¹ and I_D/I_G ~1, typical of disordered basal plane with functional groups. Samples were prepared by spin coating (1000 rpm, 30 s) ~1 ml of GO dispersion as prepared (concentration ~2.5 mg/ml) on a silicon wafer, followed by drying on a hot plate at ~60°C for 15 min.](image)
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