

Fabrication and Characterization of Graphene Oxide for Biomedical Applications

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Summary: Graphene Oxide (GO) has garnered significant attention for its potential in biomedical applications, including wound healing, drug delivery, biosensing, cancer therapy and environmental sustainability. However, the costly fabrication and characterization methods have hindered its utilization in these applications, especially in developing countries like Pakistan, where financial and resource constraints limit access to advanced materials like GO. In this study, high-quality Graphene Oxide is successfully fabricated using a cost-effective approach utilizing modified Hummer's method adapted to resource constrained laboratory conditions, offering a cost effective and scalable alternative. The synthesized GO was comprehensively characterized using FTIR, SEM, XRD, TGA, and DSC. XRD confirmed a well-ordered layered structure with characteristic peak at 10.9° (d-spacing = 8.7 Å), indicative of successful oxidation. FTIR identified key oxygenated functional groups; hydroxyl, epoxy, carbonyl and carboxyl, essential for biomedical functionality. SEM revealed a uniform particle size of approximately 10 μm , with porous regions that may enhance adsorption performance. Thermal analysis (TGA\ DSC) confirmed the presence of oxygen containing groups, validating the degree of oxidation. The promising results demonstrate that the proposed approach produces GO with properties consistent with established literature, while significantly reducing fabrication barriers. This research lays a foundation for affordable GO based solutions in wound healing, targeted drug delivery, biosensing and environmental remediation in developing countries.

Keywords: Graphene Oxide (GO), Cost-effective, Synthesis, Characterization, Biomedical Applications.

Introduction

Graphene Oxide is a bi-dimensional material composed of a single layer of carbon atoms arranged in a hexagonal pattern with oxygen- based functional groups such as Epoxy [-C(O)-C], Carbonyl [C=O] and Hydroxyl group [-OH] attached to the planar regions while Carboxyl [-COOH], alkoxy [C-O-C], and some hydroxyl [-OH] groups are attached to its edges [1]. This configuration creates a distinct hybrid system, combining sp^2 hybridization among carbon atoms and sp^3 hybridization between carbon and oxygen atoms [2]. The presence of such functional groups gives GO a set of very unique and interesting characteristics, such as making it easier for GO to interact with other materials, forming various kinds of composites [3]; its hydrophilic nature, which allows GO to disperse in many solvents, especially water, enhancing its compatibility with other materials [4]; and strong oxidizing characteristics [4]. Additionally, the tight packing of sp^2 carbon atoms acts as a near-perfect barrier to gas molecules, making it useful as a packing material, protection for sensitive electronic devices, and as a corrosion-resistant material [5].

GO's novel characteristics make it valuable in fields as diverse as biomedical engineering, sciences, environmental sciences, electronics, and healthcare [6]. In recent years, GO has been a subject

of growing interest because of its transformative potential in tackling key global and local challenges.

Tanugi and Grossman researched the potential of GO nano porous membranes for water desalination and filtration and found that it was 33% to 100% effective, depending on the pore size and applied pressure [7]. In 2019, Ali and Basheer successfully utilized GO for the adsorption of hazardous rare earth metal ions present in wastewater [8]. Xiao Xiao and their team in 2020 found that graphene-based composites presented an alternative strategy for the removal of crude oil from the ecosystem [9]. Krasteva and their team concluded that Amine NH_2 -coated GO nanoparticles were promising for the treatment of colon cancer [10]. A study in 2020 showed that GO can carry and release drugs like doxorubicin (DOX) in a controlled way, making it ideal for targeted treatment and drug delivery applications [11]. Additionally, GO possesses nearly all of the characteristics of an ideal carrier of drugs. Including biocompatibility, drug binding sites, nontoxic nature, safer elimination, and excellent drug solubility [12]. The GO fabricated by Sujiono was evaluated to have a band gap of 4.38 eV, highlighting its semiconductor properties [13].

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Despite the significance and its remarkable properties and diverse applications, the widespread implementation of GO remains a challenge due to the high cost and complexity of traditional fabrication routes, particularly in resource limited settings. To address these challenges, a significant shift towards the utilization of sustainable, ecofriendly carbon precursor or biomass derived sources as low-cost alternatives [4]. Recent studies have investigated diverse carbon rich waste materials, including the two-stage pyrolysis of sugarcane dry leaves [14], the muffled carbonization of sugarcane bagasse [15], coconut shells [13], rice straw [16], Tea waste [17], Coffee grounds [18], and the graphite recovered from spent lithium-ion batteries [19, 20]. While these approaches reduce the reliance on the costly commercially available Graphite, they introduce significant challenges such as multi step processing requirements that are hectic and complex, high carbonization temperatures ($400^{\circ}\text{C} - 800^{\circ}\text{C}$), residual metal contamination from catalysts like ferrocene, inconsistent oxidation degrees across different biomass types, and mostly limited reproducibility [14, 15, 21, 22]. All of these are the factors that are critical when GO is intended for biomedical applications where material consistency is essential. Electrolytic synthesis routes have also been investigated as greener alternatives [21, 23]. However, these methods suffer from low oxidation degrees resulting from graphite delamination during electrolysis, uneven electric field distribution, and specialized equipment requirements that limit their scalability and adoption in resource constrained settings. As a result, there still remains a critical need for a streamlined, resource optimized modification of the Hummers method that balances high structural integrity and high oxidation degrees with a reduction in chemical toxicity and infrastructure dependency. This study addresses that need by synthesizing and comprehensively characterizing GO via a modified Hummer's method using locally procured, low-cost reagents, demonstrating its feasibility in resource constrained laboratory conditions in Pakistan for potential biomedical and environment application.

Experimental

Chemicals and Reagents

The graphite powder (20 μm), Sulphuric acid 98% (H_2SO_4), Phosphoric acid 85% (H_3PO_4), Hydrogen peroxide 52% (H_2O_2), Hydrochloric acid 37% (HCL), oxidizing agent potassium permanganate 99% (KMnO_4) and Deionized water 1L were all procured from Bio Logic Traders (BLT).

Fabrication of Graphene Oxide

Modified Hummer's method was utilized for fabrication. First, 40.5 mL of Sulphuric acid (H_2SO_4) and 4.5 mL of Phosphoric acid (H_3PO_4) were mixed in a beaker and stirred. While stirring, 0.34g of graphite powder (20 μm particle size) was slowly added to the acid mixture. After 10 minutes, 1.98g of potassium permanganate (KMnO_4) was gradually introduced, and the mixture was allowed to stir for a minimum of 6 hours. After 6 hours, the solution developed a pungent smell and appeared dark green. The solution was allowed to rest for a few minutes before adding 1mL of Hydrogen peroxide (H_2O_2) slowly to remove excess debris. The solution was left to cool down for 10 minutes.

Washing of Graphene Oxide

To remove impurities, 15 mL of Hydrochloric acid (HCL) was mixed with 45mL of deionized water and then added carefully into the solution. The mixture was centrifuged at 4000 RPM for 11 minutes. After centrifugation, the supernatant was removed and the precipitate was collected. The washing process was repeated twice, though additional washes may be performed if necessary.

Drying of Graphene Oxide

The resultant Graphene Oxide GO was then placed in a hot air oven at 80°C for 24 hours to dry.

Characterization of Graphene Oxide

Scanning Electron Microscopy

Scanning Electron Microscopy (SEM) is an advanced imaging technique that produces high resolution images of a surface of a material utilizing a focused electrons beam. The images provide insight into surface morphology, layers and microstructure of the material fabricated [24].

Thermal Gravimetric Analysis

Thermo Gravimetric Analysis (TGA) is primarily used to determine the thermal stability of material. It analyzes the change in mass of material over heating the material at a specific range of temperature in a controlled environment [25]. In Graphene Oxide testing this technique is used to identify the existence of Oxygen containing functional groups through the degradation of fabricated Graphene Oxide [26]

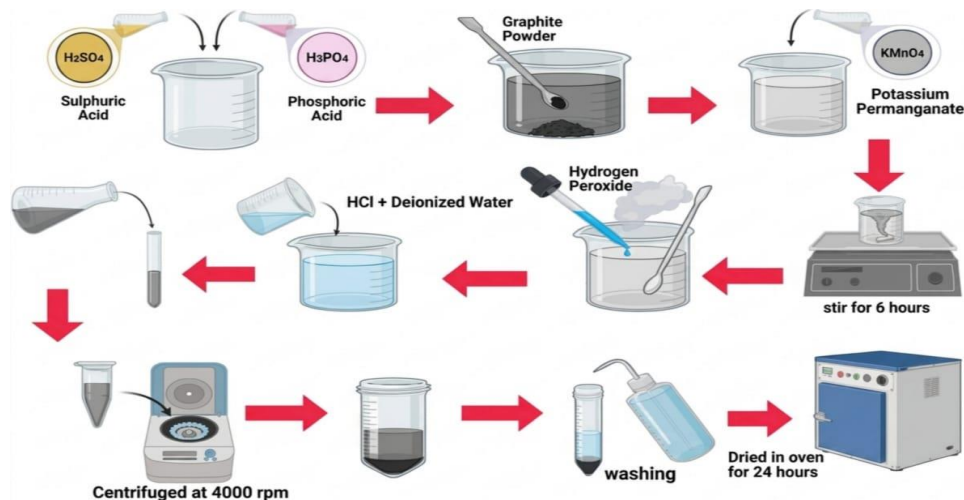


Fig 1: Schematic of Fabrication Methodology of GO based on Modified Hummers Method.

Differential Scanning Calorimetry

Differential Scanning Calorimetry (DSC) is a thermo analytical technique used to characterize the thermal properties of material such as transition temperature and change in enthalpy. In this testing energy material is heated, as the temperature changes heat flow is represented by the downward and upward peaks reflecting exothermal and endothermal activity, respectively [27]. Using DSC characterization will help us study the energy flow within our material during the heating process.

X-Ray Diffraction

X-ray diffraction (XRD) is an analytical technique used for the analysis of crystal structure, phase composition, and other structural properties of materials. It is also helpful in identifying the degree of crystallinity of the material [28]. This technique works by measuring the intensity of scattered X-rays as a function of angle [29].

Fourier Transformation Infrared Spectroscopy

FTIR measures the intensity of infrared radiation over a wavelength of light. It is helpful in the determination of functional groups associated with nanoparticles and its structural features [30, 31]. For GO, FTIR is utilized to identify the functional groups present on its surface, such as Epoxy [-C(O)-C], Carbonyl [C=O] and Hydroxyl group [-OH], Carboxyl [-COOH], alkoxyl [C-O-C] and hydroxyl [-OH] group.

Results and Discussion

The fabricated graphene oxide is in a fine, black powdered form. It appears to have a soft and flaky texture. The flakes are dry and look uniform. The Fig below shows the synthesized graphene oxide.

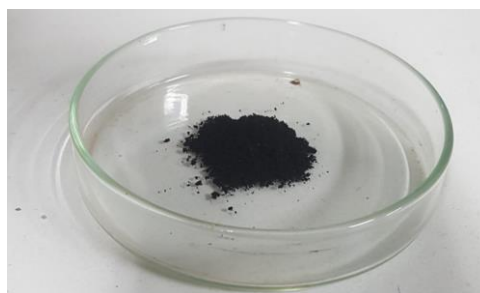


Fig 2. Image of fabricated Graphene Oxide.

Scanning Electron Microscopy

The SEM micrograph in Fig 3, captured at 1,000x magnification, shows a rough surface morphology of the synthesized graphene oxide (GO). The flakes are irregularly shaped with jagged edges and are unevenly distributed across the surface. ImageJ analysis of randomly selected flakes in Fig 4 revealed mean particle size of $12.2 \pm 2.51 \mu\text{m}$, with size ranging from 7.63 to $18.71 \mu\text{m}$, with the majority of flakes (57.8 %) falling within the $10.9 - 14.2 \mu\text{m}$ range, as shown in Fig 4. Additionally, some porous or void regions are also visible.

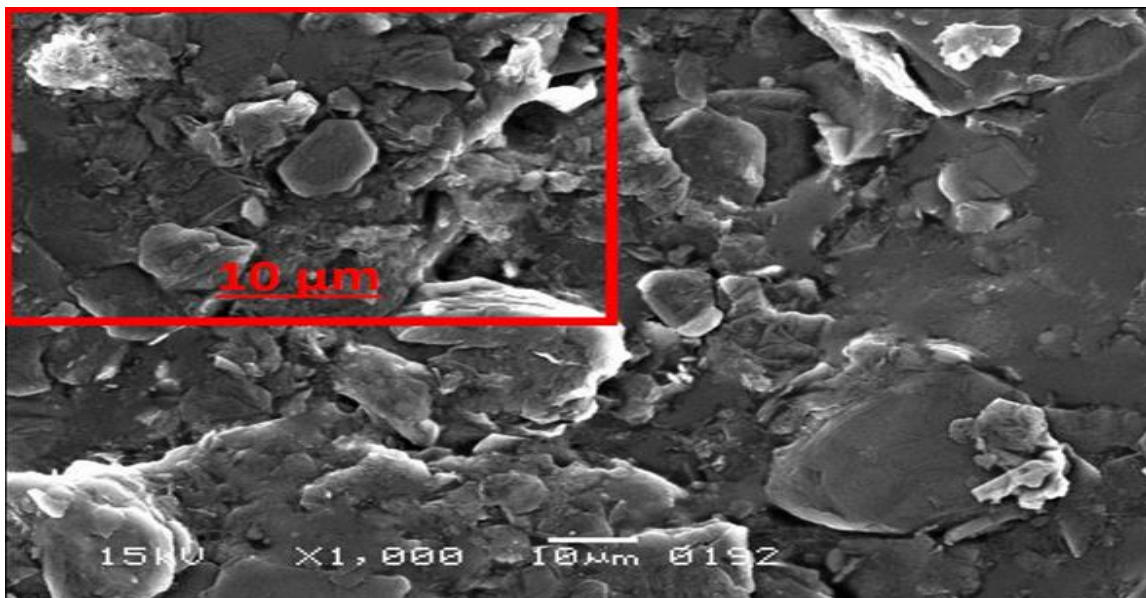


Fig. 3: SEM image of the Graphene oxide (GO).

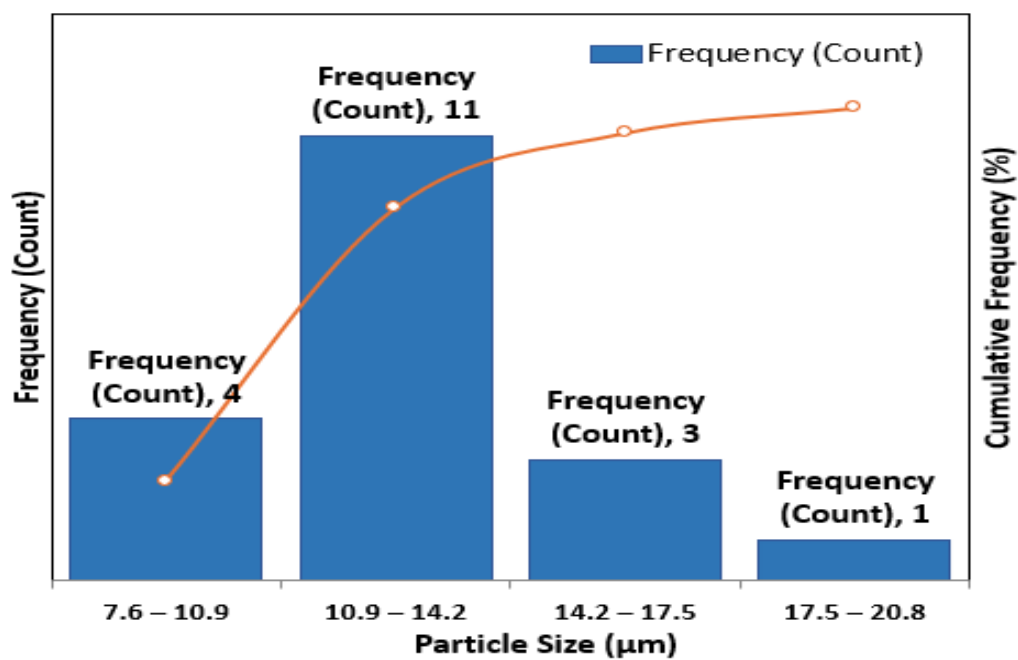


Fig 4. Particle Size Histogram of Graphene Oxide (GO)

Thermal Gravimetric Analysis

The thermogravimetric analysis curve in Fig 5 displays a progressive weight loss pattern of graphene oxides. The temperature ranges from 50°C to 500°C. An initial weight loss is observed below 150°C, followed by a more substantial weight drop between 150°C and 300°C. A slower weight loss occurs between 300°C and 400°C. And lastly, the weight seems to stabilize around 20–25% of the original mass.

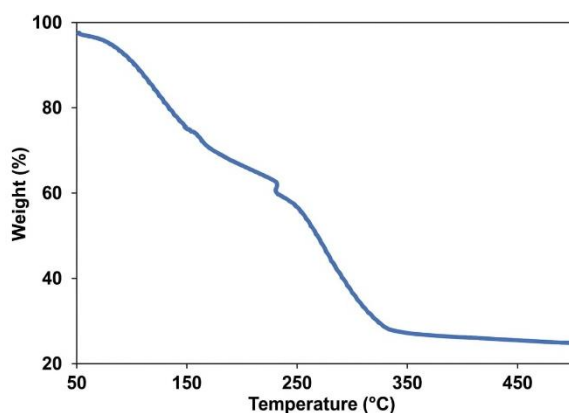


Fig. 5: TGA Curve of Fabricated Graphene Oxide (GO).

Differential Scanning Calorimetry

DSC analysis of graphene oxide illustrated in Fig 6 reveals its thermal behavior. The differential scanning calorimetry of synthesized Graphene Oxide was analyzed over the temperature range of 50 °C to

600 °C. A sharp decrease in heat flow was observed around 150 °C revealing an endothermic reaction, which was followed by a steep increase in the heat flow near 230 °C showing an exothermic reaction. Additionally, a broad exothermic peak was observed between 500 °C and 550 °C. At last after 550°C the flow of heat stabilized at (13-16) % of it.

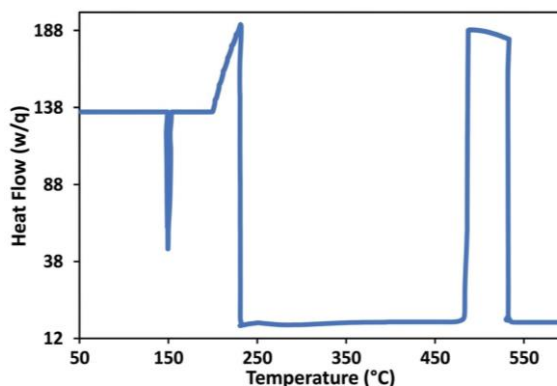


Fig. 6: DSC Analysis of Fabricated Graphene Oxide (GO).

X-Ray Diffraction

The synthesized graphene oxide XRD pattern shown in Fig 7 was observed in the 2θ range of 5° to 50°. Three distinct peaks, representing the diffraction plane, were observed in the spectrum. The first prominent peak, at 10.9°, was recorded with an interlayer spacing of 8.7 Å. The second peak appeared at 25.3° with a d-spacing of 3.5 Å, followed by a sharp peak at 44.7° with a differential spacing of 2.0 Å.

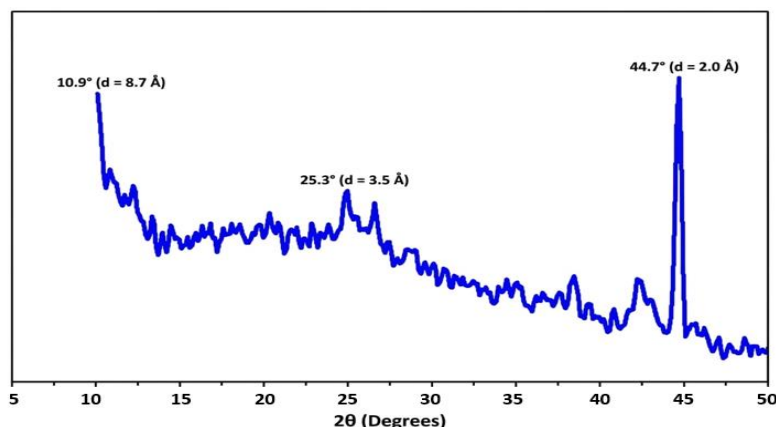


Fig 7. XRD Results of Fabricated Graphene Oxide (GO).

Fourier Transformation Infrared Spectroscopy

The FTIR analysis showcased in Fig 8 revealed a broad O–H peak at 3416.3 cm^{-1} . Peaks at 2362.4 cm^{-1} and 1733.72 cm^{-1} were assigned to O=C=O and C=O groups. Signals at 1655.6 cm^{-1} and 1597.7 cm^{-1} indicated C=C bonds. A peak at 1953.5 cm^{-1} corresponded to C=C=C stretching. Peaks at 1262.2 cm^{-1} and 1053.9 cm^{-1} indicated C–O and C–O–C groups.

The objective of this study was to synthesize and characterize graphene oxide. The characterization was achieved using SEM, TGA, DSC, XRD, and FTIR. The findings from these techniques confirm the complete oxidation and exfoliation of graphene oxide. Each characterization provides insight into the synthesized material's structural, chemical, morphological, and thermal properties.

The SEM micrograph (Fig 3) revealed a wrinkled and layered surface combined with the irregular flake-like morphology, a typical characteristic of exfoliated graphene oxide [32, 33]. Irregular, jagged edges indicate exfoliated graphene layers resulting from the oxidative treatment of graphite. Quantitative analysis of flake dimensions was performed using ImageJ software on 19 randomly selected flakes. The results revealed a mean particle size of $12.2 \pm 2.51\text{ }\mu\text{m}$, with size ranging from 7.63 to $18.71\text{ }\mu\text{m}$, as shown in Fig 4. The majority flakes (57.8%) fall within the $10.9 - 14.2\text{ }\mu\text{m}$ range, μm , confirming a moderately and consistent size distribution typical of GO synthesized via the modified hummers method. The observed size diversity is attributed to the mechanical and chemical forces exerted during the fabrication process. Additionally, porous and void regions observed between the layers indicate sheet separation caused by oxygen-containing functional groups. These porous regions have great significant from biomedical perspective. The increased surface areas and accessible void spaces are expected to enhance the adsorption capacity of GO, making it a strong candidate for the drug delivery applications such as doxorubicin (DOX), 5-fluorouracil(5-Fu), Camptothecin (CPT) delivery, as well as in bone and teeth implants where large surface area, antibacterial characteristics and biocompatibility is important [34]. The high surface area and interconnected pores also act like a microscopic sponge to selectively adsorb and remove heavy metals, bioactive waste and bacterial pollution from water [8, 35, 36, 37]. A similar surface

morphology was observed in the research conducted by Liu and their team [31].

The TGA curve of the synthesized GO illustrated in Fig 5 reveals three distinct thermal stages, each stage reflecting the specific structural changes of graphene oxide. The first stage occurring below 150°C , attributed to the evaporation of physically adsorbed water and intercalated moisture trapped between the GO layers. This is the common feature of a hydrophilic and heavily oxidized graphene oxide [26]. The second and most significant stage occurs between 150°C and 300°C corresponds to the breakdown of oxygen-containing groups, such as hydroxyl (-OH), epoxy (-C-O-O-C-), and carboxyl (-COOH). This stage is typically responsible for the largest mass loss and represents high degree of oxidation achieved in the synthesis [38]. The third stage takes place between 300°C and 400°C , is indicative of the gradual decomposition of the more thermally stable (C = O) groups and the degradation of the carbon matrix [39, 40]. Beyond 400°C , the weight stabilizes, indicating thermally stable GO, with an overall reduction from nearly 100% to 20–25%, reflecting thermal instability. The overall weight loss profile confirms a high degree of functionalization and is consistent with the findings by Aliyev and their group [26].

The DSC profile (Fig 6) of the synthesized graphene oxide demonstrates three distinct thermal events that closely correlate with the TGA findings. The endothermic (downward) peak at 150°C corresponds to the desorption of intercalated water molecules and the loss of weakly bound hydroxyl groups (-OH), consistent with the first stage mass loss in TGA. The sharp exothermic peak near 230°C is the most energetically significant event highlighting the decomposition of oxygen-containing functional groups, particularly epoxy and hydroxyl moieties, yielding reduced graphene oxide (rGO) [38, 41]. This stage releases considerable energy, reflecting the high density of volatile functional groups introduced during the modified hummers synthesis. The baseline stabilizes between 300°C and 450°C , signifying a thermally stable phase. The broad peak between 500°C and 550°C marks the high temperature degradation of the remaining carbonyl and carboxyl groups, along with progressive breakdown of carbon backbone. The stabilization of the heat flow after 550°C indicates the formation of a thermally stable graphitic residue. Taken together, the DSC results confirm the presence of multiple oxygen containing functional groups at distinct bonding energies,

validating the structural complexity of the fabricated GO. These observations are in agreement with the findings reported by Sahoo and their team [36]. The thermal stability of the synthesized GO, combining with the carbon backbone which remained intact beyond 400°C as confirmed by both TGA and DSC is an important property for biomedical applications. This stability suggest that GO based biosensor electrodes and implantable devices fabricated from the synthesized material would retain their structural integrity under physiological and operational conditions [38, 42].

The XRD results shown in Fig 7 affirm the successful synthesis of graphene oxide. The prominent peak at 10.9° ($d = 8.7 \text{ \AA}$) is characteristic of well-oxidized graphene oxide. It also confirms an increased interlayer spacing due to the intercalation of oxygen functional groups and water molecules between the carbon layers. A broad and less intense peak at 25.3° ($d = 3.5 \text{ \AA}$) indicates the presence of partially restacked graphene layers or residual reduced graphene oxide (rGO) [43]. While the sharp peak at 44.7° ($d = 2.0 \text{ \AA}$) corresponds to intact graphitic domains that resisted oxidation. These findings collectively provide strong structural evidence of successful oxidation. Raman spectroscopy would further complement these structural findings, particularly through the D band ($\sim 1350 \text{ cm}^{-1}$) [44], which reflects lattice disorder introduced during oxidation, and the G band ($\sim 1580\text{-}1595 \text{ cm}^{-1}$), which represents the in-plane vibration of sp^2 hybridized carbon and the I_D / I_G intensity ratio serving as a

quantitative measure of the degree of structural defects [45]. While the Raman analysis was not conducted in this study, it is recommended as a priority in future characterization work to further validate the structural integrity of the synthesized GO.

The FTIR spectrum illustrated in Fig 8 supports the presence of various oxygen-based functional groups, clearly reflecting the successful transformation of graphite into graphene oxide (GO). A prominent, broad absorption band centered at 3416 cm^{-1} indicates hydroxyl ($-\text{OH}$) stretching, demonstrating extensive oxidative functionalization [46, 47]. The presence of carbonyl ($\text{C}=\text{O}$) stretching at 1733 cm^{-1} and aromatic ($\text{C}=\text{C}$) skeletal vibrations between $1655\text{-}1597 \text{ cm}^{-1}$ further characterize the carboxyl and graphitic domains inherent to GO groups. These are the typical features of GO synthesized via the modified Hummers method [48, 49]. The resonance observed at 2362 cm^{-1} likely represents stretching from residual CO_2 . Additionally, epoxy and alkoxy groups are identified via $\text{C}-\text{O}$ (1262 cm^{-1}) and $\text{C}-\text{O}-\text{C}$ (1053 cm^{-1}) stretching vibrations and confirms the presence of basal plan functionalization [50, 51]. From a biomedical perspective, these oxygenated functional groups are critical as they render the GO surface highly hydrophilic and provide reactive sites for the covalent bonding sites of the therapeutic agents such as Paclitaxel. Additionally, the carboxyl and the hydroxyl groups facilitate stable dispersion in physiological buffers and promote bioconjugation with proteins or DNA for targeted gene delivery and biosensing applications [52].

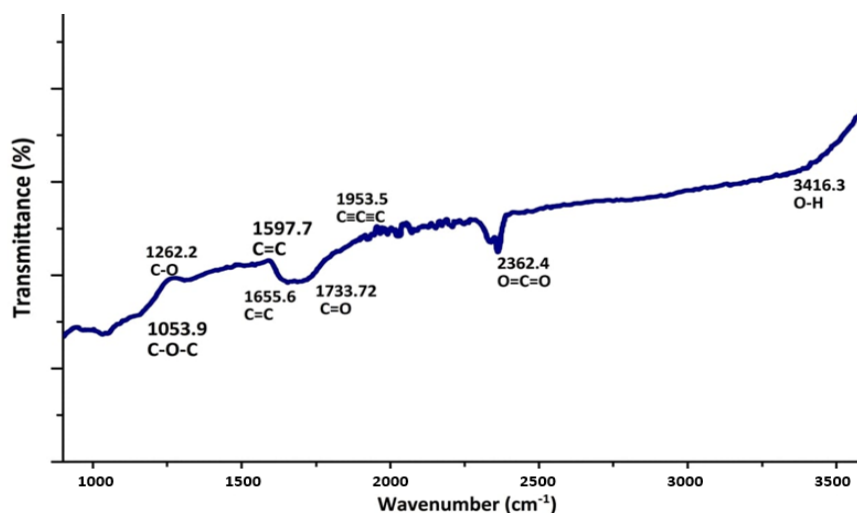


Fig 8: FTIR Spectrum of Fabricated Graphene Oxide (GO).

In summary, multiple characterization techniques were utilized to assess the quality of the synthesized graphene oxide. The results of these analyses are consistent with established literature, demonstrating the reliability of the methodology. The material's structural and functional properties, including its porous morphology, reflecting its adsorption capabilities [53], interlayer spacing, and abundant oxygen functional groups, highlight its potential across diverse fields. In biomedical sciences, the adsorption capacity and presence of binding sites make GO an ideal candidate for targeted drug delivery for diseases like cancer [54]. In energy storage, the interlayer spacing and thermal stability suggest promise for supercapacitors and Li-ion batteries [2]. Additionally, the high surface area supports environmental applications like heavy metal filtration [55]. However, the current high cost and scalability challenges limit practical implementation. Future research should focus on scaling up the production and optimizing these properties to address critical needs in affordable healthcare, water purification, and environmental remediation.

Conclusion

It has been demonstrated that the modified Hummers method successfully synthesized graphene oxide from graphite with strong oxidizing agents without releasing or exposing toxic gases. Additionally, this method yields a high fraction of graphene oxide in powdered form. Hydrocarbon functional groups enhance their hydrophilicity and electrical conductivity, facilitating further chemical modification and integration with the polymer matrix. These unique characteristics of GO make it a valuable material in fields like medicine, energy, technology, and environmental sustainability. Future studies should incorporate Raman spectroscopy and biocompatibility assessment to further validate and expand upon these findings.

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