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Electrochemical Synthesis, Characterization, and Biological Activity of Graphene and Graphene Oxide: A Comprehensive Review

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Abstract

The nanomaterials play crucial roles in the sciences due to their unique physical and chemical properties, for instance, electrical, mechanical, structural, and conductivity properties. The graphene oxide (GO) and graphene have good characteristic between different types of nanomaterials and a plethora application due to their properties. The best method used for preparing nanomaterials is electrochemical exfoliation, that prefer over other methods for the preparation of nanomaterial that because economical, faster than other methods, has less crystal size and not complicated methods eco-friendly method for creating superior graphene-based products. For the identification of graphene and graphene oxide (GO), different techniques were used: X-ray diffraction (XRD) for studying the phases and measuring the crystal size, transmission electron microscopy (TEM) for study three-dimensional image of nanomaterials, and field emission scanning electron microscopy (FESEM) for study morphological, all techniques are discussed. Biological activity is one of the key uses for graphene and graphene oxide, with special focus on its antibacterial behaviour, bio interactions, oxidative activity in biology, cytotoxicity, and biocompatibility. To provide useful insights for the logical design of graphene-based materials for upcoming biological and environmental applications, this review attempts to provide an integrated knowledge regarding the interaction between synthesis techniques, material properties, and biological activity.

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1. INTRODUCTION

Although graphene compounds, such as graphene oxide is not well known to the general public, they are predicted to be the wonder material of the twenty-first century, just as thermoplastic was thought to be essential to the development of the twentieth.(Anjali & Kizhakayil, 2024). The promise of materials made from graphene for use in many fields is due to their exceptional mechanical, optical, thermal, electrical, and chemical characteristics. The primary study areas are GO and reduced-GO (rGO)(Tarcan et al., 2020), which are excellent precursors to graphene production with higher yield and reduced synthesis costs(Kavitha, 2022). A single monomolecular layer of graphite with different oxygen-containing functions, including OH, C=O, and C-O-C groups, comprised the GO material. However, by eliminating the oxygen-containing groups and recovering a conjugated structure, rGO is a graphene-like sheet.(Khan et al., 2021)Because GO has oxygen functions, it disperses readily in organic solvents and water. Because of this advanced property, GO can be used as a beneficial ingredient in the production of ceramics and polymers, improving their electrical and mechanical qualities like conductivity, elasticity, and tensile strength.(Ahmad et al., 2018) . Additionally, because GO/rGO is an effective thermal and electrical conductor, it is important for energy conversion and storage.(Markandan et al., 2017).

The optimal way to synthesise GO/rGO has been investigated using several techniques, including the Modified/Improved Hummers synthesis method, chemical vapour deposition, and a microwave heating system.(Chen et al., 2012). Due to its sustainable development (an environmentally benign process), low cost, and increased efficiency, the electrochemical approach has currently garnered a lot of attention in the synthesis of graphene-based materials (in this review, graphene, GO, rGO, and composites)(Ramírez et al., 2021)However, according to recent research, different GO/rGO quality demands call for different parametric setups and characterisation analyses, which makes it extremely difficult to find a standard electrochemical process design that can produce different graphene-based materials without the need for significant process changes and in-depth characterisation research(Al Faruque et al., 2021). have made a similar observation, although there is a lack of consistency in the manufacture of graphene-based materials. The goal of this work is to discover a solution to the aforementioned limitation/challenge by concentrating only on the recent development of graphene-based material from the electrochemical exfoliation method.(Majumder & Gangopadhyay, 2022). The examination of carefully chosen papers from 2010 to 2020 is part of the literature analysis. It includes a variety of technical and scientific databases.(Saqib et al., 2025). The best operating conditions and characterisation analyses are used to discuss the quality of graphene-based materials (products). Lastly, the current work suggests a simple and adaptable experiment design to quickly synthesise a material based on graphene.(Razzaq et al., 2025).

2. The electrochemical exfoliation process's adaptability

Graphite conductivity is used in electrochemical processes to create graphene-based materials by activating the anions and hydrocarbons in the electrolytes into graphite under voltage and current biases to create a graphite incorporation compound.(He et al., 2022). Water that has been electrolysed to create oxygenatedwater. The graphite interconnected compound will be oxidised by the substance to create GO/rGO.(Shen et al., 2018). Single-step and two-step electrochemical methods, which comprise anodic and cathode graphite exfoliation mechanisms, are two potential alternate pathways for electrochemical exfoliation of GO/rGO from graphite.(Edward et al., 2021).

The electrochemical disintegration process of graphene-based materials has been the subject of numerous reviews. have examined the literature on GO synthesis in terms of product quality, characterisation investigations, and the operating conditions of the electrochemical exfoliation process. Their analysis recommended focusing on the basic graphite mechanism throughout the exfoliation process to acquire high-quality graphene-based material, encourage a large-scale setup, and identify potential uses for graphene-based material in developing applications.(Zhang, 2021).

provided a summary of the characterisation analyses about the current synthesis techniques for graphene/GO and its derivative, together with supplementary analyses such as yield and production cost(Patten et al., 2015). Likewise, have carried out a review study that incorporates comparable work scopes as in(Potbhare et al., 2024). In their review paper, three different techniques—two-electrode, three-electrode, and electrolyte exfoliation—were covered. To the best of the author's knowledge, no study has suggested a standard electrical exfoliation approach to meet various industrial and product demands, which makes this short review paper unique. We only included research that used platinum as the counterpart electrode in order to limit the scope of this study. Platinum is regarded as a practical and adaptable electrode that may be used with a variety of working electrode types.(ten Elshof et al., 2016).

2.1 Electrochemical exfoliation in a single step

Graphene is created by the single-step electrochemical process of intercalating electrolyte ions. Using a single-step exfoliation process (Abdelkader et al., 2015) .have successfully created monolayer and bilayer graphene. mechanism (direct)(Edward et al., 2021). In their research, a low defect of high-temperature produced graphene Ammonium sulfate was used as an electrolyte for three hours of synthesis at a voltage of 10 V to produce stability in air(Momodu et al., 2021). produced graphene from the pencil core using a comparable electrolyte solution at a voltage of 10 V and a low concentration (0.1 M). A thin layer of graphene can be produced by using ammonium sulphate as the electrolyte, according to another research(Momodu et al., 2021). produced graphene powder using three distinct electrolyte solutions (distilled water, nitric acid, and sulfuric acid) at a voltage of 10 V for 50 minutes. However created multi-layered graphene using a mixture of

titanium oxide and hydrogen fluoride at a 10 V of electricity(Momodu et al., 2021).

They found that adding titanium oxide could accelerate the rate of exfoliation and encourage graphene sheet fluorination as a composite material.(Zhao & Ci, 2020).generated graphene sheet graphite that was taken out of used Zn-C batteries. The electrochemical exfoliation procedure was carried out in a poly (sodium 4 styrenesulfonate) (PSS) solution at a voltage of 5 V. It was noted that they were able to synthesise graphene of excellent grade.(Wang & Sasaki, 2014). Graphene flakes were prepared by using a remaining battery, by using the electrode from graphite coated with parafin, the solution of the cell was an appropriate concentration of sodium hydroxide with applied voltage 3 V in a confined environment to activated exfoliation of graphite and stop excessive graphite expansion.(Liu et al., 2019). In their experiment, a strong acid such as sulfuric acid was used as the cell electrolyte solution to completely produced high result graphene with a good size of graphene. (Munuera et al., 2016). To gain one layer of graphene oxide with a good size, a high concentration of sulphonic acid must also be used to increase the exfoliation (Munuera et al., 2017). The surfactant was used for the preparation of graphene oxide (GO), which produced a single layer through add the surfactant to the solution of electrolyte. In another study. Single-step electrochemical exfoliation has been employed in a limited number of studies. (A Morshed et al., 2021)

2.2 Electrochemical exfoliation in two steps

The interconnection and oxidation/exfoliation processes are represented sequentially by a two-step electrochemical exfoliation method. used a two-step process to generate GO(Cao et al., 2017). The graphite anode was first anodically intercalated in sulfuric acid, and then it was oxidised and exfoliated in ammonium sulfate for ten minutes at a voltage of 10 V. An ideal voltage for GO synthesis was discovered. (Brisebois & Siaj, 2020). to be 2 V during the intercalation procedure and 20 V during the oxidation stage. High concentrations of sulfuric acid (95% and 65%) were used in both stages at synthesis times of 10 minutes and 1 minute, respectively. used electrolyte solutions that were comparable to those used to synthesise GO at a somewhat lower voltage (10 V). Their two-step method produced GO with a respectable oxygen content (17.7%), good quality (> 90%, monolayer), and high yield (> 70 weight per cent). (Abdelkader et al., 2015). used water electrolytic oxidation of graphite to produce a high yield of GO. The study employed diluted sulfuric acid for the oxidation procedure after using highly concentrated sulfuric acid without an oxidant agent for the intercalation process.(Hong et al., 2013). It was found that only at sulfuric acid concentrations between 40 and 60 weight per cent was severely oxidised GO (C/O < 2) attained(Rimkute et al., 2022).employed a combination of phosphoric and sulfuric acids for the intercalation process, then potassium permanganate for the oxidation step.

Based on their experimental conditions, high-quality GO was produced at a synthesis time of 6 hours and a fixed intercalation period of 4 minutes.(Iwashita & Inagaki, 1989). used varying

sulfuric acid concentrations at a 1 cm distance between two electrode sites. To guarantee the expansion of the pyrolytic graphite electrode for an effective intercalation procedure, the working electrode was first cathodically pretreated at a negative direct current (DC) of 10 V for 30 s and then 3 V for 15 minutes.(Maeda et al., 1985)Their findings demonstrated that low electrolyte concentrations (0.5 and 1.0 M sulfuric acid) could accelerate electrochemical oxidation processes at the graphite electrolyte interface, resulting in a product with greater oxidation endowment(Qiao et al., 2018). This illness could contribute to the maximum output of GO, as indicated.

Additional research has demonstrated that using sulfuric acid at low synthesis temperatures can provide high-quality single or multilayer graphene or graphene-based materials. For example, a moderate amount of GO powder was produced utilising a voltage of 2 V for a 10-minute intercalation procedure and 20 V for a 60-second oxidation phase. They employed 65% sulfuric acid as an electrode at room temperature. The as-synthesised GO powder has a single layer at 0.345 nm at the previously specified conditions.(Akpotu & Moodley, 2018). After a longer synthesis period, they were able to create a very pure GO at room temperature in a 0.1 M sulfuric acid solution. for four hours at a steady potential of 3 V. A GO sheet monolayer free of flaws and functional groups containing oxidation was produced in a different investigation(Shao et al., 2012). In their investigation, they utilised concentrated sulfuric acid at 1.6 V for 20 minutes, followed by an electrochemical reaction in 50% sulfuric acid for 3 minutes at 5 V.

3.Characterization of Graphene

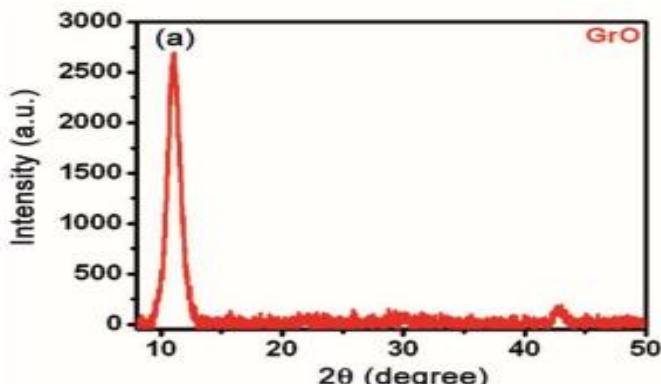
3.1 XRD technique

The Bruker D8 Advance X-ray diffractometer was used to record the X-ray diffraction (XRD) spectroscopic patterns of GrO at a scan rate of 0.02°/s(Stobinski et al., 2014). slit width of 0.1 mm with the radiation source being the CuK α line ($\lambda = 1.540598 \text{ \AA}$). Using a 514.5 nm laser as the excitation source, spectra were recorded using a Raman microscope (Renishaw in Virginia)(Surekha et al., 2020)GrO's XRD patterns are displayed in Figures 1(a) and (b). As can be observed, GrO exhibits a single diffraction peak with a 2θ value of 11.07.

$$d = \frac{\lambda}{2\sin\theta}$$

corresponding to an inter-layer spacing of 7.98 Å determined by the following formula: where d is the inter-layer spacing, θ is half of the corresponding diffraction angle, and λ is the X-ray source's wavelength.

Figure 1: XRD Patterns of GrO (Edward et al., 2021)



pristine graphite5 ($d \sim 3.34 \text{ \AA}$), which attests to the graphitic stack's growth due to the creation of Polar functionalities across graphene layers via oxidation. Notably, a tiny quantity of water molecules is constantly present as trapped species between the layers due to the hydrophilic character of the produced GrO, which co-contributes to the observed d value.(Strankowski et al., 2016). However, their contribution pales in comparison to the physical separation brought about by the introduction of functional groups and the disruption of planarity caused by sp^3 faults. Unlike the XRD peak positions, which rely on inter-layer spacing, the peak width is strongly influenced by the size of the graphitic domains that are present inside the large-scale graphene materials. Grain boundaries, or the break in graphitic stacking, divide the domains laterally, while sp^2/sp^3 domain borders divide them longitudinally. In particular, the well-known Debye-Scherer equation provides the average crystallite width (D), or the perpendicular dimension within which the graphitic ordering is maintained.

$$D = \frac{K\lambda}{\beta \cos\theta} = \frac{0.89\lambda}{\beta \cos\theta}$$

where θ is half diffraction, and β is the full peak width for the diffraction peak at half maximum height (FWHM), given in radians. angle of peak that corresponds to inter-layer spacing ($2\theta \sim 11.8$ for GrO and $2\theta \sim 24.8$ for RGrO); K is a crystallite shape-related constant that is often calculated as 0.89 for cylindrical crystals with cubic unit cells(Saini et al., 2017).

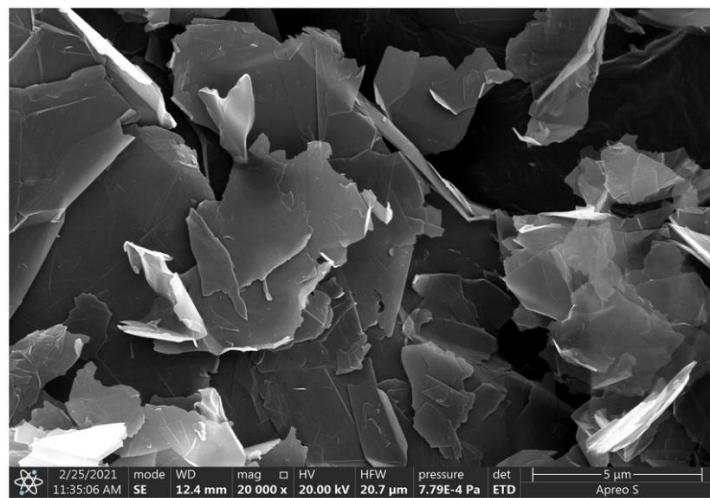
3.2 FESEM for graphene

The best techniques used for studying the morphology of nanomaterials are FESEM. The field emission scanning electron microscopy deals with the surface of nanomaterials (Chen et al., 2009).

The exfoliation process and the inherent flexibility of graphene layers are frequently blamed for the folds and ripples seen in FESEM micrographs. You can also use it to check the stability and consistency of the graphene dispersion. FESEM can show obvious changes in the form of graphene, reduced graphene oxide, or graphene

oxide. For instance, oxidised versions have sheets that are thicker and more crumpled, and the surface is rougher. (Teo et al., 2012). FESEM serves as an essential A complementary instrument to techniques such as TEM, AFM, and Raman analysis for validating effective synthesis and understanding the general shape of graphene-based nanomaterials, despite their inability to provide direct insights into atomic structure.

Figure 2: FESEM for Graphene (Banavathu et al., 2023)

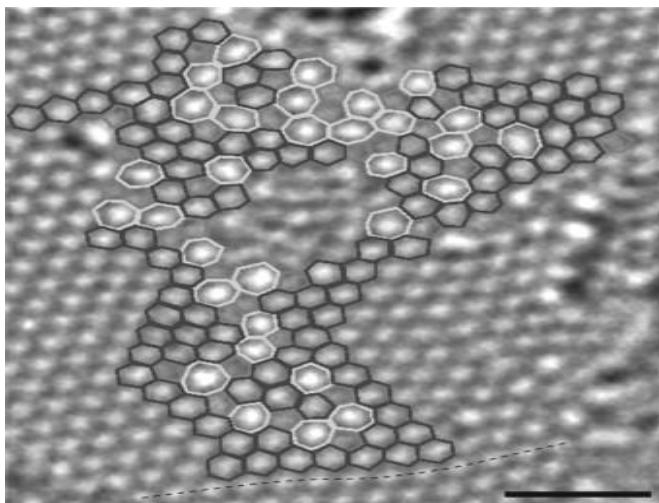


3.3 TEM for graphene

Transmission electron microscopy (TEM) is one of the most essential techniques for detailed nanoscale structural investigation of graphene (Bachmatiuk et al., 2015). The

An electron beam in TEM can go quite deep into graphene sheets, which gives us immediate information about their internal structure, transparency, and size. In transmission electron microscopy photographs, single-layer or few-layer graphene usually looks like unique, smooth, and uniform bands. However, darker spots show where the material has folded or stacked in more than one layer. (Stobinski et al., 2014). High-resolution transmission electron microscopy (HRTEM) is used for a more focused study of nanomaterials and provides a special characteristic compared to TEM. (Robertson & Warner, 2013). The TEM also provide wide range study about the number of layers, three dimensional study for nanomaterials when working together with Raman spectroscopy, XRD and TEM. (Stobinski et al., 2014).

Figure 3: FESEM for Graphene (Meyer, 2014)



4. Characterization of Graphene Oxide

4.1 XRD for graphene oxide

A significant technique for evaluating the crystalline structure and interlayer spacing

The structure of graphene oxide (GO) is X-ray diffraction (XRD)(Stobinski et al., 2014). Graphene oxide typically has a pronounced and broad peak at lower angles, generally between

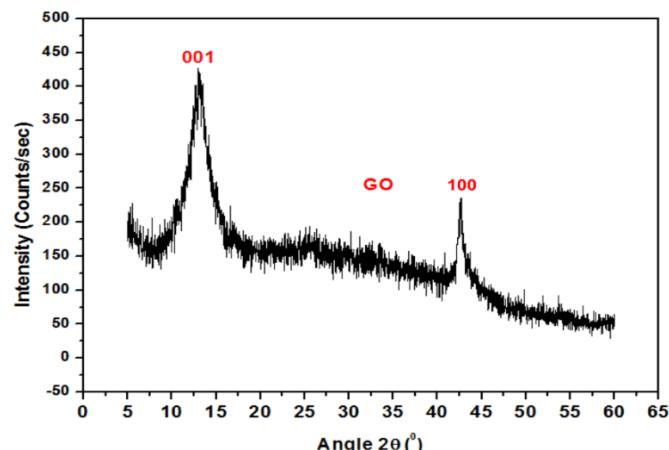
$2\theta = 10\text{--}12^\circ$, whereas pristine graphite presents a sharp diffraction peak at around 2θ

$\approx 26.5^\circ$, corresponding to the (002) plane (Kotsyubinsky et al., 2021). The presence of oxygen-containing functional groups (such as hydroxyl, epoxy, and carboxyl groups) and water molecules that are intercalated between the graphene layers causes a peak

that is due to the (001) reflection of GO. This shows that the distance between the layers has increased a lot. The broadening and lower intensity of the GO diffraction peak show that graphite is partially flaking off during oxidation and that long-range

The order is lost. Bragg's rule shows that the d-spacing of graphene oxide is usually between 0.7 and 0.9 nm, which is much bigger than that of graphite (around 0.34 nm)(Zaid et al., 2022). Therefore, the XRD measurement is very important for making sure that graphite was turned into graphene oxide and for keeping track of changes in structure during reduction or further chemical changes. When GO is turned into reduced graphene oxide (rGO), the unique GO peak goes down or disappears. A broad peak about $2\theta = 24\text{--}26^\circ$, may appear, which means that the graphene structure is starting to come back together. (Zaid et al., 2022).

Figure 4: XRD for Graphene Oxide (Gul & Alrobei, 2021)

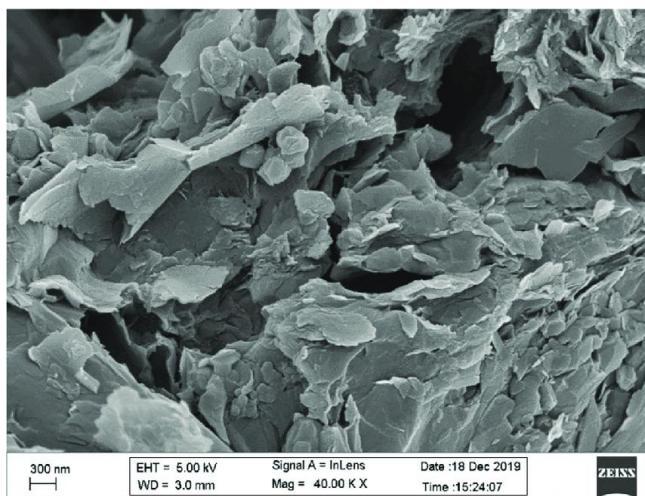


4.2 FESEM for graphene oxide

Graphene oxide (GO) surface appearance and structural characteristics are frequently examined using field emission scanning electron microscopy (FESEM). Thin, sheet-like nanostructures with a highly creased, crumpled, and folded look are commonly seen in FESEM images of GO.(Kanti & Maiya, 2022). This morphology results from the oxidation process, which distorts the carbon lattice by adding oxygen-containing functional groups to the basal planes and edges of graphene.

Because of layer stacking, the presence of interconnected functional groups, the sheets are frequently thicker and more irregular than pristine graphene.(Mahmood et al., 2023). Furthermore, the level of exfoliation and aggregation of the sheets of graphene oxide can be accessed via FESEM analysis. While agglomerated areas appear to be dense and restacked clusters, well-exfoliated GO displays translucent, loosely packed layers with crisp edges. One important sign of good oxidation is the higher surface roughness found in GO as compared to graphene. FESEM is a quick and efficient method for verifying the morphological properties of graphene oxide, even if it cannot offer atomic-level information. It is usually used in conjunction with TEM, XRD, and Raman spectrum analysis for thorough material characterization.(Sheikh et al., 2023).

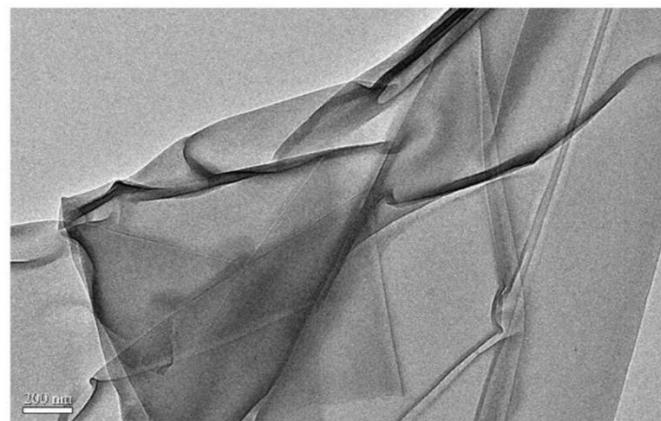
Figure 5: XRD for Graphene Oxide (Singh et al., 2021)



4.3 TEM for Graphene Oxide

A potent method for analysing the internal structure, thickness, and shape of graphene oxide (GO) at the nanoscale is transmission electron microscopy (TEM) (Bayrak, 2017). Extensive, thin, and semi-transparent sheet-like formations are frequently observed in TEM images of GO, signifying successful exfoliation into single-layer or few-layer nanosheets (Kong et al., 2017). The number of layers in transmission electron microscope images is closely related to the contrast of transparency, with dark areas indicating folded, overlapping, or multi-layered regions, while light areas indicate thin sheets of graphene oxide.(Huang et al., 2015). Wrinkles and ripples are frequently observed, resulting from the addition of oxygen-containing functional groups, which bend the sp^2 carbon structure. In addition, the region-specific electron diffraction (SAED) patterns derived from graphene oxide typically show diffuse ring patterns rather than distinct spots, indicating partial loss of crystallinity due to oxidation.(Taneja et al., 2024). The introduction of structural disorder into graphene planes can be confirmed by high-resolution transmission electron microscopy (TEM), which can show the location of defects and disrupted lattice fringes. TEM offers unmistakable evidence of oxidation and delamination and is particularly useful for identifying sheet edges, folds, and structural defects.(Rathanasamy et al., 2021). Along with X-ray diffraction, Raman spectroscopy, and phase-enhanced scanning electron microscopy (FESEM), transmission electron microscopy (TEM) is a vital characterisation technique that helps differentiate graphite oxide from pure graphene and modified graphene oxide. (Zhang et al., 2014).

Figure 6: XRD for Graphene Oxide (Song et al., 2012)



5. Biological Activity of Graphene and Graphene Oxide

The graphene oxide and graphene have their specific properties and multi-application, it's also applied in study to different types of bacterial activity due to their good physical and chemical characteristics. The structure, size of crystal, number of layers, purity and degree of oxidation all of these play important roles in the activity of graphene and graphene oxide with biological systems. (Vineeshkumar et al., 2026). The most extensively studied is the antibacterial activity of graphene and graphene oxide. Chizini boxes, containing essential materials, can contain potent antibacterial agents such as bacteria, personalities, and symbols. The basic principles of activity, physical transport, membrane transport production, active oxidative stress, and tension are present in electronic devices, which contain the best lists of graphics and microscopic membranes. Bacterial membranes can be a high-quality functional group or highly adhesive, meaning that, well, it may be possible to access the nutrient- and health-promoting components that the kidneys need.

Thanks to self-electrical provitamins, graphene oxide energy (rGO) can destroy the active activity model of chitin (ROS), which is an activator of microorganisms.(Hublikar & Ganachari, 2026). It has strong antioxidant and antioxidative effects, in addition to its antibacterial properties, depending on the concentrations and environmental factors. Graphene oxide can get rid of harmful free radicals and reduce oxidative stress at low concentrations, but it can cause oxidative damage at high concentrations through the production of reactive oxygen species. This double connector is extremely important when evaluating the biological safety of graphene materials and their interactions with living cells.(Ahmad et al., 2026).

The world of cytotoxicology and biobiology is fueled by graphene and graphene oxide (GO) through various kidney types. Customer feedback particularly highlights these factors, such as increased isolation and improved performance and function. Yagaluma's top-rated GO distribution lists for regular flights and more, and the most customer bookings are excellent. BULO, a high-quality product including polymer and biomolecule coating, is truly remarkable for its cytotoxic effects and increased biological efficacy. The reason for using high-quality panels and materials in the basic drawing is to demonstrate their absorption and bioavailability(Nekhlaoui et

al., 2026). These characteristics have strong interactions with proteins, enzymes, and nucleic acids, which may alter the structure of proteins and their biological activity. These interactions are important to understand all of the beneficial biological effects and possible risks associated with them. Generally, the biological activities of graphene and graphite oxide are highly variable and depend on surface modification, manufacturing technology, and environmental factors.

Comprehensive evaluation of safety and long-term biological effects is still necessary, although its antibacterial properties and biological interactions show promise for biological or environmental applications. In order to make the most of the positive biological activity while reducing the negative effects, future research should be focused on unifying procedures for physical evaluation and improving the design of materials.(Thakur et al., 2026).

6. DISCUSSION

By intensifying the basic drawing process, both those experiencing electrical stress and those who are effective are demonstrated. Electrochemical methods offer rapid control over fuel and electricity supply, in less than an hour or more than using traditional chemicals for conventional chemical procedures, such as the Hammersa method. Optimisation procedures allow for control of hydration, elimination of oxygen monitoring, and stabilisation. Electrochemicals are specifically used to heat the drawing of defective human waste. The properties of the methods are changing the main role in recycling well. The excellent guidelines in GO rely on the best diffraction analysis in the delivery of results of the functional group that seeks to communicate and conform to a variety of distinctive graphite characters. FESEM illustrates photographs, building structures, posters, and GO car bodies, also видобrazauть вплив окислення. Furthermore, TEM proposes improved support for Sharif, crystal, and crystal kerfs, representing a convergence of dokazom chudovogo vydsaruvannya. By providing high electrical power and superior, high-quality methods for high-functioning drawing and output writing (GO), it offers a perspective for exploration in biomedical devices. However, all these issues will pose challenges for innovators, including those in the biomedical sciences, and the need to unify their concepts. Recycling biomedical laboratory equipment addresses this problem.

7. CONCLUSION

This review highlights the effectiveness of electrochemical stripping as a durable and sustainable way to produce graphite, graphite oxide, and reduced graphite oxide. According to the intended use, both the electrochemical methods, single-step and double-step, have clear advantages in controlling the layers, the condition of corrosion, and the quality of the construction. Graphite has been characterised accurately using X-ray diffraction (XRD), field emission electron microscopy (FESEM), and transmission electron microscopy (TEM), which provides a deep understanding of the structural and morphological characteristics of graphene-based materials. Graphene and graphene oxide have important biological

activities in addition to their physical properties, such as strong antibacterial activity, modification of oxidative behaviour, and prominent biological interactions with cellular components. Although graphene-based materials show promising potential for biological and environmental applications, challenges related to cellular toxicity, long-term biological safety, and reproducibility remain. Future research should be focused on establishing consistent manufacturing procedures, improving control of material properties, and developing reliable biological evaluation methods. These efforts are necessary to make effective use of the biological activities of graphene and graphene oxide, while ensuring their stability and safety in practical applications.

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