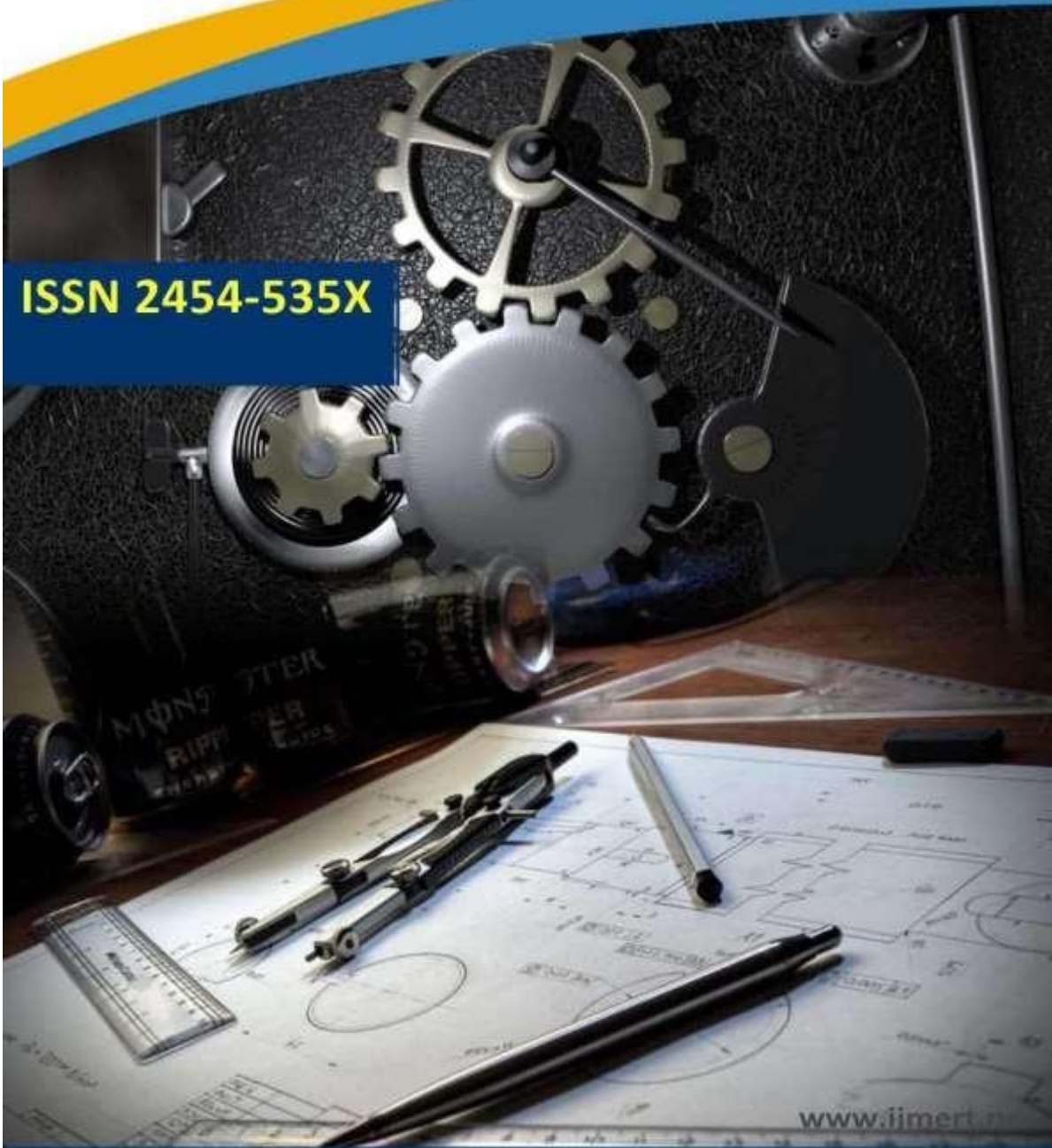




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## Design, Synthesis, and Antimicrobial Activity Against MDR Bacterial Pathogens of Graphene Oxide-Based Glass Substrate

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**ABSTRACT:** The scientific community is fascinated with graphene oxide (GO), which has unusual structural, thermal, and mechanical characteristics. It is the strongest substance ever measured and used in numerous biological applications. It is also the thinnest material known to exist in the cosmos. The GO sheets were introduced in ultrasonic dispersion and deposition on glass substrate utilizing distillation and a vacuum furnace after being created using a Gaussian 09 set of programs with a density function theory. GO is produced and then characterized by XRD, SEM, E-graph, and UV-Visible spectroscopy. According to atomic force microscopy, the layers are generally defect-free. The scattered phase was shown by scanning electron microscopy to contain tiny graphitic particles. According to X-ray photoelectron spectroscopy, ultrasonography is crucial for speeding up chemical reactions. A crista

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KEYWORDS: Na no Gra phene Oxide; B3lyp; Bacteria; Ultra sonic Dispersion; Biologica l Application.

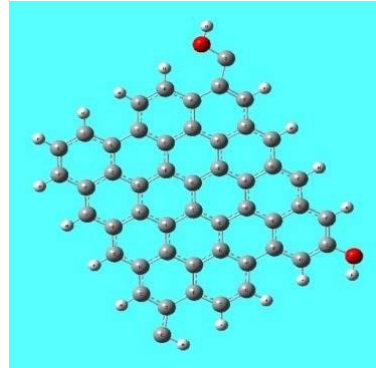
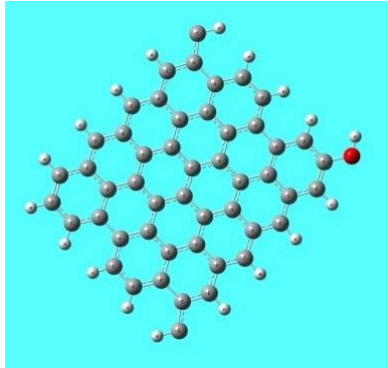
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### INTRODUCTION

ade of carbon atoms organized in a honeycomb lattice, is the ndamental omponent of graphite in all other dimensions. [1,2] A lot of interest has been cused on graphene because of its xceptional electrical, physical, and optical properties. Because it is rimarily created by exfoliating graphite, graphene can only currently be manufactured in small proportions. However, the isolation of graphene through the microcleaving of highly oriented pyrolytic graphite (HOPG) has opened up numerous fascinating opportunities for experimental studies. [3,4] However, when vast substrate areas are covered in graphene for large-scale applications, chemical methods of graphene production can offer distinct advantages over the micro cleaving of HOPG. A novel hybrid approach that combined a chemical and mechanical

## MATERIAL AND METHODS

### Theoretical Methods

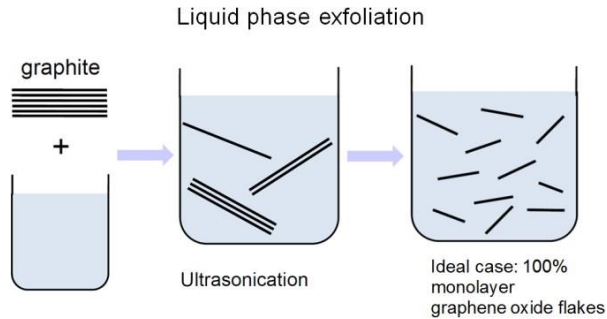


Graphene oxide were designed in the form of pure sheets by the Gaussian 09 view program and the study of chemical and optical properties using the density function theory-B3LYP through the Gaussian package and finding the best stability of the system, as shown in Figure (1).

**Figure 1.** Structures of GO in this work (Carbon≡ gray, Hydrogen≡ white, oxygen ≡red)

### Preparation Method and Characterizations

One of the simplest methods used to prepare graphene oxide is the ultrasonic dispersion method for pure graphite that is shown in Fig.2. 25 g of pure graphite was mixed and grinded using a nanometer mill and dissolved with (250 mL) Acetone C<sub>3</sub>H<sub>6</sub>O, water (250 mL) and cholate (3 g) to increase chemical reaction (120 min) to increase the dispersion of graphite particles and the reaction within the solution to form graphene oxide as shown in Figure (2). And then deposition of the product by distillation on the substrate of glass and inserted in a vacuum tube (Fig.3) at different temperatures (100, 300 oC) to increase the formation of graphene oxide for about 60 minutes. The components of graphene phases were analyzed using an X-ray diffractometer device (XRD-6000 x Shimadzu X-ray diffractometer with using an incident angle of 0.154nm with copper-K $\alpha$  radiation). Topographic Details for the upper surface layer of the graphene phase was performed using the TESCAN Vega 3SB electronic microscope with a value for voltage acceleration of 200V to 30KV and a magnification force of 6X to 100,000X. The 400–4000 cm<sup>-1</sup> range were used for the FTIR analyses by using the IR Affinity-1 Shimadzu equipment.



**Figure 2.** Stages of formation of graphene oxide.



**Figure 3.** The GSL-1600-60X vacuum tube furnace used in this work

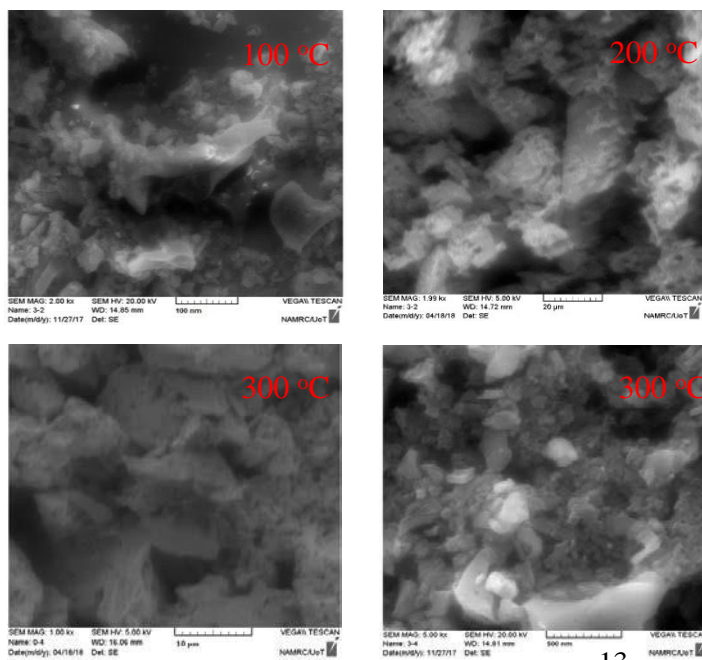
**Antimicrobial Activity Test**

The antimicrobial activity against bacteria was carry out by Kirby–Bauer Agar disc diffusion method. [8] using micropipette, (100 μl) of 24 hrs culture of each organism was spread by a sterile swab on Muller Hinton agar plates and glass discs of (7 mm) were placed on agar plates by the use of a sterile forceps after then the petri plates were let to stand for (1h) for proper diffusion and it was incubated aerobically at (37 ± 0.2°C) for an overnight., The antibacterial activity was evaluated by measuring the zone of inhibition (mm) around the test bacteria.

**RESULTS**

**Sem Result**

Figure (4) shows the photos obtained by using Scanning Electron Microscopy (magnification of 1000, 2000 and 5000 times), concerning graphene oxide at different temperatures. The grain size of each of the oxides is very similar, from just a few to a micrometer. Surface oxide graphene clearly has a good and smooth growth. moreover, a parallel arrangement displays for the respective layers. The SEM image clearly indicates that the GO sheet was stacked and formed in a regular form. Note also some papers were wrapped on each other as a result of their high elasticity, and the other was flat due to distillation and annealing at different temperatures within the vacuum tubular furnace. As this high flexibility helps to restrict the bacteria inside the infected space and increase the chemical effectiveness to avoid the occurrence of complications. the particle size for the produced GO layers was characterized by using the Nano-Brook 90Plus particle size analyzer, where according to this test, GO layer have the range of 40-68 nm.



**Figure 4.** Images of GO by using Scanning Electron Microscopy at different temperatures

### XRD Result

The crystalline analysis was performed for the composition of the prepared GO, as well as the original graphite structure by XRD test. As shown in Figure (5), the XRD pattern of the GO shows a diffraction peak (002) at  $2\theta = 24.56^\circ$ . Also, the emergence of another diffraction peak at  $2\theta = 43.06^\circ$ . Depending on the information from the previous reports, the interlayer distance is about 33 nm for graphite for the (0 0 2) peak [9,10]. Based on the results of previous tests, these detected peaks were set at (0 0 2) and (1 0 0) of GO [11]. The appeared of many XRD high intensity peaks, as shown in Figure 3 considered as evidence for the multi-layer GO formation in the current synthesis. Also, from observing the XRD patterns, it appears that the high peak (0 0 2) of graphite ( $43.06^\circ$ ) had actually disappeared for the GO sample. This may be due to the separation of different graphite layers and then oxidized in the GO plates which are certain to be GO. The graphene at 200 oC temperature had the highest intensity and the same peak value. An indication of the effect of temperature variations on the crystalline structure of GO and this is indicative of increasing the crystalline volume and the tendency towards high crystallization, as shown in Figure (5).

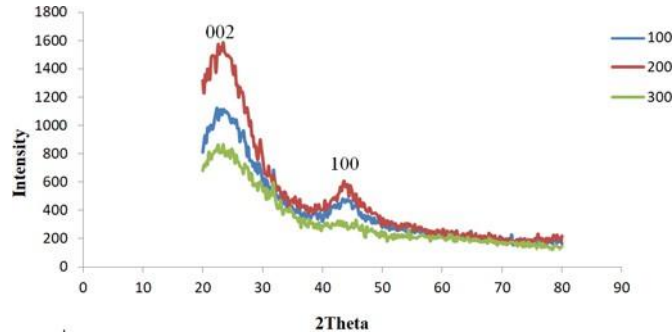
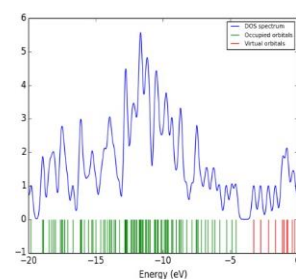
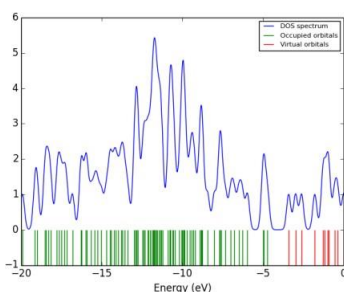
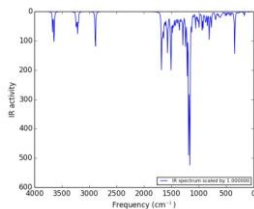


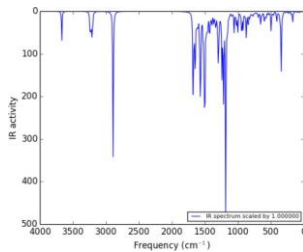
Figure 5. X-ray diffraction pattern of the GO at different temperatures

**IR-Spectrum Activity and Dos Result**

The process of vibrational spectroscopy (or IR) is based on the idea of vibration of groups of atoms when the subject subjected to the exposure of infrared light used, as well as this examination depends on the type of atoms and the type of bonds between the atoms in addition to the frequency at which the atoms vibrate, which is unique for each arrangement. Figure (6) clarify the infrared IR spectrum of GO from the B3LYP/DFT. The highest activity was found at frequency range (400 to 1900) cm-1, indicating the high activity of graphene in inhibiting the bacteria used (gram positive bacteria *S. aureus* and gram negative bacteria *E.coli*). Based on FTIR data for bulk graphite, it is clear that The GO samples were found to contain several oxygen configurations in their structure. One of the most prominent evidence is the distinct vibration patterns present for the hydroxyl group in IR test at 3750–3050 cm-1, C-H vibrations at 2920–2820 cm-1, species ketonic bands (C=O) (1750– 1650 cm-1), sp2-hybridised C=C (1630–1510 cm-1), and epoxide groups (<1000 cm-1). All of the previously mentioned bands in this work correspond to those obtained in the previous reports as they are convincing for the typical GO configuration in the current study with different types of oxygen moieties. [12,13] Figure (7) illustrates the density of states DOS diagram of the complex involves all the occupied and the virtual orbitals. The observed nonzero DOS indicates a finite number of states. From the distribution of the DOS, shows results indicate a pronounced antimicrobial activity of GO [13,14]. This is consistent with the results of HOMO and LUMO as shown in Fig (8). Where we note the increase of negative charge carriers on the positive in the samples and increase these defensive negative carriers more and more by increasing the oxygen on the pure graphene formation of GO.

Figure 6. IR-Spectrum for structures





of GO in this work.

Figure 7. The DOS for structures of GO in this work.

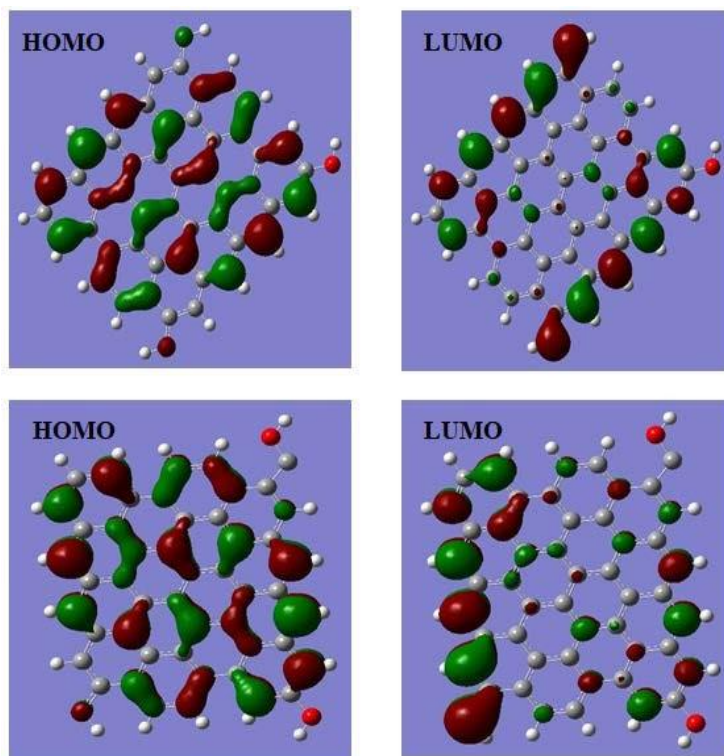


Figure 8. The 3-D distribution of HOMO and LUMO for GO in this work

**AntimicrobialActivityTestResults**

Fig. (9) shows the effect of GO (100), (200) and (300) °C discs on the growth of the MDR tested organisms namely, G+ bacteria S. aureus and G- bacteria E.coli, GO significantly reduced bacterial growth after 24 hrs displaying a clear bacteriostatic effect. After 24 hrs observation the bacteriostatic effect of GO (100), (200) and

(300) discs respectively was detected for S.aureus, with a zone of inhibition measured respectively (100) = 35 mm (200) = 38 mm (300) = 41 mm, it showed a significant growth reduction after 24 hrs and it also shows a potent effect against S.aureus compared to E.coli who was barely affected by the discs. Graphene oxide nanomaterial may cause significant membrane stress and they might break and destroy the cell membranes.

[15] leading to the death of the cell by releasing the intracellular contents. A remarkable distinction among different graphene materials is that the small GO NP can enfold bacterial cells, while larger GO NP would trap cells. The recent researches have confirmed that graphene nanoparticles can be trapped in bacterial membranes that consist of phospholipid molecules. It is possible that some GO NP may be formed by bacterial cells.[16] In this work, a small number of oxide ions have been produced because of the presence of graphene-based materials, as GSH oxidation clearly demonstrates that oxidation of graphene-based materials can be a major factor influencing when nanoparticles produced in direct contact with cellular components. The best metallic which can be compared to

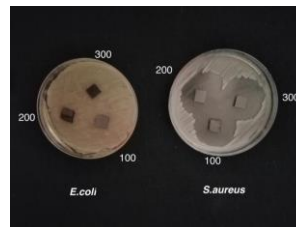


our obtained results is SWCNTs, it shows bridge conductivity features in the lipid bilayer. In this case, the electron passes from intracellular of bacterial components to the outside environments.

[17] Moreover, materials doped graphene has the ability to oxidize proteins, DNA, and bacterial lipids. In our study, we found that the oxidation of GSH by GO is strong enough to support the fact of that graphene nanoparticles can create a conductivity that be able to oxidize cellular elements and organic compounds such as thiols [18-21]. **Figure 9.** Inhibition zone of GO NP, (100) = 35 mm (200) = 38 mm (300) = 41 mm for S.aureus and it shows no action for E. coli

**CONCLUSION**

A new hybrid technique that mixed a chemical and thermal vacuumed process was used to produce the Graphene Oxide sheets. In this process the films grow directly on the surface without using of a surface catalyzed process. The infrared IR spectrum of GO from the B3LYP/DFT. The highest activity was found at frequency range (400 to 1900)cm-1, indicating the high activity of graphene in inhibiting the bacteria used The acquired results show a noticeable antimicrobial activity of graphene oxide. The effect of antimicrobials in the processed layer is displayed in 24 hours after the interaction with GO disks, where the bacterial cell membrane is destroyed under the influence of these substances, and then its demise after that. GO responsiveness is found in bacteria and fungi. In this case, G+ thick-walled bacteria die under the action of GO faster than G- thin-walled bacteria. Thus, the derived results demonstrate the future application of GO as an affordable and most effective antimicrobial carbon nanomaterial.







## ACKNOWLEDGEMENT

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