

SYNTHESIS AND CHARACTERIZATION OF GRAPHENE OXIDE AND ITS ANTIMICROBIAL ACTIVITY AGAINST *Klebsella* AND *Staphylococcus*

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ABSTRACT:

Graphene oxide has a similar layered structure to graphite, but the plane of carbon atoms in graphene oxide is heavily decorated by oxygen-containing groups, which not only expand the interlayer distance but also make the atomic-thick layers hydrophilic. As a result, these oxidized layers can be exfoliated under moderate ultrasonication. If exfoliated sheets contain only one or few layers of carbon atoms like graphene, these sheets are named graphene oxide (GO). These graphene oxides have important applications in areas related to transparent conductive film, composite materials, solar energy and biomedical applications. Present work based on Hummers' method which is most common method, used for preparing graphene oxide. The resulting graphene oxide was characterized by XRD, DLS, FESEM, and FTIR. Antibacterial activity was tested using *Klebsella* and *Staphylococcus* bacterial species. The GO exhibited stronger antibacterial activity against bacterial species.

Keywords: Hummers' method, Graphene oxide, XRD, FESEM, FTIR

[I] INTRODUCTION

Graphene oxide (GO) have recently emerged as a new carbon - based nanoscale material that provides an alternative path to graphene [1]. It is a single-atomic-layered material made of oxidizing graphite crystals which available in large quantities at inexpensive prices. Structurally, the Graphene oxide is similar to a graphene sheet with its base having Oxygen-containing groups. Since these groups have a high affinity to water molecules, it is hydrophilic and can be easily dissolved in water and other solvents allows it to be uniformly deposited onto wide ranging

substrates in the form of thin films or networks, which makes it potentially useful for microelectronics [[2]]. Graphene oxide is a poor conductor but when it undergoes treatment by heat, light or chemical reduction, most of graphene's properties are restored. One of the first commercially available graphene materials is graphene oxide. The basal planes and edges of the graphene oxide are functionalizing with exogenous groups, such as hydroxyl, epoxy group and carbonyl [3] groups which are attached at the edge [[5]].

Then oxygen containing functional groups disrupt the aromatic regions in the basal plane, so that the layer of GO consists of both aromatic regions and oxidized aliphatic six-membered rings, which leads to distorted sp^3 - hybridized geometry and results in the insulating property of GO.

Industrially produced graphene oxide could be used for wide range of application such as solar cell and hydrogen storage [[6]], paper like materials [[7]], transparent conductive films [[8]], nano electro mechanical devices [[9]], polymer composite [[10]] and biomedicine [[11]] etc.

[II] MATERIALS AND METHODS

In this work, graphene oxide was synthesized by Hummers' method [11]. The chemicals used for synthesis are Graphite flake (mesh size 300), Sulfuric acid (H_2SO_4 , 98%), potassium permanganate ($KMnO_4$, 99.9%), hydrogen peroxide (H_2O_2 30%).

2.1. Synthesis of graphene oxide

Graphene oxide was synthesized by Hummers method [13]. In a typical synthesis, graphite flakes was added to solutions which contained strong oxidizing agents (90% sulphuric acid and 10% phosphoric acid) and stirred with a magnetic stirrer. Then potassium permanganate was added slowly to the mixture during the stirring process. After stirring, the mixture slowly shifted to another bottle which contained additional water and H_2O_2 was added. The color of mixture was changed to bright yellow indicating a high oxidation level of graphite. The solution was filtered and washed several times with water to remove the remaining impurities. The washing process was carried out using a simple decantation of the supernatant with centrifugation technique at 5000 rpm for 30 minutes which results in the formation of graphene oxide (GO).

2.2. Characterization

X-ray diffraction (XRD) scans of graphite flakes, and graphene oxide were performed with Bruker's D8 advanced X-ray diffractometer using $CuK\alpha$ radiation ($\lambda=1.5418\text{\AA}$). Dynamic Light Scattering (Model no: HORIBA Nano particle analyzer SZ-

100) was used to measure the size of the particle. The surface morphology of the prepared GO was examined by a Carl Zeiss ultra 55 Field Emission Scanning Electron Microscope (FESEM). Fourier Transform Infrared Spectroscopy (FTIR) was performed to identify types of chemical bonds, i.e. functional groups in a molecule (Model no: Perkin Elmer precisely FT-IR spectrometer) over the wave number range of $4000-500\text{ cm}^{-1}$.

[III] BIOMEDICAL APPLICATION

3.1. Sources required

Materials used for antimicrobial activity of Graphene oxide was Nutrient broth 1.3 g, Nutrient agar 5.6 g, Agar-agar 0.5 g, petriplates, cotton wabs, *Klebseilla*, *Staphylococcus*. Well diffusion method was used for antimicrobial activity of Graphene oxide.

3.2. Preparation of inoculums

Nutrient broth (1.25g in 100 ml D/W10) was prepared in two conical flasks and sterilized. In one conical flask clinically isolated strain of *Klebseilla*, was inoculated and in the other conical flask clinically isolated strain of *Staphylococcus* was added. The bacterial cultures inoculated nutrient broth was kept on rotary shaker for 24 hours at 100 r.p.m.

3.3. Inoculation of test plate

Nutrient agar is prepared (8g nutrient agar 0.5g Agar Agar in 100ml distilled water) [12] and sterilized. The agar suspension is poured into sterile petri-plates and allowed to solidify. Then the two pathogenic strains *Klebsiella* and *Staphylococcus* were taken and spreaded evenly over the entire surface of the plate by swabbing in three directions. Plates were allowed to dry before applying the sample.

3.4. Preparation of GO sample

Antimicrobial activity enhancement of GO was obtained by using Graphite flakes and Graphene Oxide. GO of two different concentrations are used to know the effective concentration for its activity. 0.01grams of graphite flakes were suspended in distilled water. Two different concentrations of GO i.e., 0.01grams and

0.05grams were suspended in distilled water separately.

3.5. Well diffusion method

The wells were casted by porer on the test plates. The samples were loaded with equal volume (30 μ l) on the plates. Control plate does not contain any antibiotic. The test plates were incubated at room temperature. The activity was clearly visible from 19-24 hrs on the plates. The zone of inhibition was measured & the sample of the Graphene oxide showing maximum antimicrobial activity was noted.

[IV] RESULTS

4.1. X-ray diffraction analysis

Figure 1 shows the XRD patterns of graphite flakes, and graphene oxide. Graphite flakes exhibits a strong and sharp peak at 26.4 $^{\circ}$ in Fig.1(a), indicating a higher ordered structure, that corresponds to a basal spacing $d_{002} = 0.334$ nm. The pattern of graphene oxide, on the other hand, exhibits a 001 reflection at 9.09 $^{\circ}$ corresponding to a basal spacing of $d_{001} = 0.961$ nm. The interlayer spacing of GO was calculated to be 0.961 nm according to the diffraction peak at $2\theta = 9.09^{\circ}$. This value is higher than interlayer spacing of graphite flakes (d-spacing= 0.334nm, $2\theta = 26.4^{\circ}$), due to the presence of oxygenated functional groups and intercalated water molecules.

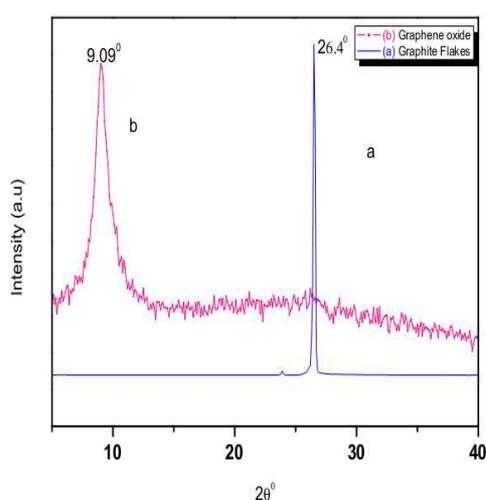
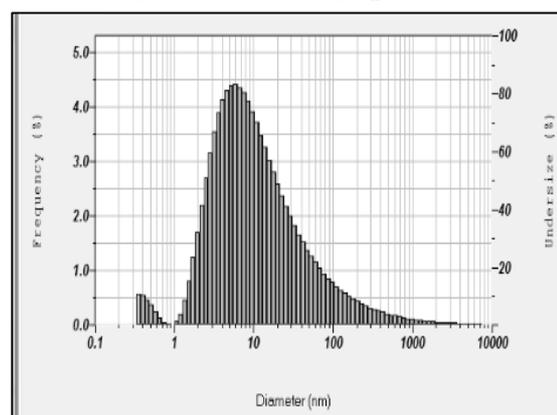


Fig. 1. X-ray diffraction patterns of (a) Graphene oxide, (b) Graphite Flakes

4.2. Dynamic Light Scattering (DLS)

The measurement of particle size distribution of graphene oxide is done by Dynamic Light Scattering (via Laser input energy of 532 nm). In the prepared sample it was observed that, particle have a wide size distribution, but the majority of them were dispersed within a narrow range, as shown in Fig (2). The average particle size from



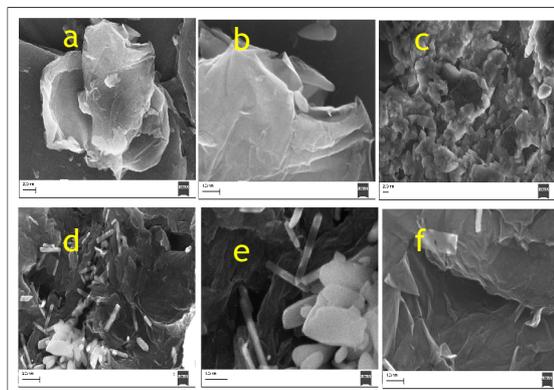
the histogram was found to be 8 nm.

Fig. 2. Particle distribution in the Dynamic Light Scattering.

4.3. Field Emission Scanning Electron Microscope (FESEM)

The grain size and surface morphology were observed by the field emission scanning electron microscope (FESEM). FESEM images of the Graphene Oxide (GO) have well defined and interlinked three-dimensional Graphene sheets, forming a porous network that resembles a loose sponge like structure as shown in Fig (3).

Fig. 3. FESEM image of (a, b, c) represents the graphene oxide, (d, e, f) represents the graphite fine powder

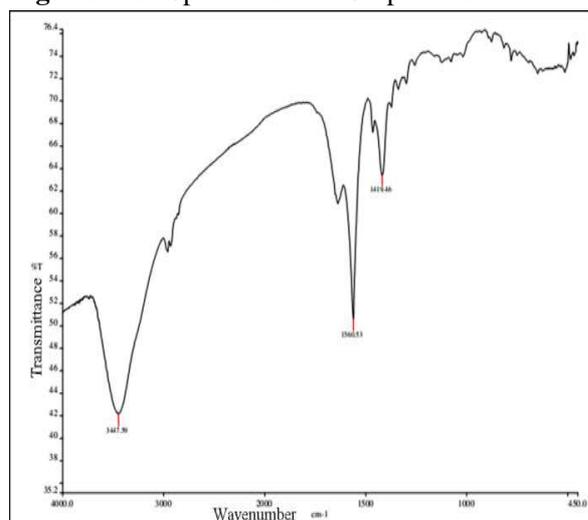


It was synthesized using graphite flakes which resemble the layers of an onion as shown in Fig 3(a), 3(b), 3(c), on other hand graphene nano rods have been observed as shown in Fig 3(d), 3(e), 3(f), when fine powder was used.

4.4. Fourier-Transform Infrared Spectroscopy (FTIR)

FT-IR spectrum of the Graphene oxide obtained in these steps confirms the successful oxidation of the graphite as shown in Fig (4). The presence of different types of oxygen functionalities in graphene oxide were confirmed at broad and wide peak at 3447 cm^{-1} can be attributed to the O-H stretching vibrations of the C-OH groups and water [14, 15]. The absorption bands at 1560 cm^{-1} can be ascribed to benzene rings [16]. The sharp intense peak at 1419 cm^{-1} can be attributed to CO-carboxylic.

Fig: 4. FT-IR Spectra for the Graphene oxide



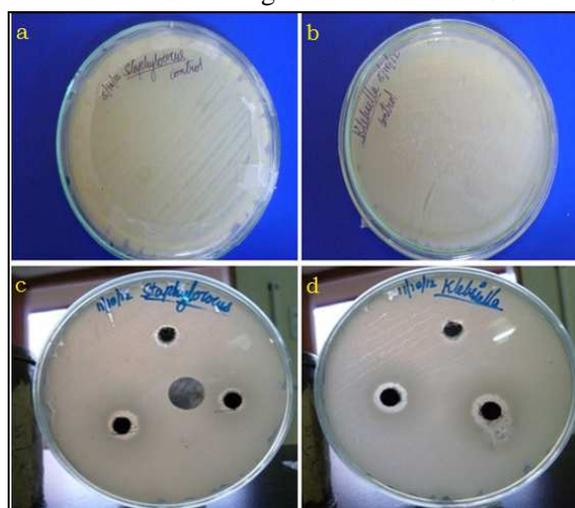
4.5. Antibacterial activity

Well diffusion method was used for the assessment of antibacterial activity. The antibacterial activity of the sample was identified by the formation of Zone of Inhibition. Zone of Inhibition is the area on an agar plate where growth of a control organism is prevented by an antibiotic usually placed on the agar surface. If the test organism is susceptible to the antibiotic, it will not grow where the antibiotic is present. The size of the zone of inhibition is a measure of the

compound's effectiveness, the larger the clear area around the antibiotic, the more effective the compound.

The activity of the sample was observed by the formation of Zone of inhibition after 24 hours. Presence of zone of inhibition confirmed inhibitory activity of GO. The Zone of Inhibitions of different bacteria is given in the figure. The control plates show the growth of bacteria in the absence of antibacterial agents. The clear zone surrounding the sample in the remaining plates shows the activity of the sample. Figure shows the petri dishes with samples of graphite flakes, 0.01gms of GO and 0.05gms of GO. The zone surrounding the sample is clear that shows complete zone of inhibition. The space surrounding the complete zone of inhibition is partial zone of inhibition where the activity decreases than complete zone of inhibition. The Zone of inhibition is more for the high concentration of GO. The results showed that the zone of inhibition increases within the concentration of GO in both the bacteria. Comparing the two, inhibitory activity of GO on *Klebsiella* was higher.

Fig: 6. (a) control of *Staphylococcus*, (b) control of *Klebsiella*, (c) Zone of Inhibition formation against *Staphylococcus* - GO (d) Zone of Inhibition formation against *Klebsiella* - GO.



[V] CONCLUSION

Modified Hummers method has been synthesized completely to produce large area graphene oxide. This method was carried out with the highest conversion level of graphite flakes to graphene oxide and shows that pure graphene oxide is formed. XRD conform its graphene oxide with hexagonal structure. The average particle size obtained from particle analyzer (DLS) was 8nm. The particle sizes were in the range of 35nm to 65 nm from FESEM. FTIR shows formation of graphene oxide. The antibacterial activity of GO was confirmed by Zone of inhibition. As the diameter of the zone of inhibition is high, we can conclude that GO is a very effective antibacterial agent.

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