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Strong inhibitory effect of antibiotics with graphene nanoparticles

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Abstract

The antimicrobial activity of carbon-based nanomaterials, such as graphene oxide, as well as their mechanisms of action, has been investigated. We made graphene oxide from high purity graphite precursors using a modified Hummer's process. X-ray diffraction (XRD), field emission scanning electron microscopy (FESEM), transmission electron microscopy (TEM), and scanning electron microscopy (SEM) have all been used to investigate the microstructures and morphology of graphene-based materials. To ensure broad surface area access to medication, Brunauer, Emmett, and Teller (BET) and Barrett, Joyner, and Halenda (BJH) methods are used to analyse the precise surface area and porosity analysis of graphene oxide. Using normal zone of inhibition, we investigated its antibacterial activity on pathogenic microorganisms. With graphene nanoparticles, a distinct inhibition zone was observed. In comparison to the nanoparticle-treated disc, the standard antibiotics Erythromycin, Streptomycin, and Tetracycline display a smaller zone of inhibition. The findings suggest that antibiotics delivered with graphene nanoparticles have a stronger inhibitory effect. On the basis of the obtained results, the effect of various concentrations will be presented.

1. Introduction

Microorganisms have begun to develop resistance to widely used antimicrobial agents as a investigation of widespread use of antibiotics. Multidrug-resistant (MDR) microorganisms (viruses, bacteria, fungi, protozoa, and others) have made traditional infectious disease care extremely complicated, vital, and dangerous around the world. As a result, it appears that the production of an alternative and effective antimicrobial agent is needed. Infections continue to be a leading cause of death and acute illness.

Antibiotic resistance is becoming more common, particularly among bacteria classified as "Gram-negative." Since these bacteria have two cell membranes, drugs have a harder time penetrating and killing them. As a observation new broad-spectrum antimicrobial nanomaterials will eliminate a wide range of pathogenic species such as viruses, bacteria, and fungi [1].

As a result, new and evolving nanoparticle-based materials in the field of antimicrobial therapies have gotten a lot of publicity around the world. Because of their wide surface area compared to their size, nanomaterials may be strategically beneficial as active antibacterial classes. If only a small dose of nanosized particles is used, they can have a high level of activity. As a result, nanomaterials may be used instead of antibiotics to treat bacterial infections. Nanomaterials as antibacterial complements to antibiotics hold a lot of promise and are attracting a lot of attention because they could fill in the gaps where antibiotics often fail [2].

Graphene is now being considered as a possible nanomaterial for the above applications. A single, flat sheet of carbon is arranged in a honeycombed lattice to form graphene. The content was produced for the first time in 2004. Graphene and its derivatives technology has been rapidly evolving since then[3].

Graphene's peculiar physical properties, such as its exceptional mechanical strength, thermal stability, and high electrical conductivity, have sparked interest in various fields of science and technology. While graphene oxide is not an antibiotic, it has been discovered to be a growth enhancer that can function as a biofilm to help bacteria bind and multiply. Graphene oxide (GO)-based materials, on the other hand, can cause oxidative stress in bacteria due to reactive oxygen species (ROS). Antimicrobial surface coatings, surface-attached stem cells for orthopaedics, antifouling for biocides, microbial fuel cells, and microbial electro-synthesis are all applications of GO-based materials [4].

Understanding graphene's bioactivity needs a thorough examination of its antibacterial properties. These model organisms are vulnerable to a variety of harmful influences, and their physiological manifestations help researchers understand toxicity mechanisms [5].

In 2010, Akhavan and Ghaderi published the first report on the toxicity of graphene against several bacterial organisms, as well as evidence that graphene oxide was more active than pristine graphene. The toxicity of graphene against bacteria has been extensively studied since then [6].

We investigated the antimicrobial activity of carbon-based nanomaterials, such as graphene oxide, as well as their mechanisms of action, in this research. We made graphene oxide from high purity graphite precursors using a modified Hummer's process. Using normal zone of inhibition, we investigated its antibacterial activity on pathogenic microorganisms. With graphene nanoparticles, a distinct inhibition zone was observed. In comparison to the nanoparticle-treated disc, the regular antibiotics have a smaller zone of inhibition. The preliminary findings suggest that antibiotics delivered with graphene nanoparticles have better inhibition behaviour. On the basis of the obtained results, the effect of various concentrations will be presented. The goal of our research is to synthesise and characterise graphene oxide nanomaterials, as well as to test their antibacterial activity in Escherichia coli bacteria. Because of their well-characterized physiology and ease of genetic manipulation, these bacteria are widely used as model organisms in modern toxicology. The toxicity mechanisms of different carbon nanomaterials have been studied using E. coli strains.

Since GO application to antibacterial mechanisms is a relatively new area, our findings may be applicable to biocatalyst and metabolite development in the near future. It will open up new possibilities for the creation of novel biosensors and the measurement of a variety of bioanalytical methods.

2. Materials and Methods

2.1 Preparation of graphene oxide (GO)

The graphene oxide (GO) was made from graphite powder using a modified Hummer's method [7], and then reduced with hydrazine hydrate to produce reduced graphene oxide [8].

The graphene oxide (GO) was made from graphite powder using a modified Hummer's method [7, 8], which can be summarised as follows: First, graphite powder (Sigma Aldrich) and sodium nitrate (Merck) were combined in a round bottom flask with a 2:1 weight ratio of the contents, and the flask was put in an ice bath for magnetic stirring. The mixture was first treated with a predetermined volume of concentrated sulfuric acid (Merck), accompanied by a gradual addition of potassium permanganate (Merck). After that, the mixture was stirred for an hour at room temperature before adding distilled de-ionized (DDI) water drop by drop. When stirring for another half hour, a small amount of warm DDI water was applied to the suspension, along with 30% hydrogen peroxide. For around 20 minutes, the resulting suspension was centrifuged. To obtain graphene oxide, the stock was washed with hydrochloric acid (HCl) and dried at room temperature under a gentle supply of flowing air (GO).

Following the procedure mentioned in the literature [7,8], the GO was chemically reduced to obtain reduced graphene oxide (R-GO). Predetermined amounts of GO (100 mg) were placed in a round bottom flask containing 100 mL filtered water to produce a yellow-brown dispersion, according to the protocol. For one hour, this dispersion was sonicated with (Thorough clean ultrasonic (India) Pvt. Ltd). Hydrazine hydrate (1.00 ml) was then applied to the suspension, which was then heated in an oil bath for 6 hours at 100 oC. The dark black suspension was filtered through a fritted glass filter (medium pore size) after cooling to room

temperature, then washed cupiously with DDI water and methanol. The black material that resulted was dried in a continuous air flow.

The GO were exfoliated before being activated (chemically and physically), as defined in the literature [9-12]. An aqueous dispersion of 1 mg/ml of GO material was prepared and sonicated to exfoliate GO. In most cases, 200 mg of GO powder was poured into a 500 mL round bottom flask with 200 mL distilled water. This solution was sonicated until it became a homogeneous/clear dispersion with no visible particulates. High surface area graphene oxide is the product of this process. For the KOH activation, a colloidal suspension of GO (1 mg/ml) was prepared in distilled water, then a 1 M aqueous solution of KOH was added drop-by-drop. The suspension was thoroughly stirred for 4 hours at room temperature before being static soaked for another 20 hours. The KOH and GO were mixed in a 16:1 (w/w) ratio. The mixture was dried overnight at 110 oC in a vacuum oven after filtering through a polycarbonate membrane (0.2 µm, Whatman).

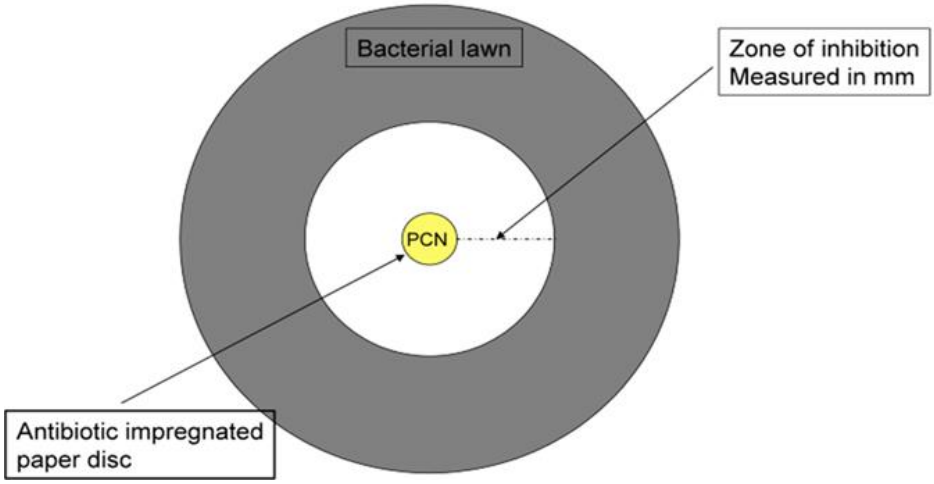
The temperature of the GO and KOH mixture was increased from 25 to 800 °C at a rate of 5 °C/min under a steady flow of nitrogen gas for physical activation. For the next two hours, the material was subjected to a steady flow of carbon dioxide gas while maintaining a temperature of 800 °C. After allowing the powder to cool to room temperature, it was washed with acetic acid (10 wt%) and de-ionized water until it reached a pH of 7. The mixture was then dried overnight in a vacuum oven at 110°C, followed by 2 hours of thermal annealing at 800°C to obtain high surface area GO powders.

2.2 Instrumentation

At room temperature in a N2 setting, the surface area and total pore volume of GO powders were measured using a surface area and pore size analyzer (model: Gemini-V, Micromeritics, USA).

XRD patterns of GO were reported using CuK radiation in the Bragg angle (2) range of 5o to 70o with a scan rate of 4o/min and a phase width of 0.02o on a high resolution X-ray diffractometer (D8-Discover, Bruker, USA). FESEM images of GO were captured using a Supra PV 55 model FESEM instrument with a 3 kV accelerating voltage. TEM images of GO were obtained using a transmission electron microscope (Tecnai G2T30, U-TWIN) with a LiB6 filament as an electron source and a 50-100 kV working voltage range. Carbon-coated copper grids were used to prepare the samples for TEM.

The Disc Diffusion method (Kirby-Bauer Disc Method) is used to increase the biological activity of graphene oxide [13]. The agar diffusion process is another name for this method. The concentration of antibiotic decreases as the material diffuses from the filter paper to the agar containing the bacteria. The antibiotic is reduced to the extent that it no longer inhibits microbial growth at a certain distance from each disc. The presence of growth-inhibition zones demonstrates an antibiotic's efficacy (Fig.1).



The antimicrobial substances diffused from these zones of inhibition (ZOIs), which appear as transparent areas surrounding the disc. To begin, we placed the antibiotic discs on the bacterial lawn without graphene oxide as a control. The antibiotic discs were then coated with 50 g/ml graphene oxide. To ensure good touch, gently tap each antibiotic disc onto the surface of the agar with a sterile stick or toothpick. Plates should be incubated at 37°C for 24-48 hours. Check each plate for the presence of inhibition zones. The rulers were used to measure the diameter of each zone in millimetres. Compare the diameter of each zone to the Clinical and Laboratory Standards Institute's standard map (CLSI). Antimicrobial Susceptibility Testing Performance Standards

A ruler may be used to calculate the diameter of the ZOI, and the effects of such an experiment form an antibiogram. The agar diffusion method employs commercially available filter paper discs that each contain a particular antibiotic concentration. The susceptibility range of an organism is based on the relative effectiveness of various antibiotics.

It's important to note that chemotherapeutic agents aren't selected solely on the basis of which produces the greatest ZOI. Because of the essence of the growth-inhibition agents, this is the case. The density or viscosity of the culture medium, the antibiotic's rate of diffusion, the antibiotic's concentration on the filter disc, the organism's sensitivity to the antibiotic, and the antibiotic's contact with the medium can all influence the size of the region. Furthermore, an agent with a significant antibiotic effect may not be therapeutically beneficial because it may have significant adverse effects in the system for which it is intended. The disc diffusion method is a straightforward method for determining whether or not substances have substantial antibiotic activity. This knowledge, along with a variety of pharmacological considerations, is used to determine which antibiotic should be used for treatment. The disc-diffusion assay has a range of advantages over other methods, including its flexibility, low cost, ability to evaluate large numbers of microorganisms and antimicrobial agents, and ease of interpretation of the findings. Furthermore, multiple studies have shown that an antibiotherapy dependent on the antibiogram

of the causative agent is of great importance in patients suffering from bacterial infection. This is due to the fact that in vitro data and in vivo evolution are highly correlated [14].

3. Result and Discussion

SEM experiments were conducted to investigate surface morphology after various physic-chemical techniques were used to characterise the GO powder. Figure-2 shows field emission scanning electron microscopy (FE-SEM) images of GO, which display smooth and flat surfaces with agglomerates.

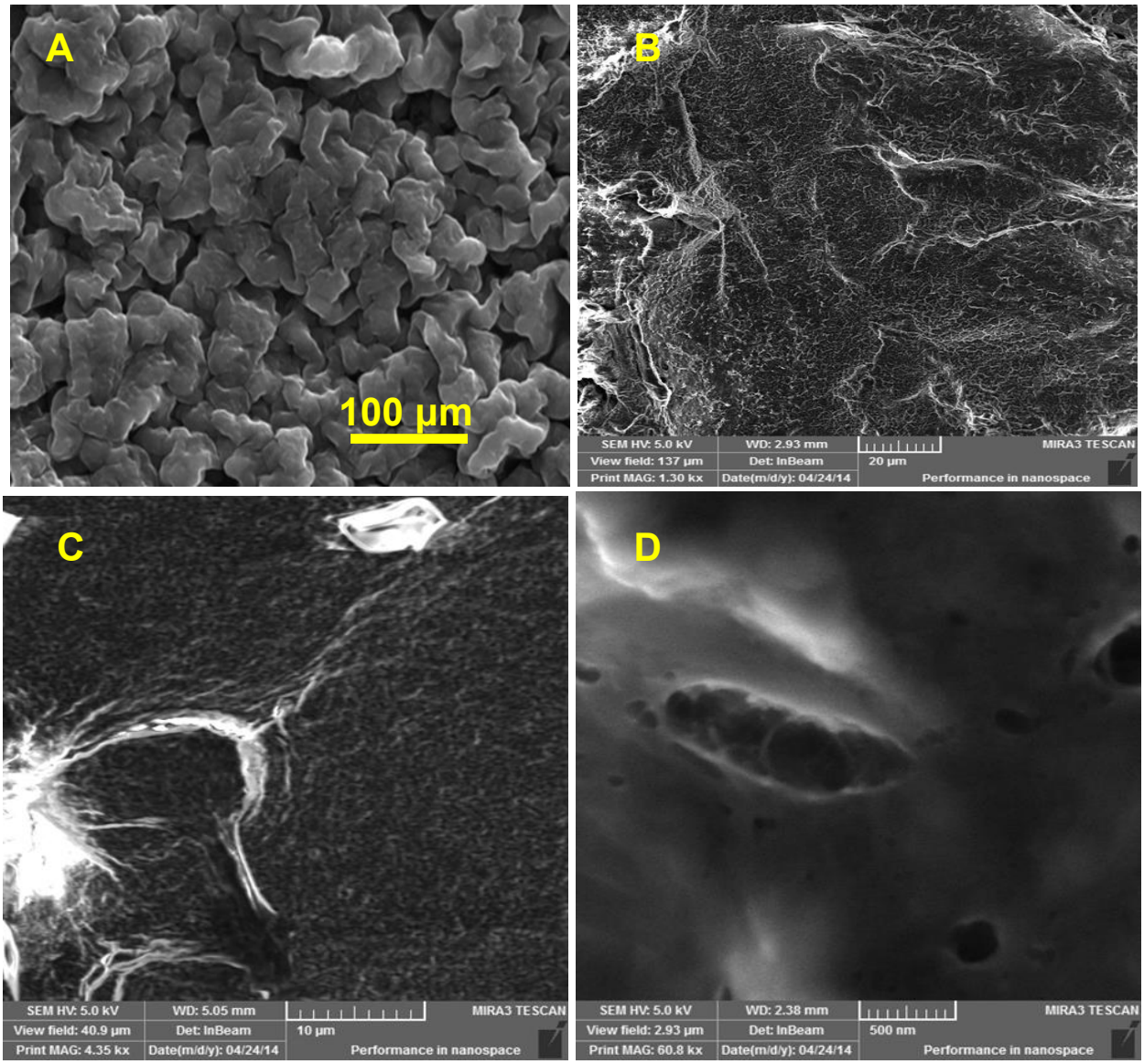


Figure-2: FESEM images of graphene oxide powders depicted with different magnifications.

Three dimensional (3D) framework embedded with crumpled flakes exhibits distorted sheet like entities. The 3D morphology formed by physical cross-linking of crumpled sheets creates pore structure and can effectively prevent the restacking of the sheets. The graphene oxide flakes are

found to be scattered, crumpled/defective. The magnified images show graphene sheets are in the network, close to one another.

Figure 3 shows TEM photos of GO sheets that are partly folded and wrinkled, resembling silk veil waves due to the wide surface area occupied by thin graphene sheets bonded with oxygen, and aggregation due to extensive exfoliation. The three-dimensional networks formed by graphene oxide sheets reflect defective/disordered structure caused by the formation of meso- and microporosity. Figure 3's inset shows selected region electron diffraction (SAED) patterns that display diffuse diffraction patterns with no bright spots, confirming the graphene oxide powder's non-crystalline/amorphous structure.

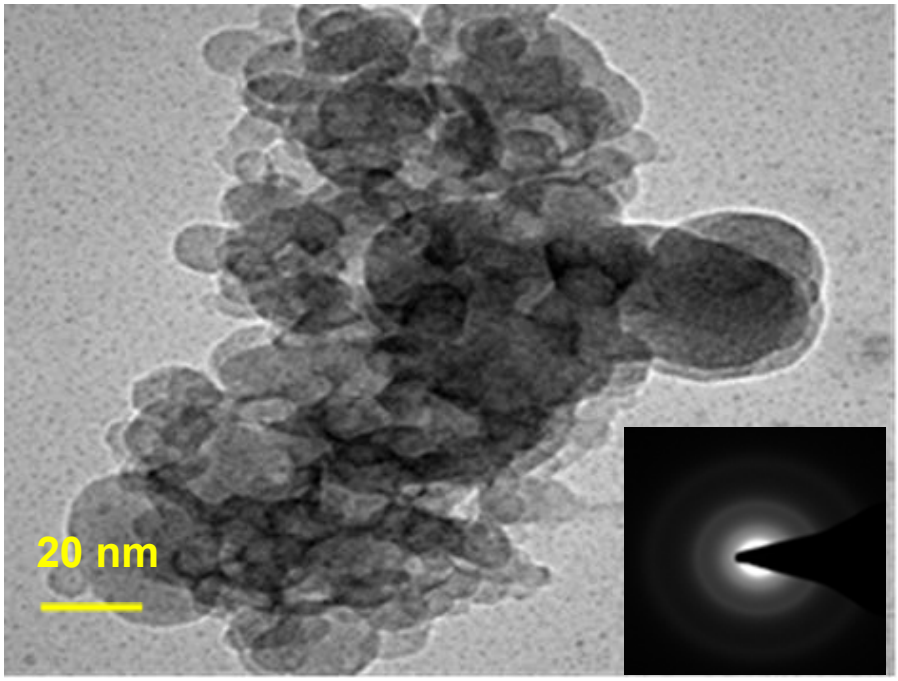


Figure-3: The TEM images of alcohol dispersed graphene oxide nano sheets

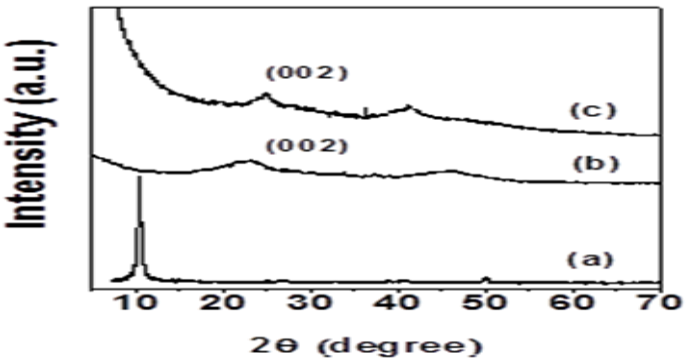


Figure-4: XRD spectra of (a) graphite, (b) graphene oxide and (c) reduced graphene oxide

In Figure-4, the XRD spectra of graphite, graphene oxide, and reduced graphene oxide have been plotted as a single set.

The graphite XRD spectra (a) revealed a distinct peak at 11°. The XRD spectra of graphene oxide (b) and reduced graphene oxide (c) are also shown for comparison. The strong diffuse peak suggests that the resulting graphene oxide powders are non-crystalline/amorphous. Due to interlamellar water trapped between hydrophilic GO sheets, the XRD spectra of GO show a distinct and wide peak at 25.10°, corresponding to a d-spacing (in this case, the interlayer gap between sheets) of approximately 7.15 [15,16].

According to the IUPAC classification of adsorption isotherms, Figure-5 shows mainly type-IV isotherms with a minor contribution from type-I isotherms, indicating the presence of predominant mesopores with a minor contribution from micro-porosity. The proportion of micro-porosity is indicated by the initial abrupt absorption of quantity of nitrogen gas below the relative pressure P/Po=0.1 (Fig.-5).
A moderate rise in nitrogen gas adsorption over a wider range of relative pressure suggests a significant amount of mesoporosity, with a specific SBET of 221 m2/g.

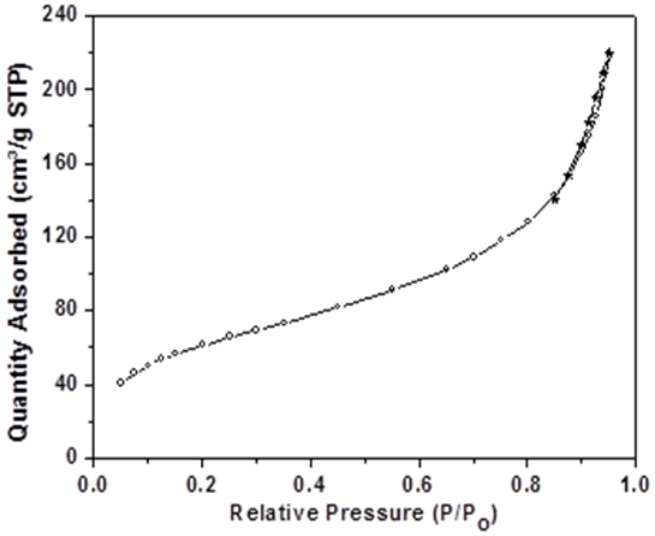


Figure-5: N_2-adsorption-desorption isotherms of GO

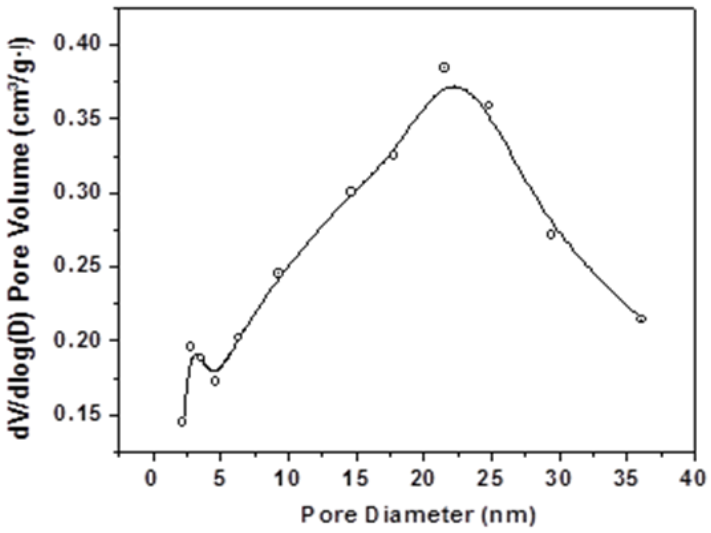


Figure-6: Pore-size distribution curve of GO powder obtained from BJH analysis

Barrett-Joyner-Halenda (BJH) analysis of graphene oxide (GO) materials yielded the pore-size distribution. The pore size distribution curve for graphene oxide powders shows a wider peak centred around 20 nm, followed by a small peak at 3 nm, indicating the presence of a significant amount of micro-pores in the nanomaterial powder formed by graphene oxide.

The effect of GO with various antibiotic agents on the formation of Zones of Inhibition (in mm) for each antibiotic has been observed, and measurement data is provided in Table-1.

Table-1: Effect of GO with different antibiotic agents on the formation of Zone of Inhibition (in mm) for each Antibiotic

Name of Antibiotic	**Zone of Inhibition (mm) before treated with GO**	**Zone of Inhibition (mm) after treated with GO (50 µ/ml)**	**Sensitivity ratings**
Erythromycin Inhibit protein synthesis (50s subunit inhibitor)	16.5	16.8	Intermediate
Streptomycin Inhibit protein synthesis (30s Subunit inhibitor)	15.8	17.0	Sensitive

Tetracycline Inhibit Protein synthesis (30s subunit inhibitor)	24	27.8	Sensitive

• The results indicate that graphene oxide treatment has no major effect on erythromycin. It acts as an intermediate antibiotic, preventing bacteria from growing.
• The zone of inhibition in Streptomycin was smaller (15.8 mm) than the first one, but it increased (17 mm) after treatment with GO, indicating better results after nanomaterial treatment.
• In all three antibiotics, tetracycline has the highest zone of inhibition (24 mm), which increases dramatically following treatment with GO (27.8 mm).
The following section depicts one possible reason for this behaviour:
If bacteria have an outer-membrane-Gram-negative-bacteria or do not have an outer-membrane-Gram-positive-bacteria, when an external barrier composed of GO-based materials develops around the surface of the bacteria, it suppresses nutrients that are necessary for microbial growth while simultaneously producing oxidative stress, which causes bacteria to die [17].

4. Conclusion

A updated Hummer's method was used to successfully synthesise graphene oxide. The microstructures and morphology of graphene oxide are studied using XRD, FESEM, and TEM. The basic surface area and porosity analysis of graphene oxide were investigated in order to ensure that a large surface area is usable for medical applications. The antibacterial effect of GO on pathogenic microorganisms was investigated. For Tetracycline, graphene nanoparticles showed the largest inhibition region, while the other two antibiotics showed a smaller inhibition zone. As a result, Tetracycline is the best antibiotic to use in conjunction with graphene oxide. As a result, graphene oxides can be used as a stronger antibacterial agent for Gram negative bacteria.

Since the application of graphene to antibacterial mechanisms is a relatively new area, our findings may be used for biocatalysts and metabolite development in the near future. It will open up new possibilities for the creation of novel biosensors and the measurement of a variety of bioanalytical methods.

Refereance

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