Graphene and Graphene Oxide and their synthesis, characterization and study of anti-microbial property

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Abstract –

Graphene and related two-dimensional nanomaterials including graphene oxide (GO) have received considerable attention in research regarding their basic properties and potential applications. Given the potential widespread usage, research on the ecological impact of this emerging class of nanomaterial has begun to increase rapidly 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. 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, used for preparing graphene oxide. The resulting graphene oxide was characterized by XRD, DLS, FESEM, and FTIR. Antibacterial activity was tested using Klebseilla bacterial specie. The GO exhibited stronger antibacterial activity against bacterial species.

Keywords: Graphene oxide, nanomaterial, Bacterial

1. Introduction

Infectious diseases caused by pathogenic microorganisms such as viruses, bacteria and fungi are still one of the world's most challenging global health issues [1]. While morbidity and mortality from infections decreased considerably from the late 1940s onwards due to the commercialization and use of penicillin, issues related to the emergence of many microorganisms that have become resistant to antibiotics have made the search for the treatment of infectious diseases again of high importance [2]. For example, some of the Gram-positive (e.g. Staphylococcus aureus) and Gram-negative (Escherichia coli, Pseudomonas aeruginosa) bacteria are the common multi-drug resistant (MDR) pathogens and important causes of

various often hospital-acquired infections [4]. According to published data in 2011, 25000 patients die annually in the EU as a result of infections caused by antibiotic-resistant bacteria, with two-thirds of these deaths due to Gram-negative pathogens. The report entitled "Review on Antimicrobial Resistance" published in December 2014 by J. O-Neill estimates a death quote attributable to antimicrobial resistance (AMR) of 10 million in 2050 [5]. The costs incurred by drug resistant infections amount to an estimated €1.5 billion annually, due to the increase in healthcare expenditure costs. The situation is all the more serious as antimicrobials have become an essential tool for modern medicine and many surgical operations could not be performed without them [6]. The demand for developing new antimicrobial drugs and therapies for combating bacterial infections has thus become crucial.

Next to the conventional treatments of pathogenic bacteria with antibiotics, other antimicrobial agents such as topical antiseptics, [7] iodine-containing solutions, [8] or silver ion containing preparations can be used. While these approaches have the advantage of surpassing any bacterial resistance mechanism, they are not harmless and generally do not target only bacteria but also normal cells. Absorption of antiseptics such as iodine compounds can cause many adverse effects, such as psychological disorders to skin reactions or acidosis, but also metabolic disorders such as hyperthyroidism. This made iodine not recommended for the treatment of infected wounds. Silver preparations such as silver nitrate or silver sulfadiazine are also used as antimicrobial agents. Taking again the example of wound infections, such compounds are not capable of penetrating deep inside and are ineffective in the treatment of deeply infected wounds and burns. This and other infection related problems have motivated researchers to concentrate on the development of novel, inexpensive and efficient antimicrobial treatment strategies for fighting pathogenic infections. These strategies are becoming even more important over the years, as, despite extensive efforts in research and enormous investment of resources, the speed of antibiotic development has not kept up with the development of resistance. The application of materials science and nanotechnology to medicine has shown impressive potential in tackling different aspects of microbial infections.

Several works on the antibacterial properties of graphene, graphene oxide have been developed by many scientists, but till now, no more reports are available on the application of these materials to make anti-microbial agent. Only a few studies associated with the selective killing of pathogenic microorganisms over non-pathogenic ones are provided. In light of the increasing spread of antibiotic resistant bacteria associated with their severe threat against public health worldwide, it might be important to investigate other pathogenic species and phenotypes to illustrate the broad range of bactericidal properties of graphene nanostructures. Indeed, most of their action has still to be validated on other bacterial strains. To overcome this problem, exploration of graphene, graphene oxide material, which can be synthesized by an

environment friendly, cost effective synthesis approach, is highly desirable. Synthesizing the reported graphene, graphene oxide using different synthesis methodology to get better phosphorescence intensity and duration is also required.

2.2. Experimental Section

The chemicals used for synthesis are Graphite flake (mesh size 300), Sulfuric acid (H_2S0_4, 98%), potassium permanganate ($KMnO_4$, 99.9%), hydrogen peroxide ($H_2O_230\%$).

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).

Characterization

X-ray diffraction (XRD) scans of graphite flakes, and graphene oxide were performed with Bruker's D8 advanced X-ray diffractometer using CuKα radiation (λ=1.5418 A). 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 4000-500cm^{-1}.

BIOMEDICALAPPLICATION

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*, *Staphylococus*. Well diffusion method was used for antimicrobial activity of Graphene oxide.

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 Staphylococus was added. The bacterial cultures inoculated nutrient broth was kept on rotary shaker for 24 hours at 100r.p.m.

Inoculation of test plate

Nutrient agar is prepared [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.

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.

Well diffusion method

The wells were casted by porer on the test plates. The samples were loaded with equal volume (50µl) on the plates. Control plate does not contain any antibiotic. The test plates were incubated at room temperature. The activity was clearly visible from19-24 hrs on the plates. The zone of inhibition was measured & the sample of the Graphene oxide showing maximum antimicrobial activity was noted.

1.3. Result and discussion

1.3.1 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^{o} in Fig.1, 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^{o} corresponding to a basal spacing of d_{001} = 0.961nm.The interlayer spacing of GO was calculated to be 0.961 nm according to the diffraction peak at 2Θ =90.9º. This value is higher than interlayer spacing of graphite flakes (d-spacing= 0.334nm, 2Θ =26.4°), due to the presence of oxygenated functional groups and intercalated water molecules.

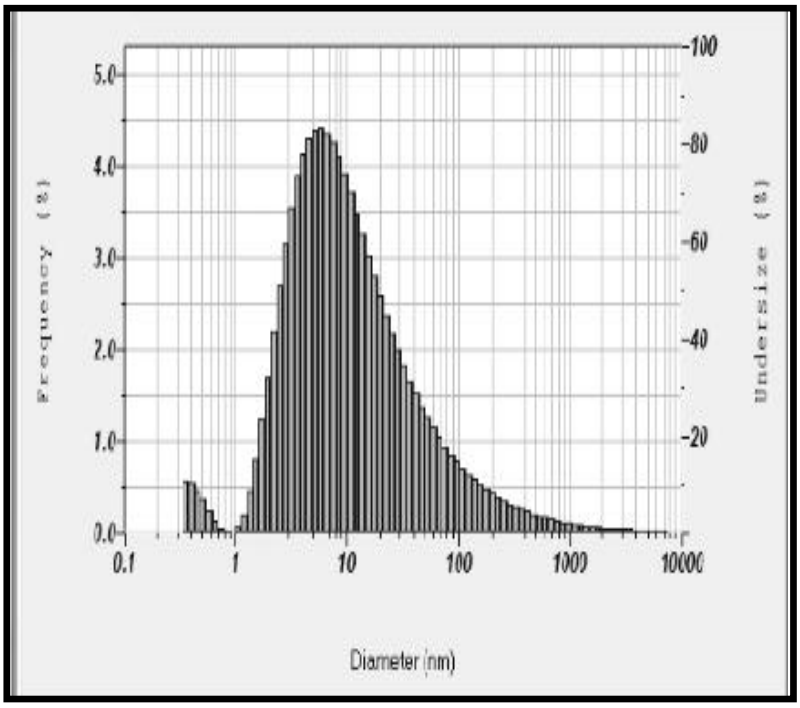
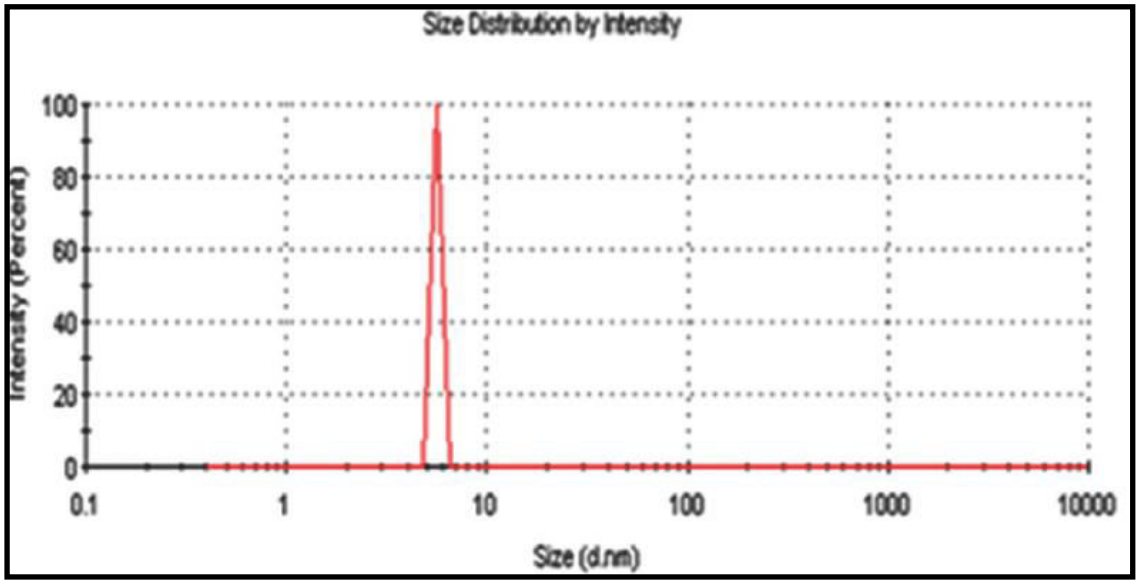


Fig: 1 X-ray diffraction patterns of Graphene oxide

2.3.2 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 8nm.

Fig: 2 Particle distributions in the Dynamic Light Scattering



2.3.3 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). It was synthesized using graphite flakes which resemble the layers of an onion as shown in Fig 3 on other hand graphene nano rods have been observed as when fine powder was used.

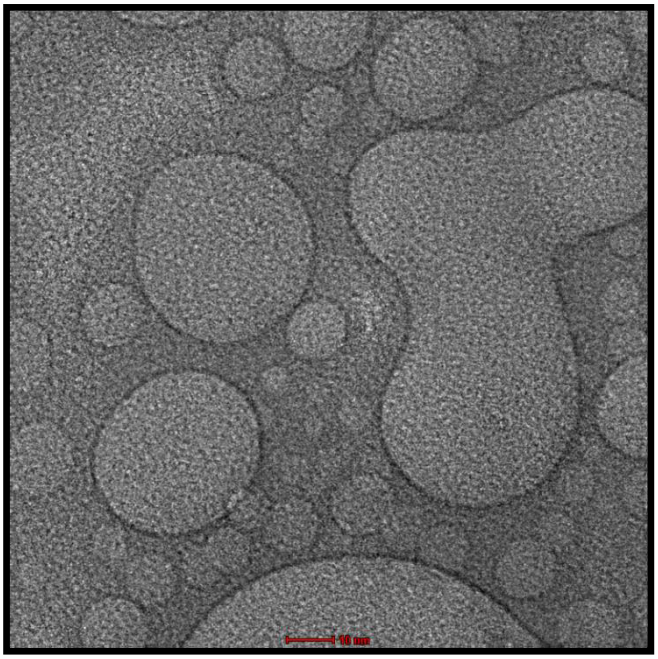


Fig: 3 FESEM image of represents the graphene oxide

1.3.4 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.

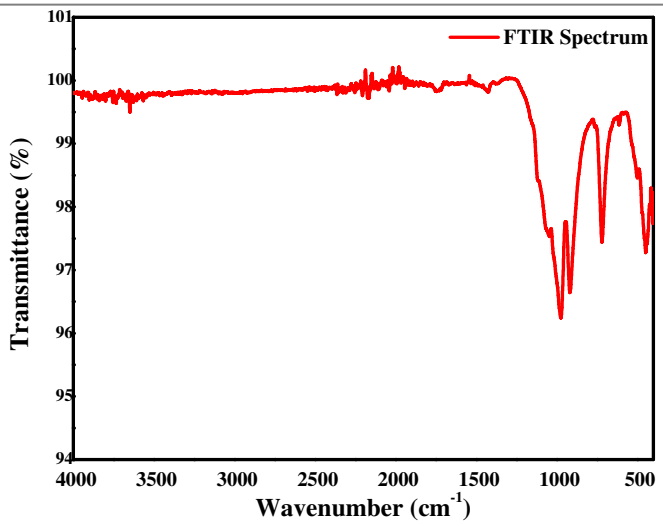


Fig: 4.FT-IR Spectra for the Graphene oxide

1.4 Antibacterial activity

Well diffusion method was used for the investigation of antibacterial activity. The antibacterial activity of the graphene oxide 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 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 0.05 gms 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 *Klebseilla* was higher.

1.5. 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 to hexagonal structure .The average particle size obtained from particle analyzer (DLS) was 8 nm. The particle sizes were in the range of 45 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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