ANTIBACTERIAL ACTIVITY OF GRAPHENE OXIDE NANOSHEETS PREPARED BY ELECTROCHEMICAL EXFOLIATION METHOD

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A B S T R A C T

The antibacterial activity is capable of great significance in case of therapeutic treatments. There is an increasing demand for antibacterial materials. The graphene is a multifunctional material due to its unique physical and chemical properties. The antibacterial activity of graphene oxide (GO) nanosheets was examined against the Escherichia coli (*E.coli*) and Staphylococcus aureus (*S.aureus*). The electrochemical exfoliation method was used to produce GO nanosheets. The resulting sample of GO nanosheets was characterized by x-ray diffraction and transmission electron microscopy with selected-area diffraction (SAD) pattern. Ultraviolet–visible and fluorescence spectroscopy were used to study the optical properties. The diffraction pattern rings were coincides with the principal (002) and (100) peaks of GO. The clear SAD image shows hexagonal symmetry due to the layered structure (sheets) of GO. The interlayer spacing between two graphene sheets (d) was 0.339 nm. Results of antibacterial activity shows higher zone of inhibition towards *E.coli* as compared to *S.aureus* hence GO nanosheets can be used for antibacterial application.

Keywords: Graphene oxide, Nanosheets, Electrochemical exfoliation method Antibacterial activity.

1. Introduction

Graphene is a potential candidate for biological applications due to its excellent electronic property and biocompatibility. It has unique two dimensional (2D) hexagonal lattice structure of sp^2 hybridized carbon atom [1 –3]. It has many exceptional physical and chemical properties compared to other carbon allotrope that is fullerecences, carbon nanotubes and graphite. Due to these unique properties and biocompatibility, graphene have many applications in biotechnology [4-6]. It exhibited some properties such as thermal, mechanical and electrical properties and also has excellent mobility of charge carriers and high surface area. It possesses the excellent antimicrobial activity. Fan's group firstly found it in 2010 [7]. The graphene oxide (GO) and reduced graphene oxide (rGO) nanowalls have shows effect against gram negative and gram positive microbes through direct contact and rGO exhibited stronger antimicrobial activity than GO [8]. The rGO nanosheets can suppress the proliferation of microbes on their surfaces even in an environment that is highly suitable for microbial growth [9]. GO and other derivatives of graphene can be used in biotechnology due to their interactions with biomolecules and there is no cytotoxicity on human cells (23;28;29). The Go nanosheets can extract phospholipids from *E.coli* membrane and destroy the membrane integrity, thus killing bacteria.

Li et al [10] reported for gram negative *E.coli* and gram positive *S.aureus* that the antimicrobial action of large area monolayer graphene film on conductor Cu, semiconductor Ge and insulator SiO_2. Their results shows that the growth of both bacteria inhibited by graphene film on Cu and Ge. But graphene film on insulator SiO_2 does not inhibited the same.

Notley et al [11] carried out the work on understanding the interaction between graphene and bacteria. They reported the preparation of graphene by using Hummer's method. The electron mobility property of graphene is

effectively dominated upon reduction and oxidation reactions. Mishra et al [12] evaluated the prepared GO nanoparticles and their antibacterial activity. The antibacterial activity is tested against gram positive and gram-negative bacterial stains. Badiei et al [13] have synthesized the GO by oxidation of graphene powder by Hummer's method and investigated the antibacterial activity. They are evaluated the antibacterial activity of graphene against *E.coli* by colony forming count (CFU) method. Their results confirmed that the sample shows excellent antibacterial activity against *E. coli*. Chen et al [14] carried out the research on interaction mechanism between GO and typical phytopathogens. The antimicrobial activity of GO against two bacterial pathogens (*pseudomonas syringae* and *Xanthomonas campestris athovars undulosa*) and two fungal pathogens (*Fusarium graminearum* and *Fusarium oxysporum*) are investigated. The results showed that GO had a powerful effect on the reproduction of all four pathogens. Gurunathan et al [15] investigated the antibacterial effect of GO and rGO in case of *Pseudomonas aerugonosa*. In this work, the novel reducing agent, betamercaptoethanol (BME) is used for synthesis of graphene to avoid the use of toxic material. The two types of graphene based materials and their antibacterial activity towards a bacterial model *Pseudomonas aeruginosa* is studied and compared to uncover the effects of GO and rGO on health of the human.

This research work deals with the synthesis of GO by electrochemical exfoliation method. The prepared GO was evaluated for the antibacterial activity with gram positive *S.aureus* and gram negative *E. coli* bacterial stains.

2. Material and Methods

In the present work GO nanosheets were prepared by modified electrochemical exfoliation method. The copper electrode acts as cathode and graphite rod as an anode electrode. For this method ghraphite rod and copper electrode were inserted in to an ionic solution with separation of 5 cm. The ionic solution was prepared by taking 4.8 gm sulfuric acid (99.99 % SD fine) diluted in 100 ml double distilled water [16]. The process was carried out in a direct current (DC) bias (10V) arrangement at room temperature (303 K). Exfoliated graphene sheets were collected through cellulose nitrate filter paper and washed with doubled distilled water. The obtained sample was then dried at 50 °C for 2 hrs. The GO nanosheets were obtained by heating at 500 °C for 8 hrs.

The structural properties of as synthesized GO nanosheets were characterized through X ray diffraction (XRD, Rigaku ,Miniflex-II) using CuKa radiation (=1.54 Å), The morphology was examined by high-resolution transmission electron microscopy (HR-TEM, Tecnai F-30107; Philips) with selected area diffraction pattern (SADP) analysis. Optical characterization was done through ultraviolet–visible (UV–Vis; PerkinElmer) and fluorescence spectroscopy (FL spectrophotometer model F-7000; Hitachi).

The antibacterial activity of GO nanosheets was carried out using disc diffusion method against *E.coli* and *S.aureus* bacterial stains. Small watman filter paper discs of uniform size 5 mm were used. Entire work was carried out strictly in aseptic conditions using laminar air flower in between two burners. The petriplates were incubated for 24 hrs and average diameter of inhibition of zone was calculated.

3. Results and discussion

The XRD pattern of the GO nanosheets is shown in figure 1. The pattern shows well supporting structural and phase purity of GO. The two prominent peaks in the XRD pattern, (002) and (100), are the characteristic peaks of graphene. The sharp peak observed at 2= 26.3° shows a highly organized structure related to interlayer spacing of 0.339 nm [16]. This is dependable with the layer separating of regular graphite. The broad peak observed at 2= 44.2° may be assigned to a lower degree of crystallization and the presence of some defects. This might be because of adsorption of oxygen molecules with amorphous carbon introduce on the sheet surface.

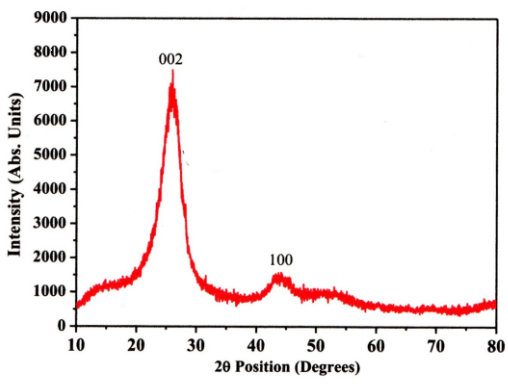

Figure 1. XRD pattern of GO nanosheets.

The structure of GO nanosheets is imagined specifically by HR-TEM, as shown in figure 2 (highlighted by red box). The inter-layer spacing between two graphene sheets (denoted by d) was found to be 0.339 nm. The inset of figure 2 demonstrates a SADP picture of the GO nanosheets. Which is very well agree with the results that are obtained from XRD analysis. The rings in the diffraction pattern are match with the principal (002) and (100) graphene peaks. The clear SADP picture is because of the sheet structure of graphene, demonstrating the hexagonal symmetry [17].

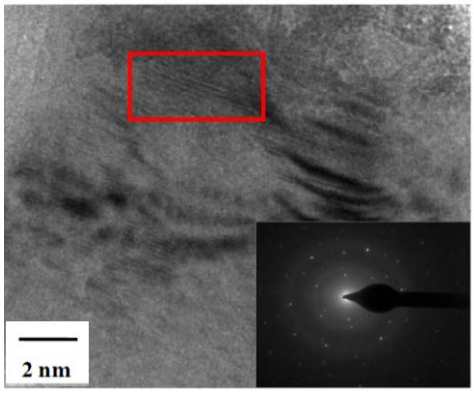

Figure 2. HR-TEM image of GO nanosheets.

The UV–Vis absorption spectra of GO nanosheets is as shown in figure 3. The spectrum provides a tentative idea about the layered structure of GO nanosheets. The spectrum in the range 250-500 nm can be assigned to the □ □* transition of aromatic C-C bond and smaller peak at 630 nm corresponding to n →□□ * transition of C and O bonds [18]. Whereas, GO nanosheets reduction and restoration of the aromatic network clearly observed due to broad peak in visible region.

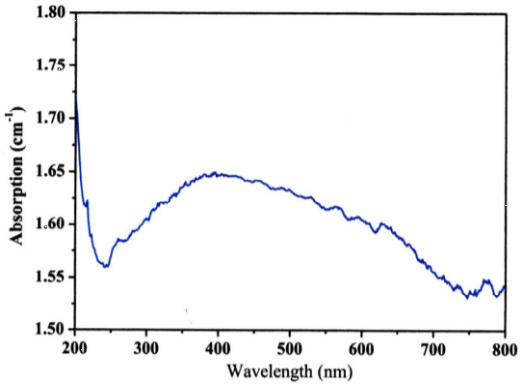

Figure 3. UV–Vis absorption spectra of GO nanosheets.

Figure 4 shows the room temperature PL emission spectra of GO nanosheets in the wavelength range between 300-800 nm at the excitation wavelength of 254 nm.

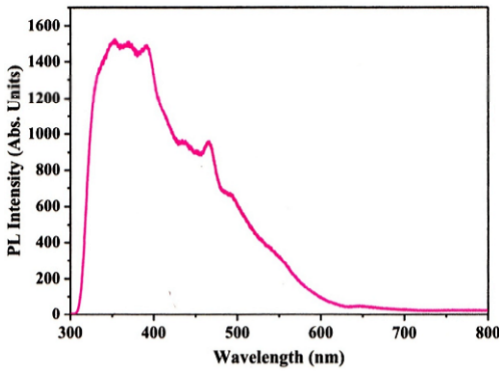

Figure 4. PL emission spectra of GO nanosheets.

The broad emission peak shows in UV region. The electrons relax to the ground state which emitting ~380 nm. The confinement of graphene in the graphite plane leads to the appearance of edge states near the Fermi level in the electronic band structure. These edge states are not very prominent nanosheets due to the saturation of dangling bonds at the edges by O-H group and appear as bump ~430-500 nm.
Figure 5 (a and b) shows the antibacterial activity of GO nanosheets towards *E.coli* and *S.aureus*.

Figure 5. Antibacterial activity of GO nanosheets against *E.coli* and *S.aureus*.

The first indication is that the GO nanosheets are used as an antimicrobial activity and point to some synergetic effect of its concentration. The GO nanosheets shows good antibacterial activity with increase in zone size is as shown in table 1.

Table 1. Antimicrobial activity (zone of inhibition, mm) of GO nanosheets at different concentrations against *E.coli* and *S.aureus*.

Biological entity	Concentrations of GO nanosheets	Inhibition zone diameter
Escherichia coli (E.coli)	0.25	11
	0.5	14
	1	16
	1.5	18
Staphylococcus aureus (S.aureus)	0.25	11
	0.5	12
	1	14
	1.5	16

Zone of inhibition (ZIO) is the area on an agar plate where the bacterial growth prevented in the presence of antibacterial compound. The ZOI is calculated simply by ruler method.

As the concentration of GO nanosheets varied from 0.25 to 1.5 gm/ml, the inhibition zone diameter was obtained in case of *E.Coli* with diameter from 11 to 18 mm, respectively whereas, in case of *S.aureus*, from 11 to 16 mm, respectively. The maximum inhibition zone diameter was observed in case of *E.Coli.*

The strength rigidity and shape for the cell can be provided by bacterial cell wall. Also it can protect the cell from mechanical damage and osmotic rupture. The electrostatic interaction is responsible for attachment of nanoparticles on the bacterial cell wall. Thus rupturing the cell walls thereby increase in the permeability. It causes the leakage of cytoplasm that leading to death of bacterial cell. Due to thin layer of peptidoglycon and cell membrane brust, the cell walls of *E.Coli* are broken easily [19]. Thus, this analysis revels that the antibacterial properties are influenced not only by the crystallinity but also by the size.

4. Conclusions

The GO nanosheets were synthesized using electrochemical exfoliation method with one of the copper electrode. The XRD pattern was well supporting the structural and phase purity of GO. The interlayer spacing between two graphene sheets (d) was observed as 0.339 nm from HR-TEM. SADP image of the GO nanosheets are agreed with the results obtained from XRD analysis. GO nanosheets reduction and restoration of the aromatic network clearly observed from UV-Vis due to broad peak in visible region. The confinement of graphene in the graphite plane observed from PL emission leads to the appearance of edge states near the Fermi level in the electronic band structure. The GO nanosheets shows good antibacterial activity with increase in zone size. Antibacterial activity of GO nanosheets was confirmed by using different stains of bacteria where *E.coli* gave best results..

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