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RESEARCH ARTICLE

# Synthesis and Characterization of Graphene Oxide Nanosheets by Electrochemical Exfoliation Method and its Antibiofilm Activity against *Pseudomonas aeruginosa*

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## Abstract

In this work, the Graphene oxide (GO) nanosheets were synthesized by a electrochemical exfoliation method. The morphological, structure and optical properties were investigated by using Field emission scanning electron microscope (FE-SEM), X-ray diffraction (XRD), Raman spectroscopy, FTIR and UV-VIS spectroscopy. FE-SEM images of GO nanosheets confirmed the presence of layered structure. The crystalline size and number of layers of GO nanosheets were estimated by XRD analysis. The UV-VIS spectrum of GO nanosheets shows maximum and shoulder absorption peaks. The oxidation of graphene was confirmed via FTIR analysis by the appearance of functional groups of oxygen. Raman analysis showed the formation of peaks D, G and 2D. The antibiofilm activity of different concentrations of GO nansheets against *Pseudomonas aeruginosa* (*P. aeruginosa*) as a model of more antibiotics resistant Gram-negative bacteria was investigated. These results confirm that the GO nanosheets material damages the biofilm of P. aeruginosa. Therefore, It can be considered as an anti-bacterial material.

**Keywords**: *Graphene Oxide, Nanosheet, Electrochemical Exfoliation, Antibiofilm, Pseudomonas aeruginosa.*

## Introduction

Nanotechnology includes the application of nanoscale materials with distinctive properties compared to bulk materials of the same structure [1]. The nanotechnology of carbon-based material is a favourable field for study and research because of the possibility of its application in numerous fields such as sensors, water treatment, alternative energy and nanoelectronics [2, 3]. GO material is easily dissolved in water, hydrophilic, and other solvents [4].

Graphene oxide is a carbon material with promising future in the field of biological application especially as antibacterial efficacy [5]. Graphene oxide nanosheets are two-dimensional, characterized by being a single atomic material consisting of oxidized graphite particles [6].GO nanosheets have a similar structure to the graphene structure, but with functional groups containing oxygen such as carbonyl, hydroxyl and carboxyl groups. These groups have a high affinity to water molecules [7]. On the other hand, antibiotic resistance is one of the most serious challenges to health, development and food security in the world.

One of the main reasons of bacterial resistance is surrounding it with a biofilm [8]. The structures of biofilms include the presence of many microorganisms [9].Biofilm is a complex accumulation of surface-based microscopic organisms.This polymeric cluster contains extracellular polysaccharides, DNA, fats and proteins [9, 10].These polymer clusters are formed in the form of matrix that act as a barrier that protects bacteria from immune defenses and antibiotic therapy [11].

The acute chronic infections such as urinary tract infections, infections associated with implantation, catheterization, upper respiratory tract infections and cystic fibrosis by *P.aeruginosa* are caused by pathogenic factors in biofilms [12, 13].*P. aeruginosa* is a gram-negative pathogenic bacteria that attacks an immune-weakened host. The bacterium form biofilms and thus exhibit high resistance to antibacterial agents. Furthermore, *P. aeruginosa* have the ability to colonize and cause acute and chronic infections [14].Because of the low effect of antibiotics on biofilm, it is necessary to look for therapeutic substitutions [15].

So the trend was towards nanomaterials to reduce the risk of biofilms. In this paper, graphene oxide nanosheets were prepared, characterize and used as anti-biofilm active nanomaterial.

## Materials and Methods

### Synthesis of GO Nanosheets

The GO nanosheet is prepared by chemical exfoliation method. The high pure graphite rods were used as cathode and anode (Fig.1) and the distance between the rods was 3 cm where they placed in a beaker of 100 mL containing an acids solution adjusted to pH 4.The acids solution consists of $H_2SO_4$, HCl, $CH_3COOH$, $H_2O_2$ and $HNO_3$. Ten volts was applied to the rods for a full day where the graphite flakes begin to expand, dissociate, and spread into the solution until the mixture color is converted to bright-yellow.

Then the solution was sonicated for two and half hours. To shake and disassemble the molecules of the solution. Moreover, the solution was centrifuged 3 times with distilled water (DW) at 3500 rpm for 15 min to separate the large microparticles and to remove the residual acid. Thereafter, the suspension was dried in the vacuum oven for 3 hours to obtain GO powder.

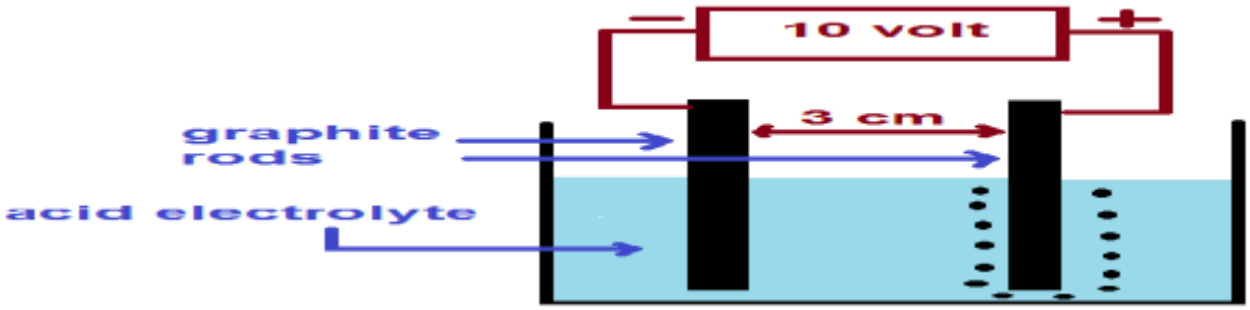


**Fig.1: Scheme of electrochemical exfoliation cell**

### Anti-Biofilm Formation Assay

Concentrations of 100, 150, 200, 250, 300, 350 and 400 mg /ml of GO nanosheets were suspended in distilled water, separately. From each concentration, 10 µl was added to single well in the microplate, that involves 180 µl of brain heart infusion (BHI) broth and 10 µl *P. aeruginosa* culture ($1.5 \times 10^8$ cells/ml). The Control consists of 190 µl of BHI broth and 10 µl *P. aeruginosa* culture. Thereafter, the microplate was incubated for 24 h at 37 °C. After that, the content of microplate was removed and washed the bacteria four times with PBS (pH 7). Biofilms formation by bacteria were fixed by 95% ethanol and stained with 1% (w/v) crystal violet .Rinsed off 5 times with DW to remove excess stain and kept to dry. Then, 200 µl of 33% glacial acetic acid was add and read it after 15 minutes by microplate reader at 590 nm. The absorbance considered the value of bacterial adhesion on the surface of nanoparticles and formation of te biofilm. Average of triplicate reads of each concentration were calculated [16].

## Results and Discussions

### FE-SEM

FE-SEM image of prepared GO (Fig.2) displayed several layers arranged on top of each other as papers-like structure, with undulant and folded sheets. Moreover, the morphology offers thin and homogeneous GO film.

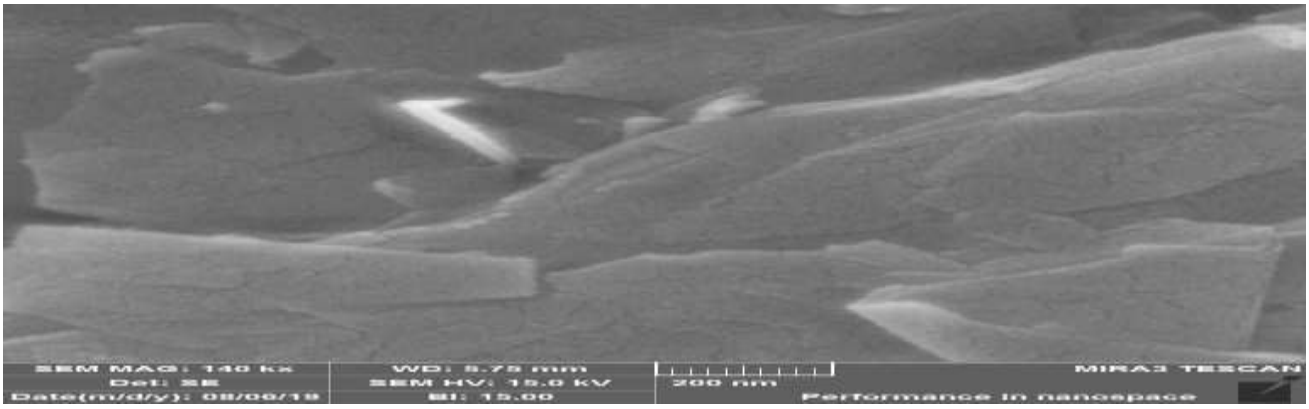


**Fig. 2: FESEM image of GO nanosheets**

## UV-VIS Analysis

UV-Visible spectra of GO nanosheets suspension in Fig.3. The intense absorption peak at 234 nm is attributed to the π→π* electronic transition of C-C bond, while the shoulder peak at 316 nm due to n-π* transitions of aromatic C-O bonds [17].

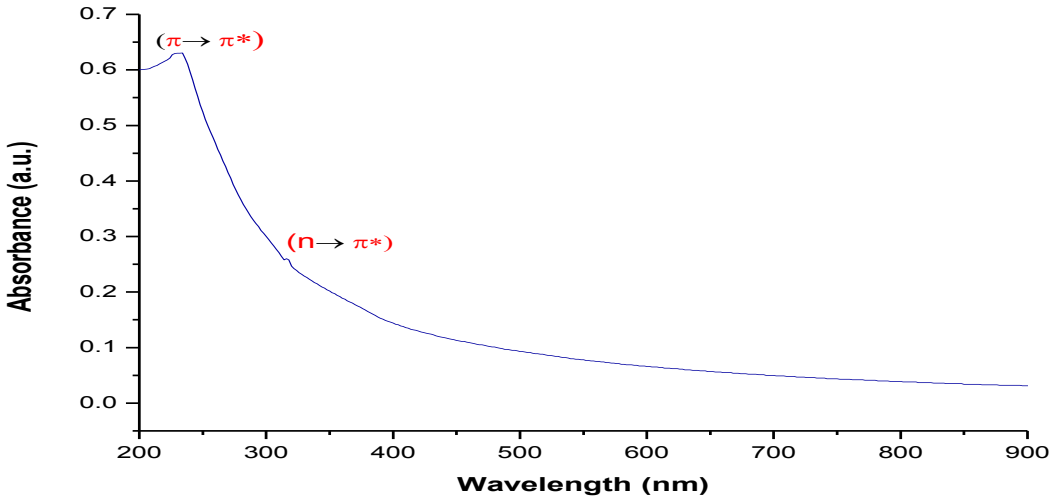


**Fig.3: UV-Visible spectra of GO nanosheets**

## RAMAN Analysis

Two peaks were observed in the Raman analysis of GO nanosheets. The first peak is the D band, which represents a disorder or defect in the GO structure, and the second peak is G band refers to the nature of the graphitic (graphitic region). Figure (4) displays the Raman spectrum of GO Nanosheets, where the vibration (G band) of GO is at 1608.23 nm and the D band of GO is at 1345 nm. The 2D peak shows around 2729.3 nm. Raman spectroscopy is a widely employed analysis to characterize the structural and electronic properties of carbon based materials, and studied the bonding nature of GO nanosheets as a nondestructive technique [18].

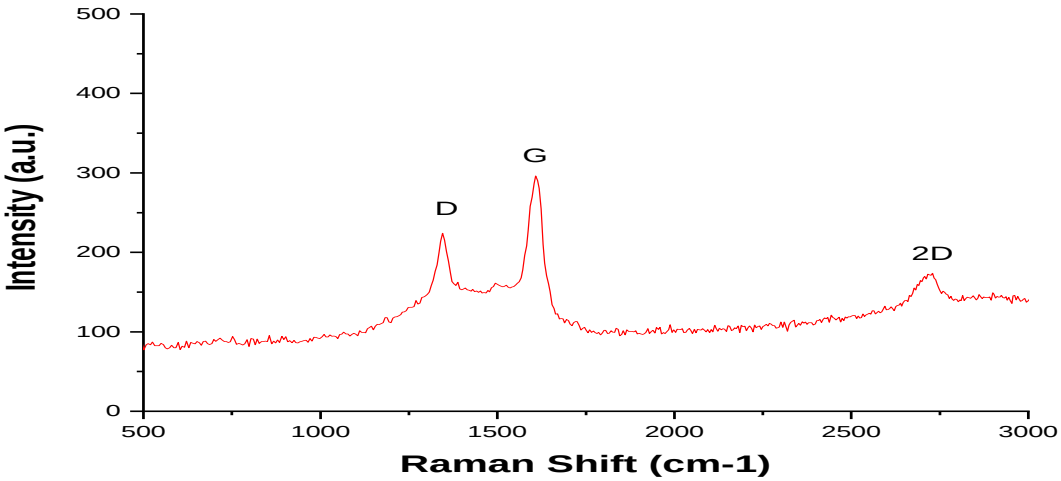


**Fig.4: Raman spectra of synthesized GO nanosheets**

## FTIR Analysis

The FTIR spectrum of GO displys a wide peak at 3420 $cm^{-1}$ which belongs to the hydroxyl group (O-H bond) and at 2896 $cm^{-1}$belongs to C-H bond, as shown in Fig. 5. A sharp peak at 2362 $cm^{-1}$ indicates carbon dioxide. Moreover, absorption peaks at the edges of GO (C = C) and (C = O)were observed at 1720 $cm^{-1}$ and 1620 $cm^{-1}$ [19].The peak at 1512 $cm^{-1}$ is related to the N-O stretching (nitro compound).Finally, the absorption peak at 1130 $cm^{-1}$is correspond to the stretching vibration of C-O .The presence of these functional groups confirms the high oxidation of graphene.

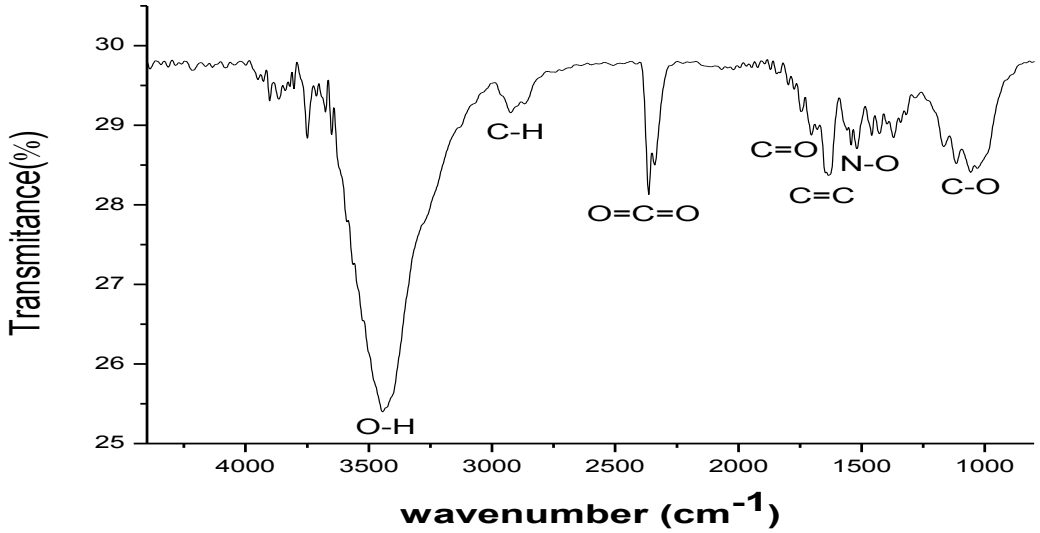


**Fig. 5: FTIR Spectra of GO nanosheets**

## XRD Analysis

XRD analysis of GO prepared by electrochemical exfoliation method shows main diffraction peak showed at 2θ =12.04° with an interlayer distance of 0.74 nm (Fig. 6). The crystalline size and the number of layers are found tobe 6.8 nm and ~9 respectively.

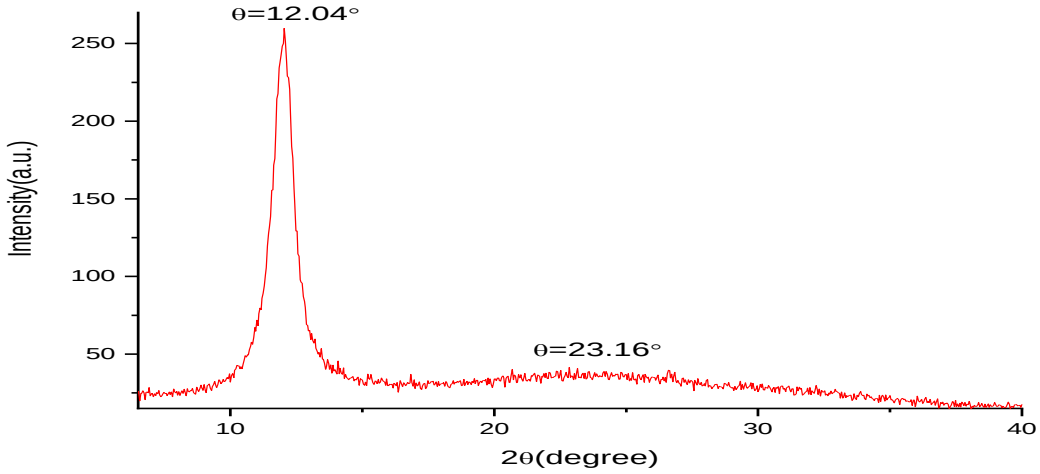


**Fig.6: XRD Spectra of GO nanosheets**

## Antibiofilm Activity of GO Nanosheets

The antibiofilm activity of different GO concentrations against *P. aeruginosa* was determined by biofilm formation assay. The results indicate that GO nanosheets, prepared by electrochemical exfoliations method, are effective to inhibit bacterial film proliferation whenever GO concentrations increased. The highest inhibition of the bacterial biofilm against *P.aeruginosa* was 400 mg/ml, while the lowest inhibition was 100 mg/ml as shown in Fig.7. Biofilms play an important role in the progression of bacterial infections. These biofilms cause antibiotic resistance and responsible for destroying the host immune system. The anti-bacterial properties of GO are connected with some physical and chemical factors such as the presence of functional groups of the oxygen and dispersibility of nanosheets, etc.) [20].Therefore, GO nanosheets are anti-biofilm material because the sharp edges of GO nanosheets penetrate and rupture the membrane of cell leading to cell destruction and death by cytoplasmic leak. The interactions between GO nanosheets and biofilms lead to deformation of the membrane and the formation of bilayer sprinkled lipid due to the enormous drag forces of graphene oxide nanosheets which consequently break down the biofilm [21, 22].

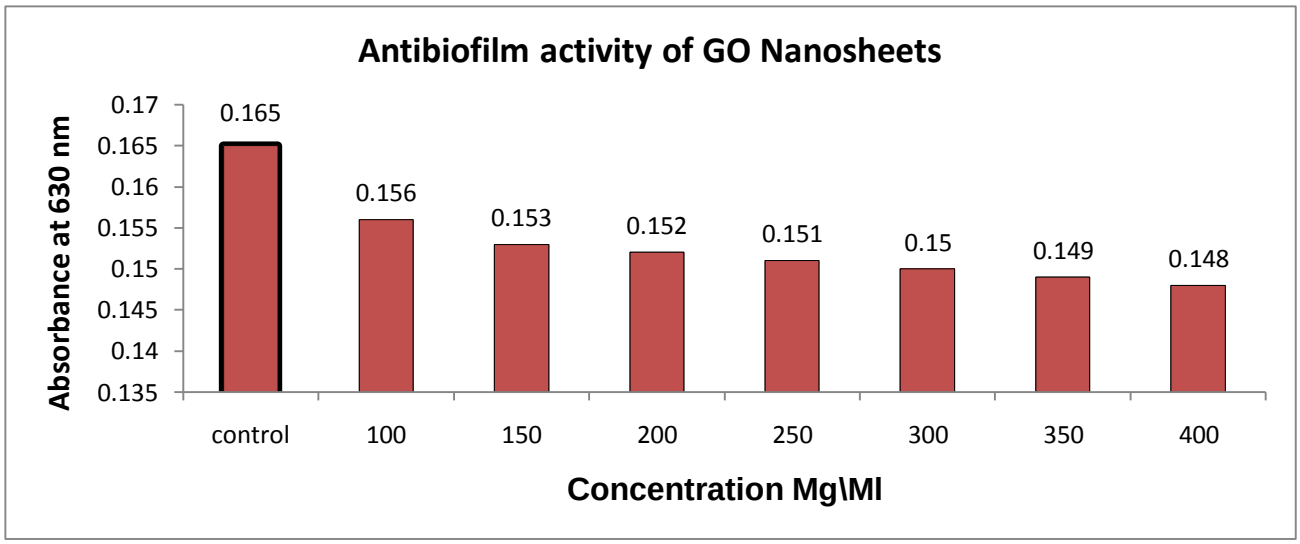


**Fig.7: Antibiofilm activity of GO nanosheets against *P. aeruginosa*. Control includes of BHI broth and *P. aeruginosa* culture**

## Conclusions

GO nanosheets have been successfully prepared by simple and cheap electrochemical exfoliation method. GO nanosheets exhibit anti-biofilm properties and thus it may be inhibit P.aeruginosa bacteria growth or kill them.

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