Antimicrobial Activity of Graphene Oxide and Reduced Graphene Oxide Nanoparticles

Su Myat Htay[1], Ye Myint Aung[2]

Abstract

Graphene oxide (GO) and reduced graphene oxide (rGO) were found to have superior characteristics for a range of applications. The properties of the synthesized GO and rGO were investigated using X-Ray Diffraction (XRD), Fourier Transform Infrared Spectroscopy (FT IR), Raman Analysis, UV-Vis, Scanning Electron Microscope (SEM), Field Emission Scanning Electron Microscope (FE SEM) and Energy Dispersive X-ray Spectroscopy (EDX) methods. After being synthesized, the GO underwent chemical reduction using hydrazine hydrate utilizing a modified Hummer's technique. Different morphologies, attributes, functional groups and crystallinities were present in GO and rGO. It was examined whether GO and rGO had any antibacterial effect against *Escherichia coli* (*E.coli*) and *Staphylococcus aureus* (*S.aureus*) by using agar well diffusion method. The 20-25 mm-wide inhibitory zones were noted.

Key words: graphene oxide, reduced graphene oxide, modified Hummer method, hydrazine hydrate

Introduction

Graphite, one of many allotropes of carbon, can provide great potential in many applications such as in electronic and functional nanocomposites (Hidayah et al., 2017). Single atomic plane layer of graphite is called graphene. Graphene Oxide (GO) is prepared from oxidation of graphite powder by Modified Hummer's Method (Mindivan, 2016). The oxidation of graphite in protonated solvents leads to graphite oxide, which consists of multiple stacked layers of graphene oxide. GO has a similar hexagonal carbon structure to graphene but also contains hydroxyl, alkoxy, carbonyl, carboxylic acid, and other oxygen-base functional groups (Smith *et al*., 2019). GO and rGO are different carbon compounds that have received a lot of attention recently as potential antibacterial agents in tissue engineering biomaterials with low toxicity to living cells (Gahnim *et al*., 2018).

Materials and Methods

Sample Collections

In this research, graphite powder (extra pure) purchased from China Aladdin Industry Corporation, sodium nitrate($NaNO_3$), sulphuric acid (H_2SO_4), potassium permanganate ($KMnO_4$), hydrogen peroxide (H_2O_2) and hydrochloric acid (HCl) were purchased from local chemical shop. All chemicals used were of analytical reagent grade.

Preparation of Graphene Oxide (GO)

Graphene oxide was prepared from graphite powder using Hmmer's Method. Firstly, 5g of graphite and 5 g of $NaNO_3$ were added to 120 mL of concentrated sulphuric acid (H_2SO_4). The mixture was ultrasonicated for 1 hour and maintained the temperature approximately 5 ºC using ice bath. After that 15 g of $KMnO_4$ was added slowly. Then a mixture was stirred by ultrasonication using ultrasonic bath for 1 hour for a homogeneous mixture. After that, 250 mL distilled water was added gradually to

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mixture followed by ultrasonication for 1 hour. The brown colour of the mixture was observed. The colour of the mixture changed to light brown. Subsequently, another 100 mL DI water was added to mixture and temperature was increased to 90 ºC and stirred for 1 hour. Finally a solution of 50 mL hydrogen peroxide and 100 mL distilled water was added to obtain a light yellow suspension. When hydrogen peroxide was added, the residual $KMnO_4$ and MnO_2 formed in the solution was also reduced to colourless soluble salts and the colour changes from brown to light yellow.

Preparation of Reduced Graphene Oxide (rGO)

100 mL of distilled water were mixed with 0.1 g of GO to synthesize rGO. The mixture was after that heated at 95 ˚C for 12 h with 1 mL of hydrazine hydrate added. The rGO was then extracted from the mixture as a black powder after filtering. Multiple repetitions of distilled water washing were performed on the finished product.

Characterization of Graphene Oxide and Reduced Graphene Oxide

The prepared graphene oxide and reduced graphene oxide were characterized by using modern techniques. Synthesized GO and rGO were characterized by modern analysis (X-Ray Diffraction (XRD), Fourier Transform Infrared Spectroscopy (FT IR), Raman Analysis, UV-Vis, Scanning Electron Microscope (SEM), Field Emission Scanning Electron Microscope (FE SEM), and Energy Dispersive X-ray Spectroscopy (EDX) method.

Screening of Antimicrobial Activity of GO and rGO

Antimicrobial activity of GO and rGO were screened by agar well diffusion method at the Biological Resources and Biotechnology Development Center, Pathein University, Ayeyawaddy Region.

Preparation of Agar Well Method

The pathogenic test organisms were incubated in nutrient broth at an appropriate temperature for 24 h. The assay medium containing glucose (1.0 g), yeast extract (0.3 g), peptone (0.2 g), agar (1.6 g), and 100 mL of distilled were placed in a beaker and the content was heated for 30 min. The assay medium was put into a sterilized conical flask and plugged with cotton wool and then autoclave at 121 °C for 15 min. After cooling down to 40 °C, one drop of suspended strain was inoculated to the assay medium with the help of a sterilized disposable pipette near the burner. About 20 mL of medium was poured into the sterilized Petri-dishes and allowed to set the medium. Two wells of 8 mm diameter each were cut out in the inoculated agar to place the sample to be tested. The volume of the sample placed in a well was 0.1 mL. The Petri-dishes were then incubated at room temperature for 24 h, and the diameter of the clear inhibition zone around the well if appeared was measured with calipers in diameter. The antimicrobial activity was determined by measuring the clear zones around the wells (Balouiri *et al*., 2016).

Results and Discussion

XRD Analysis

The crystalline nature and phase structure of the synthesized GO and rGO were determined by X-ray Diffraction (XRD) analysis. The XRD spectrum of GO is illustrated in Figure 1, GO exhibit a strong and sharp peak at $2\theta = 10.48^{o}$ corresponding to the (001) plane. This indicates the formation of highly oxidized GO

sample. The larger interlayer distance of GO must be due to the formation of oxygen containing functional groups such as hydroxyl, epoxy, and carbonyl in graphene layers. The peak corresponding to GO at 2θ= 10.48º is completely disappeared in XRD pattern of rGO which is due to the removal of functional groups and indicate complete deoxygenation and exfoliation to rGO. This peak confirms that the oxygen containing functional groups of GO could completely be removed by reduction through hydrazind hydrate.

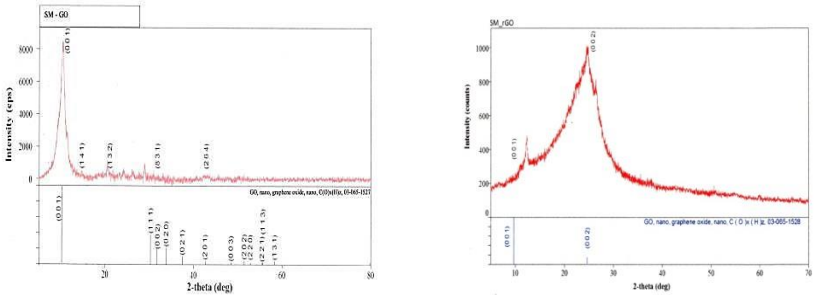

Figure 1 XRD diffractogram of GO and rGO

FT IR Analysis

The presence of different types of oxygen functional groups in GO and rGO were confirmed by FT IR spectroscopy. The FT IR spectrum GO was shown in Figure 2. The peak at 3581 cm^{-1} confirmed the presence of O-H bond (hydroxyl group) in grapheme oxide. The spectral data at 1722 cm^{-1} represented the C=O stretching and showed the carboxyl groups are situated at the edges of GO. The peak located at 1612 cm^{-1} was associated with aromatic C=C bonds. Additionally, the peaks at 1388, 1118 and 1049 cm^{-1} were ascribed to the C-O stretching (epoxy) group, 925 cm^{-1} whereas C-O-C bending respectively. Among oxygen groups, the hydroxyl and epoxy groups are located at the basal plane while the carboxyl groups are found at the edge of the sheet. This conjoining of enormous oxygen-containing groups makes GO hydrophilic and highly stable in polar solvents. After the GO was reduced, hydroxyl and alkoxy groups were significantly decreased and the C=C ring stretching at 1555 cm^{-1} was present. Appearance of the peak at 1555 cm^{-1} corresponds to the C=C stretch verify high degree of reduction of GO.

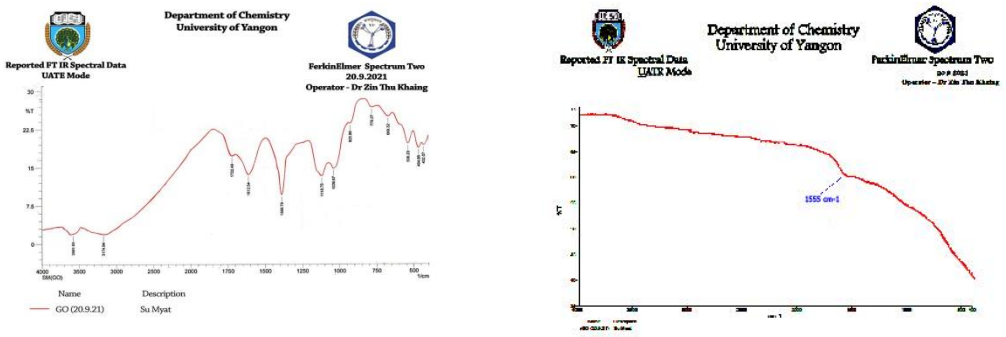

Figure 2 FT IR spectra of synthesized GO and rGO

Raman Analysis

The Raman spectra of GO and rGO reveal two prominent peaks at G band and D band. The Raman spectrum of GO revealed two prominent peaks at G band and D

band of graphene-based materials. These bands are related to the graphitic structure and local disorder. The D band is the amount of disorder in graphene and showing oxygenated groups in GO. As for grapheme oxide, the peaks at 1579 cm^{-1} (G band) and 1354 cm^{-1} (D band) are clearly present. The ratio between the D and G bands (ID/IG) can be used to estimate the degree of structural disorder. ID/IG ratio of GO was determined to be 1 whereas it is 1.16 for rGO indicates fewer defects and more disorder.

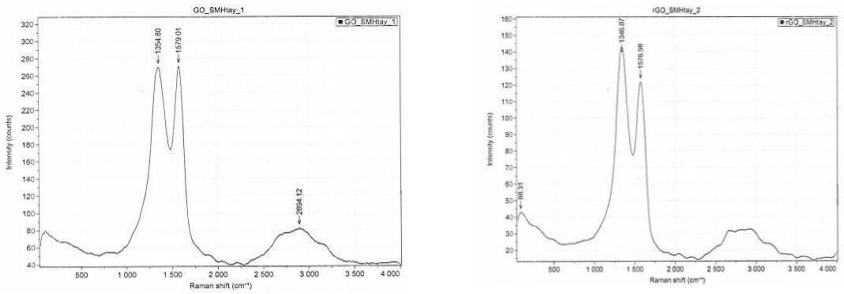

Figure 3 Raman spectra of synthesized GO and rGO

UV-visible Analysis

The UV-visible spectrum of graphene oxide and reduced graphene oxide were shown in Figure 4. The strong absorption band at 200-300 nm attributing to π-π* transition of aromatic C-C bonds to n-π* transitions of C=O bonds in sp3 hybridization. The UV-vis spectrum of GO illustrated an absorption peak at wavelength 230 nm, which demonstrated the π-π transition of skeletal C=C bonds. In rGO, the aromatic C-C bonds are red shifted to 280 nm indicating the restoration of a π-conjugation network.

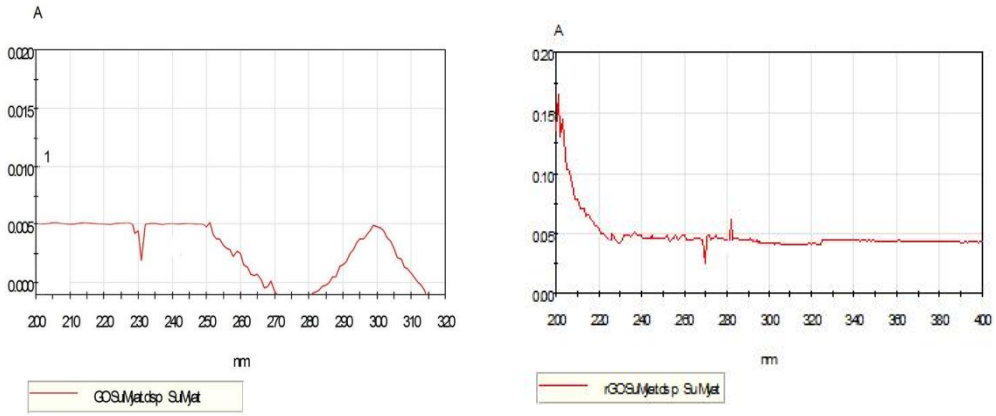

Figure 4 UV absorption spectra of synthesized GO and rGO

SEM Analysis

SEM analysis was used to study the change in morphology and surface features of the functionalized graphene oxide and reduced graphene oxide. The morphologies of GO were observed as platelet-like crumbled and wrinkle-like structure which was result of deformation upon the exfoliation and restacking processes. The various functional groups including oxygen tied on GO sample during the oxidation of graphite by modified hummer's method. These functional groups are hydroxyl and epoxy groups on the basal plane, with a small amount of carboxyl, carbonyl at the edges. As the results of SEM analysis, the graphene layers were fully oxidized to GO. The micrograph of rGO where chemical reduction by using

hydrazine hydrate, the surface contained crumpled thin sheets which accumulated to form disordered structure materials.

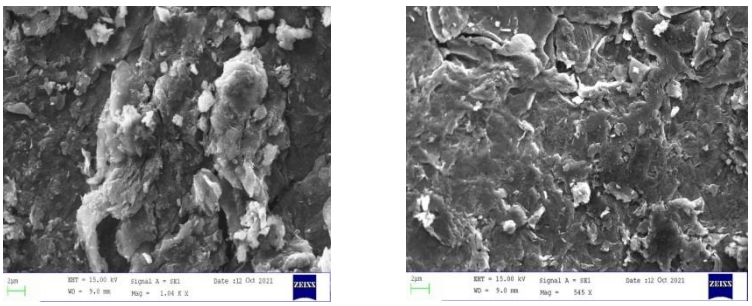

Figure 5 SEM micrographs of synthesized GO and rGO

FE SEM Analysis

Field emission scanning electron microscopy (FE SEM) is used to visualize very small topographic details on the surface or entire or fractioned objects. FE SEM image of GO was obtained on a Aligent 8500, USA and show in Figure 6. At the FE SEM image of GO, crystal and folding appearance of GO showing the sheets were slightly amalgamated with each other was observed. After oxidation of graphite, GO has spacing between the sheets and a flat structure. As the high magnification of FE SEM of GO, it can be seen that the edges of the sheets are sharp with uniform inner surface morphology.

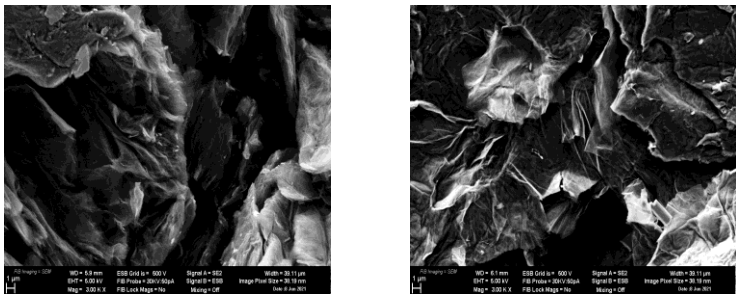

Figure 6 FE SEM micrographs of synthesized GO and rGO

EDX Analysis

The carbon/oxygen (C/O) ratio is indication of the degree of graphene oxidation, and also assesses the nature of oxygen functionalities. The degree of oxidation of the prepared graphene oxide can be estimated from C/O ratio. The EDX spectrum was shown in Figure 7.The maximum weight percentage of carbon and oxygen related to hydroxyl and carbonyl groups in GO was 69.75 % and 30.25 % . C/O ration of GO was found to 2.30. The sample of GO prepared in this work exhibited a C/O atomic ratio of 2.3, consistent with the range reported for well-oxidized graphene. The rGO contains 86.65 % and 13.35 % of carbon and oxygen shown in Table 3.14. Before the reduction of GO, the atomic ratio of carbon to oxygen was 2.3, there was still 30.25 % oxygen content remaining. After the reduction of GO, the atomic ratio of carbon to oxygen was 6.4, there was still 13.35 % oxygen content remaining. It was revealed that there was a decrease in oxygen content and an increase in carbon content when compared to that of GO.

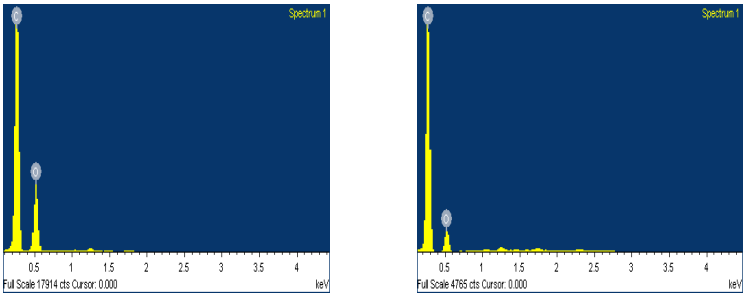

Figure 7 EDX spectra of synthesized GO and rGO

Antimicrobial of GO and rGO

The antimicrobial activity of GO and rGO were investigated *Bacillus Pumilus, Bacillus subtilis, Candida Albicans, Escherichia coli, Pseudomonas Aeruginosa* and *Staphylococcus aureus* respectively. The measurable zone diameter including the well diameter shows the degree of antimicrobial activity. The larger zone diameter, the more activity on the organism. The high activity on *Escherichia coli* and *Staphylococcus aureus*. According to antimicrobial activity the prepared GO and rGO can be used for antimicrobial agent.

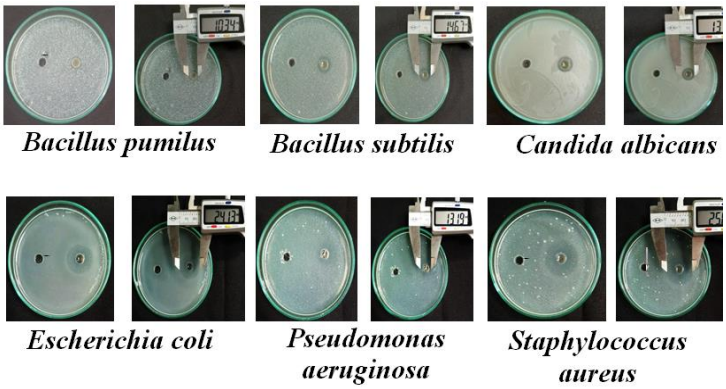

Figure 8 Antimicrobial activity of GO

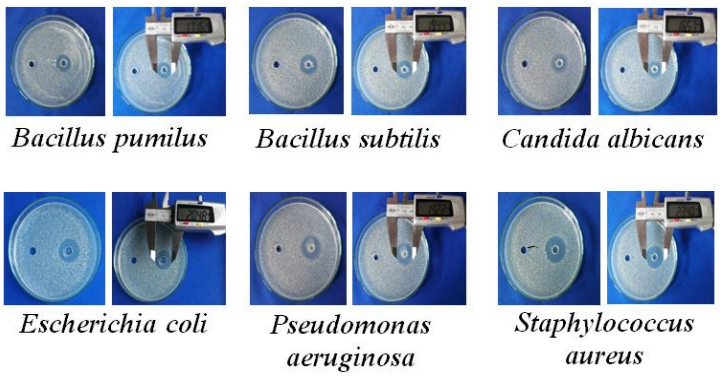

Figure 9 Antimicrobial activity of rGO

Conclusion

Graphene oxide was successfully prepared by oxidizing graphite by a Modified Hummer's Method. Chemical method results as an efficient method for the synthesis of GO by providing high yields as required for various applications. The prepared GO and rGO were confirmed by XRD, FT IR, Raman, UV-visible

spectroscopy , SEM, FE SEM and EDX result. The antibacterial activity of GO and rGO nanoparticles were observed against *E.coli* and *S aureus* bacterial. It can be used as antibacterial material for biomedical and pharmaceutical applications.These unique properties possessed by graphene oxide and reduced graphene oxide could open up possibilities to satisfy the needs in various applications.

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