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Research Article

Bioactive potential of Graphene derivatives

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ABSTRACT

Graphene and graphene oxide (GO) layers have become a hotspot so far and have been actively investigated to build new composite materials. These novel nanomaterials have great potential in applications such as electrochemical devices, energy storage catalysis, and adsorption of enzyme, cell imaging and drug delivery, as well as biosensors. In this project, we have synthesized graphene oxide and reduced graphene oxide (rGO) by chemical method and characterized with UV- Vis spectroscopy, FTIR, Raman spectroscopy and XRD. The antibacterial activity of GO and rGO aqueous dispersions toward selected microorganisms were compared. Colony counting method results show that GO has the highest antibacterial activities, followed by rGO under the same dispersion concentration.

Keywords: Graphene Oxide; Reduced Graphene Oxide; Colony counting; Antibacterial activity

INTRODUCTION

Over the years, the NCI has been screening terrestrial plants and marine organisms worldwide for bioactivity and has come up with a number of hot prospects, a number of which are in clinical trials (Anbuselvi et al., 2009; Priya et al., 2011; Chellaram *et al*, 2009 and 2011). Schiff bases are very much important in synthetic organic and inorganic chemistry because of the sterric and electronic properties (Gladis *et al*, 2011).Graphene is a flat monolayer of carbon atoms tightly packed into a two-dimensional (2D) honeycomb lattice and is a basic building block for graphitic materials. Graphene oxide is often referred to as a disordered material (Keim and Novoselov, 2007). But it is this inherent disorder, induced by the presence of functional groups, which provides opportunities for tailoring its chemical functionality (Kian P.L *et al,* 2010). Graphene and its derivatives have attracted great research interest for their potential applications in electronics, optical, thermal, mechanical, energy, materials and biomedical areas (Novoselov KS, et al 2004, Zhang Y et al 2005, Geim AK et al 2007, Jiao LY et al 2009, Geim AK 2009, Stankovich S et al 2006, Bunch JS et al 2007, Xu Y et al 2008, Dong XC et al 2009).

Graphene is strong, extremely conductive, flexible, and transparent material, and is the preferred alternative to silicon. The reduced form of graphene oxide (GO), a compound of carbon, oxygen, and hydrogen in variable ratios, is an attractive alternative to graphene because of its similar structure and components. GO can be chemically or thermally reduced to achieve graphene-like properties (Yang D et al, 2010).The antibacterial activity of GO and rGO has been attributed to membrane stress induced by sharp edges of graphene nanosheets, which may result in physical damages on cell membranes, leading to the loss of bacterial membrane integrity and the leakage of RNA (Hu Y.J et al, 2010, Akhavan O and Ghaderi, 2010). To better understand the antimicrobial mechanism, (Shaobin Liu et al, 2010) compared the antibacterial activity of four types of graphene-based materials (graphite (Gt), graphite oxide (GtO), graphene oxide (GO), and reduced graphene oxide (rGO)) toward a bacterial model, *E.coli*. Under similar concentration and incubation conditions, GO dispersion shows the highest antibacterial activity, sequentially followed by rGO, Gt, and GtO. Such graphene-based nanomaterials effectively inhibited the growth of *E.coli* while showing minimal cytotoxicity (Hu Y.J et al, 2010).

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MATERIALS & METHODS

Preparation of Graphene Oxide (GO)

Chemical synthesis of graphene oxide from graphite is becoming a promising method because of its scalability, high volume production and ease of chemical modification. GO nanoparticles were synthesized using modified hummer's method. A solution of 5 g of graphite, 100 ml of concentrated sulphuric acid (H_2SO_4 98%), and 2.5 g sodium nitrate ($NaNO_3$) were prepared. 20 g of potassium permanganate ($KMnO_4$) was then be

slowly added to the solution, while keeping the mixture in an ice bath to maintain a temperature of 35°C. The solution was continuously stirred using a standard magnetic stirrer. After an hour the solution became quite viscous and was no. The solution was held in an ice bath till it reaches 10°C. The solution was then transferred into a bigger container and diluted with 1 L deionized (DI) water. The solution was dark brown at this point. Hydrogen peroxide (H_2O_2 30%) was slowly added until the solution turns green. To aid filtration, 50 ml of concentrated hydrochloric acid (HCL 37.5%) was added to the mixture, and the solution was stirred for 30 min. Graphene oxide was recovered from the solution after washing it with distilled water till the neutral pH is reached.

Preparation of Reduced Graphene Oxide

The GO sheets were chemically reduced. 100mg of GO was dispersed in 100 mL of water and sonicated for 1 h. 200mg of $NaBH_4$ was added to the dispersion. The mixture was stirred for 30 min and heated at 125°C for 3 h. During the reduction process, the yellow-brown solution gradually yielded a black precipitate. The black solid was isolated by centrifugation, washed with water, and then finally dried.

Characterization of Nanoparticles

Some of the techniques used will be UV-Visible Spectroscopy (UV), X-Ray Diffraction (XRD), Fourier transform infrared spectroscopy (FTIR) and Raman Spectroscopy.

Antimicrobial Study

The antimicrobial effect of graphene oxide and reduced graphene oxide were evaluated against *Escherichia coli, Staphylococcus aureus, Bacillus subtilis* and *Pseudomonas aeruginosa.* Two test tubes with 10ml of nutrient broth was inoculated with 100µl of bacterial culture and added with 1ml of 80mg/ml GO and rGO respectively. 1 ml of distilled water was added to the nutrient broth and bacterial culture to form control. About 2ml of the culture was harvested separately and stored at 4°C after a time interval of 0,2,4 and 8 hours. Later, all the samples were diluted and plated. The plates were incubated at 37°C overnight. The next day, colonies formed in the plates were counted using a digital colony counter. The readings were tabulated. The same procedure was followed for all four bacteria.

RESULTS AND DISCUSSION

Go and rGO Dispersions

GO, and rGO were prepared as described in the Materials and Methods section. The GO dispersion was stable after standing still for several days. This can be attributed to the large amount of hydrophilic functional groups, such as carboxyl, hydroxyl, and epoxy groups, on GO nanosheets (Stankovich et al, 2006). Figure 5 is an SEM image of GO. GO sheets are smooth with small wrinkles at the edges. The rGO dispersion was obtained by chemically reducing the GO dispersion using sodium borohydrate (Stankovich et al, 2006). After reduction, the surface of rGO nanosheets became hydrophobic, and some black particles started precipitating (Stankovich et al, 2006). The strong van der waals forces among rGO nanosheets would facilitate the aggregation of rGO particles. Thus, plenty of rGO particles precipitate after the rGO dispersion stood still for 2h.

Characterisation

Figure 1 shows the dispersion characteristic of prepared samples in water. Dispersions of the sample were characterized by UV-Vis spectrometry. The UV-Vis spectra were obtained at 233nm. Oxidized GO still possess a layered structure, but is much lighter in color than graphite because of the loss of electronic conjugation brought about during the oxidation. The vials with parent graphite contain visible precipitates, indicating poor dispersion.

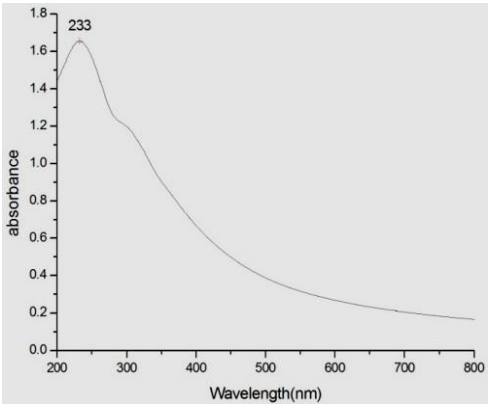

Figure 1: UV-Vis spectrum of grapheme

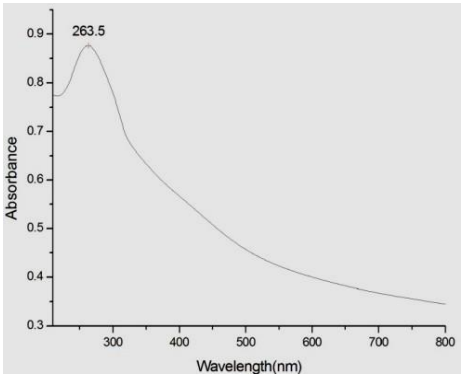

Figure 2: UV-Vis spectrum of oxide reduced graphene oxide

zThe reduced graphene oxide nanoplatelets can be dispersed into a homogeneous suspension in water via ultrasonic vibration, though the dispersion can be stable for only a few hours because of its hydrophobic nature. The UV-vis spectra were obtained at 263.5nm.

Figure 3 shows powder XRD results of graphite, GO sample and reduced GO. Raw graphite showed the very strong 002 peak at 26.44°. Complete oxidation is monitored by the total disappearance of the 0.34 nm inter graphene spacing and the appearance of a new

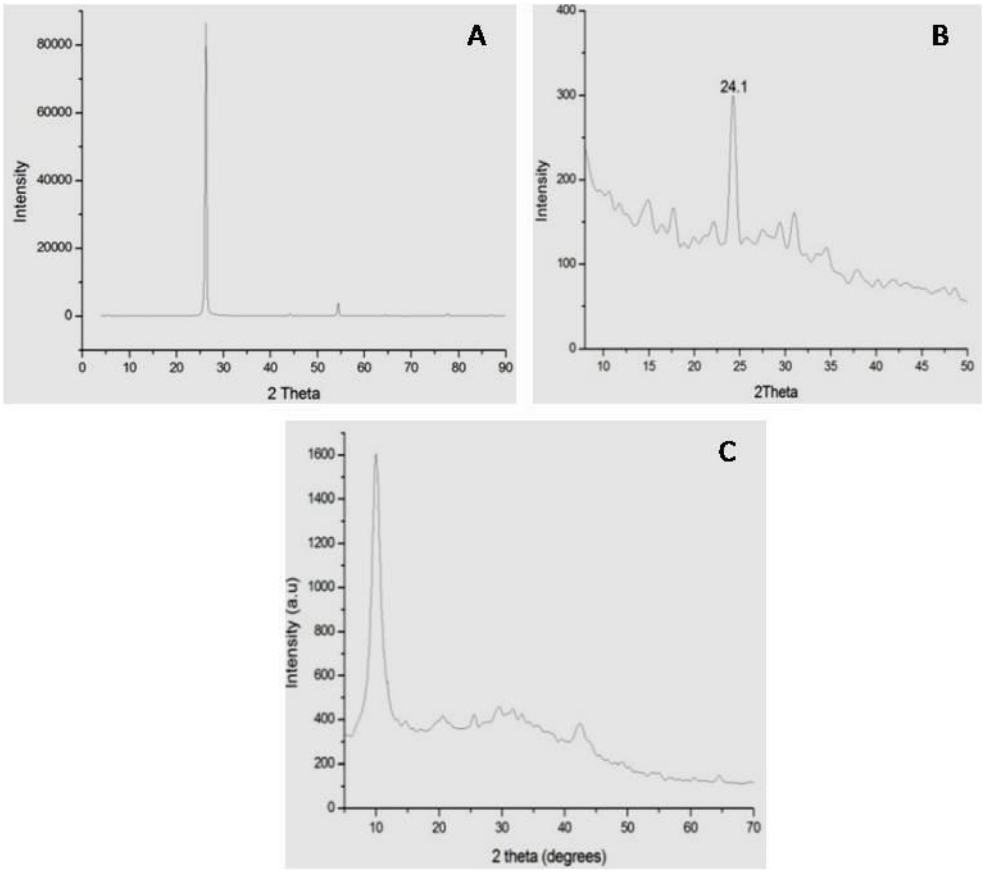

Figure 3: XRD pattern of A) raw graphite B) graphene oxide C) reduced graphene oxide

one with 0.78 nm d-spacing. Such d-spacing is significantly larger than that of single layer pristine graphene (~0.34 nm). Because the presence of oxygen-containing functional groups attached on both sides of the graphene sheet and the atomic scale roughness arising from structural defects (sp3 bonding) generated on the originally atomically flat graphene sheet, individual graphene oxide sheets are expected to be thicker than individual pristine graphene sheets. Besides, hydrogen peroxide molecules are expected to insert into the graphite layers, which will also expand the inter graphene spacing. After reduction, we discern a gradual change in the patterns to finally achieve, a randomly ordered carbonaceous layered solid, with basal spacing of 0.34 nm instead of 0.78 nm for the parent GO, indicating that the bulk of the oxygen containing functional groups is removed from GO.

Figure 4 shows FTIR spectra of graphene oxide and reduced graphene oxide. The presence of different type of oxygen functionalities in graphene oxide was confirmed at 3400 cm^{-1} (O-H stretching vibrations), at 1720 cm^{-1} (stretching vibrations from C=O), at 1600 cm^{-1} (skeletal vibrations from unoxidized graphitic domains), at 1220 cm^{-1} (C-OH stretching vibrations), and at 1060 cm^{-1} (C-O stretching vibrations) (Xu, Y. et al, 2008). FTIR peak of reduced graphene oxide presents that O-H stretching vibrations observed at 3400 cm^{-1} was significantly reduced due to deoxygenation. However, stretching vibrations from C=O at 1720 cm^{-1} were still observed and C-O stretching vibrations at 1060 cm^{-1} became sharper, which were caused by remaining carboxyl groups even after reduction (Li, D. et al., 2008).

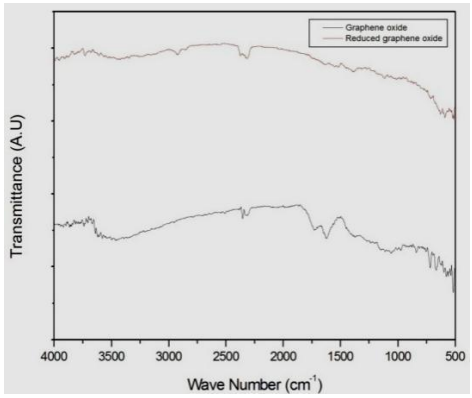

Figure 4: FTIR spectra of graphene oxide and reduced graphene oxide

Raman spectroscopy is known as an efficient method to examine the ordered/disordered crystal structures of carbonaceous materials, such as graphene. The famous characteristics of Raman spectra of carbon materials are the D and G bands (approx.1350 and 1580 cm^{-1}) which are usually attributed to the local defects/disorders (especially located at the edges of graphene and graphite platelets) and the sp^2 graphitized structure, respectively. Therefore, smaller I_D/I_G peak intensity ratios are assigned to lower defects/disorders in a graphitized structure such as graphene. The Raman spectra shown in Figure 5 display the D and G lines at

about 1346 and 1595 cm^{-1}, respectively. After reduction, the G band of reduced GO is broadened and I_D/I_G peak intensity ratio has been increased. This phenomenon can be attributed to the significant decrease of the size of the in-plane sp^2 domains due to oxidation and partially disordered graphite crystal structure of reduced graphene oxide.

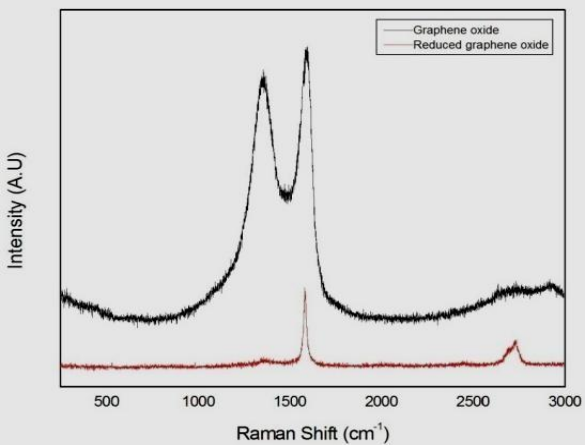

Figure 5: Raman spectra of graphene oxide and reduced graphene oxide

Antimicrobial Study

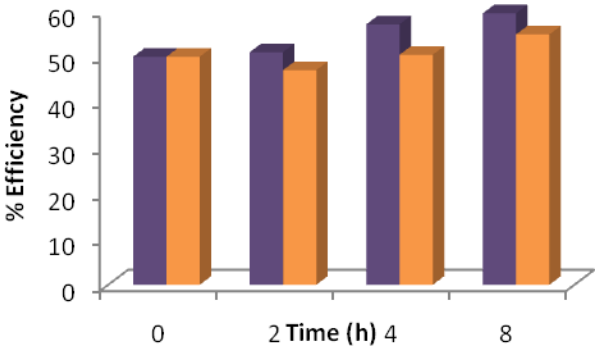

Figure 6: Graphical representation of % efficiency of GO and rGO against *E.coli*

Figure 6 shows the efficiency of GO and rGO against *E.coli* as the time increases. The amount of colonies formed in GO and rGO incubated sample are much lesser than colonies formed in control. GO has better antimicrobial activity than rGO as it has more efficiency.

The death rate of bacterial cells was determined by the colony counting method described in the Materials and Methods section. The time dependent antibacterial behaviour of two materials (GO and rGO) were studied. GO and rGO dispersions (80 µg/mL) were incubated with bacterial cultures for up to 8 h. The colonies formed were counted at different time (0h, 2h, 4h, 8h) intervals. Figure 7 shows the efficiency of GO and rGO against *B.subtilis* as the time increases as the time increases. The amount of colonies formed in GO and rGO incubated sample are much lesser than colonies formed in control. GO has better antimicrobial activity than rGO as it has more efficiency.

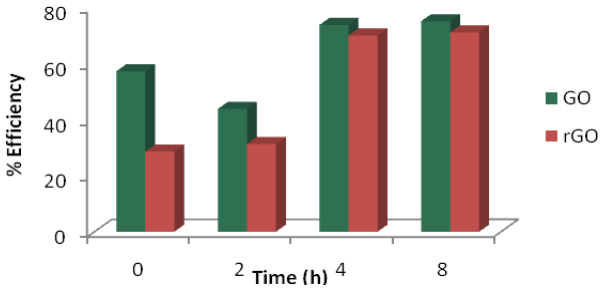

Figure 7: Graphical representation of % efficiency of GO and rGO against B.subtilis

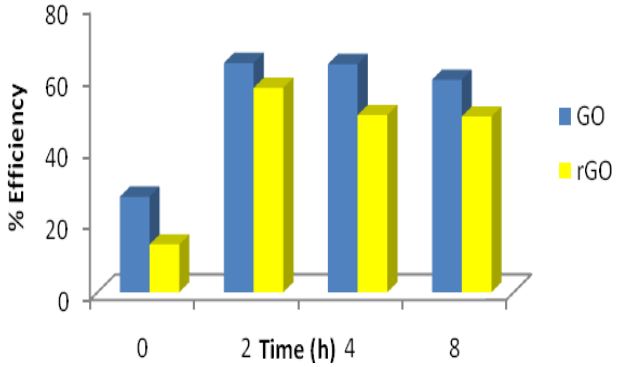

Figure 8: Graphical representation of % efficiency of GO and rGO against *S.aureus*

Figure 8 shows the increase in the efficiency of GO and rGO against *S.aureus* as the time increases. The amount of colonies formed in GO and rGO incubated sample are much lesser than colonies formed in control. GO has better antimicrobial activity than rGO.

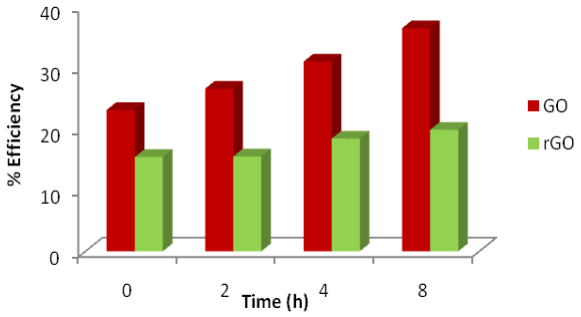

Figure 9: Graphical representation of % efficiency of GO and rGO against *P.aeruginosa*

Figure 9 shows the efficiency of GO and rGO against *P. aeruginosa* as the time increases. The amount of colonies formed in GO and rGO incubated sample are much lesser than colonies formed in control. GO has better antimicrobial activity than rGO as it has more efficiency.

The antibacterial activity of GO and rGO aqueous dispersions toward selected microorganisms were compared. Colony counting method results show that GO has the highest antibacterial activities, followed by rGO under the same dispersion concentration. Their antibacterial activities are time dependent. Most of bacterial inactivation happens in the first hour of incubation, and cell death rate increases continuously with the

increase of time. The bacterial cytotoxicity may be attributed to both membrane and oxidative stress. A three step antibacterial mechanism is applicable to graphene-based materials. In general, graphene materials, which contain a higher density of functional groups and are smaller in size, have more chances to interact with bacterial cells, resulting in cell deposition. By direct contact, graphene can induce membrane stress by disrupting and damaging cell membranes, leading to cell death. On the other hand, they display strong time dependent oxidization capacity. With the knowledge obtained in this study, we envision that physicochemical properties of graphene-based materials, such as the density of functional groups, size and conductivity, can be better tailored to either reducing their risks or increasing their application potentials.

CONCLUSION

In summary, graphene-based nanomaterials have been found to be excellent antibacterial materials. Given the superior antibacterial effect of GO and the fact that GO can be mass-produced and easily processed to make freestanding with low cost, we expect this new carbon nanomaterial could offer new opportunities for the development of antibacterial materials.

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