INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

www.ijabpt.com Volume-5, Issue-3, July-Sept -2014 Coden : IJABPT Copyrights@2014

ISSN : 0976-4550

Received: 12th Feb-2014 *Revised: 10th March-2014* *Accepted: 11th March-2014*

Research article

HIGHLY MICROBIAL RESISTANT GRAPHEME OXIDE NANOPARTICLES: SYNTHESIS, CHARACTERIZATION AND ITS ANTIBACTERIAL ACTIVITY

Vijaylaxmee Mishra*, Richa Sharma

Department of Biotechnology and Allied Sciences, Jayoti Vidyapeeth Women's University, Jaipur -303122, Rajasthan

Department of Physics Science, University of Rajasthan, Jaipur

*Corresponding author- richa.phd.15@gmail.com, +91-8890225097

ABSTRACT: The present work deigned to prepare graphene oxide nanoparticles and their antimicrobial activity has been evaluated. Graphene oxide is a singal layer of carbon arranged in a hexagonal pattern the basal planes and the edges of graphene oxide nanoparticles contain functional exogenous groups such as hydroxyl, carbonyl and epoxy group, which not only expand the interlayer distance but also make the atomic thick layer hydrophilic. Most important application in area related to transparent conductive film, composite materials, solar energy and biomedical application. Present work based on Hummer's method which is most common used for preparing graphene oxide. The result graphene oxide was characterized by UV-Vis Spectra and SEM. The graphene oxide nanoparticles absorption peak was occurred at 289nm in UV-Vis spectra. SEM analysis showed the average particles size of 50-60nm corresponding to Hummer's method respectively. Its antbacterial activity tested against gram negative and gram positive bacterial *(Bacillus subtilis, Enterobacter aerogenes, and Staphylococcus epidermis)* strain. Graphene oxide nanoparticles of Hummer's method showed the best inhibitory effect against *Staphylococcus epidermis* in comparison to other bacterial strain.

Keyword: Hummer's method, SEM, UV-Vis spectra, antibacterial activity.

INTRODUCTION

Graphene oxides have currently emerged as a new carbon- based a nanoscale particle that provides an alternative path to grapheme (By Y. Zhu. 2010). It's a single atomic layered material made up of oxidize graphite crystals which available in large quantities at inexpensive prices and other nanomaterials at very low prices. Structurally, the grapheme oxide is very similar to a graphene sheet with its base having oxygen containing groups (J.Guo et al, 2012). Since these groups have a high affinity to water molecules, it is hydrophilic and can be easily dissolved in water and other solvents allows it to be informally deposited on to wide ranging substrates in the form of thin films on networks, which makes it potentially useful for micro-electronics (M.Yousefi. (2012). Graphene oxide is a poor conductor but when it undergoes treatment by heat, or chemical reduction, mostly graphene's properties are restored one of the first commercially available materials is graphene oxide. The basal planes of the grapheme oxide are functionalizing with hydroxyl, carbonyl groups and exogenous groups which are attached at the edge (M. R. Das et al, 2011). Then oxygen containing functional groups disrupt the aromatic regions in the basal planes, so that the layer of graphene oxide consist of aromatic regions and oxidized aliphatic 6-membered rings, which leads to dissolved sp^3- hybridized property of graphene oxide (S. M. S. Chauhan and S. Mishra, 2011). Industrially produced graphene oxide could be used for wide range of application such as solar cell and hydrogen storage, transparent conductive films (S. Bykkam et al, 2013), Polymer composite (Shaobin Liu. (2012), Paper like materials, biomedicine (Xinjuam Liu. (2012), nano electro mechanical devices (Yancai Li. (2013) etc.

METHODS AND METERIAL

Synthesis of Grapheme Oxide Nanoparticles

In the objective of present study was to investigate the graphene oxide was synthesized by Hummer's method. The chemicals used for synthesis are Graphite flakes, Hydrogen peroxide, Sulphuric acid, and Potassium permanganate synthesis of graphene oxide. A solution of strong oxidizing agent was prepared (H_2SO_4 and $KMnO_4$) and graphite flakes were added to it. The mixture was continuously stirred and potassium permanganate was added to it. After mixing properly mixture was transferred to another flask containing water and H_2O_2.

The colour of the solution changed to bright yellow indicating oxidation of graphite. This mixture was then centrifuged techniques at 10,000rpm for 15 minutes and washed 3-4 times with distilled water to remove all the impurities. Which result in the synthesis of graphene oxide nanoparticles.

Characterization

UV-visible absorption spectra were measured using shima-dzu UV-1800 ENG24OV. SOFT, spectrophotometer. The surface morphology of the prepared grapheme oxide nanoparticles was done by using a Carl ZEISS-SMART SEM (Scanning Electron Microscopy) machine.

Biomedical Application

The sources of required for the testing of antibacterial activity is Nutrient broth, Nutrient agar media, Sabourand dextrose agar, Sabourand dextrose broth and microbial cultures. Bacterial (*Bacillus subtilis*, *Staphylococcus epidermis*, *Enterobacter aerogenes*) cultures were used for antimicrobial activity. Disc diffusion method was used for antibacterial activity.

Preparations of inoculums

Nutrient broth (1.25gm/100ml D.H_2O) was prepared in conical flasks and sterilized. In conical flasks clinically isolated strain of (*Bacillus subtilis, Staphylococcus epidermis, Enterobacter aerogenes*) was inoculated. The bacterial cultures inoculated nutrient broth was kept on rotary shaker 24hr. at 37˚C.

Preparation of disc

The sterile disc approximately 6mm in diameter of whatman filter paper discs were prepared by applying 100µg/ml of synthesized graphene oxide nanoparticles for bacterial cultures and also applying for Standard antibiotics streptomycin sulphate. The disc was dried in hot air oven until it gets fully dry.

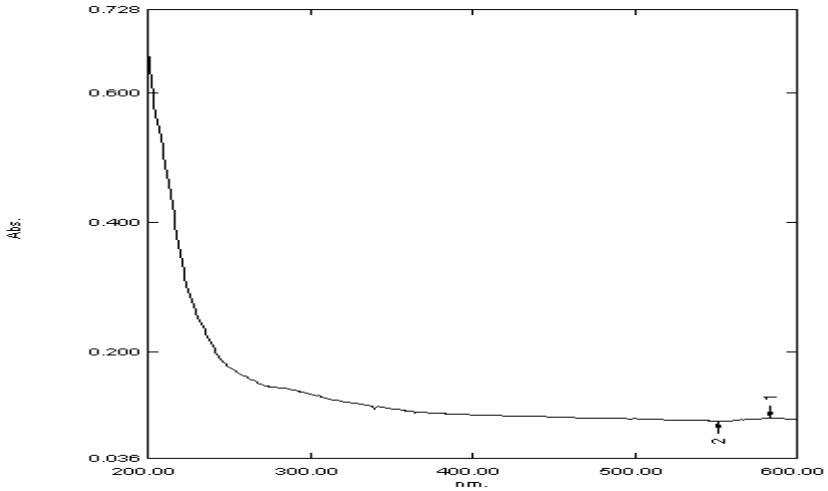
Inoculation of test plate

Nutrient agar media is prepared and sterilized. The agar suspension is poured into sterile petri-plates and allowed to solidify. Than the pathogenic bacterial strains *Bacillus subtilis*, *Staphylococcus epidermis*, and *Enterobacter aerogenes* fresh overnight cultures were spreaded evenly over the entire surface of the plate by swabbing in three directions. The sterile discs so prepared were kept in the centre of all petri plates after they were fully dried and incubated at 37˚C for bacterial cultures. The standard antibiotic discs Streptomycin sulphate purchases from CDH (laboratory reagent) New Delhi were used. The activity was clearly visible in 24hr. for bacterial cultures. The zone of inhibition was noted for all the petri plates.

RESULTS

UV-Vis Spectroscopy Analysis

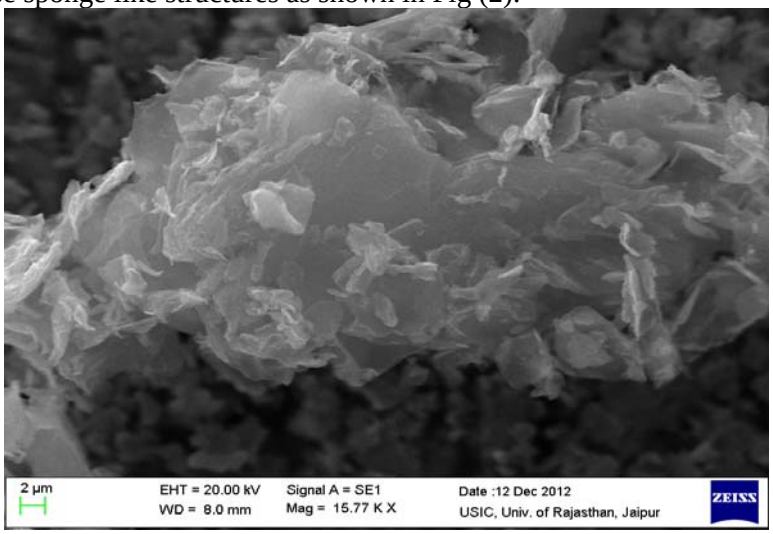
Formation and stability of graphene oxide nanoparticles in sterile distilled water is conformed using UV-Vis spectroscopy in a range of wavelength from 250nm to 300nm Fig (1). The synthesis of graphene oxide nanoparticles but after 20min. the surface Plasmon resonance of graphene occurs at 289nm and steadily increasing with the time of reaction without much change in the peak wavelength.



Figures: 1 UV- Vis absorption spectra of Graphene oxide nanoparticles was synthesized by Hummer's method. The chemical used for synthesis are graphite flake, H_2SO_4, $KMnO_4$ and H_2O_2 synthesis of graphene oxide nanoparticles.

Scanning Electron Microscopy Analysis

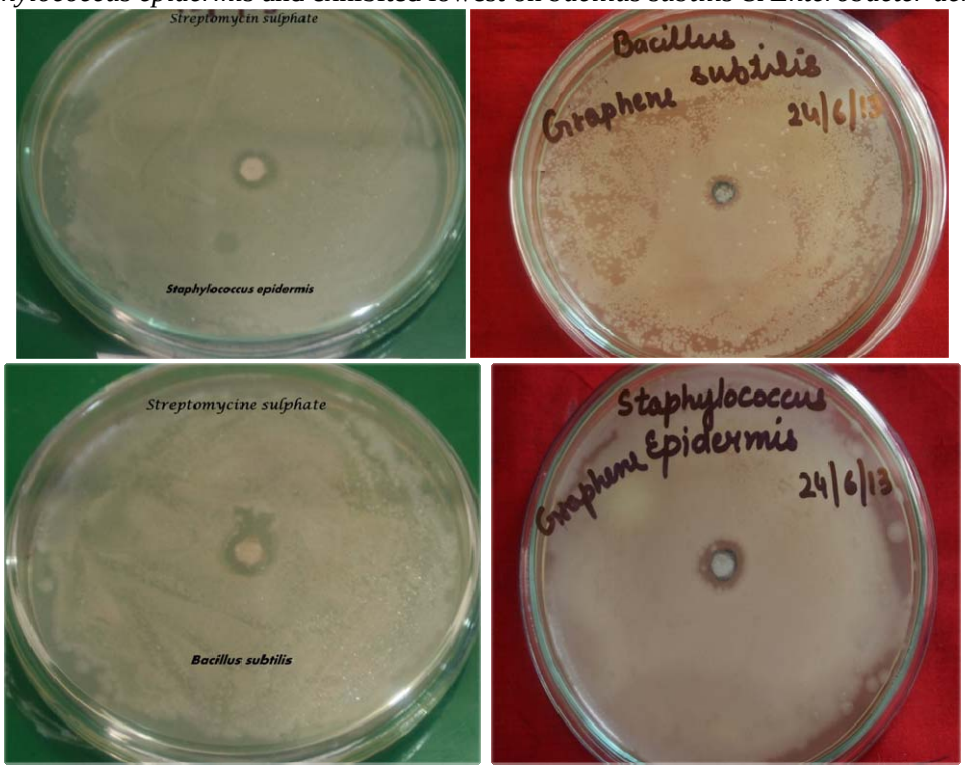
The suspended graphene oxide nanoparticles in sterile distilled water were used for scanning electron microscopy analysis by fabricating a drop of suspension on to a clean electric stubs and allowing water to completely evaporate. The SEM image of graphene oxide nanoparticles have well defined interlinked one dimensional graphene sheets, forming a porous network that resemble a loose sponge like structures as shown in Fig (2).



Figures: 2. SEM Analysis of graphene oxide nanoparticles at University of Rajasthan, Jaipur.

Antibacterial Activity Studies

Screening of antibacterial activity of synthesized graphene oxide nanoparticles through the hummer's method was examined against microbial pathogens. The activity of sample was identified by the formation of zone of inhibition after 24hr. at 37˚C. The presence of result zone of inhibition conformed inhibitory antibacterial activity of graphene oxide nanoparticles. Different type of bacterial zone of inhibition is given in the Fig 3. Graphene oxide nanoparticles highest toxicity against *Staphylococcus epidermis* and exhibited lowest on *bacillus subtilis* & *Enterobacter aerogenes.*



Figures: 3. Antibacterial activity of 100µg/ml (Bacterial cultures) graphene oxide nanoparticles and antibiotics. Comparative graphical representation of inhibition zones.

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 size of zone of inhibition is a measure of the compounds effectiveness, the larger clear area around the antibiotic, the more effective the compound. The reason could be that the smaller size of the nanoparticles which leads to increased membrane permeability and cell distruction.

Table 1. The antibacterial activity of graphene oxide nanoparticles synthesized using by hummer's method and reference drugs Streptomycin sulphate.

Tested organism	Zone of Inhibition (mm)	
	GO-NPs (100µg/ml)	Antibiotic (100µg/ml)
Staphylococcus epidermis	12mm	10mm
Bacillus subtilis	9mm	15mm
Eterobacter aerogenes	7mm	12mm

DISCUSSION

In the present work, we discoused on the functionalized groups on graphene oxide surface due to the oxidation process can be obtained. Graphene oxide nanoparticles have been synthesized by the reduction ions of aqueaus solution of graphite ions using the hummer's method. The effect of concentration of reducing substances, temperature, size and shape of the graphene oxide nanoparticles was synthesized. UV-Spectrum of Graphene oxide nanoparticles the peak at 289nm correspond to an mode of graphene oxide and related to the vibration of sp^2-bonded carbon atom and this is also supported by SEM analysis. Usually surface plasmon bond shifts depending on the particles shape, size, chemical surrounding, absorbed species on the surface and dielectric constant.

CONCLUSION

Preparation of high quality graphene materials in a cost effective manner and on the desired scale is essential for many applications. We have demonstrated the synthesis of graphene oxide nanoparticles in solution phase of Hummer's method. This method was carried out with the highest conversion level of graphite flakes to graphene oxide is formed. This technique showed that the best peak 289nm as well as revealed the structure. SEM showed the average size of nanoparticles was in the range of 50-60nm. The results showed that graphene oxide nanoparticles presented good antibacterial activity was conformed by zone of inhibition as the diameter of the zone of inhibition is high, against common pathogens. It can be concluded that the graphene oxide nanoparticles is a very effective antibacterial agent against common human pathogenic microorganism.

ACKNOWLEDGEMENTS

The authors are grateful to the Dr. Pankaj Garg founder and advisor of Jayoti Vidyapeeth Women's University, Jaipur, India for providing the technical facility in this work.

REFERENCES

By Y. Zhu. (2010). Graphene and Graphene Oxide: Synthesis, Properties and Applicatin. Adv. Mater; XX: 1-19.

J.Guo, R.wang, W.W.Tjiu, J. Pan and T. Liu (2012), Synthesis of Fe Nanoparticles @ Graphene Composites for Environmental Application. J. Harzar. Mater; 63-73.

M.Yousefi. (2012). Synthesis and Characterization of Silver Nanoparticles/ Graphene Oxide Nanocomposites an Antimicrobial Agent. 4th Int. Confer. Nano. 1358-1360.

M. R. Das, R. K. Sarma, R. Saikia, V.S. Kale, M.V. Shelke and P.Sengupta (2011). Synthesis of Silver Nanoparticles in an Aqueous Suspension of Graphene Oxide Sheets and Its Antimicrobial Activity. Col. & Sur. B: Bio. 83: 16-22.

S. M. S. Chauhan and S. Mishra (2011). Use of Graphite Oxide and Graphene Oxide as Catalysts in the Synthesis of Dipyrromethane and Calix (4) Pyrrole. Molecules; 16: 7256-7266.

S. Bykkam, V. K. Rao, CH. S. Chakra and T. Thumgunta (2013). Synthesis and Characterization of Graphene Oxide and Its Antimicrobial Activity Agents Klebseilla and Staphylococcus. Int. J. Adv. Biotech. & Res. 1(4): 142-146.

Shaobin Liu. (2012). Lateral Dimension Dependant Antibacterial Activity of Graphene Oxide Sheets. Langmuir; 28: 12364-12372.

Xinjuam Liu. (2012). UV-assisted Photocatalytic Synthesis of ZnO-reduced Graphene Oxide Composites with Enhanced Photocatalytic Activity in Reduction of Cr (VI). Chem. Eng. J. 183: 238-243.

Yancai Li. (2013). A High Performance Enzymes-free Glucose Sensor Based on the Graphene-CuO Nanocomposites. Int. J. Electro. Sci. 8: 6332-6342.