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One Pot Hydrothermal Synthesis Characterizations Of Silver Nanoparticles On Reduced Graphene Oxide For Its Enhanced Antibacterial And Antioxidant Properties.

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Abstract: *Graphene-based nanocomposite have significant applicability in catalysis, electronics, medicine, and energy. In this report silver nanoparticles (AgNPs) with Reduced Graphene Oxide (RGO) - nanocomposite was prepared by a one-pot hydrothermal process using silver nitrate as a precursor. Under hydrothermal process Graphene oxide (GO) was reduced to reduced graphene oxide (RGO), without using chemical reagents. As synthesized (Ag-RGO) nanocomposite was characterized by XRD, UV Vis-spectroscopy, Scanning electron microscope, and Raman spectroscopy. Antimicrobial activities of the composite were investigated against both Gram-positive and Gram-negative bacteria. The results demonstrate that Ag-RGO nanocomposite was a strong bactericide against Gram-negative bacteria. Antioxidant activity was evaluated for bare GO, Ag and Ag-RGO nanocomposite by DPPH radical scavenging assay. It was observed that Ag/RGO nanocomposite has enhanced antioxidant activity than bare GO and Ag.*

Keywords*: Nanocomposite, hydrothermal, antibacterial, antioxidant, Ag-RGO.*

I. Introduction

Graphene, the wonderful material composed of mono–atomic sp^2 bonded carbon atoms, which forms honey comb crystal lattice structures containing functional groups such as hydroxyl and epoxide on the two sides and carboxylic groups are present at the edges, it is the thinnest material in the world, and basic building block of many metal nanocomposite. Silver is a noble metal with remarkable optical properties, and excellent antibacterial activity [1]. Large size of metal (Ag) particles significantly suppresses its activity [2]. Graphene is a supporting carbon material for the AgNPs to be decorated on it, the large surface area and high electrical conductivity of the graphene have received significant attention. GO possess antibacterial activity against bacterial species [3]. The introduction of AgNPs on to GO prevent the aggregation of AgNps, and increases the bactericidal effect of the composite. Recently, many nanocomposites such as AgNPs-RGO ZnO [4], AgNPs incorporated chitosan in RGO ZnO [5], were explored as antibacterial agents, but to our knowledge, for the first time, the silver nano particles loaded on the RGO without using reducing agents were exhibited excellent antibacterial and antioxidant properties.

Recently AgNps decorated on reduced graphene oxide sheets has attracted many researchers due to their applications in the area of biology catalysis [6] Ag-RGO nanocomposite has been synthesized by various methods, such as ultrasonic irradiation [7] microwave irradiation [8] Ag mirror reaction [9] electrostatic force directed assembly [10] These methods and have multi-steps and require strong reducing agents. Pruneanu et al prepared AgNps on few-layers graphene by radio frequency catalytic chemical vapor deposition [11]. Won B K et al synthesized Ag-RGO nanocomposite by adding $NaBH_4$ as reducing agent [12].

Herein, Ag-reduced graphene oxide nanocomposite (Ag-RGO) was prepared eco-friendly by a one-pot hydrothermal process using graphene oxide and $AgNO_3$ as starting materials. Ag^+ ion was co-ordinated with negatively charged oxygen-containing functional groups on RGO sheets and forms the composite. The AgNPs are synonymously decorated on the surface of the RGO sheets. The prepared nanocomposite was characterized by XRD, Raman spectroscopy, SEM, HR-TEM, and UV-vis spectroscopy. The synthesized Ag-RGO nanocomposite exhibited enhanced antimicrobial properties towards gram $-^{ve}$ bacteria and possesses strong antioxidant properties.

II. Experimental

2.1 MATERIALS

All the chemicals were analytical grade. Graphite powder, sodium nitrate, potassium permanganate, Hydrogen peroxide, sulphuric acid, hydrochloric acid, $AgNO_3$ was purchased from Sigma-Aldrich. All reagents were used without further purification.

2.2 PREPARATION OF Ag-RGO NANOCOMPOSITE

In this work, Graphene oxide was prepared by modified hummer's method. Ag with reduced graphene oxide composite was prepared by the following procedure. 3mL of GO dispersion was allowed to sonicate for 1h. Then (50 mM) of required amount of $AgNO_3$ solution was slowly added into the GO suspension under continuous stirring for 1h. The contents were stirred for 2 h; the obtained slurry was transferred into a 50 mL Teflon–lined stainless steel autoclave and heated to 150 °C for 5h. After the reaction, the superannuated liquid was discarded and the precipitate was cooled to room temperature washed with water, centrifuged and dried in an oven at 60 °C and labeled as Ag-RGO composite.

2.3 CHARACTERIZATION

The prepared composite was characterized by its crystal phases using an X-ray powder diffractometer (X'Pert PRO PAN Analytical diffractometer) of Cu Kα radiation (kα-50.15406 nm) with the scanning rate of 0.01° /step. Morphology of the composite was studied by scanning electron microscope (SEM). Working at a 20 kV accelerating voltage. UV-Vis spectra were recorded using JASCO-V-530 spectrophotometer. TEM image was obtained on a (PHILIPS CM 200) transmission electron microscope at an acceleration voltage of 200 kV. The composite was dispersed in ethanol solution and then transferred onto Cu/lacey carbon TEM grids. Raman spectra were obtained on a micro-Raman spectrometer employing a 514 nm laser beam.

2.4 ANTIBACTERIAL ASSESMENT

In this study, *E. coli* (ATCC-23848), *P. vulgaris* (ATCC-27853), *S. aureus* (ATCC-25923) and *B. subtilis* (ATCC-11774), were used as the test organisms to evaluate the antimicrobial activity. The Antimicrobial activities of the prepared composite were examined, by agar well diffusion method. The microorganisms were grown overnight at 37˚C in 10 ml of Mueller-Hinton Broth for 24 hours. Mueller Hinton agar medium was seeded with 100 µl of the bacterial inoculum (1×10^8 CFU/ml). The impregnated circles containing the test (100 µg/ml) were set on the agar medium seeded with test microorganisms. The plates were then incubated at 37˚C for 24 hours to permit the growth of the microorganisms.

The cultures were adjusted with sterile saline solution to obtain turbidity comparable to the standard (1.0×10^8 CFU/ml). Petri dishes containing Mueller-Hinton agar were inoculated with the microbial suspensions. Chloramphenicol disc was used (standard antibacterial agent). The plates were incubated overnight at 37˚C for 24 hours and the diameter of any resulting zones of inhibition (mm) was measured. The plates containing the microorganisms and the composite were incubated at 37ºC for 24-48 h. The plates were examined; the diameter of the zone of inhibition was measured for each organism and expressed in millimeter.

2.5 Antioxidant activity.

Free radicals are causing various human diseases such as diabetes, atherosclerosis, cancer, hypertension, alzheimer's parkinsonism and cirrhosis During oxidation the reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced in the cell and leads to tissue damage and cell death. Antioxidant plays an important role in the prevention and treatment of diseases by removing free radical intermediates and inhibits other oxidation reactions by being oxidized themselves. The radical scavenging activity of GO, Ag and Ag-RGO composite was examined against the model stable radicals 2, 2-diphenyl-1-picrylhydrazyl (DPPH). The scavenging sites are associated with the pristine graph.

2.6 DPPH radical scavenging assay

DPPH free radical scavenging activity was examined for the prepared composite. Concentrations (50-200 µg/ml) were added, at an equal volume of a methanolic solution of DPPH. The mixture was allowed to react at room temperature in the dark for 30 minutes. Ascorbic acid was used as a standard control. After 30 minutes, the absorbance (A) was measured at 518 nm and converted into the percentage antioxidant activity using the equation: $\% = [(A_o - A_1)/A_o] \times 100$. Where A_o was the absorbance of the control and A_1 was the absorbance in the presence of composite.

III. Result And Discussion

3.1 X-Ray diffraction analysis

The X-Ray diffraction analyses were performed to examine the phase structure of GO and Ag-RGO nanocomposite. The synthesized GO displays a characteristic (002) peak at 11.9° after the hydrothermal process, the peak (002) shifted to a higher angle at about 23.3°, which clearly indicated the reduction of GO sheets. The diffraction peaks of the Ag-RGO nanocomposite are indexed as 38.1°, 44.42°, 64.5°,77.5°, corresponding to the crystal planes (111), (200), (220), and (311) indicated the presence of Ag metal in the composite (JCPDS card: 04-0783 [13].

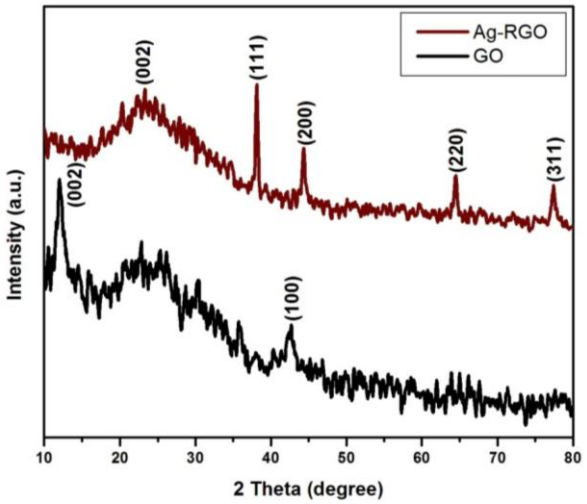


Fig.1. XRD patterns of GO and Ag-RGO nanocomposite.

3.2 RAMAN ANALYSIS

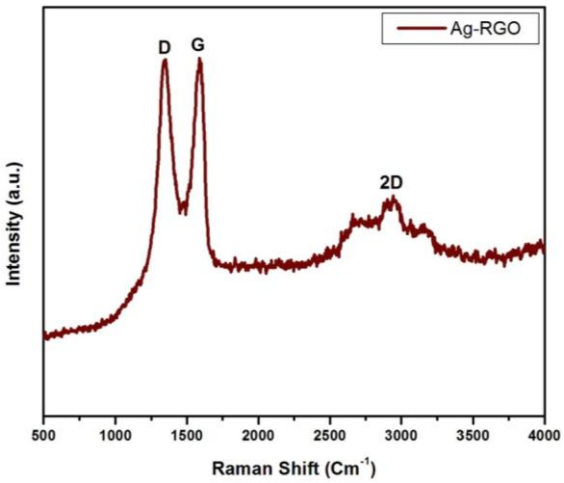


Fig.2. Raman spectra of Ag-RGO nanocomposite

The Fig.2 shows the Raman spectra of Ag-RGO nanocomposite. The reduction of GO to RGO was confirmed by this spectra. It is noted that the composite exhibits a G band at 1587cm^{-1} and D band at 1349 cm^{-1}, respectively. The intensity disorder ratio is higher after hydrothermal treatment (ID/IG=0.99) which further confirmed the reduction [14]. Raman spectra confirmed the formation of crystalline Ag with RGO during hydrothermal treatment.

3.3 UV-Visible spectra

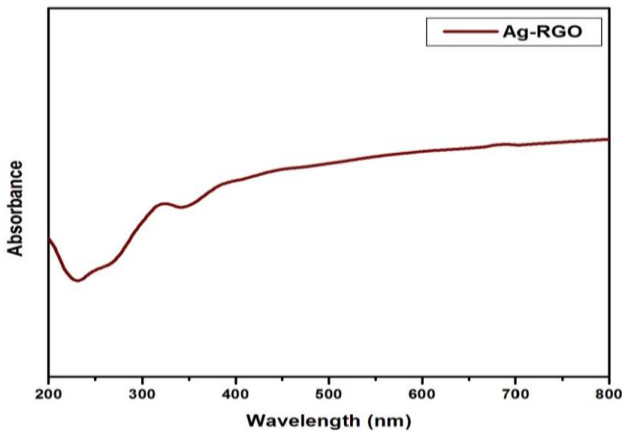


Fig. 3 Shows the UV-Vis absorption spectra of synthesized nanocomposite. UV-Vis absorption spectra of Ag-RGO nanocomposite indicated that the absorption peak of Ag nanoparticles was located at 325nm

3.4. SEM ANALYSIS

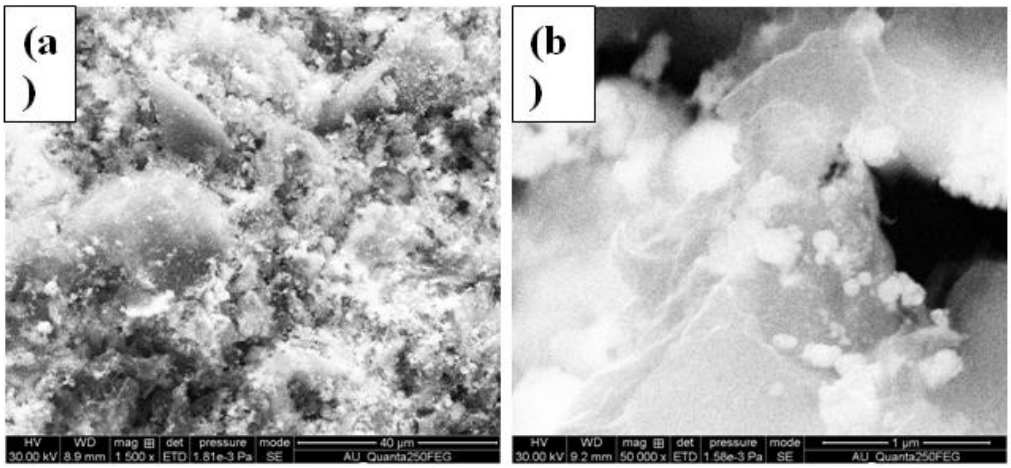


Fig.4. (a,b) SEM images of Ag-RGO nanocomposite.

The morphology of the synthesized composite was observed by Scanning electron microscope (SEM). Fig. 4 (a,b) displays the SEM images of the nanocomposite. It exhibits a uniform decoration of AgNPs on the RGO sheets. The average particle size is about 21 nm. The re-stacking property of RGO sheets was prevented by the uniform distribution of AgNPs (15).

3.5 TEM ANALYSIS

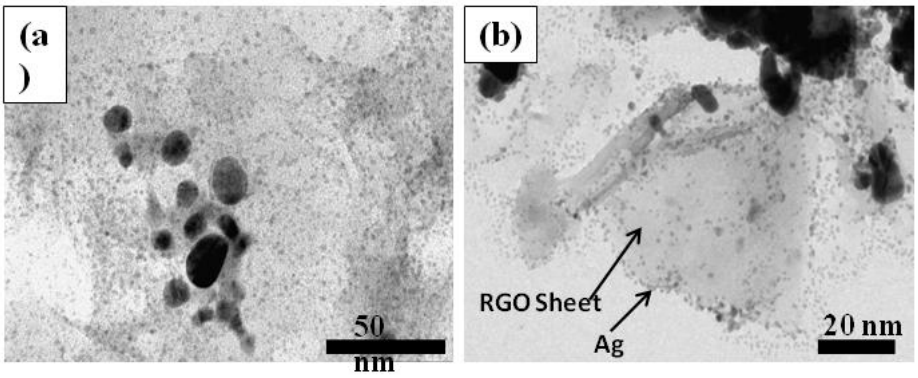
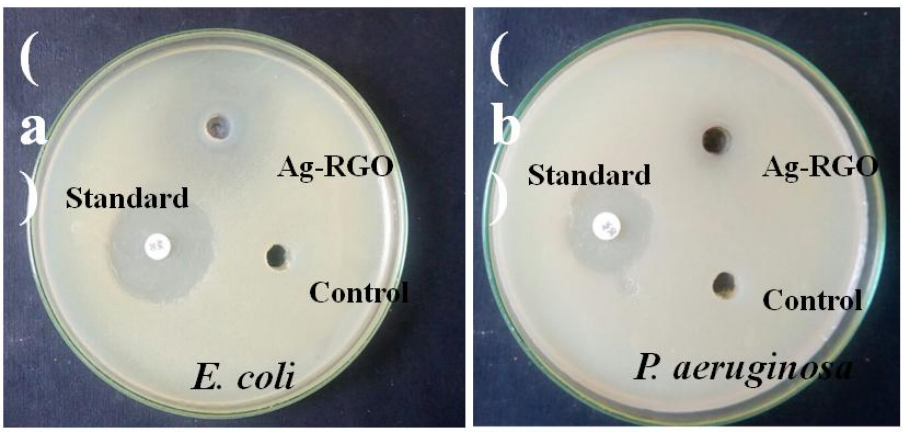


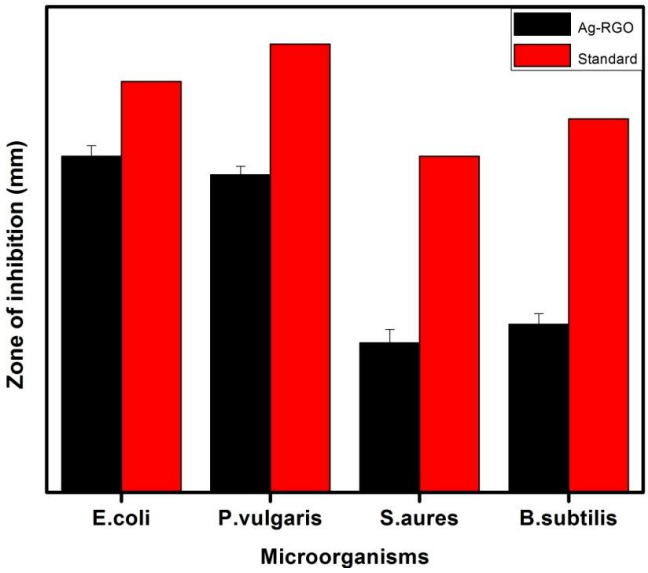
Fig. 5. HR-TEM images of Ag-RGO nanocomposite.

The TEM images of the synthesized nanocomposite clearly showed that the metal particles are monodispersed, spherical in nature; very few places have agglomeration [16]. The nanoparticles are crystalline in nature.

3.6 ANTIBACTERIAL ACTIVITY

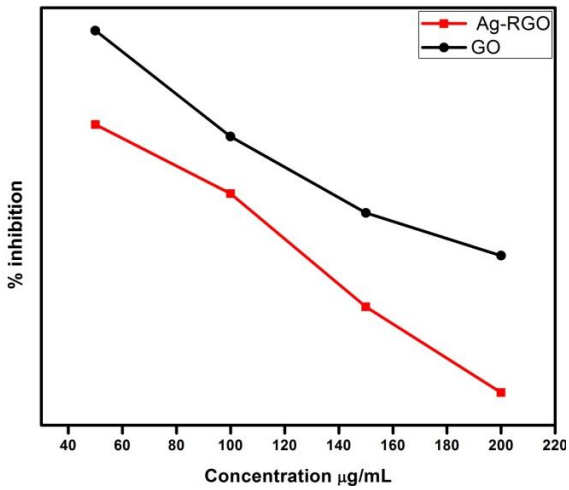


The composite Ag-RGO was examined for antimicrobial activity against microorganisms using Chloramphenicol as a control. Maximum zone of inhibition was found to be gram $-^{ive}$ bacteria *E.Coli* (18mm), *P.vulgaris* (17mm).The mechanism of the antibacterial efficacy is explained as the positive charge on Ag+ ion depletes the negatively charged cell membrane of the microorganisms through the electrostatic attractions [17]. The composite accumulates in the bacterial membrane and cause the progressive release of lipopolysaccharide molecules and protein, which degrades the membrane structure and cause the death of the microorganism [18]. The mechanism of the interaction between the components is still unclear.



3.7 DPPH radical scavenging assay

DPPH free radical was widely used to examine the scavenging ability of the composite. The antioxidants present in the composite transfer an electron to neutralize the DPPH radical. It converts DPPH radical into 1, 1- diphenyl-2-picryl hydrazine [19] the reducing property of the composite is the significant indication for its antioxidant capacity. This reduction is determined by the color change. The activity of the DPPH radical was detected and compared with ascorbic acid the standard antioxidant.



IV. Conclusion

One-step hydrothermal synthesis was successfully developed for the preparation of Ag-RGO nanocomposite. The novelty of this method is that it did not require any reducing or stabilizing agent to reduce the graphene oxide. Hydrothermal method plays an important role in reduction of graphene oxide to RGO.The synthesized nanocomposite exhibited excellent antimicrobial activity towards gram -ive bacteria than bare GO. The nanocomposite possesses strong antioxidant property. It was examined by DPPH free radical assay. The graph showed that GO has a little antioxidant effect, whereas Ag-RGO has a strong effect. Our present work

conclusively states that Ag-RGO nanocomposite is a potential candidate for the antimicrobial and antioxidant applications.

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