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Invention of GO-ZnO Nanocomposite and Antibacterial Activity of Five Waterborne Pathogenic Bacteria Species at Low Concentrations

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ABSTRACT

The GO-ZnO nanocomposite was prepared by a facile wet chemical method to achieve superior antibacterial properties without damaging other species. The crystal phase, surface area, microscopic analysis of the GO-ZnO nanocomposites were studied by P-XRD, Raman spectroscopy, FE-SEM, HR-TEM, SAED and EDAX. In the composite, ZnO nanoparticles, with a size of about 5-10 nm, homogeneously anchored onto GO sheets. Antibacterial activity was evaluated using *Aeromonas*, *Bacillus subtilis*, *Klebsiella* and *Pseudomonas aeuroginsa* bacterial species. The results showed that the composite has a much stronger ability to kill bacteria at low concentrations.

1. Introduction

Bacterial infection has become one of the world's largest public health issues, reported in millions of people every year [1]. Antibacterial drugs are now widely used to address this issue. However, the abuse of traditional antibiotics has led to the issue of antibiotic resistance, which makes it extremely difficult to treat the infection. To overcome this, several nontraditional antibacterial agents, such as carbon nanotubes [2], metal nanoparticles [3], and metal oxide nanoparticles [4], have been explored. Nanoparticles are emerging nanosized colloids of concern because of their potential negative impact on aquatic systems and water quality. At present, however, little is known about interactions between nanoparticles and microbes. The impact of nanoparticles on the growth of bacteria and viruses, especially in binary (bacteria–viruses) systems, remains largely unknown [5]. Antimicrobial properties have been demonstrated for metallic nanoparticles, metal oxide powders and nanoparticles [6].

Recently, graphene oxide (GO) is an oxidized derivative of graphene, a fascinating carbon material that has spurred significant interest in the last 10 years. GO contains a large number of oxygen bonds, such as hydroxyl and epoxy functional groups on the hexagonal network of carbon atoms and carboxyl groups at the edges [7]. These graphene related materials exhibit unique electronic, thermal, and mechanical properties, and hold great promises in potential applications, such as nanoelectronics, conductive thin films, supercapacitors, nanosensors and nanomedine [6, 8]. Several inorganic nanoparticles such as Ag, Au, Fe_3O_4, CdS, TiO_2, SnO_2 and ZnO are used to hybridize with GO or graphene to obtain antibacterial materials, controlled targeted drug carriers, optoelectronic materials, electrode materials and photo catalytic materials [9–18].

Zinc oxide (ZnO) has the intrinsic advantage of broad antibacterial activities against bacteria, fungus and virus [19-23]. In recent years, ZnO nanoparticles have been found to show much better antibacterial ability than the counterpart microsized ZnO particles [19]. At present, developing ZnO NPs with excellent antibacterial properties and less toxicity to other species is still an attractive challenge.

In this study, we synthesized GO-ZnO nanocomposites by using a wet chemical method under alkaline conditions. The antibacterial activity of the GO-ZnO nanocomposite was studied against disk diffusion method. Antibacterial activity was tested using *Aeromonas, Bacillus subtilis, Klebsiella and Pseudomonas aeuroginsa* species and waterborne pathogenic bacteria species. The results showed that the composite has a much stronger ability to kill bacteria at low concentrations.

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2. Experimental Methods

2.1 Chemicals

Natural graphite flakes (100 mesh) were commercially obtained from Sigma-Aldrich. In addition, zinc acetate ($ZnC_4H_6O_4{\cdot}2H_2O$), hydrochloric acid (HCl), sulfuric acid (H_2SO_4), hydrogen peroxide (H_2O_2), sodium hydroxide (NaOH), sodium nitrate ($NaNO_3$), Potassium permanganate ($KMnO_4$) chemicals were purchased from the Merck and used without further purification. The ethanol was supplied by China Medicine Co Ltd and throughout the experiment doubled distilled water was used.

2.2 Synthesis of Graphene Oxide

Graphene oxide was prepared by modified hummers method [24]. In a typical procedure, about 5 g of graphite flakes were added to 115 mL of concentrated (98%) H_2SO_4 in an ice bath with stirring for 30 min. A 15 g of $KMnO_4$ was added slowly to the above mixture with stirring and cooling for 30 min. Subsequently, 2.5 g of $NaNO_3$ was added with continuous stirring for 1 hr. So that the temperature of the mixture maintained below 15 °C during that time. The temperature of mixture then raised to 40 °C with water bath, and the mixture was continuously stirred for 30 min. After that, the mixture was diluted by 800~1000 mL of distilled water, the temperature of which then raised to 98 °C. The mixture was then added by H_2O_2 (30%) until gas evolution ceased followed by filtering. The color of the dispersion turned from black to yellow. The product was washed repeatedly with 1 M HCl (5%) and distilled water until the pH value of the product arrived at near 7. Then the product was dried in a vacuum oven at 60 °C to obtain graphene oxide.

2.3 Invention of Graphene Oxide-Zinc Oxide Nanocomposite

In this work, we synthesis process of the GO-ZnO nanocomposite reported in the previous study [25]. The graphene oxide was dissolved in ethanol (2 mg/ 1 mL) and sonicated for 1 hr under ambient condition. The suspension is stable for several months with no precipitation and while stirring, 0.880 mg of zinc acetate ($ZnC_4H_6O_4{\cdot}2H_2O$) was added into the mixture. Afterwards, 1 M NaOH solution was added to the mixture and the pH of the solution was adjusted by 10, after being stirred for 30 min, the mixture was reacting for 24 hrs at 140 °C under N_2 atmosphere. The

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prepared composites were then centrifuged and washed by ethanol and double distilled water for several times. The product was dried in a vacuum oven for 24 hrs at 60 °C. The chemical route synthesis is shown in Fig. 1.

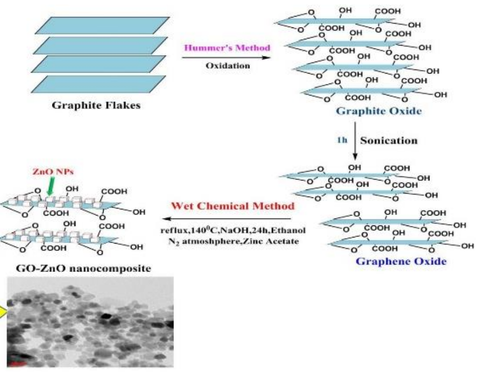

Fig. 1 Illustration for the invention of GO-ZnO nanocomposite via in wet chemical method

2.4 Characterization

The resulting GO-ZnO nanocomposites were characterized by various techniques including X-ray diffractometer (XRD) Bruker D8 using CuKα1 (1.5406 Å) and Kα2 (1.54439 Å) radiations. Raman spectra were recorded using a WiTec alpha 200 SNOM system. Morphology of as-obtained products was studied by field emission scanning electron microscope (FESEM) imaging with energy dispersive X-ray spectroscopy (EDAX) or (EDS) using a Carl Zeiss model Ultra 55 microscope operating at 5 and 20 kV. Structure analysis was conducted by transmission electron microscope (TEM) measurements on a Tecnai G2FEI F12I at 200 kV.

2.5 Antibacterial Activity

The antibacterial activity of the GO-ZnO nanocomposite was investigated by the disk diffusion assay. In this method, Luria Bertani (LB) /agar (10 g of LB and 6 g of agar dissolved in 100 mL of distilled water) were used to cultivate bacteria. The agar suspension is poured into sterile Petri-plates and allowed to solidify (Nutrient agar). Filter paper discs (5 mm in diameter) were soaked in 5 µL of the sample (GO-ZnO) at low concentrations 0.5, 0.6, 0.7, 0.8, 0.9 and 1 mg were suspended in double distilled water separately and placed on the inoculated plates and allowed to dry for 15 min, then incubated at 37 °C for 24 hrs. Then, the zone of inhibition was observed and measured for analysis of microorganism.

3. Results and Discussion

The crystalline nature and orientation of the as-synthesized GO-ZnO nanocomposite was analyzed by powder X-ray diffraction (P-XRD) as shown in Fig. 2.

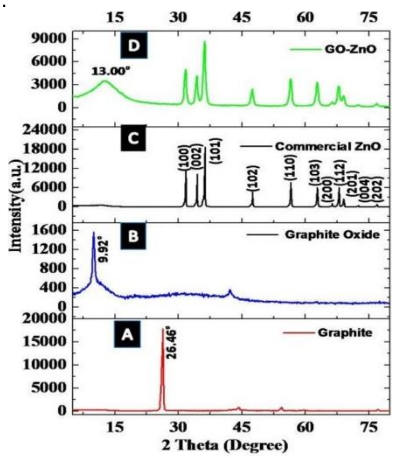

Fig. 2 Powder XRD pattern of a) graphite, b) graphite oxide, c) commercial ZnO and d) GO-ZnO composite

For graphite, an intense crystalline peak around 26.46° was observed, which represents the characteristic peak of the hexagonal graphite. After oxidation, the previously mentioned peak disappears and a weak peak appears at 9.92°, which corresponds to graphite oxide. All the samples exhibited analogous diffraction peaks in terms of ZnO framework. The dominant peaks located at 31.73, 34.37, 36.21, 47.48, 56.53,62.77, 66.30, 67.86, 69.00, 72.46, and 76.86° are indexed to (100), (002), (101), (102), (110), (103), (200), (112), (201), (004), and (202) crystallographic planes of hexagonal wurtzite ZnO (JCPDS No. 89-1397) consistent with GO-ZnO composite. The small broader diffraction peak at 13° was observed in the GO-ZnO composite, which is similar to the diffraction patterns of GO [26].

Raman spectroscopy is a powerful apparatus to characterize the crystalline excellence of graphene oxide. The presence of both carbon and ZnO can be confirmed from the Raman spectra. In Fig. 3, shows the spectra for ZnO displays a peak at 342 cm^{-1} which was assigned to the second order Raman spectrum arising from zone –boundary photons of hexagonal ZnO. The intensity peak at 452 cm^{-1} corresponds to E2 (HI) mode, which was the characteristic peak of the hexagonal wurtzite phase ZnO. The peak at 583 cm^{-1} was assigned to E1 longitudinal optical (LO) mode, attributed to oxygen deficiency defects in ZnO [27]. The peak at 1149 cm^{-1} was due to the multiple –photon scattering processes [28]. The intensity of these peaks was reduced in composites as compared to that in ZnO due to the interaction of the ZnO and GO.

From the Fig. 3, the prominent peaks of GO-ZnO at 1361 and 1607 Cm^{-1} correspond to the well documented D band and G band, respectively, where G band refers to the sp^2 carbon-type structure and D band refers to the presence of disorder in the graphene structure [29] for GO and nanocomposites suggested that the structure of GO was maintained in the composites. It was reported that pristine graphite showed a sharp G band varying from 1570 to 1584 cm^{-1} and a weak D band from 1328 to 1352 cm^{-1} [30-33]. The G band peak for GO was shifted toward longer wave number compared to that of pristine graphite, which was due to the presence of isolated double bonds in GO that resonate at frequencies higher than that of the G band of graphite [34, 35]. The peak at 2703 cm^{-1} was corresponding to the overtone of the D band, and the peak at 2923 cm^{-1} was associated with the D+G band [36]. It was worth noting that a G band up-shift from 1584 to 1607 cm^{-1} was observed for GO-ZnO nanocomposite with graphite oxide, which was generally viewed as an evidence for chemical doping of the carbon materials [37].

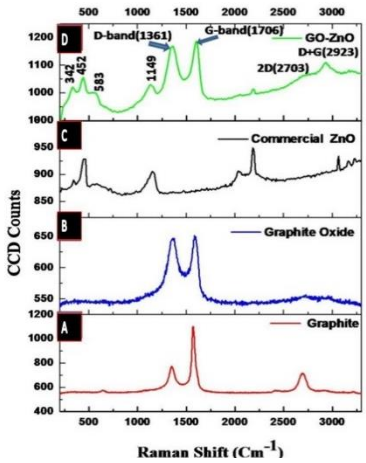

Fig. 3 Raman spectrum of a) graphite, b) graphite oxide, c) commercial ZnO and d) GO-ZnO composite

GO-ZnO was prepared by wet chemical method under nitrogen atmosphere at 140 °C for 24 hrs. The morphology of the synthesized GO-ZnO composite was studied by FE-SEM images (Fig. 4(a) low magnification and Fig. 4(b) high magnification). It is clearly observed that the surface of curled GO nanosheet is packed densely by ZnO nanoparticles, which displays a good combination between GO and ZnO. N_2 adsorption-desorption isotherms of GO and GO-ZnO nanocomposite is shown in Fig. 4. Specific surface areas of GO and GO-ZnO were measured by the multipoint BET method. Surface area of GO-ZnO (158.0 m^2g^{-1}) was lower than that of GO (186.5 $m^2 g^{-1}$) due to the high density and low surface area of ZnO cubic voids.

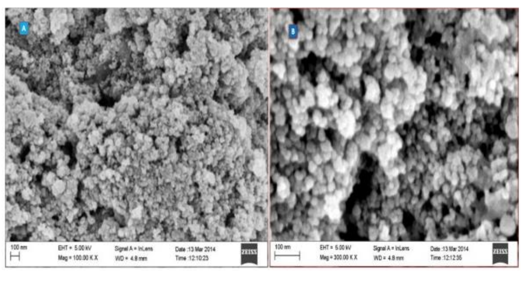

Fig. 4 a), b) Magnification FESEM images of GO-ZnO nanocomposite

The TEM image of GO-ZnO in Fig. 5((a) and (b)) clearly indicates that the GO sheets are decorated by ZnO nanoparticles. The GO sheets act as bridges for the connection between different ZnO nanoparticles, which can significantly increase the separation of photo-generated carriers and enhance the photocatalytic performance. From Fig. 5((a) and (b)) a white particle indicates the hexagonal arrangement of graphene oxide and black particles indicate the cubic ZnO nanoparticle.

Selective area electron diffraction (SAED) pattern of hybrid material shows the fusion of super lattices characteristic of the layer structure of any kind of materials. When we selectively focused the electron beam on a hybrid material, we found that apart from the super lattices we also observed cubic arrangement of ZnO (inside Fig. 5(b)) and hexagonal arrangement of GO (inside Fig. 5(a)). Fig. 5(c) shows the energy dispersive absorption x-ray spectrum (EDAX) results of the GO-ZnO nanocomposite. Zn, O, C and Cu elements are observed. The Cu element is observed because the EDAX test was performed using HRTEM with a Cu mesh substrate.

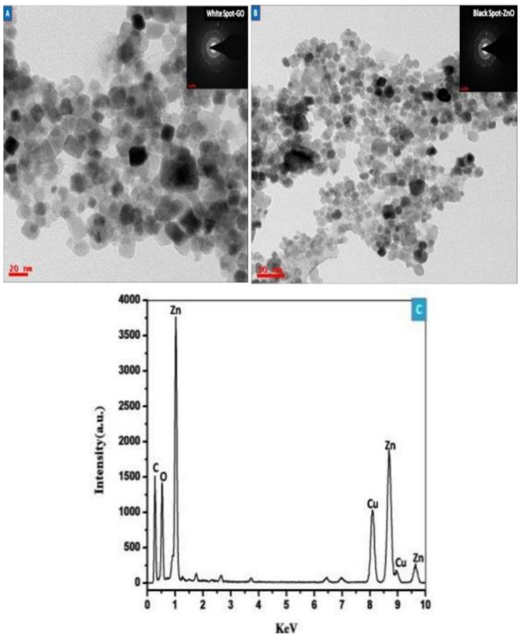

Fig. 5 a), b) TEM images of GO-ZnO nanocomposite. The inside SAED pattern of the Graphene Oxide and ZnO, c) EDAX spectra of GO-ZnO nanocomposite

An HR-TEM image of the GO-ZnO nanocomposite shows springs/curled in 5 nm range, which is usually observed only in the case of material that is crystalline. From the P-XRD it is observed the crystalline nature of the GO-ZnO nanocomposite as well as graphene oxide was confirmed by HRTEM. When focused on GO and ZnO individually, HRTEM results observed the springs on the both layers as in hexagonal arrangement (GO) and cubic form which are shown in Fig. 6.

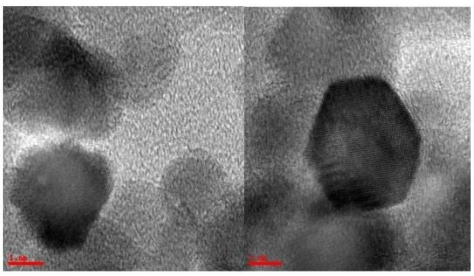

Fig. 6 HRTEM images of ZnO-GO invention materials

The agar disc diffusion method was employed to determine the antimicrobial activities of the GO-ZnO nanocomposites (Fig.7). Disc-assay was found to be a simple, cheap and reproducible practical method [38]. The inhibition zone obtained indicates maximum antibacterial activity of the prepared test sample. Results obtained in previous studies [39] also support the antibacterial potential of GO-ZnO nanocomposites. The Fig.7 showed that, with the increase of the concentration of the GO-ZnO nanocomposite, the growths of five bacterial species were inhibited more and more severely. The results indicate that the inhibitory efficiency of the GO-ZnO nanocomposite was primarily dependent on its concentration. Overall, the synthesized GO-ZnO nanocomposite can be effectively used to kill the bacteria at low concentrations.

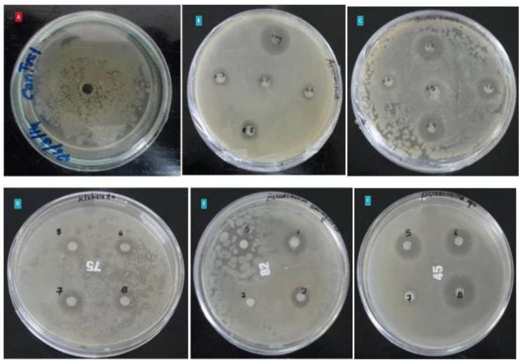

Fig. 7 Phtographs of the inhibition zone by disk diffusion method: a) Control plates with filter paper disk without any nanoparticle; b) *Aeromonas*, c) *Bacillus subtilis*, d) *Klebsiella*, e) *Pseudomonas aeuroginosa* and f) *Pseudomonas* species at low concentraions (0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 mg) of GO-ZnO nanocomposite

4. Conclusion

A fashionable GO-ZnO nanocomposite has been developed by wet chemical method and characterized by the P-XRD, Raman spectroscopy, SEM and TEM. The process parameter for the size control of ZnO on graphene Oxide has been investigated. The composite exhibits antibacterial activity in *Aeromonas, Bacillus subtilis, Klebsiella, Pseudomonas* species and *Pseudomonas aeuroginsa* bacterial species. The results showed that the composite has a much stronger ability to kill bacteria at low concentrations. The increases of the concentration of the GO-ZnO nanocomposite, the growth of five bacterial species were inhibited more and more severely. With the knowledge obtained in this study, we visualize that physicochemical properties of graphene-based materials, such as the density of functional groups and metal oxide, size, and conductivity, can be increasing their application potentials.

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