

Preparation of silver nanoparticles loaded graphene oxide nanosheets for antibacterial activity

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Abstract. A simple, facile method to fabricate successfully silver nanoparticle (AgNPs) decorated on graphene oxide (GO) layers via grafted thiol groups. Samples were prepared with different concentrations of AgNO₃. Resulting AgNPs were quasi-spherical in shape and attached on the layers of GO. Physical properties were confirmed by X-ray diffraction (XRD), zeta potential, dynamic light scattering (DLS), Fourier transform infrared (FTIR) spectra, thermogravimetric analyzer (TGA), transmission electron microscopy (TEM) and field emission scanning electron microscopy (FE-SEM). Antimicrobial test was effectively showed using MRSA (*Staphylococcus aureus*). The GO-Ag NPs with appropriate Ag NPs content of 0.2M AgNO₃ exhibited the strongest antibacterial activity at 48.77% inhibition after 4 hours incubation.

1. Introduction

Antibiotic resistance is one of the biggest threats to modern society. The medicines used to treat dangerous bacterial infections are rapidly losing effectiveness that creating side effects. Nowadays, scientists have found that bacteria are less prone to develop resistance against silver nanoparticles (Ag NPs) than conventional antibiotics[1]. Recently, graphene oxide (GO) is also the potential materials which becomes popular with many applications, one of them is antibacterial treatment. The principle of antimicrobial may come from GO traps bacteria while Ag kills bacteria[2, 3]. Hence, the combination of GO and Ag NPs may suggest to be an efficient material due to biocompatibility, simple synthesis and low cost. In this work, Ag NPs was decorated onto GO sheets via grafted thiol groups. Despite of myriad studies are carried in literature but there are less report and quest about the difference between graphene oxide and graphene oxide – silver composite bacteria treatment. Herein, to clarify this matter, our main focus of this study is to evaluate the characterization of these two kind materials and analyze their antibacterial properties.

2. Materials and methods

2.1. Chemicals

Graphite powder, NaSH, Propidium iodide were purchased from Sigma-Aldrich, St. Louis, Missouri, USA. Potassium permanganate (KMnO₄) powder was purchased from Nihon Shiyaku Industries .Ltd, Osaka, Japan. Sulfuric Acid (H₂SO₄) (95-98%) solution was purchased from Scharlab S.L., Barcelona, Spain. AgNO₃ was purchased from Mallinckrodt Baker Inc., Paris.

S. aureus (ATCC 25178) was obtained from Bioresource Collection and Research Center, Taiwan. Bacteria were cultivated in agar disc and keep in fridge. Before using was growth overnight in Difco™ Nutrient Broth under aerobic conditions at 37°C using a FIRSTEK S300R orbital shaker incubator for 12 hours.

2.2. Preparation of GO-Ag nanocomposites

Graphene oxide was prepared from graphite powder by modified Hummer's method[4]. GO (0.5g) was dispersed in 30mL deionized water (DI) in 50mL beaker while ultrasonic for 20 minutes. NaSH (8g) was gradually added by stirring and then was ultrasonic at 40°C. The mixture was then



maintained under stirring for 20 hour at 55⁰C to produce thiol groups on the GO surface. The product was filtered and washed with DI water and dried in the vacuum at 50⁰ for 3h. The thiolate GO powder 0.1g was dispersed in DI water 30mL by sonication for 30 minutes. Then series of aqueous solution of 0.1M and 0.2M, 0.25M AgNO₃ was added to thiolate GO solution under stirring, respectively. Subsequently, 50mL 0.1M NaOH was added and stirred for 20 hours. The GO-Ag powder was obtained by centrifugation at 10000rpm for several times, and then dried in vacuum in 40⁰C. Power products was then using dialysis tubing in order to remove unreacted Ag and loose bound of Ag NPs. Finally, we get GO-Ag with concentration named GO-Ag 0.1M and GO-Ag 0.2M, GO-Ag 0.25M.

2.3. Characterization of GO and GO-Ag nanocomposites

The latex of composite suspension were determined for transmission electron microscopy (TEM, JEM 2000EXII, JEOL, Tokyo, Japan) and Energy-dispersive X-ray spectroscopy (EDX, model JSM-7500F, Hitachi High- Technologies Corp., Tokyo, Japan), laser light scattering (Zetasizer, 2000 HAS, Mavelvern, Worcestershire, UK), UV-visible spectrophotometer (V-650, Jasco, Tokyo, Japan). The latex of composite were dried and the powders measured by Fourier Transform Infrared Spectroscopy (FTIR), X-ray diffraction (XRD, model D5005D, Siemens AG, Munich, Germany).

2.4. Growth curves of bacteria strains after incubators with samples

Bacteria was centrifuged in 10.000rpm for 5 min by using Hitachi, the supernatant was discarded, precipitate was collected and diluted with 12ML PBS, 450μL of the above bacterial suspension was then transfer into 48 well-plate. Following this addition, 50 μL of each sample was added (PBS is used as a control sample) and kept in incubator for 1 hour. Consequently, 10μl of sample mixture was taken out and changed into 96 well-plates, 90μL NB was added afterwards. All treatments were performed two times in triplicate, measured by BioTek microplate reader with OD 600nm after each an hour during 4 hour. The inhibition efficiency was calculated by using following equation[5]:

$$\text{Inhibition (\%)} = \left(1 - \frac{OD_{\text{sample treatment}}}{OD_{\text{control}}} \right) \times 100 \quad (1)$$

Results were expressed as mean ± standard deviation. Comparative studies of means were performed using excel software with n=3.

3. Results and Discussion

3.1. Physicochemical characteristics of GO and GO-Ag nanocomposites

The Ag NPs attached on the graphene was confirmed by UV-visible spectra. Figure 1(a) shifted the peak at 410nm which was consistent with the surface plasmon resonance phenomena of Ag NPs formulation[6] while pristine GO exhibited a typical peak around 247nm corresponding to the C=C aromatic bonding[7]. The XRD was used in order to examine the GO and GO-Ag crystalline structure. As shown on Figure 1(b), the graphene oxide powder exhibited a sharp diffraction peak (001) at $2\theta = 11.7^\circ$. This results indicated the successful synthesis and matched with references data for GO sythesis [3, 8-12]. GO-Ag composite shifted the peak values at about 38.1^o, 44.3^o, 64.5^o and 77.5^o which are assigned to the (111), (200), (220) and (311) crystal lattice planes of face-centered cubic (fcc) Ag nanoparticles, respectively while the peak of GO disappeared, that was reported because the metal-nanoparticles was attached onto the inlayers may lead to the sheeting the signals of graphene oxide peaks [13]. Prominently, the sharp peak of 38.1^o confirmed that the nanoparticles are composed of pure crystalline Ag [14, 15]. In Figure 1(c), FTIR illustrated the functional groups that contains on GO and GO-Ag sample. GO spectrum were observed at 3404, 1718, 1625 and 1055 cm⁻¹. Those spectra associated with the stretching vibration of C-OH (hydroxyl), the stretching vibration of C=O, the vibration of C=C (possibly due to the skeletal vibration of un-oxidized graphite domains), and C-O stretch located, respectively. Based on the presence of those oxygen-containing groups such as carboxyl, hydroxyl and epoxy, it confirmed that the result of oxidation process is GO. This FTIR results are also similar with previous studies [8]. Besides, after decoration with Ag NPs, compare with the spectrum of graphene oxide, the -OH groups became stretched out while the intensity related to

the bonding signal slightly decreased due to the interactions between Ag^+ ions and the oxygen-containing groups on the GO sheets, which may suggest the nucleation sites for anchoring and growth of AgNPs [16]. Remarkably, there has a bonding of thiol group (C-SH) at 910 appeared in Ag-GO sample.

Figure 2(a), (b), (c), (d) shows the TEM images of GO, GO-Ag 0.1M, GO-Ag 0.2M and GO-Ag 0.25M samples, respectively. Fig 2. (a) shows the typical flaky structure like winkle, wavy, transparent pure graphene oxide. At Figure 2(b) and (c) have displayed obviously small dots stick on the layer of graphene layers while at Figure 2(d) the dots tend to aggregate each other which form high ratio volume phenomena. Energy-dispersive X-rays spectroscopy was used to detect the element composition of sample. At Figure 3, the signal of Ag peak was clearly observe by EDS spectrum which indicates the presence of Ag NPs therein GO-Ag samples.

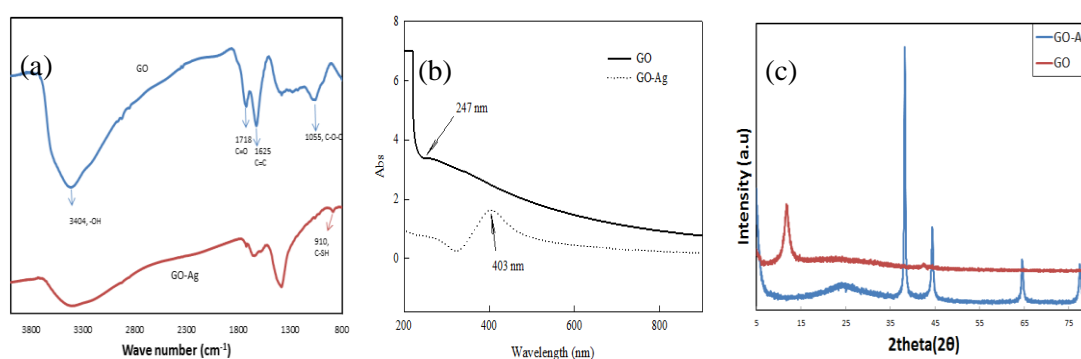


Figure 1(a) illustrates FT-IR, (b)UV-visible and,(c)XRD patterns of GO and GO-Ag

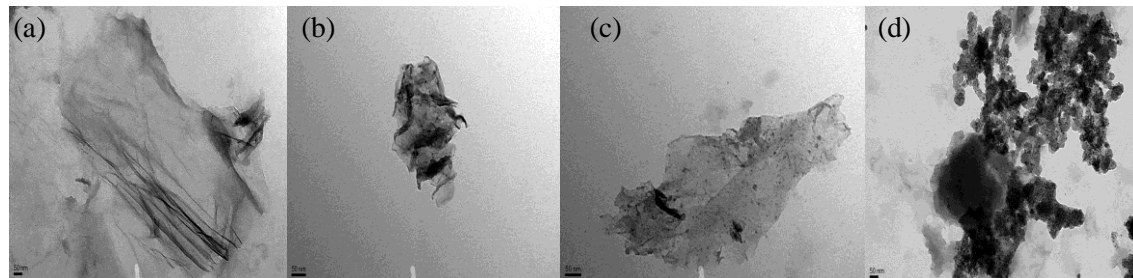


Figure 2(a) TEM images of pure-GO, (b)GO-Ag 0.1M, (c)0.2M, (d)0.25M

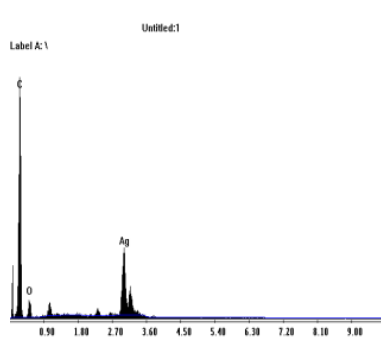


Figure 3. EDS spectrum of GO-Ag nanocomposite confirming the presence of Ag, C and O

3.2. Antibacterial of GO and GO-Ag nanocomposites

Elisa reading with 600nm absorbance was performed by imbedded of each sample (PBS was used as a control) after each hour reading during 4 hours. Time curves growth was described in Figure3 and inhibition results in details has shown in Table 1. Obviously, GO-Ag 0.2M which was the most

significant decreased after time incubation. For more details, table 1 showed GO had no inhibition at all while GO-SH still had antibacterial activity even lower than other GO-Ag and gradually increased from GO-Ag 0.1M to GO-Ag 0.2M from 10.8%, 16.7% and 48.77%, respectively. Interestingly, we find that the inhibition of GO-Ag 0.25M was 19.21% lower than GO-Ag 0.2M 48.77% even though the AgNO_3 amount is higher. This is because Ag NPs in GO-Ag 0.25M tends to agglomerate reducing the contact surface area of Ag NPs with bacteria. This suits with TEM images about the distribution of Ag NPs on GO sheets.

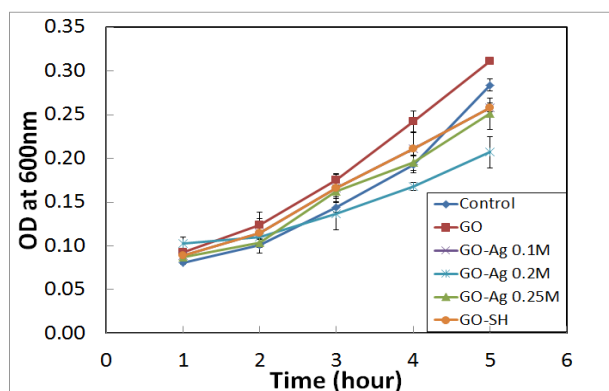


Figure 4. Illustrates growth curves of optical density measurements of bacteria at a wavelength of 600nm

4. Conclusion

The graphene oxide loading silver nanoparticle based on thiol groups was successfully fabricated. The XRD, FTIR, UV-visible spectra results were confirmed the property of sample. The silver was decorated well-distributed to the layer of graphene oxide by TEM observation. The difference of silver ratio contributed to the antibacterial activity at concentration. At 0.2M AgNO_3 was obtained the effectively antimicrobial at 48.77% higher than that one in other concentrations. That is reasonable results because many studies consider that too much silver may lead to agglomeration[17]. The synthesized GO-Ag NPs may have potential in antibacterial in particular and in biomedical in general.

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