Preparation and antibacterial activity of graphene oxide/cuprous oxide/zinc oxide nanocomposite

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Abstract

In this study, an antibacterial GO/Cu2O/ZnO nanocomposite was synthesized by a hydrothermal synthesis method, and its phase and microstructure were characterized by a series of test methods. The results showed that the synthesized cuprous oxide nanoparticles and the added zinc oxide nanoparticles were uniformly dispersed on the surface of graphene oxide, and did not cause the agglomeration of the nanoparticles. The graphene oxide successfully made enhanced the effective surface area of the metal oxide nanoparticles due to its adsorption capacity and chargeability. Thereby enhancing the antibacterial activity of the nanocomposite, reaching a 100% antibacterial rate.

1. Introduction

Graphene oxide is a raw material for functionalized graphene. It contains a large number of oxygen-containing functional groups such as hydroxyl, carboxyl and epoxy bonds, and can be divided into single layer or multilayer [1–6]. Since 2010, based on the excellent antibacterial properties of graphene, a large number of researches on graphene and its composite antibacterial materials have been reported, further confirming the great potential of graphene materials in antibacterial applications [7–11]. Graphene oxide is a raw material for functionalized graphene. It contains a large number of oxygen-containing functional groups such as hydroxyl, carboxyl and epoxy bonds, and can be divided into single layer or multilayer [12–16]. Because of GO has a high surface area and its surface is rich in various functional group-contains, it can be used to uniformly disperse metal nanoparticles on its surface, thereby reducing nanoparticle agglomeration, and it is a good carrier for nanocomposite preparation [11]. Researches on the antibacterial properties of graphene oxide have become increasingly common, but the antibacterial properties of nanocomposites loaded with two or more metal oxides using graphene oxide as a carrier are still few.

As a photosensitive antibacterial material, zinc oxide (ZnO) has certain antibacterial advantages [17, 18], antifungal material [19]. The bactericidal mechanism of nano-ZnO is manifested by the interaction between the released reactive oxygen species and bacterial surface proteins or bacterial genetic material in cells [20]. The material selected as an antimicrobial agent must have two characteristics, one is effective sterilization, and the other is good bio-compatibility [21]. However, ZnO nanoparticles are easy to agglomerate in solution due to their surface effects and intermolecular forces [22]. Therefore, using surfactant, dispersant, etc can uniformly disperse zinc oxide nanoparticles and reduce the agglomeration between particles [23].

The test results of Nguyen Huu Hieu et al proves that the nanocomposite of graphene oxide and zinc oxide nanoparticles has better antibacterial properties than a single component. At the same time, it is found that when the mass ratio of graphene oxide to zinc oxide nanoparticles is 1:1, the anti- Staphylococcus aureus performance is the strongest, and the anti- E. coli activity is higher. It can be used as a potential antibacterial material and has a wide range of applications [24].
Cu2O has become a popular antibacterial agent and is widely used because of its simple preparation method, low price, and good antibacterial performance. Cu2O interacts and kills bacteria by releasing copper ions and producing active oxygen [25]. Therefore, the morphology distribution, dispersion of Cu2O nanoparticles and the release ability of copper ions have a great influence on their antibacterial properties. For example, Feng et al conducted related studies on the cytotoxicity of Cu2O nanoparticles with different morphologies [26].

Zhao Qingyang et al used the large specific surface area of reduced graphene oxide to adsorb cuprous oxide to make it uniformly dispersed. At the same time, it effectively slows down the release of copper ions and improves the ability of cuprous oxide to release active oxygen. The synergistic effect between the two increases the inactivation rate of the nanocomposite to Escherichia coli and Staphylococcus aureus to about 70% and 65%, respectively, compared with a single component [27].

To sum up, GO can not only be served as a carrier for the dispersion of metal nanoparticles, but also achieve the long-term release of metal nanoparticles. In addition, through the synergistic effect between them, the long-term and efficient antibacterial performance and stability of composite nanoparticles can be achieved.

In this study, zinc oxide and cuprous oxide nanoparticles were dispersed on the surface of graphene oxide, and the synergy between the three nanoparticles was used to improve the antibacterial activity of the nanocomposites. Zinc oxide and cuprous oxide nanoparticles were chemically loaded on graphene oxide sheets. The phase and micro-structure of the synthesized GO/Cu2O/ZnO nanocomposite was characterized by XRD, FT-IR, SEM, TEM and other methods. In addition, the antibacterial activity of composite nanoparticles was also studied.

2. Experimental procedures

2.1. Materials preparation

The reagents were as follows, Copper sulfate pentahydrate \([\text{CuSO}_4 \cdot 5\text{H}_2\text{O}]\) (99%), Sodium dodecyl sulfonate (SDS) and ascorbic acid \([\text{C}_6\text{H}_8\text{O}_6]\), Sodium hydroxide \([\text{NaOH}]\) (99%), were purchased from Nanjing Chemical Reagent Co., Ltd. And Single layer of graphene oxide (GO), zinc oxide \([\text{ZnO}]\), were acquired from Xuzhou Jie Innovation Material Technology Co., Ltd. The purchased raw materials are used directly without secondary purification. Skymen JP-020 ultrasonic cleaner was used, and the ultrasonic frequency is 40KHz.

All materials were prepared and characterized using distilled water as the medium.

At room temperature, 0.075 g of copper sulfate pentahydrate, 3.85 g of sodium dodecyl sulfonate, 0.03 g of graphene oxide, 0.05 g of spherical zinc oxide were added into a 500 ml Erlenmeyer flask, then 300ml distilled water was added and ultrasound for 1 h; it was placed on a magnetic stirrer, choosing a large rotor, and stirring vigorously; 10 ml of 0.2 mol l^{-1} ascorbic acid was measured into the Erlenmeyer flask, and finally 15 ml of 1 mol l^{-1} sodium hydroxide was measured into the Erlenmeyer flask, and controlled the pH value was 9.0; the stirring was continued for 2 h, and precipitation was found in the flask, the stirring was stopped, the rotor was taken out, and the layer was allowed to stand still. The precipitation was washed 3~5 times with distilled water and anhydrous ethanol, and vacuum dry at 60 °C for several hours to obtain GO/Cu2O/ZnO nanocomposites. The preparation process was shown in figure 1.

2.2. Tests and characterization

X-ray diffraction (XRD) was used to determine the phase composition of the nanocomposite. It used a copper target as a radiation source (\(\lambda = 1.540 56\ \text{Å}\)), with a step length of 0.02° and a 2θ angle measurement range of 5° ≤ 2θ ≤ 80° to obtain phase data. Using Fourier transform spectrometer to measure the sample was tested in the range of 4000–400 cm\(^{-1}\), and the infrared spectrum was obtained. Under the same conditions, the antibacterial activity of the nanocomposite was tested by using the plate method and the inhibition band method through gram-positive bacteria (Staphylococcus aureus) and gram-negative bacteria (E. coli = Escherichia coli).

3. Results and discussions

3.1. Physical analysis

The XRD test results of GO/Cu2O/ZnO nanocomposite were shown in figure 2. The (8.60°) peak marked with a circle in the figure belonged to graphene oxide (002) [28]. The peaks (38.52°, 40.89°, 61.92°, 65.25 and 73.09°) marked with triangles in the figure corresponded to the (111), (200), (220) and (311) crystal planes formed by Cu2O nanoparticles (JCPDS card 05-0667.) [18]. The peaks (32.11°, 33.89°, 48.73°, 54.56, 59.55, and 67.38°) marked with squares in the figure corresponded to (100), (002), (102), (110), (103) and (112) crystal planes formed by ZnO nanoparticles (JCPDS card number 36-1451.) [29].

Figure 3 illustrated FT-IR spectrum of the synthesized GO/Cu2O/ZnO nanocomposite. The broad peak at 3487.3 cm\(^{-1}\) was the vibration absorption peak of the hydroxyl group. The peak at 1176.57 cm\(^{-1}\) was attributed
Figure 1. The preparation process of GO/Cu$_2$O/ZnO nanocomposites.

Figure 2. XRD patterns of the synthesized GO/Cu$_2$O/ZnO nanocomposite.

Figure 3. FT-IR spectrum of the synthesized GO/Cu$_2$O/ZnO nanocomposites.
to the vibration absorption peak of the epoxy group. The peaks at 1064.19 cm$^{-1}$ and 1042.10 cm$^{-1}$ were the C–H vibration absorption peaks in the methyl and methylene groups of graphene oxide [30]. The frequency bands of 2922.06 cm$^{-1}$, 2852.90 cm$^{-1}$, 1465.43 cm$^{-1}$, 1414.85 cm$^{-1}$ and 616.16 cm$^{-1}$ were produced by the bending vibration of Cu–O. Which confirmed the successful formation of Cu$_2$O. The frequency band at 532.24 cm$^{-1}$ was the characteristic absorption caused by the vibration and bending of Zn–O.

Figures 4(a) and (b) shows the EDS diagram of the nanocomposite powder, and figure 4(c) shows the histogram of the nanoparticle size distributed on the surface of GO. The results illustrate that the prepared nanocomposite is heterogeneous, which may be due to the conditions during the reaction were not sufficient, resulting in the failure of some single nanoparticles to be dispersed on the surface of GO, thus forming agglomerations.

### 3.2. Structural analysis

The SEM micrographs of as-synthesized single layer of GO and GO/Cu$_2$O/ZnO nanocomposites are shown in figures 5(a) and (b). The surface morphology of GO was smooth, wavy and textured. The surface of graphene oxide—marked by the red circle—corresponds to attached nanoparticles and part of the graphene oxide is stacked and the texture becomes weaker. The attachment of Cu$_2$O and ZnO nanoparticles on graphene oxide sheets was observed. The surface appeared to be rough, which indicates that Cu$_2$O and ZnO nanoparticles have been attached on surface of graphene nanosheets.

Figure 6 was a transmission electron microscope (TEM) image of the synthesized GO/Cu$_2$O/ZnO nanocomposite, in which a batch of nanocomposite was developed from Cu$_2$O, ZnO, GO nanoparticles, forming evenly dispersed metal oxide nanoparticles morphology. Among the two metal oxide nanoparticles, the
The diameter of zinc oxide nanoparticles was 20 nm–30 nm, and the diameter of cuprous oxide nanoparticles 5nm-10nm. And they were not agglomerated in the graphene sheet, increasing their effective specific surface area, which could also greatly enhance the antibacterial effect of the nanocomposite.

Most of the nanoparticles could be observed through graphene oxide nanosheets, which indicated that GO could be used as a dispersion carrier for metal oxide nanoparticles. In addition, almost no agglomeration of nanoparticles was shown, which meant that GO had a positive effect on the dispersion of metal oxide nanoparticles.

Figure 7 was a High Resolution Transmission Electron Microscope (HRTEM) image of the synthesized GO/Cu2O/ZnO nanocomposite. Figures 7(a) and (b) show that Cu2O and ZnO nanoparticles uniformly dispersed on GO basal plane. The image of lattice fringe was calculated to be 0.281 nm and 0.213 nm which have been pictured in figures 7(c) and (d). These value also persists with the XRD results of the composite [6].

3.3. Antibacterial activity

In this study, the antibacterial properties of composite nanopowders were tested by the flat plate method and the Inhibition zone method. The specific test methods were carried out in accordance with the GB/T 21510-2008 ‘antibacterial properties of nano-inorganic materials’. The preserved strains were activated, and the bacterial growth curve was drawn by spectrophotometer method to determine the bacterial growth cycle, and the stable bacterial growth period was selected as antibacterial experimental bacteria [31]. Luria-bertani medium (LB medium) was selected as the experimental medium according to the strains tested. The formula of LB medium and phosphate buffer was formulated as follows:

- **LB medium** (per 100 ml distilled water): 1g sodium chloride, 1 g peptone, yeast extract 0.5 g, sodium hydroxide (1 mol l⁻¹) 0.4ml, AGAR 1.4 g.
- **Phosphate buffer** (per 100 ml distilled water): 0.8 g sodium chloride, 0.020 g potassium chloride, 0.027 g potassium dihydrogen phosphate, 0.355 g disodium hydrogen phosphate dodecahydrate.

The formulas refer to the international standard 'Method for Testing antibacterial Properties of Inorganic Materials (GB/T 21510-2008)'. The LB medium and phosphate buffer were adjusted to pH 7.2 – 7.4 using sodium hydroxide, which should be autoclave sterilized at 121 °C for more than 20 min before use.

The specific operation steps taken in this study are as follows:

The LB culture solution was poured into the petri dish (diameter: 100 mm) and cooled for use. The activated bacterial solution (about 10⁸ cfu ml⁻¹) was diluted to about 10⁵ cfu ml⁻¹ with phosphate buffer solution (PBS), and then 0.01g of GO/Cu₂O/ZnO powder sample was weighed and mixed with 1 ml of diluted bacterial...
solution in a test tube, shaken well, and contacted at a constant temperature for 12 h. In addition, 0.01 g control sample was weighed for the same treatment. After that, the upper layer of the bacteria liquid of each sample was evenly coated on the surface of the culture medium, and then the culture dish was placed in a constant temperature incubator at 37 °C for 24 h. Finally, the culture dish was taken out, and the bacteriostasis rate of each group was calculated by colony counting method.

The bacteriostasis rate R was calculated according to Formula (1):

\[ R = \frac{A - B}{A} \times 100\% \]  

(1)

Where: R = antibacterial rate, %
A = Number of colonies in the control group, per
B = Number of bacterial colonies in experimental group, per

Inhibition zone, to complete the inhibition zone test, the filter paper was cut into a disc shape, immersed it in deionized water with a sample concentration of 1 mg ml⁻¹, and dried it. 200 μl of activated E. coli was evenly dispersed on the agar plate by a triangular expansion machine. Then the soaked filter paper discs were put into the above plate, and then incubating it in a biochemical incubator at 37 °C for 24 h to check the inhibition zone. The zone of inhibition was measured by the rounded area on the disc-shaped filter paper without bacterial growth [6].

The antibacterial activity of GO/Cu₂O/ZnO nanocomposite was illustrated in figures 8 and 9, the results of the plate test shown that the sterilization rate of the nanocomposite to the two kinds of bacteria reached 100%. The test results of the inhibition circle method illustrated that the nanocomposite had a strong inhibitory effect on both bacteria, but it also expounded a stronger inhibitory effect on gram-positive bacteria, which may be due to the different cellular structures of the two bacteria, and caused this phenomenon. Finally, we conclude that the synergy among the various components in GO/Cu₂O/ZnO nanocomposite enhanced its antibacterial activity.

Many references have recorded the preparation of antibacterial materials and their antibacterial properties. The following table compares the antibacterial properties of some materials with the materials in this study. The
results in the comparison table show that the composite antibacterial material prepared in this study has more excellent antibacterial properties compared to other antibacterial materials.

3.4. Antibacterial mechanism
Compared with single component GO, GO composites exhibit better antibacterial properties and bio-safety [36]. In addition to the antibacterial mechanism of a single component, the antibacterial mechanism of composite materials must also be considered for the synergistic coupling between the components. GO and metal oxide composite materials can prevent the latter from agglomerating and disperse it evenly on the surface of GO, increasing its contact area with bacteria, and GO can promote the long-term release of metal ions and increase the long-term antibacterial properties of the composite material [36–39]. The nanocomposite powder prepared in this study uses the physical cutting antibacterial mechanism of GO, and Zn\(^{2+}\) and Cu\(^{2+}\) are combined with thiol-containing proteins and enzymes on the surface of bacterial cells to cause cell rupture and membrane damage mechanism [38]; GO and metal oxides after the components are compounded, a synergistic coupling effect is produced, which can further induce the production of reactive oxygen free radicals (ROS) and enhance oxidative stress [40].

### Table 1. Antibacterial performance comparison.

| Materials             | Antibacterial ingredients | Antibacterial rate       | References |
|-----------------------|---------------------------|--------------------------|------------|
| GO/Cu\(_2\)O/ZnO      | GO/Cu\(_2\)O/ZnO          | 100\% (E. coli) and S. aureus) | [32]       |
| Fibers                | ZIF-8                     | 100\% (E. coli)          | [33]       |
| ZIF-67@SA ZnO/PVA     | ZIF-67 particles          | near 100\% (E. coli)     | [33]       |
| nanofiber mat         | ZnO                       | 84.8 $\pm$ 2.5\% (E. coli) | [33]       |
| Cu\(_2\)O/ZnO         |                           | 90.23\% (E. coli)        | [33]       |
| composite film        | Cu\(_2\)O/ZnO             | 88.78\% (S. aureus)      | [34]       |
| Cu\(_2\)O-Ag/ZnO      | Cu\(_2\)O/Ag/ZnO          | 70.2\% (E. coli)         | [35]       |
|                       |                           | 86.3\% (S. aureus)       | [35]       |
4. Conclusion

The synthesized Cu2O and the ZnO nanoparticles added in the synthesis process were successfully dispersed in the graphene oxide sheet by the hydrothermal synthesis method. The micro-structure analysis of the sample shown that the two metal oxides had been uniformly supported on graphene oxide. The diameter of the zinc oxide nanoparticles added was 20 nm–30 nm, but the diameter of the Cu2O nanoparticles was 5 nm–10 nm. The antibacterial activity expounded that the nanocomposite had an inhibitory rate of 100% against *Staphylococcus aureus* and *Escherichia coli*. Compared with the introduction of single metal oxide nanoparticles on GO nanosheets, this paper proposes to use Cu2O and ZnO two metal oxide nanoparticles to enhance the antibacterial activity and obtain a more excellent antibacterial effect, which can be used in biomedicine and antibacterial coatings. It can be vigorously promote in other fields.

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Data availability statement

The data generated and/or analysed during the current study are not publicly available for legal/ethical reasons but are available from the corresponding author on reasonable request.

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