ISSN1027-5495. Functional Materials, 30, No.4 (2023), p. 620-624
doi:http://dx.doi.org/10.15407/fm30.04.620

Preparation of nano-sized zinc oxide by adjusting the reaction system alkalinity and its antibacterial properties

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Received October 12,2023

This paper uses the precipitation method to prepare multi morphology nano-zinc oxide materials. Using sodium lignosulfonate (CMN) as a structural directing agent and zinc acetate (ZnAC) and sodium hydroxide (NaOH) as raw materials, the influence of alkalinity on the preparation of nano-zinc oxide during the reaction process was studied by adjusting the NaOH concentration through the controlled variable method. The purity and morphology of the prepared nano-zinc oxide were characterized. The antibacterial properties were also studied. The experimental results show that the nano-zinc oxide prepared by this method has good antibacterial characteristics and can be used as a potential antibacterial agent.

Keywords: nano-zinc oxide; alkalinity; morphology; antibacterial performance

Отримання нанорозмірного оксиду цинку шляхом регулювання лужності реакційної системи та його антибактеріальни властивості. *Junli Xue, Wenchong Zhang, Lin Zhu, Xueling Cao*

Використовується метод преципітації для отримання мультиморфологічних матеріалів нанооксиду цинку. Використовуючи лігносульфонат натрію (CMN) як структурний напрямний агент і ацетат цинку (ZnAC) і гідроксид натрію (NaOH) як сировину, вплив лужності на отримання нанооксиду цинку під час реакційного процесу вивчали шляхом регулювання концентрації NaOH через метод контрольованої змінної. Охарактеризовано чистоту та морфологію отриманого нанооксиду цинку. Також вивчалися антибактеріальні властивості. Експериментальні результати показують, що нанооксид цинку, отриманий цим методом, має хороші антибактеріальні характеристики і може бути використаний як потенційний антибактеріальний засіб.

1. Introduction

Zinc oxide (ZnO) is a semiconductor material with a wide band gap and large laser binding energy. Due to its special physical, chemical and photoelectric properties [1], its broad application prospects have stimulated the research interest of many scholars. So far, ZnO materials have been widely used in semiconductor devices, photocatalysis, antibacterial, medical and health fields [2, 3]. It is well known that the properties of materials are related to the composition, structure, particle size and surface morphology of materials. Generally, nano-zinc oxide has the advantages of larger specific surface area, higher chemical activity, higher

product fineness, and chemical purity compared to ordinary ZnO [4]. However, nano-zinc oxide itself is prone to agglomeration, with strong surface polarity, and is not easily uniformly dispersed in organic media, which greatly limits the utilization of material nano effects [5]. Therefore, dispersion and surface modification of nano-zinc oxide powders have become necessary treatment methods for nanomaterials before their application in the matrix.

ZnO has excellent physical, chemical and photoelectric properties [6], and has been widely used in semiconductor devices [7], photocatalysis [8], medical and health [9] and other fields. Among them, the application of nanometer-scale antibacterial properties of zinc oxide has become widespread [10-13]. In this paper, sodium lignosulfonate (dispersant CMN) was used as the structure-directing agent, zinc acetate and sodium hydroxide were used as the main reactants, and zinc oxide nanoparticles with different morphological characteristics were prepared by adjusting the pH value of the reaction. The antibacterial properties of zinc oxide nanoparticles with better morphological characteristics and properties were tested, which proved that they had good antibacterial properties.

2. Experimental

Reagents and instruments

A transmission electron microscope (Japan Electronics Co., Ltd.) and an X-ray diffractometer (Bruker, Germany) were used.

Zinc oxide (Sinopharm Group Chemical Reagent Co., Ltd.); sodium hydroxide (Tianjin Dingshengxin Chemical); sodium lignosulfonate (Tianjin Damao Chemical Reagent Factory) and other analytically pure reagents were used.

Synthesis of nano-zinc oxide

First, 1 g sodium lignosulfonate, and 6 mL 4 mol/L sodium hydroxide aqueous solution were sonicated under the ice bath by ultrasound; 1 mol/L zinc acetate was added into the solution of sodium lignosulfonate drop by drop. Then, after sonication of the solution for 1 h under ice bath, it was heated and stirred at 80° for 5h. After the reaction, the solution was washed with water for many times, then centrifugated, washed until the supernatant was clear; the precipitate was collected, dried at 80° and calcined at 450° for 2 h to obtain the white product.

Measurement of growth curve

10mL EP tubes labeled 0-7 were taken; 6mL liquid medium and 2mL cultured bacterial solution were added into the EP tubes, respectively. Then 10 mg/mL nano-zinc oxide stock liquid was added into EP tube No. 0-7, and the amount was 0 mL, 6.5 mL, 25 mL, 50 mL, 100 mL, 200 mL and 400 mL, respectively; after homogenizing, the absorbance was measured by scanning the baseline with 3 mL liquid medium at the wavelength of 600 nm with an ultraviolet spectrophotometer.

Determination of protein standard curve

NaCl solution, bright blue dye and bovine serum albumin (BSA) were added to 6 EP tubes, and after the reaction of the above 6 groups of solutions for 5 min, the baseline was scanned with 3 mL bright blue and 300 mL 0.1mol/L NaCl solution at a wavelength of 488 nm by an ultraviolet spectrophotometer. The absorbance of 6 groups of solution was measured and the standard curve was made.

3. Results and discussion

Sodium hydroxide, as the main precipitant for preparing nano-zinc oxide, has a significant impact on the morphology and purity of nano-zinc oxide. Therefore, the reaction conditions were optimized by adjusting the concentration of sodium hydroxide, and the optimal concentration of sodium hydroxide for preparing nano-zinc oxide was selected through SEM and XRD characterizations. Fig. 1A, B, C shows the XRD spectra of nano-zinc oxide prepared at different concentrations of sodium hydroxide. Fig. 1A shows the powder prepared at a concentration of 2mol/L sodium hydroxide, Fig. 1B shows the powder prepared at a concentration of 4mol/L sodium hydroxide, and Fig. 1C shows the powder prepared at a concentration of 6mol/L sodium hydroxide. It can be seen from the figures that under the action of three different concentrations of sodium hydroxide solutions, the prepared nano-zinc oxide exhibits all characteristic peaks of the zinc oxide standard spectrum. But when the concentration of sodium hydroxide is 4mol/L, the spectrum has the least number of impurity peaks, higher purity and crystallinity. Therefore, 4mol/L is the optimal concentration of sodium hydroxide for the reaction. All peaks in Fig. 1 are characteristic diffraction peaks of ZnO, consistent with the XRD standard spec-

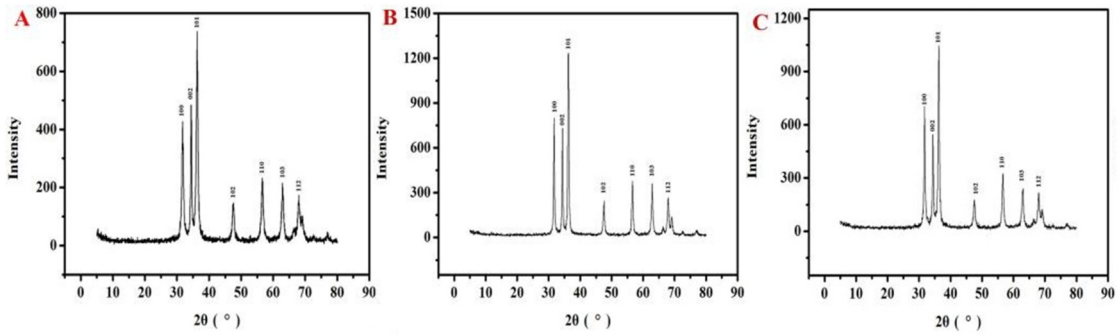

Fig. 1. XRD spectra of nano-zinc oxide prepared at different sodium hydroxide concentrations (A: 2mol/L sodium hydroxide; B: 4mol/L sodium hydroxide; C: 6mol/L sodium hydroxide)

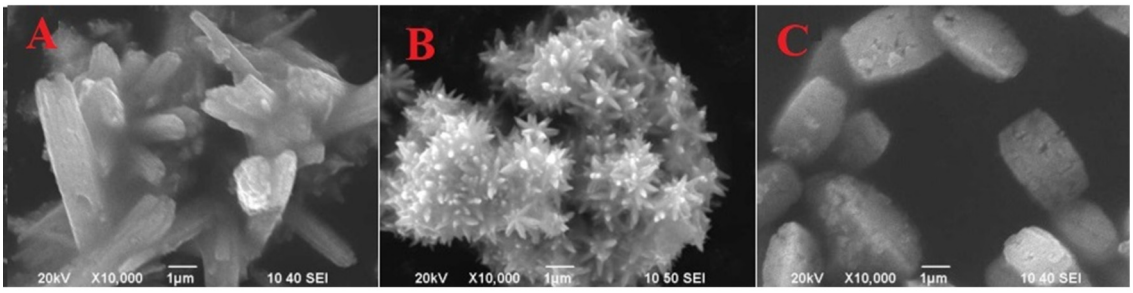

Fig. 2. TEM of nano-zinc oxide prepared at different sodium hydroxide concentrations with 10000x magnification (A: 2mol/L sodium hydroxide; B: 4mol/L sodium hydroxide; C: 6mol/L sodium hydroxide)

trum JCPDS36-1451. Therefore, the prepared powder is indeed nano-zinc oxide.

Fig. 2 shows the SEM diagram of nano-zinc oxide prepared at concentrations of 2mol/L, 4mol/L and 6mol/L sodium hydroxide. It can be seen from the figure that although the concentration of sodium hydroxide is different, the prepared nano-zinc oxide has a regular morphology and uniform distribution. When the concentration is 4mol/L, the nano-zinc oxide presents a hexagonal snowflake-like morphology; at a concentration of 6mol/L, the nano-zinc oxide presents a blocky morphology; the morphology of the nano-zinc oxide formed at a concentration of 2mol/L is a rod-like structure; the differences in morphology may be caused by the volume shrinkage due to the sintering and decomposition of the impurities in the zinc oxide.

As shown in Fig.1, when the concentration of sodium hydroxide is 4mol/L, all peaks are characteristic diffraction peaks of ZnO, which are consistent with the XRD standard spectrum JCPDS36-1451; the absence of additional peaks indicates that the nano-zinc oxide prepared under this alkaline condition has high purity and good crystallinity. The nano-zinc oxide prepared under optimal alkaline conditions was tested by scanning electron microscopy (SEM) and transmission electron microscopy (TEM), as shown in Fig. 3. It can be seen that the morphology of nano-zinc oxide prepared under optimal alkaline conditions is petal-shaped, and the particles are uniform, and the dispersion is not agglomerated. It is proved that under this condition, the ionic polarity of zinc oxide is effectively inhibited, which provides a reference value for large-scale industrial production.

In order to study the antibacterial properties of nano-zinc oxide, *Escherichia coli* and *Bacillus subtilis* were selected as research objects, and their growth curves within 36 hours were measured respectively, as shown in Fig. 4. It can be observed from Fig. 4A that the number of *Escherichia coli* colonies is not effectively inhibited with an increase in culture growth time; the curve shows an upward trend and becomes stable with increasing time, which is in line with the growth curve trend of *Escherichia coli* in natural environment, indicating that nano-zinc oxide has no obvious inhibition effect on *Escherichia coli*. Fig. 4B shows a downward trend of the curve, i.e. nano-zinc oxide has a significant inhibitory effect on *Bacillus subtilis*, and the inhibitory effect is enhanced with increasing time; a relatively balanced state is reached after 10-15 hours. The results showed that the optimal inhibitory time of nano-zinc oxide against *Bacillus subtilis* was 15 hours. It was verified that nano-zinc oxide had a weak inhibitory effect on gram-negative bacteria, such as *Escherichia coli*, and a strong inhibi-

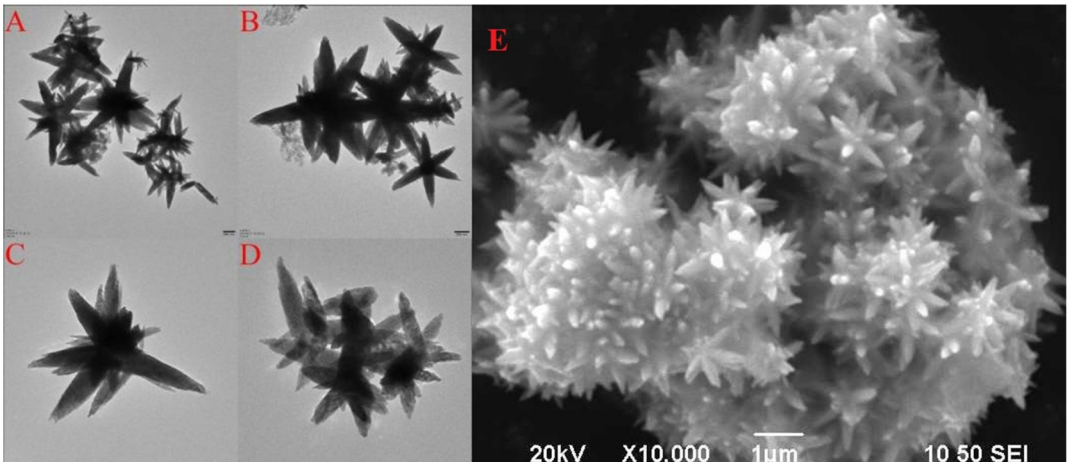

Fig. 3. TEM of nano-zinc oxide prepared under optimal alkaline conditions (A: 8000x magnification; B: 10000x magnification; C: 15000x magnification; D: 30000x magnification) and E: SEM of nano-zinc oxide

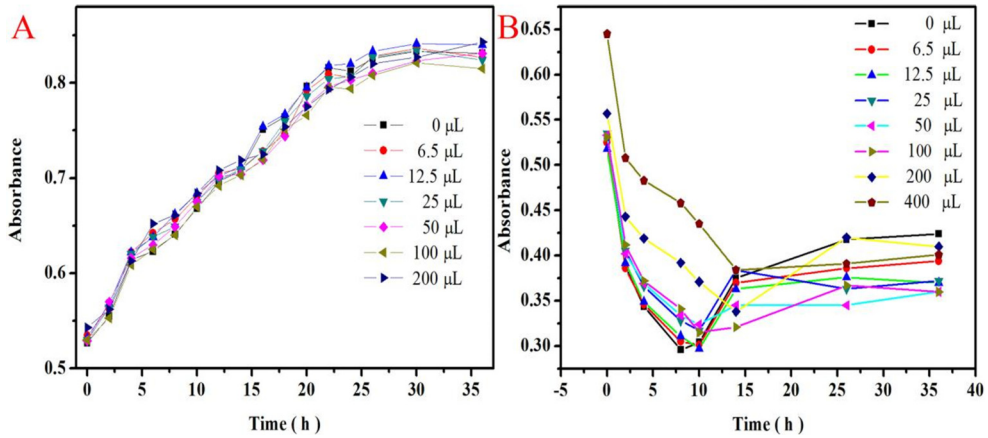

Fig. 4. Growth curve (A is *Escherichia coli;* B is *Bacillus subtilis*)

tory effect on Gram-positive bacteria, such as *Bacillus subtilis*.

In order to further verify the antibacterial performance of nano-zinc oxide, the protein content in the bacterial solution after 14 hours was detected, as shown in Fig. 5; Fig. 5A shows the protein content in a bacterial solution of *Escherichia coli*, and Fig. 5B shows the protein content in a bacterial solution of *Bacillus subtilis*. Fig. 5 shows that the protein content gradually increases with increasing volume of *Bacillus subtilis* liquid added; however, the protein content in a liquid sample with the same volume of *Bacillus subtilis* gradually decreases with increasing time, indicating that the abundance of *Bacillus subtilis* is constantly decreasing with increasing time. The protein content of the samples added with the *Escherichia coli* bacteria solution was almost unchanged with increasing time, which more reliably confirmed that nano-zinc oxide did have antibacterial properties, and the inhibition effect on gram-positive bacteria was stronger than that on gram-negative bacteria.

4. Conclusion

By controlling the amount of sodium hydroxide added, we explored the effect of alkalinity of the reaction system on the morphology and purity of the prepared nano-zinc oxide. The optimal alkalinity was determined when preparing nanozinc oxide; the dispersant CMN served as a structure-forming agent, and when preparing nanozinc oxide powder by precipitation, 4 mol/l sodium hydroxide was added in an amount of 6 ml; nanozinc oxide obtained after calcination has good dispersibility and high purity. The antibacterial properties of *Escherichia coli* and *Bacillus subtilis* were tested. It was proved that the nano-zinc oxide prepared by this method has good antibacterial properties, and the antibacterial properties of *Bacillus subtilis* are stronger than that of *Escherichia coli*.

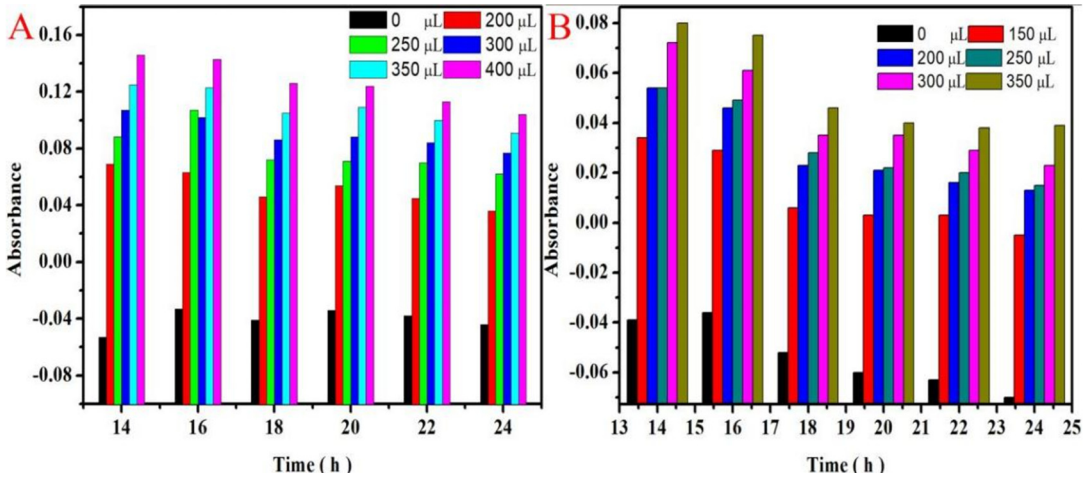

Fig. 5. Changes in protein content (A is *Escherichia coli;* B is *Bacillus subtilis*)

Acknowledgments

The present work was supported by Hainan Province Science and Technology Special Fund (Nos. ZDYF2023GXJS001) and Hainan Provincial Natural Science Foundation of Chine (Hos 222QN333)

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