ISSN 1027-5495. Functional Materials, 27, No.2 (2020), p. 311-314.
doi:https://doi.org/10.15407/fm27.02.311

Preparation of ZnO nanoparticles with antibacterial properties

Xueling Cao, Lin Zhu, Yageng Bai

Department of Chemical and Pharmaceutical Engineering, Jilin Institute of Chemical Technology, 45 Chengde Str., 132022 Jilin, P. R. China

Received January 20, 2020

In this work, the antibacterial zinc oxide nanoparticles with lignin as stabilizer were studied. On the one hand zinc oxide nanoparticles were synthesized in aqueous solution with lignin as reductant and stabilizer. The influence of calcination before and after calcination was mainly investigated by X-ray diffraction, scanning electron microscopy and transmission electron microscopy. On the other hand the antibacterial properties of zinc oxide nanoparticles were studied.

Keywords: precipitation method, sodium lignosulfonate, nano zinc oxide, antibacterial agent.

Изучены антибактериальные наночастицы оксида цинка с лигнином в качестве стабилизатора. Наночастицы оксида цинка синтезированы в водном растворе с лигнином в качестве восстановителя и стабилизатора. Влияние прокаливания до и после прокаливания исследовано методами рентгеновской дифракции, сканирующей электронной микроскопии и просвечивающей электронной микроскопии. Изучены антибактериальные свойства наночастиц оксида цинка.

Одерхжання наночастинок ZnO з антибактеріальними властивостями. *Xueling Cao, Lin Zhu, Yageng Bai*

Вивчено антибактеріальні наночастинки оксиду цинку з лігніном як стабілізатора. Наночастинки оксиду цинку синтезовано у водному розчині з лігніном в якості відновлювача та стабілізатора. Вплив процесу прожарювання до і після нього переважно досліджено за допомогою рентгенівської дифракції, скануючої електронної мікроскопії та мікроскопії пропускання електронів. Вивчалися антибактеріальні властивості наночастинок оксиду цинку.

1. Introduction

ZnO nanoparticle is a kind of metal nanomaterial, which has unique and good properties such as electron, optics, catalysis, antibacterial, surface modification and so on. In recent decades, it has attracted many researchers' attention, and these properties largely depend on their size, shape and chemical environment [1]. ZnO nanoparticles are considered to be biosafety, nontoxic and biocompatible. Therefore, ZnO nanoparticles have advantages over other metal oxide nanoparticles (such as TiO_2) in the development of metal oxide based antimicrobial agents under environmental conditions, because their activity does not need photoactivation [2]. The antibacterial activity and stability of ZnO nanoparticles can be improved by adding additives in the synthesis process. Lignin is the most abundant renewable and biodegradable natural resource in the world after cellulose; it is one of the main research directions in the field of renewable resources. The researchers found that lignin contains a large number of active hydroxyl, aldehyde and other reducing groups, which belongs to polymer materials and has a special three-dimen-

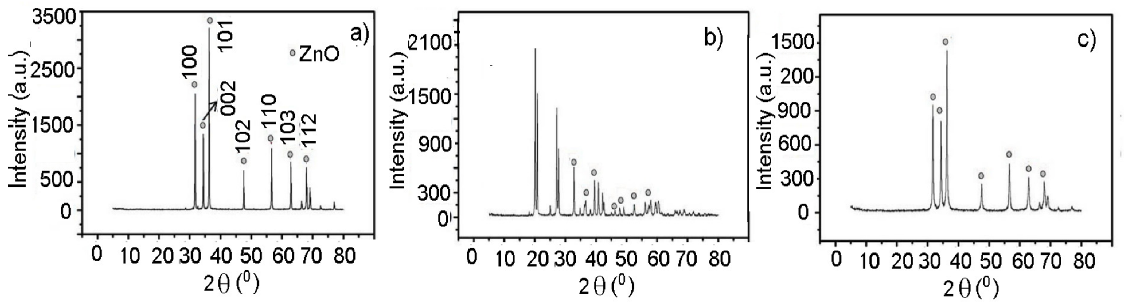

Fig. 1. The X-ray diffraction patterns of ZnO and ZnO nanoparticle (A) ZnO. (B) ZnO nanoparticle without calcination. (B) ZnO nanoparticle with calcination.

sional spatial structure [3]. Lignin has the function of green reduction and stabilization of nano metal particles, so as to avoids the use of toxic and harmful chemicals and necessary modification process [4]. At the same time, lignocellulosic fibers have a large number of active groups, due to which lignin also has the properties of oxidation resistance, UV resistance and antibacterial [5]. In the process of synthesizing composite materials, lignocellulosic fibers not only become stabilizers and reducers, but can also impart or enhance the functionality of composite materials. Based on this, nano lignin or different nanocomposites will significantly increase the added value of its application.

In this paper, sodium lignosulfonate was selected as stabilizer and reductant, sodium hydroxide and zinc acetate are used as raw materials to synthesize nanometer zinc oxide. The influence of nanometer zinc oxide on the morphology of nanometer is explored by calcination at high temperature, and the antibacterial property of nanometer zinc oxide sample is studied.

2. Experimental

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

A number of sterile qualitative filter paper discs with a diameter of 6 mm were prepared. The distilled water, 10 mg/mL lignin solution, 10 mg/mL nanometer zinc oxide suspension, 1 mg/mL nanometer zinc oxide suspension and 0.1 mg/mL nanometer zinc oxide suspension prepared respectively. Several filter paper discs were soaked into 5 samples, and then taken out after soaking for 10 min, dried and sterilized by high-pressure steam;

6 g Peptone, 3 g yeast extract, 6 g NaCl, 16 g agar and 600 mL distilled water were shaken well, adjusted the pH value to 7.2–7.4 with a 6 M sodium hydroxide solution, put it into the autoclave, sterilized at 120° for 30 min; then it was taken out and cooled. Cultured *Bacillus subtilis* was added accurately into the above liquid to make a solid culture medium, and 5 discs of filter paper with samples were inserted into each culture dish. The samples were put into a constant temperature incubator at 37° for 24 h, and the growth of the bacteriostatic circle was observed.

3. Results and discussion

The optimized reaction conditions were used to prepare ZnO nanoparticles with sodium lignosulfonate as stabilizer and reductant, and sodium hydroxide and zinc acetate as reactants. During the study of reaction conditions, we found that high temperature calcination was very important for the whole preparation process. Fig. 1 shows the XRD patterns of pure zinc oxide, zinc oxide nanoparticles without calcination and calcined zinc oxide nanoparticles. Fig. 1a shows peaks at the positions of $2\theta =$ 31.63 deg (100), 34.50 deg (002), 36.25 deg (101), 47.50 deg (102), 56.50 deg (110), 62.80 deg (103) and 67.92 deg (112), which consistent with the a standard wurtzite-type ZnO structure (JCPDS 36-1451) [6]. It can be seen from the Fig. 1b that there are not only the characteristic diffraction peaks of ZnO but also the characteristic diffraction peaks of Zn^{+} in the XRD spectrum of the non-calcined sample; this indicates that the

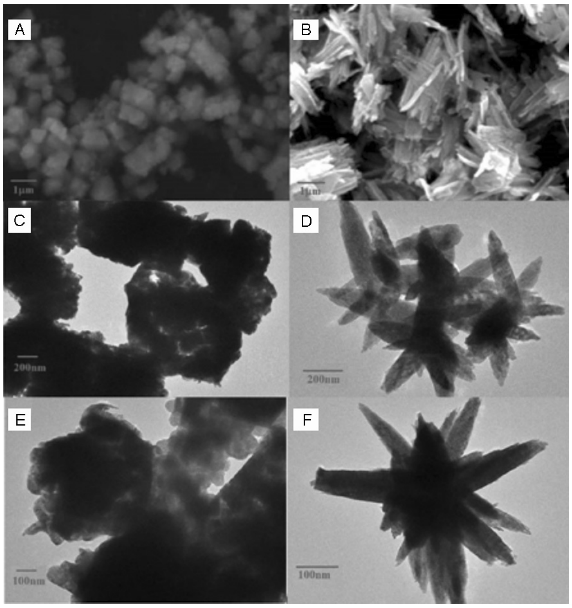

Fig. 2. (a) SEM images of ZnO nanoparticle without calcination (1μm). (b) SEM images of ZnO nanoparticle with calcination (1μm). (c) TEM images of ZnO nanoparticle without calcination (200 nm). (d) TEM images of ZnO nanoparticle with calcination (200 nm). (e) TEM images of ZnO nanoparticle without calcination (100 nm). (f) TEM images of ZnO nanoparticle with calcination (100 nm).

sample before calcination was not pure ZnO, but contained Zn^{+} impurities. After calcination, the impurity peaks of Zn^{+} disappeared, and all peaks were the characteristic diffraction peaks of ZnO (Fig. 1c), which were consistent with the Fig. 1a. It showed that after calcination, Zn^{+} was converted into ZnO completely without other impurities, and the crystallinity was very high. The major XRD diffraction peaks of (100), (002) and (101) were strong, that also suggested a high level of crystallinity of the as-grown powder [7].

The ZnO nanoparticles were characterized by electron microscopy (SEM and TEM). It could be seen from morphology in Fig. 2 that the morphology of the synthesized ZnO nanoparticles was different depending on whether they were calcined or not. When the ZnO nanoparticles were not calcined, they had massive structure and compact aggregation; after calcination, the prepared ZnO nanoparticles had an ordered structure of rods (Fig. 2a and Fig. 2b). Furthermore, the morphology of ZnO nanoparticles before and after calcination could be seen more clearly by TEM (Fig. 2c, d, e and f). Before calcination, the particles were massive, which was consistent with the results of SEM, but after calcination, they have a beautiful flower shape. This may be due to the decomposition of the impurity Zn^{+} in ZnO after burning, resulting in volume shrinkage.

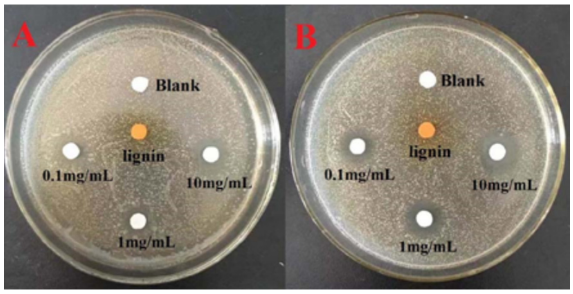

Fig. 3. Inhibition circles of ZnO nanoparticles against *Bacillus subtilis* (a) ZnO nanoparticle without calcination. (b) ZnO nanoparticle withcalcination.

Then the antibacterial activity of ZnO nanoparticles was tested. The filter paper was soaked in the suspension of ZnO nanoparticles, and bactericidal activity was determined in the form of the inhibition zone. Gram-positive *Bacillus subtilis* was selected as the antibactrial target (Fig. 3) to evaluate the antibacterial potential of ZnO nanoparticles before and after calcination. After 24 h, clear zones of inhibition were observed against *Bacillus subtilis* with three different concentrations of ZnO nanoparticles that were calcined at 450°. On the other hand, the ZnO nanoparticles without calcination showed comparatively lesser diffusion when tested against *Bacillus subtilis*, but still clear zones of inhibition were observed. Before calcination, zinc oxide nanoparticles have impurities of zinc hydroxide. However, zinc ions are known to inhibit many activities in bacteria, such as glycolysis, transmembrane proton translocation and acid resistance. Although it is reported that zinc ions are antibacterial [8], they can help to improve the antibacterial activity of zinc oxide [9]. Therefore, it could also be seen obvious bacteriostatic circles for *Bacillus subtilis*.

However, zinc ions are known to inhibit many activities in bacteria, such as glycolysis, transmembrane proton translocation and acid resistance. It is reported that zinc ion is antibacterial rather than bactericidal [8, 9], so it could also be seen that there were obvious bacteriostatic circles for *Bacillus subtilis* [10].

In general, the published reports clearly show that the regular shaped ZnO nanoparticles have significantly higher antibacterial activity than the bulk ZnO nanoparticles, and their activity does not need any photoactivation. The properties of regular spherical or different shape nanoparticles depend largely on their size and morphology [11]. Although zinc oxide with different structure and morphology can be effectively synthesized by different synthesis methods, it will be more convenient for practical operation to control the morphology by post-treatment. In this paper, the calcination of ZnO nanoparticles can not only eliminate the impurities, but also form regular flower shaped particles, which improves its antibacterial activity.

4. Conclusions

Lignin/zinc oxide nanoparticles were simply prepared by the precipitation method. It was proved that high temperature calcination could purify and optimize the morphology of ZnO nanoparticles. This method could provide zinc oxide nanoparticles with greater antibacterial activity. The experimental results show that it has high antibacterial activity against Gram-positive bacteria.

References

1. O.Yamamoto, M.Komatsu, J.Sawai et al., *J. Mater. Sci. Med.*, **15**, 847 (2004).
2. H.L.Liu, T.C.Yang, *Proc. Biochem.*, **39**, 475 (2003).
3. H.Sakagami, T.Kushida, T.Oizumi et al., *Therapeut*, **128**, 91 (2010).
4. Z.Shen, Y.Luo, Q.Wang et al., *ACS Appl. Mater. Inter.*, **6**, 16147 (2014).
5. S.Shankar, J.W.Rhim, K.Won, *Int. J. Biol. Macromole.*, **107**, 1724 (2018).
6. K.R.Raghupathi, R.T.Koodali, A.C.Manna, *Langmuir*, **27**, 4020 (2011).
7. S.Mahmud, *J. Alloy. Compd.*, **509**, 4035 (2011).
8. T.J.McCarthy, J.J.Zeelie, D.J.Krause, *J. Clin. Pharm. Ther.*, **17**, 51 (1992).
9. G.Applerot, N.Perkas, G.Amirian et al., *Appl. Surf. Sci.*, **2565**, 53 (2009).
10. P. Hajokova, P. Spatenka, J. Horsky, et al., *Plasma. Process Polym.*, 4, S397, (2007).
11. X.Wang, F.Yang, W.Yang et al., *Chem. Commun.*, **42**, 4419 (2007).