The preparation of Ag-TiO₂ and the study on its bacteriostatic properties

Qi Yao CUI, Hong Hao SUN*

School of Bioengineering and Food, Key Laboratory of Fermentation Engineering (Ministry of Education), National "111" Center for Cellular Regulation and Molecular Pharmaceutics Hubei University of Technology, Key Laboratory of Industrial Microbiology in Hubei, Hubei University of Technology, Wuhan, Hubei 430068, China.

Corresponding E-mail: 1048923282@qq.com

Abstract. The silver was deposited on the surface of titanium dioxide (TiO₂) here, and the silver was presented in its elemental form. The antibacterial composite material prepared by liquid phase precipitation method in this study has a 98.69% yield with silver loaded efficiency of 98%. The content of silver in the composite material was 2.8% determined by titration. Antimicrobial effect of the synthesized composite material was carried out against gram-negative bacteria (E. coli) and gram-positive bacteria (S. aureus) by using the zone of inhibition method. The synthesized Ag-TiO₂ showed enhanced bactericidal activities in compare with TiO₂.

1. Introduction

Bacteria, fungi and other pathogenic microorganisms exist extensively and highly susceptible to reproduction, affecting people’s living environment and physical health. With the rapid development of nanotechnology and materials science, various new inorganic antimicrobials have been developed and applied in coatings, fibers and medical fields. TiO₂ is a well-known photocatalytic agent that has been extensively used for destroying microorganisms including bacteria, viruses and fungi [1-2]. The silver-supported nano TiO₂ has obvious advantages over other inorganic antimicrobials [3]. The loading silver on TiO₂ particles can enlarge the applied range of light to visible light and improve the performance of photocatalytic sterilization [4]. The composite materials combined the photocatalysis of nano TiO₂ and the excellent broad-spectrum antibacterial properties of silver, its antibacterial ability was significantly improved compared to nano TiO₂ [5]. The Ag NPs is liable to aggregation and oxidation, which significantly affects its practical application [6]. The instability of Ag NPs could be improved by loading on the nano TiO₂. Because of its good thermal stability, broad-spectrum and persistent antibacterial properties, the composite material is dominant in compound antibacterial agents. In this paper, Ag-TiO₂ composite nanoparticles was prepared with uniform size and good dispersibility. It could be used as bactericidal component in practical application.

2. Materials and Experiment

2.1 Materials

Nanocrystalline TiO₂, silver nitrate (AgNO₃), concentrated nitric acid (HNO₃), sodium hydroxide
(NaOH), glucose were procured from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). KSCN and NH4Fe(SO4)2•12H2O were obtained from Aladdin (Shanghai China). Ultrapure water (18.2 MU cm) used for all experiments was obtained from a Milli-Q system (Millipore, Bedford, MA).

2.2 Preparation of Ag-TiO2
Nano TiO2 (12.5 g) was added to a three-necked flask then 70 ml ultrapure water was added to the flask with stirring for 30 min at room temperature. The pH was adjusted to acidity by adding 0.5 ml concentrated nitric acid and keep stirring the mixture for another 30 min. 0.5 g AgNO3 was added into the flask after was mixed with 20 ml ultra-water, then stirred the mixture for 30 min. After that, 1.5 ml NaOH solution (10 M) was added to the flask to give a pH 5-6 and continued to stir for another 2 h. The pH of the solution was further adjusted to 11 by adding 10 ml NaOH solution (10 M), stirred the mixture for another 30 min. 6 ml glucose solution (0.5 M) were added to the mixture and stirred for 12 h at 70℃. The product was washed by ultrapure water through suction filtration after the reaction. The silver content in filtrate was measured by titration. The filter cake was dried in oven to give a final product.

2.3 Antimicrobial Test
E. coli strain and S. aureus strain were used in this experiment. The concentration of bacterial cells was diluted to approximately 105 CFU ml−1 with sterilized PBS solution. The antibacterial activity of the composite material against E. coli and S. aureus was determined by qualitative evaluation using the zone of inhibition method. For the qualitative evaluation (zone of inhibition test), 20 ml of liquid nutrient agar was poured onto disposable sterilized petri dishes and allowed to solidify. Then 200 μl of the bacterial PBS solution (105 CFU ml−1) was streaked over the plate and spread uniformly. Then two sterilized oxford cup were placed over solidified agar gel in the petri dishes, after that, 240 μl titanium dioxide suspension and Ag-TiO2 suspension were added in the two oxford cup respectively. The plates were incubated at 37 ℃ for 48 h and the antagonistic activity was estimated by a clear zone of inhibition around the oxford cup.

3. Results and Discussion
3.1 Characterization
The nanocomposites were characterized by DLS and TEM. Hydrodynamic diameter (Dh) measurements were conducted by dynamic light scattering (DLS) with a Malvern Zetasizer Nano ZS90 (Malvern, UK) instrument using a He–Ne laser at a wavelength of 632.8 nm. The average size of the TiO2 measured by DSL was 305 nm, and the average size of Ag-TiO2 composite nanoparticles was 355 nm as shown in figure 1, the increase of the hydrodynamic diameter has indicated that the AgNPs were successfully deposited on the surface.

The Ag-TiO2 composite nanoparticles were examined in a transmission-electron microscope (TEM). Transmission electron microscopy (TEM) images were taken on a JEM-2100F transmission electron microscope at an accelerating voltage of 200 kV. The Figure 2C and Figure 2D showed the TEM images of silver deposited on TiO2. From the Figure 2, we can see small silver nanoparticles with the diameter of 20 nm were loaded on the surface of the TiO2 particles with average diameter around 300nm.
3.2 Antibacterial activity evaluation

The antibacterial activity is evidenced by the inhibition zones of bacteria (E. coli and S. aureus) growth around the resultant substrates as shown in the Figure 3. For Ag-TiO2, there was an inhibition zone with a diameter of 15 mm for E. coli (see Figure 3a) and an inhibition zone with a diameter of 34 mm for S. aureus (see Figure 3b) respectively, the composite material has obvious bacteriostatic effect. But the TiO2 did not show clear inhibition zones on both petri dishes, the result demonstrated that the composite material could inhibit the bacterial growth and have much stronger bacteriostatic performance than neat TiO2. The test result also shows that the S. aureus is more sensitive to Ag-TiO2 composite nanoparticles than E. coli.

4. Conclusions

The antibacterial composite material reported in this paper was prepared by liquid phase precipitation method, the method had simple operations and the Ag NPs were successfully deposited on the surface of TiO2. The nanoparticles prepared here have small particle size and good dispersibility. The composite material reported in this article have excellent bacteriostatic both on E. coli and S. aureus. The composite antibacterial material would have a vast range of prospects in textile, medicine and other fields.

Acknowledgements

The authors would like to thank Hubei Province Outstanding youth science and technology innovation team in institutions of higher education (T201705), Hubei Province Natural Science Fund Project (2014CFA080), the National Natural Science Foundation of China (21401051, 21501054), Chutian Scholars Fund Project (2013) from the Education Department of Hubei Province, and Hundred Talents Program (2013) from the Organization Department of Hubei Province for financial support.
References
[1] Daghrir R, Drogui P and Robert D 2013 Modified TiO$_2$ for environmental photocatalytic applications: A review. *Ind. Eng. Chem. Res.* 52 3581–99
[2] Nakano R, Hara M, Ishiguro H, Yao Y, Ochiai T, Nakata, K, Murakami T, Kajioka J, Sunada K and Hashimoto K 2013 Broad spectrum microbicidal activity of photocatalysis by TiO$_2$. *Catalysts.* 3 310–23
[3] Keleher J, Bashant J, Heldt N, Johnson L and Li Y 2002 Photocatalytic preparation of silver-coated TiO$_2$ particles for antibacterial applications. *World J. Microbiol. Biotechnol.* 18 133–9
[4] Franci G, Falanga A, Galdiero S, Palomba L, Rai M, Morelli G and Galdiero M 2015 Silver Nanoparticles as Potential Antibacterial Agents. *Molecules.* 20 8856–74
[5] Romero L, Piccirillo C, Castro P M L, Bowman C, Warwick M. E. A and Binions R 2015 Titanium Dioxide Thin Films Deposited by Electric Field-Assisted CVD: Effect on Antimicrobial and Photocatalytic Properties. *Chem. Vap. Deposition.* 21 63–70
[6] Kong H, Jang J 2008 Antibacterial Properties of Novel Poly(methyl methacrylate) Nanofiber Containing Silver Nanoparticles. *Langmuir.* 24 (5) 2051-56