

Synthesis of Ag/TiO₂ Core/Shell Nanoparticles with Antibacterial Properties

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Monodispersed Ag/TiO₂ core/shell nanoparticles were synthesized in solution *via* colloid-seeded deposition process using Ag nanoparticles as colloid seeds and Ti(SO₄)₂ as Ti-source respectively. Silver nitrate was reduced to Ag nanoparticles with N₂H₄·H₂O in the presence of CTAB as stabilizing agent. The titania sols hydrolyzed by the Ti(SO₄)₂ solution deposited on the surface of Ag nanoparticles to form the Ag/TiO₂ core/shell nanoparticles. Inductively coupled plasma atomic emission spectrometry (ICP-AES) showed low amount of Ag ion leaching from the Ag/TiO₂ core/shell nanoparticles. The Ag/TiO₂ core/shell nanoparticles indicated excellent antibacterial effects against *Escherichia coli* and maintained long-term antibacterial property.

Key Words : Nanocomposites, Spectroscopy, X-ray techniques

Introduction

Due to the worldwide outbreak of infectious diseases caused by different pathogenic bacteria in recent years, pharmaceutical companies and researchers are searching for new antibacterial materials containing various natural and inorganic substances.¹⁻³

Most antibacterial investigations are conducted in aqueous environments (agar-plate incubation begins with the production of an aqueous suspension of microbial/antimicrobial agents). Good dispersion of nanomaterials is a prerequisite for sufficient contact and interaction between nanomaterials and microbial species. Ag nanoparticles have been known to exhibit bactericidal property because of its strong cytotoxicity towards a broad range of micro-organisms, however they are hardly usable by themselves since they are poor at dispersion stability and tend to aggregate in the medium. At the same time, silver reagent is hardly cost-effective. For the economic and efficient reason, a common challenge is to obtain highly dispersed Ag composite nanoparticles. In an earlier report, it has shown that Ag nanoparticles deposited on TiO₂ nanostructures enhance their bactericidal property.⁴ Such a structure, though effective, results in exposing both metal Ag to reactants and the surrounding medium. Corrosion or dissolution of the noble metal Ag nanoparticles during the operation of a bactericidal reaction is likely to limit the use of metal Ag. A better synthetic design can significantly improve the stable performance and maintain the excellent antibacterial effects of metal-oxide composite. Many attempts have been made to prepare metal Ag core/shell nanoparticles by coating Ag with different chemical compositions.⁵⁻⁷ The Ag core/shell nanoparticles would provide a new possibility to be good candidates for antibacterial materials due to their unique structures and at the same time the shell to protect Ag nanoparticles and stabilize them against chemical corrosion. It could be reasonably envisioned that Ag cores would release Ag ion slowly

through the outer porous coating layer, thereby producing and maintaining the excellent antibacterial effects.

Although a certain number of studies had covered reports on Ag-TiO₂ nanocomposite materials with high antibacterial properties,^{8,9} the Ag/TiO₂ core/shell nanoparticles with a facile method have so far hardly been explored. If Ag/TiO₂ core/shell nanoparticles can be prepared through a simple method, this method would be the discovery of a novel antibacterial agent. There existed two reports concerning the synthesis of Ag/TiO₂ core/shell nanoparticles, in which organic titanium reagents were adopted as titania precursor. Such methods posed a big challenge to large-scale preparations.¹⁰⁻¹¹ Following the two drawbacks of research above-mentioned, the present study is to prepare Ag/TiO₂ core/shell nanoparticles using the colloid-seeded deposition reaction on a large scale, and then investigate their release of Ag ion and antibacterial properties.

Materials and Methods

Ti(SO₄)₂, AgNO₃, N₂H₄·H₂O, and CTAB were purchased from Shanghai Chemical Reagents Company, and put into use without further purification.

Morphological studies of the nanoparticles were carried out using the transmission electron microscopy (TEM) (JEM-2100, JEOL, Japan). For TEM observations, 1 drop of properly diluted sample was placed on a copper grid and air-dried before examination. The X-ray diffraction (XRD) patterns of the products were recorded by a Phillip X'pert pro Advance Powder X-ray diffractometer (Cu K α radiation). The UV-vis absorption spectra were recorded on a Shimadzu UV3100 spectrophotometer (Shimadzu, Japan). The Ag⁺ concentration was detected by ICP-AES (ICP, Thermo IRIS Intrepid II, America) equipped with CID detector.

Ag nanoparticles were prepared as follows. N₂H₄·H₂O solution (100 mM, 0.5 g) was added to the CTAB solution (1 mM, 20 g) stirring at the room temperature. AgNO₃

solution (50 mM, 0.5 g) was added to the above mixed solution stirring further for 10 min at the room temperature to disperse the Ag nanoparticle. Ag/TiO₂ core/shell nanoparticles were prepared as follows. The Ag nanoparticles were used as the seeds in the followed growth process of Ag/TiO₂ core/shell nanoparticles. A fresh Ti(SO₄)₂ solution (1 mM, 25 g) was added into the above Ag nanoparticles suspension drop by drop stirring vigorously for several hours at 15 °C. The resulting dispersion was allowed to age at the same temperature for several hours. A couple of drops of the solid deposits were taken out for TEM observation.

TiO₂ colloidal suspension was prepared in a procedure similar to the one for the Ag/TiO₂ core/shell colloids, except for the omission of AgNO₃ addition. The slow hydrolysis of Ti(SO₄)₂ produced a transparent sol of TiO₂.

Analysis of Ag ions released from the core/shell nanoparticles was carried out using ICP-AES. The core/shell nanoparticles were dispersed in distilled water placing for 10 days and centrifugated before ICP measurement. Each sample was tested at least three times. The corresponding standard curve of silver ion was constructed by ICP-AES in standard AgNO₃ solutions.

Results and Discussion

The Ag/TiO₂ core/shell nanoparticles were prepared by hydrolysis of Ti(SO₄)₂ solutions. The temperature of hydrolysis was crucial to the formation of a TiO₂ shell around the Ag nanoparticle core. After several attempts we found stable colloids of core/shell nanoparticles were generated at 15 °C. The characterization of the Ag and the core/shell nanoparticles were subject to different analytical techniques.

The TEM images of two different sets of nanoparticles were presented in Figure 1. As shown in Figure 1(a), the Ag nanoparticles were roughly in the shape of a globe, while the core/shell nanoparticles, as demonstrated in Figure 1(b), were highly monodispersed cone-shaped *via* colloid-seeded deposition reaction of Ti(SO₄)₂.¹² Of them, the latter seemed to be much larger than the former in size. As the TEM images showed, the core/shell nanoparticles were about 15 nm in diameter. Among these core/shell nanoparticles, the 2–4 nm Ag nanoparticles were located at the edge of particles rather than in the middle of particles. The Ag nanoparticles were encapsulated within a nearly 5-nm-thick titania layer for the thicker area. This configuration was advantageous for the release of Ag ion.

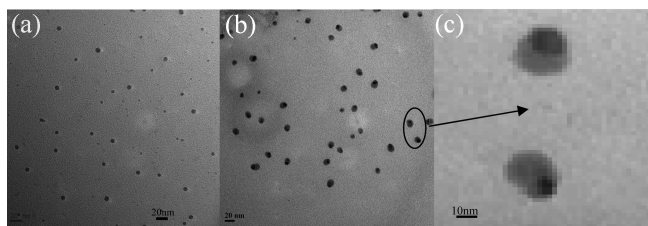


Figure 1. TEM images of (a) Ag nanoparticles, (b) Ag/TiO₂ core/shell nanoparticles, (c) Enlargement of Ag/TiO₂ core/shell nanoparticles.

X-ray diffraction analysis was used for the identification of structures and analysis of crystal phase. Figure 2 showed the wide-angle XRD patterns of the two type nanoparticles. The diffraction peaks in Figure 2(a) could be attributed to Ag, which exhibits the diffraction peaks at $2\theta = 38.1, 44.2, 64.4,$ and 77.4° respectively, corresponding to the (111), (200), (220) and (311) crystalline planes of Ag crystal. The core/shell nanoparticles were found to keep the diffraction peaks characteristic of Ag crystal, whereas no peak was detected assigned to titania crystal, suggesting that the titania shell was amorphous. The absence of Ag₂O reflections indicated that Ag crystal was not oxidized during the reaction due to the protection of TiO₂.

The UV-vis absorption spectra of the Ag and the core/shell nanoparticles suspensions were revealed in Figure 3. Both exhibited a strong absorption in the visible zone. This visible absorption occurred from the surface plasmon band of Ag core, and was strongly influenced by the oxide shell. The maximum peak corresponding to the plasmon of the Ag nanoparticles remained around 408 nm, a characteristic of Ag colloidal dispersion. In addition, the suspension was light red (fresh). Similar surface plasmon absorption, induced by

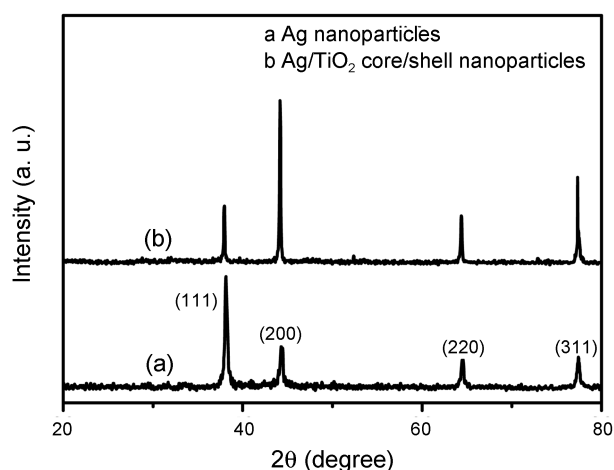


Figure 2. X-ray diffraction patterns of (a) Ag nanoparticles and (b) Ag/TiO₂ core/shell nanoparticles.

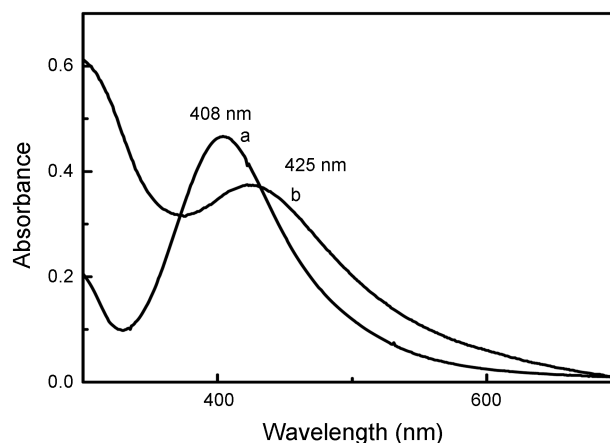


Figure 3. UV-vis spectra of (a) Ag nanoparticles and (b) Ag/TiO₂ core/shell nanoparticles.

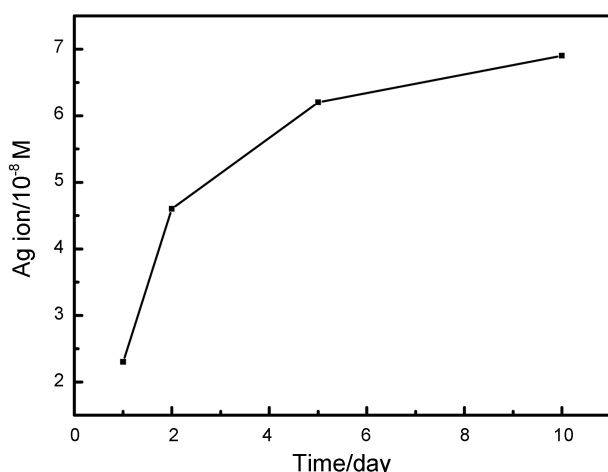


Figure 4. The Ag ion release curve of the Ag/TiO₂ core/shell nanoparticles.

the Ag nanoparticles, was observed for the Ag/TiO₂ core/shell nanoparticles at a wavelength longer than that of the suspension of the Ag nanoparticles. The surface plasmon absorption band of the Ag/TiO₂ core/shell nanoparticles was at 425 nm, with the color being light yellow. This absorption shift caused by a high refractive index of TiO₂ shell strongly suggests a complete coverage of the Ag nanoparticles with TiO₂.^{10,11}

ICP was used to estimate Ag ion leaching from the Ag/TiO₂ core/shell nanoparticles. Figure 4 showed release profiles of the Ag/TiO₂ nanoparticles depending on time. As we could see that the quantities of released Ag ion increased in initial two days. After that, the release rate of Ag ion is relatively slow. Namely, the Ag/TiO₂ core/shell nanoparticles display a better control of the release. Released concentration of Ag ion is 0.023 ppm, 0.046 ppm, 0.062 ppm and 0.069 ppm at one day, two day, five day and ten day after centrifugation respectively. It could be reasonably envisioned that Ag cores would release Ag ion slowly through the outer porous titanium dioxide shell, as a result, leaching of the Ag ion was more efficiently controlled.

Antibacterial properties of the Ag/TiO₂ core/shell nanoparticles were investigated by inhibition testing against *E. coli*. Nutrient agar was poured into disposable sterilized Petri dishes and allowed to solidify. 200 mL of *E. coli* bacterial water (about 10⁶ CFU/mL) was streaked uniformly over each plate. Round pieces (1 cm × 1 cm) of conventional filter paper, the TiO₂ nanoparticles sheet, and the Ag/TiO₂ core/shell nanoparticles sheet were gently placed on the

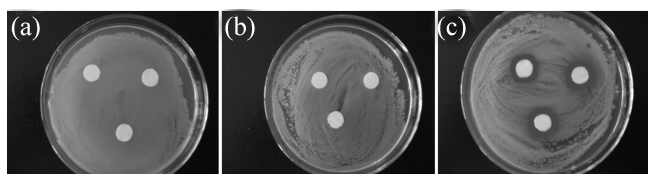


Figure 5. The results of inhibition testing. a, b, c, conventional filter paper, TiO₂ nanoparticles sheet, Ag/TiO₂ core/shell nanoparticles sheets, respectively.

solidified agar gel in separate Petri dishes. Then, the plates were incubated at 37 °C for 24 h. The results, as shown in Figure 5 revealed an obvious zone of inhibition around the Ag/TiO₂ core/shell nanoparticles sheet, suggesting the antibacterial property of the Ag/TiO₂ core/shell nanoparticles. There was no growth detected in the TiO₂ nanoparticles sheet (Figure 5(b)) as well as in the regular printing paper (Figure 5(a)). The justification was that the TiO₂ nanoparticles were the amorphous sample.¹³ This experiment revealed clearly the antibacterial properties of the Ag/TiO₂ core/shell nanoparticles. Therefore, such core/shell Ag/TiO₂ nanoparticles could be useful and effective in bactericidal applications, and would present a reasonable alternative for the development of new bactericides.

The mechanism of the antibacterial effect of silver nanoparticles had been reported in scientific papers.¹⁴ It could be concluded that Ag ion from Ag cores could be released through porous TiO₂ shells. It may be reasonable to presume that such core/shell structures will benefit preventing corrosion and prolonging the release time of Ag ions and preserving the sustained antibacterial behavior.

This work integrates nanotechnology and bacteriology, leading to possible advances in the formulation of new types of bactericides. However, future studies on the biocidal influence of this nanomaterial on other Gram-positive and Gram-negative bacteria are needed in order to make a full evaluation of its possible use as a new bactericidal material.

Conclusions

In conclusion, The monodispersed Ag/TiO₂ core/shell nanoparticles were synthesized in solution *via* colloid-seeded deposition process. Ag ion released from the Ag/TiO₂ core/shell nanoparticles by ICP-AES detection showed this structure could control release of Ag ion. The antibacterial properties of above core/shell nanoparticles against *E. coli* by inhibition testing indicate that this material acquires excellent antibacterial effects. The antibacterial capability of the core/shell nanoparticles is attributed to its structural feature of Ag nanoparticles.

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