

Preparation and antibacterial activity of Ag–TiO₂ composite film by liquid phase deposition (LPD) method

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MS received 24 June 2007; revised 4 October 2007

Abstract. A liquid phase deposition (LPD) method has been devised for the deposition of Ag–TiO₂ thin films on ceramic tiles with glazed surface at a low temperature. The Ag–TiO₂ thin films obtained were well-adhered, homogenous and coloured by interference of reflected light. The films were characterized by X-ray diffraction and scanning electron microscopy. From these analyses, it was found that silver ions were trapped in TiO₂ matrix and their reduction could be achieved at 600°C annealing temperature. The antibacterial activity against *S. aureus* and *E. coli* has been studied applying the so called antibacterial-drop test. The Ag–TiO₂ thin films exhibited a high antibacterial activity. AAS was used for the quantitative determination of silver ion concentration releasing from the Ag–TiO₂ thin film. The releasing rate of silver ions from the Ag–TiO₂ film was 0.118 µg/ml during 192 h. The antibacterial effect of Ag–TiO₂ thin film before and after aging in a weathering chamber for 48 h was compared and the results show that the antibacterial activity is not compromised after weathering.

Keywords. Ag–TiO₂ thin films; liquid phase deposition method; antibacterial activity.

1. Introduction

Ceramic tiles possess excellent decorative and chemical durability, and are widely used in various areas such as in the hospital environment and in every household. Unfortunately, ceramic tile itself does not have antibacterial activity and microorganisms easily breed on its surface, especially in moist environment. The existence and breeding of microorganisms on the surface of ceramic tiles may harm people's health, therefore, preparation of the antibacterial film on glazed surface of ceramic tiles as well as the investigation of its antibacterial activity is of practical significance.

TiO₂ thin films possess deodorizing, antibacterial and self-cleaning function under ultraviolet light (Matsubara *et al* 1995; Negishi *et al* 1995; Kikushi *et al* 1997). However, its disadvantage is that the band-gap energy of TiO₂ is ~3.2 eV, therefore, UV illumination is necessary to photoactivate this semiconductor.

Silver or silver ions have long been known to be excellent materials for breeding spectrum antibacterial agents (Kang *et al* 2000; Kawashita *et al* 2000). According to various studies, it is believed that Ag reacts with proteins by combing the –SH groups of enzymes, which leads to the inactivation of the proteins. Feng *et al* (2000) studied

the antibacterial mechanism of Ag⁺ on bacteria. Ag⁺ makes DNA molecules to loose their replication abilities. Silver-doped materials are chemically durable and release Ag⁺ for a long time (Toshikazu 1999). If silver nanoparticles are immobilized in the TiO₂ film on glazed surface of ceramic tiles, the release time of silver ions can be delayed for a long time so that the ceramic tile with this film will be of great potential for antibacterial application. This excellent antibacterial activity is not restricted by UV illumination.

TiO₂-based films have been prepared by a number of techniques such as sputtering, spray pyrolysis (Yanagi *et al* 1997), sol–gel processing (Yoko *et al* 1991) and chemical vapour deposition (CVD) (Lee *et al* 1994; Schvisky *et al* 2000). These methods usually require the cost-competitive techniques and expensive devices in those application areas for film deposition. Thus, TiO₂-based thin films on ceramic tiles were not easily achieved using these techniques. A successful deposition process can be achieved by a liquid phase deposition (LPD) method (Deki *et al* 1996; Nagayama *et al* 1998) in a soft-solution at a low temperature. This results in quality improvement, lower costs and environmental-friendly processing. However, to-date Ag-doped TiO₂ thin films on glazed surface of ceramic tiles prepared by this method as well as its antibacterial activity have never been reported.

In our work, Ag–TiO₂ thin films on glazed surface of ceramic tiles having excellent antibacterial activity were

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directly obtained by liquid phase deposition method (LPD). Here, we report the microstructure of as-prepared Ag-TiO₂ thin films by XRD and SEM. Mainly, the antibacterial activity of Ag-TiO₂ films on glazed surface of ceramic tiles are also investigated.

2. Experimental

2.1 Preparation and characterization of Ag/TiO₂ films

The precursor aqueous solution was freshly prepared by mixing 500 ml of ammonium hexafluorotitanate (IV) solution (0.034 mol L⁻¹), 500 ml of boric acid (0.102 mol L⁻¹) solution and 10 ml of silver nitrate (0.01 mol L⁻¹) solution. The ceramic tile with glazed surface was cut into pieces of 50 × 50 × 4 mm³ in size and washed ultrasonically with ethanol and deionized water. The as-cleaned specimens were immersed into the precursor aqueous solution and maintained at a constant temperature of 25–30°C for 18–54 h. Then, the specimens were rinsed with deionized water and dried at ambient temperature. Heat treatment for the deposited films was performed under air flow for 1 h at various temperatures.

The crystal phase of the as-prepared TiO₂ thin film and Ag-TiO₂ thin film was identified using an X-ray diffractometer (XRD, CuK α , Rigaku, Japan, $\lambda = 0.1541$ nm). Data were collected from 20–90°, 2θ at a scan rate of 1° min⁻¹. The surface morphology measurements were performed using a scanning electron microscope (SEM, JEOL JSM-6300, Japan) equipped with an energy-dispersive X-ray analyser, after the surfaces were coated with gold film.

2.2 Determination of antibacterial effect of deposited films

The antibacterial activities of films obtained against *S. aureus* and *E. coli* were studied using the so-called antibacterial drop-test. *S. aureus* ATCC6538 and *E. coli* O157 were used as the experimental bacteria and cultured on the culture medium at 37°C for 18–24 h. The cultured bacteria were added in 10 ml saline solution to reach approximately the concentration of 10⁸ CFU/ml. A portion of the saline solution containing the bacteria was diluted to 10⁶ CFU/ml for the 'drop-method' antibacterial experiments. The samples were placed in the sterilized petri dishes. Then 100 μ l of saline solution with bacteria was added dropwise onto the surface of each deposited film, and undeposited piece of ceramic tile was used as a blank. The surface of the samples was covered by an antistaling film. The samples were laid at ambient temperature for 4, 8, 12, 16, 20 and 24 h. After each time period the bacteria containing drops were washed from the surface of the films by using 5 ml phosphate buffer solution (PBS) in the sterilized petri dish. Then 10 μ l each of bacteria suspension was dispersed on the culture medium. The num-

ber of surviving bacteria on the petri dishes were counted after incubation for 24 h at 37°C.

AAS was used for the quantitative determination of the silver ion concentration releasing from the Ag-TiO₂ films. The ceramic pieces with Ag-TiO₂ film, 50 × 50 × 4 mm³ in size, were immersed in 50 ml doubly distilled water. The liquids were then taken out at the designated time intervals from the samples and the concentration of Ag⁺ was measured by atomic absorption spectrophotometer (316MC, Shanghai Analytical Instrument Factory, China). The stability of the Ag-TiO₂ films was tested in a weather chamber (Atlas Electric Device Co., Chicago, USA). During the test, a water spray was activated for 5 min in 30 min cycle. The simulated solar irradiation was directed at the film surface with an intensity of 0.56 W/m² at 340 nm. After 48 h, the samples were removed and dried at ambient temperature before being subjected to an antibacterial test. The antibacterial activity of the film after weathering was then compared to the one before weathering.

3. Results and discussion

3.1 Deposition conditions and properties of film

The deposition conditions for the preparation of Ag-TiO₂ films were examined using pieces of ceramic tiles with glazed surface as substrate. The molar ratio of ammonium hexafluorotitanate and boric acid was 1 : 3, when the concentration of ammonium hexafluorotitanate solution was over 0.1 mol L⁻¹, the deposited films were easily peeled off in a heating treatment. At a concentration of ammonium hexafluorotitanate solution below 0.01 mol L⁻¹, neither precipitation nor film formation was observed. At 0.034 mol L⁻¹, the films were uniform and even as well as hardly peeled off in a heating treatment. The thickness of Ag-TiO₂ films increased with increasing deposition time and it showed reflection-interference colours such as silver white, golden yellow, pink, blue and green, successively. The films thicker than 200 nm easily peeled off after heat treatment. These results are similar to those reported by us earlier (Hu *et al* 2006). On the other hand, the films were dark brown in colour below 550°C annealing conditions. When they were kept at room temperature to expose to natural light for a few days they turned dark, because TiO₂ films were not completely densified and Ag⁺ ions were not completely trapped in the TiO₂ matrix under below 550°C annealing conditions. Previous researchers have reported that silver is unstable at low annealing temperature (Ritzer *et al* 1997). The films treated at over 750°C were brown yellow in colour. But the films treated at 600°C showed reflection-interference colours mentioned above.

The surface morphology of as-prepared Ag-TiO₂ films was examined by SEM and TiO₂ film was examined as a blank (figure 1). Both the films were directly obtained on

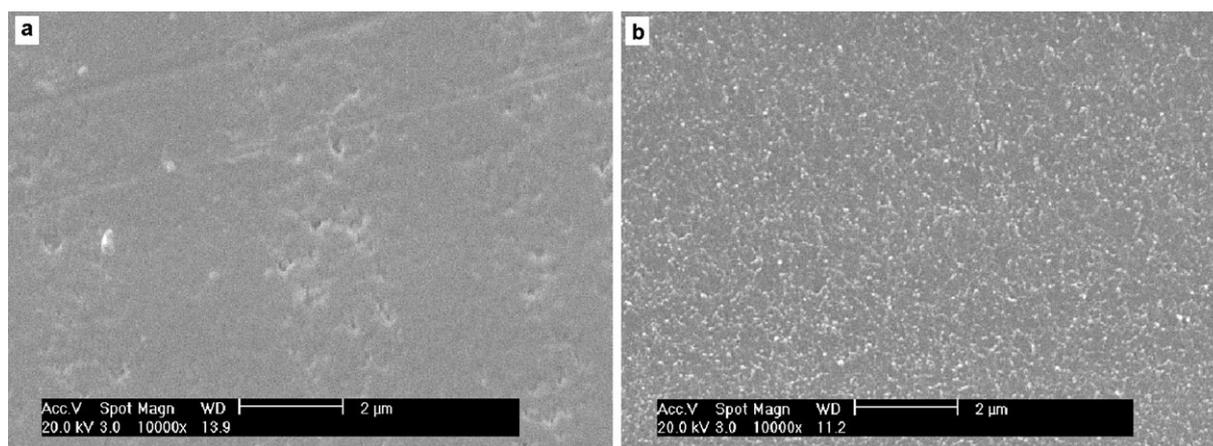


Figure 1. SEM images of the as-deposited (a) TiO₂ film and (b) Ag/TiO₂ film.

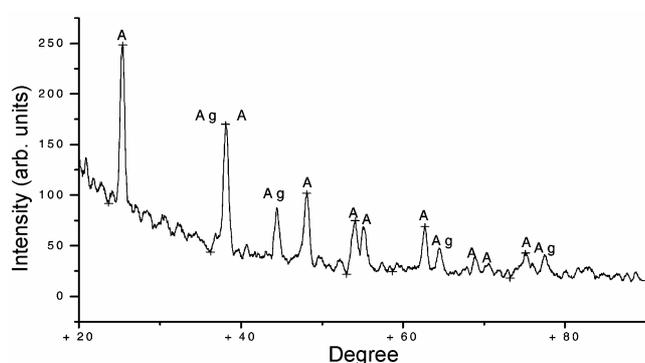


Figure 2. X-ray diffraction patterns of Ag/TiO₂ film heat-treated at 600°C (A: anatase).

pieces of ceramic tiles using the deposition process by heat treatment at 600°C, but obvious difference in surface morphology was found. The TiO₂ film consisted of fine particles with a diameter of several tens of nanometer, but in morphology of Ag-TiO₂ film, the metallic silver agglomerates to small spheroids (Ag⁺ to Ag formed during the annealing process) embedded in the TiO₂ matrix. The mean diameter of Ag small spheroids is 100 nm. The Ag-TiO₂ film is homogeneous and constructed of small particles showing no cracks as compared with the TiO₂ film.

Figure 2 shows the X-ray diffraction pattern in 2θ range 20°–90° of the Ag-TiO₂ film calcined at 600°C. The diffraction peaks for the anatase phase of TiO₂ and Ag have been marked with 'A' and 'Ag', respectively. The four distinct diffraction peaks are clearly observed at ca. $2\theta = 38.1^\circ$, 44.3° , 64.5° and 77.4° being assigned to (111), (200), (220) and (311) reflections of Ag, respectively. The other diffraction peaks are observed at ca. $2\theta = 25.3^\circ$, 48.12° , 53.94° , 55.04° and 62.74° being assigned to (101), (200), (220), (105), (211) and (204) reflections of anatase phase of TiO₂. The mean size of

silver particles was estimated by analysing the broadening of the (111) reflection. The mean particle sizes can be calculated by the Scherrer equation:

$$D = k\lambda/(\beta\cos\theta),$$

$K = 0.89$, $\lambda = 0.1541$ nm, θ is the half-diffraction angle, β the half-peak width, D the diameter of crystalline particle. The estimated average crystal size of Ag particle embedded in TiO₂ matrix is 95 nm for the films heat treated at 600°C for 1 h, which coincides well with those of the SEM analysis.

3.2 Antibacterial activity of Ag-TiO₂ film and mechanism

Nanometer Ag particles show excellent bactericidal effects and do not cause adverse health effects (Lee *et al* 2003). Therefore, loading the biocompatible TiO₂ with a tiny amount of silver in terms of economic importance and reusability, is thus attracting more and more attention (Sökmen *et al* 2001; Zhang *et al* 2003). In the present study, we examined the antibacterial activity of the Ag-TiO₂ film prepared in this study without UV irradiation in comparison with TiO₂ film.

The results of the antibacterial effect are evaluated by film attachment method of TiO₂ thin films and Ag-TiO₂ thin films against *S. aureus* and *E. coli*. The results are shown in figures 3–5. In the case of *E. coli*, the antibacterial rate of the TiO₂ thin film is about 48%, but Ag-TiO₂ thin film has 99% antibacterial activity after 24 h. In case of *S. aureus*, the antibacterial rate of the TiO₂ thin film is about 42%, but Ag-TiO₂ thin film has 91% antibacterial activity after 24 h. This reveals that Ag-TiO₂ thin films are more effective antibacterial materials than TiO₂ thin films under conditions without UV-ray illumination. The significant difference in antibacterial effects between TiO₂ thin films and Ag-TiO₂ thin films may be attributed

to the difference in antibacterial mechanisms between both films. It is the photo-catalytic property of the TiO₂ thin films that makes it to have a sterilizing capability. Under the illumination of ultraviolet light from natural light, the TiO₂ thin films can spontaneously decompose positive-negative, electron e^- and cavity h^+ , which can form the electron-cavity, the cavities oxidize the OH⁻ and H₂O which are absorbed on the surfaces of the TiO₂ thin films to \cdot OH. The oxidizing activity of \cdot OH can decompose the cell wall and the cell membrane of *S. aureus* and *E. coli* attached on TiO₂ thin films. The leakage of intracellular molecules will result in a change in the cell viability. But the percent of ultraviolet light from natural light is only 5% which results in the lower antibacterial activity.

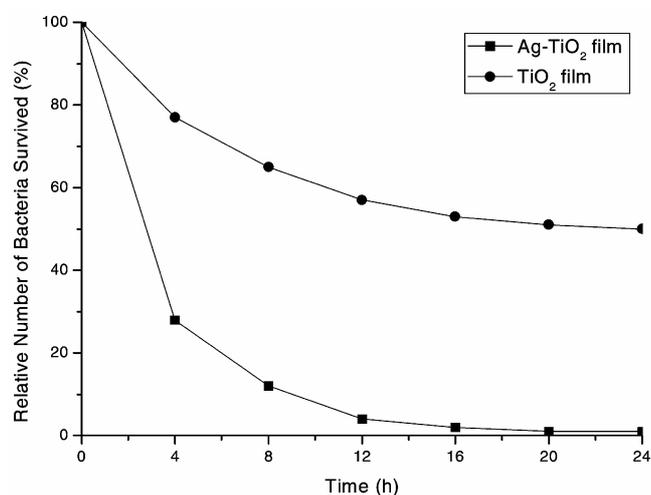


Figure 3. In case of *E. coli*, relative number of bacteria survived for TiO₂ film and Ag/TiO₂ film.

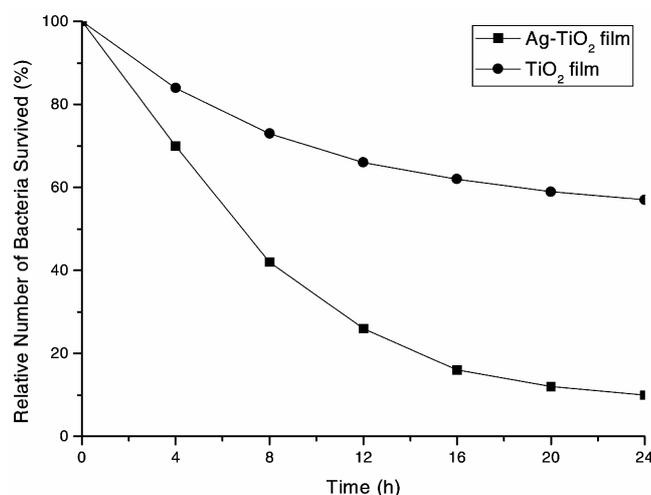


Figure 4. In case of *S. aureus*, relative number of bacteria survived for TiO₂ film and Ag/TiO₂ film.

The antibacterial activity of Ag-TiO₂ thin films is directly related to Ag nanoparticles. For the antibacterial mechanism of Ag, there are two different theories. One theory advocates that metal silver can react with water and release silver ions, and silver ions combine with sulphhydryl in bacteria, resulting in the blocking of breathing and finally the death of the bacteria (Zhang 1996). The other theory holds that silver can react with the oxygen dissolved in water and generate activated oxygen O[•] which can decompose the bacteria (Vallee and Ulmer 1972; Rahn and Landry 1973; Ritchie and Jones 1990; Peterwig 1996). We carried out the Ag⁺ ions releasing experiment and AAS was used for the quantitative determination of the silver ion concentration releasing from Ag-TiO₂ films in water. Figure 6 shows that the releasing concentration of silver ions of the sample stably increases with the increase in sampling time. The releasing concentration of silver ions from the Ag-TiO₂ film is 0.118 μg/ml during 192 h. These imply that silver ions are released from the surface of Ag-TiO₂ thin films and these ions kill the microorganisms. This mechanism implies that the antibacterial material would only have a limited period of effectiveness. Another potential problem is that the compound released into the environment may increase drug resistance throughout the microbial realm (Shi *et al* 2004). Therefore, the stability of Ag-TiO₂ thin films was carried out in this study.

The stability experiment of Ag-TiO₂ thin films was processed by exposing the antibacterial films to simulated weathering conditions. After processing simulated weathering with water spray and UV irradiation for 48 h, Ag-TiO₂ thin films were again tested for antibacterial activity by film attachment method and the viable cell number as a function of time was monitored. A comparison of antibacterial activity of Ag-TiO₂ thin films before and after weathering is given in figure 7. The results show that the antibacterial activity is not compromised after weathering.

The application of ceramic tiles with Ag-TiO₂ thin films as antibacterial materials can be expected for environmental protection, especially in medical facilities, and in pharmaceutical and medical device factories, where the bacteria are needed to control.

4. Conclusions

In the present study, it has been found that transparent Ag-TiO₂ thin films were obtained on glazed surface of ceramic tiles by a liquid phase deposition method (LPD). The Ag-TiO₂ thin films obtained adhered well, were homogenous and coloured by interference of reflected light. XRD and SEM experiments indicated that silver nanoparticles were completely trapped in TiO₂ matrix and reduction could be achieved at 600°C annealing temperature. Ag-TiO₂ thin films show high antibacterial activity eliminating the *E. coli* and *S. aureus*. One reason is that



Figure 5. Test results on *E. coli* after 24 h: (a) incubated with a ceramic tile, (b) incubated with TiO₂ film and (c) incubated with Ag-TiO₂ film.

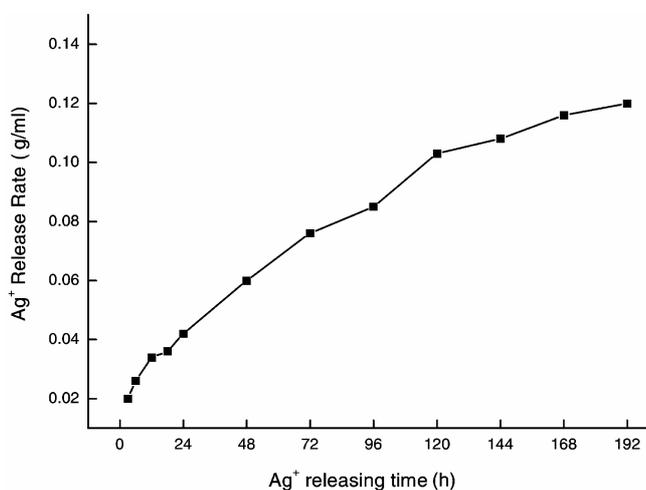


Figure 6. Atomic absorption spectroscopy of silver ion releasing rate from the Ag-TiO₂ film with releasing time.

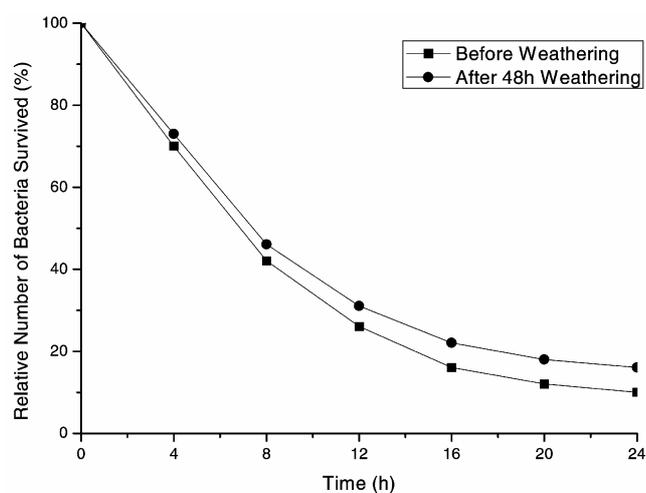


Figure 7. Comparison of the antibacterial effect of the Ag/TiO₂ film against *S. aureus* before and after aging in a weathering chamber for 48 h.

the silver ion is released from the Ag-TiO₂ thin films in a certain extent. The antibacterial functionality of Ag-TiO₂ thin films was not compromised even after aging in a weathering chamber. Ag-TiO₂ thin films are believed to be useful as antibacterial materials.

Acknowledgement

The authors are indebted to the Chinese Natural Science Foundation for financial support.

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