

High Bactericidal Ability of Silver (Ag)-Coated TiO₂ Films via Doctor Blade Technique

Rungnapa Tongpool*, Suppharat Inphonlek, Jidaporn Ninlapat,
Preeyawis Na Ubol and Kongthip Setwong

National Metal and Materials Technology Centre (MTEC)
114, Paholyothin Rd., Klong 1, Klong Luang, Pathumthani 12120, Thailand

*Corresponding author. E-mail: rungnapt@mtec.or.th

ABSTRACT

Films and nanoparticles of P25, precipitated (ppt.) TiO₂, 1.5%Ag-loaded TiO₂ and 1.5%Ag-loaded P25 were used as photocatalysts in bactericidal reactions. P25 powder was obtained commercially while nanoparticles of ppt. TiO₂, Ag-TiO₂ and Ag-P25 were prepared by hydrolysis and co-precipitation reactions. Brookite crystal structure was found more in Ag-TiO₂ powder than in TiO₂ powder, indicating that Ag ions influenced the crystallization of TiO₂. All the films were fabricated by doctor blade technique. The Ag-doped samples showed highest bactericidal activities. However after being washed, the doped powder became ineffective. Unlike the doped powder, the doped films still performed well after being washed, indicating that Ag was strongly adhered to the films. This work succeeded in preparing highly-effective bactericidal films via a simple doctor blade technique.

Keywords: TiO₂, bactericidal, Ag, film, dissolution

INTRODUCTION

Disinfection is necessary in drinking and household water. It is an important function of swimming pool, floor tile, window and clothes. It has been known that, when exposed to UV light, TiO₂ generates holes which directly oxidise organic materials or react with water and oxygen, producing hydroxyl (OH) radicals which are highly reactive. These radicals damage the cell wall (Kuhn et al., 2003) and cell membrane of bacteria (Zhang et al., 2003). It causes abnormal cell division and physicochemical alteration of the cell membrane (Amezaga-Madrid et al., 2003]. Silver (Ag) and silver ions have been known to be able to kill germ. They could be reused, handled more easily and more economically if impregnated on substrates. Since TiO₂ is environmental friendly, Ag coated TiO₂ (Ag-TiO₂) has been widely studied (Sokmen et al., 2001; Zhang et al., 2003). Several work reported that Ag-TiO₂ particles have high bactericidal abilities. However, we found that the dissolved Ag ions (Sokmen et al., 2001), not photocatalytic reaction, were the reason for bacteria reduction. The dissolution of Ag ions results in system contamination and life-time shortening of the photocatalysts. Various work incorporated Ag in TiO₂ films

via sol-gel process (Chang et al., 2006; Colmenares et al., 2006; Ge et al., 2006; Rengaraj and Li, 2006). This process was slow and complicated and the obtained films were expensive and had low surface area. Ag-TiO₂ films from sputtering or vapor deposition require sophisticated equipment. This work proposed high bactericidal ability Ag-TiO₂ films where Ag ions were strongly adhered, using a simple doctor blade technique.

MATERIALS AND METHODS

TiO₂ nanoparticles (denoted as ppt. TiO₂) was precipitated from hydrolysis reaction of tetraisopropyl orthotitanate (Ti(OiPr)₄, Fluka) and calcined at 450°C for 1 h. The Ag-loaded TiO₂, denoted as Ag-TiO₂, was obtained from co-precipitation of AgNO₃ (BDH, AnalaR) solution and Ti(OiPr)₄ and then calcined at 450°C for 1 h. The Ag-loaded P25 (a commercial TiO₂, J.J. Degussa), denoted as Ag-P25, was obtained from mixing P25 and AgNO₃ solution and then calcined at 450°C for 1 h. The molar ratios of Ag in TiO₂ and P25 were 1.5%.

The obtained powder of 1 g was mixed with 1 ml of glycerol (Aldrich Chemical Co., Inc., 99%) and then the slurry was cast on a cover glass, using doctor blade technique. The films were dried and calcined at 450°C for 30 min.

Structural properties of the samples were obtained by an X-ray diffractometer (XRD, JEOL JDX-3530). Particle sizes of the samples were investigated by transmission electron microscope (TEM, JEOL JEM 1220). Surface area of the samples was obtained by BET surface area analysis, using surface area analyzer (Quantachrome Instrument, Autosorb-1).

Before bactericidal study, the powder samples were washed 8 times with 5 ml of distilled water. Then 1 ml of *E. coli* (ATCC 25922) solution was mixed with 1 mg of the powder samples. The mixtures were irradiated with 365-nm UV light (UVP Inc., UVLS 26) for 1 min at the distance of 26 cm from the lamp. Bacteria solution without TiO₂ was also irradiated. Then they were diluted with 0.85% NaCl (Fluka, > 99.5%) and poured onto plate count agar (Oxoid, UK). The colonies were counted after incubation at 37°C for 24 h. Bactericidal ability was determined from % reduction of *E. coli* number as shown in the Equation (1);

$$\% \text{ Reduction} = (n_0 - n) / n_0 \times 100 \quad \dots\dots\dots(1)$$

where n_0 is a concentration of *E. coli* solution without UV exposure and n is concentration of *E. coli* solution after UV exposure.

As the films can be easily detached from the cover glass, they were washed by immersing in 3 ml of water for 2 days. Then the films were sterilized, using UV light of 254 nm for 15 min. *E. coli* solution of 0.1 ml was dropped onto the film and the film was irradiated with 365-nm UV light for 1 min at the distance of 26 cm from the lamp. Then, the film was cultured in nutrient broth (Oxoid, UK) at 37°C for 24 h. The liquid-cultures of 1 ml was taken to a sterilized tube and mixed with 10 μ l

of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) solution (5 mg/ml), and incubated at 37°C for 4 h. Then 1 ml of 0.04 M HCl in isopropanol was added to all the tubes to dissolve the blue-violet crystals. The obtained solution of 200 μ l was taken into a 96-well plate (Nunc, Wiesbaden, Germany) and the absorption at 570 nm was measured, using a microplate reader (Bio-Tek EIA reader, U.S.A). The absorption is proportional to the number of *E. coli*. The *E. coli* on a cover glass without UV exposure was denoted as an untreated sample. Bactericidal ability was determined from Equation (2);

$$\% \text{ Reduction} = (x_0 - x) / x_0 \times 100 \quad \dots\dots\dots(2)$$

where x_0 is the absorption of the solution from untreated samples (no UV exposure) and x is the absorption of the solutions from the UV-exposed samples.

RESULTS AND DISCUSSION

According to JCPDS-International Centre for Diffraction Data (2003), P25 and Ag-P25 nanoparticles contain anatase and a small amount of rutile phase, noted from the peak height at the 2-theta of 27.5° (rutile, Fig. 1). The peak height ratios of the 2-theta of 27.5° (rutile) and 25.4° (anatase) is 0.13 for both P25 and Ag-P25, indicating no change in the phase content. The ppt. TiO_2 and Ag- TiO_2 nanoparticles consist of anatase and a small amount of brookite crystal form, noted from the peak height at the 2-theta of 30.9°. The peak height ratios of the 2-theta of 30.9 (brookite) and 25.4 (anatase) is 0.08 and 0.18 for TiO_2 and Ag- TiO_2 , respectively, indicating an increase of the brookite content in Ag- TiO_2 . The phase change occurs probably because the doping process was carried out in a liquid phase where atomic arrangement is possible and the Ag ions might be responsible for this. Since Ag and Ag compound (AgO , Ag_2O , Ag_2O_2) peaks superimpose on TiO_2 peaks (indicated by arrows), they are difficult to be distinguished. However, we found that the calcined doped samples became blackened when left in the room or after being washed. This occurs because the silver changed to silver oxide by oxidation and light (Kim et al., 2003).

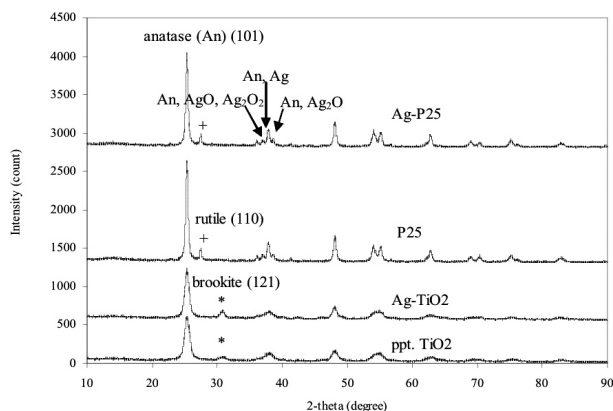


Figure 1. XRD patterns of the sample powders.

The synthesized ppt. TiO_2 has much higher surface area than the commercial P25 (Table 1), correlating to the smaller particle sizes of the ppt. TiO_2 . The surface areas of the doped powder were lower than those of the undoped powder, correlating to the larger sizes of the doped samples. Zhang et al (2003) also reported the reduction in surface area of the powder after being doped. Since ionic radii of Ag ($\text{Ag}^+ = 1.26^\circ\text{\AA}$ and $\text{Ag}^{+2} = 0.89^\circ\text{\AA}$) are much larger than that of Ti^{+4} ($0.68^\circ\text{\AA}$), small spheres of Ag compound adhered on the surface of TiO_2 particles (Fig. 2).

Table 1. surface area and particle size of nanoparticle samples.

Sample	Surface area ($\text{m}^2 \text{g}^{-1}$)	Particle size (nm)	size (nm)
ppt. TiO_2	126.07+1.10	9.4+2.5	9.43+2.51
1.5%Ag- TiO_2	79.07+0.57	11.3+2.5	
P25	56.07+0.77	23.3+3.9	18.70+8.27
1.5%Ag-P25	49.37+0.83	25.1+8.9	10.06+2.03

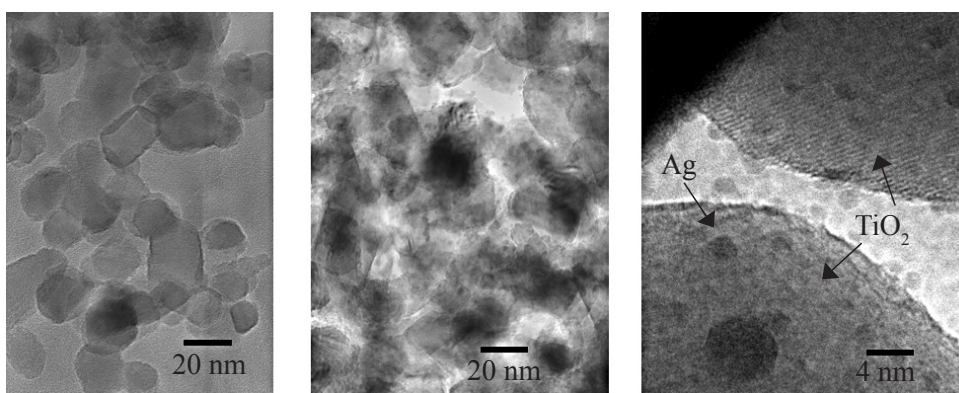


Figure 2. TEM images of (a) P25 (b) Ag-P25 powder and (c) Ag-P25 with high resolution.

Bactericidal activities of the samples are shown in Figs. 3 and 4. The ppt. TiO_2 powder showed higher bacterial reduction than did P25 as a result of higher surface area. The effect of surface area is also demonstrated by the difference in % reduction obtained from the undoped powder which has high surface area and the undoped films which have low surface area. The former shows higher activity than the latter. The Ag-doped samples showed the highest bactericidal activities as % reduction of bacteria number was as high as 98-100%. The Ag-doped powder performed worse after being washed, implying that the Ag was dissolved out. Unlike the doped powder, the washed doped films perform well as the unwashed do, implying that the Ag was strongly adhered onto the films. Bactericidal activities of the doped films were much higher (> 11 times) than the undoped films and the

cover glass, indicating that the photocatalytic activity of TiO_2 was much enhanced by Ag.

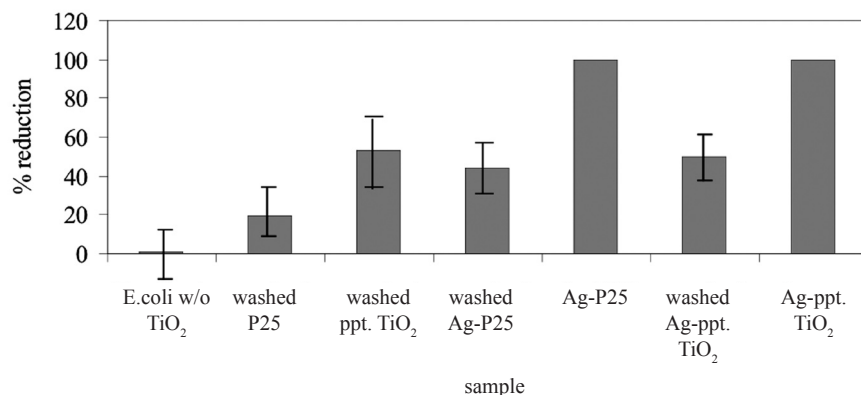


Figure 3. Reduction of *E. coli* on the powder samples.

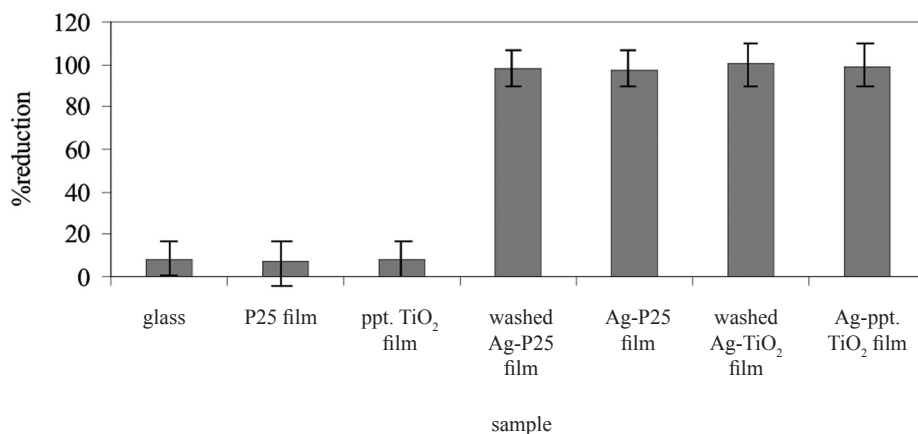
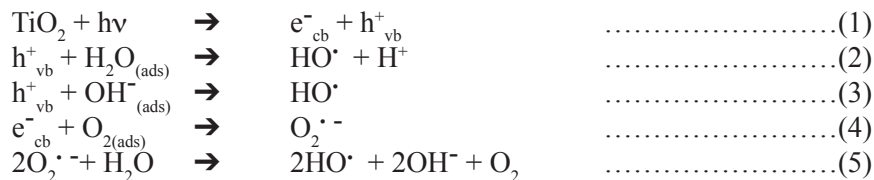


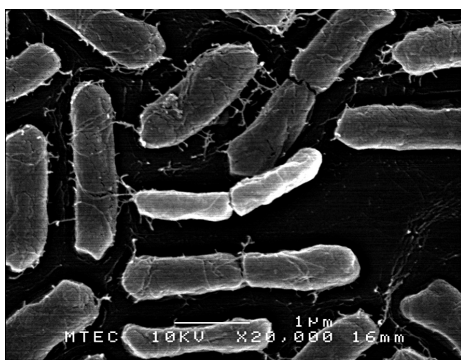
Figure 4. Reduction of *E. coli* on the film samples.

This is because of the fact that when exposed to UV, conduction band electrons and valence band holes in TiO_2 are generated (Equation (1)) and recombined in a short time (Tachikawa et al., 2004). In the presence of Ag on TiO_2 particle, photogenerated electrons from TiO_2 were transferred to Ag clusters, as Ag has higher work function than TiO_2 , inhibiting the electron-hole pair recombination (Zhang et al., 2003). As a result, the holes have longer life time to oxidize organic materials and to react with adsorbed water and hydroxyl groups, producing hydroxyl radicals (Equations (2) and (3)) (Ireland et al., 1993). Some of the photogenerated electrons can react with adsorb oxygen on TiO_2 surface, generating superoxide ions (Equation (4)) which decompose to hydroxyl radicals (Equation (5)). Hydroxyl radicals are very reactive

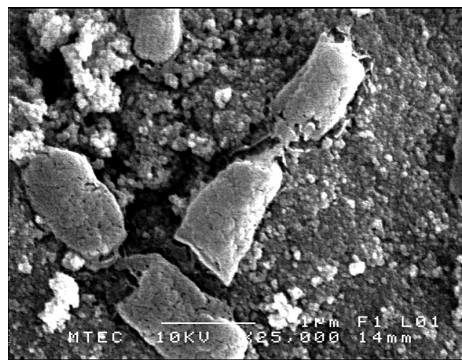
and cause the death of the cells (Fig. 5).



It is possible that the Ag itself (on the films) caused the cell damage as Zhang and Yu (2005) reported that Ag^+ ions present during the photocatalysis can degrade adsorbed species on the catalyst. In addition to that, during the cell incubation, Ag might dissolve out in a small amount and cause the cell death. However, we found that the longer the illumination, the more the cell damage (Fig. 6). Bare in mind that *E. coli* cells were in contact with Ag-TiO₂ film surface all the time. This confirms that UV light influences the bactericidal ability of the samples.

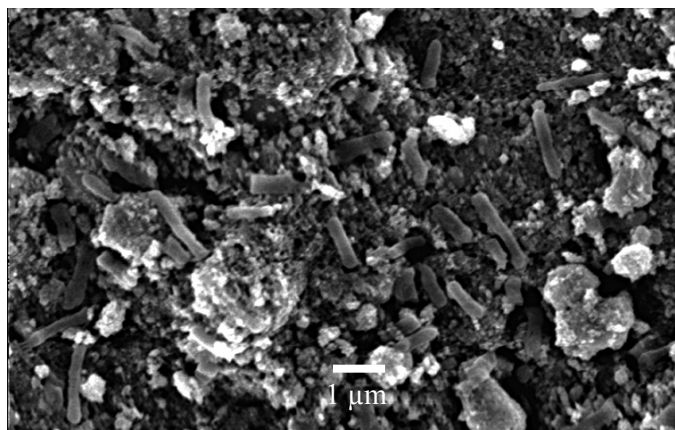


(a)



(b)

Figure 5. SEM images of *E. coli* on (a) cover glass without UV exposure and (b) Ag-TiO₂ film with UV exposure.



(a)

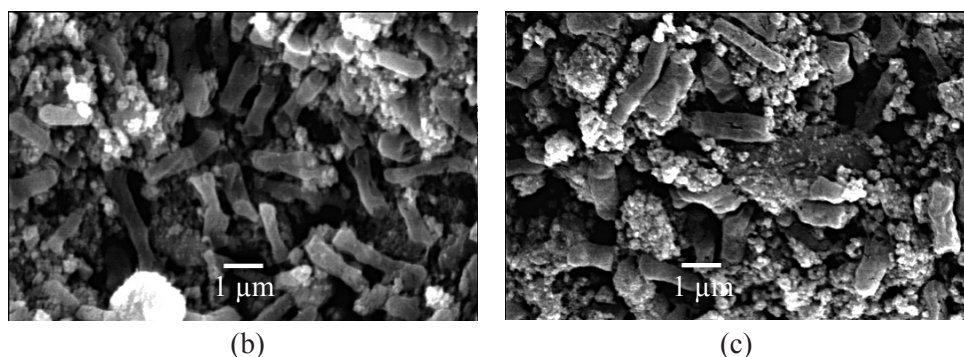


Figure 6. SEM images of *E. coli* on Ag-TiO₂ films (a) without UV exposure, (b) with 15-sec exposure and (c) with 30-sec exposure.

CONCLUSION

It was found that there was more brookite structure in Ag-TiO₂ powder than in TiO₂ powder, indicating that Ag influenced crystallization of TiO₂. Although the doped powder had lower surface area, they were more reactive than the undoped samples, indicating that Ag played an important role in bactericidal ability. There was Ag dissolution from the Ag-doped powder during the washing process, leading to a decrease in bactericidal ability. Unlike the doped powder, the doped films showed high reactivity after being washed, eleven times higher those without Ag. This work was a success in preparing effective doped films via doctor blade technique.

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