

Synthesis of Silver Doped Titanium Dioxide by Wet-Ball Milling Sol–Gel Method for Antibacterial Application

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Abstract Titanium dioxide (TiO₂) has been extensively studied as photo-catalyst for water treatment, air purification and antibacterial applications due to its challenging properties such as chemical stability, environmental friendly and strong photocatalytic activity. However, the limitation of TiO₂ on its dependent to ultraviolet radiation for photocatalytic activity is still aroused. In this study, silver doped titanium dioxide (Ag-TiO₂) was synthesized by wet-ball milling sol–gel method (WBMS). Ag-TiO₂ molar ratio was varied from 0% to 10% to study the effect of silver content on the synthesized Ag-TiO₂ characteristics and the ability to apply on antibacterial applications. The objective of this work was to find an optimal concentration of Ag in Ag-TiO₂. Characterization of the particle size, morphology, and surface area of synthesized Ag-TiO₂ were discussed by techniques of transmission electron microscopy (TEM) and Brunauer-Emmett-Teller (BET). Photocatalytic activity was investigated from degradation of methylene blue. Antibacterial activity was conducted by finding minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) tests performed on *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) under dark condition and under visible light. The results demonstrated that the doping of Ag inhibited crystal growth of Ag-TiO₂. The smallest particle size and the highest surface area were obtained from 5% Ag-TiO₂. Also, it was found that methylene blue degradation rate increased to the highest number of $1.62 \times 10^{-3} \text{ min}^{-1}$ when Ag concentration reached 5%, and methylene blue degradation rate reduced when Ag concentration was higher than 5%. The antibacterial activity of Ag-TiO₂ was better than TiO₂. The optimal concentration of 3-5% Ag-TiO₂ was observed from the MIC and MBC tests.

1 Introduction

Titanium dioxide (TiO₂) nanoparticle is photocatalyst has been widely researched in waste treatment applications due to its strong oxidizing property, non-toxicity and long-term photo stability (Zaleska, 2008). It has been also in the research interest for the antibacterial applications. However, the limitation of using TiO₂ upon its dependency on UV light is prominent. To overcome this limitation of TiO₂ nanoparticles, as well as enhancing the effectiveness of its photocatalytic and antibacterial activity, silver doped TiO₂ (Ag-TiO₂) nanoparticle is proposed. In this study, Ag-TiO₂ was synthesized by wet ball milling sol-gel method (Phomma *et al.*, 2017). Effect of silver concentration on the photocatalytic activity and antibacterial activity was investigated. Characterization of the particle size and morphology of synthesized Ag-TiO₂ were determined.

2 Experimental

2.1 Ag-TiO₂ Preparation

Ag-TiO₂ was synthesized by wet-ball milling sol–gel method, modified from the previous report (Phomma *et al.*, 2017). The substitution reaction of Titanium (IV) isopropoxide 97%wt (TTIP) with methanol (MeOH) were carried out with a molar ratio of 1:15. The solution pH was adjusted to 2 by adding 2 ml of HNO₃. The various amount of Ag was added to the TiO₂ solution in concentration of 3%, 5%, 6% and 10% by mole. The yellow colloid of Ag-TiO₂ was obtained. Citric acid was used to reduce Ag⁺ into Ag⁰. The Ag-TiO₂ gel was then observed. Washing Ag-TiO₂ gel with DI water by centrifuged at 10,000 rpm for 15 min, then the yellow gel was dried at 110°C for 24 hours. The yellow powder, then,

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appeared. The yellow powder was dry ground with 10 mm-ball milling at 300 rpm for 20 min and consequently wet ground by 2 mm-ball milling at 500 rpm for 3 hours in IPA. The Ag-TiO₂ solution was dried again at 110°C for 24 hours and was calcined at 400°C for 4 hours with a heating rate of 5°C/min. After all the processes mentioned, Ag-TiO₂ nanoparticle powder was synthesized. The nomenclature 3% Ag-TiO₂, 5% Ag-TiO₂, 6% Ag-TiO₂ and 10% Ag-TiO₂ are used to represent the various concentration of silver doped.

2.2 Characterization of synthesized Ag-TiO₂

The morphology and particle size distribution of Ag-TiO₂ were studied by high resolution transmission electron microscopy (HRTEM, JEOL JEM-2100) operated at 200 kV. Brunauer-Emmett-Teller (BET) was used to determine surface area of Ag-TiO₂ particles. The degradation of methylene blue (MB), (Ajax Finechem), under UV radiation was used as a model system to evaluate the photocatalytic activity of Ag-TiO₂. The photocatalytic reaction was carried out in the in-house photocatalytic chamber. The initial concentration of MB (C₀) was prepared at 5 ppm. A 10-mg of Ag-TiO₂ was mixed with 40 ml of MB solution and then stirred in the photocatalytic chamber. The mixture was kept in the dark for 1 h to ensure the saturation of MB on the surface of the catalysts. The MB solution was irradiated by 0.25 UV light Wm⁻² (λ=351 nm). The sample was collected at every hour for 24 hour. The concentration of MB in suspension was analyzed using a UV-Vis spectrophotometer at wavelength of 635 nm. The kinetic constants of reaction rate were determined according to the pseudo-first-order kinetic model as follows:

$$\ln(C/C_0) = -kt \quad (1)$$

where t is the irradiation time

k is kinetic constant (min⁻¹), and

C is the concentration of the MB (mg/L).

The antibacterial activity of the synthesized Ag-TiO₂ was investigated using the standard microdilution method. Minimum inhibitory concentration (MIC) that inhibited the growth of bacterial strain was determined on 96-well microdilution plates. Initial concentration of microorganisms (Staphylococcus aureus; S. aureus 8739 as a gram positive or Escherichia coli; E. coli 6538P as a gram negative bacteria) was 105 cfu/ml. Preparation of Ag-TiO₂ solution by dissolved 10 mg Ag-TiO₂ in 10% dimethyl sulfoxide (DMSO) and make a serial dilution of Ag-TiO₂ powder with Mueller-Hinton Broth (MHB). End point were determined when no turbidity in the 96-well was observed after 24 hours under dark and UV light condition of incubation at 37°C and 95% relative humidity. Minimum bacterial concentration (MBC) was

also determined by counting colony on agar plates after incubated at 37°C for 24 hours.

3 Results and Discussions

From TEM images, as shown in Figure 1, silver distribution was observed as black particles scattering on white powder. The scattered silver particles came close to each other more when the silver concentration was higher. The aggregation of particles seemed to be more obvious with higher silver amount added.

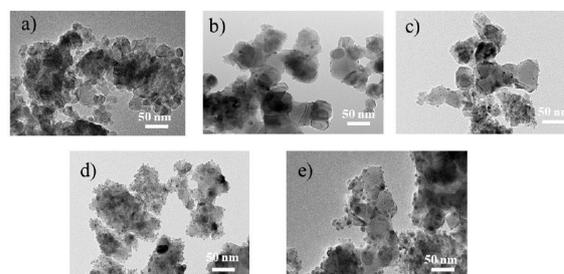


Figure 1. TEM images of synthesized TiO₂(a), 3%Ag-TiO₂(b), 5% Ag-TiO₂(c), 6% Ag-TiO₂(d) and 10% Ag-TiO₂ (e)

As demonstrated in Table 1, particle size of Ag-TiO₂ increased from 16.4±5.2 nm, when there was no Ag added, to 34.0±9.1 nm when it was 3% Ag-TiO₂. Higher concentration of silver, however, did not cause further increasing of the particle size. It stayed in a range of 21 – 28 nm. This pattern might be well-explained owing to the larger size of Ag⁺(ca.126 pm) when compared to the size of Ti⁴⁺(ca.68 pm). With small amount of silver added, the possibility of silver doped in the lattice of TiO₂ is greater, and expand the particle size of the Ag-TiO₂. When more silver was added, however, silver ion rather stayed on the outer surface of the TiO₂ than doped into the structure. The Ag⁺ dispersed on surface of TiO₂ effect the density of surface increased defect at the surface of anatase grain which favors the rutile nucleation (Chao *et al.*, 2003). Then, surface area of 3% Ag-TiO₂ decreased from 18.62 to 13.97 m²/g as shown in Table1 due to large particle size of 3% AgTiO₂. Increasing of molar ratio of Ag from 5% to 6%, the particle size decreased from 23.0±7.4 nm to 21.8±7.2 nm. Since the Ag doping in optimum molar ratio usually hinders the growth of particle size (Ahmad *et al.*, 2007). The highest surface area was obtained at 5% AgTiO₂. Moreover, when molar ratio of Ag increased from 6% to 10%, the particle size was increased to 28.5±9.6 nm relative with decreasing of surface area. Then, 10% Ag-TiO₂ showed agglomeration of nanoparticle since it presented high surface energy of the particle which the particle size of TiO₂ is not limit of growth.

Table 1. Particle size and BET surface area of synthesized TiO₂ and Ag-TiO₂

Sample	Particle size (nm)	Surface area (S _{BET} m ² /g)
TiO ₂	16.4±5.2	18.62
3% Ag-TiO ₂	34.0±9.1	13.97
5% Ag-TiO ₂	23.0±7.4	32.12
6% Ag-TiO ₂	21.8±7.2	27.52
10% Ag-TiO ₂	28.5±9.6	30.25

Figure 2 showed photocatalytic activity of TiO₂ and Ag-TiO₂ with the degradation of MB solution under UV light and calculated the kinetic constant rate (*k*) as shown in Table 2. It was shown that the photocatalytic activity of Ag-TiO₂ were higher than TiO₂ powder with kinetic constant rate increased from 1.03x10⁻³ min⁻¹ to 1.62x10⁻³ min⁻¹. The highest photocatalytic activity with the kinetic constant of 1.62x10⁻³ min⁻¹ was obtained with 5% Ag-TiO₂. This result was compliant to the highest surface area of 5% Ag-TiO₂ as discussed earlier. When Ag-TiO₂ was increased from 6% to 10%, the photocatalytic activity decreased as can be seen by the decreasing of kinetic constant to 1.53x10⁻³ min⁻¹. Since a large silver nanoparticle hindered UV absorption onto TiO₂, in consequence, the degradation of MB was slower.

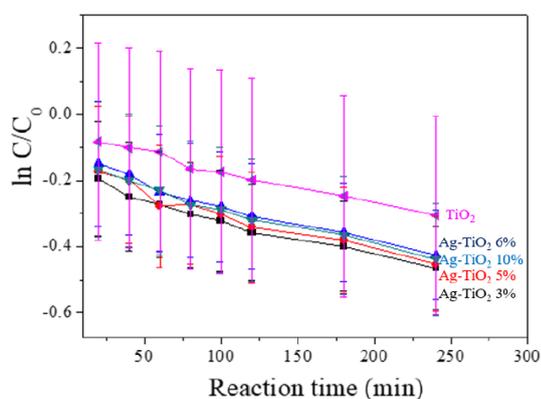


Figure 2. Photocatalytic reaction rates of MB degradation with different silver concentration in Ag-TiO₂

Table 2. Kinetic constant of MB degradation

Sample	Kinetic constant rate (kx10 ⁻³ min ⁻¹)
TiO ₂	1.03
3% Ag-TiO ₂	1.12
5% Ag-TiO ₂	1.62
6% Ag-TiO ₂	1.57
10% Ag-TiO ₂	1.53

The MIC of TiO₂ and Ag-TiO₂ with different concentrations was estimated by using Staphylococcus aureus (*S. aureus*) 8739 as a gram positive and Escherichia coli (*E. coli*) 6538P as a gram negative bacteria. Table 3 show MIC of TiO₂ and Ag-TiO₂ with different concentrations under dark and UV light condition. The results showed that neither MIC nor MBC of microorganism growth could not be found in case of using TiO₂ under dark condition. This is obvious that the

antibacterial applications using TiO₂ solely cannot be achieved due to its dependence to UV light.

Table 3. MIC and MBC for *S. aureus* and *E. coli*

Sample	MIC (mg/ml)				MBC (mg/ml)			
	<i>E. coli</i>		<i>S. aureus</i>		<i>E. coli</i>		<i>S. aureus</i>	
	Dark	UV light	Dark	UV light	Dark	UV light	Dark	UV light
TiO ₂	-	1.250	-	1.250	-	10.000	-	10.000
3% Ag-TiO ₂	0.625	0.625	1.250	1.250	2.500	1.250	2.500	2.500
5% Ag-TiO ₂	0.625	0.625	1.250	1.250	1.250	1.250	1.250	2.500
6% Ag-TiO ₂	0.625	1.250	1.250	1.250	1.250	2.500	2.500	2.500
10% Ag-TiO ₂	0.312	0.625	0.625	1.250	0.625	0.625	5.000	2.500

Considering MIC obtained when using different concentrations of Ag-TiO₂, it was clearly seen that the bacteria growth can be inhibited even in the dark condition when silver is doped to TiO₂. MIC of *E. coli* and *S. aureus* were the same when 3% Ag-TiO₂, 5% Ag-TiO₂, and 6% Ag-TiO₂ were used with both dark and UV-light conditions. However, the MIC was decreased to almost half when 10% Ag-TiO₂ was applied. It might be able to explain that 10% Ag-TiO₂ showed the highest percentage of silver nanoparticles which affect directly to bacteria cell and cause its death.

The MBC is smaller when Ag-TiO₂ was used in UV-light condition, indicating that the synergistic effect of bacteria degradation by titanium and silver was presented. The MBC of *E. coli* and *S. aureus* under UV-light condition of TiO₂ showed the highest amount of 10 mg/ml to kill bacteria. On the other hand, Ag-TiO₂ gave lower amount of concentration to kill bacteria. Therefore, Ag-TiO₂ showed the higher antibacterial activity than TiO₂. MBC of *E. coli* of 3% Ag-TiO₂ under UV light was at 0.625 mg/ml which is twice lesser than that of the dark condition at 1.250 mg/ml. As MBC of *S. aureus* under dark and UV light conditions were the same at 2.500 mg/ml. It suggested that *S. aureus* is more resistant than *E. coli*. As can be seen when 5% Ag-TiO₂ was used, MBC of *E. coli* was less than that of *S. aureus*. When increasing silver concentration to 6% Ag-TiO₂ - 10% Ag-TiO₂, however, MBC of *E. coli* and *S. aureus* were the same. The lowest MBC of *E. coli* is showed when using 3% Ag-TiO₂ - 5% Ag-TiO₂ and MBC of *S. aureus* also the same value at 2.500 mg/ml. It was proved that the antibacterial activity of Ag-TiO₂ is higher than that of TiO₂. Looking back to the images shown in Figure 1, it can be seen that Ag nanoparticle tends to deposit on TiO₂ surface. The deposited Ag can be oxidized with oxygen to generate Ag⁺ ion. Ag⁺ ion can attack the cell wall of bacteria and diffuse into the cytoplasm leading to the cell death. Moreover, TiO₂ makes photocatalytic reaction happened and generate reactive oxygen species that can kill bacteria. Furthermore, from the results we obtained the higher value MBC of *S. aureus* than *E. coli* is because the difference in cell wall structure between gram positive and gram negative bacteria. Because gram negative consists of a thin peptidoglycan layer (PG) So, gram negative bacteria can be attacked easier than gram positive.

4 Conclusion

Doping silver to TiO₂ nanoparticles by WBMS method are successful. It results in modifying the lattice structure of TiO₂ nanoparticle and cause the different size and surface area depending on the concentration of silver doping. The synthesized Ag-TiO₂ nanoparticle shows higher performance on antibacterial applications when compared to TiO₂. The optimal amount of Ag in TiO₂ is 5% by mole. It shows the highest photocatalytic activity with kinetic constant rate $1.62 \times 10^{-3} \text{ min}^{-1}$. Antibacterial activities of 3% Ag-TiO₂ and 5% Ag-TiO₂ for E. coli and S. aureus are the highest and higher than those of TiO₂ as well.

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