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Photocatalytic disinfection of *P.aeruginosa* bacterial Ag-doped TiO_2 film

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Abstract

TiO_2 and TiO_2-Ag composites films were prepared by sol–gel method and coated on glass fibre roving. The surface morphology and properties of synthesized composites films were characterized by X-ray diffraction (XRD), scanning electron microscopy (SEM), energy dispersive x-ray spectroscopy (EDS), Fourier transform infrared spectroscopy (FT-IR) and UV-vis diffuse reflectance spectroscopy (DRS). The antibacterial activity studies of TiO_2 and TiO_2 composite films were evaluated by photocatalytic reaction against *P.aeruginosa* bacteria. The results shown that pure TiO_2 and TiO_2 porous (TiO_2-PEG) films have disinfection efficiency 57% and 93% within 15 min under UV irradiation, respectively. TiO_2-1Ag film has highest antibacterial effect under UV irradiation and that disinfection efficiency is 100% within 10 min. It has been found that Ag doped TiO_2 films have the higher disinfection efficiency than that of pure TiO_2 due to the effect of silver species.

Keywords: Ag doped TiO_2; thin film; P.aeruginosa bacterial; photocatalytic; porous film; Sol-gel method

1. Introduction

Titanium dioxide (TiO_2) is an excellent photocatalyst. It is widely used as a photocatalyst because it is relatively highly efficient, cheap, non-toxic, chemically and biologically inert and photo stable. TiO_2 in the anatase phase has been used as an excellent photocatalyst and it is well application for purification [1]. This process is performed by activation of photocatalyst using ultraviolet or visible light to produce primarily hydroxyl and superoxide radicals which are the active sites on TiO_2 surfaces for oxidizing

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organic compounds and antibacterial to water vapour and carbon dioxide [2]. However, TiO_2 powders used for industry are difficult for separation and removal of TiO_2 powders from air or water. Therefore, many studies have shown that TiO_2 film coated on many kinds of substrates such as [3], stainless steel [4] and polymer [5] in order to recycle them more easily. And many studies have shown that TiO_2 coated on glass and tiles for purification, antibacterial [6-7] and self-cleaning [8] under UV-light in living area.

In order to improve photocatalytic activity, efforts have been made to increase surface activation sites by making porous microstructure [9-11]. TiO_2 film has been synthesized by many techniques such as introducing TEA [12], nanocarbon spheres [13] and PEG [14] into the precursor solution. However, the efficiency of TiO_2 photocatalytic is low for its application [15]. The effective way to improve the TiO_2 photocatalytic activity is to introduce transition metal ions into TiO_2. A wide range of transition metal ions have been reported to be used as electron acceptor to decrease the e^- - h^+ recombination such as Ag [16-17] and then that is known as the most interesting antibacterial materials [18].

In this study, we are interested in Ag doped TiO_2 because Ag doping into TiO_2 prevents the recombination of electron-hole pairs and improves it antibacterial activities. Polyethylene glycol (PEG) was introduced into TiO_2 for increase surface activation site. Therefore, in this study prepared porous Ag doped TiO_2 films on glass fibre roving were prepared by sol-gel and dip coating methods. Experimental study of the effect of Ag and PEG doping on photocatalytic activity against *P.aeruginosa* bacteria under UV irradiations was carried out compared to those of undoped TiO_2 and TiO_2-Ag composite films.

2. Experimental

2.1. Raw Materials

Materials used for synthesis of pure TiO_2 and Ag/TiO_2 composite films were titanium (IV) isopropoxide (99%) (Fluka Sigma-Aldrich), silver nitrate ($AgNO_3$, VWR Prolabo, United Kingdom), PEG4000, ethanol (99%) and nitric acid were both AR grade.

2.2. Film Preparation

Pure TiO_2 and TiO_2 composite films coated on glass fibre roving were prepared by sol-gel method. Titanium (IV) isopropoxide (Fluka Sigma-Aldrich) of 10 ml and 2 g of PEG4000 were added into 100 ml ethanol (95%) and it was mixed with the solution prepared by dropping silver nitrate ($AgNO_3$, VWR Prolabo, United Kingdom) in 50 ml ethanol. The pH of mixed solution was adjusted to about 3 and it was vigorously stirred at room temperature for 1h until sol was formed. Glass fibre roving was coated with as prepared sol by dip coating and oven drying at 100 °C for 12 h before calcinations at 500 °C for 1h with a heating rate of 10 °C /min.

2.3. Materials Characterization

The surface morphologies of pure TiO_2 and TiO_2 composites films coated on glass fibre roving were observed by using a scanning electron microscope (JSM-5800 LV, JEOL). Microstructures of TiO_2 composites were determined from X-ray diffraction (Phillips, Cu-k_∞ radiation of wave length 1.5418 Å). The data were taken in the range of 10-70 (2θ). The average crystallite size was determined from the X-ray diffraction pattern using Scherer's equation [19]. The band-gap energy and the optical absorption spectra were measured at wavelength in the range of 200-800 nm by UV-visible diffuse reflectance spectroscopy (UV-2401, Shimadzu, Japan) equipped with an integrating sphere and a powdered $BaSO_4$ as reference. The dispersion of Ti in the film was characterized by EDS (EDS: Oxford ISIS 300) attached to the scanning electron microscope. Fourier-transformed infrared spectrophotometer (FT-IR, Bruker

Equinox 55) in KBr pellets and infrared spectra were recorded in the wavelength between 4000-400 cm^{-1} was used to assess the presence of functional groups in different TiO_2.

2.4. Antibacterial Activity Study

P. aeruginosa which is a Gram-negative, aerobic and rod shaped bacteria was utilized for studying antibacterial activity of the synthesized film. *P. aeruginosa* cells were grown aerobically in 4 ml of tryticase soy broth at 35 °C for 24 h. This component was a 10 serial dilution by 0.85% sodium chloride solution. The colony forming units (CFU) were determined by spreading on Macconkey agar (Difco MacConKey Agar) plate technique. The plates were incubated for 24 h at 35 °C, and the numbers of colonies were counted. The number of bacterial colonies to determine the growth inhibition rates with the equations (1). [20]

$$R\,(\%) \quad = \quad (A - B) / A \qquad (1)$$

Where R = the growth inhibition rates, A = the number of bacterial colonies from control sample, and B = the number of bacterial colonies after treated.

The initial bacterial cell concentration was about 1×10^3 CFU/ml and pipette solution 2 ml onto TiO_2 coated glass fibre roving. The antibacterial activity tests were performed under UV irradiations for 0, 15, 30, 45 and 60 min. The solution of 100 µl after treatment was sampled, spread onto Macconkey agar plate and incubated at 35 °C for 24 h. Then the number of colony was counted. All glassware was sterilized by autoclave at 121 °C for 15 min before using in the test.

3. Result and Discussion

3.1. Characterization

Fig.1 shows the XRD patterns of Pure TiO_2 and TiO_2 composites powders. The crystal structures of the powders were clarified to be a single phase of anatase by XRD measurements. Pure TiO_2 and TiO_2 composites powders are well crystallized. The crystallite size calculated using the Scherrer's equation and determined from the broadening of the anatase (101) peak (2θ=25.3°) were 14.7 nm and 25.3 nm for pure TiO_2 and TiO_2-Ag, respectively. The crystallite size of TiO_2-Ag powder tends to slightly increase as compared to pure TiO_2. These results indicate that the during drying and calcining process Ag^+ ions spreading on the surface anatase grains would gradually be reduced to Ag^0 is effect to the crystallite size of anatase phase increase [21]. The crystallite size of TiO_2-Ag-PEG powder was 16.6 nm. That of TiO_2-Ag-PEG powders smaller than TiO_2-Ag. PEG is stabilizer and it benefits to reduce Ag^+ to Ag^0 during films calcination.

Fig. 2 shows SEM images of Pure TiO_2 and TiO_2 composites films prepared by sol-gel method and dipped coating on glass fibre roving. Fig. 2a and 2b indicate SEM images of the surface of glass fibre roving with the different magnifications. Fig. 2c and 2f shows morphologies of the pure TiO_2 and TiO_2 porous films coated on glass fibre roving. It can be estimated that TiO_2 doped PEG films has large specific surface area compared to those of other samples. The porous structures of TiO_2 films are formed by CO_2 gas immigrated from organic compound and PEG combustion during calcinations which PEG started to decompose at around 250 °C in air and that a temperature of 300 °C allowed complete removal of PEG from a TiO_2 film [22].

EDS analyses shown in Fig.3 confirm the presence of Ti in pure TiO_2 and TiO_2 composite films coated on glass fibre roving. Ti was well dispersion on glass fibre. The Si, Ca and Na peaks as shown in Fig. 3 refer to the substrate composite observed with a scanning electron microscope (SEM).

The UV-visible diffuse reflectance spectra of the samples are shown in Fig.4. It can be seen that all samples were strong absorptions in the ultraviolet region. The absorption edge of TiO_2 composites moved to longer wavelength in comparison with pure TiO_2 indicating the band gap was decreased by doping with Ag. The calculated band gap energy of pure TiO_2 and TiO_2-1Ag are 2.98 and 2.74 respectively. The photocatalytic reaction over *P. Aeruginosa* disinfection of TiO_2-Ag should be high activity due to the electron–hole pair separation efficiency induced by enhancing the charge pair separation and inhibiting their recombination by the Ag dopant [23].

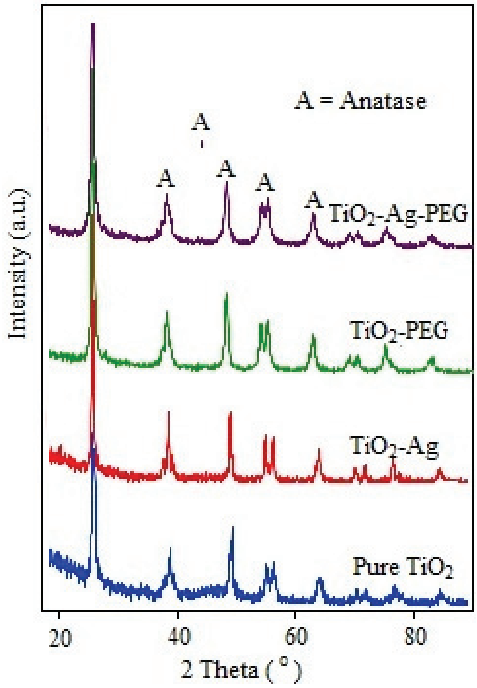

Fig. 1. XRD patterns of pure TiO_2, TiO_2-1Ag, TiO_2-PEG and TiO_2-1Ag-PEG films coated on glass fibre roving

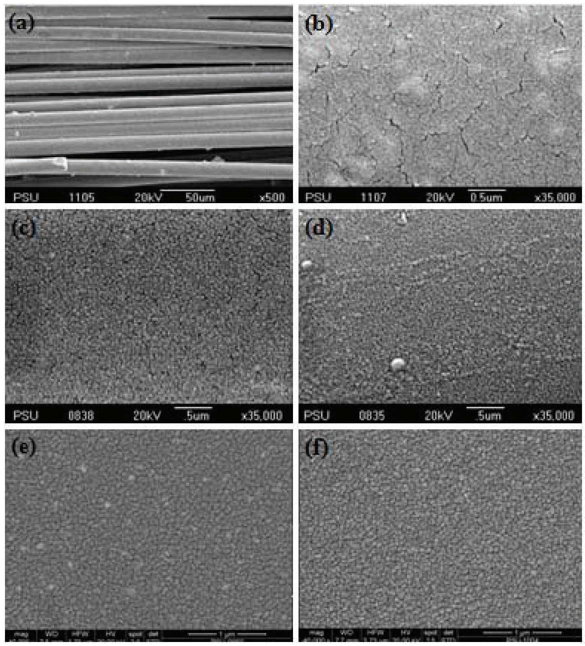

Fig. 2. SEM images of (a) and (b) surface of glass fibre roving with the different magnifications (c) pure TiO_2, (d) TiO_2-1Ag, (e) TiO_2-PEG and (f) TiO_2-1Ag-PEG films coated on glass fibre roving

Results of Fourier transform infrared spectroscopy for pure TiO_2 and TiO_2 composites films calcined at the temperature of 500°C in air for 1h are shown in Fig.5 The spectra show several peaks observed such as 3433, 1643 and 478 cm^{-1}. The spectra at 3413 and 1626 cm^{-1} are -OH stretching of TiO_2 surfaces (Ti-OH) and O-H bond of hydroxyl group, respectively [24]. The 535 cm^{-1} spectrum is Ti-O bond of anatase phase [25]

3.2. Antibacterial Activity

Fig. 6 demonstrates the photographs of disinfection of *P.aeruginosa* in the various systems. In the TiO_2-1Ag and TiO_2-1Ag-PEG, the *P.aeruginosa* was almost disinfected compared with the Pure TiO_2. And then Pure TiO_2 has the higher disinfection efficiency than that *P.aeruginosa* treated under UV irradiation without TiO_2. Because TiO_2 produces hydroxyl and superoxide after absorb UV light. It attacks polyunsaturated phospholipids in *P.aeruginosa.* The lipid peroxidation reaction that subsequently causes a breakdown of the cell membrane structure and therefore its associated functions is the mechanism underlying cell death [26]. Moreover, it is oxidation of intracellular coenzyme A cause of decreases in respiratory activities that led to cell death [27-28].

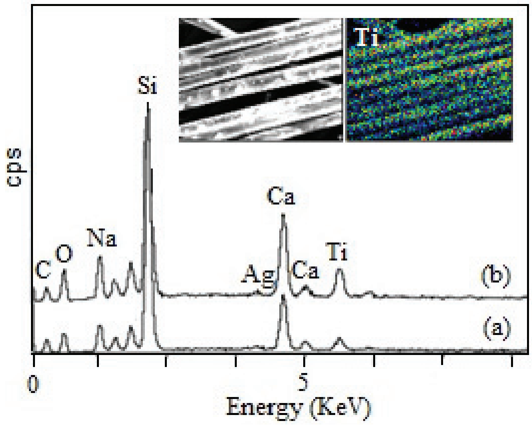

Fig. 3. The EDS spectra of (a) pure TiO_2 and (b) TiO_2-1Ag powders

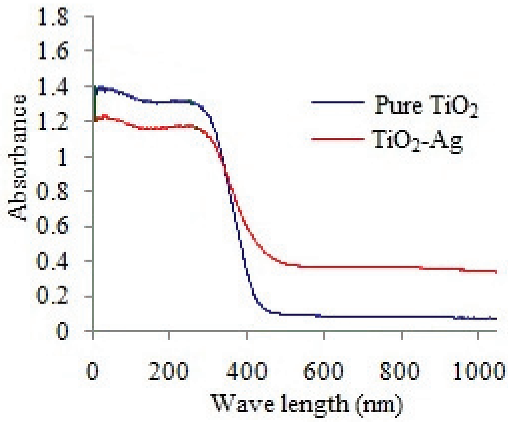

Fig. 4. The UV-VIS spectra of pure TiO_2 and TiO_2-1Ag powders

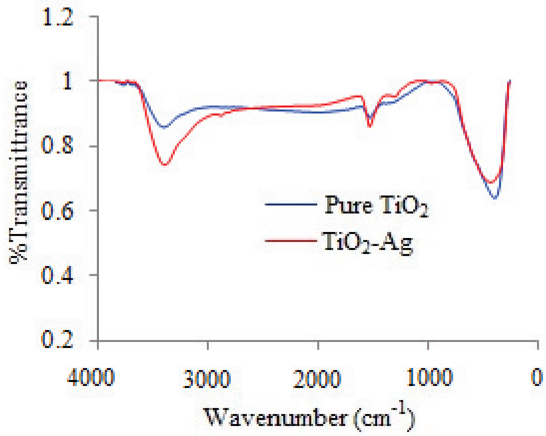

Fig. 5. FT-IR spectra of Pure TiO_2 and TiO_2-Ag calcined at the temperature of 500 °C in air

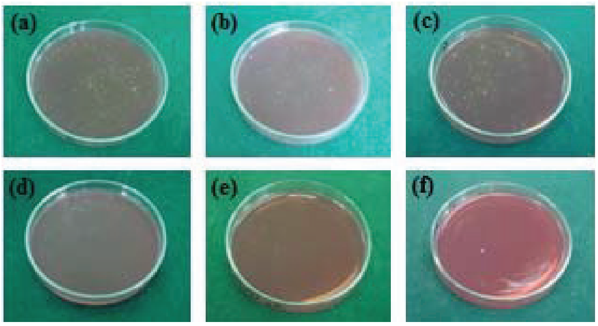

Fig. 6. Photographs of *P.aeruginosa* bacteria grown on agar plate (a) control (b) treated under UV irradiation without TiO_2 and treated with (c) pure TiO_2, (d) TiO_2-1Ag, (e) TiO_2-PEG and (f) TiO_2-1Ag-PEG film for 15 min

The antibacterial activity of pure TiO_2 and TiO_2 composites films were evaluated by photocatalytic reaction against *P.aeruginosa* bacteria. Based on the growth inhibition rate shown in Fig. 7, it can be seen that TiO_2-1Ag and TiO_2-1Ag-PEG have strong antibacterial effect under UV irradiation. It is clear that the disinfection efficiency is 100% and 99% within 15 min under UV irradiation, respectively. While those of pure TiO_2 and TiO_2-PEG films are about 57% and 93%, respectively. PEG addition in film seem to have a slightly effect on photoactivity due to its porous structure discussed above. For long treated time, Pure TiO_2 and TiO_2 composite films tend to completely kill this kind of bacterial.

The Ag doped TiO_2 films have the higher disinfection efficiency than that of pure TiO_2 due to Silver species co-existed, Ag^+, Ag^0 and metal Ag acting as photogenerated electrons trapping sites prevent the electron-hole pairs recombine rapidly after photo-excitation leading to enhancement of photocatalytic activity [29]. And the absorption edge of TiO_2 composite moved to longer wavelength in comparison with pure TiO_2. This mechanism produces more hydroxyl radicals for disinfection. Moreover, silver itself is a well antibacterial agent. The mechanisms of silver in TiO_2 films are the interaction of respiratory enzymes damaged by silver or silver oxide and cell membrane damaged by reactive oxygen species (ROS) when cells contact to film surfaces. It was shown that Ag^+ ions prevent DNA replication and affect the structure, leading to the damage of the cell membrane [30]. Silver ions are also photoactive in the UV-A and UV-C irradiations, leading to enhance UV inactivation of bacteria.

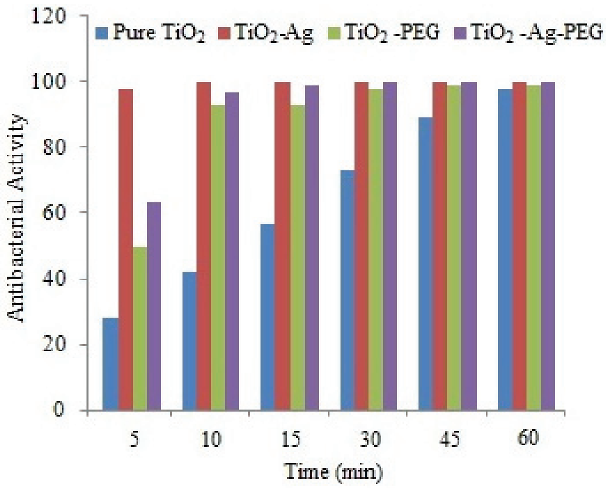

Fig. 7. Antibacterial efficiency under UV irradiation as a function of time for Pure TiO_2, TiO_2-Ag, TiO_2-PEG and TiO_2-Ag-PEG

4. Summary

In this work, pure TiO_2 and TiO_2 composites films were prepared by sol-gel method and dipped coating on glass fibre roving. It was found that Ag doping affects to crystallite size and antibacterial efficiency**.** XRD pattern of pure TiO_2 and TiO_2 composite films photocatalyst shows single phase of anatase. The absorption edge of TiO_2 composite moved to longer wavelength in comparison with pure TiO_2 indicating the band gap was decreased by doping with Ag. It was found that TiO_2-1Ag film has highest antibacterial effect under UV irradiation and their disinfection efficiency is 100% within 10 min while TiO_2-PEG and TiO_2-Ag-PEG have disinfection efficiencies 93% and 99% for 15 min. PEG may affect on enhance surface area of films to make them porous but it may influence on t density reduction of photo induced electrons on glass fiber roving.

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