Preparation and Characterization of Antibacterial TiO_2/Poly[acrylamide-*co*-(acrylic acid)] Composite Hydrogel

K. Boonpavanitchakul, D. Yiamsawas and W. Kangwansupamonkon

National Nanotechnology Center, National Science and Technology Development Agency
111 Thailand Science Park, Paholyothin Road, Klong 1, Klong Luang, Pathumthani 12120, Thailand.
wiyong@nanotec.or.th

ABSTRACT

Photocatalytically antibacterial TiO_2/poly[acrylamide-*co*-(acrylic acid)] composite hydrogels were prepared from acrylamide (AAm) and Acrylic acid (AAc) copolymer by free-radical aqueous polymerization, using *N,N'*-methylene-bisacrylamide (*N*-MBA) as a crosslinker, ammonium persulfate (APS) as an initiator, and a commercially available TiO_2 photocatalyst (Degussa P-25) and then neutralized with sodium hydroxide solution .

In this work, a comparative study on the antibacterial effects of TiO_2 containing hygrogel prepared by different amounts of TiO_2 nanoparticles was reported. An antibacterial activity of TiO_2-hydrogel composite against gram negative *Escherichia coli (E. coli)* and gram positive *Staphylococcus aureus (S. aureus)* was investigated. Its antibacterial performance was observed under UV light with irradiation time and dark conditions. The results demonstrated that the antibacterial activity depended on amounts of TiO_2 nanoparticles and the antibacterial activity of TiO_2 hydrogel composite increased under UV irradiation at 1 $mWcm^{-2}$ for 1, 3 and 5 hours, respectively. The particulates of pristine TiO_2(P-25) and TiO_2 composite hydrogel were characterized using Brunauer-Emmett-Teller (BET), TEM, SEM & EDX techniques.

Keywords: hydrogel, composite, photocatalytic, TiO_2 nanoparticles , antibacterial

1 INTRODUCTION

Hydrogels have a network structure and suitable degree of crosslinking [1]. Such a polymer is able to absorb much more water than its mass, ranging from hundreds to thousands of times. These polymers have been extensively applied as sorbents in such personal care products, as infant diapers, feminine hygiene products, and incontinence products. These polymers also attract attention for a variety of more-specialized applications, including matrices for enzyme immobilization, biosorbents in preparative chromatography, materials for agricultural mulches, and matrices for controlled release devices [2].

Photocatalytic materials such as titanium dioxide (TiO_2), zinc oxide (ZnO), cadmium sulfide (CdS), and zinc sulfide (ZnS) have been widely studied since 1972 by Fujishima and Honda [3]. TiO_2, one of the most powerful photocatalytic materials, possesses high activity, strong oxidizing powers,and long-term stability. [4-6] TiO_2 can generate strong oxidizing power when illuminated under ultraviolet (UV) light with wavelengths of less than 385 nm [7-9]. The photon energy generates an electron hole pair on the TiO_2 surface. The hole in the valence band can then react with water or hydroxide ions adsorbed on the surface to produce hydroxyl radical ($OH^{\bullet}$), and the electron in the conduction band can reduce O_2 to produce superoxide ions (O_2^{-}). Both holes and $OH^{\bullet}$ are extremely reactive upon contacting organic compounds, [5] and transform these compounds nontoxic materials. This property can be applied for killing bacterial. It is well known that the TiO_2 in anatase form is capable of oxidizing and decomposing various organisms including virus, bacteria, fungi, algae, and cancer cell [10-11]. Shieh et al. have reported an antibacterial performance of TiO_2 against *Escherichia coli* that could reach 99.99% bacterial reduction under an activation by visible light [12-13].

Therefore, In this work, we report the synthesis of superabsorbent composites by copolymerization reaction of acylic acid (AAc) and acrylamide (AAm) on TiO_2 nano-particles (Degussa P-25) and a comparative study on the antibacterial effects of TiO_2 containing composite hydrogel prepared by 5 and 20 %wt by monomer of TiO_2 nano-particles was reported. A detailed characterization of these TiO_2 composite hydrogel and the determination of their antibacterial activities using *Escherichia coli (E.coli)* and *Staphylococcus aureus (S. aureus)* were presented in this article.

2 EXPERIMENTAL

2.1 Materials

Monomers acrylamide (AAm) and acrylic acid (AAc) were commercially obtained from Siam Chemical Industry Co., Ltd. Initiator ammonium persulfate (APS), *co*-initiator *N,N,N',N'*-tetra-methylethylenediamine (TEMED), crosslinking agent *N,N'*-methylenebisacrylamide (*N*-MBA), sodium hydroxide (NaOH) were all purchased from Fluka and TiO_2 nanoparticles (Degussa P-25). All chemicals were used without further purification. Deionized water was used for the preparation and swelling study of hydrogel in this research.

2.2 Preparation of TiO_2 composite hydrogel

Monomers AAm and AAc were dissolved in distilled water at room temperature. Then the solution of AAm and AAc was placed in a 500 mL four-necked round-bottom flask, equipped with a mechanical stirrer set at 200 rpm and a gas inlet tubing for feeding nitrogen gas. The reaction temperature was controlled at 60 °C. The mixture was stirred by a small-bladed propeller with 200 rpm under the nitrogen atmosphere for 30 min. The 2.5 x 10^{-2} M crosslinking agent (*N*-MBA) was added into the reaction flask and the reaction was stirred for 10 min. The solution of TiO_2 nanoparticles (Degussa P-25) was sonicated for 5 min; the monomer solution was added into the reaction flask while stirring for 10 min. Then 2.5 x 10^{-3} M of APS initiator was added. After stirring for 5 min, the mixture was added with 0.5 mL of TEMED co-initiator. Solution polymerization continued for 30 min. The resulting polymer was dehydrated with methanol, cut into small pieces, dried at 50 °C for 24 h in a vacuum oven to a constant weight and then milled before characterization.

2.3 Characterization

Scanning Electron Microscopy (SEM) images were taken with a Hitachi S3400 (Japan) operating at an acceleration voltage of 20 kV. SEM specimens were prepared by placing powder of TiO_2 composite hydrogel on carbon tape, followed by coating with gold, Transmission Electron Microscopy (TEM) image of the sample was recorded using a JEOL JEM-1230 operating at 200 kV. The sample was prepared by dispersing 2–3 drops of TiO_2 composite hydrogel on a copper grid and dried at room temperature after removal of excess solution using a filter paper and The surface area, pore volume and pore size of TiO_2 nanoparticles (Degussa P-25) was determined using a Brunauer-Emmett-Teller (BET) technique by BEL, BELSORP-max, Japan.

2.4 Antibacterial activity by reduction test method

Antibacterial activity of TiO_2 composite hydrogel was performed using a method from ASTM E 2149-01: Determining the Antimicrobial Activity of Immobilized Antimicrobial Agents Under Dynamic Contact Conditions. All samples were sterilized by autoclaveing at 121°C for 15 minutes. Each microbe at the initial concentration of 10^5 CFU/mL in buffer solution was mixed with 0.1g TiO_2 composite hydrogel and a control containing only hydrogel without TiO_2 and then the swollen hydrogels were exposed to UV radiation by a UV lamp at the wavelength of 365 nm, radiation intensity of 1 mW cm^{-2}, and without exposure to UV radiation for dark conditions and stired at 37°C for 1, 3 and 5 hours. The distance between each light source and sample was set at 10 cm. After light exposure, appropriate dilutions of each microbe were spread on TSA medium using spiral plate and then incubated at 37 °C for 24 hours, Bacterial colonies were counted by a cell counter. Each measurement was performanced three times. Bacterial reduction percentage is defined as:

$$\%Bacteria\ \ reduction = \frac{A-B}{A}\times 100$$

where A is a number of microorganisms of untreated bacteria and B is number of microorganisms of treated bacteria with TiO_2 composite hydrogel.

3 RESULTS AND DISCUSSION

Table 1 shows that BET technique of TiO_2 (P-25) was determined, and the average pore diameter was found to be 32 nm. A TEM image of TiO_2 (P-25) nanoparticles is shown in Figure 1. The image demonstrates regularly shaped particles with diameters in the rage of 50 nm. The surface morphologies of hydrogel without TiO_2 and 5%, 20%wt TiO_2/poly[AAm-*co*-(AAc)] composite observed from SEM are shown in Figure 2. The SEM micrographs indicate that TiO_2 particles appear on the surface and the inner of the TiO_2/poly[AAm-*co*-(AAc)] composites. The TiO_2 particle distribution increases with increase in the TiO_2 content. However, the fine and nanoscaled TiO_2 particles are susceptible to agglomerate when its content is 20%wt of TiO_2/poly[AAm-*co*-(AAc)] composite as shown in Figure 2(c).

Table1: Properties of TiO_2 (P-25) by the BET technique.

TiO_2	S_{BET} (m^2 g^{-1})	Pore volume (mL g^{-1})	Mean pore diameter (nm)
P-25	54	0.43	31.77

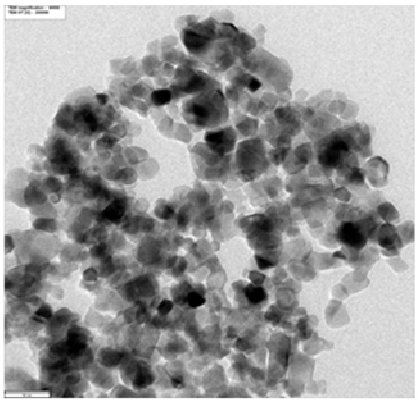


Figure 1 : TEM image of TiO_2 (P-25). The sample was taken at a magnification of 200,000 and operating at 200 kV. The scale bar in this case correspond to 50 nm.

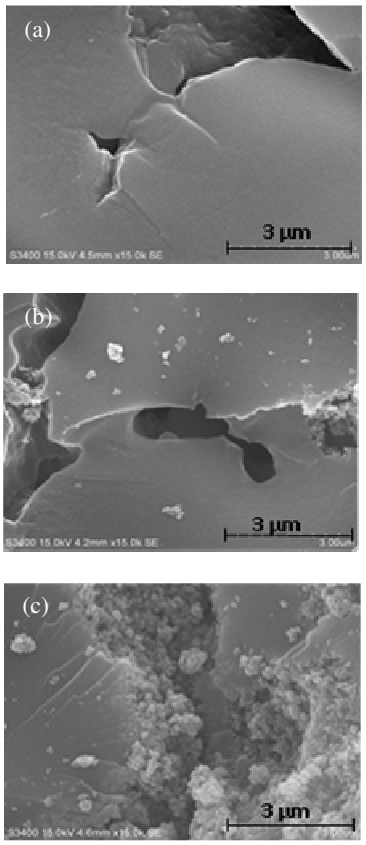
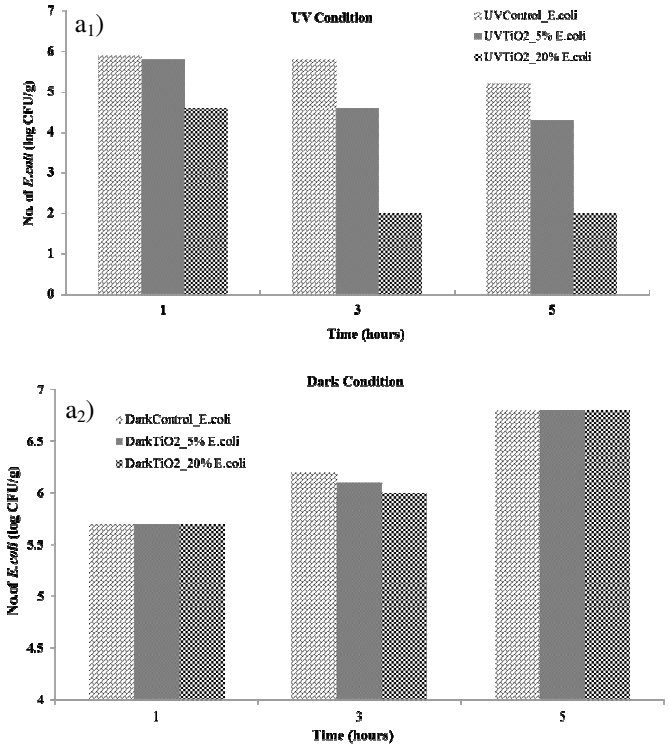


Figure 2 : Morphologies of TiO_2 composite hydrogels with a magnification of 15,000 (a) hydrogel without TiO_2 (b) 5% wt and (c) 20% wt of of TiO_2/poly[AAm-*co*-(AAc)] composites.



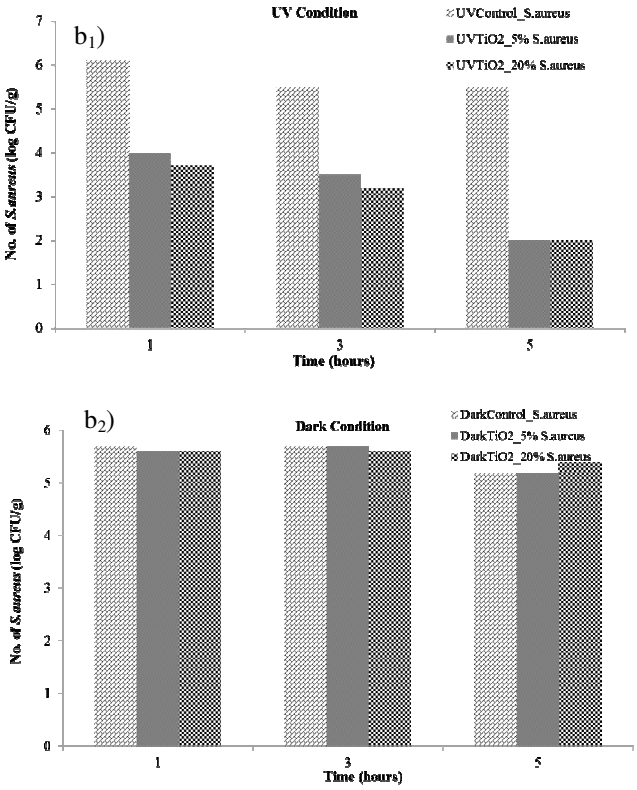


Figure 3 : Antibacterial activity of 5, 20%wt TiO_2/ poly[acrylamide-*co*-(acrylic acid)] composite hydrogel and control, hydrogel without TiO_2 against (a_1-a_2) *E.coli* and (b_1-b_2) *S. aureus* in dark conditions: without any lighting and under UV irradiation at 1 $mWcm^{-2}$ for 1, 3 and 5 hours, respectively.

The antibacterial effects, evaluated by the plate counting method, of hydrogel without TiO_2, 5%wt and 20%wt TiO_2/ poly[acrylamide-*co*-(acrylic acid)] composite hydrogels against the *E. coli* and *S. aureus.* From Fig. 3 a_2 and b_2, it can be seen that with 5%wt and 20%wt TiO_2/ poly[acrylamide-*co*-(acrylic acid)] composite hydrogels irradiated with a UV intensity of 1 $mWcm^{-2}$, the number of viable cell count decreased with increasing irradiation time (5 hours < 3 hours < 1 hour) of the Gram-negative *E.coli* and Gram-positive *S.aureus*, respectively. On the other hand, in all of the TiO_2-hydrogel sample no reduction of initial bacterial was observed without UV irradiation.

4. CONCLUSIONS

This study indicates photocatalytic activity of TiO_2/ poly[acrylamide-*co*-(acrylic acid)] composite hydrogels as a possible method for antibacterial application. Its effectiveness was clearly confirmed against two types of bacterial (*S.aureus* ATCC 6538 and *E.coli* ATCC 25922) by reduction test method. The results demonstrated that the the number of viable bacteria decreased with increasing irradiation time and increasing amounts of TiO_2 nanoparticles.

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[1]National Nanotechnology Center, National Science and Technology Development Agency, 111 Thailand Science Park, Paholyothin Road, Klong 1, Klong Luang, Pathumthani 12120, Thailand., Ph: (66) 2564-7100, Fax: (66) 2564-6981, wiyong@nanotec.or.th