

Preparation of Nano-TiO₂/Polystyrene Hybride Microspheres and Their Antibacterial Properties

Guohua Jiang,^{1,2} Jianfang Zeng²

¹Key Laboratory of Advanced Textile Materials and Manufacturing Technology (ATMT), Ministry of Education, Zhejiang Sci-Tech University,

Hangzhou 310018, People's Republic of China

²Department of Materials Engineering, College of Materials and Textile, Zhejiang Sci-Tech University, Hangzhou 310018, People's Republic of China

Received 29 June 2009; accepted 21 September 2009

DOI 10.1002/app.31484

Published online 10 December 2009 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: This study provided a facile method to prepare nano-TiO₂/polystyrene hybride microspheres in ethanol solution. The formation of titanium dioxide (TiO₂) nanoparticles and hybrid microspheres were verified by FTIR, SEM, transmission electron microscopy, thermogravimetric analysis, and X-ray powder diffraction. Monodispersed colloid TiO₂ nanoparticles with small particle sizes were obtained, and the average particle size could be effectively controlled from about

10 nm. The antibacterial activity of the organic microspheres and hybride microspheres was also investigated against *Escherichia coli*. They were able to efficiently inhibit the growth and the multiplication of *E. coli* under the UV. © 2009 Wiley Periodicals, Inc. *J Appl Polym Sci* 116: 779–784, 2010

Key words: casting; nanocomposite; microspheres; antibacterial

INTRODUCTION

The incidence of fungal and bacterial infections has increased dramatically in recent years.¹ The widespread use of antifungal and antibacterial drugs and their resistance against fungal and bacterial infections has led to serious health hazards. The study of wide spectrum antifungal and antibacterial materials has recently attracted significant attention.^{2–4} Considerable efforts have been devoted to design and fabrication of organic–inorganic hybrid materials, which often exhibit improved physical and chemical

properties.^{5–8} The organic–inorganic hybrid materials present the properties of both the inorganic nanoparticles and the polymer by combining thermal stability, mechanical strength, or optical properties with flexibility and the ability to form films.

The use of semiconducting photocatalytic materials as antibacterial agents working based on the interaction of light with the dispersed metallic nanoparticles have attracted great interest due to their high and broad photocatalytic activities. Among various semiconducting photocatalytic materials being developed for antibacterial applications, titanium dioxide (TiO₂) has received great attentions because of its chemical stability and high reactivity under UV light irradiation.^{9,10} When TiO₂ is exposed to ultraviolet light ($\lambda < 400$ nm), holes (h_{vb}^+), and excited electrons (e_{cb}^-) are generated. The hole is capable of oxidizing water or hydroxide anions into hydroxyl radicals (\bullet OH). \bullet OH is known to be powerful, indiscriminate oxidizing agents to degrade a wide range of organic pollutants, such as, aromatics and aliphatics, dyes, pesticides, and herbicides.⁹ With high photo-reactivity, low cost, nontoxic nature, and chemical stability, TiO₂ is promising for eliminating microorganisms in self-cleaning and self-sterilizing materials.

Polystyrene (PS) is probably the most versatile class of polymers due to the great variety of raw materials that can be used in various fields with the advantages of nonpollution and nontoxicity.¹⁰ Nano-sized additives are used as an effective strategy to alter and enhance the properties of PS. In this study, we report a one-step route by combining the

Correspondence to: G. Jiang (polymer_jiang@hotmail.com).

Contract grant sponsor: National Natural Science Foundation of China; contract grant number: 20604024.

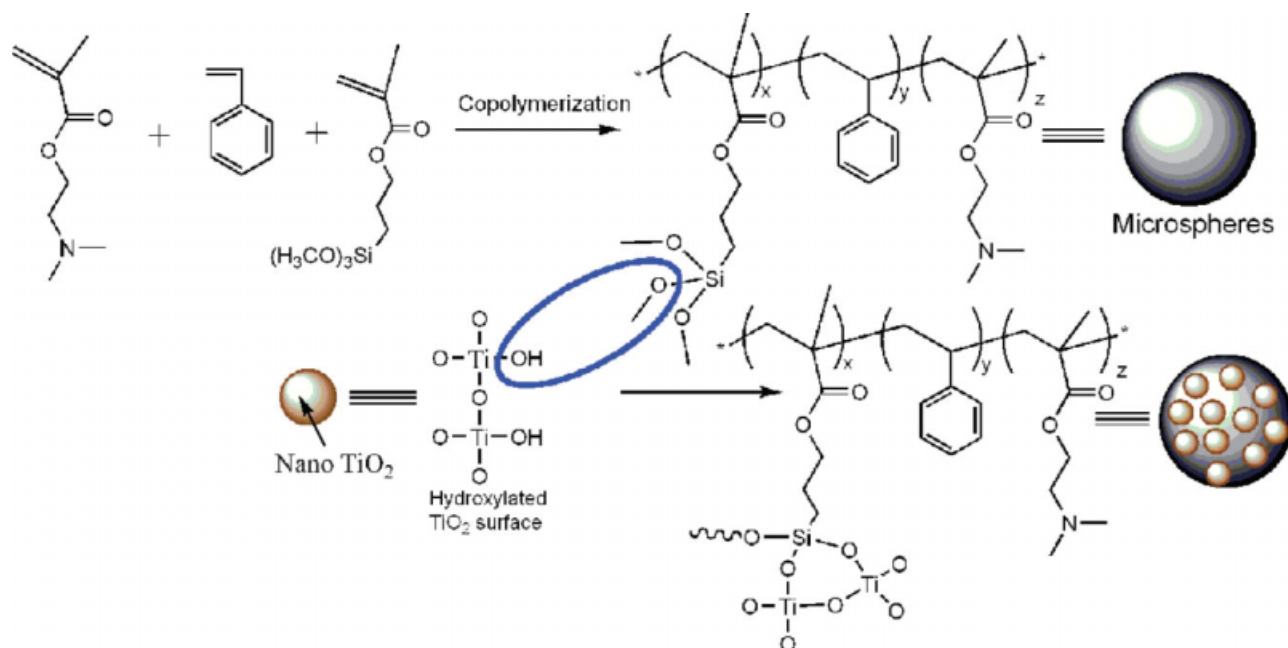
Contract grant sponsor: TORAY Research Fund; contract grant number: 0901056-J.

Contract grant sponsor: Scientific Research Foundation of Zhejiang Sci-Tech University; contract grant number: 0901808-Y.

Contract grant sponsor: Key Laboratory of Advanced Textile Materials and Manufacturing Technology (ATMT), Ministry of Education (Training Foundation for the Excellent Young Talents); contract grant number: 2008QN10.

Contract grant sponsor: Program for Changjiang Scholars and Innovative Research Team in University; contract grant number: PCSIRT: 0654.

Contract grant sponsor: Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry.



Scheme 1 Schematic representation of the procedure for fabrication of nano-TiO₂/polystyrene hybrid microspheres. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

polymerization of styrene in the presence of *N,N*-dimethaminoethyl methacrylate (DMAEMA) and methacryloxypropyl trimethoxysilane (MPS) as comonomers to form latex particles and the coating of TiO₂ nanoparticles onto the polymer microspheres (PMS). During the process, TiO₂ was added to enhance the stability of the system because of its charged and hydrophilic properties. TiO₂ nanoparticles were coated on the surface of the polymer particles by the hydrolysis interaction between hydroxyl groups of particles and methoxy groups of MPS. Because *Escherichia coli* had been used as a biological indicator of water system, *E. coli* was taken as a model in our study. The antibacterial activity of the films against *E. coli* with UV light irradiation was examined in this study, whereas most researches have been done using UV light.

EXPERIMENTAL PART

Materials

Titanium (IV) isopropoxide (95%), anhydrous ethanol, hydrogen chloride (HCl) 2,2-azoisobutyronitrile (AIBN, 95%) were purchased from East China Chemical Co. (Shanghai, China) and used as received. DMAEMA and MPS were purchased from Sinopharm Chemical Reagent Co. (Shengyang, China). Styrene (St) was freshly distilled before use. AIBN recrystallized from ethanol before use.

Synthesis of anatase TiO₂ nanoparticles

TiO₂ colloid nanoparticle suspension was prepared with the sol-gel method according to the procedure

previously reported.¹¹ Briefly, 1 mL of titanium isopropoxide was added into 9 mL anhydrous ethanol, and shaken subsequently to obtain the transparent solution in which the concentration of titanium isopropoxide was 10%. Hundred milliliter of distilled water was adjusted to pH 1.45 using concentrated HCl and then transferred to the conical flask. As mentioned earlier, titanium isopropoxide solution (0.6 mL) was subsequently added into the conical flask slowly with vigorous stirring using a magnetic stir bar. The dropping rate of the titanium isopropoxide solution was 0.05 mL every 30 s. After the addition of titanium isopropoxide, the solution was further agitated for another 3 h and then stored in the refrigerator at the temperature of 4°C for further use.

Preparation of organic and hybrid microspheres (PMS and TiO₂-HMS)

In a three-neck flask was charged 80 mL of anhydrous ethanol, 18 mL of St, 1–3 mL of DMAEMA, and 1–3 mL MPS. 2,2-azoisobutyronitrile (AIBN, 0.04 g, 0.24 mmol, CP), 2 wt % relative to St, DMAEMA, and MPS, dissolved in 20 mL of ethanol was added into flask after complete dissolution of the monomers. The temperature of the thermostat shaker was rapidly increased to 70°C within 30 min. Initially, homogeneous reaction mixtures turned milky white within 5–10 min after reaching 70°C, depending on the formulation. For preparation nano-TiO₂/polystyrene hybrid microspheres, 2 mL of TiO₂ colloid nanoparticle suspension was added

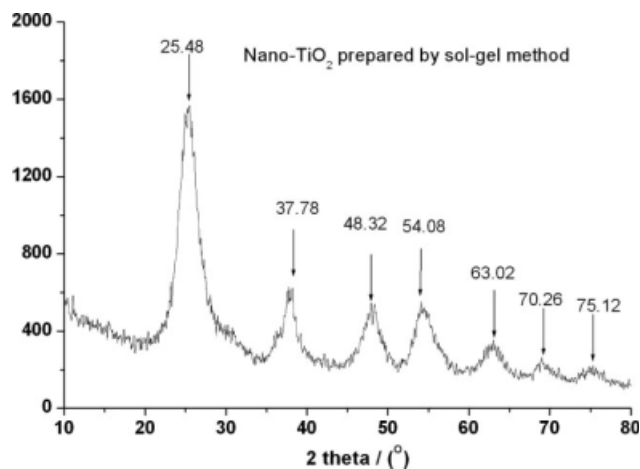


Figure 1 XRD pattern of TiO₂ nanoparticles. Peaks which are indicated by the solid arrows correspond to the anatase structure.

into the flask after 0.5 h. The resultant mixture was further reacted for 12 h to obtain PMS and TiO₂-coated hybride microspheres (TiO₂-HMS), Scheme 1.

Antibacterial properties of organic and hybride microspheres

For the antibacterial assay, the samples (PMS and TiO₂-HMS) was investigated both under the low-intensity UV light and in the dark. For the antibacterial assay under the UV light, the UV lamp was used as the source of UV light, the wave length of which was 365 nm. Sample was first dispersed in acetone using an ultrasonic bath to remove the oily dirt, and again washed with ethanol for 15 min and water for 15 min with ultrasonic bath to further wash away the impurities in the sample. It was then dried under reduced pressure at 30°C for 24 h to constant weight. The sample was diluted in PBS (pH

= 7.2) using an ultrasonic bath to provide a concentration of 0.5 mg/mL for the tests. Sample suspension (3 mL) was added in well plates and make the sample disperse uniformly. Then 3 mL of *E. coli* suspension in PBS with an initial cell concentration of 5×10^5 cells/mL was pipetted into the well plates and covered the samples completely. The well plates were 6.8 cm away from the UV lamp where the UV measured by UV radiometer strength was 100 μ W/cm². The whole experimental setup was placed in 37°C incubator, which was separated from any ambient light. At predetermined time, cells were pipetted out from the well plates, and consecutive dilutions were performed by taking 1 mL of the previous solution and mixed with 9 mL of PBS. The mixture was left standing at room temperature for 15 min. A series of 10-fold dilutions in neutralizing broth were prepared and plated out in trypticase soy agar. The plates were incubated at 37°C for 24 h and counted for colony forming units. The neutralization broth contained a mixture of neutralizing agents that was demonstrated to inactivate the antimicrobial activity from the sample residues that were carried forward into the 10-fold dilutions. All experiments were carried out in duplicate.

The results are expressed as follows:

Log reduction = Log initial count of inoculum – Log survivor count at 2 h exposure time

% Kill = $100 \times (\text{Count of inoculum} - \text{Survivor count at 2 h exposure time}) / \text{Initial count of inoculum}$

For the antibacterial assay in the dark, the procedure was exactly the same as above except that the UV lamp was removed.

Characterization

The TiO₂ crystal structure was investigated by X-ray powder diffraction (XRD), Rigaku, Japan. The XRD

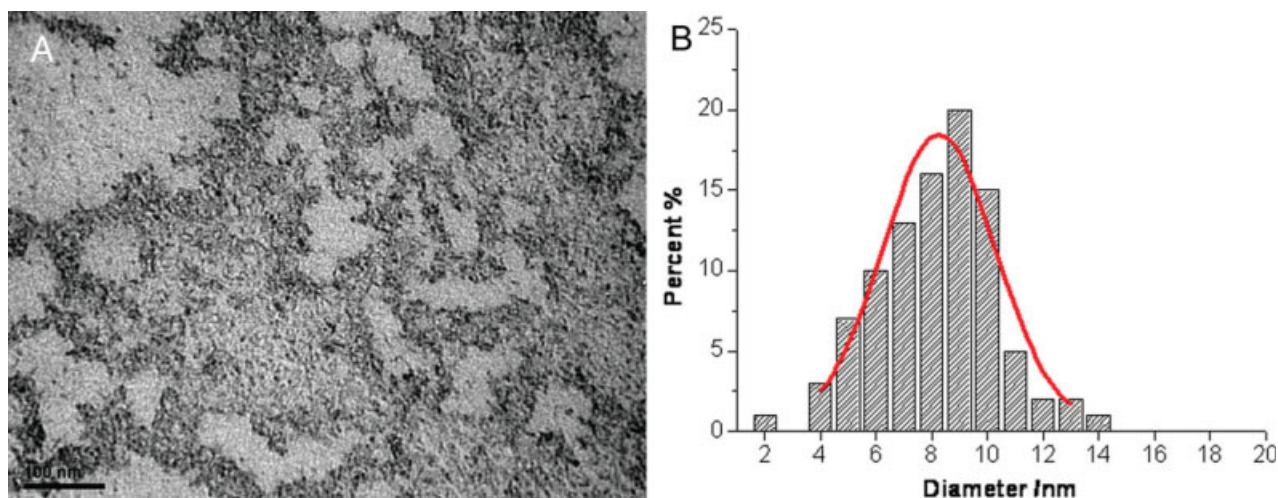


Figure 2 TEM image (A) and histograms of size distribution (B) of synthesized TiO₂ nanoparticles. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

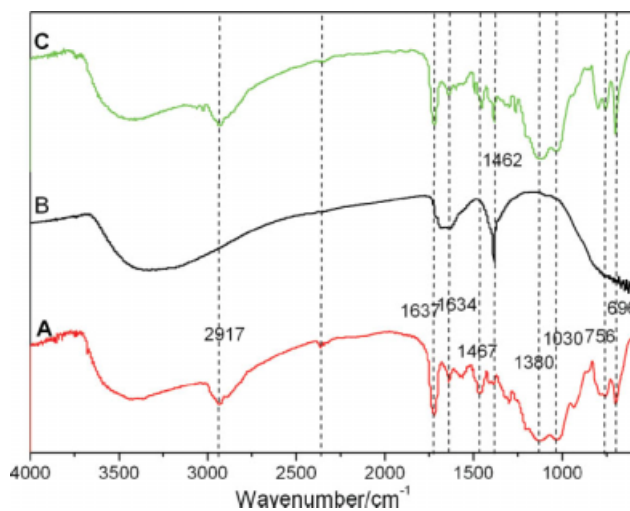


Figure 3 FTIR spectra of copolymers (A, PMS), nano-TiO₂ (B), and nano-TiO₂-coating microspheres (C, TiO₂-HMS). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

pattern was obtained with CuK_α radiation at 40 kV and 80 mA over the range of 10° < 2θ < 80°. The size and morphology of TiO₂ nanoparticles were observed using transmission electron microscopy (TEM), JEM-1200EX, operating at 80 kV. The chemical composition and structure of the TiO₂ colloid, PMS, and TiO₂-HMS were recorded on a Nicole Avatar 360 FTIR using KBr pellets for samples. Spectra were recorded with a resolution of 4 cm⁻¹ from 4000 cm⁻¹ to 400 cm⁻¹ by taking 32 scans. Final spectra were the result of subtraction of the KBr spectrum to the samples. In both cases, the background spectrum corresponds with the air spectrum at each one of the time periods taken into consideration. Thermogravimetric analysis (TGA) was performed on a Netzsch STA 409 PG/PC instrument (Germany) at a heating rate of 20°C/min from 40 to

500°C in a flow of nitrogen. The antimicrobial activity of PMS and TiO₂-HMS against *E. coli* was investigated both under the low-intensity UV light (black-light bulb-6W, Haining minghui light source Co., China; λ = 365 nm) and in the dark.

RESULTS AND DISCUSSION

The TiO₂ nanoparticles were synthesized via the simple sol-gel method. TiO₂ colloid particles were dried into powder under decompression for X-ray diffraction (XRD) analysis. Several obvious peaks are observed at 2θ values equals 25.48, 37.78, 48.32, 54.48, 63.02, 70.26, and 75.12, which correspond to the anatase structure.¹¹ It demonstrates that TiO₂ nanoparticles prepared by this method take the anatase crystalline structure without a significant amount of any other phase (Fig. 1). Using the Scherrer equation, $D = k\lambda/\beta\cos\theta$,¹² the average crystallize size obtained from XRD data is found to be ~ 9 nm. The TEM images of TiO₂ nanoparticles (Fig. 2) indicated that the size of TiO₂ nanoparticles is rather small (few exceed 20 nm) and most of TiO₂ nanoparticles are located in the range of 5–10 nm.

In the FTIR spectra of nano-TiO₂, PMS, and TiO₂-HMS, benzene ring folding appeared at 696 cm⁻¹, C–H bending of the benzene ring at 756 cm⁻¹, and C–C stretching of the benzene ring at 1467 cm⁻¹ and 1637 cm⁻¹. All the absorption bands of the benzene ring and TiO₂ in the hybrid material remained uncharged. The band at 1030 cm⁻¹ is attributed to stretching vibrations of Si–O–C bonds. When comparing the FTIR cures of PMS [Fig. 3(A)] and TiO₂-HMS [Fig. 3(C)] at 1030 and 1380 cm⁻¹, the decrease of relative density of the band at 1030 cm⁻¹ indicates that the bond of Si–O–C is broken during the hydrolysis of MPS.¹³ Therefore, using MPS as a comonomer is able to improve the combination of

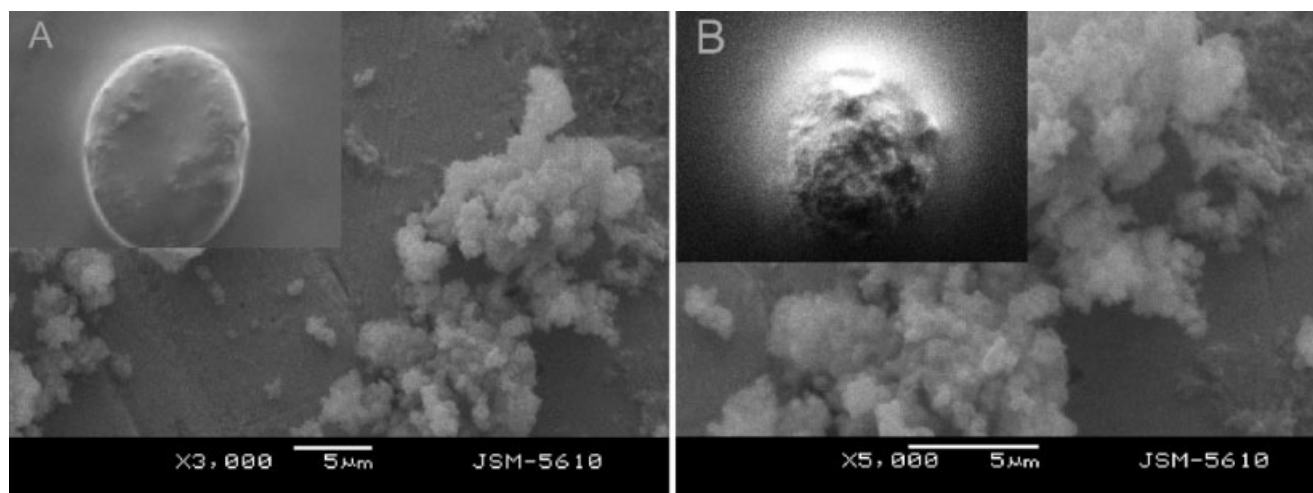


Figure 4 SEM image of organic microspheres (A) and nano-TiO₂-coating microspheres (B).

TiO₂ with the polymer. Because of the tendency for MPS groups to gather on the surface of latex particles, it is favorable for "anchoring" TiO₂ nanoparticles on the surface of polymer microspheres.

The morphology of the as-prepared samples investigated by field-emission scanning electron microscopy. We found the as-prepared sample consisted of spherical particles (Fig. 4). The size of these spheres is around 10 μm. When compared with the PMS, the surface of TiO₂-HMS was relatively rough.

The primary of thermograms of nano-TiO₂, PMS, and PMS-TiO₂ are shown in Figure 5. From Figure 5, there are initial stages of TGA test which come from the moisture loss and decomposition of hydroxyl groups on the surface of particles. The nano-TiO₂ has high thermal stability, which has 20% weight loss at the 500°C. In the case of PMS, the decompositions is almost absolute at 450°C. The TiO₂-HMS have 80% weight loss at the 500°C, which demonstrates that there is nano-TiO₂ present in the hybride.

TiO₂ is a highly active photocatalyst and very strong antibacterial agent under the irradiation of UV light. However, it shows little antibacterial activity in the dark. The antibacterial assays against *E. coli* under the UV light and in the dark were both performed. *E. coli* is the most characterized bacterium, has been used as a model bacterial system for various antimicrobial testing programs.¹⁴

Figure 6 shows the results of antibacterial assay under the UV light and in the dark. The PMS exhibits low antibacterial activity under the UV light and in the dark. The percent kill for *E. coli* is around 5.7% ± 2.0% and 10.5% ± 2.5% in dark and under UV light, respectively. However, the TiO₂-HMS shows much stronger antibacterial property. More

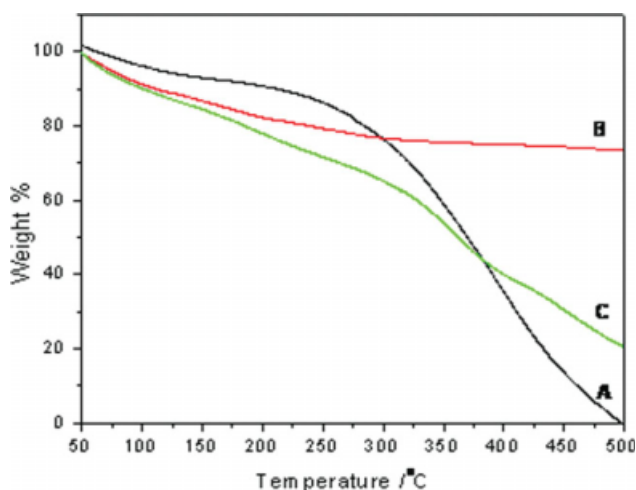


Figure 5 TGA curves of organic microsphere (A), nano-TiO₂ (B), and hybride microspheres (C). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

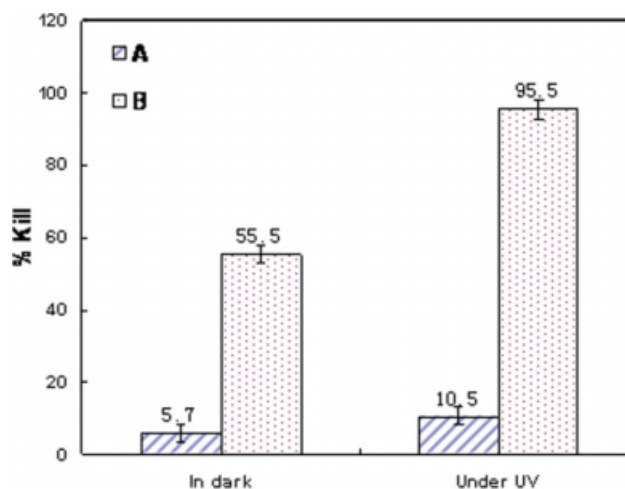


Figure 6 The results of antibacterial assay against *E. coli* under the UV light and in the dark for the sample of organic sphere (A) and TiO₂-coated hybrid microspheres (B) after 2 h. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

than 95% of the bacteria were killed by the TiO₂-HMS under the UV light after only 2 h. Even in the dark condition, more than 50% of the bacteria killed by the TiO₂-HMS after 2 h. The higher antibacterial activity of TiO₂-HMS under the UV light is from the photocatalytic activity of nano-TiO₂. Under the irradiation of UV light, the photocatalyst initially produces electron and hole pairs that will migrate to the surface. The holes will subsequently react with adsorbed water or OH⁻ to generate highly active oxygen species, such as, hydroxy radicals and the electrons will react with oxygen to mainly to produce the superoxide ions. All of the above active oxygen species will oxidize the organic matter that is adsorbed on the surface of TiO₂. If the bacteria was adsorbed on the TiO₂ surface, the structure of the bacteria will also be destroyed by the oxidation reaction. Therefore, it is an ideal candidate to serve as a highly efficient contact-active bactericidal agent.

CONCLUSIONS

This study provided a facile method to prepare nano-TiO₂/polystyrene hybride microspheres in ethanol solution. The formation of TiO₂ nanoparticles and hybrid microspheres were verified by FTIR, SEM, TEM, TGA, and XRD techniques. Mono-dispersed colloid TiO₂ nanoparticles with small particle sizes were obtained, and the average particle size could be effectively controlled from about 10 nm. The antibacterial activity of the PMS and TiO₂-HMS was also investigated against *E. coli*. They were able to efficiently inhibit the growth and multiplication of *E. coli* under the UV. Such hybride microspheres may have great potential in the

preclinical sterilization and the reduction of infection caused by implanted medical device.

References

1. Tandon, V. K.; Maurya, H. K.; Mishra, N. N.; Shukla, P. K. *Eur J Med Chem* 2009, 44, 3130.
2. Lee, D.; Cohen, R. E.; Rubner, M. F. *Langmuir* 2005, 21, 9651.
3. Hinman, M. M.; Rosenberg, T. A.; Balli, D.; Black-Schaefer, C.; Chovan, L. E.; Kalvin, D.; Merta, P. J.; Nilius, A. M.; Pratt, S. D.; Soni, N. B.; Wagenaar, F. L.; Weitzberg, M.; Wagner, R.; Beutel, B. A. *J Med Chem* 2006, 49, 4842.
4. Zhang, Y.; Peng, H.; Huang, W.; Zhou, Y.; Zhang, X.; Yan, D. *J Phys Chem C* 2008, 112, 2330.
5. Sun, X.; Dong, S.; Wang, E. *J Am Chem Soc* 2005, 127, 13102.
6. Chen, C.-H.; Crisostomo, V. M. B.; Li, W.-N.; Xu, L.; Suib, S. L. *J Am Chem Soc* 2008, 130, 14390.
7. Lin, C.-L.; Yeh, M.-Y.; Chen, C.-H.; Sudhakar, S.; Luo, S.-J.; Hsu, Y.-C.; Huang, C.-Y.; Ho, K.-C.; Luh, T.-Y. *Chem Mater* 2006, 18, 4157.
8. Akhavan, O. *J Colloid Interface Sci* 2009, 336, 117.
9. Zhang, W.; Chen, Y.; Yu, S.; Chen, S.; Yin, Y. *Thin Solid Films* 2008, 516, 4690.
10. Lu, H. W.; Lu, Q. H.; Chen, W. T.; Xu, H. J.; Yin, J. *Mater Lett* 2004, 58, 29.
11. Yuan, W.; Ji, J.; Fu, J. *J Biomed Mater Res B Appl Biomater* 2007, 85, 556.
12. Klug, H. P.; Alexander, L. E. *X-Ray Diffraction Procedures for Polycrystalline and Amorphous Materials*; Wiley: New York, 1974; p 966.
13. Pantoja, M.; Díaz-Benito, B.; Velasco, F.; Abenojar, J.; del Real, J. C. *Appl Surf Sci* 2009, 255, 6386.
14. Yuan, W.; Jiang, G.; Che, J.; Qi, X.; Xu, R.; Chang, M. W.; Chen, Y.; Lim, S. Y.; Dai, J.; Chan-Park, M. B. *J Phys Chem C* 2008, 112, 18754.