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Radiolytic Synthesis of Ag-Loaded Polystyrene (Ag-PS) Nanoparticles and Their Antimicrobial Efficiency Against *Staphylococcus aureus* and *Klebsiella pneumoniase*

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Abstract: Ag nanoparticles were distributed onto polystyrene nanoparticle (PS-Ag) beads using two synthetic methodologies. In the first methodology, polystyrene (PS) beads were prepared via emulsion polymerization, with Ag nanoparticles subsequently loaded onto the surface of the PS beads. The polymerization of styrene was radiolytically induced in an ethanol (EtOH)/water medium, generating PS beads. Subsequently, Ag nanoparticles were loaded onto the PS beads via the reduction of Ag ions. The results from the morphological studies, using field emission transmission electron microscopy (FE-TEM), reveal the PS particles were spherical and nanosized, and the average size of the PS spherical particles decreased with increasing volume % of water in the polymerization medium. The size of the PS spherical particles increases with increasing radiation dose for the polymerization. Also, the amount of Ag nanoparticle loading could be increased by increasing the irradiation dose for the reduction of the Ag ions. In the second methodology, the polymerization of styrene and reduction of Ag ions were simultaneously performed by irradiating a solution containing styrene and Ag ions in an EtOH/water medium. Interestingly, the Ag nanoparticles were preferentially homogeneously distributed within the PS particles (not on the surface of the PS particles). Thus, Ag nanoparticles were distributed onto the surface of the PS particles using the first approach, but into the PS clusters of the particles via the second. The antimicrobial efficiency of a cloth coated with the Ag-PS composite nanoparticles was tested against bacteria, such as *Staphylococcus aureus* and *Klebsiella pneumoniase*, for 100 water washing cycles.

Keywords: polymerization, reduction, Ag nanoparticles, Ag-PS composite nanoparticles, antimicrobial efficiency, *Staphylococcus aureus*, *Klebsiella pneumoniase*.

Introduction

Polymer-metal composite materials have been extensively studied as organic-inorganic composites. As an example, composite particles with polymer as core and inorganic components (metal or metal oxides) as shell have been synthesized and found to have the synergic characteristics of inorganic materials and polymers.[1,2] They have potential applications for optical,[3] catalytic,[4] and magnetic materials,[5-7] etc. Spherical polymeric particles (beads) were coated

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with fine metal particles by mechanical or chemical method. Mechanical methods such as stirred mixing or high-shear milling has been used for the preparation of microspheres containing metal particles.[8] In chemical methods, polymeric particles are synthesized by suspension-polymerization[9,10] or by emulsion-polymerization,[11] followed by deposition of metal particles[12] on the surface of the polymeric particles. Thus far, studies on radiation induced preparation of composite organic-inorganic particles are scarce.

Previously,[13] polymer particles were synthesized by a radiation-induced polymerization (radiolytic polymerization) of glycidyl methacrylate with epoxide group for immobilizing the protein moieties. Also, reports are available on the preparation of metallic nanoparticles (e.g., Ag, Au, Pd, and Pt) using radiation induced reduction of the respective metal ions.[14-16] To the best of our knowledge, a comparative study on the use of radiation for loading or distribution of metal nanoparticles over the surface or within polymer particles has not been attempted. It has been demonstrated that Ag nanoparticles are potentially useful for applications such as a catalyst for reduction of aromatic nitro compounds,[17] surface-enhanced agent,[18,19] a chiral-enhanced agent,[20,21] an additive for chiral separation,[22] and an antimicrobial agent,[23] etc.

In this investigation, Ag-loaded polystyrene (Ag-PS) nanoparticles were synthesized using γ-irradiation. The first method ("Method-I") involves two steps of radiation-induced (radiolytic) reactions. First, polystyrene (PS) nanoparticles were synthesized by radiolytic polymerization of styrene in the presence of poly(vinyl pyrrolidine) (PVP). Then, the Ag nanoparticles were loaded on the surface of the PS particles by radiolytic reduction of Ag ions.

In the second method ("Method-II"), the Ag-PS nanoparticles were synthesized in a single step, where both radiolytic reactions (radiolytic polymerization of PS nanoparticles and formation Ag nanoparticles were formed by irrading a solution containing Ag ion and styrene in EtOH/water mixture. PS-Ag composite nanoparticles prepared by Method-I and Method-II were characterized by energy filtered transmission electron microscopy (EF-TEM), field emission transmission electron microscopy (FE-TEM), energy dispersive X-ray spectroscopy (EDXS), and X-ray diffraction (XRD) analysis. The antimicrobial efficiency of the PS-Ag nanoparticles was evaluated for bacteria such as *Staphylococcus aureus* and *Klebsiella pneumoniase*.

Experimental

Chemicals. The styrene (99%) was obtained from Sigma-Aldrich Co. Silver nitrate ($AgNO_3$) was obtained from Kojima Chemicals Co. Ltd. (Japan). The poly(vinyl pyrrolidine) (PVP, M=40,000) was obtained from Junsei Co. Ltd. (Japan). All other chemicals were in reagent grade, and were used without further purification.

Radiolytic Synthesis of Ag-PS Nanoparticle.

Method-I (Two Step Process): First, polystyrene nanoparticles were synthesized by the following procedure. The PVP (1.0 g), as a surfactant, was dissolved in 200 mL of EtOH/H_2O mixture, to which styrene (1.0 g, 1.1 mol) was added. The solution was stirred for 30 min under nitrogen atmosphere, and irradiated by γ-ray from Co-60 source for 3 h. Then the radiolytic loading (or coating) of Ag nanoparticle on the surface of the PS nanoparticle was performed. Briefly, the $AgNO_3$ (0.6 g, 1.0×10^{-2} M) solution was prepared in PS colloids of 184 mL. Nitrogen gas was bubbled through the solution for 30 min to remove oxygen, and then the solution was irradiated by γ-ray from the same source as above.

Method-II (One-Step Process): Ag-PS nanoparticles were synthesized in one step (one pot) by the following procedure. The PVP (1.0 g), styrene (1.0 g, 1.1 mol), and $AgNO_3$ (0.6 g, 6.5 mmol) were dissolved in 100 mL of EtOH/water mixture (60/40 vol%). The solution was stirred for 30 min under nitrogen atmosphere, and then was irradiated by γ-ray from the same Co-60 source for 3 h. The yellowish brown Ag-PS nanoparticles were produced.

Antimicrobial Efficiency Test of Ag-PS Nanoparticles Against *Staphylococcus aureus* and *Klebsiella pneumoniase*. The antimicrobial efficiency of the Ag-PS nanoparticles was examined according to the protocol "KS K 0693-2001". First, the Ag-PS nanoparticles were coated on a cloth. Then the Ag-PS-coated cloth of 0.4 g was immersed in the bacteria solution of 0.2 mL. The bacteria solution was then stored for 18 h at 37 °C for the bacteria incubation before measuring the bacteria concentration. The antimicrobial efficiency was determined by

$$\text{Antimicrobial efficiency } (\%)=\frac{M_b-M_a}{M_b}\times 100 \quad (1)$$

where M_a and M_b are the concentrations of the bacteria before and after the incubation.[24,25] The initial concentrations of *Staphylococcus aureus* ATCC 6538 and *Klebsiella pneumoniase* ATCC 4352 were 1.3×10^5 and 1.5×10^5 CFU (Colony Forming Unit)/mL, respectively. To test the "After-washing" antimicrobial efficiency, the Ag-PS-coated cloth was washed at 40±3 °C, and dried at room temperature according to the protocol "KS K 0432-1999".

Characterization of Ag-PS Nanoparticles. The size and morphology of the Ag-PS nanoparticles were analyzed using EF-TEM (Carl Zeiss (Leo), EM912 Omega EF-TEM, Germany), FE-TEM (Hitachi, S-4700, Japan), and XRD (Rigaku, Dmax, Rint-2200, Japan).

Results and Discussion

Polymerization of styrene was performed using γ-radiation as the initiation source. There are few advantages for using radiation for initiation. Polymerization happens in the absence of the initiator and hence the polymer (PS) is pure

Table I. Effects of Solvent Composition on the Size of Polystyrene Particles Produced by Radiation-Induced Polymerization[a]

Vol% EtOH	Vol% H_2O	Particle Diameter[b] (nm)	Note
100	500	-	-
80	20	-[c]	Figure 1(1)
60	40	257-260	Figure 1(b)
50	50	98-253	Figure 1(c)
40	60	80-106	Figure 1(d)
20	80	73-98	Figure 1(e)
0	100	60-90	Figure 1(f)

[a]Reaction condition: 0.5 wt% of styrene to the total solution; total irradiation dose=5 kGy; room temperature; 0.5 wt% of PVP to the total solution. [b]Determined by EF-TEM. [c]Can not determined by EF-TEM.

and free from end fragments of the initiator. Radiolytic polymerization of styrene was carried out using a γ-irradiation in the presence of PVP in EtOH/H_2O. The composition of EtOH to water was varied and the size of PS particles formed under these conditions was determined by FE-TEM and EF-TEM. Table I displays the size of PS particles obtained with different solvent composition as medium of the reaction. It can be seen that size of PS particles gradually decreases as the vol% of water increases. It is envisaged that as vol% of water increases in the medium, the solubility of the PS decreases and thus PS particles precipitates at the early stages aryl nucleation of smaller particles.

Table II. Effects of Irradiation Dose on the Size of Polystyrene Particles Produced by Radiolytic Polymerization and Radiolytic Reduction of Ag Ions[a]

Total Irradiation Dose (kGy)	Particle Diameter[b] (nm)	Note	Note[c]
5	230-260	Figure 2(a)	Figure 2(a-1)
10	250-300	Figure 2(b)	Figure 2(b-1)
30	380-454	Figure 2(c)	Figure 2(c-1)

[a]Reaction condition: 0.5 wt% of styrene to the total solution; EtOH/H_2O=60/40 vol%; room temperature; 0.5 wt% of PVP to the total solution. [b]Determined by EF-TEM. [c]Radiolytic reduction of Ag ions.

Figure 1 show the EF-TEM images of the PS particles produced by radiolytic polymerization at room temperature. PS particles prepared in water (absence of EtOH) are gelated and hence size could not be determined. Likewise, the PS particles formed with 80/20 EtOH/H_2O are also partially gelled (see, (a) of Figure 1). This might also be due to high solubility of the styrene in the medium and PVP interfering the formation of individual particles.

The majority of the PS particles are aggregated (Figure 1). This may have been caused by the interactions between the PVP's on the surface of the PS particles and the radicals generated through γ-irradiation. Table II shows the effect of the irradiation dose on the size of the PS particles produced by Method-I at room temperature in 60/40 EtOH/water. The EF-TEM images of the PS particles are shown in Figures 2(a)-2(c). The average particle size of the PS particles grad-

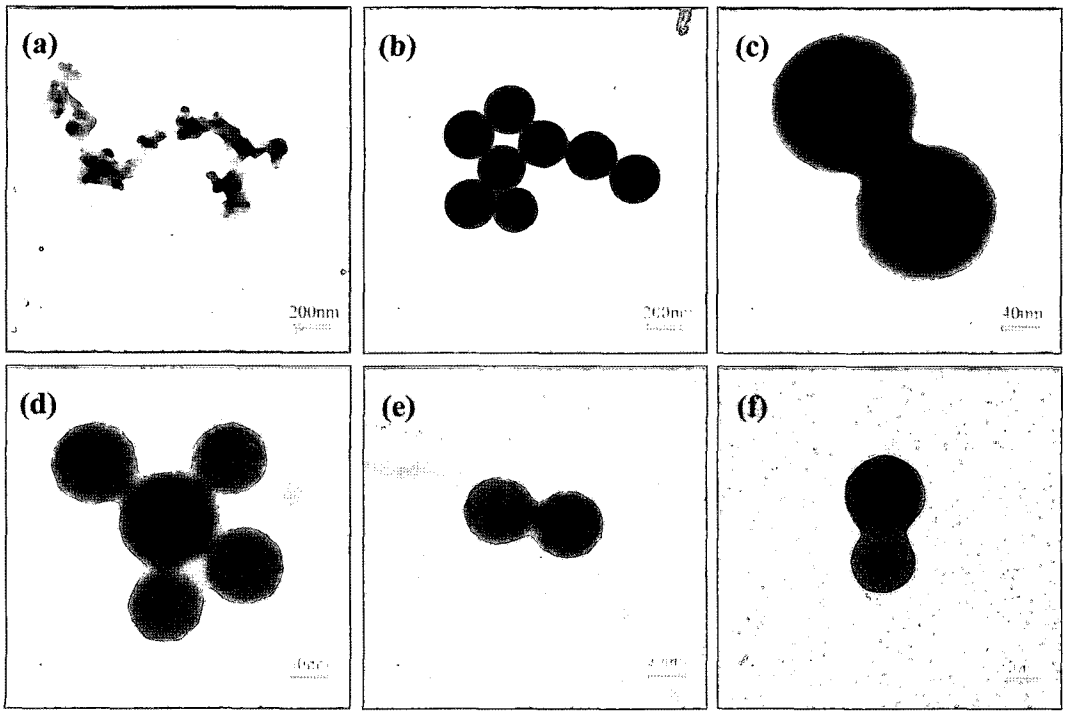

Figure 1. EF-TEM image of PS nanoparticles produced by radiolytic polymerization in EtOH/water.

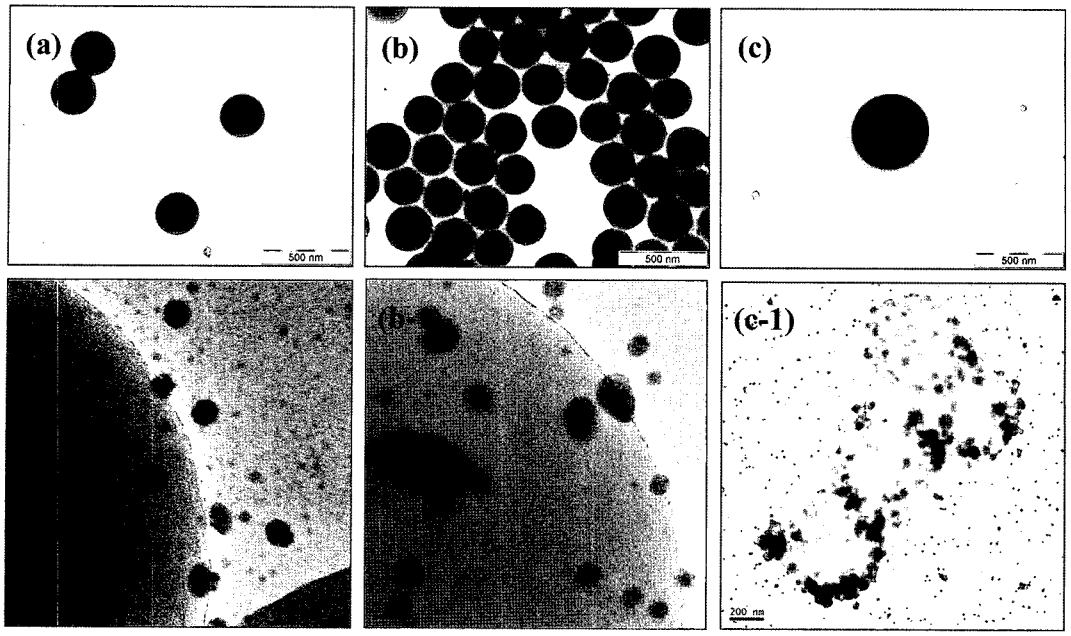

Figure 2. TEM images of PS nanoparticles and PS-Ag nanocomposites prepared by γ-irradiation at irradiation dose of 5 kGy (a, a-1), 10 kGy (b, b-1), and 30 kGy (c, c-1) in 60/40 EtOH/water.

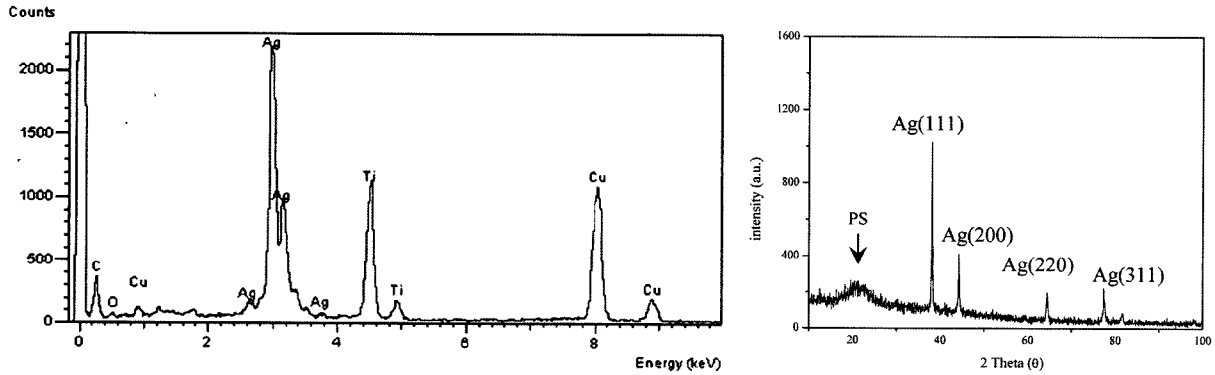

Figure 3. EDXS and XRD spectra of Ag-PS particles shown in Figure 2(c-1).

ually increases as the irradiation dose increases from 5 to 30 kGy. Figures 2(a-1), 2(b-1), and 2(c-1) shows the FE-TEM images of Ag-PS particles produced by radiolytic reduction of Ag ions at the total irradiation doses of 5, 10, and 30 kGy, respectively in the presence of PS particles, which were synthesized by Method-I. FE-TEM images of Ag composite nanoparticles of (Figures 2(a-1), 2(b-1), and 2(c-1)) reveal that Ag nanoparticles are of 10-20 nm sized and the Ag nanoparticle are loaded on the surface of the spherical beads of PS particles. It is known that hydroxyl radicals (OH radicals) are formed during γ-irradiation. As the radiation dose increases larger amount of (OH) may be formed. Hence, the surface of the PS particles may be more hydrophilic with larger number of OH radicals. As a result, larger extent of Ag nanoparticles is loaded on the PS particles as the irradiation dose increases. Previous results indicated that the Ag nanoparticles are dispersed better in nylon matrix than in PET matrix, which may be due to the more hydrophilic nature of PET over nylon 6.[26] Ag nanoparticles are densely formed on PS particles when formed with 30 kGy. Figure 3 shows the energy dispersive X-ray spectroscopy (EDXS) spectra and XRD pattern of the Ag-PS composite particles prepared with 30 kGy irradiation (Figure 2(c-1)). These results confirm the presence of Ag nanoparticles on the surface of the particles.

Ag-PS composite particles were also synthesized by the one-step procedure (Method-II) as described earlier, and their FE-TEM images are shown in Figure 4. Examination of the FE-TEM image reveals that the particles are somewhat uniform in size (around 250 nm). Unlike the results from the two-step procedure (Method-I), the Ag particles were found within PS particles (not just on the surface of

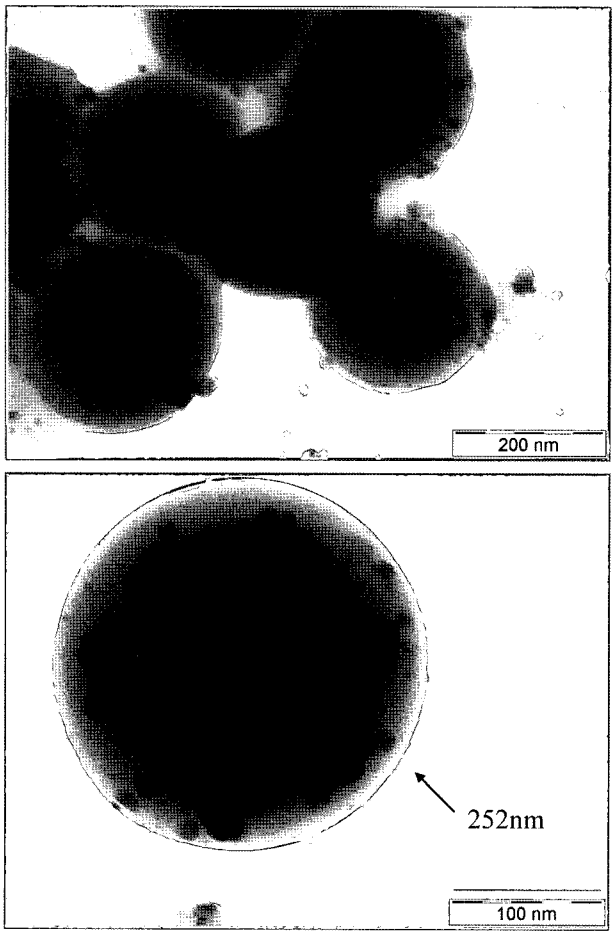

Figure 4. FE-TEM images of Ag-PS particles produced by one-step radiolytic reaction (Method-II) at the total irradiation dose of 30 kGy in 60/40 EtOH/water.

Table III. "After-washing" Antimicrobial Efficiency of Ag-PS Particles Shown in Figure 4 against *Staphylococcus aureus* (ATCC 6538) and *Klebsiella pneumoniase* (ATCC 4352)

No. of Washing Cycles	Antimicrobial Efficiency (%) Against	
	ATCC 6538	ATCC 4352
20	99.9	99.9
50	99.9	99.9
70	99.9	99.9
100	99.9	99.9

the particles) as shown in Figure 4. The average size of Ag particles are of 12-25 nm in size.

The antimicrobial efficiency of Ag-PS composite was tested against two model bacteria (*Staphylococcus aureus* and *Klebsiella pneumoniase*), and the results are shown in Table III. The test procedure was described in the experimental section. The antimicrobial efficiency of the cloth coated with the PS-Ag composite nanoparticles were 99.9% after 20 cycles of washing, and remained constant at the same level even after 100 washing cycles.

Conclusions

Ag-loaded polystyrene (PS-Ag) composite nanoparticles were prepared by two different approaches (two step and single step processes). The two-step method results in loading of Ag particles on the surface of the PS particles, while the one-step method distributes the Ag particles within the PS particles. When coated on a cloth, the PS-Ag nanoparticles produced by the one-step method showed an excellent antimicrobial activity. A cloth coated with those particles shows a high antimicrobial efficiency of 99.9% against two bacteria, *Staphylococcus aureus* and *Klebsiella pneumoniase*.

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References

(1) I. B. Jang, J. H. Sung, H. J. Choi, and I. Chin, *Synthetic Metals*, **152**, 9 (2005).
(2) Z. Huang, F. Tang, and L. Zhang, *Thin Solid Films*, **471**, 105 (2005).
(3) A. Pich, J. Hain, Y. Prots, and H.-J. Adlera, *Polymer*, **46**, 7931 (2005).
(4) T. Endo, T. Yoshimura, and K. Esumi, *J. Colloid Interf. Sci.*, **286**, 602 (2005).
(5) W.-J. Liu, W.-D. He, Y.-M. Wang, D. Wang, and Z.-C. Zhang, *Polymer*, **46**, 8366 (2005).
(6) H. Nathani and R. D. K. Misra, *Mater. Sci. Eng. B*, **113**, 228 (2004).
(7) Z. L. Liua, Z. H. Dinga, K. L. Yaoa, J. Taoa, G. H. Dua, Q. H. Lua, X. Wanga, F. L. Gongb, and X. Chenc, *J. Magn. Magn. Mater.*, **265**, 98 (2003).
(8) T. Iwasaki, M. Satoh, and T. Ito, *J. Mater. Process. Technol.*, **146**, 330 (2004).
(9) B. S. Lee, S. Mahajan, and K. D. Janda, *Tetrahedron*, **61**, 3081 (2005).
(10) J.-W. Shim, J.-W. Kim, S.-H. Han, I.-S. Chang, H.-K. Kim, H.-H. Kang, O.-S. Lee, and K.-D. Suh, *Colloid. Surface A*, **207**, 105 (2002).
(11) C.-S. Chern and T.-C. Yu, *Polymer*, **46**, 1899 (2005).
(12) Y. Wang and C. Pan, *Eur. Polym. J.*, **37**, 699 (2001).
(13) S.-H. Choi, K. P. Lee, and H. D. Kang, *J. Appl. Polym. Sci.*, **88**, 1153 (2003).
(14) K. Torigoe, H. Remita, P. Beaunier, and J. Belloni, *Radiat. Phys. Chem.*, **64**, 215 (2002).
(15) A. Sárkány, Z. Papp, I. Sajó, and Z. Schay, *Solid State Ionics*, **176**, 209 (2005).
(16) S. Seino, T. Kinoshita, Y. Otome, T. Maki, T. Nakagawa, K. Okitsu, Y. Mizukoshi, T. Nakayama, T. Sekino, K. Niihara,

and T. A. Yamamoto, *Scripta Materialia*, **51**, 467 (2004).
(17) N. Pradhan, A. Pal, and T. Pal, *Colloid Surface A*, **196**, 247 (2002).
(18) S.-H. Choi and H. G. Park, *Appl. Surf. Sci.*, **243**, 76 (2005).
(19) S. Sugawa, K. Sayama, K. Okabe, and H. Arakawa, *Energ. Convers. Manage.*, **36**, 665 (1995).
(20) T. Li, H. G. Park, H.-S. Lee, and S.-H. Choi, *Nano Tech.*, **15**, S660 (2004).
(21) S.-H. Choi, S. H. Lee, Y. M. Hwang, K. P. Lee, and H. D. Kang, *Radiat. Phys. Chem.*, **67**, 517 (2003).
(22) S.-H. Choi, H. J. Noh, and K. P. Lee, *Bull. Korean Chem. Soc.*, **26**, 1549 (2005).
(23) S.-D. Oh, S. Lee, S.-H. Choi, I.-S. Lee, Y.-M. Lee, J.-H. Chun, and H.-J. Park, *Colloid Surface A*, **275**, 228 (2006).
(24) S.-D. Oh, B.-S. Byun, S. Lee, and S.-H. Choi, *Macromol. Res.*, **14**, 194 (2006).
(25) http//www.fiti.re.kr.
(26) S.-H. Choi, K. P. Lee, and S. B. Park, *Stud. Surf. Sci. Catal.*, **146**, 93 (2003).