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Preparation of Ag-PS and Ag-PSS Particles by γ-Irradiation and Their Antimicrobial Efficiency against *Staphylococcus aureus* ATCC 6538 and *Klebsiella pneumoniae* ATCC 4352

Seong-Dae Oh, Bok-Soo Byun, Seungho Lee, and Seong-Ho Choi*

Department of Chemistry, Hannam University, Daejeon 306-791, Korea

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Abstract: Polystyrene, PS, particles of 450 nm diameter and poly(styrene-*co*-styrene sulfonate), PSS, particles of 140-160 nm diameter were prepared by emulsifier-free emulsion polymerization. The surfaces of the PS and PSS particles were coated with Ag nanoparticles for the application of antimicrobial agents by reduction of Ag ions using γ-irradiation. The Ag-PS and Ag-PSS were characterized by High-Resolution Transmittance Electron Microscopy (HR-TEM), Field-Emission Scanning Electron Microscopy (FE-SEM), and Energy Dispersive X-ray Spectroscopy (EDXS). The HR-TEM and EDXS data showed that the Ag nanoparticles were loaded on the surface of the PS and PSS particles, respectively. The antimicrobial efficiency of the Ag-PS and Ag-PSS particles (0.4 g) with ca.100 ppm Ag, which was coated onto yarn (KS K 0905-1996 rule), was tested against Staphylococcus aureus ATCC 6538 and *Klebsiella pneumoniae* ATCC 4352 after 100 washing cycles (KS K 0432-1999 rule). The antimicrobial efficiency of the Ag-PS particles against *Staphylococcus aureus* ATCC 6538 and *Klebsiella pneumoniae* ATCC 4352 was 99.9% after 100 cycles washing., confirming that the Ag-PS particles can be used as antimicrobial agents.

Keywords: γ-irradiation, emulsifier-free emulsion polymerization, Ag nanoparticle, Ag-PS particles, Ag-PSS particles, antimicrobial efficiency, *Staphylococcus aureus* ATCC 6538, *Klebsiella pneumoniase* ATCC 4352.

Introduction

The conventional emulsion polymerization has been widely applied in polymer materials preparation. Many biomedicine materials can also be prepared through this method.[1] However, the residuary of emulsifier in materials will greatly influence the purification and performance of the products. Moreover, the environmental pollution coming from the usage of emulsifier is also more severity. So the emulsifier-free emulsion polymerization has been more attentions in recent years. Compared to the conventional emulsion polymerization, it provides one or more of the following advantages:[2,3] no emulsifier migration during film formation, mono-disperse particle size distribution, and excellent shear stability. It is also used for some medical and biochemical purposes because of cleanness of the disperse medium and functionality due to the on-surface groups.

On the other hand, the silver compounds have been applied for their medicinal properties for centuries[4] and it has been known for long time that silver is an effective antimicrobial agent. Gibbard first systematically investigated the antimicrobial activity of silver.[5] He found that if silver was used to be coating cloth or paper, it becomes inactive. Today, silver sulfadiazine is used for topical treatment of burn-wounds,[6] and silver nitrate is still used as a prophylaxis in neonatal ophthalmic.[7] Silver nanoparticles were used as a catalyst for reduction of aromatic nitro compounds,[8] as a surface-enhanced agents,[9] as chiral-enhanced agents,[10,11] as the additive of chiral separation,[12] and etc.[13]

In a previous paper,[14] the Ag and Ag-SiO_2 nanoparticles were synthesized by γ-irradiation, and tested their antibacterial and antifungal efficiency against *Salmonella enterica serovar* Typhimurium and *Botrytis* cinerea. Test results showed the Ag-SiO_2 nanoparticles have strong potential as an antifungal as well as an antibacterial agent. At the presence of 50 ppm of the Ag-SiO_2 particles, the *Salmonella enterica serovar Typhimurium* grew much slower, and at 100 ppm, they did not grow fully even after 58 hrs. The antifungal efficiency of the Ag-SiO_2 nanoparticles against *Botrytis* cinerea was about 65.0, 99.9, and 99.9% at the concentration of the Ag-SiO_2 particles of 10, 50, and 100 ppm, respectively. However, the Ag-SiO_2 nanoparticle was not applied a coating to cloth because the SiO_2 nanoparticles were rigid and hydrophilic properties. In order to overcome

*Corresponding Author. E-mail: shchoi@hannam.ac.kr

rigid and hydrophilic properties, the polystyrene was selected the matrix for loading Ag nanoparticle.

In this study, Ag-PS and Ag-PSS particles were prepared by γ-irradiation by reduction of silver ions in PS and PSS colloids prepared by emulsifier-free emulsion polymerization in order to apply antimicrobial agents to coating cloth. The products were characterized by high-resolution transmittance electron microscopy (HR-TEM), field-emission scanning electron microscopy (FE-SEM), energy dispersive X-ray spectroscopy (EDXS). In addition, the antimicrobial efficiency of the Ag-PS and Ag-PSS particles was examined against *Staphylococcus aureus* ATCC 6538 and *Klebsiella pneumoniase* ATCC 4352, respectively.

Experimental

Chemicals. Styrene (99%) and potassium persulfate ($K_2S_2O_8$) were obtained from Sigma-Aldrich Co. Sodium styrene sulfonate (NaSS) was purchased from Tokyo-Kasei (Japan). Silver nitrate ($AgNO_3$) was obtained from Kojima Chemicals Co. Ltd. (Japan). All other chemicals were in reagent grade, and were used without further purification.

Synthesis of PS and PSS Particles by Emulsifier-Free Emulsion Polymerization. The polystyrene (PS) particles were prepared as follows: At first, potassium persulfate (KPS) was dissolved completely in deionized water (800 mL). A styrene was added KPS solution, and polymerized at 75 °C for 24 hrs by stirring of 350 rpm under nitrogen atmosphere. The detail reaction condition was described as shown in Table I. The conversion yield (%) was determined according to the published paper.[15]

The poly(styrene-*co*-styrene sulfonate) (PSS) particles were prepared as following manner: the potassium hydroxide (0.1 g) and KPS was dissolved completely in deionized water (800 mL). A styrene and NaSS (1.0 g) was added KPS solution, and polymerized at 85 °C for 24 hrs by stirring of 350 rpm. The reaction condition was described as shown in Table I.

Synthesis of Ag-PS and Ag-PSS Particles by γ-Irradiation. Briefly, the $AgNO_3$ of 1.0×10^{-2} M was prepared in PS or PSS colloids of 184 mL in the presence of PVP. Nitrogen gas was bubbled through the solution for 30 min to remove oxygen, and the solution was irradiated by γ-ray from Co-60 source.

Table I. Effect of the Co-monomer (Sodium Styrene Sulfonate, NaSS) Content on the Particle Size in Styrene/KPS/H_2O System (Emulsifier-Free Emulsion Polymerization)[a]

Co-monomer[b] Ratio (wt%)	Conversion (%)	Particle Diameter (nm)	Note
0	93.0	450	Figure 1(a)
0.5	93.5	160	Figure 1(a)
1.0	93.7	155	-
1.5	94.0	140	-

[a]Reaction condition: styrene of 10 wt%; KPS of 5.2×10^{-4} mol/L; the reaction temperature of 75 °C; the reaction time of 24 hrs.
[b]The weight percent of co-monomer (NaSS) to the total solution.

Antimicrobial Efficiency Examination of Ag-PS and Ag-PSS Particles against *Staphylococcus aureus* ATCC 6538 and *Klebsiella pneumoniase* ATCC 4352. The antimicrobial efficiency examination was performed according to the KS K 0693:2001 rule. First, the cloth (KS K 0905-1996) was coated with Ag-PS or Ag-PSS particle. The initial concentration of *Staphylococcus aureus* ATCC 6538 and *Klebsiella pneumoniase* ATCC 4352 was 1.3×10^5 CFU/mL and 1.5×10^5 CFU/mL, respectively. CFU means Colony Forming Units. The Ag-PS and Ag-PSS coated cloth of 0.4 g was added the initial virus solution of 0.2 mL. The samples were incubated at 37 °C for 18 hrs, respectively. The decrease ratio of virus (%), antimicrobial efficiency (%), were calculated as follows

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

where M_a and M_b is the initial average concentration (CFU) of virus and the average concentration (CFU) of virus after incubation, respectively.

On the other hand, the antimicrobial efficiency examination after washing was also examined as according to the rule of KS K 0432-1999. The Ag-PS and Ag-PSS coated cloth were washed at 40±3 °C, and dried at room temperature. The antimicrobial efficiency was determined as the above same method.

Characterization. FTIR spectra (Perkin Elmer, Spectrum 1000, USA) were recorded and compared to PS and PSS particles. Particle size and morphology of Ag-PS and Ag-PSS particles were investigated by a FE-SEM (Hitachi, S-4700, Japan) and HR-TEM (JEOL, JEM-2010, USA).

Results and Discussion

Table I shows the effects of comonomer on particle size based on St/KPS/H_2O system. The conversion of monomer and comonomer was above 93%. The particle diameter of PS prepared by emulsifier-free emulsion polymerization was 460 nm (see, Figure 1(a)). The particle diameters of PSS were in the range of 140-160 nm. The particle diameter (nm) was decreased with increasing comonomer weight (%). The size distribution of PS and PSS particles prepared emulsifier-free emulsion polymerization were very narrow (mono-dispersed) as shown in Figure 1(a) and (b). The size of PS particle was larger than that of PSS particle prepared by emulsifier-free emulsion polymerization.

For an emulsifier-free polymerization, the use comonomer was to either introduction of the suitable functional group onto the surface of particle or function as surfactant. The

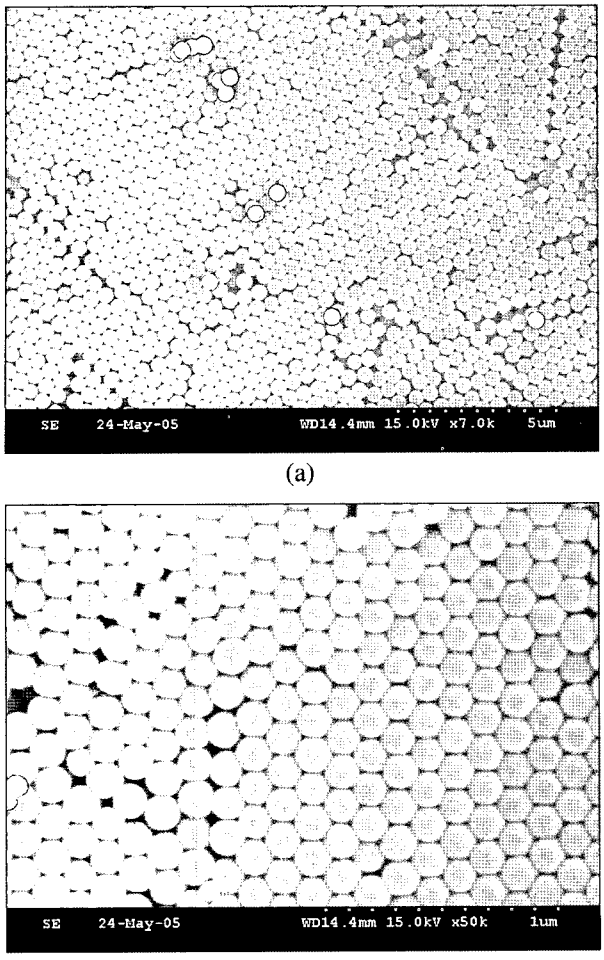


Figure 1. FE-SEM images of PS (a) and PSS (b) synthesized by emulsifier-free emulsion polymerization.

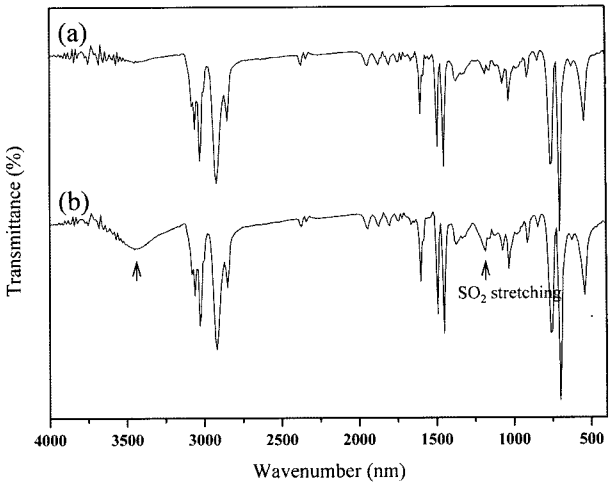


Figure 2. FTIR spectra of PS (a) and PSS (b) synthesized by emulsifier-free emulsion polymerization.

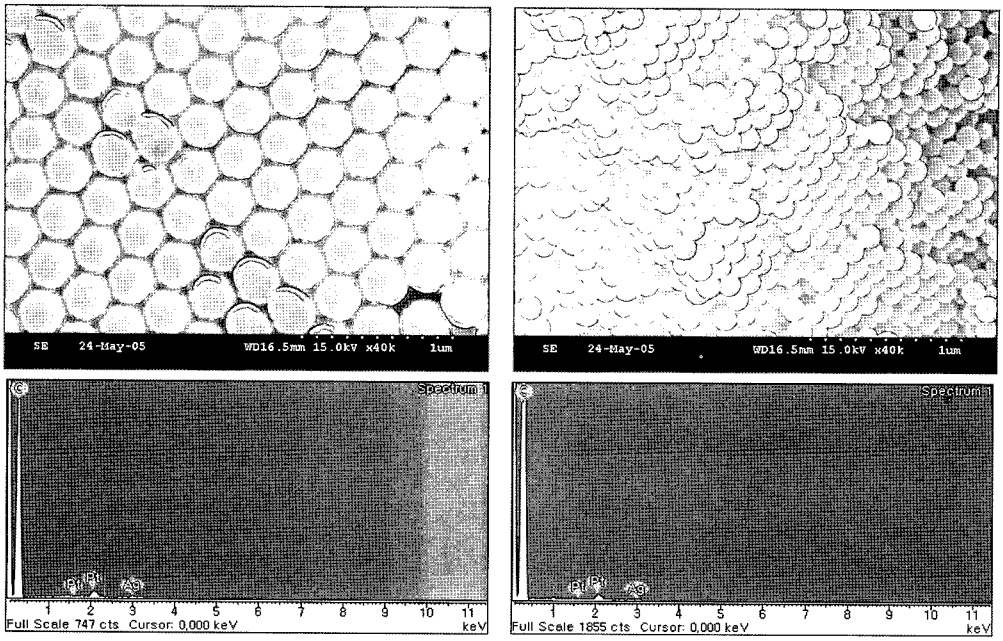
reaction states of a comonomer in an emulsifier-free polymerization could be at the water phase of the system, in the interior or on the surface of the particle. Comonomer, which is existed on the surface of particle, can enhance the stability of particle during polymerization. Adding a hydrophilic comonomer leads to higher concentration of comonomer in aqueous solution at the easily stage of polymerization and therefore higher primary particle, which could results in smaller nanoparticle. We might thus expect that nanoparticles prepared in the presence of hydrophilic comonomer with higher solubility could obtain higher number primary particles and therefore smaller resulting nanoparticles.

In order to know the existence of hydrophilic comonomer on the surface of particles, the PS and PSS was analyzed by FTIR spectroscopy as shown Figure 2. In FTIR spectrum of PSS particle, the peaks at 1180 cm^{-1} could be assigned to the symmetric SO_2 stretch. The characteristic peak around 3500 cm^{-1} due to -OH stretching was appeared. FTIR results means the hydrophilic comonomer was existed on the surface of polystyrene particles.

Figure 3 shows the FE-SEM image and EDXS data of Ag-PS and Ag-PSS particles prepared by γ-irradiation. In FE-SEM images, the Ag nanoparticles were not founded on the surface of the PS and PSS particles. On the other hand, the EDXS data appeared the Ag peaks. This means the Ag nanoparticles were existed on the surface of PS and PSS particles.

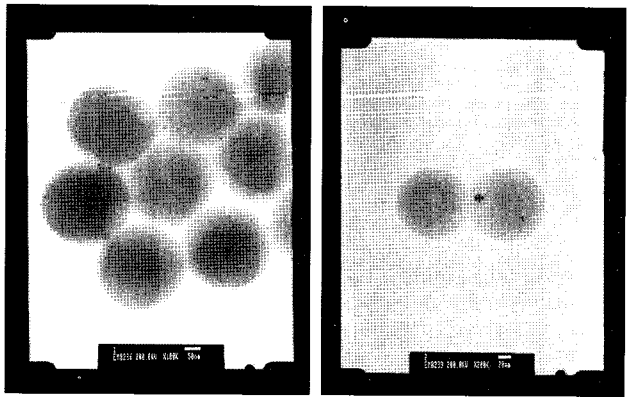
Figure 4 shows HR-TEM images of Ag-PS (a) and Ag-PSS (b) prepared by γ-irradiation. In FE-SEM images of Ag-PS, aggregated Ag nanoparticles were founded on the surface of the PS particles. In HR-TEM images of Ag-PS, the Ag nanoparticles (of ~7 nm) were placed on the surface of PS particle (of ~450 nm). The Ag nanoparticles placed on the surface of PS particles was narrow in size distribution. On the other hand, the HR-TEM image of Ag-PSS particle, a little of the Ag nanoparticles (of ~20 nm) were obtained on the surface of PSS particle (of ~160 nm). In order to know the complex formation of the silver ions and sulfonated group, the $AgNO_3$ was reacted with sodium dodecysulfate (SDS) as the colloids stabilizer in water, and the solution was irradiated by γ-ray. The SDS-stabilized Ag nanoparticles (20-50 nm) were obtained. These results may be considered that the Ag nanoparticles were partly generated because silver ions were complexed with sulfonated group of PSS, and then the silver complexes were reduced by hydrated electron reduced by γ-irradiation.

In order to test the antimicrobial efficiency of the Ag-PS and Ag-PSS particles, *Staphylococcus aureus* ATCC 6538 and *Klebsiella pneumoniase* ATCC 4352 was chosen as a model viruses, and was incubated as above described Experimental section.[16] Table II shows the antimicrobial efficiency against *Staphylococcus aureus* ATCC 6538 and *Klebsiella pneumoniase* ATCC 4352 for Ag-PS and Ag-PSS coating cloth. The antimicrobial efficiency against ATCC 6538 and



(a) (b)

Figure 3. FE-SEM images and EDXS spectra of Ag-PS (a) and Ag-PSS (b) particles prepared by γ-irradiation.



(a) (b)

Figure 4. FE-TEM images of Ag-PS (a) and Ag-PSS (b) prepared by γ-irradiation.

ATCC 4352 for Ag-PS coating cloth after 100 cycle washing was 99.9%. However, the antimicrobial efficiency against ATCC 6538 and ATCC 4352 for Ag-PSS coating cloth after 100 cycle washing was 35 and 30%, respectively.

On the other hand, the antimicrobial efficiency against *Staphylococcus aureus* ATCC 6538 and *Klebsiella pneumoniase* ATCC 4352 for Pd-PS and Pd-PSS coating cloth was determined.

Conclusions

By using a new γ-irradiation method, Ag-PS and Ag-PSS particles were synthesized. From a FE-SEM measurement of the Ag-PS and Ag-PSS particles, Ag nanoparticles were placed on the surface of the PS and PSS particles, respectively.

Table II. Antimicrobial Efficiency of Ag-PS and Ag-PSS against *Staphylococcus aureus* ATCC 6538 and *Klebsiella pneumoniase* ATCC 4352[a]

Number of Washing Cycles[b]	Antimicrobial Efficiency (%) of Ag-PS against		Antimicrobial Efficiency (%) of Ag-PSS against	
	ATCC 6538	ATCC 4352	ATCC 6538	ATCC 4352
20	99.9	99.9	55	46
50	99.9	99.9	50	45
70	99.9	99.9	40	38
100	99.9	99.9	35	30

[a]Antimicrobial activity was measured according to the protocol "KS K 0693:2001".
[b]"After-washing" antimicrobial activity was measured according to the protocol "KS K 0432-1999".

The antimicrobial efficiency of Ag-PS and Ag-PSS particles coating cloth against *Staphylococcus aureus* ATCC 6538 and *Klebsiella pneumoniase* ATCC 4352 was examined. The antimicrobial efficiency of Ag-PS and Ag-PSS coating cloth after 100 cycle washing was 99.9%. The Ag-PS and Ag-PSS particles can be used for antimicrobial coating material for cloth.

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