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SHORT COMMUNICATION

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Polymer/Ag composite microspheres produced by water-in-oil-in-water emulsion polymerization and their application for a preservative

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Abstract Nanosized Ag particles were entrapped successfully in multihollow porous poly(methyl methacrylate) (PMMA) microspheres by water-in-oil-in-water emulsion polymerization. The structure of the PMMA/Ag microspheres prepared was characterized by scanning electron microscopy and X-ray powder diffraction analysis. It was found that the Ag nanoparticles were impregnated in the inner voids of the microspheres and they had a face-centered cubic structure. In the preservation test, the PMMA/Ag microspheres showed a powerful antibacterial performance, indicating that the Ag ions released effectively through the nanosized pore channel of the PMMA wall.

Keywords Ag particles · Multihollow · Water-in-oil-in-water · Antibacterial · Nanosized pore

Introduction

Recently, the incorporation of metal nanoparticles into a polymer matrix has proved to be an effective and low-cost approach to improve the performance of already existing polymers [1, 2, 3]. A number of different methods have been proposed to prepare metal/polymer nanocomposites. Usually, metal nanoparticles are mixed mechanically with polymers to form composites [4]. In this case, it is very difficult to obtain a uniform dispersion of metal particles in the polymer matrix because of the easy aggregation of metal particles and the high viscosity of the polymer. Therefore, more recently, more attention has been paid to the in situ synthesis of metal particles in polymer matrices, where metal ions are reduced directly in the polymer matrices. Several metallic particles, such as Au, Ag, and Cu, have been synthesized successfully by reducing in polymer matrices [5, 6, 7]. Other polymer/metal nanocomposites have also been synthesized by using unique methods [8, 9].

This study proposes a completely different approach to produce polymer/Ag composite microspheres. Ag nanoparticles were synthesized and incorporated into multihollow porous polymer microspheres. In this procedure, we employed water-in-oil-in-water (W/O/W) emulsion polymerization. The W/O/W emulsion polymerization is useful for the production of a multihollow structure where many voids are located in the rigid polymer particles [10, 11, 12]. Using W/O/W emulsion polymerization, Ag-containing multihollow polymer microspheres could be produced successfully. Moreover, a porous structure was induced along with the polymer wall. Through the pore channel of the wall structure, the Ag nanoparticles were expected to show their biological activity. In the present contribution, we report a convenient procedure for the preparation of polymer/Ag composite microspheres. The morphology of the multihollow porous polymer microspheres was characterized in the presence of Ag nanoparticles. Finally, the importance of the morphological structure of the

composite microspheres was emphasized by considering the preservation performance of Ag nanoparticles.

Experimental

Materials

Silver nitrate (Aldrich Chemical Co.), hydrazine (35% in water, Aldrich), and Tween 20 (polysorbate 20, Uniquema Americas) were used for the synthesis of Ag nanoparticles. Methyl methacrylate (MMA, Aldrich), ethylene glycol dimethacrylate (EGDMA, Aldrich), Arlacel P135, a polyethylene glycol(30) dipolyhydroxystearate (Uniquema Americas), and 2,2′-azobis(2,4-dimethylvaleronitrile) (ADVN, Wako Pure Chemicals) were used as received. Poly(vinyl alcohol) (PVA, $M_w = 8.8\times10^4$–9.2×10^4 g mol^{-1}, degree of saponification 87–89%) was purchased from Kuraray Co.

Synthesis of Ag nanoparticles

Ag nanoparticles were synthesized by the reduction of $AgNO_3$. $AgNO_3$ (0.5 g) was dissolved in 0.1 wt% Tween 20 aqueous solution (100 g). While stirring vigorously, 0.005 wt% hydrazine aqueous solution was added dropwise slowly at room temperature. The reaction was carried out for 6 h. Then, Tween 20 and other unreactants were removed by repeated centrifugation/redispersion processes.

W/O/W emulsion polymerization

W/O/W emulsion polymerization was carried out as described elsewhere. First, the Ag nanoparticles were dispersed finely in water by 30-min sonication at room temperature. The concentration of Ag was controlled to 0.2 wt% in the final yield of the microspheres. The aqueous solution containing Ag nanoparticles was mixed with the oil phase composed of MMA, EGDMA, toluene, Arlacel P135, and ADVN. Then, the mixture was homogenized with an MX-5 homogenizer (Nihonseiki Co., Japan) at 1×10^4 rpm for 5 min at room temperature, resulting in a fine water-in-oil (W/O) emulsion.The W/O emulsion was continuously homogenized mildly in 1 wt% PVA aqueous solution at 5.0×10^2 rpm for 5 min. Then, the W/O/W emulsion prepared was transferred into a double-walled glass reactor equipped with a mechanical stirrer, a reflux condenser, thermocouples, and a nitrogen gas inlet system. After deaerating with nitrogen gas, the polymerization was carried out at 60 °C for 10h. Polymerization in the aqueous phase was inhibited by adding a small amount of sodium nitrite (0.01 g) [12]. The Ag-containing microcapsules produced were repeatedly washed by centrifugation/redispersion in water and dried. A typical recipe is summarized in Table 1.

Characterizations

The overall image of the microspheres was observed with an optical microscope (OM, Nikon Microphot FAX). The images of the surface and the inner part of the microspheres were observed with a field-emission scanning electron microscope (JSM-6330F, JEOL). The structural state of the Ag nanoparticles in the poly(methyl methacrylate) (PMMA) microspheres was identified by X-ray diffraction (XRD) patterns obtained at a scanning rate of 4°min^{-1}, using a Rigaku D/Max-2200 (copper radiation, 40 kV, 100 mA, a nickel filter) in the range $2\theta = 10$–80°. The conversion was measured gravimetrically as described elsewhere [11, 12].

Table 1 A standard recipe for water-in-oil-in-water emulsion polymerization

Emulsifying step	Ingredient	Weight (g)
Water-in-oil emulsion[a]	Methyl methacrylate	10
	Ethylene glycol dimethacrylate	10 (variable)
	Arlacel P135	0.3
	2,2′-Azobis (2,4-dimethylvaleronitrile)	0.2
	Toluene[c]	2
	Colloidal Ag[d]	0.04
	Distilled deionized water	7
Water-in-oil-in-water emulsion[b]	Water-in-oil emulsion	30
	Poly(vinyl alcohol)	3
	$NaNO_2$	0.01
	Distilled deionized water	266.99

[a] 1.0×10^4 rpm, 5 min, 25 °C
[b] 5.0×10^2 rpm, 5 min, 25 °C
[c] Toluene was added to induce a pore structure (20 wt% against total oil weight)
[d] Net weight of colloidal Ag

Preservation test

The preservation efficacy of the composite microspheres was tested to evaluate the antimicrobial property [13, 14]. Samples were prepared by dispersing the PMMA/Ag composite microspheres in viscous aqueous solutions containing 0.01wt% of neutralized poly(acrylic acid). A mixed culture of microorganisms, *Pseudomonas aeruginosa* (ATCC9027), *Staphylococcus aureus* (ATCC6538), and *Escherichia coli* (ATCC8739) was obtained on tryptone soya broth after 24 h incubation at 32 °C. Then, 20 g samples were inoculated with 0.2 g of the microorganism suspensions to adjust the initial concentration of bacteria to 10^6 cfu g^{-1}. The inocula were mixed homogeneously with the samples and stored at 32 °C. The microbial counts were carried out using the pour plate count method.

Results and discussion

In a previous study [11], a stable W/O/W emulsion system was constructed using a surfactant mixture composed of macromolecular surfactants: Arlacel P135 as a lipophilic primary surfactant and PVA as a hydrophilic secondary surfactant. Therefore, the W/O/W emulsions prepared in this study could retain the initial morphology of multiple emulsions throughout the polymerization process. OM and SEM photographs of the multihollow porous PMMA microspheres obtained after the polymerization are shown in Fig. 1. The microspheres were produced in the size range of 10 μm. In addition to the intrinsic multihollow morphology from W/O/W emulsion polymerization, a porous structure was imparted to the PMMA wall, as shown in Fig. 1c. The porous structure was induced successfully by the cross-linking of the PMMA phase in the presence of inert solvent, toluene. This happens because the phase separation induced by the collapse of growing MMA–EGDMA copolymer chains is more favorable at low

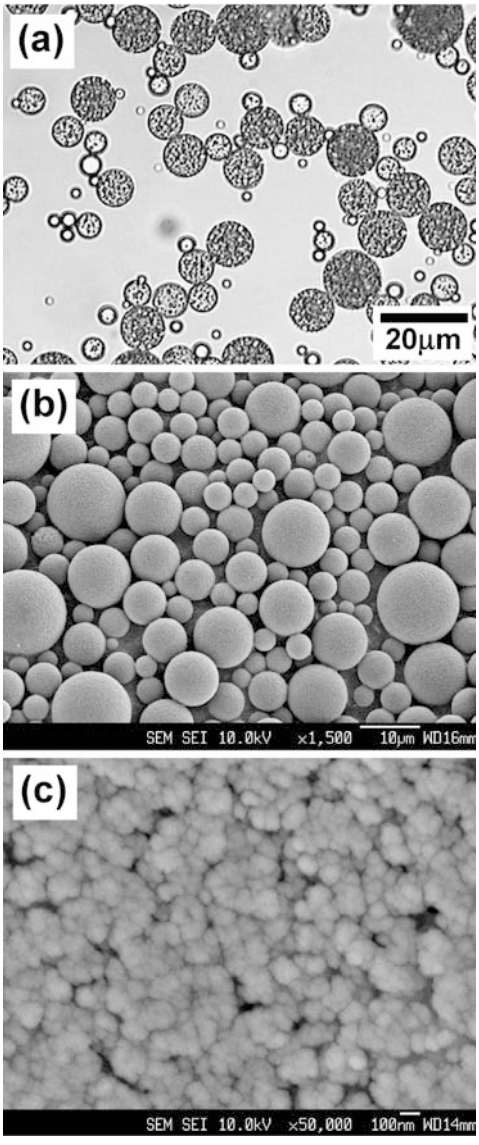

Fig. 1 Optical microscope (*OM*) and scanning electron microscope (*SEM*) photographs of multihollow porous poly(methyl methacrylate) (*PMMA*) microspheres: **a** OM image, **b** SEM image, and **c** SEM surface image with high magnification

viscosity [15, 16]. It is expected that the pores generated can act as a channel for the encapsulated materials to make contact with the external environment in the microbial application.

SEM photographs of the multihollow porous PMMA microspheres containing Ag nanoparticles are shown in Fig. 2. In the observation of the fracture surface shown in Fig. 2a, it was verified that the microcapsules contained a number of small voids in their inner phase, which is attributed to the internal water droplets before the polymerization of W/O/W multiple emulsions. The Ag nanoparticles entrapped in the

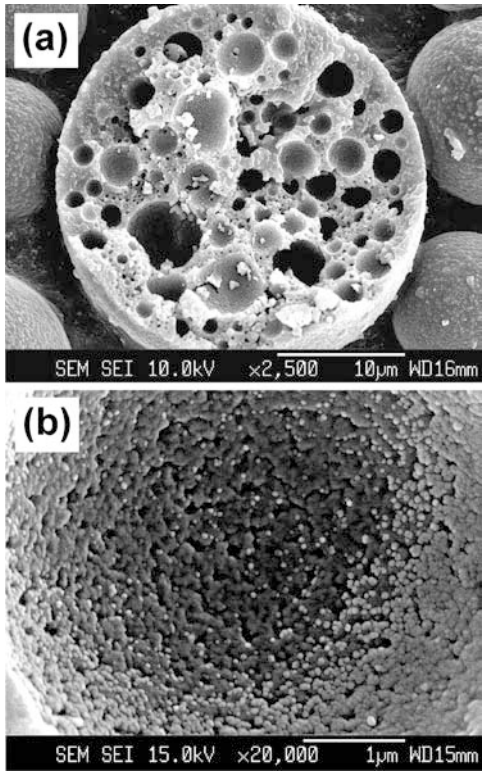

Fig. 2 SEM photographs of Ag-containing multihollow porous PMMA microspheres: **a** inner void image and **b** distribution of colloidal Ag on the surface of an inner void. The microspheres contain 2×10^3 ppm Ag

internal voids could be observed at high magnification of the void surface. Ag particles about 20 nm in size were packed well along with the surface of the void. The actual concentration of Ag nanoparticles in the microspheres was 0.19 wt%, which was measured by atomic absorption spectroscopy. The high loading efficiency is closely related to the surfactant system composed of macromolecular surfactants for W/O/W emulsion polymerization: Arlacel P135 as a liphophilic primary surfactant and PVA as a hydrophilic secondary surfactant. In a previous study, it was found that the W/O/W emulsions prepared with the surfactant system had a lower breakdown rate [11, 17].

The presence of Ag nanoparticles in the internal water phase had no serious influence on the formation of a multihollow porous structure. In order to confirm the effect of Ag nanoparticles on the polymerization process, the polymerization kinetics were observed in the presence of Ag nanoparticles. The conversion–polymerization time curves are shown in Fig. 3. Irrelative to the presence of Ag nanoparticles, the polymerization kinetics were the same, which indicates that Ag nanoparticles in the internal water droplets did not have any effect on the polymerization procedure.

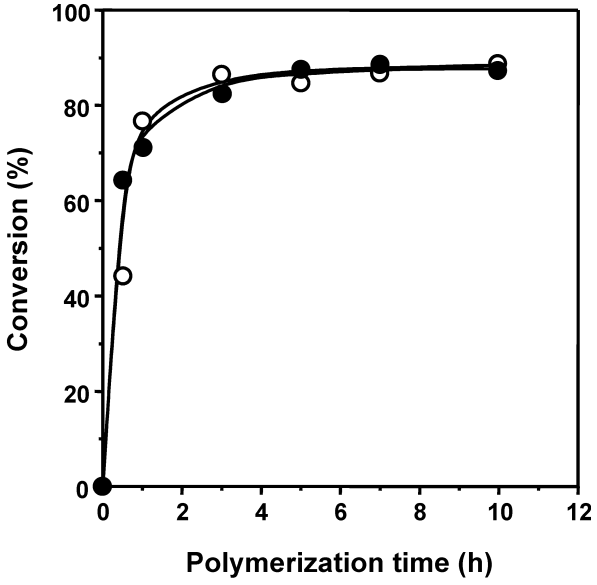

Fig. 3 Conversion–polymerization time curves of multihollow porous PMMA microspheres in the absence (*closed circles*) and in the presence (*open circles*) of Ag (2×10^3 ppm in the microspheres)

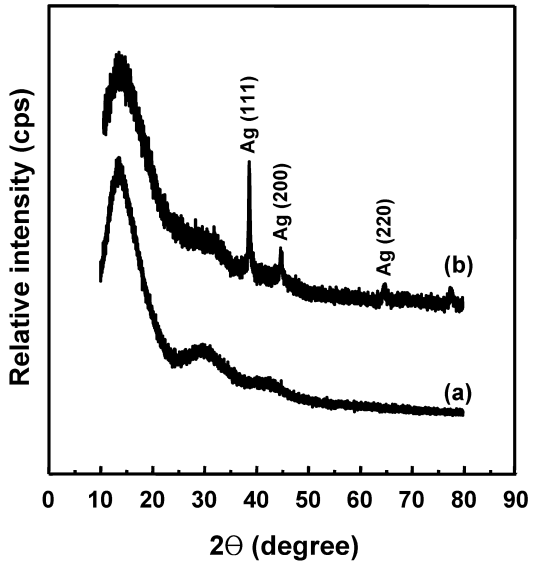

Fig. 4 X-ray diffraction patterns of multihollow porous PMMA microspheres in the absence (*a*) and in the presence (*b*) of Ag (2×10^3 ppm in the microspheres)

The phase of the PMMA/Ag microspheres was characterized by XRD and is shown in Fig. 4. Unlike PMMA microspheres, the PMMA/Ag composite microspheres showed two characteristic XRD patterns. In addition to the PMMA XRD pattern, another three peaks were detected, which correspond to the face-centered cubic Ag phase (111, 200, 220). Referring to Fig. 4, the value of the Ag lattice constant has been estimated to be $a = 4.081$ Å, a value which is consistent with $a = 4.0862$ Å reported by the JCPDS file number 4-0783. This XRD result verifies well that the microspheres are composites composed of PMMA and Ag.

Table 2 Preservation performance of poly(methyl methacrylate)/Ag composite microspheres

Ag concentration (ppm)[a]	Number of bacteria (cfu g^{-1})		
	0 days[b]	7 days	28 days
0	1.0×10^6	4.0×10^5	5.6×10^5
25	1.0×10^6	1.5×10^3	$< 1.0\times10^2$
100	1.0×10^6	$< 1.0\times10^2$	$< 1.0\times10^2$

[a]The concentration of Ag in the formulations that contain the poly(methyl methacrylate)/Ag composite microspheres
[b]Initial number of bacteria

It is widely known that Ag has many biological functions [18, 19]. In order to evaluate the biological function in the form of polymer composites, the antibacterial performance of PMMA/Ag microspheres was tested in the aqueous dispersion and is summarized in Table 2. The preservation performance was evaluated by counting the number of bacteria in the sample with storage time at 32 °C. In the absence of Ag, the number of bacteria remained constant. However, on adding Ag to the test formulations, the number of bacteria decreased dramatically. Moreover, as the concentration of Ag increased, the number of bacteria decreased more sharply. Within 1 week, most of the initially inoculated bacteria disappeared. From this result, it is evident that the PMMA/Ag microspheres have powerful antibacterial activity. Even though the Ag nanoparticles were entrapped in the internal voids of the microspheres, the ionization of Ag metals seems to happen effectively through the nanosized pore channels of the PMMA wall.

From these results, we concluded that a new antibacterial colloidal system could be constructed by employing the W/O/W emulsion polymerization technique. The structure-controlled polymer phase of the microspheres played an important role in that Ag nanoparticles were entrapped in the internal voids and they displayed antibacterial activity through the pore channel of the polymer wall.

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References

1. McEuen PL (1997) Science 278:1729
2. Zhu ZK, Yin J, Cao F, Shang XY, Lu QH (2000) Adv Mater 12:1055
3. Ramos J. Millan A, Palacio F (2000) Polymer 41:8461
4. Ghosh K, Maiti SN (1996) J Appl Polym Sci 60:323
5. Hatchett DW, Josowicz M, Janata J, Baer DR (1999) Chem Mater 11:2989
6. Gotoh Y, Igarashi R, Ohkoshi Y, Nagura M, Akamatsu K, Deki S (2000) J Mater Chem 10:2548
7. Huang CJ, Yen CC, Chang TC (1991) J Appl Polym Sci 42:2237
8. Schmid G (1994) Colloids and surfaces. VCH, Weinheim
9. Zhang Z, Zhang L, Wang S, Chen W, Lei Y (2001) Polymer 42:8315
10. Kim JW, Joe YG, Suh KD (1999) Colloid Polym Sci 277:252
11. Kim JW, Ko JY, Jun JB, Chang IS, Kang HH, Suh KD (2003) Colloid Polym Sci 281:157
12. Kim JW, Lee CH, Jun JB, Suh KD (2001) Colloid Surf A 194:57
13. Cosmetic Toiletry Fragrance Association (1993) Microbiology guidelines. CTFA, Washington, DC
14. Farrington JK (1994) Appl Environ Microbiol 60:4553
15. Kim JW, Ryu JH, Suh KD (2001) Colloid Polym Sci 279:146
16. Wang QC, Svec F, Frechet JMJ (1994) J Polym Sci Part A Polym Chem 32:2577
17. Jager-Lezer N, Terrisse I, Bruneau, F, Tokgoz S, Ferreira L, Clausse D, Seiller M, Grossiord JL (1997) J Controlled Release 45:1
18. Dowling JP, Donnelly K, McConnell ML, Eloy R, Arnaud MN (2001) Thin Solid Films 398:602
19. Joyce-Wörmann RM, Hentschel T, Müstedt H (2000) Adv Eng Mater 2:380