Preparation and Characterization of Core-Shell Nanoparticles Hardened by Gamma-Ray

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Running Head: Nanoparticles Prepared with Gamma-Ray

Abstract. Core-shell nanoparticles have been prepared by irradiation of gamma-ray on block copolymer micelles consisting of hydrophilic polyacrylic acid and hydrophobic polyisoprene with each 40 monomer units. The structure was determined by means of dynamic light scattering (DLS), small angle X-ray scattering (SAXS) and atomic force microscopy (AFM). The size distribution of the core-shell nanoparticles determined by DLS and AFM was very narrow. The average diameter of the particles decreased from 48 nm for the original micelles to 26 nm by the irradiation of 30 kGy. The core size determined by SAXS combined with DLS was roughly constant of 10 nm, irrespective of irradiation dose, whereas the shell thickness of the micelles was twice as large as core size, and decreased with increasing irradiation dose.

Introduction

Micro- and nanosize materials with unique morphology and functions have become more and more important in medical applications [1-3]. Polymeric materials with nanoscale structure have also shown significant potential for use, such as water-miscible carriers [4] and/or contaminants removers [5]. Recently, a new method for manufacturing particles with nano-structure was reported by several scientists, who employed self-assembly of amphiphilic diblock and triblock copolymers in aqueous solutions [6-10]. These particles can be obtained by crosslinking among chain segments composed of the outer hydrophilic parts of the copolymers in the form of micelles to obtain a core-shell structure. Generally, the crosslinking has been accomplished by using chemical agents [6-8]. For example, a reagent which has more than two amino groups, such as polydiamine, is employed as a crosslinker of the diblock copolymers with carboxyl groups. However, the residues of such chemical agents are hard to be recycled, and have often been found poisonous when applying the core-shell nanoparticles to the medical field.

Liu et al. [9] and Nardin et al. [10] prepared nanoparticles based on micelles consisting of photocrosslinkable block copolymers by means of UV-irradiation crosslinking. Disadvantages of this method are that enough time (more than 24 hours) is often required for hardening the particles, and the photocrosslinkable materials are conventionally unstable under daylight. On the other hand, it is well known that the high-energy irradiation induces decomposition and crosslinking of a variety of polymers, which is employed for gelation and/or hardening of a number of polymers [11]. Up to now we are not aware of any reports that a non-toxic polymer is converted into a toxic one by the irradiation. In various fields, gamma-ray irradiation modifies lots of materials (e.g. smart adhesive plasters [12] and contact lenses [13]) to be more acceptable by virtue of their harmless feature.

In this paper, we tried to induce crosslinking on micelles consisting of polyisoprene and polyacrylic acid (PI-b-PAA) by gamma-ray irradiation as an alternative of the chemical reagents. Acrylic acid was chosen for the hydrophilic block because vinyl polymers having an α hydrogen on each monomer is generally classified as the radiation crosslinking polymer, though gamma-ray irradiation also induces breaking of the polymer chains [11]. For example, Kadlubowski et al. prepared nanogel from PAA using electron beam [14]. It is expected that the radicals generated by gamma ray react mainly with the shell portion in a dilute aqueous solution because the most part of the energy is absorbed by water. Furthermore, the reactivity of PAA due to the irradiation is much higher than that of PI [11]. Thus, it is expected that the gamma-ray irradiation induces inhomogeneous crosslinking and breaking of the segments in the copolymer micelles. It is expected that nano-capsule will be obtained from the product because PI core can be removed by ozonolysis [6b]. To determine the structure of the irradiated micelles, we used a combination of measurements of dynamic light scattering (DLS), atomic force microscopy (AFM) and small angle X-ray scattering (SAXS).

Experimental Section

Materials. Polyisoprene with a living anion was obtained by the polymerization of 7.0 mmol isoprene using 0.18 mmol sec-buthyllithium in n-hexane (Kanto) as an initiator at room temperature under nitrogen atmosphere. 0.18 mmol 1,1-Diphenylethylene (Wako) and tetrahydrofuran (Kanto) including 0.23 mmol of LiCl (Aldrich 99.99+%), was added to the anion solution sequentially. Seven mmol trimethylsilylacrylate (Aldrich) diluted with tetrahydrofuran was poured into the mixture of the resultant mixture at −78°C. Finally, the polymerization was terminated with ethanol. Dilute hydrochloric acid was added to the copolymers in order to hydrolyze the trimethylsilyl groups. The prepared diblock copolymers were intentionally designed as PAA and PI with each 40 monomer units. The product was reprecipitated into methanol and then washed with cyclohexane to remove origoisoprene.

After the resultant block copolymers were dried in vacuum for 24 hours, 0.3 wt% aqueous dispersion designated as NRT0 was prepared to form micelles, as shown in Figure 1. The dispersion of the micelles was irradiated under nitrogen atmosphere by the gamma-ray generated from a ^{60}Co source with a dose rate being 10 kGy /h in Takasaki Radiation Chemistry Research Establishment of Japan Atomic Energy

Research Institute (JAERI). Three samples irradiated with 10 kGy, 20 kGy, and 30 kGy were chosen for this study and designated as NRT10, NRT20, and NRT30, respectively.

Measurements. AFM images were observed for the samples prepared under the same condition expect for the radiation dose. A small amount of the dispersions was dropped on the surface of highly ordered pyrolytic graphite (HOPG) and dried well to deposit particles on HOPG. The images were taken by using a Digital Nanoscope III (Veeco Instruments) operating in the tapping mode in both the dry and wet conditions; we employed an Si cantilever (NCH; TOYO Co., Ltd) at the dry condition and an Si_3N_4 cantilever (DNP-S; TOYO Co., Ltd) in the wet condition. However, we could not decide R_a in the wet condition because the size of the nanoparticles was considerably dependent on the tapping force, probably because of the very soft surface. Thus, we used the AFM images only in the dry condition for the determination of the number-average diameter $R_{a.}$ R_a and the standard deviation were determined for more than 50 particles observed on the AFM images.

DLS measurements were carried out for the dispersions whose concentrations were 0.05 and 0.025 wt% at 25 ^{o}C at the scattering angles of 45° and 90° using a laboratory made light scattering photometer [15] with a green diode laser, BWT-50, B&W having 532 nm wavelength and a Brookhaven BI-9000 correlator. Each suspension was made clean by centrifugation at 1500 g for 30 min just before measurements. The diffusion coefficient D for each sample was almost independent of the concentration and the angle in the experimental range. Hydrodynamic radius R_h at infinite dilution was determined from D by using the Stokes-Einstein equation.

SAXS experiments were performed by using a synchrotron radiation small-angle X-ray scattering spectrometer installed at BL40-B2 Line of the 8GeV storage ring at the SPring-8 at the Japan Synchrotron Radiation Research Institute (JASRI). The wavelength was set at 0.15 nm and the sample to detector distance was 100 cm. The samples were poured in a quartz-glass capillary cell of 1.5 mm diameter, which was placed into a cell holder. A two-dimensional detector was used to collect the scattered X-ray. The experimental temperature was 25.0 ± 0.5 ^{o}C.

When a core-shell sphere structure has a electron density (e_C) in the core with the radius R_C and that (e_S) in the shell with the outer radius R_O and the dispersing phase is filled with solution with the electron density (e_0), the scattering factor of a spherical particle with core-shell structure can be expressed by the relation [16]

$$I(q) \propto P(q) = \\ \left\{(4\pi)(\Delta e_C - \Delta e_S)/q^3\left[\sin(qR_C) - qR_C\cos(qR_C) + p(\sin(qR_o) - qR_S\cos(qR_o))\right]\right\}^2 \tag{1}$$

where $\Delta e_C = e_C - e_0$, $\Delta e_S = e_S - e_0$, $p = \Delta e_S/(\Delta e_C - \Delta e_S)$ and q is the magnitude of the scattering vector q=$(4\pi/\lambda)\sin(\theta/2)$. For the fitting of the experimental data to eq (1), we used the outer radius R_h as R_o. Therefore, only two adjustable parameters, average R_C and p, were employed as the fitting parameters.

Results and Discussion

Small spherical particles were observed under AFM in all the irradiated dispersions of the nanoparticles. Figure 2 shows the typical AFM images taken in the dry condition for NRT30. The average radius determined from AFM (R_a) and that from DLS (R_h) were listed in Table I. R_h and R_a obtained for each irradiated sample agree fairly well with each other and linearly decrease with increasing irradiation dose, as illustrated in Figure 3. The average diameter of the particles decreased from 48 nm for the original micelles to 26 nm by the irradiation of 30 kGy. The small standard deviations of R_h and R_a in Table I indicates a narrow size distribution. The scattering intensity decreased with increasing irradiation dose and the intensity for NRT 30 at 90 ° was about 10% smaller than that for NRT0. This result shows that scission of PAA chain was observed but is not remarkable for this system.

Figure 4 shows a plot for the logarithm of the small angle X-ray scattering intensity I(q) vs q at each irradiation dose. The observed scattering curves fit eq (1) fairly well, as shown by solid curves. The slight deviations between the fitting curves and experimental data at high q regime could be attributed to the distribution of size and p for the micelles. The parameters determined by a nonlinear least-square method, are listed in Table I. In contrast to R_h or R_a, the outer radius R_C is roughly constant of 9.7~10.0nm, irrespective of irradiation dose, and R_C/R_o increases from 0.20 for the original micelles to 0.39 by the irradiation of 30 kGy. Because ratio of PI chain length to total chain length is estimated to be 0.45, the smaller values of R_C/R_o of 0.20~0.39 suggests that the outer PAA portion had an extended conformation whereas the inner PI portion shrinked in the micelles, and the thickness of outer PAA portion decreased with increasing the irradiated dose. The extremely small value of p for the micelles suggests that the electron densities of dispersing water and the swollen PAA layer are similar each other. The enhancement of p with increasing irradiation dose should be due to the increase of electron density by crosslinking of PAA portion.

In the present work, we applied gamma-ray irradiation to obtain shell-crosslinked micelles. It can be concluded that cross-linking were induced mainly in the outer PAA portion of the micelles while the total molecular weight of the micelle decreased very gently. It is interesting to further investigate the permeability of molecules through shell portion.

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References

(1) Stupp, S. I.; Braun, P.V. Science 1997, 277, 1242.

(2) Bhatia, S. N.; Balis, U. J.; Yarmush, M. L.; Toner, M. FASEB J. 1999, 13, 1884.

(3) Bruchez, M. Jr.; Moronne, M.; Gin, P.; Weiss, S.; Alivisatos, A. P. Science 1998, 281, 2013.

(4) Prokop, A.; Kozlov, E.; Carlesso, G.; Davidson, J. M. Adv. Polym. Sci. 2002, 160, 119.

(5) Lee, Y.; Huang, H.; Sayre, L. M. J. Am. Chem. Soc. 1996, 118, 7241.

(6) (a) Huang, H.; Remsen, E. E.; Wooley, K. L. Chem. Comm. 1998, 1415.

(b) Huang, H.; Remsen, E. E.; Kowalewski, T.; Wooley, K. L. J. Am. Chem. Soc. 1999, 121, 3805.

(c) Zhang, Q.; Remsen, E. E.; Wooley, K. L. J. Am. Chem. Soc. 2000, 122, 3642.

(d) Ma, Q.; Remsen, E. E.; Kowalewski, T.; Schaefer, J.; Wooley, K. L. Nano Lett. 2001, 1, 651.

(7) Sanji, T.; Nakatsuka, Y.; Kitayama, F.; Sakurai, H. Chem. Comm. 1999, 2201.

(8) Li, T.; Ning, F.; Xie, J.; Chen, D.; Jiang, M. Polym. J. 2002, 34, 529.

(9) Guo, A.; Liu, G. J.; Tao, J. Macromolecules 1996, 29, 2487.

(10) Nardin, C.; Hirt, T.; Leukel , J.; Meier, W. Langmuir 2000, 16, 1035.

(11) Tabata, Y.; Ito, Y.; Tagawa, S. (Eds) CRC Handbook of Radiation Chemistry. CRC Press, Boston (1991).

(12) Yoshii, F.; Makuuchi, K.; Darwis, D.; Iriawan, T.; Razzak, M.T.; Rosiak, J. M. Radiat. Phys. Chem. 1995, 46, 169.

(13) Carenza, M. Radiat. Phys. Chem. 1992, 39, 485.

(14) Kadlubowski, S.; Grobelny, J.; Olejniczak, W.; Cichomski, M.; Ulanski, P. Macromolecules, **2003,** 36, 2484. and references therein.

(15) Isojima, T.; Fujii, S.; Kubota, K.; Hamano, K. J. Chem. Phys., **1999**,111, 9839.

(16) Chu,: B.; Wu, G.; Schneider, D. K. J. Polym. Sci. B **1994**, 32, 2605.

Figure captions

Figure 1. Schematic illustration of irradiation process of core-shell nanoparticles.

Figure 2. Tapping mode AFM image of nanoparticles (NRT-30) in the dry condition.

Figure 3. Dose dependence of R_h (triangles) and R_a (circle) of nanoparticles.

Figure 4. Small angle X-ray scattering profiles for nanoparticle suspension irradiated with gamma-ray at the indicated dose. Solid, dash, and dash-dot line are the calculated values using eq (1) with the parameters in Table I for NRT0, NRT10, and NRT30, respectively,.

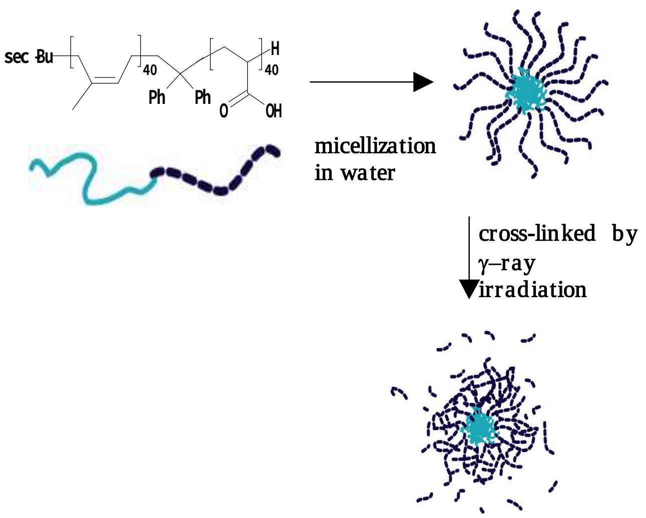

Figure 1

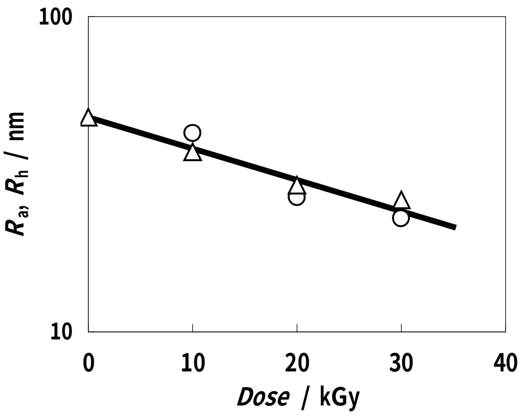

Figure 3

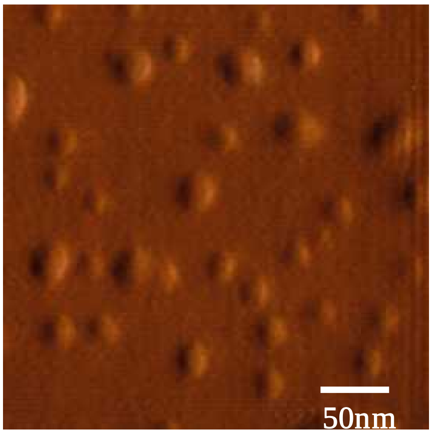

Figure 2

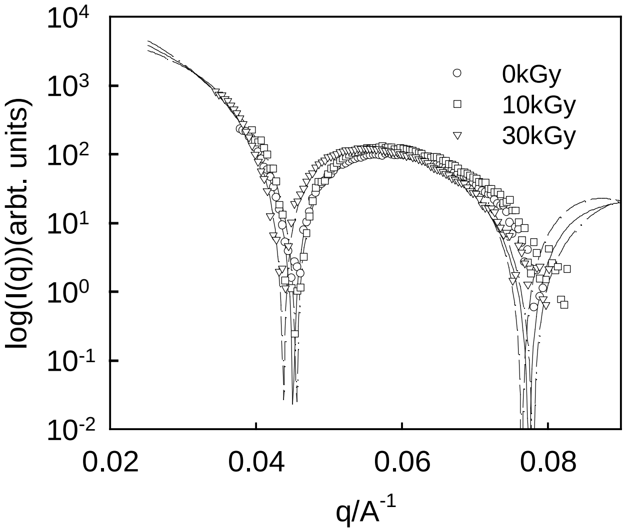

Figure 4

Table 1. Characteristics of nanoparticles

Sample name	Radiation dose (kGy)	R_a (nm)	R_h (nm)	R_c (nm)	R_c/ R_o (-)	p (-)
NRT0	0	-	48.0±11.2	9.7±2.2	0.20	0.0030
NRT10	10	42.7±6.4	37.3±11.4	9.9±3.0	0.27	0.0079
NRT20	20	26.7±3.3	29.2±7.1	-	-	-
NRT30	30	22.9 ±4.6	26.2±3.7	10.0 ±1.2	0.39	0.0384

R_a, the number-average radius observed by AFM in the dry condition; R_h, the hydrodynamic radius determined by DLS. R_C, the radius of the core. R_C/R_o, core radius/outer radius ratio. $p = \Delta e_S / (\Delta e_C - \Delta e_S)$. The errors denote the standard deviations.