Synthesis of Amphiphilic Blockcopolymer Using Mechanically Produced Macromonomers Possessing Anhydrate as a Terminal Group and Its Application to Polymeric Micelles

Shin-ichi Kondo[1,*], Machi Omoto[1], Yuka Sawama[1], Yasushi Sasai[1], Kenjiro Tatematsu[2], Yukinori Yamauchi[3] and Masayuki Kuzuya[4]

[1]*Laboratory of Pharmaceutical Physical Chemistry, Gifu Pharmaceutical University, 1-25-4 Daigaku-Nishi, Gifu 501-1196, Japan*

[2]*Laboratory of Radiochemistry, Gifu Pharmaceutical University, 5-6-1 Mitahora-Higashi, Gifu 502-8585, Japan*

[3]*Department of Pharmaceutical Physical Chemistry, College of Pharmaceutical Sciences, Matsuyama University, 4-2 Bunkyo-cho, Matsuyama, Ehime 790-8578, Japan*

[4]*Department of Health and Welfare, Faculty of Human Welfare, Chubu Gakuin University, 2-1 Kirigaoka, Seki-shi, Gifu 501-3993, Japan*

Abstract: We have synthesized macromonomers by mechanochemical reaction of poly(benzyl methacrylate) (PBzMA) and maleic anhydride (MA). The ESR spectrum of the fractured sample of PBzMA and MA showed a broad singlet, which was apparently different from the spectrum of PBzMA mechanoradical. The amphiphilic blockcopolymer was synthesized with macromonomer of PBzMA and amino-terminated polyethyleneglycol (α-methyl-ω-aminopropoxy polyoxyethylene, MEPA). The number average molecular weight of the produced amphiphilic blockcopolymer was 33,000. Polymeric micelles were readily prepared from the present amphiphilic blockcopolymer by a dialysis method. The mean diameter of the micelles measured by dynamic light scattering was about 146 nm. It was shown that the present macromonomer mechanically produced can be used for the synthesis of amphiphilic bockcopolymer to form polymeric micelles.

Keywords: Polymeric micelle, macromonomer, maleic anhydride, mechanoradical.

INTROUCTION

Amphiphilic blockcopolymers consisting of a hydrophilic block and a hydrophobic block can form core-shell micelles in a solvent that is selective for one of the blocks, due to the association of the insoluble block. The micelles formed are referred to “polymeric micelles.” Polymeric micelles have attracted much attention as possible drug delivery systems because of their ability to solubilize hydrophobic molecules, their nanoscale size, their good thermodynamic solution stability, their ability to release various drugs over an extended period of time, and their slow clearance by the reticuloendothelial system [1-10]. Polyethylene glycol (PEG) is often used as a hydrophilic block because of its high water solubility, and very low toxicity and immunogenicity.

A macromonomer is a polymer possessing a functional group that can participate in further polymerization. The macromonomer technique has widely been appreciated as one of the most convenient and promising methods to prepare well-defined polymers, such as a block copolymer, a branched polymer and so on [11-13].

In the previous paper, we reported the first example of the synthesis of macromonomers in the solid-state by the mechanochemical reaction of poly(methyl methacrylate) (PMMA) and maleic anhydride (MA) [14]. In this method the PMMA mechanoradicals, which are produced by mechanical fracture of polymer due to the polymer main-chain scission, can react with MA in the solid-state. However, the MA radicals produced cannot react with other MA molecules in the particle, since MA does not homopolymerize. As a consequent the macromonomers possessing acid anhydride as a terminal group would be synthesized.

In this paper, we aimed to the synthesis of amphiphilic blockcopolymer possessing PEG as a hydrophilic block by using the mechanochemically produced macromonomer and the formation of polymeric micelles by the use of the amphiphilic blockcopolymer. We synthesized the macromonomer of poly(benzylmethacrylate) (PBzMA) by mechanochemical solid-state reaction in the presence of MA to apply for a hydrophobic moiety of amphiphilic

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*Address correspondence to this author at the Laboratory of Pharmaceutical Physical Chemistry, Gifu Pharmaceutical University, 1-25-4 Daigaku-Nishi, Gifu 501-1196, Japan; Tel: +81-58-230-8114; Fax: +81-58-230-8114;
E-mail: skondo@gifu-pu.ac.jp

blockcopolymer. The macromonomer of PBzMA was reacted with amino-terminated PEG to obtain its amphiphilic blockcopolymer. The polymeric micelle formation by the use of the amphiphilic blockcopolymer was also confirmed by dynamic light scattering.

EXPERIMENTAL

Materials

Benzyl methacrylate was purchased from Tokyo Chemical Industry Co., Ltd. MA was purified by sublimation. All other chemicals were of special reagent grade.

Synthesis of PBzMA

Radical initiated solution polymerization of benzyl methacrylate was carried out according to a conventional method. A mixture of benzyl methacrylate (0.9 g) and 2,2'-azobisisobutyronitrile (AIBN) (0.9 mg) in *N,N*-dimethylformamide (DMF) (3 ml) was warmed at 60 ºC in a sealed glass-made tube under nitrogen for 24 h. The content was poured into a large amount of methanol. The precipitated polymer was collected and dried *in vacuo* to yield 0.82 g (91 %).

Mechanochemical Reaction of PBzMA and MA

The mixture of PBzMA (90 mg) and MA (10 mg) was mechanically fractured under anaerobic condition (e.g. in nitrogen) by vibratory ball milling apparatus (Shimadzu Co. Ltd.) equipped with a stainless steel ball (6.0 mmϕ, 890 mg) in a stainless steel twin-shell blender (7.8 mmϕ, 24 mm long) at room temperature for a prescribed period. The frequency of vibratory ball milling was 60 Hz. Residual oxygen in this system was removed with a model 1000 Oxygen Trap (Chromatography Research Supplies). The oxygen concentration was monitored with an oxygen analyzer (Toray Engineering Co. Ltd., LC-750/PC-120) and kept below 10 ppm. PBzMA (100mg) was also fractured in the same procedure described above.

Synthesis of Amphiphilic Blockcopolymer

The macromonomer (100 mg) of PBzMA and MA was dissolved in DMF (1 ml). To this solution was added α-methyl-ω-aminopropoxy polyoxyethylene (MEPA) (100mg, Sunbright MEPA-12T, MW 12,000) in DMF (2 ml). The mixture was stirred at room temperature for 15 h. This solution was poured into H_2O (100 ml). The precipitate was collected and dried *in vacuo* to yield 55 mg (28 %) of amphiphilic blockcopolymer.

ESR Spectral Measurement

ESR spectra were recorded on a JES-RE1X (JEOL) spectrometer with an X-band and 100-kHz field modulation. Care was taken to ensure that no saturation occurred and that the line shape was not distorted by excessive modulation amplitude. From a plot of the square root of the microwave power versus the signal peak height, a microwave power level of 0.04 mW was chosen. The ESR spectral intensity was determined by double integration. The radical concentration (spin numbers /g) was calculated from the spectral intensities with the aid of calibrated lines obtained from the spectral intensities of the PMMA sample impregnated with 1,1-diphenyl-2-picrylhydrazyl (DPPH).

Molecular Weight Measurement

Molecular weight of polymer was measured by a gel permeation chromatograph (GPC, Shimadzu LC-6A), equipped with a Refractive Index Detector (Shimadzu RID-6A), and a gel column (Shodex KD-80M, KD-802 and KD-G), under the following conditions: elution solvent, DMF containing 10 mM LiBr; flow rate, 0.7 ml/min; column temperature, 40 ºC. The data analysis was performed with Chromato-PRO-GPC (Runtime Instruments Co.). The calibration for the molecular weight determination was carried out with a standard specimen of polystyrene.

Preparation of Polymeric Micelles

The block copolymer (10 mg) was first dissolved in dimethyl sulfoxide (DMSO) (10 ml). This solution was transferred through a 0.45 μm filter (GL Chromatodisc 13N, GL Sciences Inc.) into a pre-swollen semi-permeable membrane (Spectra/Por, Spectrum Laboratories, Inc.; molecular weight cutoff 3,500 g/mol), and dialyzed against 2000 ml of water for 24 h to form the micelles. The dialysate water was exchanged at 2, 4, 6, and 8 h.

Dynamic Light Scattering Measurement

Dynamic light scattering measurements were performed using a dynamic light scattering spectrophotometer (DLS-5500G, Photal, Otsuka Electronics) with a He/Ne laser. A scattering angle of 90º was used in this study. The hydrodynamic diameters of micelles and the polydispersity factor of micelles, represented as μ_2/Γ^2, were calculated using the Stokes-Einstein equation and the cumulant method.

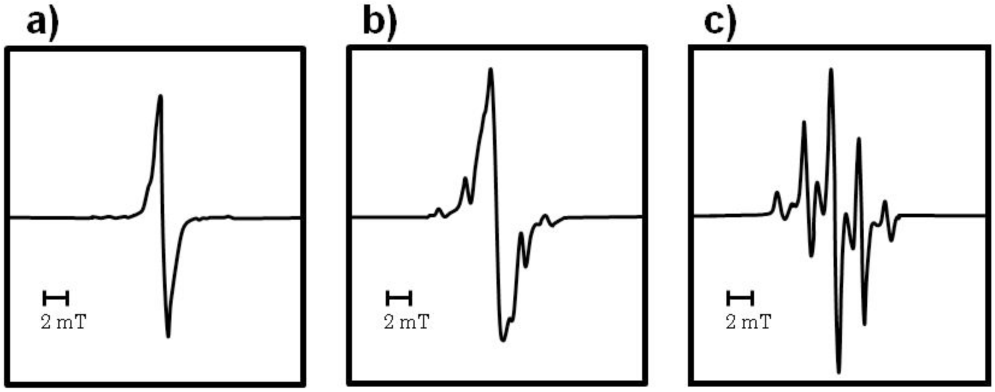

Figure 1: ESR spectra of the fractured samples of PBzMA with MA for 30 min (**a**) and 60 min (**b**) compared with that of PBzMA for 30 min (**c**).

RESULTS

ESR Spectra Observed in Mechanochemical Reaction of PBzMA and MA

Figure **1** shows the ESR spectra of the fractured samples of PBzMA with MA by vibratory ball milling for 30 and 60 min. Although the ESR spectrum of the fractured sample of PBzMA with MA at 30-min vibratory milling showed a broad singlet, the spectral feature at 60-min vibratory milling showed the overlapped spectrum of broad singlet and nine-line spectrum ascribed to PBzMA mechanoradicals (Figure **1**). The similar results were obtained in the case of the mechanochemical reaction of poly(methyl methacrylate) and MA as previously reported [14].

Progressive Changes in Radical Concentration and Molecular Weight

Figure **2** shows the progressive changes in radical concentrations in the course of vibratory ball milling of PBzMA with or without MA. In each case the radical concentration gradually increased to a maximum, but after reaching the maximum value, the radical concentration slowly decreased. The radical concentration of the fractured sample of PBzMA with MA, however, was apparently smaller than that of without MA. On the other hand, the decrease rate of molecular weight in the course of the mechanochemical reaction of PBzMA with MA was almost the same that without MA (Figure **3**).

Synthesis of Amphiphilic Blockcopolymer

The fractured samples of PBzMA and MA were reacted with amino-terminated PEG, α-methyl-ω-aminopropoxy polyethylene (MEPA), to synthesis amphiphilic blockcopolymers, PBzMA-MEPA. Polymers possessing larger molecular weight would be produced in this reaction due to the coupling reaction between macromonomers and MEPA. As show in Figure **4**, the molecular weight distribution of PBzMA-MEPA (solid line) apparently shifted to the larger molecular weight region than that of the untreated sample (dotted line), which indicated that the coupling reaction between macromonomer and MEPA successfully proceeded. The number average molecular weight (Mn) of PBzMA-MEPA was about 33,000, and that of the untreated sample about 28,000.

Dynamic Light Scattering Measurement of Polymeric Micelles

Polymeric micelles consisting of PBzMA-MEPA were prepared by dialysis method (see Experimental). The change in light scattering intensity of the dialysis solution was measured. A considerable increase in

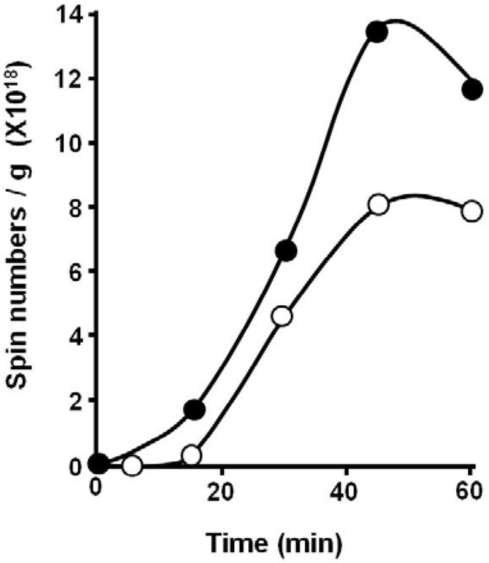

Figure 2: Progressive changes in radical concentrations in the course of vibratory ball milling of PBzMA with or without MA.

●: without MA, ○: with MA.

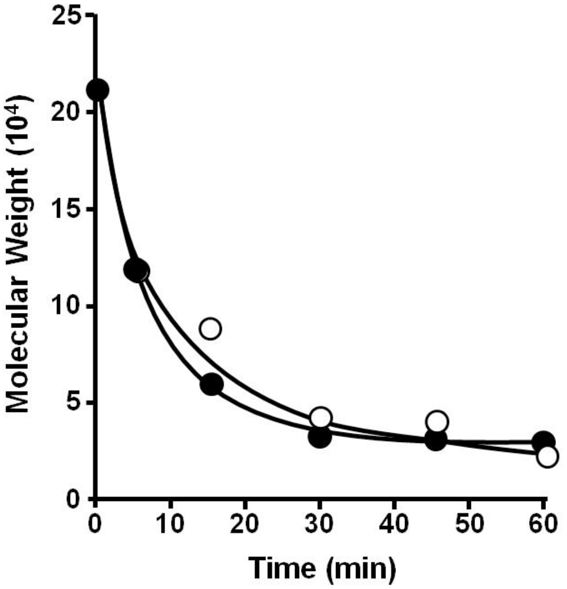

Figure 3: Progressive changes in molecular weight in the course of vibratory ball milling of PBzMA with or without MA.

●: without MA, ○: with MA.

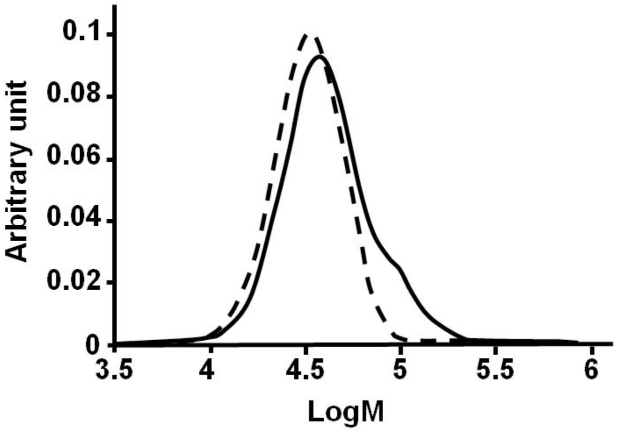

Figure 4: Molecular weight distribution of PBzMA before and after coupling reaction with MEPA.

Solid line: after coupling reaction, dotted line: before.

light scattering intensity was observed after dialysis of PBzMA-MEPA solution, although the solution was visibly transparent. This result suggested that polymeric micelles were formed from the present blockcopolymer.

The diameter distribution of PBzMA-MEPA was measured by dynamic light scattering (DLS) in water, and the results are shown in Figure **5**. PBzMA-MEPA showed a unimodal size distribution, and its mean diameter (number average diameter) was found to be 146 nm.

To obtain the insight on micellar shape, the angular dependence of light scattering from the micelle solution was estimated from the DLS measurements. Figure **6** shows the dependence of the scaled characteristic line width (Γ/K^2) on the scattering angle. The value of Γ/K^2 was almost constant against angle.

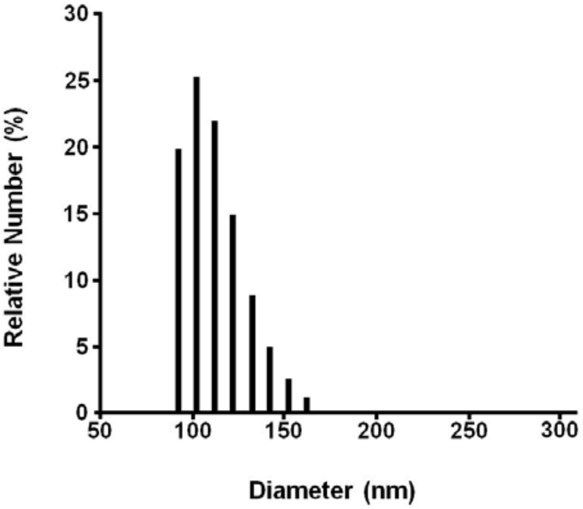

Figure 5: Diameter distribution of PBzMA-MEPA micelle in water.

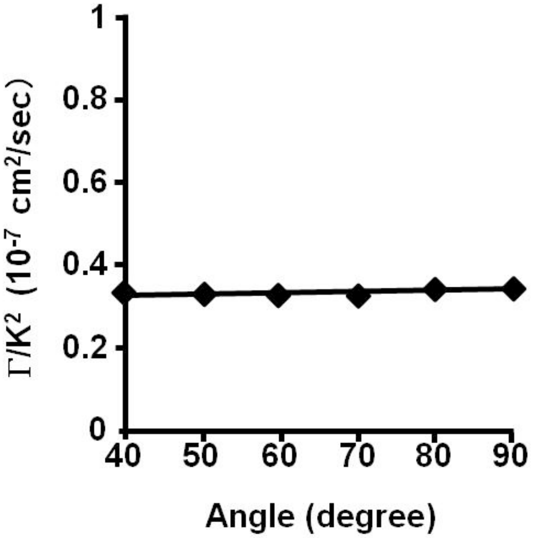

Figure 6: Plots of the scaled average characteristic line width (Γ/K^2) against angle of PBzMA-MEPA micelle.

DISCUSSION

We have synthesized macromonomers by mechanochemical reaction of PBzMA and MA. The ESR spectrum of the fractured sample of PBzMA and MA showed a broad singlet, which was apparently different from the spectrum of PBzMA mechanoradical (Figure **1**). Therefore, PBzMA mechanoradical would react with MA.

The progressive changes in radical concentrations and molecular weights in the course of vibratory ball milling of PBzMA with or without MA were also examined (Figures **2** and **3**). The decrease rate of molecular weight in the course of the mechanochemical reaction of PBzMA with MA was almost the same that without MA (Figure **3**). The changes in molecular weight would correlate to the amount of produced radicals, so that the total amount of produced radicals would be almost the same in both cases. Therefore, the smaller radical concentration of the fractured sample of PBzMA with MA indicated that the radical quenching reaction steeply proceeded in the presence of MA, which could be ascribed to the unstability of MA radical at room temperature.

The amphiphilic blockcopolymer was synthesized with the macromonomer of PBzMA and MEPA. The number average molecular weight of the produced amphiphilic blockcopolymer was larger than that of the macromonomer (Figure **4**). Polymeric micelles were readily prepared from the present amphiphilic blockcopolymer by a dialysis method.

The mean diameter of the micelles measured by dynamic light scattering was about 146 nm (Figure **5**). A narrow diameter distribution was observed in the polymeric micelles obtained. As the molecular weight of amphiphilic block copolymer used was 33,000 g/mol, the mean diameter of unimer might be 1 ~ 2 nm. The mean diameter of PBzMA-MEPA was much larger than that expected for unimer, so that it was suggested that many molecules aggregated to form micelles. Similar results were obtained for other polymeric micelles [2-6, 25].

Depending on the composition of the blockcopolymer, two types of micelles can be distinguished: star and crew-cut. The star micelle is usually made from blockcopolymers in which the corona-forming blocks are much longer than the core-forming blocks, while the crew-cut micelle is made from copolymers in which the core-forming blocks are much longer. It is known that a multitude of different micellar shapes (spheres, cylinders, vesicles, compound vesicles) exists for crew-cut micelles [15-20]. When a unitary solvent, such as water, is used as a selective solvent to form star micelles, spherical micelles are obtained and other micellar shapes not formed within our knowledge [1-10, 17, 18, 21-24]. The PBzMA-MEPA possessed much longer hydrophilic blocks than hydrophobic blocks. Therefore, a spherical micelle would be formed by the aggregation of PBzMA-MEPA. It is known that spherical micelles show no angle dependence of the scaled characteristic line width [26]. As shown in Figure **6**, the value of Γ/K^2 was almost constant against angle, indicating that the polymeric micelles were spherical as expected.

This method can provide macromonomers by the mechanochemical reaction of polymer in the presence of MA, so that it seems to be applicable for a wide variety of polymers. The nature of drug release from polymeric micelles prepared by this method will be the subject of a forthcoming paper.

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