Novel Synthesis of Macromonomers by Mechanochemical Reaction for the Application to Polymeric Micelles

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Abstract: We have presented the first example of the synthesis of macromonomers by mechanochemical reaction of polymethylmethacrylate (PMMA) and maleic anhydride (MA). The mechanochemical reaction of PMMA and MA was carried out by vibratory ball milling under anaerobic condition. The ESR spectrum of the fractured sample of PMMA and MA showed a broad singlet, which was apparently different from the spectrum of PMMA mechanoradical. Therefore, PMMA mechanoradical would react with MA. We underwent the UV-labeling of the fractured samples of PMMA and MA to confirm the formation of macromonomers. The gel permeation chromatograms of UV-labeled compounds derived from this fractured sample showed a broad peak in a polymer region with refractive index detector and UV detector, which indicates that macromonomers bounding MA would be produced. This method seems to be applicable for a wide variety of polymers to synthesize macromonomers possessing MA.

KEYWORDS: Mechanoradical, ESR, vibratory milling, maleic anhydride.

INTRODUCTION

Much attention is now being paid to polymeric micelles as microcontainers for drug delivery systems. Polymeric micelles are fabricated by the self-assembling of amphiphilic block copolymers, and characterized by their unique core-shell architecture, where hydrophobic segments are segregated from the aqueous exterior to form an inner core surrounded by hydrophilic segments. Polymeric micelles have several interesting properties as follows: the ability to solubilize hydrophobic molecules, nanoscale size, good thermodynamic solution stability, the ability to release drugs over an extended period of time, and the slow clearance by the reticuloendothelial system [1-10].

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]. The coupling reaction between different macromonomers can also give a block copolymer.

We have reported a series of studies on mechanochemical solid-state polymerization for the development of polymeric prodrugs and amphiphilic block copolymers for polymeric micelles [14-21]. Mechanochemical reaction is a chemical reaction induced by mechanical energy, such as fracture, compression and so on. It has long been known that the mechanochemical reaction of polymer, synthetic and natural, gives rise to mechanically induced radicals, so-called mechanoradicals, due to the polymer main-chain scission, when the reaction is conducted at a temperature lower than the glass transition temperature (*Tg*) of polymers [22]. On the other hand, maleic anhydride (MA) is a conventional vinyl monomer. In general, radical homopolymerization of MA does not proceed, although its radical copolymerization with various monomers easily occurs. If a polymer could be fractured with MA in the solid-state, the mechanoradical produced would react with MA. The MA radical, however, would not undergo a propagation reaction (polymerization) to be surrounded by MA monomers in the solid-state, so that the macromonomer possessing MA at the end site would be produced. In this paper, we report the synthesis of macromonomers in the solid-state by the mechanochemical reaction of poly(methylmethacrylate) (PMMA) and MA. PMMA is one of the most widely used synthetic polymers. It is also known that only the electron spin resonance (ESR) spectrum of the tertiary end-chain radical is observed at room temperature by the mechanochemical reaction of PMMA [22-26]. We investigated the progressive changes in ESR spectrum observed in the mechanochemical reaction of PMMA

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and MA together with the changes in molecular weight. The formation of macromonomers was also confirmed by the UV-labeling of the fractured sample.

EXPERIMENTAL

Materials

PMMA (M_w = 800,000), purchased from Nacalai Tesque, Inc., was purified repeatedly by dissolving it in $CHCl_3$ and precipitating in excess methanol. It was then dried *in vacuo* at 70 ºC for 5h and screened with a 100 – 140 mesh sieve. MA was purified by sublimation. All other chemicals were of special reagent grade.

Mechanochemical Reaction of PMMA and MA

The mixture of PMMA (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. PMMA (100mg) was also fractured in the same procedure described above.

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 / or a Tunable Absorbance Detector (Waters 486), a gel column (Shodex KD-800M and KD-80M), and a data analyzer (Shimadzu, Chromatopac C-R4A), under the following conditions: elution solvent, tetrahydrofuran (THF); flow rate, 0.7 ml/min; column temperature, 40 ºC. The calibration for the molecular weight determination was carried out with a standard specimen of polystyrene.

UV-labeling of Fractured Sample

The fractured samples were dissolved in the mixture of THF (0.15ml) and DMF (0.1ml), and then potassium carbonate (10mg) was added to this solution. After the reaction mixture was stirred for 2 h at room temperature, solutions of *p*-bromophenacyl bromide (100μl, 400 mmol/l DMF solution) and 18-crown-6-ether (100μl, 1.5 mmol/l DMF solution) were added to the supernatant. This solution was kept at room temperature for 2h, and applied to GPC equipped with RI and UV detectors.

RESULTS AND DISCUSSION

ESR Spectra Observed in Mechanochemical Reaction of PMMA and MA

Figure **1** shows the progressive changes in observed ESR spectra of the fractured samples of PMMA with or without MA by vibratory ball milling. The spectral feature of PMMA fractured without MA (Figure **1a**) remained unchanged at various stages of vibratory milling as reported in a previous paper [18], which is most characterized by five major lines with four shoulders (nine-line spectrum). It is well known that this nine-line spectrum is ascribed to a tertiary radical (single end-chain radical shown in Chart **1**) [22-26]. On the other hand, the ESR spectra of the fractured sample of PMMA with MA at 5-min vibratory milling showed a broad singlet, and the spectral feature gradually changed with the fractured time (Figure **1b**). The peaks indicated with arrows in 30-min vibratory milling were corresponding to the nine-line spectrum ascribed to PMMA mechanoradicals. We separately carried out the mechanochemical reaction of PMMA with succinic anhydride, which is a saturated compound of MA, at various milling times. The ESR spectrum observed in mechanochemical reaction of PMMA with succinic anhydride was a nine-line spectrum at various stages of vibratory milling as well as that of fractured PMMA, and the radical concentration was as almost same as that of fractured PMMA. These results suggest that PMMA mechanoradical might react with the double bond of MA. It is also shown that PMMA mechanoradicals effectively reacted with MA at the earlier stage but not very much at the latter stage in this experimental condition, although there were excess amount of MA at the latter stage to react with PMMA mechanoradical from the point of view of radical concentration (*vide infra*).

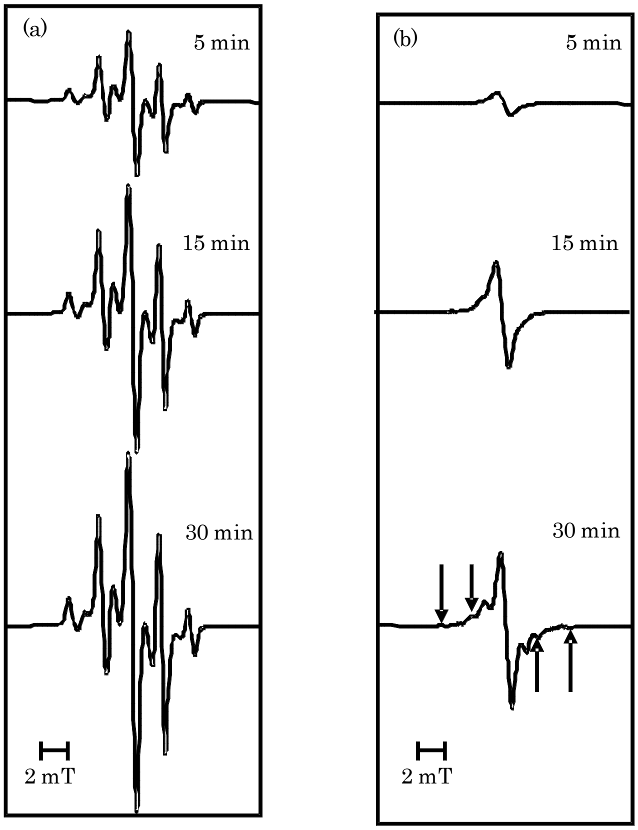
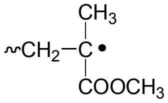


Figure 1: Progressive changes in observed ESR spectra of the fractured samples of PMMA (a) without or (b) with MA by vibratory ball milling.



$$\sim CH_2-\overset{\displaystyle CH_3}{\underset{\displaystyle COOCH_3}{C}}\bullet$$

Chart 1:

It is difficult to assign the radical structure produced by the mechanochemical reaction of PMMA with MA from these data obtained. Fukaya *et al.* observed the ESR spectra of MA radicals produced by γ-irradiation at 77 K [27], which were not a broad singlet and apparently different from those obtained in this experiment. Tion *et al.* reported that MA radicals produced by mechanochemical reaction of copolymer of MMA and MA (poly(MMA-co-MA)) at -55 °C disappeared with spectral changes at 0 °C [28]. Therefore, the radical observed in this experiment might not be MA radical, and could be a transferred radical from MA radical.

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 PMMA 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 PMMA with MA, however, was apparently smaller than that of without MA. On the other hand, the molecular weight at 60-min vibratory ball milling was almost the same in both cases, although the decrease rate of molecular weight in the course of the mechanochemical reaction of PMMA with MA was slightly slower than that of without MA (Figure **3**). The changes in molecular weight would correlate to the amount of radical produced, so that the total amount of radical produced would be almost the same in both cases. Therefore, the radical quenching reaction steeply proceeded in the presence of MA, which could be ascribed to the unstability of MA radical at room temperature.

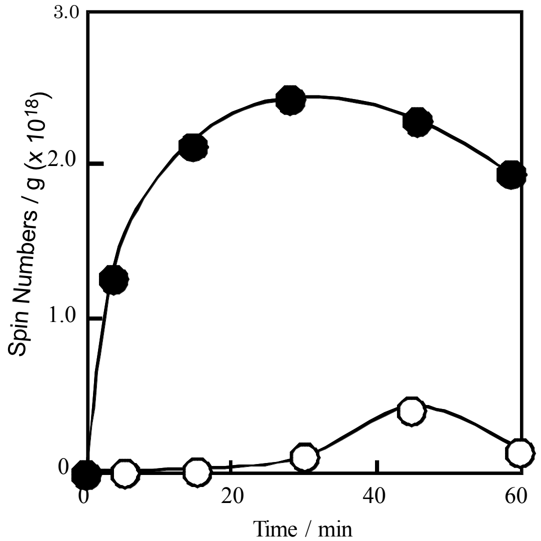


Figure 2: Progressive changes in radical concentrations in the course of vibratory ball milling of PMMA with or without MA. ●: without MA, ○: with MA.

Confirmation of Macromonomers

To confirm the formation of macromonomers, the fractured sample of PMMA and MA was reacted with *p*-bromophenacyl bromide to obtain the UV-labeled compound (Scheme **1**). As a comparison, MA and fractured PMMA were also treated with *p*-bromophenacyl bromide in the same procedure.

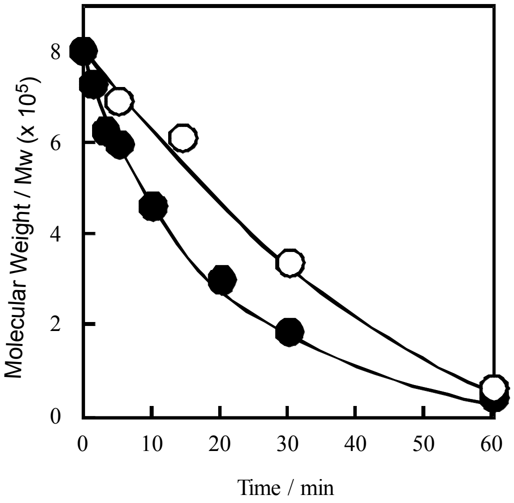


Figure 3: Progressive changes in molecular weight in the course of vibratory ball milling of PMMA with or without MA. ●: without MA, ○: with MA.

Figure **4** shows the gel permeation chromatograms (GPCs) of UV-labeled samples with refractive index (RI) detector and UV detector. The peaks over 30 min are ascribed to the low molecular weight compounds, such as UV-labeled MA, *p*-bromophenacyl bromide and solvent. Although a broad peak around 28 min, which was ascribed to a polymer (Mn = 11,000, Mn: number average molecular weight), was observed with RI detector in the fractured PMMA, no peak was detected with UV detector. Therefore, PMMA did not react with *p*-bromophenacyl bromide. On the other hand, the fractured sample of PMMA and MA showed a broad peak around 28 min with RI and UV detector. These results suggest that the macromonomer possessing MA would be synthesized by the mechanochemical reaction of PMMA and MA.

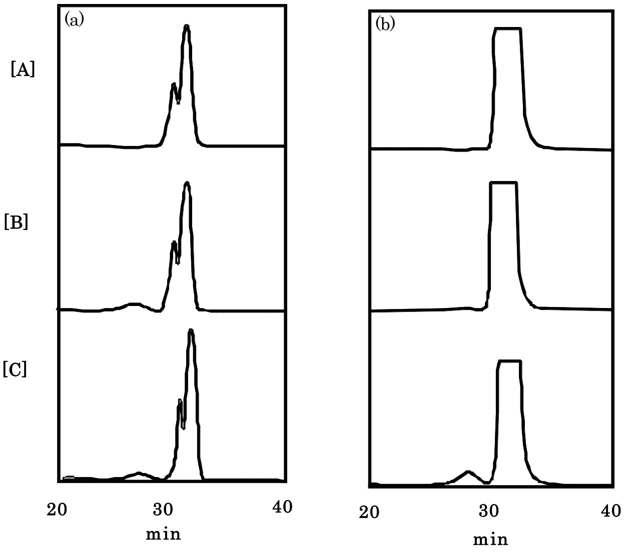
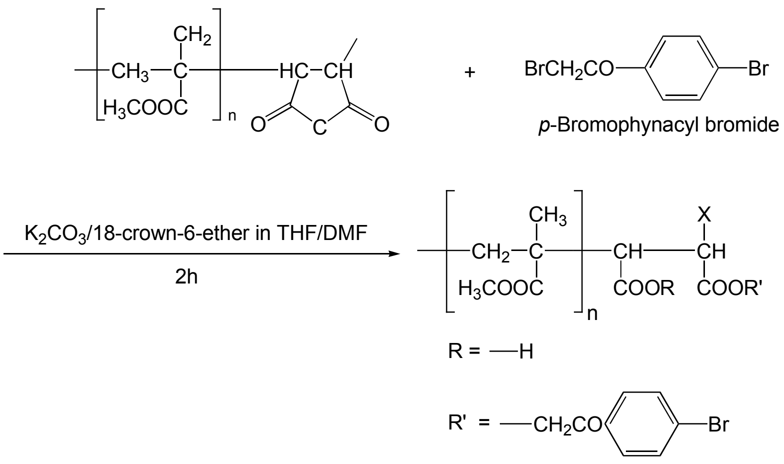


Figure 4: Gel permeation chromatograms (GPCs) of UV-labeled samples with (a) refractive index (RI) detector and (b) UV detector. [A]: MA, [B]: PMMA without MA, [C]: PMMA with MA

The fractured samples of PMMA and MA were also reacted with 1,4-phenylenediamine. Polymers possessing larger molecular weight would be produced in this reaction due to the coupling reaction between macromonomers via 1,4-phenylenediamine. As show in Figure **5**, the molecular weight distribution of the treated sample (solid line) apparently shifted to the larger molecular weight region than that of the untreated sample (dotted line), which indicates that the



Scheme 1:

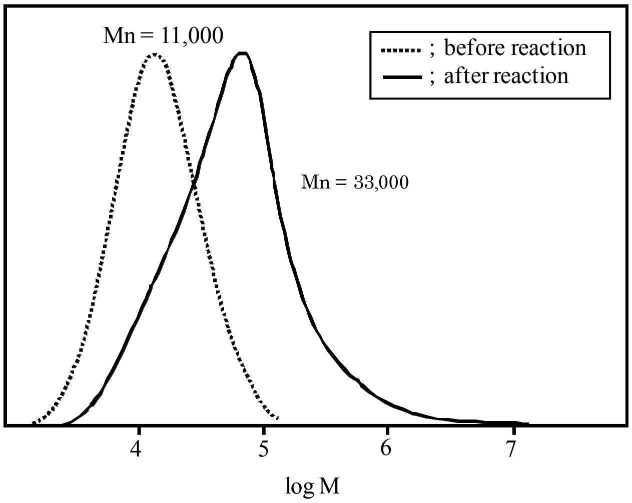


Figure 5: Molecular weight distribution before and after coupling reaction.

coupling reaction between macromonomers proceeded. The number average molecular weight (Mn) of the treated sample was about 33,000, and that of the untreated sample about 11,000. These results suggested the existence of macromonomers possessing MAs at both ends.

CONCLUSION

We have presented the first example of the synthesis of macromonomers by mechanochemical reaction of PMMA and MA. The ESR spectrum of the fractured sample of PMMA and MA showed a broad singlet, which was apparently different from the spectrum of PMMA mechanoradical. Therefore, PMMA mechanoradical would react with MA. The formation of macromonomers was confirmed by the UV-labeling of the fractured samples. The UV-labeled compounds derived from the fractured sample of PMMA and MA showed a broad peak in the polymer region with UV detector, although PMMA was not derived to a UV-labeled compound. In other words, macromonomers tethering MA would be produced. This result is also supported by the coupling reaction between macromonomers via 1,4-phenylenediamine.

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. Polyethylene glycol (PEG) is widely used as a hydrophilic segment of amphiphilic block copolymer applied to polymeric micelle. Recently functionalized PEG, such as amino-terminated PEG, can be purchased. It is hoped that the macromonomer produced by this method can be applied to the synthesis of amphiphilic block copolymer containing PEG segment for polymeric micelle.

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