

Original Contributions

Production of submicron-size monodisperse polymer particles having aldehyde groups by seeded aldol condensation polymerization*)

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Abstract: Submicron-size monodisperse polystyrene/polyglutaraldehyde composite particles having aldehyde groups at the surfaces were produced by seeded aldol condensation polymerization of glutaraldehyde in the presence of polystyrene particles prepared by emulsifier-free emulsion polymerization. This technique is expected to be useful for the production of size-controlled polymer particles having aldehyde groups.

Key words: Polymer emulsion – seeded polymerization – glutaraldehyde – aldol condensation polymerization – aldehyde group

Introduction

Recently, there has been much interest in applying polymer particles as carriers of proteins for bioreactors, biosensors and bioseparators. We have also been trying to immobilize proteins such as enzymes and antibodies onto submicron-size polymer particles having functional groups at the surfaces [1–3]. For this purpose, aldehyde groups are useful for the covalent immobilization of bioreactive proteins under mild conditions [4–6]. Polymer particles having aldehyde groups have been produced by emulsion homopolymerization [7] and copolymerization of acrolein [8]. In the copolymerization, the concentration of aldehyde groups at particle surfaces can be controlled by changing monomer composition. However, the particle size is also changed with the monomer composition. It is quite difficult to control the particle size and the aldehyde concentration at the same time. This often causes a serious problem in their applications.

In this article, in order to solve this problem, we will propose the seeded aldol condensation polymerization technique of glutaraldehyde in the presence of polymer particles of which size and monodispersity can be preliminarily controlled.

Experimental

Materials

Styrene (S) was distilled under reduced pressure in a nitrogen atmosphere. Glutaraldehyde (GLA) (25% aqueous soln.) was of analytical grade and used as received. Potassium persulfate (KPS) of reagent grade was purified by recrystallization. Deionized water was distilled with a Pyrex distillator. Other chemicals were of analytical grade.

Preparation of seed particles

Emulsifier-free emulsion polymerization of S was carried out at 70 °C for 48 h under a nitrogen atmosphere at a stirring rate of 100 rpm in a round-bottomed four-necked flask under the conditions listed in Table 1. The prepared polystyrene (PS) particles were washed twice with water.

Seeded aldol condensation polymerization

PS/polyglutaraldehyde (PGLA) composite particles were produced by seeded aldol condensation polymerization of GLA in the presence of PS seed

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Table 1. Preparation of PS seed particles by emulsifier-free emulsion polymerization^{a)}

Ingredient	
Styrene (g)	160
KPS (mg)	1280
Water (g)	1440

^{a)} 70 °C, 48 h, N₂.

Table 2. Production of PS/PGLA composite particles by seeded aldol condensation polymerization^{a)} in the presence of PS seed particles

Ingredient	
PS seed emulsion ^{b)} (ml)	100
GLA ^{c)} (ml)	20

^{a)} 30 °C, 6 h, pH 11 (with NaOH).

^{b)} Solid, 100 g/l.

^{c)} 25% aqueous solution.

particles at 30 °C for 6 h at pH 11.0 under the conditions listed in Table 2. Prior to the polymerization, the mixture of PS seed emulsion and GLA was stirred at pH 3 for about 30 min to stabilize the temperature and to make GLA absorb into PS seed particles. The conversion of GLA was measured by gas chromatography (Yanagimoto MFG. Co., Ltd., G 2800) with nitrogen as a carrier gas. Chromosorb W AW-DMCS (stationary phase, 25% polyethyleneglycol) was used as the column packing. The temperatures of the gas chromatographic measurement were: injector, 200 °C; column, 120 °C; detector, 200 °C. After the polymerization the produced PS/PGLA composite particles were washed three times with water using centrifuge at 13,000 g for 15 min.

Determination of aldehyde groups

The amount of aldehyde groups at the particle surfaces was determined using the 2.5 pH method proposed by Smith et al. [9], as follows. They measured the amount of aldehyde groups in water/alcohol solution. We preliminarily confirmed that the method can be applied for the determination of the amount of aldehyde groups, using known concentrations of GLA aqueous solu-

tions. The pH values of the purified composite emulsion (solid content, ca 6%) (30 ml) and 1 N hydroxylamine hydrochloride aqueous solution (30 ml) were both adjusted to 2.50 with 1 N HCl solution, and they were mixed. The mixture was stirred for 1 h at room temperature. The pH value of the mixture fell below 2.50 due to HCl liberated from the reaction between aldehyde groups and hydroxylamine hydrochlorides. The potentiometric titration was carried out with 0.05 N NaOH solution. The amount of aldehyde groups was calculated from the amount of NaOH required to adjust the pH value to 2.50.

Transmission electron microscopic (TEM) observation

A Nihon Denshi JEM-200CX was used for TEM observation. The polymer emulsions were diluted down to about 50 ppm, and a drop was placed onto a film-coated grid and allowed to air dry at room temperature in a desiccator. TEM examination was carried out with great care to minimize beam damage.

FT-IR measurement

Infrared spectroscopy (Shimadzu FT-IR 4200) was used for the detection of aldehyde groups in the polymer particles. The infrared spectra of the samples were obtained by a KBr method.

X-ray photoelectron spectroscopy (XPS) measurement

The PS/PGLA composite particles after reaction with hydroxylamine hydrochloride were washed three times with water and powdered by freeze drying. The particles dried at room temperature under reduced pressure by continuous operation of diffusion pump just before XPS measurement were spread on an indium plate with a spatula.

Surface analysis by XPS was carried out with a Kratos XSAM-800 apparatus. The pressure in the measurement chamber was ca 8×10^{-10} Torr. MgK α radiation was used from an x-ray tube operated at 12.5 kV and 12 mA. A Digital Equipment Corporation DS-800 computer system was used for spectrometer control and data handling.

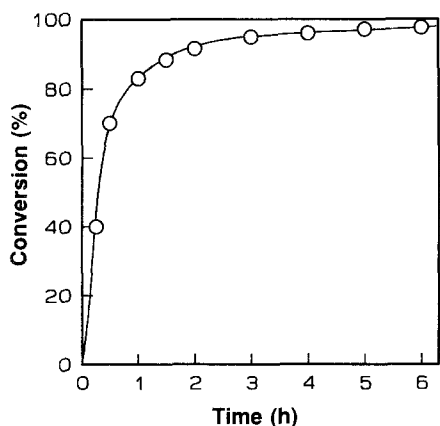


Fig. 1. Time-conversion curve of seeded aldol condensation polymerization of GLA at 30 °C in the presence of PS particles at pH 11.0

Results and discussion

Figure 1 shows the time-conversion curve of aldol condensation polymerization of GLA at pH 11.0 in the presence of PS seed particles at 30 °C. The polymerization proceeded smoothly and almost completed within several hours.

Figure 2 shows TEM photographs of PS seed particles (a) and unpurified PS/PGLA composite particles (b). In the latter photograph, the generation of new PGLA particles was not observed. PS/PGLA composite particles were highly monodisperse because of the monodispersity of PS seed particles. As shown in the recipe listed in Table 2, if all of GLA polymerize and accumulate at the surface of PS particles, the particle size must increase

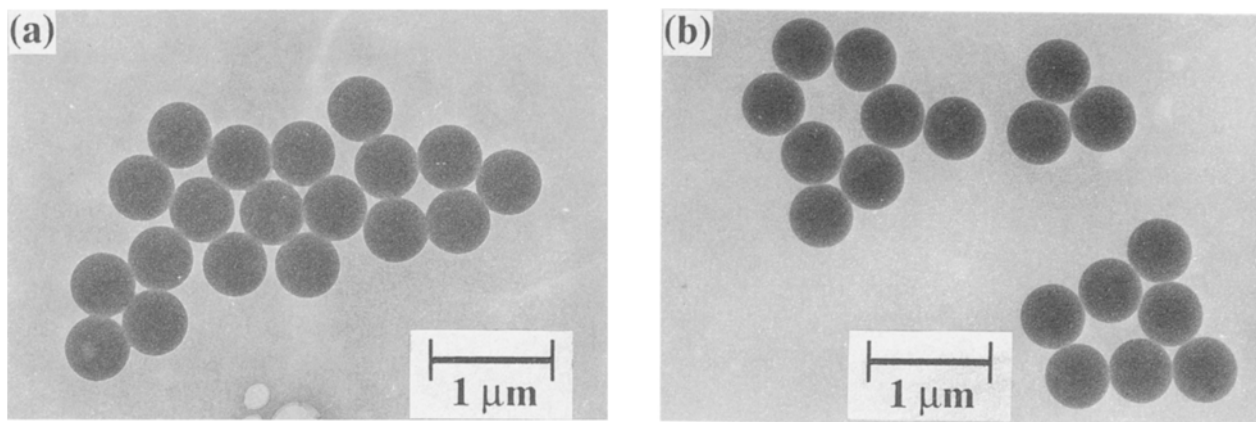


Fig. 2. TEM photographs of PS seed particles (a) and unpurified PS/PGLA composite particles (b)

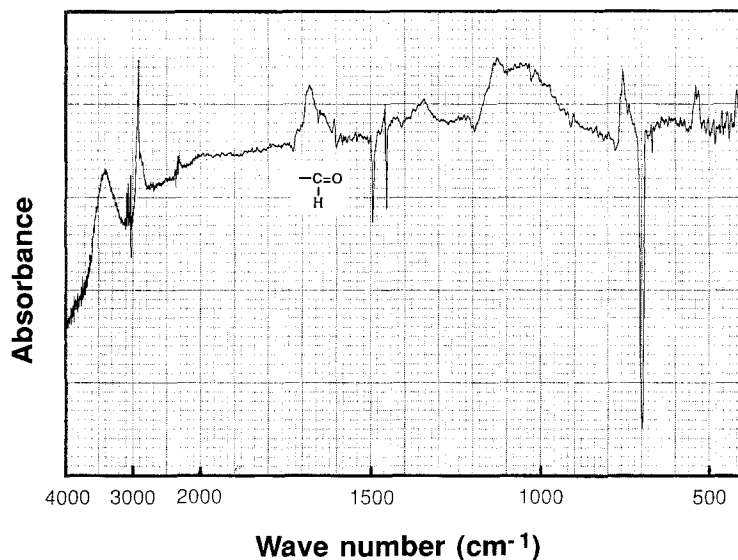


Fig. 3. Differential spectrum obtained by subtracting the spectrum of PS seed from that of the purified PS/PGLA composite particles

from 0.5 to 0.6 μm . However, the increase could not be acknowledged. This suggests that the polymerization of GLA proceeds mainly in the medium and all of PGLA produced does not accumulate on the PS particles.

Figure 3 shows the differential spectrum obtained by subtracting the absorbance contribution of PS seed from the spectrum of purified PS/PGLA composite particles. The spectrum indicates that PS/PGLA composite particles have aldehyde groups (1680 cm^{-1} : stretching of $\text{C}=\text{O}$) and the crosslinked structure (1100 cm^{-1} : stretching of $\text{C}-\text{O}$ of hydroxyl group and the ether linkage in

hemiacetal formed between aldehyde group and hydroxyl group in PGLA) (see Eq. 1). Besides the crosslinked structure due to the ether linkage, PGLA in the composite particles may form a cross-linked structure due to aldol condensation.

As shown in Fig. 4, the peaks due to the aldehyde groups were disappeared after the reaction with hydroxylamine hydrochloride (see Eq. 2). Here, oxime would be formed by the reaction between aldehyde groups and hydroxylamine hydrochloride, but its absorbance was not clear.

Figure 5 shows a part of XPS spectrum for purified PS/PGLA composite particles after the reaction

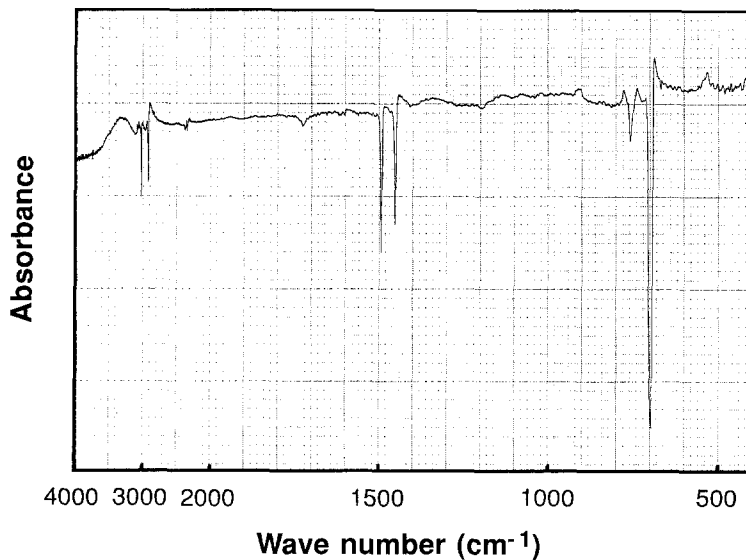
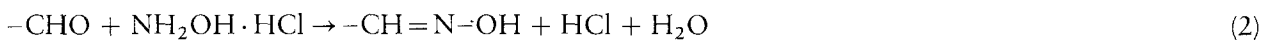
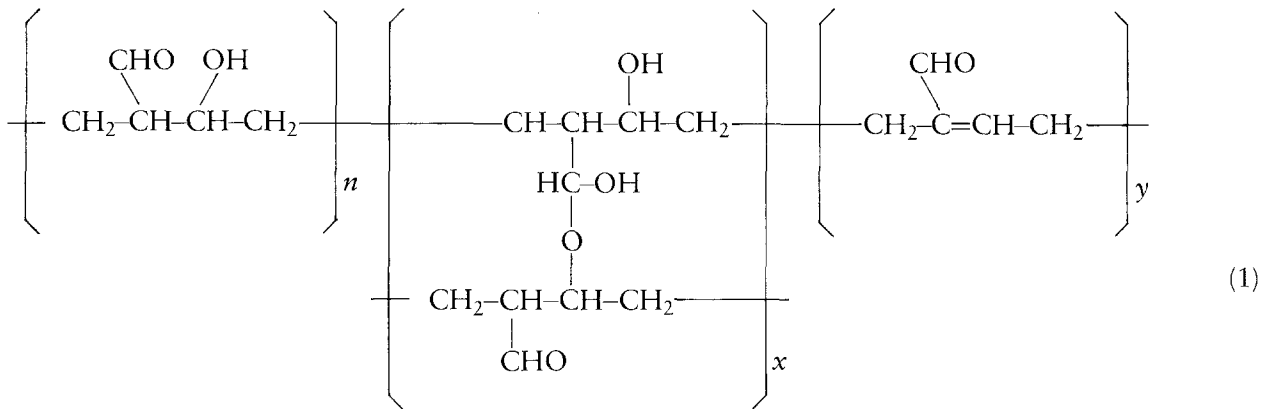


Fig. 4. Differential spectrum obtained by subtracting the spectrum of PS seed from that of the purified PS/PGLA composite particles after reaction with hydroxylamine hydrochloride

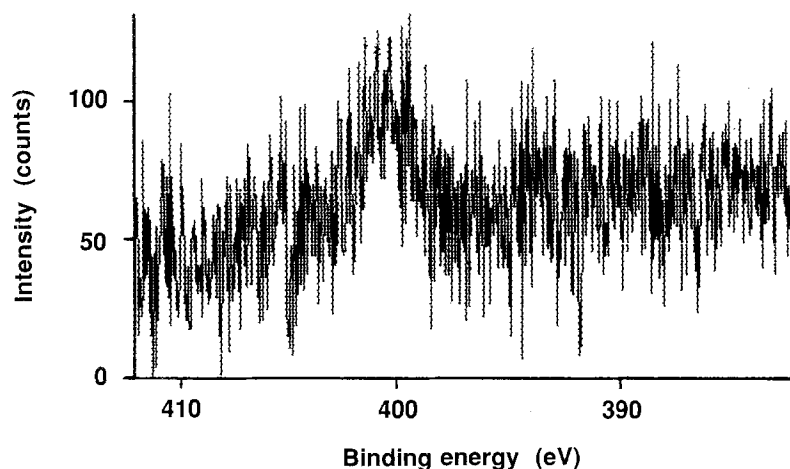


Fig. 5. XPS N_{1s} spectrum for the purified PS/PGLA composite particles after reaction with hydroxylamine hydrochloride

with hydroxylamine hydrochloride. The peak due to N_{1s} was observed at ca 400 eV of binding energy. This indicates that oxime was introduced onto PS/PGLA composite particles by the reaction.

The amount of aldehyde groups in the purified PS/PGLA composite particles by serum replacement with water three times was determined to be 4.8×10^{-6} mol/m². Its value was little decreased by repeating the serum replacement an additional four times. This also suggests that PGLA at the purified composite particles has a crosslinked structure. The apparent occupied area of one aldehyde group calculated from the value was 34 Å². This indicates that the PS/PGLA composite particles had a sufficient amount of aldehyde groups at the surfaces as reactive groups for the covalent immobilization of proteins.

From these results, it is proposed that size-controlled monodisperse polymer particles having aldehyde groups can be produced by seeded aldol condensation polymerization of GLA in the presence of monodisperse polymer particles, of which the size and monodispersity are preliminarily controlled.

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