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Preparation of Poly(γ-benzyl-L-glutamate) Microspheres by the Precipitation Polymerization

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ABSTRACT: Poly(γ-benzyl-L-glutamate) microspheres were prepared from γ-benzyl-L-glutamate *N*-carboxy-α-amino acid by precipitation polymerization in polystyrene solution. The effect of the polystyrene concentration, characteristics of solvents and weight ratio of polystyrene/monomer and monomer/initiator was investigated. The particle size of the microspheres was decreased with increasing the concentration of polystyrene and increased with increasing the weight ratio of polystyrene/monomer. It was found that the microspheres are distributed and uniformly spherical particles, but are slightly elongated shape. Particle size and average molecular weight of the microspheres were distributed in the region of 0.75—1.48 μm, 2.7—3.4 × 10^4 respectively.

KEY WORDS Microsphere / Poly(amino acid) / Poly(γ-benzyl-L-glutamate) / Poly(L-glutamic acid) / Precipitation Polymerization /

Since Leuch[1] has presented synthetic method of peptides based on synthetic amino acids, basic research and its study on application[2–5] of synthetic peptide and proteins as biomaterials are actively progressing. As it is possible to mass production, developed the synthesis technology of protein, There are various application fields as functional material such as food, medicine etc. More systematic study is demanded because poly(amino acid)s are excellent biodegradable and biocompatible polymer and it is used widely in the field of biomedical application.[6–8]

The controlled release systems designed with a biodegradable poly(amino acid)s are desirable because they do not need to be retrieved when drug delivery is complete. Poly(glutamic acid) microspheres have the application as a biodegradable drug carrier having reactive carboxyl groups, which is able to attach drugs and targetting moieties. Many biodegradable polymers such as gelatin, albumine, and aliphatic polyesters have been investigated as matrices of the microsphere.[9–11] In general, poly(amino acid) microspheres cannot prepare by suspension and emulsion polymerization but these can be obtained by solvent evaporation method.[8] In solvent evaporation system, it is inevitable the pollution of particle surface by the addition of stabilizer.

In this study, a newly developed method for obtaining small and pure microspheres has been proposed. For the purpose of the microspheres synthesis by the newly suggested method, γ-benzyl-L-glutamate *N*-carboxy-α-amino acid and initiator. Poly(γ-benzyl-L-glutamate) microspheres were prepared by precipitation polymerization method[12,13] which can obtain at high yield and controll the particle size. The effect of concentration, of polystyrene solution characteristic of solvents, weight ratio of polystyrene/monomer, and monomer/ initiator on the particle size of the microspheres were investigated.

EXPERIMENTAL

Materials

γ-Benzyl-L-glutamate (γ-BLG), Triphosgen and polystyrene (PS, M_w: 108000, M_w/M_n: 1.51) were used without further purification. Ethyl acetate (EA), tetrahydrofuran (THF) were dried over CaH_2 and distilled before use. Triethylamine initiator was refluxed with sodium for 8 h and distilled from sodium. Acetone, diethylether, and *n*-hexane were purified according to the method conventional.

Preparation of Poly(γ-benzyl-L-glutamate) Microspheres

γ-Benzyl-L-glutamate *N*-carboxy-α-amino acid (γ-BLG NCA) was obtained by modified phosgen method.[14] Poly(γ-benzyl-L-glutamate, P(γ-BLG)) microspheres were prepared from γ-BLG NCA by precipitation polymerization in polystyrene solution. The formulation of P(γ-BLG) microsphere preparation was listed in Table I.

Given amount of γ-BLG NCA dissolved in ethyl acetate or ethyl acetate/aceton was added into highly swollen polystyrene by ethyl acetate. Polymerization was carried out by adding 5×10^{-3} mol L^{-1} of triethylamine initiator in shaking bath maintained at 30°C for 3 day. In order to remove polystyrene, the reaction mixture was dissolved in excess THF, and then the microspheres was collected by centrifugation. This procedure was repeated over several times until disappear the characteristic absorption peaks by polystyrene at IR spectrum.

Characterization of the Microspheres

The shape and size distribution of the microspheres were observed by scanning electron micrography (SEM, Hitachi S-2500C). The microspheres were mounted and sputter coated with gold using a sputter coater. Particle size distribution of the microspheres was estimated from average size of 150 particles. The intrinsic viscosity of P(γ-BLG) was determined in dichloroacetic acid at 25°C using Ubbhelod viscometer and the average molecular

Table I. Preparation of P(γ-BLG) microspheres in polystyrene solution

Run No.	PS	Solvents EA/acetone	Weight ratio		Particle size	Conversion
	wt%	v/v%	PS/M	M/I	μm	%
A-1	20	100/0	4	1000	Aggregated	95.2±0.5
A-2	30				Aggregated	90.3±0.2
A-3	40				0.75—1.20	92.3±0.3
A-4	50				0.45—0.86	78.6±0.5
B-1	40	100/0	4	1000	0.75—1.20	92.3±0.3
B-2		90/10			0.78—1.23	86.7±0.5
B-3		80/20			0.87—1.48	87.2±0.3
B-4		70/30			Aggregated	93.5±0.4
C-1	40	100/0	2	1000	0.58—1.10	67.8±0.3
C-2			3		0.65—1.20	80.6±0.4
C-3			4		0.75—1.20	92.3±0.3
C-4			5		0.97—1.62	90.3±0.4
D-1	40	100/0	4	50	1.10—1.56	94.2±0.2
D-2				100	0.95—1.42	89.2±0.3
D-3				300	0.83—1.29	85.4±0.5
D-4				1000	0.75—1.20	92.3±0.3

PS, polystyrene; M, monomer; I, initiator; EA, ethyl acetate.

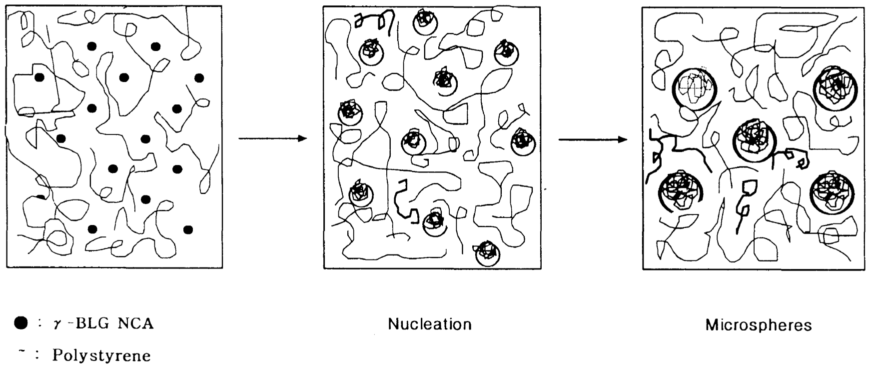

Figure 1. Schematic diagram of the microsphere formation.

weight was estimated by Doty equation.[15]

$$[\eta]=2.78\times10^{-5}M_n{}^{0.67}$$

The molecular weight distribution of the microspheres was determined by gel permeation chromatography (GPC, Waters 750). The instrument consisted of two columns (HGS-20, HSG-50S). The sample solution in *N*,*N*-dimethylformamide (DMF) was eluted at a flow rate of 1 mL min^{-1} and the columns were calibrated by using polystyrene standard.

RESULTS AND DISCUSSION

P(γ-BLG) microspheres were prepared from γ-BLG NCA by the precipitation polymerization in polystyrene solution. From the FT-IR spectra, it was confirmed that γ-BLG NCA was synthesized by the appearance of the absorption peaks at 1820, 1860 cm^{-1} which is assigned to the anhydride linkage. And also, P(γ-BLG) was synthesized by the appearance of the peaks at 1660, 1545 cm^{-1} which is assigned to amide I and amide II bond, respectively.

In the preparation of P(γ-BLG)microspheres, γ-BLG NCA monomer forms a nucleus in the highly swollen polystyrene chain. The swelling solvent used in a good solvent for the polystyrene and a non-solvent for the P(γ-BLG). As the space of polymerization is restricted by polystyrene chain, small compact microspheres will be formed without inter-molecular reaction between each particles. Figure 1 shows a schematic diagram of the microspheres formation suggested.

It was observed the particle shape and size of the microspheres by scanning electron micrography (SEM). The micrographs of the microspheres prepared by precipitation polymerization in ethyl acetate is shown in Figure 2. It could be seen that the particles are spherical but are not uniform shape. Figure 2 shows that the particle size distribution is in the range of 0.75—1.20 μm and average particle size is around 1.0 μm.

In the precipitation polymerization system, the influence of solvents is very important. In this study, we decided to select ethyl acetate which is good solvent for monomer (γ-BLG NCA) and poor solvent for polymer [P(γ-BLG)]. For the increasing of hydrophilicity, volume ratios of acetone to ethyl acetate were changed. The preparation of the microspheres in selected solvents was summarized in Table II.

The microspheres with particle size of 0.45—1.62 μm

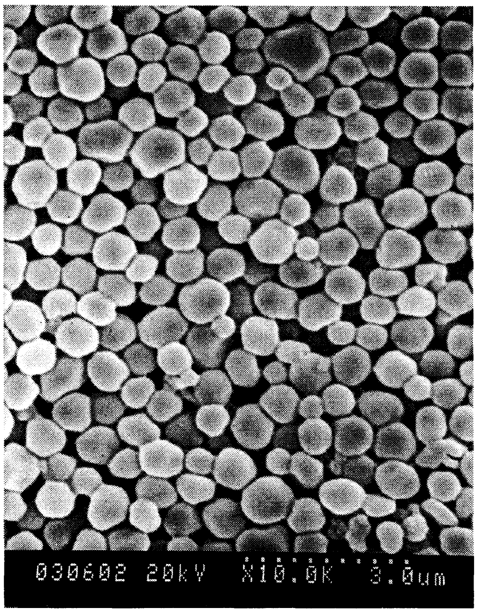

Figure 2. Scanning electron micrograph of P(γ-BLG) microspheres prepred in polystyrene solution (Run No. B-1).

Table II. Molecular weights of P(γ-BLG) microspheres prepared in EA/acetone of the different volume ratio

Run No.	Solvents EA/acetone	$[\eta]^a$	Molecular weight^b		M_w/M_n
	v/v%	dL g^{-1}	M_n ($\times 10^4$)	M_w ($\times 10^4$)	
B-1	100/0	1.6	1.4	2.7	1.9
B-2	90/10	2.1	1.6	2.9	1.8
B-3	80/20	2.5	2.3	3.4	1.4
B-4	70/30	3.5	2.9	5.6	1.9

[a] Intrinsic viscosities were determined in DMF at 30°C. [b] Molecular weights were determined by GPC.

were obtained and in high yield of 67.8—92.3% in this polymerization system. It is shown that the particle size depend on the solvent and it increases with increasing the volume ratio of acetone to ethyl acetate. When the volume ratio of acetone was more than 30% (v/v), the particles were aggregated. This result is thought for coagulation which is caused by interaction between polymer particles with increasing of the polarity of the medium.

The concentration of polystyrene solution was changed in the range of 20—50 wt% in order to observe the effect of the concentration of polystyrene solution on the particle size of the microspheres. The particle size distribution of microspheres prepared according to the concentration of polystyrene solution is shown in Figure 3. The particles were not formed in the polystyrene solution of 20 wt% and 30 wt% but were formed in the concentration of 40 wt% and 50 wt%. The particle size of the microspheres shows tendency to reduce according to increasing of the concentration of polystyrene solution. The restriction of the intermolecular reaction of the microspheres is a very promising and effective method for obtaining small compact pure microspheres. The effect of the weight ratio of PS/M on particle size of the microspheres is shown in Figure 4. The particle size of

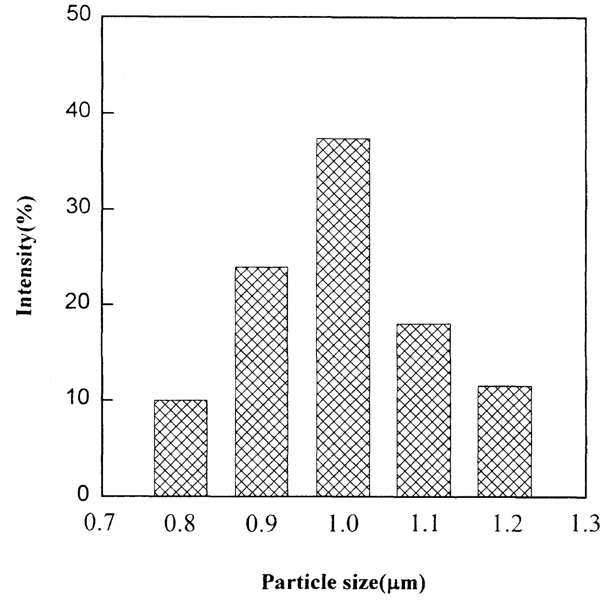

Figure 3. Particle size distribution of P(γ-BLG) microspheres prepared in polystyrene solution (Run No. B-1).

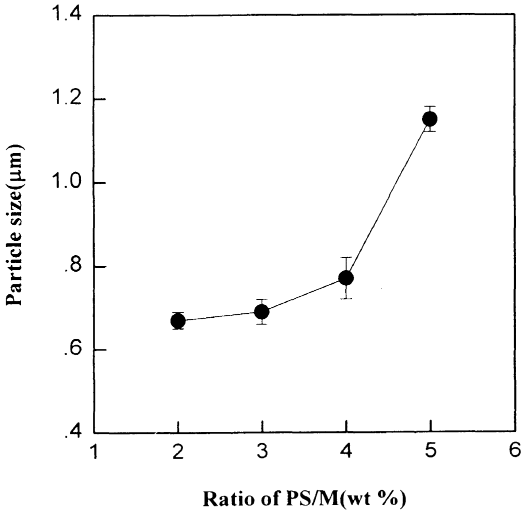

Figure 4. Effect of the weight ratio of PS/M on particle size of P(γ-BLG) microspheres.

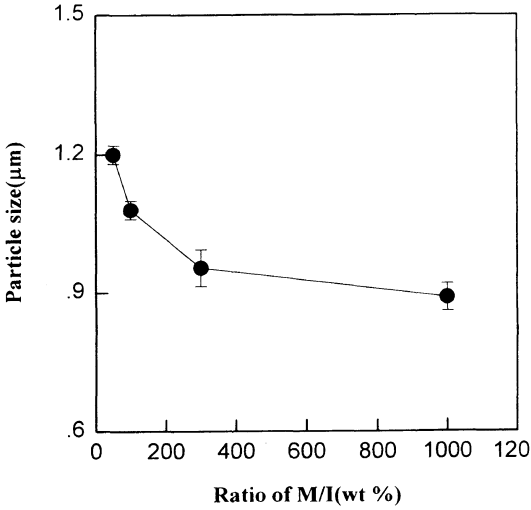

Figure 5. Effect of the weight ratio of M/I on particle size of P(γ-BLG) microspheres.

microspheres was increased significantly with increasing the weight ratio of PS/M. This is indicated that inter- and intra-molecular reaction increase relatively according to increasing of the weight ratio of PS/M.

The particle size of microspheres prepared according to changing the weight ratio of M/I is shown in Figure 5. In the weight ratio of M/I from 50 to 100, the particle size of microspheres was changed significantly and in the weight ratio of that from 100 to 1000, it almost was not changed.

CONCLUSION

P(γ-BLG)microspheres were prepared by precipitation polymerization in polystyrene solution. In this polymerization system, it was selected ethyl acetate which is good solvent for the monomer and poor solvent for polymer. The microspheres were obtained comparatively high yield of 86.7—93.5% with particle size of 0.75—1.48 μm and average molecular weight of $2.9—3.4 \times 10^4$. The particle size of microspheres was increased with increasing the volume ratio of acetone to ethyl acetate and decreased with increasing of the polystyrene concentration. The particle size of those also increased significantly in the weight ratio of polystyrene to monomer from 3 to 5 and almost did not change in the weight ratio of monomer to initiator from 100 to 1000.

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