Colloid Polym Sci (2007) 285:1487–1493
DOI 10.1007/s00396-007-1713-x

ORIGINAL CONTRIBUTION

Synthesis of monodisperse polystyrene microspheres by dispersion polymerization using sodium polyaspartate

Takuro Nakashima · Yuri Yamada · Hidekazu Yoshizawa

Received: 20 February 2007 / Revised: 29 May 2007 / Accepted: 3 June 2007 / Published online: 26 July 2007

Abstract We prepared monodisperse polystyrene microspheres by dispersion polymerization using sodium polyaspartate (PAspNa) as a dispersion stabilizer in an ethanol/water medium. The influence of reaction parameters, i.e., the volume fraction of ethanol in the medium, stabilizer concentration, and the monomer concentration, on the average diameter of the prepared polystyrene microspheres and its distribution were investigated. Polystyrene microspheres were successfully prepared, and the average diameter of the prepared monodisperse polystyrene microspheres was controlled by adjusting the reaction parameters. The zeta potential of the microspheres and the time course of conversion, the particle diameter and its distribution, and particle numbers were also examined. It was found that PAspNa as a dispersion stabilizer provides an environmentally benign process for the preparation of monodisperse polymer microspheres by dispersion polymerization.

Keywords Sodium polyaspartate · Dispersion polymerization · Monodisperse particle · Biodegradable polymer · Amino acid

T. Nakashima · Y. Yamada · H. Yoshizawa (✉)
Department of Material and Energy Science,
Graduate School of Environmental Science
Okayama University,
3-1-1 Tsushima-Naka,
Okayama 700-8530, Japan
e-mail: yhide@cc.okayama-u.ac.jp

Introduction

Monodisperse polymer microspheres have been applied to standard particles for calibrating instruments, spacers of liquid-crystal display panels, carrier particles for liquid chromatography, and particles for biomedical analyses. Dispersion polymerization has been widely used for the preparation of monodisperse polymer microspheres with diameters ranging from approx. 0.1 up to approx. 15 μm [1, 2]. In dispersion polymerization, the microspheres are formed in a reaction mixture that is initially homogeneous in the presence of a suitable polymer dispersion stabilizer, such as polyacrylic acid (PAA) or poly(*N*-vinylpyrrolidone) (PVP) [3, 4]. However, these stabilizers are not biodegradable and difficult to recover. They also contaminate the polymer microsphere surface, and wastewater containing them requires subsequent treatment for their removal.

Sodium polyaspartate (PAspNa) is a typical hydrophilic biodegradable polymers and has a chemical structure similar to that of PAA. PAspNa is synthesized by polycondensation of L-aspartic acid (L-Asp) [5]. It has biocompatibility and therefore can be used in medicines, cosmetics, and food. It is also easy to introduce various side chains by aminolysis with various amines in the synthesis process to design new polymers having more functions.

In this work, we studied the dispersion polymerization of styrene with PAspNa as a dispersion stabilizer. This paper describes the effects of different reaction parameters such as the volume fraction of ethanol (EtOH) in the medium, stabilizer concentration, and monomer concentration on the average diameter and coefficient of variation (CV) of the prepared polystyrene microspheres. The zeta potential of prepared polystyrene microspheres and the time course of

conversion, particle diameter and its distribution, and particle numbers were also investigated.

Experimental

Materials

All chemicals were purchased from Wako Pure Chemical Industry. Styrene (99%, with 3.0×10^{-3}% 4-*tert*-butylpyrocatechol stabilizer) was distilled under reduced pressure in a nitrogen atmosphere to remove the inhibitor. 2, 2′-*azobis*(isobutyronitrile) (AIBN) was purified by recrystallization from methanol. The water used as a polymerization solvent was purified with a Millipore Milli-Q water purification system.

Synthesis of polysuccinimide

L-Asp was mixed with phosphoric acid under the conditions listed in Table 1. The mixture was heated for 8 h under reduced pressure in an oil bath at 453 K. The product was dissolved in *N*,*N*-dimethylformamide (DMF), and the solution was poured into the purified water. The precipitate polysuccinimide (PSI) was filtered, rinsed with water until the suspension neutral pH was reached, and dried in an oven in vacuo at 333 K. Then, the molecular weights of the PSI were determined by gel permeation chromatography (GPC) measurement using DMF as a solvent. For the GPC measurement, the apparatus and the conditions used for the GPC measurement were as follows. The apparatus for the GPC measurement was HLC-8120GPC (TOSOH). The columns used for the measurement were guard column G0003, Super AWM-H ×2 and Super Aw 2500 (TOSOH). The eluent was DMF dissolving 0.01 mol/L lithium bromide. The flow rate of the eluent was 0.6 mL/min. The temperature of the columns was 313 K. The detector for the measurement was the refractive index. The determined molecular weight of PSI was 4.25×10^4. The reference material for the measurement was polystyrene. ^{1}H magnetic resonance imaging (MRI) spectra were measured with a JEOL FT NMR System JMN-AL300 (Scheme 1).

Table 1 Preparation of polysuccinimide by polycondensation

L-Asp (g)	Phosphoric acid (g)	Reaction temp (K)	Reaction time (h)	Mw of PSI[a]	Mw of PAspNa[b]
46.6	19.8	453	8	42,500	60,000

[a] Determined by DMF-GPC
[b] Calculated from Mw of PSI

Synthesis of sodium polyaspartate

The synthesized PSI was dispersed in the purified water, and an aqueous sodium hydroxide solution was added in drops so as not to exceed pH 10 of the solution. The aqueous solution was then neutralized and concentrated under reduced pressure at 313 K. The concentrated solution was precipitated into methanol, and white PAspNa powder was recovered by filtration [6]. The molecular weight of PAspNa was 6.0×10^4 calculated from the molecular weight of PSI. ^{1}H MRI spectra were measured with a JEOL FT NMR System JMN-AL300 (Scheme 1).

Microsphere preparation

Polystyrene microspheres were prepared by dispersion polymerization under the conditions listed in Table 2. Prepared microspheres were observed with a HITACHI S-3500N scanning electron microscope (SEM), and the average diameter and CV were analyzed by WinRoof (Mitani-corp, ver. 3.53).

Time course of conversion, particle diameter and its distribution, and particle numbers

A small amount of PAspNa was withdrawn at different polymerization intervals. The samples were dissolved in methanol with a small amount of 4-*tert*-butylcatechol to terminate the polymerization. The concentrations of residual styrene monomer dissolved in methanol solution were determined by high-performance liquid chromatography to calculate the conversion. The samples were also measured with the SEM to determine the particle diameters of the prepared microspheres at different polymerization intervals. Particle numbers were calculated from the conversion and particle diameters.

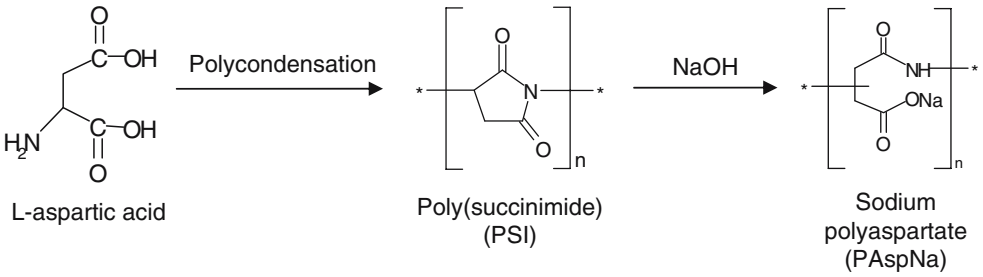
Results and discussion

Characterization of synthesized PAspNa

The formation of synthesized PSI was characterized by ^{1}H MRI. The two major resonances corresponding to the methine and methylene protons of the succinimide unit were observed in the ^{1}H MRI spectrum at δ=2.7 and 3.2 ppm and δ=5.3 ppm, respectively [7].

The formation of synthesized PAspNa was characterized by ^{1}H MRI. The resonances at δ=4.5 and 4.7 ppm and δ= 2.5–2.9 ppm were assigned to the methine and methylene protons of the aspartic acid unit, respectively. The two chemical shift values of the methine groups show the different ring-opening manner, i.e., α- and β-openings. The

Scheme 1 Synthesis of PSI and PAspNa



^{1}H MRI spectra indicate that the β-opening predominantly occurred rather than the α-opening [7].

Kinetics of dispersion polymerization of styrene with PAspNa

Figure 1 shows a SEM image of the reaction products synthesized with PAspNa at an EtOH volume fraction of 60 vol.%. The monodisperse polystyrene microspheres were clearly observed, and the particle diameter was approx. 1.7 μm.

Figure 2 shows the time courses of styrene conversion, particle diameter, CV, and particle numbers of polystyrene microsphere with PAspNa vs polymerization time in the polymerizations at an EtOH volume fraction of 60 vol.%. PAspNa concentration was 3.4 mg/mL.

The final conversion attained a value higher than 80%, and the particle diameter increased with time as the conversion of styrene increased. The CV decreased with time. This is because the polydispersed small particle nuclei aggregated in the initial stage of polymerization to form primary particles as seen in Fig. 2. In addition, primary particles aggregated to form secondary particles.

Particle numbers decreased with time until the particles stabilized and then became constant. In addition, the time course of particle diameter shows the particle diameter increased even after the particle numbers became constant. This was caused not by the aggregation of secondary particles but by the absorption of monomer and/or particle nuclei formed during the middle stage of polymerization in the solution. The polymerization mechanism observed is similar to that in previous studies on dispersion polymerization [8, 9]. The above findings suggest that the polymerization kinetics of this process can be discussed in terms of previously reported dispersion polymerization.

Table 2 Recipe for the preparation of monodisperse polystyrene microspheres by dispersion polymerization

Ingredients	
Styrene (g)	1.35
AIBN (g)	0.107
PAspNa (g)	0.15
Aqueous ethanol solution (mL)	45

343 K; 6 h; N_2; in flask with stirring rate, 360 rpm
AIBN 2,2′-Azobisisobutyronitrile, *PAspNa* sodium polyaspartate

Effect of EtOH volume fraction on average diameter and CV

Figure 3 shows the effect of the EtOH volume fraction in the polymerization medium on the average diameter and CV. The EtOH volume fraction was between 0 and 70 vol.% with 3.4 mg/mL dispersion stabilizer. The feed of styrene monomer was completely dissolved in the EtOH/water solvent when the EtOH content was above 40 vol.%, and the solution before the reaction was homogeneous.

The particle diameter increased from about 350 nm to 1.6 μm as the EtOH volume fraction increased. Because the solubility of styrene increased with increasing EtOH volume fraction, the critical chain length in precipitation increased. Therefore, polymer microspheres with larger diameters were formed. In addition, monodisperse polystyrene microspheres were obtained at all EtOH volume

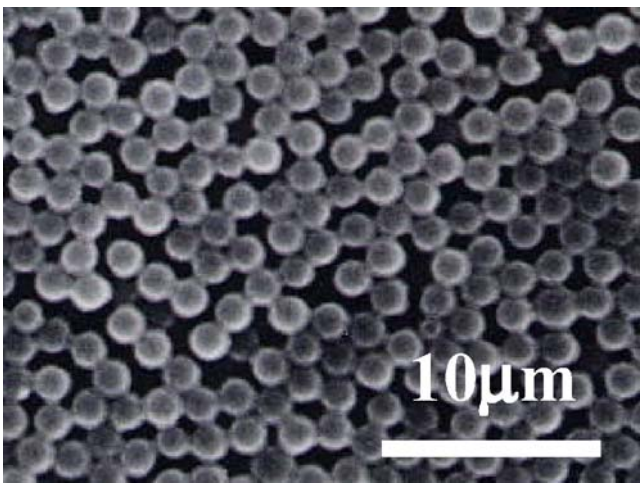


Fig. 1 SEM image of polymeric microspheres synthesized with PAspNa. Volume fraction of EtOH=60 vol.%

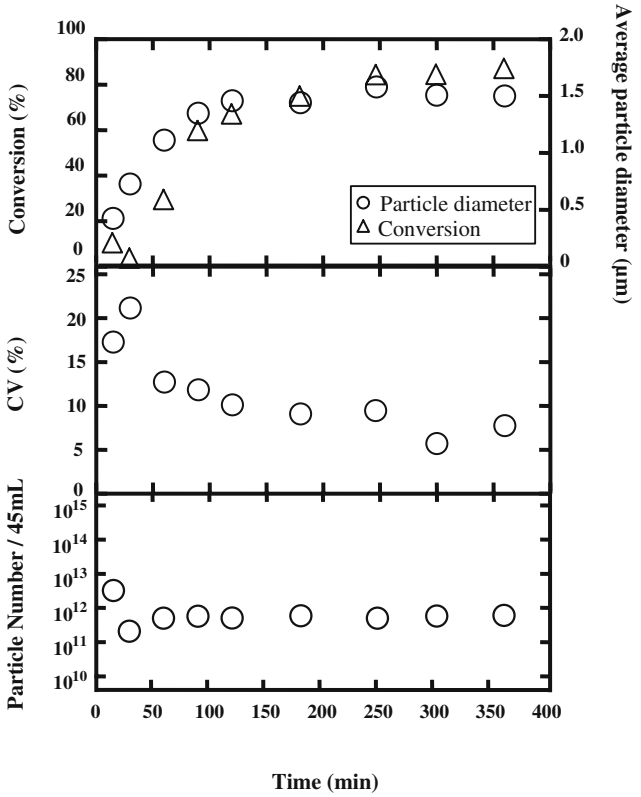


Fig. 2 Time course of conversion, particle diameter, CV, and particle number. Concentration of dispersion stabilizer=3.33 mg/mL. Volume fraction of EtOH=60 vol.%

fractions except 40 vol.%. Bimodal distribution of the particle diameter was observed at an EtOH volume fraction of 40 vol.% in a reproducible fashion, which caused higher CV. We are unsure of the reason for the mechanism of the formation as yet. Tentatively, we can say that the size determination of the microspheres is very sensitive to not only the volume fraction of the solvent but also the hydrophilicity of the dispersion stabilizer.

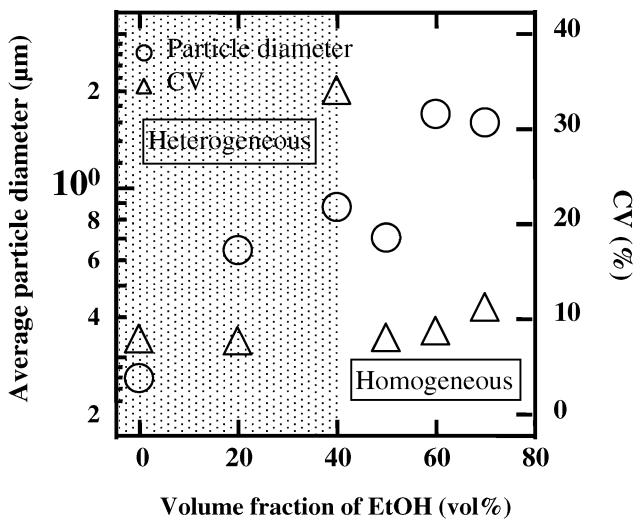


Fig. 3 Effect of EtOH volume fraction on particle diameter. Concentration of dispersion stabilizer=3.33 mg/mL

Effect of dispersion stabilizer concentration on average diameter and CV

Figure 4 shows the effect of dispersion stabilizer concentration on the average diameter and CV. In this study, the dispersion stabilizer concentration was varied from 2.2×10^{-1} to 6.7 mg/mL. The particle diameter decreased with increasing concentration of dispersion stabilizer, whereas the distribution became monodisperse as the concentration increased. This is because the high initial stabilizer concentration results in increasing amounts of stabilizer adsorbed on the nuclei and thereby in better protection against aggregation processes. On the other hand, at low initial stabilizer concentration, i.e., less than 6.7×10^{-1} mg/mL, the amount of stabilizer adsorbed on the nuclei is not sufficient to protect them from aggregation processes. Furthermore, as shown in Fig. 4, the particle diameter decreased linearly with increasing dispersion stabilizer concentration from 6.7×10^{-1} to 6.7 mg/mL. The scaling relationship is

$$[\text{Particle diameter}] \propto [\text{Dispersion stabilizer concentration}]^{-0.29} \quad (1)$$

Paine et al., in their work with PVP as a dispersion stabilizer, reported similar dependency for PVP concentrations from 4 to 40 mg/mL. They showed that the dependency of the particle diameter on PVP concentration is approx. −0.3 power [10–12].

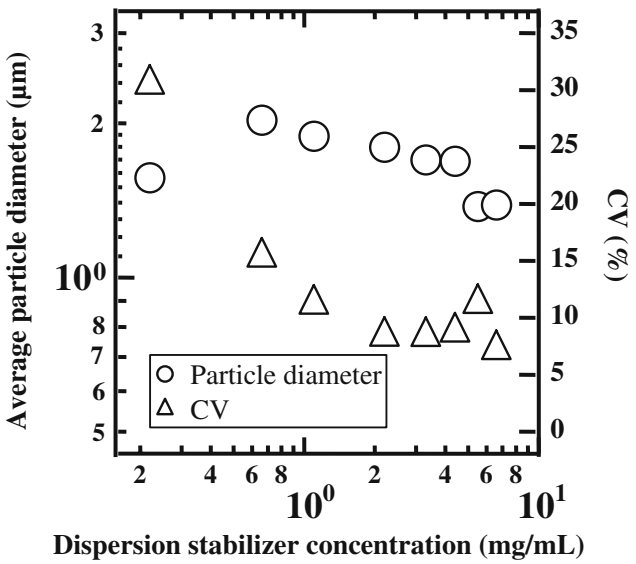


Fig. 4 Effect of dispersion stabilizer concentration on particle diameter. Volume fraction of EtOH=60 vol.%

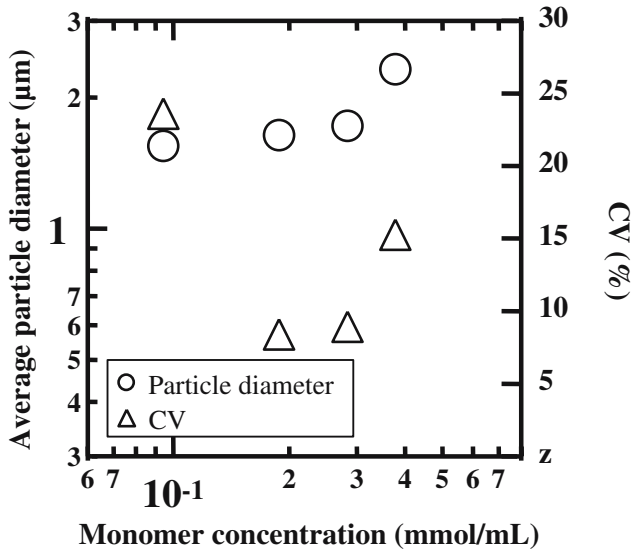


Fig. 5 Effect of monomer concentration on average diameter and CV concentration of dispersion stabilizer=3.33 mg/mL. Volume fraction of EtOH=60 vol.%

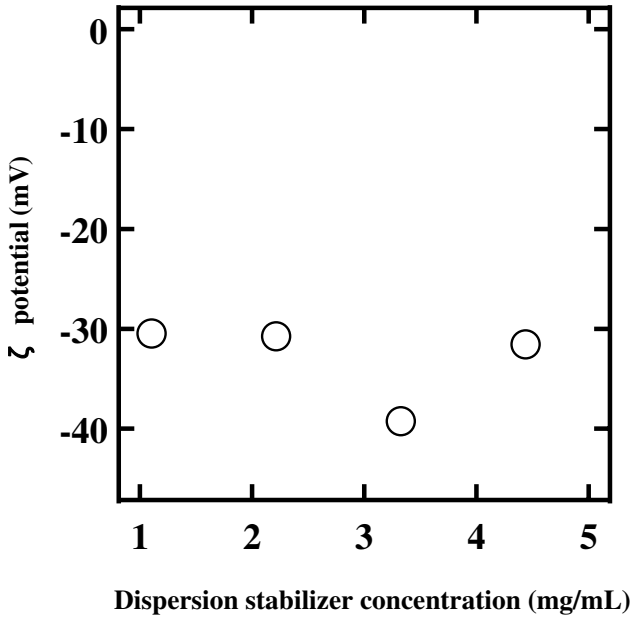


Fig. 6 Effect of dispersion stabilizer concentration on zeta potential. Volume fraction of EtOH=60 vol.%

Effect of monomer concentration on average diameter and CV

Figure 5 shows the effect of styrene concentration on the average diameter and CV with 3.4 mg/mL dispersion stabilizer. In this study, the styrene concentrations were between 0.96×10^{-1} and 3.9×10^{-1} mmol/mL in a 60% EtOH volume fraction medium. The solution before the reaction was homogeneous because styrene completely dissolved in the medium under these concentrations. The particle diameter increased from approx. 1.5 up to approx. 2.3 μm with increasing monomer concentration. Previous studies have also reported an increase in particle diameter as the monomer concentration increased [4, 13]. According to those studies, the effect of monomer concentration on particle diameter is mainly due to changes in the initial solvency of the medium. By increasing the monomer concentration, the solvency of the continuous phase increases, and the critical chain length in precipitation increases. In addition, the adsorption rate of the dispersion stabilizer on the particles surface decreases as the monomer concentration increases. These caused the forming nuclei to become larger and to aggregate more frequent. Therefore, the higher monomer concentration leads to decreasing the number of microspheres and increasing the particle diameter.

Zeta potential of formed polystyrene microsphere surface

Figure 6 shows the effect of dispersion stabilizer concentration on zeta potential. In this study, the concentration of the dispersion stabilizer was changed from 0 to 4.4 mg/mL. The polymer microsphere surfaces were negatively charged. Furthermore, the zeta potential stayed constant at each stabilizer concentration.

Figure 7 shows the effect of pH on the zeta potential for polymer microspheres washed in water. The pH of the microsphere-dispersed solution was changed from 4 to 10. The zeta potential decreased as pH increased below 8 and became constant when it was above 8. This suggests that Na^+ of the carboxyl group derived from PAspNa onto polymer microsphere surfaces ion-exchanged to H^+.

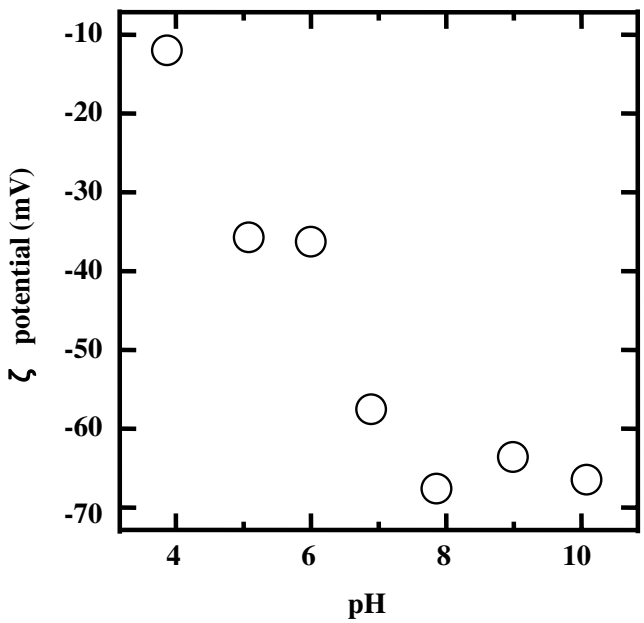


Fig. 7 Effect of pH on zeta potential. Concentration of dispersion stabilizer=3.33 mg/mL. Volume fraction of EtOH=60 vol.%

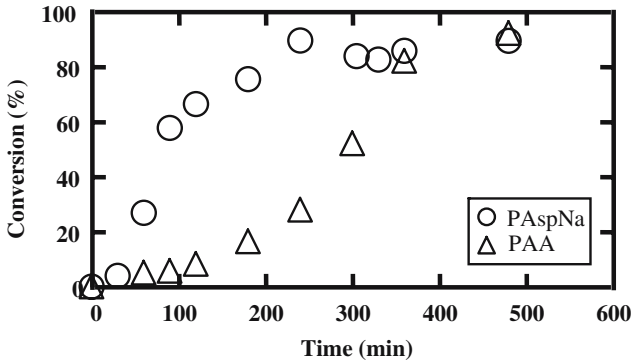


Fig. 8. Time course of conversion for PAspNa and PAA as the dispersion stabilizer. Concentration of dispersion stabilizer=3.33 (PAspNa) and 10 mg/mL (PAA). Volume fraction of EtOH=60 vol.%

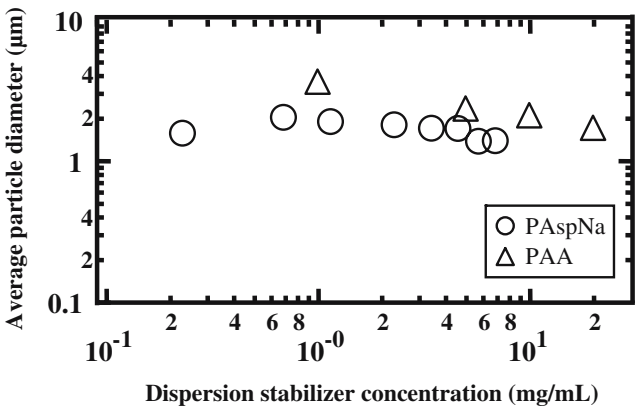


Fig. 10 Effect of dispersion stabilizer concentration on particle diameter for PAspNa and PAA. Concentration of dispersion stabilizer=3.33 (PAspNa) and 10 mg/mL (PAA), volume fraction of EtOH=60 (PAspNa) and 70 vol.% (PAA)

Comparison of PAspNa with PAA

The features of the dispersion polymerization of styrene with PAspNa as the dispersion stabilizer were compared with those of PAA [1]. We chose PAA as a comparative dispersion stabilizer for several reasons. One is that like PAspNa, PAA has carboxyl groups. Another is that the dispersion polymerization with PAA is carried out in a water and EtOH mixture, not in a water solvent.

Figure 8 shows the conversion of styrene vs polymerization time. The PAspNa concentration was 3.4 mg/mL, and the PAA concentration was 10 mg/mL. The molecular weight of PAA was 2.0×10^5 [1]. The polymerization rate of styrene with PAspNa was faster than that with PAA at an EtOH volume fraction of 80 vol.%. This shows that the dispersion polymerization of styrene with PAspNa can be carried out at a higher water volume fraction than that with PAA.

Figure 9 shows the effect of EtOH volume fraction in the polymerization solvent on the average diameter of polymer microspheres prepared with a water-soluble polymer. The effect of EtOH volume fraction on the average diameter was discussed here only under the condition that monodisperse microspheres were prepared with PAA or PAspNa (CV was below several percents in all runs). The dispersion polymerization with PAA in the solution with the EtOH volume fraction between 70 and 100 vol.% containing 10 mg/mL dispersion stabilizer provided the monodisperse microspheres from 1.8 to 3.9 μm in diameter [1]. On the other hand, monodisperse microspheres were produced by dispersion polymerization with PAspNa at the EtOH volume fraction of less than 60 vol.%. The size of prepared monodisperse microspheres in the PAspNa system varied

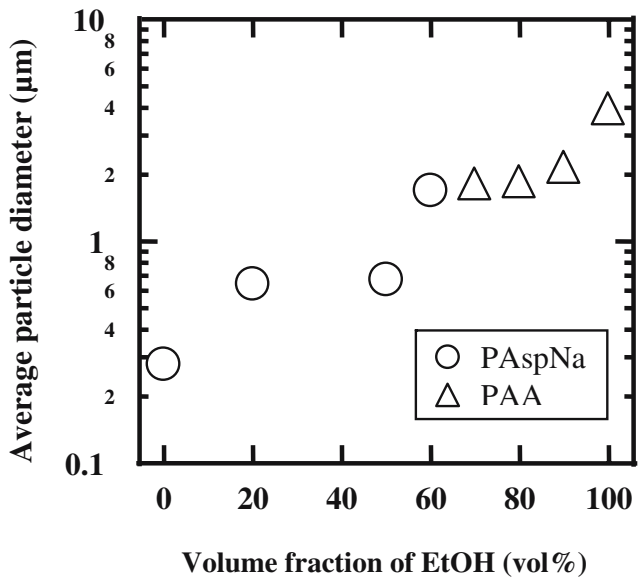


Fig. 9 Effect of ethanol volume fraction in polymerization solvent on average diameter of polymer microspheres prepared with PAspNa or PAA. Concentration of dispersion stabilizer=3.33 (PAspNa) and 10 mg/mL (PAA)

Table 3 The conditions for preparing monodisperse microspheres in EtOH/water-mixed solution with PAA or PAspNa as a dispersion stabilizer

Ingredients	PAA (Mw: 2.0×10^5; non-biodegradable)	PAspNa (Mw: 6.0×10^4; biodegradable)
Ethanol volume fraction in polymerization solvent (vol.%)	70	60
Concentration of dispersion stabilizer (mg/mL)	10.0	3.33
Concentration of monomer (mmol/mL)	1.93	2.89×10^{-1}
Average diameter (μm)	1.80	1.69

from 0.28 to 1.7 μm with the EtOH volume fraction between 0 and 60 vol.% with 3.3 mg/mL dispersion stabilizer.

Figure 10 shows the effect of dispersion stabilizer concentration on the average diameter for PAspNa and PAA. The dependence of the average particle diameter of the microspheres on dispersion stabilizer concentration was found to be the same in both cases; however, the microspheres with PAspNa were smaller than those with PAA at each dispersion stabilizer concentration.

Table 3 summarizes the conditions for preparing monodisperse microspheres in EtOH/water-mixed solution with PAA or PAspNa. Under each condition, polymer microspheres with almost the same diameter can be prepared. The important point is that monodisperse microspheres can be prepared using PAspNa, which is biodegradable. Furthermore, the dispersion stabilizer concentration of PAspNa for preparing monodisperse microspheres is about one third of that of dispersion polymerization with PAA. In addition, the monomer concentration and EtOH volume fraction for preparing monodisperse microspheres in the PAspNa system is lower. This means that dispersion polymerization using PAspNa as a dispersion stabilizer is an environmentally friendly process because monodisperse microspheres can be prepared at lower dispersion stabilizer and monomer concentrations and at lower EtOH volume fractions in the solution. The difference in the function as a dispersion stabilizer was assumed the difference of hydrophilicity of these dispersion stabilizers. PAspNa has higher hydrophilic property than PAA. That is, PAspNa has more affinity to the solution at lower EtOH volume fractions. Therefore, the polymerization rate was accelerated when PAspNa was used as the dispersion stabilizer.

Conclusion

We synthesized PAspNa by polycondensation with L-Asp. We obtained monodisperse polystyrene microspheres with PAspNa in dispersion polymerization. It was confirmed that this polymerization process is almost the same as the common dispersion polymerization. In addition, PAspNa acts as a dispersion stabilizer in this polymerization. The particle diameter increased as the EtOH volume fraction increased, and with increasing dispersion stabilizer concentration, the particle diameter and its distribution decreased. The dispersion polymerization of styrene with PAspNa can be carried out at a higher water volume fraction than that with PAA, and monodisperse microspheres can be prepared at a lower EtOH volume fraction and dispersion stabilizer concentration in the PAspNa system than in the PAA system.

References

1. Okubo M, Ikegami K, Yamamoto Y (1989) Colloid Polym Sci 267:193
2. Serizawa T, Chen MQ, Akashi M (1998) Langmuir 14:1278
3. Wang, Dimonie VL, Sudol , El-Aasser MS (2002) J Appl Polym Sci 84:2710
4. Bamnolker H, Margel S (1996) J Polym Sci A Polym Chem 34:1857
5. Neri P, Antoni G, Benvenuti F, Cocola F, Gazzei G (1973) J Med Chem 16:893
6. Shinoda H, Asou Y, Suetsugu A, Tanaka K (2003) Macromol Biosci 3:34
7. Tomida M, Nakato T (1997) Polymer 38:4733
8. Sudol ED (1997) Dispersion polymerization. In: Asua JM (ed) Polymeric dispersions: principles and applications. Kluwer, Dordrecht, pp 141–154
9. Cawse JL (1997) Dispersion polymerization. In: Lovell PA, El-Aasser MS (eds) Emulsion polymerization and emulsion polymers. Wiley, Chichester, pp 743–761
10. Yasuda M, Seki H, Yokoyama H, Ogino H, Ishimi K, Ishikawa H (2001) Macromol 34:3261
11. Paine AJ, Luymes W, McNulty J (1990) Macromolecules 23:3104
12. Paine AJ (1990) Macromolecules 23:3109
13. Tseng CM, Lu YY, El-Aasser MS, Vanderhoff JW (1986) J Polym Sci A Polym Chem 24:2995