Poly(styrene-co-Acrolein) Latex Particles: Copolymerization and Characteristics

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Synopsis

Reactive microspheres suitable for binding proteins were prepared using emulsifier-free emulsion copolymerizations of styrene (St) and acrolein (AL) with various molar ratios of monomers St and AL. A maximum polymerization rate was obtained when the molar ratio of the monomers was 1 : 1. The diameter of the latex particles increased with increase in the amount of monomer AL. The amounts of aldehyde groups on the latex particle surfaces were determined by conductometric titration. The binding capacity of the copolymer latexes with spacer molecules was also examined.

INTRODUCTION

Immunomicrospheres have been widely used in the biomedical and biochemical fields.[1] Several types of polymeric microspheres with various functional groups on their surfaces have been synthesized as carriers of proteins.[2-4] Recently polyacrolein (PA) microspheres were synthesized by aqueous polymerization under alkaline conditions and by aqueous radical polymerization.[5,6] PA microspheres have many aldehyde groups on their surfaces, and aldehyde groups can be used for the covalent binding of amino group-carrying biological materials, e.g., proteins, drugs, and enzymes, by the formation of Schiff base at room temperature. Polyacrolein microspheres, however, have some problems: Monodisperse particle size cannot be achieved easily, and the specific gravity of the particles is too high to keep them in suspension for a long time without sedimentation.

A copolymerization of acrolein with styrene seems to be, on the contrary, advantageous, because the copolymer particles obtained have a lower specific gravity than the polyacrolein particles, and are expected to be highly monodisperse. In this paper, poly(styrene-co-acrolein) latex particles were synthesized by emulsifier-free emulsion copolymerization. The amount of aldehyde groups localized on the latex particle surfaces (surface —CHO) was determined by conductometric titration. The polymerization process was examined in detail. The binding capacity of the latex particles with spacer molecules (6-amino-*n*-caproic acid) was also examined.

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Journal of Applied Polymer Science, Vol. 40, 89–98 (1990)
 CCC 0021-8995/90/1-20089-10$04.00

EXPERIMENTALS

Materials

Styrene (St) and acrolein (AL) were purified by distillation in vacuo. 6-Amino-*n*-caproic acid (6-ACA), hydroxylamine hydrochloride and sodium borohydride were of analytical grade, and used without further purification. Sodium borohydride was used as a reducing agent for the introduction of spacer groups into latex particles. 1-Ethyl-3-(3-dimethylamino-propyl) carbodiimide hydrochloride (EDC) was used for the coupling of proteins with latex particles.[7] Other reagents were commercially available. Water was doubly distilled before use.

Preparation of Latex Particles

A series of poly(styrene-co-acrolein) (PSt/AL) latexes were prepared by an emulsifier-free emulsion copolymerization. The polymerization recipes are listed in Table I. All polymerization reactions were carried out in a 250-mL round-bottomed four-necked flask. The prescribed amounts of St and AL were copolymerized in 125 mL of water by using KPS (125 mg) as initiator. The reaction mixture was stirred at 200 rpm for 8 h while the reaction temperature was kept at 55°C and N_2 gas was continuously passed through the flask. The overall conversions were determined gravimetrically. The purification procedure used to clean the latex was as follows: Each latex emulsion was centrifuged at 15,000 rpm for 10 min, and the particle precipitate was washed two times with water. Then the latex particles were resuspended in water and mixed with ion-exchange resin. One day later, the mixture was filtered and the ion-exchange resin was discarded. Finally, the latex suspension was dialyzed for 5 days against water.

The average diameter of the latex particles was estimated from electron micrographs with a JEM-100 CX electron microscope. The dispersion parame-

TABLE I
Copolymerization of the PSt/AL Latexes[a]

No.	St (mL)	AL (mL)	KPS (mg)	H_2O (mL)	Conversion (%)
1	17.5	0.0	125	125	2.2
2	15.8	1.0	125	125	14
3	14.9	1.5	125	125	20
4	13.2	2.5	125	125	46
5	12.3	3.0	125	125	36
6	8.8	5.0	125	125	69
7	5.3	7.0	125	125	66
8	3.5	8.0	125	125	55
9	1.8	9.0	125	125	41
10	0.0	10.0	125	125	27

[a] 55°C, 8 h. St = styrene; AL = acrolein; KPS = potassium peroxydisulfate.

ter ($\hat{\delta}/\bar{\chi}$) was calculated with

$$\hat{\delta} = \left[\sum_{i=1}^{n} \frac{(\chi_i - \bar{\chi})^2}{(n-1)} \right]^{1/2} \quad (1)$$

where $\hat{\delta}$ is mean square deviation, χ_i is the diameter of the particles, and $\bar{\chi}$ is the average diameter of the particles.

The composition of the PSt/AL latex was calculated from the percentage of oxygen in elemental analyses.

Determination of Aldehyde Groups

The determination of the amount of aldehyde groups localized on the latex surface (surface — CHO) was performed by conductometric titration of the latex using a conductometer (Model DDS-11A, Shanghai Second Analytical Instruments Factory, Shanghai, China). An excess amount of 2% aqueous hydroxylamine hydrochloride (~ 0.5 mL) was added to 50 mL of latex suspension (solid content: ~ 100 mg) and stirred at 10°C for 20 min. Then a 0.01N NaOH aqueous solution was used as titrant. The amount of surface aldehyde groups was calculated from the break in curves and expressed in terms of milliequivalent of surface aldehyde groups per 1 g of the polymer latex as follows:

$$-\text{CHO (mmol/g)} = N \cdot V/W \quad (2)$$

where N and V are the equivalent concentration and volume (mL) of NaOH titrant, respectively. W is the solid content weight of the latex particles (g).

Introduction of Spacer Groups into Latexes

6-ACA (1 g) was dissolved in 30 mL of H_2O, and 10 mL of latex suspension (500 mg solid) was dropped into the solution. The pH of the solution was adjusted to 9.0 with 0.1N NaOH and stirred at room temperature for 32 h. Ethanolamine (2 mL) was added to block unmodified aldehyde groups in the latex particles. After stirring at room temperature for 4 h, the reaction solution was further stirred overnight at 4°C. Then the suspension mixture was purified by centrifugation. After the addition of sodium borohydride (1 g) the suspension was continuously stirred for 4 h to reduce the Schiff base formed between the aldehyde groups and primary amino groups. After centrifugation, the spacer-carrying latex particles obtained were resuspended in H_2O. The concentration of the latex suspension was determined gravimetrically. Quantities of 6-ACA bound to the latex particles were determined by conductometric titration.

According to the previous paper,[7] the spacer-carrying latex particles were modified with proteins.

RESULTS AND DISCUSSION

Copolymerization Reaction of St and AL

Acrolein (AL) can be copolymerized with styrene (St) to make the particle hydrophilic and introduce active aldehyde groups on the particle surface. The PSt/AL latex particles have excellent monodispersities as exemplified in Figure 1. The infrared spectrum of PSt/AL latex indicated that AL was introduced into the copolymer latex (1720 cm^{-1}, stretching of C=O of aldehyde group; 2740 cm^{-1}, asymmetric stretching of C—H of aldehyde group). Polymerizations were carried out with various molar ratios of monomers St and AL at constant initiator concentration and other polymerization condition as listed in Table I. In the emulsifier-free emulsion polymerization system, the polymerization reaction is induced in the aqueous phase by initiator radicals. Therefore, the rate of emulsifier-free homopolymerization of hydrophobic styrene was very slow at 55°C. With the addition of hydrophilic monomer AL as the comonomer of styrene, the overall conversions increased markedly as shown in Figure 2. But Figure 2 also shows that an optimium polymerization rate was obtained when the molar ratio of the monomers was 1 : 1. The polymerization rate decreased with change in the molar ratio of monomers. For St (M_1) and AL (M_2) copolymerization, the monomer reactivity ratios were $r_1 = 0.25$ and $r_2 = 0.25$,[7] and the copolymerization reaction tended to be alternative in dioxane. However, the alternating copolymer was not obtained completely in our polymerization system. The content of AL in copolymeric latex increased with the increase in the amount of monomer AL (Fig. 2). Figure 3 shows that the increase in the amount of AL led to a large increase in the latex particle size, indicating that AL made the particle surface hydrophilic. This was favorable for the formation of larger particles in water.

Copolymerizations of St and AL with a 1 : 1 molar ratio were carried out in methanol–water medium. Methanol is miscible with both water and monomer

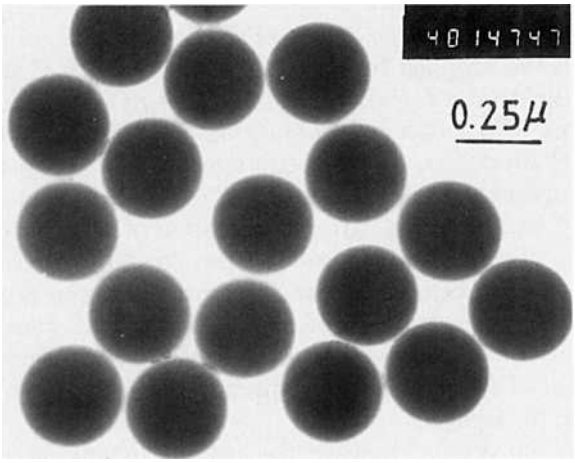


Fig. 1. Electron micrograph of PSt/AL latex particles (sample no. 14).

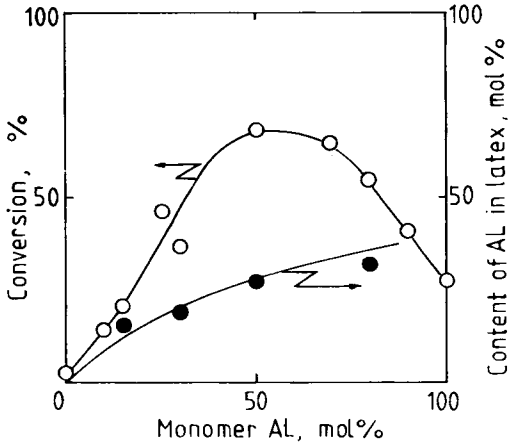


Fig. 2. Relationships between amount of monomer AL and conversion and composition of PSt/AL latex particles.

St, and is a nonsolvent for copolymers. The effects of methanol content in methanol–water system on the particle diameter, dispersion parameters and conversions are shown in Table II. The particle diameter and monodispersity of latex particles estimated 8 h after the onset of the polymerization increased with the increase in methanol content until about 50 vol %, but the conversion decreased with the increase in methanol content. In the emulsifier-free emulsion copolymerization of St and AL, the tendency was opposite to the emulsion homopolymerization of styrene reported by Homola et al.[9] and Okubo et al.[10] The particle size was reported to decrease slightly with an increase in methanol content in the emulsifier-free emulsion polymerization of

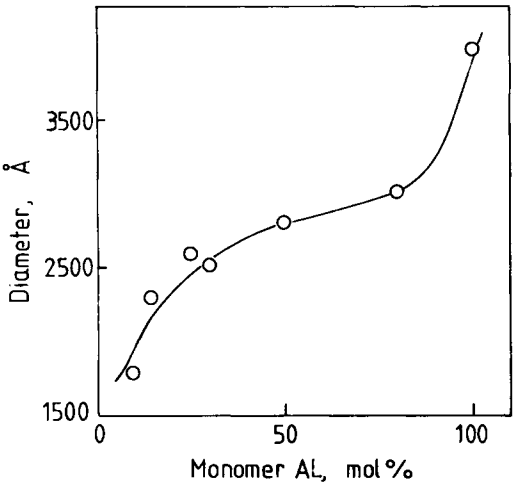


Fig. 3. Effects of amount of monomer AL on the diameters of PSt/AL latex particles.

TABLE II
The Effects of MeOH on the Copolymerization Behaviors[a]

No.	Content of MeOH (vol %)	Average diameter (Å)	Dispersion parameter	Conversion (%)
11	0	2300	0.027	54
12	12	2600	0.045	85
13	24	2700	0.029	75
14	48	3000	0.018	51
15	72	Polydispersion	—	—

[a] Polymerization reaction time: 7.5 h.

styrene in methanol–water.[9] In the emulsifier-free emulsion polymerization of styrene in acetone–water system,[10] the conversion was remarkably increased with an increase in acetone content when the acetone content was less than 40%. The particle size decreased with the increase in acetone content.

Analysis of Surface Aldehyde Groups

It was reported[5,6] that the aldehyde content of polyacrolein (PA) microspheres was determined from the percentage of nitrogen obtained by the combustion analysis of the oxime formed by the reaction of PA with aqueous hydroxylamine hydrochloride. We found that the concentration of aldehyde groups can be estimated easily by the conductometric titration. In the reaction system of aldehyde groups and hydroxylamine hydrochloride (Scheme 1), $k_1 \gg k_2$, so that the concentration of strong acid, HCl, can be determined by the conductometric titration:

$$RCHO + HO\text{-}NH_2HCl \underset{k_2}{\overset{k_1}{\rightleftharpoons}} R\text{-}CH{=}N\text{-}OH + H_2O + HCl \quad (I)$$

Scheme 1.

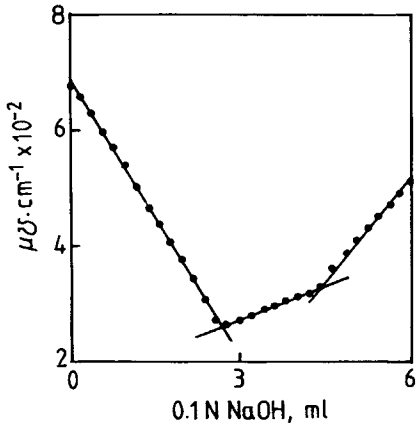


Fig. 4. Conductometric titration curve of monomer AL.

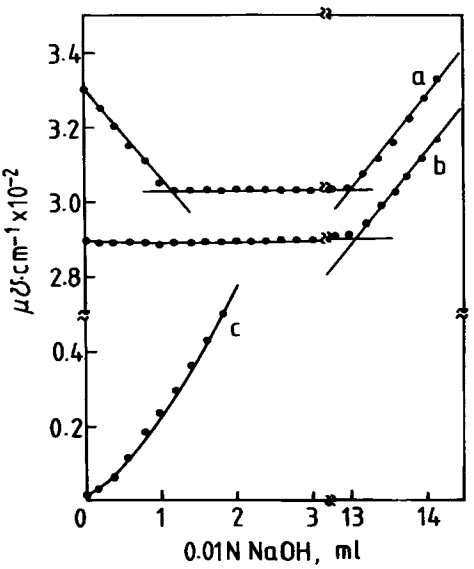


Fig. 5. Conductometric titration curves of: (a) PSt/AL latex + hydroxylamine hydrochloride; (b) hydroxylamine hydrochloride; (c) PSt/AL latex.

A conductometric titration curve for monomer AL carried out by the method mentioned above (Fig. 4) proved that the determination of aldehyde groups by conductometric titration was reliable and accurate. Twenty microliters of acrolein (3.0×10^{-4} mol) was determined to be 2.98×10^{-4} mol by conductometric titration after stirring for 20 min with hydroxylamine hydrochloride at 10°C. The two breaks in the titration curve were due to HCl released by the formation of oxime and excess $HONH_2 \cdot HCl$, respectively. Typical conductometric curve of PSt/AL latex particles is shown in Figure 5(a). The conductometric titration curve shows three straight lines with different slopes. The concentration of the surface aldehyde groups can be determined by the amount of NaOH solution added between the Y-axis and the intersection point at the end of the first descending leg of the curve. Figure 5 also indicates that the determination of aldehyde groups was not disturbed by hydroxylamine hydrochloride or the latex particle itself.

The aldehyde groups of PSt/AL latex particles are not only localized on the surface, but also buried inside of the latex particles. The results obtained, therefore, would be markedly dependent on the reaction time of PSt/Al latex with hydroxylamine hydrochloride. We determined the amount of aldehyde groups of same PSt/AL latex samples reacted with hydroxylamine hydrochloride after different reaction times at 10°C. The results are shown in Table III. It showed that surface aldehyde groups can react with hydroxylamine hydrochloride within 10–20 min completely. And the aldehyde groups buried inside of the latex cannot react with hydroxylamine hydrochloride within 1 h. With an increase in reaction time, the aldehyde groups buried inside react gradually with hydroxylamine hydrochloride. The optimium reaction time of the latex particle with hydroxylamine hydrochloride for determination of surface aldehyde groups on latex surfaces seems to be 15–20 min.

TABLE III
Effects of Reaction Time on the Amount of Aldehyde Groups in PSt/AL Latex Particles[a]

Reaction time (min)	Amount of aldehyde groups determined (mmol/g)
5	0.10
20	0.13
60	0.13
24 h	0.66

[a] Reaction temperature: 10°C.

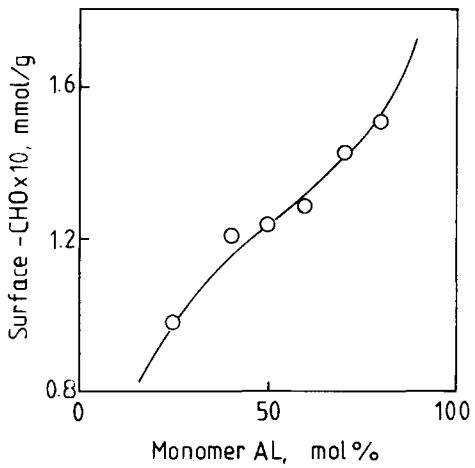


Fig. 6. Relationship between amounts of monomer AL and surface aldehyde groups.

The amounts of surface aldehyde groups on the surface area of the PSt/AL latex particles synthesized from different molar ratio of St and AL were determined by conductometric titration as described above. The results are shown in Figure 6. The amount of surface aldehyde groups increased with increase in the amount of monomer AL. The determination of surface aldehyde groups by conductometric titration was simple, rapid, and accurate. This method is also suitable for the quantitative determination of aldehyde groups of other coloured and turbid polymers and organic compounds.

Binding Capacity of Latexes with Spacer Molecules

PSt/AL latex particles can covalently bind various amino ligands, e.g., proteins, enzymes, and drugs directly through their aldehyde groups. In order to increase the biological activity of the ligand-carrying latex particles, spacer groups, such as 6-amino-*n*-caproic acid (6-ACA) or hexamethylenediamine, are usually introduced between the latex particle and the ligand.[11] The binding capacity of PSt/AL latex particles with spacer molecule, 6-ACA, was examined. The relationship between the amount of spacer 6-ACA bound onto latex surfaces and the composition of latex particles is shown in Figure 7. The figure

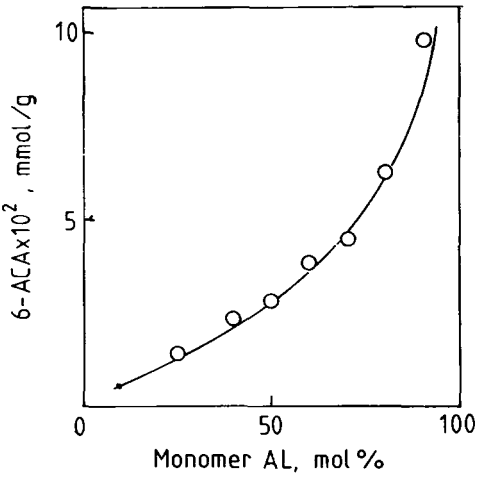
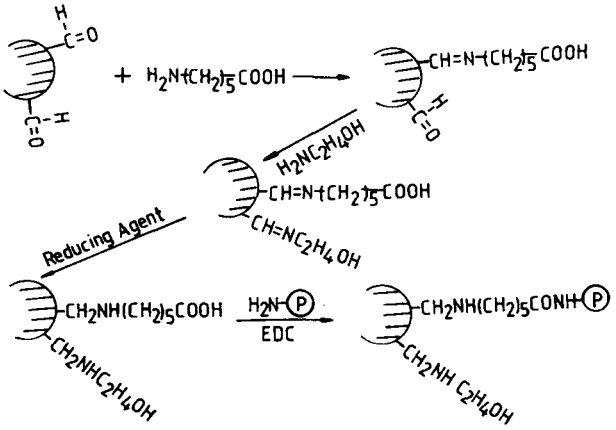


Fig. 7. Binding capacity of various PSt/AL latexes with 6-ACA spacer.

clearly shows that the binding capacity of PSt/AL latex with 6-ACA increased with the increase in the amount of monomer AL supplied in copolymerization. The 6-ACA-carrying latex particles were successfully modified with human serum albumin (HSA) and anti-HSA-F $(ab')_2$, respectively, by a water-soluble carbodiimide method using EDC at pH 5.0. The reaction process of modification of the reactive latex particles with proteins used is shown in Scheme 2:



Scheme 2.

The modified latex obtained was quite useful for diagnosis of HSA in the dilute regions, which reported elsewhere.[7]

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Received March 10, 1989
Accepted April 6, 1989