

PREPARATION AND CHARACTERIZATION OF AFFINITY POLYMER BASIC MICROSPHERES BY SOAP-FREE EMULSION POLYMERIZATION*

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Abstract Poly(styrene-*co*-glycidyl methacrylate) latex microspheres with uniform size and high-density epoxy groups on the surface were prepared by soap-free emulsion polymerization with batch wise operation mode in the presence of 2,2'-azobis(2-methylpropionamidine) dihydrochloride as an initiator. The kinetics of soap-free emulsion polymerization and the effects of polymerization factors were examined. In addition, the optimum polymerization conditions of poly(styrene-*co*-glycidyl methacrylate) latex microspheres for immobilization of biomolecules were obtained.

Keywords: GMA; Soap-free emulsion polymerization; 2,2'-Azobis(2-methylpropionamidine) dihydrochloride; Batch wise operation.

INTRODUCTION

Recently, polymer latex microspheres have received increasing attention in many fields including biochemistry^[1], medicine^[2], materials science^[3, 4], *etc.* Especially in biomedical field^[5], these polymer latex microspheres can be used as a support for the immobilization of biomolecules such as protein, antibody and enzyme through physical adsorption or covalent binding. Polystyrene latexes have been first and largely used in the biomedical field especially due to the hydrophobic nature of the polymer favoring irreversible adsorption of biomolecules^[6]. Styrene is usually copolymerized with specific functional monomers containing acetal^[7, 8], carboxylate^[9, 10], aldehyde^[11], chloromethyl^[12, 13], hydroxyl^[14], *etc.* Styrene/glycidyl methacrylate seemed to be an ideal candidate as a family of carrier materials for biomolecule immobilization. Epoxy group carrying copolymers exhibited some significant advantages as a potential carrier matrix, *i.e.*, easy and stable covalent linkages with different groups such as amino, thiol, and phenolic ones under mild experimental conditions^[15–18]. These monodisperse latex microspheres were accessible *via* controlled soap-free emulsion polymerization. The main advantage of a soap-free emulsion polymerization for preparing functional polymer particles over a conventional emulsion polymerization is that it provides stable latex particles free of added surface-active agents, and the particles produced have a well monodispersity if the process is controlled in a proper way^[19]. Furthermore, the concentration and distribution of functional groups such as epoxy groups on the surface of microspheres are the key factors influencing the biological molecule loading and activity of immobilized biomolecules^[20]. The concentration of available epoxy groups of the poly(styrene-*co*-glycidyl methacrylate) latex microspheres prepared by soap-free emulsion polymerization initiated by potassium or ammonium persulfate using batch wise operation mode is rather low. So, in order to achieve covalent coupling of biological molecules to the surface of the polymer latex microspheres, how to prepare the polymer latex microsphere with large surface area and appropriate distribution of functional groups seems significant.

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In this study, poly(styrene-co-glycidyl methacrylate) latex microspheres were prepared by soap-free emulsion polymerization with batch wise operation mode in the presence of 2,2'-azobis(2-methylpropionamidine) dihydrochloride as an initiator. The kinetics of soap-free emulsion polymerization and the effects of polymerization factors were examined, and the properties of the polymer latex microspheres were characterised.

EXPERIMENTAL

Materials

Styrene (St) was purchased from Shanghai Lingfeng Chemical Reagent Co., Ltd, washed with 10% NaOH alkaline solution, distilled under reduced pressure; glycidyl methacrylate (GMA) was purchased from Suzhou Guangfu Synthetic Material Factory. All the monomers were stored at 8°C before use. 2,2'-Azobis(2-methylpropionamidine) dihydrochloride (V-50) was purchased from J&K Chemical Ltd. Potassium persulphate (KPS) was obtained from Degussa-AJ (Shanghai) Initiators Co., Ltd. Phosphate buffer (pH 6.86) was obtained from Degussa-AJ (Shanghai) Initiators Co., Ltd. All reagents except monomers were used without further purification, and all materials except buffer were analytical grade.

Preparation of Polymer Latex Microsphere

The poly(styrene-co-glycidyl methacrylate) latex microspheres were prepared by soap-free emulsion polymerization with batch wise operation mode carried out in a three-necked 250 mL reactor, equipped with a stirrer, a spiral condenser and a nitrogen inlet. When the temperature reached up 75°C, the mixture of all the monomers, phosphate buffer and distilled and deionized water (DDIW) were added into the reactor, which was bubbled with nitrogen gas for 15 min and stirred at 300 r/min. After the initiator was fed to the reactor, the polymerization was carried out at 75°C for 4–6 h. The latex was cooled to room temperature and filtered through 200-μ sieve to remove any coagulation. The recipe range for the preparation of poly(styrene-co-glycidyl methacrylate) latex microspheres is listed in Table 1.

Table 1. Recipe range for the preparation of poly(styrene-co-glycidyl methacrylate) latex microspheres

Materials	Amount range (g)
H ₂ O	120
V-50	0.1–0.3
St	3–5
GMA	1–3
Buffer (pH 6.86)	0–50

Characterisation of Poly(styrene-co-glycidyl methacrylate) Latex Microspheres

Determination of the stability of latex

The coagulation ratio of the poly(styrene-co-glycidyl methacrylate) latex was defined as follows:

$$\text{Coagulation ratio (wt\%)} = \frac{\text{coagulation weight } (m_1)}{\text{latex total weight } (m_2)} \times 100\% \quad (1)$$

The less coagulation ratio means the better stability of latex.

Latex microsphere size and surface morphology analysis

The poly(styrene-co-glycidyl methacrylate) latex microsphere size and size distribution (polydispersity index U) were determined by malvern zetasizer 3000 HSA zeta potential and particle size analyzer (malvern instruments, UK). Before measurements, the latex was diluted with DDIW at 1:5000 volume ratio.

The number of particles N_p was calculated by the following equation^[21, 22]:

$$N_p = \frac{6 \times S}{\pi \times D_p^3 \times \rho} \quad (2)$$

S was the solid content and D_p was the diameter of the particle; the average density of the present copolymer ρ can be calculated by formula (3):

$$\frac{1}{\rho} = \sum \frac{w_i}{\rho_i} \quad (3)$$

Here w_i and ρ_i were the mass fraction (%) and density (g/cm^3) of the copolymer, respectively.

The surface morphology of the latex microspheres was observed by transmission electron microscopy (TEM, JEM-200CM).

FTIR spectra

The FTIR spectra of the poly(styrene-*co*-glycidyl methacrylate) latex microspheres were obtained using an FTIR spectrophotometer (Nicolet 5700, America). Poly(styrene-*co*-glycidyl methacrylate) latex microspheres were repeatedly centrifuged and washed, then the dried latex microspheres and KBr were thoroughly mixed. The mixture (KBr content 98 wt%–99 wt%) was pressed to form a tablet, and the spectrum was recorded.

Determination of the epoxy groups content

The concentration of available epoxy groups of the poly(styrene-*co*-glycidyl methacrylate) latex microspheres was determined by the pyridine-HCl method as described previously^[23]. Before determination, the latex was repeatedly centrifuged and dialysed for 3 days in flowing DDIW to remove the unreacted functional monomers.

Determination of the number of epoxy groups per microsphere

The number of epoxy groups per particle N_{ep} was calculated as follows:

$$N_{ep} = \frac{X \times 10^{-3} \times N_A}{N_p} \quad (4)$$

X was the available epoxy value determined by the pyridine-HCl method as described above, N_A was the Avogadro constant.

RESULTS AND DISCUSSION

Properties of Poly(styrene-*co*-glycidyl methacrylate) Latex Microspheres

In our study, the epoxide groups containing poly(styrene-*co*-glycidyl methacrylate) latex microspheres were prepared from St and GMA *via* soap-free emulsion polymerization in the presence of an initiator V-50. The chemical structure of poly(styrene-*co*-glycidyl methacrylate) latex microspheres is presented in Fig. 1.

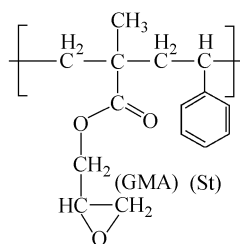


Fig. 1 Chemical structure of poly(styrene-*co*-glycidyl methacrylate) latex microspheres

FTIR spectrum of poly(styrene-*co*-glycidyl methacrylate) latex microspheres is presented in Fig. 2. The vibration at 1727 cm^{-1} represents the ester configuration of GMA. The epoxide groups give the stretching bands at 906 cm^{-1} and 756 cm^{-1} , indicating the existence of epoxy groups in the poly(styrene-*co*-glycidyl methacrylate) (PSG) latex microspheres.

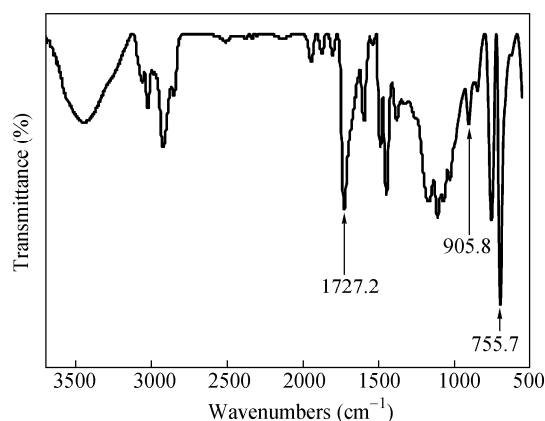


Fig. 2 FT-IR spectrum of the PSG microspheres

Soap-Free Emulsion Polymerization of St and GMA

In the soap-free emulsion polymerization, different systems have different polymerization performance. In this study, the kinetics of soap-free emulsion polymerization and the effects of polymerization factors were emphatically examined.

Effect of the concentration of initiator

In the emulsion polymerization, the kinetic role of water-soluble initiator which locates only in the water phase is now comparatively well understood. However, it must be noted that in soap-free emulsion polymerization, the kinetic role of initiator is very complicated and is needed to be clarified for complete understanding. The poly(styrene-co-glycidyl methacrylate) latex microspheres were prepared by soap-free emulsion polymerization at 75°C with batch-feed operation mode, using V-50 as an initiator. The polymerization of St and GMA was conducted with various concentrations of initiator. The concentration of initiator directly influenced the rate of polymerization and the properties of the final latex. The conversion-time curves obtained are shown in Fig. 3.

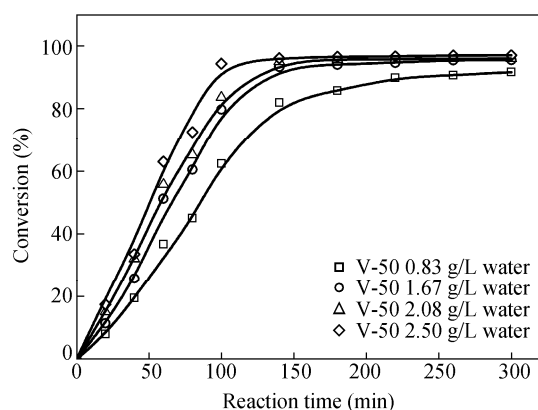


Fig. 3 Curves of conversion versus polymerization time at different concentrations of initiator 5.0% solids content, buffer (pH 6.86) 0.167 g/mL water, St:GMA = 4:2 (*W:W*); Reaction temperature, reaction time and agitation rates were 75°C, 4 h and 300 r/min, respectively.

It was clear that the rate and the conversion of polymerization were greatly dependent on the concentration of initiator. The rate of copolymerization increased with the increase of the concentration of initiator. This is because the higher the concentration of initiator was, the higher the generating rate of radical was. The increase of the concentration of free radicals resulted in the formation of more latex particles, so the rate of the

polymerization was increased. Through increasing the amount of the initiator the rate of polymerization was rapidly increased, and the final conversion was well above 90% after about 200 min. When the amount of V-50 was less than 0.83 g/L in water, the rate of polymerization was slow so that the final conversion was low.

Effect of the polymerization temperature

The polymerization temperature has important influence on the rate of the polymerization. Commonly, the rate of polymerization increases with the increase of the temperature. Adjusting the polymerization temperature, the conversion- time curves at different temperatures were obtained. The result is listed in Fig. 4.

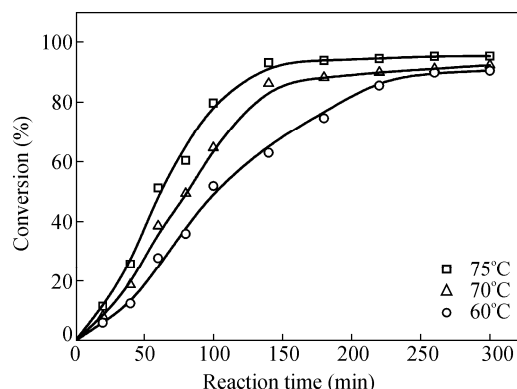


Fig. 4 Curves of conversion versus time at different polymerization temperatures 5.0% solids content, V-50 1.67 g/L water, buffer (pH 6.86) 0.167 g/mL water, St:GMA = 4:2 (*W:W*); Reaction time and agitation rate were 4 h and 300 r/min, respectively.

It was showed that the rate of polymerization obviously decreased with the decrease of the temperature. The lower polymerization temperature made the decomposition rate of V-50 much slower, which might decrease the generating rate of free radicals formed *via* the charge transfer of initiator. Therefore, the polymerization rate and the final conversion of monomers obtained were low. However, it must be noted that if the temperature was too high, the polymerization was difficult to control, which resulted in the formation of coagulation. For the sake of the final properties of the latex microspheres, the polymerization temperature had better to be below 80°C.

Effect of the ratio of comonomers and the concentration of functional groups

The poly(styrene-*co*-glycidyl methacrylate) latex microspheres were prepared by soap-free emulsion polymerization at 75°C, using V-50 as an initiator. In order to prevent the coagulation of the latex microspheres and to obtain the uniform copolymer, the monomers were fed with batch wise operation mode in all runs. Adjusting the ratio of comonomers, the characteristics of PSG latex with GMA content from *ca.* 14 wt % to *ca.* 44 wt % were examined. The results are showed in Table 2.

Table 2. Characteristics of PSG latex with different monomer recipes^a

Run no.	St:GMA (<i>W:W</i>)	D_p (nm)	Particle size distribution, U	Epoxy value (mmol/g)	Number of epoxy groups per microsphere ($\times 10^8$)
A1	5:4	409.1	1.0162	3.84	15.83
A2	4:3	366.9	1.0093	3.81	11.35
A3	2:1	346.4	1.0003	3.27	8.20
A4	3:1	332.3	1.0067	2.31	5.11
A5	4:1	286.0	1.0049	2.30	3.24
A6	5:1	209.1	1.0076	1.97	1.09
A7	6:1	198.3	1.0025	1.75	0.82

^a 5.0% solids content, buffer (pH 6.86) 0.167 g/mL water, initiator V-50 1.67 g/L water; Reaction temperature, reaction time and agitation rate were 75°C, 4 h and 300 r/min, respectively.

It's clear from Table 2 that the concentration of epoxy groups on the surface of microspheres was strongly dependent on the content of functional monomer GMA. Not only the available epoxy value, but also the number of epoxy groups per microsphere increased with the increase of GMA content, so the concentration and distribution of epoxy groups on the surface of latex microspheres could be effectively controlled by adjusting the functional monomer GMA content.

In the St/GMA polymerization system the particle diameter rose almost linearly with the increase of the amount of GMA, though the particle size distribution index U varied quite little. The increase in diameter with GMA content was difficult to rationalize. A higher GMA content would provide a higher polar content in the latex which might be expected to favor stabilization of smaller particles, especially if some hydrolysis of epoxide groups occurs as might indeed be possible. However, free radical ring opening of a low level of epoxide groups during emulsion polymerization was also possible, potentially introducing cross-links, and this might be of significance in controlling the particle size^[24].

The ratio of comonomers also had a great influence on the morphology of microspheres. Figure 5 showed the TEM images of samples with GMA content of 42.9% and 16.7%, respectively. PSG latex microspheres with relatively low GMA content 16.7% were perfectly spherical with smooth surface, and with the increase of GMA content the hydrophilicity of microspheres became stronger, accordingly, the microspheres had a rough surface. This result accorded with the earlier report^[25].

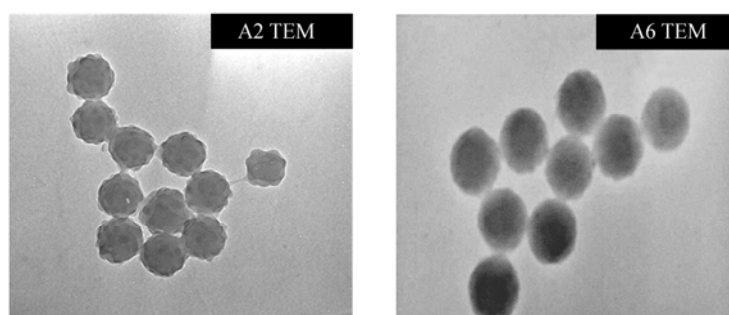


Fig. 5 Transmission electron micrographs of microspheres in experiments A2 and A6

Effect of type and concentration of initiator

The type of initiator had obvious influence on the concentration and distribution of epoxy groups. In the previous studies, the persulfate initiator was extensively used, however, in this study V-50 was chosen as an initiator. The use of V-50 as an initiator was due to the following two reasons. One is to maintain the pH of the final latex close to the neutral value compared with that of the persulfate initiator system for preventing the ring opening of GMA, and the other is to obtain the positively charged particles for future application in the field of nanobiotechnology. Since the isoelectric points of many proteins are less than pH 7, they are negatively charged at neutral pH and above neutral pH. To maintain the stability of epoxy groups of latex microspheres and the stability of proteins, the neutral pH is necessary for biomolecule immobilization in the next study^[26]. The result of the comparative experiment of V-50 and KPS is listed in Table 3. The polymerization system initiated by V-50 obtained PSG latex microspheres with higher surface epoxy values compared to the system initiated by KPS, and also had small particle size 346 nm and narrow size distribution.

Table 3. Characteristics of latex microspheres with different initiators^a

Initiator	D_p (nm)	Particle size distribution, U	Sample pH value	Epoxy value (mmol/g)	Number of epoxy groups per microsphere ($\times 10^8$)
KPS	322	1.048	3.45	1.34	2.70
V-50	346	1.0003	6.86	3.27	8.17

^a 5.0% solids content, buffer pH (6.86) 0.167 g/mL water, St:GMA = 4:2 ($W:W$), initiator 1.67 g/L water, batch wise operation mode; Reaction temperature, reaction time and agitation rates were 75°C, 4 h and 300 r/min, respectively.

The concentration of initiator had important influence not only on the kinetics of polymerization but also on the stability, particle size and distribution of the final latex microspheres. The effect of initiator concentration is shown in Table 4. Because a large number of nuclei were formed at the initial stage with the increase of the concentration of initiator, meanwhile, the number of free radicals and the growing chains increased, resulting in the increase of particle number and the decline of particle size. Simultaneously, the charge on the surface of particles enhanced, which improved the stability of the latex. However, if the concentration of initiator was too high, it might cause the zeta potential falling down and the coagulation taking place.

Table 4. Characteristics of latex with different initiator concentrations^a

Run no.	V-50 (g/L water)	D_p (nm)	Particle size distribution, U	Condensation ratio (wt%)
B1	0.83	375	1.085	4.6×10^{-3}
B2	1.67	346	1.0003	3.1×10^{-3}
B3	2.50	339	1.022	5.2×10^{-3}

^a 5.0% solids content, buffer pH (6.86) 0.167 g/mL water, St:GMA = 4:2 ($W:W$), initiator V-50, batch wise operation mode; Reaction temperature, reaction time and agitation rates were 75°C, 4 h and 300 r/min, respectively.

Effect of the feed operation mode

For our particular needs to produce monodisperse particles with as high concentration of functional groups as possible while retaining reasonably small particle size and narrow size distribution, the concentration of epoxy groups on the latex microsphere surface was a key parameter. The feed operation mode of functional groups mainly influenced the available concentration of epoxy groups, the results of different operation modes are shown in Table 5. Using one-step feeding operation mode parts of the functional monomer GMA copolymerized with St within the microspheres, resulting in a relatively low available concentration of functional groups on the surface of microspheres, so the final microspheres obtained had a low epoxy value. Correspondingly, *via* batch wise feeding operation mode, perhaps the adding of functional monomers into the polymerization system later could be effectively distributed on the surface of the microspheres, therefore, the microspheres thus produced had a relatively high concentration of epoxy groups compared to the former. But if the concentration of functional monomers added into the system later was too high, it may easily induce the formation of the new nuclei. Inevitably, the bimodal of particle size distribution would occur.

Table 5. The results comparison of different operation modes^a

Run no.	Operation mode	Reaction time (h)	D_p (nm)	Particle size distribution, U	Epoxy value (mmol/g)
C1	One-step	4	349	1.0196	1.73
C2	Two-step ¹	2 + 2	346	1.0003	3.27
C3	Two-step ²	3 + 3	/	Bimodal distribution	/

^a 5.0% solids content, buffer (pH 6.86) 0.167 g/mL water, St:GMA = 4:2 ($W:W$), initiator V-50 1.67 g/L water; Reaction temperature and agitation rates were 75°C and 300 r/min; ¹ The reaction time was 4 h, at the beginning half of the GMA was added and 2 h later the other half was added; ² The reaction time was 6 h, at the beginning half of the GMA was added and 3 h later the other half was added.

Effect of buffer solution

In the St/GMA polymerization system, a phosphate buffer of pH value 6.86 was used for the purpose to keep the system near neutral environment during the polymerization process and also to prevent the epoxy groups from the ring opening. The effect of buffer content on the latex characteristics is listed in Table 6. The latex particles produced by the polymerization without buffer had a rough surface and a relatively broad particle size distribution. The presence of buffer in polymerization could improve the surface morphology in some extent and obtained the particles with uniform size. In addition, the buffer would provide a higher concentration of electrolyte in the latex, which might keep the particles away from each other due to the static repulsion effect, so a better stability of system would be expected. However, if the ionic strength exceeded a high-point, the zeta potential around the particles would fall down, leading to the decrease of the latex stability. Therefore, buffer content should be controlled properly.

Table 6. Characteristics of latex with different buffer contents ^a

Run no.	Buffer content (g/mL water)	D_p (nm)	Particle size distribution, U	Epoxy value (mmol/g)
D1	0	332	1.0219	1.02
D2	0.083	374	1.0675	1.44
D3	0.167	346	1.0003	3.27
D4	0.333	358	1.0136	2.31

^a 5.0% solids content, St:GMA = 4:2 ($W:W$), initiator 1.67 g/L water, batch wise operation mode; Reaction temperature, reaction time and agitation rates were 75°C, 4 h and 300 r/min

CONCLUSIONS

In this study, poly(styrene-*co*-glycidyl methacrylate) latex microspheres were prepared by soap-free emulsion polymerization with batch wise operation mode in the presence of 2,2'-azobis(2-methylpropionamidine)) dihydrochloride as an initiator. The diameter of PSG latex microspheres thus produced was about 300 nm and had a well monodispersity with particle size distribution index $U < 1.05$, the concentration of epoxy groups on the surface of the microspheres could reach 3 mmol/g. On the basis of these results, using V-50 as an initiator and batch wise operation mode should be an effective method to control the available concentration of functional epoxy groups.

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