Colloid Polym Sci 279:589–596 (2001)
© Springer-Verlag 2001

ORIGINAL CONTRIBUTION

S.-J. Fang
K. Fujimoto
S. Kondo
K. Shiraki
H. Kawaguchi

Amphoteric initiators suitable for emulsifier-free emulsion polymerization and the properties of the resulting latices

Received: 28 July 2000
Accepted: 1 November 2000

S.-J. Fang · H. Kawaguchi (✉)
Department of Applied Chemistry
Faculty of Science & Technology
Keio University, 3-14-1, Hiyoshi
Kohoku-ku, Yokohama 223-8522, Japan
e-mail: haruma@applc.keio.ac.jp
Fax: +81-45-5645095

S.-J. Fang
Institute of Polymer Science & Engineering
Zhejiang University
Hangzhou 310027, China

S. Kondo · K. Shiraki
Central Laboratory
Wako Pure Chemicals Industries Ltd.
Kawagoe, Japan

Abstract The soap-free emulsion polymerizations of styrene (St) and acrylamide (AAm) or methyl methacrylate (MMA) were carried out in the presence of three kinds of amphoteric initiators, and the polymerization kinetics and the colloidal properties of the latices produced were emphatically investigated. It was found that the number of carbon atoms between the amidino and carboxyl groups in each initiator exhibited an appreciable effect on the dissociation as well as on the solubility of the initiator in water, and therefore, that the properties of the colloidal particles depended on the structure of the initiators used. All the copolymerization runs, except for the polymerization using 2,2′-azobis(*N*-(2-caboxyethyl)-2-methylpropionamidine) under a strongly alkali condition, gave amphoteric latices, which indicated higher critical flocculation concentrations at lower or higher pHs than at a medium pH. The surface charge density measured by titration for poly(St/MMA) particles was about 3–10 times as high as that for the poly(St/AAm) ones, though these were prepared under the same conditions other than the monomer composition. The influence of the polymerization pH on both the polymerization rate and the surface charge density of the resulting latices was negligible even if the constants of the decomposition rate and the dissociation of the amphoteric initiators strongly depended on the pH of the medium.

Key words Emulsifier-free emulsion polymerization · Amphoteric initiator · Amphoteric latex · Styrene · Methyl methacrylate

Introduction

Monodispersed polymer microspheres prepared without any emulsifier are more advantageous to various applications owing to their clear and well-characterizable surfaces [1–3]. Such microspheres have been proved to be excellent carrier particles for protein separation and DNA diagnosis [4–7].

As is well known, in the absence of emulsifier, we have to design the polymerization recipes taking into account the issues, i.e., how to initially induce the particle nucleation and to finally form a stable microsphere dispersion. Actually, the adoption of a high concentration of ionic initiators or/and the addition of a water-soluble monomer into the recipes are more efficient methods to achieve the previously aims [8, 9]. In the previously mentioned methods the decomposed initiator fragments contribute to particle nucleation and stabilization through the incorporation of the charged groups to the polymer chain ends, and the hydrophilic portions staying at the particle surfaces also provide steric interactions for stabilization. It is regarded as an alternative policy to control the colloidal and surface properties of latex particles by adjusting the nature and concentration of initiators in such polymerization systems. Generally, the structure of polymer chains at the particle surfaces is considered as the most important factor affecting the properties and applications of the final latices [10, 11].

A water-soluble amphoteric initiator containing two kinds of dissociable groups is expected to induce the unique properties of the resulting latices, compared to those initiators containing a single ionic group. The incorporation of amphoteric initiator fragments towards the particle surfaces easily results in the formation of amphoteric latex particles that will find potential applications.

In this work, therefore, a series of homologous amphoteric initiators were selected as initiator systems in the emulsifier-free emulsion polymerizations of styrene (St) and methyl methacrylate (MMA) or acrylamide (AAm), and the kinetics and the properties of the resulting latices were investigated.

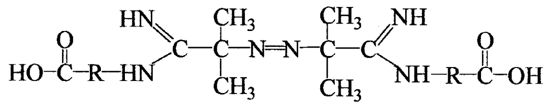
Experimental

Materials and procedure

MMA and St were purified by distillation under reduced pressure and AAm was recrystallized before use. Three kinds of homologous amphoteric azoinitiators, as schematically illustrated in Fig. 1, were received from Wako Pure Chemicals Industries and were used without further purification. NaOH and HCl were utilized to adjust the pH in all the experiments. Water was deionized and distilled and the other reagents were used as received. The basic polymerization recipes used in this study are indicated in Table 1. The procedures of polymerization and the purification of resulting latices were the same as the previously reported [12].

Characterization

The total monomer conversion, the particle size and the surface charge density were evaluated as in previous work [12]. The electrophoretic mobility of the latex particles was measured at an ionic strength of 10^{-2} M and 20 °C. The particle morphology was observed by scanning electron microscopy (SEM). The critical flocculation concentrations of various latices were determined using a spectrophotometer at a wavelength of 700 nm at 25 °C [13]. The weight-average molecular weight and its distribution in the final polymers were measured by gel permeation chromatography (Jasco Corporation, Japan) with a UV-970 detector at a wavelength of 270 nm in tetrahydrofuran as a solvent. The monodispersed polystyrene was utilized to give a standard curve of the molecular weight versus the retention volume before measurement. In addition, the first-order decomposition rate constants of the amphoteric initiators were determined by evaluating the UV absorption of residual amounts of decomposed initiators using a UV-2000 spectrophotometer at a wavelength of 370 nm.



Code*:	2CE	1CE	CM
R :	CH_2CH_2	$CH(CH_3)$	CH_2
Solubility:	15.3	0.8	0.4 (g/100gH_2O, 25°C)

*** 2CE: 2, 2'-Azobis(N-(2-caboxyethyl)-2-methylpropionamidine)**
1CE: 2, 2'-Azobis(N-(1-caboxyethyl)-2-methylpropionamidine)
CM: 2, 2'-Azobis(N-caboxymethyl-2-methylpropionamidine)

Fig. 1 Schematic illustration of various amphoteric initiators

Results and discussion

Physicochemical properties of amphoteric initiators

Decomposition rate constant

The first-order decomposition rate constants of amphoteric initiators in water were measured as a function of pH and the results are shown in Fig. 2. The rate constants of 2,2′-azobis(*N*-caboxymethyl-2-methylpropionamidine) (CM), 2,2′-azobis(*N*-(1-caboxyethyl)-2-

Table 1 The polymerization recipes used in this study using 2,2′-azobis(*N*-(2-caboxyethyl)-2-methylpropionamidine) (*2CE*), 2,2′-azobis(*N*-(1-caboxyethyl)-2-methylpropionamidine) (*1CE*) and 2,2′-azobis(*N*-caboxymethyl-2-methylpropionamidine) (*CM*) initiators. Polymerization conditions: 70 °C; 300 rpm; pH 3–10; monomers styrene (*St*), methyl methacrylate (*MMA*) and acrylamide (*AAm*)

Code ingredients	Amounts		
	1	2	3
St (g)	7.5	5.8	5.8
MMA (g)		1.7	
AAm (g)			1.7
Initiator (mol/l)		5.2×10^{-3}	
H_2O (g)		67.5	

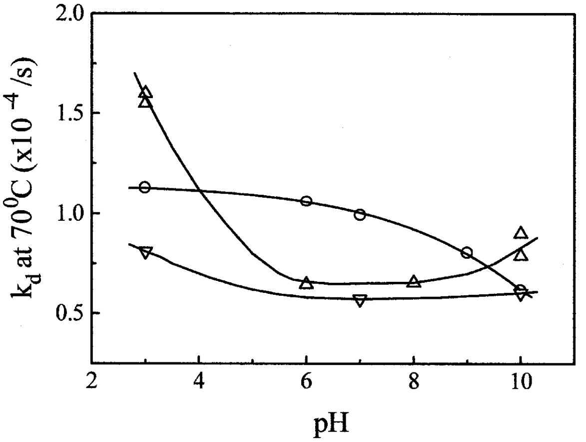


Fig. 2 pH dependence of decomposition rate constants of various amphoteric initiators at 70 °C in purified water. 2,2′-azobis(*N*-(2-caboxyethyl)-2-methylpropionamidine) (*2CE*) (○), 2,2′-azobis(*N*-(1-caboxyethyl)-2-methylpropionamidine) (*1CE*) (△), 2,2′-azobis(*N*-caboxymethyl-2-methylpropionamidine) (*CM*) (▽)

methylpropionamidine) (1CE) and 2,2′-azobis(*N*-(2-caboxyethyl)-2-methylpropionamidine) (2CE) in water are dependent on the pH of the medium although the mode of pH dependence was different in each case. It is clearly seen that the effect of pH was less pronounced on CM decomposition in water at 70 °C, which only slightly decreased with increasing pH and became constant above pH 7. In contrast, the rate constant of 1CE decomposition in water was strongly affected by the pH, i.e., the constant showed a significant decrease to reach the minimum value around pH 6, approximately half the value at pH 3, and then increased with increasing pH. Among the three amphoteric initiators, the rate constant of CM decomposition appeared the smallest in the whole pH range. The rate constant of 2CE decomposition was larger than that of 1CE only at pHs from 4 to 9.

As is known, the decomposition of 2CE in water at 70 °C around pH 10 is accompanied by the degradation of the amidino group. To understand whether similar behavior occurs in the decomposition of CM and 1CE in water under the conditions described previously, we consecutively examined the decomposed mixture of two initiators by means of NMR and found only the residue of corresponding initiator fragments and that no additional novel groups were produced. Therefore, it could be assumed that CM and 1CE amphoteric initiators followed a normal course of decomposition at 70 °C in water around pH 10. The lowest level of decomposition of these in the medium pH range seems to be attributed to the lowest repulsion of fragments at both sides of the azo group which have no charge in the medium pH range.

Dissociation behavior

The dissociation properties of the three kinds of amphoteric initiators in water, obtained from potentiometric titrations, are shown in Fig. 3. Similar dissociation behavior of CM and 1CE in water was observed, and no net ionization appeared in the pH range between 4 and 9, whereas the no-net ionization pH range was between 5 and 9 for 2CE. In addition, the p*K*s for CM and 1CE were evaluated to be about 3.5 and 9.5 for the acid and base, respectively. It was observed that the two initiators had much lower net ionization than 2CE at pHs lower than 5 and higher than 10. These could be caused by the difference in molecular structure among the three amphoteric initiators. Namely, it means that the amidino and carboxyl groups in CM and 1CE probably induce a relatively easy self-ionization owing to a single carbon atom separating them, which enables the formation of a six-membered-ring structure including an ionic bond. Such initiators were also less water soluble than 2CE because they have lower polarity owing to self-ionization.

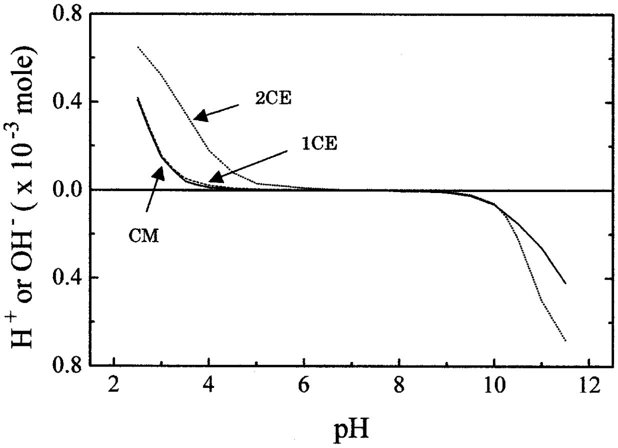


Fig. 3 Dissociation of amphoteric initiators in purified water. Titration condition: 3.5×10^{-4} mol in 50 ml water, 20 °C

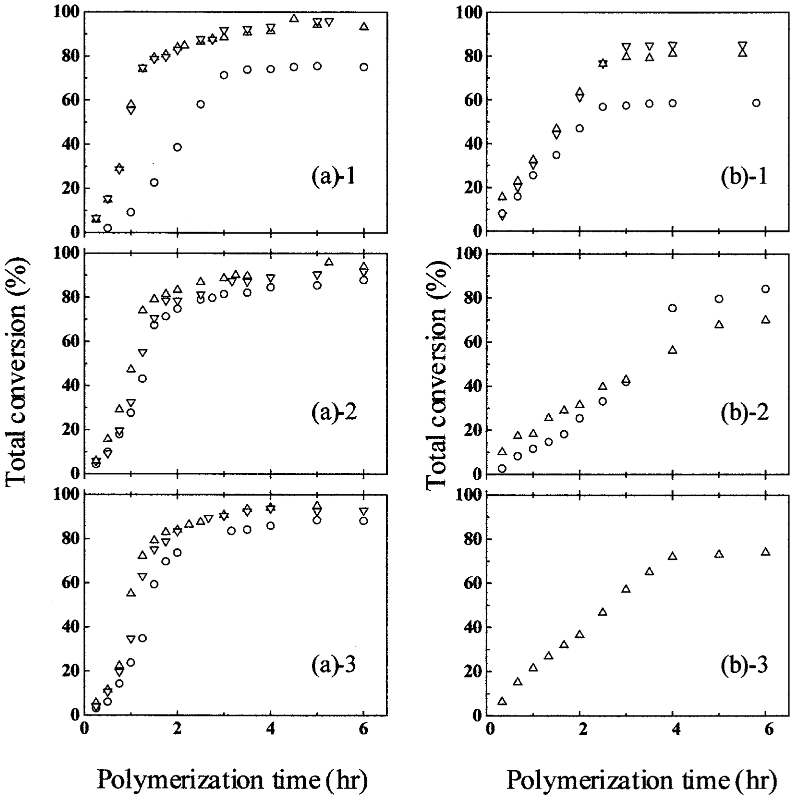
Polymerization kinetics

Conversion

Since the dissociation and decomposition rates of the amphoteric initiators used in this study are dependent on the pH of the medium, we first examined the effect of the polymerization pH on the kinetics in detail. The conversion versus time curves for the copolymerizations of St/AAm and St/MMA using various amphoteric initiators at different pHs are illustrated in Fig. 4. AAm monomer is a water-soluble one and MMA has a limited solubility in water. Here they were chosen as the comonomer to be copolymerized with St in order to compare their kinetics behavior of polymerization in the presence of amphoteric initiators. As for the St/AAm copolymerization system, the pH dependence of the conversion was negligible except for the polymerization using 2CE at pH 10, at which 2CE suffered hydrolysis [14]. Almost all of the runs could give high final conversions at a usual initiator concentration. This implies that the three amphoteric initiators appeared to have favorable efficiency to initiate the emulsifier-free emulsion polymerization of St/AAm. Moreover, it was seen that a high conversion in the case of CM used was obtained though CM exhibited the lowest decomposition rate among the three amphoteric initiators. The result can be explained by the radicals produced by the composition of CM having much higher efficiency to initiate the polymerization owing to the much stronger hydrophobicity of CM.

The St/MMA polymerizations (Fig. 4b) gave lower final conversions than the St/AAm polymerization under the same condition. The results are probably attributed to the lower solubility of MMA than AAm in water, so in the former system the number of polymer particles produced and the rate of polymerization in the

Fig. 4a, b Conversion versus time curves for emulsifier-free emulsion copolymerizations using different amphoteric initiators. Initiator concentration 5.2 × 10^{-3} mol/l, T = 70 °C. **a** styrene/(*St*)/acrylamide (*AAm*) system, **b** St/methyl methacrylate (*MMA*) system; *1*-2CE, *2*-1CE, *3*-CM; pH 3.0 (△), pH 5.8 (▽), pH 10.0 (○)



water phase were smaller than those in the latter. The number of polymer particles was 2.3 × 10^{16} particles/l for the St/AAm polymerization system and 6.5 × 10^{15} particles/l for the St/MMA one using 2CE as an initiator with a concentration of 5.2 × 10^{-3} mol/l at pH 3. The polymerization rate and the final conversion in the St/MMA polymerization using 2CE at pH 10 were lower than those at pHs lower than 10, as in the St/AAm system. In the two systems using CM and 1CE initiators, the pH dependence of the polymerization rate or conversion was small.

The size and number of polymer particles

The number of polymer particles is plotted as a function of the polymerization conversion in Fig. 5 for the St/MMA polymerization system using the amphoteric initiators at pH 3. It can be seen that in all cases the particle number decreased gradually with an increase in conversion. This reveals that the aggregation of polymer particles was prolonged till a high conversion in the present systems. The larger the solubility of the amphoteric initiator, the higher the conversion at which the number of polymer particles became constant. This can be explained by the ability of the initiator fragment to stabilize the primary particles. At the initial stage of polymerization a large number of primary particles were produced and were then stabilized by means of the coagulation of one another [15]. The constant numbers of final particles prepared using 2CE, 1CE or CM at a concentration of 5.2 × 10^{-3} mol/l were about 3.1, 0.6 and 0.35 × 10^{15} particles/l, respectively. Apparently, much smaller numbers of polymer particles were formed in the polymerizations using less water-soluble initiators which have low ability to stabilize particles.

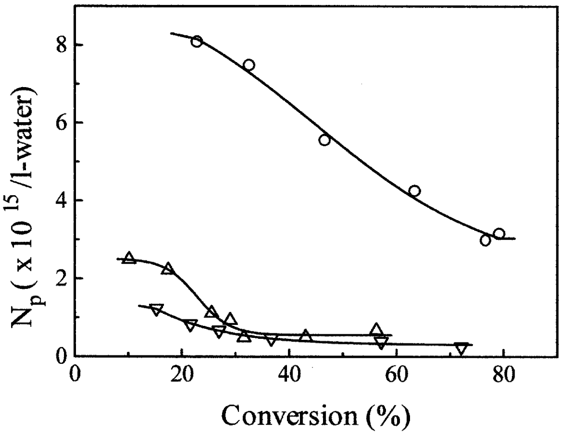


Fig. 5 The number of polymer particles during the course of copolymerization of St and MMA using various amphoteric initiators. Initiator concentration 5.2 × 10^{-3} mol/l, pH 3.0, T = 70 °C; 2CE (○), ICE (△), CM (▽)

In order to compare the difference between the St/MMA and St/AAm polymerizations, we also evaluated the number of polymer particles for the St/AAm system by carefully excluding water-soluble polymers from the latex system via repeated displacement of supernatant by centrifugation. It was noted that the number of St/AAm particles became constant after the total conversion was about 10%, differing from the St/MMA system. This can be explained by the feature of the hydrophilic monomer-including polymerization system in which more primary particles were stabilized owing to the water-soluble chains originating from the hydrophilic monomer (AAm) and the ionic initiator residues anchored onto the surfaces. Thus, a constant number of particles was really reached at the early stage of polymerization.

The sizes of the polymer particles obtained with amphoteric initiators at various pHs are presented in Table 2. As for the St/AAm system, regardless of the amphoteric initiators, the sizes of the particles were approximately independent of the polymerization pH. However, as for the St/MMA system, the particle size was affected by both of the polymerization pH and the type of amphoteric initiators. Because in the former system AAm units incorporated into the particles are probably located at the interface of water and polymer particles, a water-soluble layer thus formed should contribute predominantly to the stabilization of polymer particles owing to steric interaction. However, in the latter one, the MMA monomer has a limited solubility in water, so the preformed hydrophilic chains were inefficient in providing particle stabilization. Therefore the additional ionic interaction was necessary to finally keep the particles stable. This means that the properties of the initiator fragments played a critical role in the stabilization of the particles produced in the emulsifier-free emulsion polymerization of St and a slightly water-soluble monomer MMA. As expected, the lower the initiator solubility in water, the larger the sizes of polymer particles.

We expected that the number of polymer particles formed in the polymerization at pH 3 would be greater than the number prepared at a pH other than 3 because the rate of radical formation (the rate of initiator decomposition) was high at pH 3 and the cationized initiator fragment would serve for surface stabilization. This is confirmed in Table 2.

Molecular weight and its distribution

In order to understand the behavior of polymerization in the presence of the amphoteric initiators, the average molecular weights of St homopolymers and St/MMA copolymers were evaluated and are listed in Table 2. These were calculated from the standard curve of molecular weight versus the retention volume of monodispersed polystyrene.

As is well known, particle nucleation in an emulsifier-free emulsion system is more prolonged than in a general emulsion system. After nucleation, the compartmentalization of the polymerization reaction inside the latex particles leads to the isolation of free radicals and hence reduces the probability of bimolecular termination. From Table 2, the molecular weights of the three polystyrene

Table 2 Characterization of latex particles prepared with various amphoteric initiators. Polymerization conditions: initiator concentration 5.2×10^{-3} mol/l, $T = 70$ °C

Code[a]	Initiator	pH	X (%)	D_w (nm)	M_w ($\times 10^5$)	M_w/M_n	Stability[b]
St-1	2CE	3.0	80.3		3.78	3.96	○
-2	1CE	3.0	56.6		1.89	2.95	❍
-3	CM	3.0	60.5		6.47	7.09	❍
SM-1	2CE	3.0	81.7	358.3	2.32	4.13	○
-2		5.8	84.8	394.0	2.52	4.33	❍
-3		10.0	58.9	498.3	2.68	5.01	❍
-4	1CE	3.0	70.0	538.8	1.96	3.53	❍
-5		10.0	84.3	645.0	2.71	4.36	△
-6	CM	3.0	82.4	796.6	2.23	3.96	△
-7		5.8	90.6	871.2	3.50	5.33	△
-8		10.0	93.4	684.9	5.48	6.10	△
SA-1	2CE	3.0		194.0			○
-2		5.8		202.6			○
-3		10.0		232.1			○
-4	1CE	3.0		227.2			○
-5		5.8		234.6			○
-6		10.0		252.1			○
-7	CM	3.0		160.4			○
-8		5.8		200.2			○
-9		10.0		238.2			○

[a] Polymerization system: St; St/MMA (*SM*); St/AAm (*SA*)

[b] ○ excellent; ❍ good; △ fair

samples produced with the amphoteric initiators were near the molecular weight of polystyrene prepared with potassium persulfate, which was about 3.5×10^5 under the same polymerization condition. It can also be seen that their polydispersities, much larger than 2, were dependent upon the types of initiators and the largest was recorded in the case using CM, which has the lowest solubility in water. The results indicate that a disproportionation-dominated mechanism is likely to be operative in the present St polymerization systems using amphoteric initiators [16]. In such situations the number-average molecular weight was almost halved and the weight-average molecular weight was essentially unchanged.

Table 2 also illustrates that the weight-average molecular weights and their polydispersity of the St/MMA polymers produced with various amphoteric initiators were in the range $2–6 \times 10^5$ and $3–6 \times 10^5$, respectively. In the emulsifier-free emulsion polymerization of St and MMA using the amphoteric initiators, particle nucleation and polymerization inside the particles simultaneously progressed until high conversion so that both contributions to the molecular-weight distribution further led to the larger polydispersities. The weight-average molecular weights of the copolymers slightly increased with polymerization pH and the effect of the amphoteric initiator types on the molecular weight became unpronounced, compared with the St homopolymerization system. Furthermore, the corresponding polydispersity became larger with increasing polymerization pH.

Properties of resulting latices

Particle morphology

The latex particles prepared with various amphoteric initiators at polymerization pH 3 were observed by SEM. Figure 6 shows that SEM views of poly(St/MMA) particles, in which the latex particles were almost spherical and monodispersed, being like the morphology of particles prepared using a general ionic initiator.

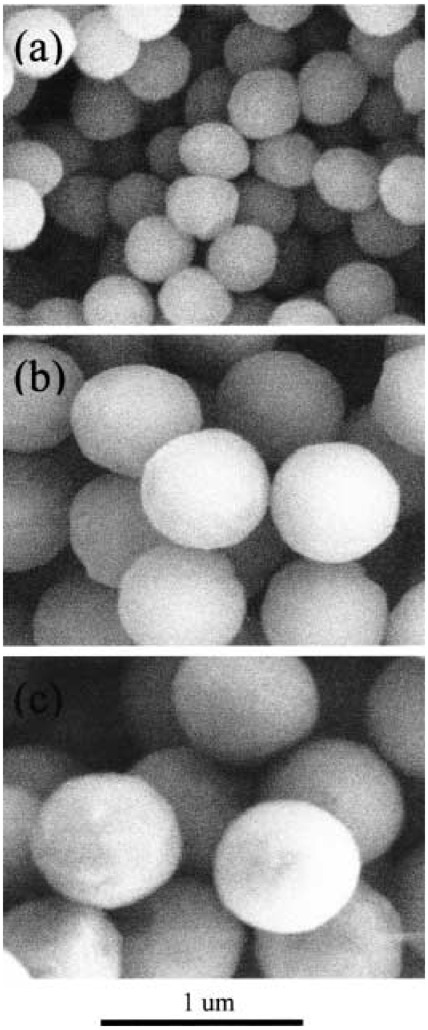


Fig. 6a–c Morphology of poly(St/MMA) latex particles prepared with all kinds of amphoteric initiators. Initiator concentration 5.2×10^{-3} mol/l, pH 3.0, $T = 70$ °C. **a** 2CE, **b** 1CE, **c** CM

Electrophoretic mobility

The pH dependence of the electrophoretic mobility of the particles of poly(St/MMA) as well as of poly(St/AAm) latices are shown in Fig. 7. The particles were prepared with various amphoteric initiators at the same pH and initiator concentration. We can see from Fig. 7 that the latex particles had negative or positive potentials depending on the electrophoretic pH. In fact, it was found that all the latex particles prepared with the three amphoteric initiators in the pH range 3–10, were of amphoteric nature, except for the particles prepared with 2CE at pH 10. In both cases using CM or 1CE initiator the amphoteric groups were not degraded and could be incorporated into the particle surfaces even under a strongly alkali state, i.e., around pH 10.

Regarding the poly(St/AAm) particle systems, the isoelectric points of the particles prepared at pH 3 with 1CE or CM were almost the same at about 3.5, whereas that of particles with 2CE was close to 4. However, in the poly(St/MMA) particle systems, the isoelectric point of the particles prepared with 1CE was about 4, slightly higher than that with 2CE (3.5). The cause cannot be clearly understood now. The poly(St/MMA) particles had about 3 times larger electrophoretic mobility than those for poly(St/AAm) particles at pHs below 2.5 and higher than 10. These results seem to be in accordance with the number of initiator fragments anchored onto the surfaces of the poly(St/MMA) and poly(St/AAm) particles (see Surface charge density). In addition, at electrophoretic

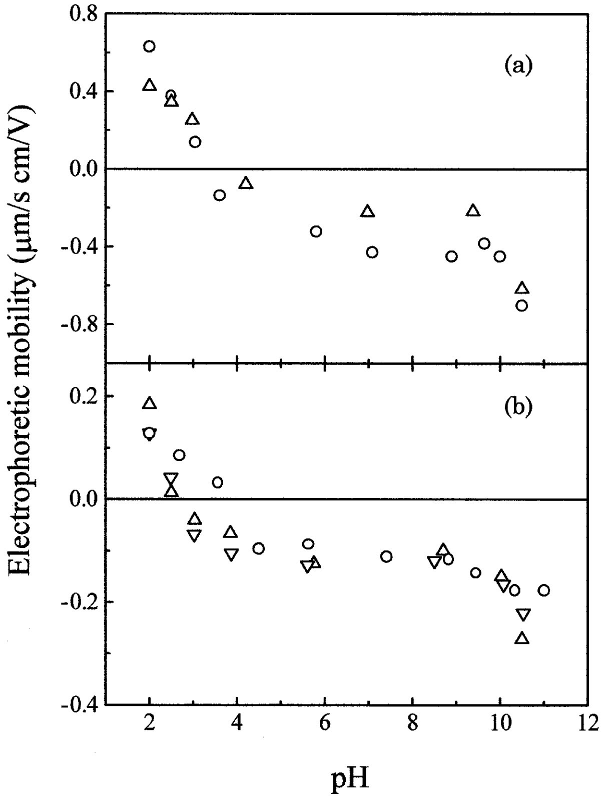


Fig. 7 pH dependence of electrophoretic mobility of latex particles prepared with various amphoteric initiators at pH 3.0. (*a*) Poly(St/MMA): 1CE (△), 2CE (○); (*b*) P(St/AAm): 1CE (△), 2CE (○), CM (▽)

pH from 5 to 9 the electrophoretic mobilities were constant for the particles prepared with 1CE and 2CE. Apparently, the pH dependence of the electrophoretic mobility of the particles prepared with the three kinds of initiators corresponds to that of their dissociation.

Surface charge density

The surface charge densities of the polymer particles prepared with the amphoteric initiators at various polymerization pHs for the St/MMA and St/AAm systems are shown in Table 3. The number of ionic groups on the surface of either poly(St/AAm) or poly(St/MMA) particles was almost independent of the polymerization pH and the kinds of amphoteric initiators if they were used at the same concentration. For the St/AAm polymerization, the CM system gave fewer ionic groups than the 2CE and 1CE systems under corresponding conditions. For the St/MMA polymerization, the 2CE system gave a slightly larger number of ionic groups than the other two systems of 1CE and CM, the latter two having almost the same number. However, the surface charge density increased in the sequence 2CE, 1CE and CM. This was probably caused by their different solubility in water. 2CE was highly water-soluble and was supposed to have a higher stabilization force than the others. If the polymerizations were carried out under the same initiator concentration and polymerization pH, the poly(St/MMA) particles had about 3 times higher charge density than the poly(St/AAm) particles in the 2CE and 1CE systems as well as about 5–10 times in the CM system.

Table 3 The surface charge density (*SCD*) (x10^{-2} molecules/nm^2) of various latex particles prepared with various amphoteric initiators at different polymerization pHs and the number of surface ionic groups measured by conductometric titration (μEq/gpolymer) (*SIG*)

Initiator	pH	Poly(St/MMA)		Poly(St/AAm)	
		SIG	SCD	SIG	SCD
2CE	3.0	9.0	33.8	5.7	11.1
	5.8	10.6	43.8	4.4	9.0
	10.0	10.8	56.4	11.6	27.2
1CE	3.0	6.4	36.2	4.3	10.2
	5.8			4.2	10.4
	10.0	6.2	41.9	4.3	11.4
CM	3.0	6.0	50.1	3.4	5.7
	5.8	6.0	54.8	3.6	7.6
	10.0	6.8	48.9	3.8	9.4

Critical flocculation concentration

The pH dependence of the critical flocculation concentration of poly(St/MMA) latex particles prepared with amphoteric initiators at a polymerization pH of 3 is shown in Fig. 8. It clearly indicates that the critical

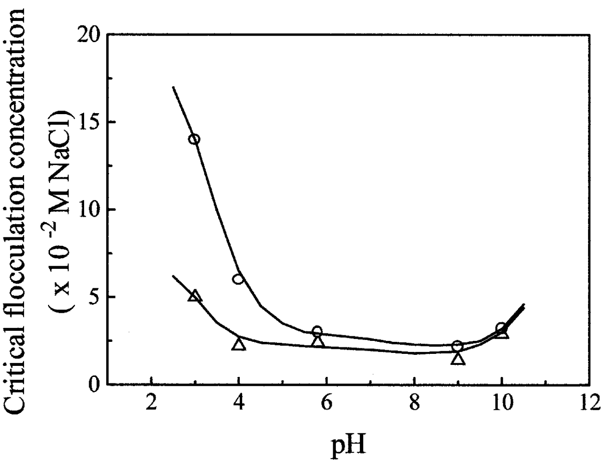


Fig. 8 pH dependence of the critical flocculation concentration of poly(St/MMA) latices prepared with various amphoteric initiators. Initiator concentration 5.2 x 10^{-3} mole/l, pH 3.0, $T = 70$ °C; 2CE (○), 1CE (△)

flocculation concentration initially decreased with increasing pH of the medium and took a minimum value at pH 6–8, then slightly increased with an increase in the pH of the medium. The result was attributed to the fact that the surfaces of the poly(St/MMA) particles produced with the amphoteric initiators were covered with dissociable initiator fragments, which can induce the interparticle electrorepulsive force to stabilize the polymer particles. The stabilization of latex particles is sensitive to the surface conditions [17, 18]. For the poly(St/MMA) latex particles prepared by emulsifier-free polymerization, initiator fragments naturally incorporated towards the particle surfaces for stabilization. Therefore, in a lower or higher pH range, the poly(St/MMA) latex particles exhibited a much larger critical flocculation concentration because initiator fragments were highly ionized.

Conclusions

A set of amphoteric initiators were efficient to initiate the emulsifier-free emulsion polymerizations so that stable amphoteric latices were produced. The constants of the decomposition rate and the dissociation of the amphoteric initiators depended upon the pH of the medium. Using amphoteric initiators the polymers had comparable weight-average molecular weights as polystyrene prepared with potassium persulfate. The polymerization rates in the St/MMA system were lower than in the St/AAm one. Both the conversions, except for the case with 2CE and pH 10, and the number of ionic groups on the surfaces were independent of the polymerization pH. The electrophoretic mobility versus electrophoretic pH curves for the St/MMA and the St/AAm colloidal particles indicated the amphoteric nature of the particles, which originated from the initiators used. The surface charge density of poly(St/MMA) latex particles was about 3–10 times, depending on the type of amphoteric initiators, higher than that of poly(St/AAm) particles which were prepared under corresponding conditions.

Acknowledgements The authors gratefully acknowledge T. Okukawa and H. Tsuji from Wako Pure Chemical Industries Ltd., Japan, for helpful discussions. S.-J. Fang is grateful to the Japan Society for the Promotion of Science for a fellowship. This work was specially supported by the Ministry of Education, Science, Sports and Culture of Japan, and also by the SRF for ROCS, the Ministry of Education of China.

References

1. Kawaguchi H, Fujimoto K (1999) Bioseparation 7:252
2. Tamai H, Sakurai H, Hirota Y, Nishiyama F, Yasuda H (1995) J Appl Polym Sci 56:441
3. Bastos D, Delas Nieves FJ (1996) Colloid Polym Sci 274:1081
4. Inomata Y, Wada T, Handa H, Fujimoto K, Kawaguchi H (1994) J Biomater Sci Polym Ed 5:293
5. Kondo A, Imura K, Nakama K, et al (1994) Ferment Bioeng 78:241
6. Arshady R (1993) Biomaterials 14:5
7. Hatakeyama M, Iwato S, Fujimoto K, Handa H, Kawaguchi H (1998) Colloids Surf 10:161
8. Ohtsuka Y, Kawaguchi H, Sugi Y (1981) J Appl Polym Sci 26:1637
9. Ottewill RH (1982) In: Piirma I (ed) Emulsion polymerization. Academic, New York, pp 1–47
10. Tamai H, Fujii A, Suzuwa T (1987) J Colloid Interface Sci 116:37
11. Kawaguchi H, Hoshino H, Ohtsuka Y (1983) Colloids Surf 6:271
12. Fang *S*-J, Fujimoto K, Kondo S, Shiraki K, Kawaguchi H (2000) Colloid Polym Sci 278:864
13. Hoshino F, Fujimoto T, Kawaguchi H, Ohtsuka Y (1987) Polym J 19:241
14. Wako Pure Chemical Industries, Japan (1999) Technical documentation on 2,2′-azobis(*N*-(2-caboxyethyl)-2-methylpropionamidine) amphoteric initiator
15. Fitch RM, Tsai CH (1971) In: Fitch RM (ed) Polymer colloids. Premium, New York, pp 73–116
16. Lichti G, Gilbert RG, Napper DH (1982) In: Piirma I (ed) Emulsion polymerization. Academic, New York, pp 94–145
17. Kawaguchi H, Hoshino H, Amagasa H, Ohtsuka Y (1984) J Colloid Interface Sci 97:465
18. Duracher D, Sauzedde F, Elaissari A, Pichot C, Nabear L (1998) Colloid Polym Sci 276:920