Colloid Polym Sci (2007) 285:819–826
DOI 10.1007/s00396-006-1631-3

ORIGINAL CONTRIBUTION

Preparation of pH-sensitive hydrogel microspheres of poly(acrylamide-*co*-methacrylic acid) with sharp pH–volume transition

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Received: 21 October 2006 / Accepted: 4 December 2006 / Published online: 26 January 2007

Abstract Monodisperse hydrogel microsphere of polyacrylamide (AAm)-methacrylic acid (MAc) cross-linked by *N*,*N*′-methylene-*bis*(acrylamide) (MB) with sharp pH–volume transition was prepared in ethanol. The dynamic light scattering (DLS) was employed to evaluate the pH sensitivity of these microspheres. The effects of main factors: composition of copolymer, cross-linked degree, and initial total concentration or solid content of comonomers were investigated. Osmotic pressure and deformation of cross-linked polymer network were considered as the two dominant factors influencing the characteristics of pH–volume transition. High content of MAc and cross-linked degree increased the osmotic pressure, thereby moving the onset of pH–volume transition to higher pH. Association/dissociation of poly-MAc segments in the domains contributed to the free energy of hydrogel–solvent mixing. As soon as pH was high enough to overcome the osmotic pressure, the dissociated poly-MAc segments simultaneously decreased the osmotic pressure and free energy of hydrogel–solvent mixing, thereby allowing the sharp and large volume transition. As a result, microspheres were prepared with pH–volume transition of almost 12 times to their original volume within a narrow range of pH variation, ca. 0.5.

Keywords Hydrogel microsphere · pH-sensitive ·
Poly(acrylamide/methacrylic acid) ·
Sharp pH–volume transition

Introduction

Microcapsules have been widely applied in various fields as the controlled releasing of medicines [1], bioactive substances [2], catalysts [3], cosmetics [4], etc., and, even more, as the microchambers for the controlled reactions [5]. Conventional concept of microcapsules includes two parts, i.e., the released core and the controlling wall materials. The diffusion rate of core molecules passing through wall material, the physical dissolution or breakage of wall materials, and the chemically hydrolyzed disruption of wall materials were usually the main factors utilized to controlled-release core substances. Therefore, these controlled-releasing behaviors exactly should be called the sustained release. Recently, with the development of chemotherapeutic and DNA repairing technique, the microcapsules that enable to fine control the releasing of medicines were

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Electronic supplementary material The online version of this article (doi:10.1007/s00396-006-1631-3) contains supplementary material, which is available to authorized users.

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boomingly developed, namely, the microcapsules released the medicines only under the specific conditions such as the ionic strength including pH and temperature. A small stimulus triggering drastic releasing was pursued. Accordingly, a new concept of controlled releasing was suggested, i.e., nonwall microcapsule. A hydrogel microsphere that consists of materials with the characteristic of deswollen–swollen volume change in response to pH or temperature change [6] is a typical example. The transition of hydrophobicity–hydrophilicity is a well factor to capture and excel a molecule from a microsphere without any wall hindrance. Hence, a sharp controlled releasing is expected. For example, when the thermosensitive poly(*N*-isopropyl acrylamide) (PNIPAM) was grafted on the surface of polystyrene (PS) particles [7, 8], the particles showed sharp hydrodynamic diameter changes in response to a small variation of temperature over the LCST of PNIPAM, and, corresponding to the diameter contraction, the loaded dye molecules were promptly released by the shell of PNIPAM.

pH sensitivity is another applicable factor [9], and compared with thermosensitivity, it gives more choices both for the materials and applying environment. pH-sensitive hydrogel microspheres are one of the examples. They have been prepared, and their pH sensitivity has been investigated too [10–15], but how to obtain the microspheres with sharp pH–volume transition has not been studied. Therefore, in this paper, we will address on the preparation of pH-sensitive hydrogel microspheres with sharp pH–volume transition.

Experimental

Materials

All the reagents and solvents used in this paper were purchased from Wako Pure Chemical Industries, Japan. Monomer methacrylic acid (MAc) was purified by distillation under the reduced pressure. Monomer acrylamide (AAm), cross-linking agent *N*,*N*′-methylene-*bis*(acrylamide) (MB), initiator dimethyl 2,2′-*azo*-*bis*(isobutyrate) (DMAIB), and solvent ethanol (EtOH, dehydrated) were used without further purification.

Methods

The batch polymerization was performed in a 300-ml four-neck, round-bottom separate-type reactor equipped with a stirrer, a condenser, and a nitrogen inlet and a rubber stopper for adding the initiator with a syringe. The formulated MAc, AAm, and MB were dissolved in ethanol and added into the reactor. Nitrogen was bubbled to deoxidize the mixture of reagents for 1 h before elevating the temperature, and the nitrogen blanket was maintained throughout the polymerization. The initiator solution (0.1 g/5 g ethanol) was injected into the reactor when the temperature attained the polymerization temperature, 60 °C. Agitation rate was ca. 200 rpm, and the polymerization time was ca. 14 h for all experiments if no specific comment.

Characterization

The conversion of monomer was determined by gravimetry. The prepared dispersions were cleaned by performing trice the recycling of centrifugation (10,000 rpm/5 min)–redispersion in ethanol centrifugation and then dried under vacuum at room temperature. The above samples (powders) were also used for the observation of scanning electron microscopy (SEM) (SEMEDX type-N, Hitachi High-Tech, Tokyo, Japan) by which the SEM diameters of particles were determined. Pt was used as the sputtering material to coat the SEM samples. The diameters of about 200 microspheres in an SEM photo were measured for one sample by using a computer program. The number-average diameter and the average root-square deviation of the diameter were used for the characterization of the diameter and its distribution.

A dynamic light scattering (DLS) instrument with a He–Ne laser source at 90° (Photal ELS-8000, Otsuka Electronic, Osaka, Japan) was employed to measure the hydrodynamic diameters of microspheres at 20 °C and to calculate the diameters and their distributions automatically based on Cumulant’s calculation method, and among them, the scattering strength diameter (relative to the weight-average and number-average diameter) was selected to evaluate the volume change. Individual measurements were automatically made every 30 s by the machine; however, to exclude the occasional causes, as a run of detection, 100 individual measurements were continuously performed, and their averaged results were drawn in one diagram. Moreover, to insure the measurements of small-size species, the range of measuring diameters was specified at 10–4,000 nm within the declared measurable range of diameters of the machine (6–6,000 nm). The 0.5-wt% aqueous solution used for DLS measurements was prepared as following. The dry sample treated with the method described above was mixed with DDI water in a 30-ml screw bottle and put in an ultrasonic automatic washer to disperse for 1 h (Iuchi US-4, SMT, Fukuoka, Japan). 1 M HCl (NaOH) was used to adjust the pH of dispersion. The pH value of pH-adjusted dispersion was measured with a pH meter (MP220, Mettler-Toledo AG, CH8603, Schwertzenbach, Switzerland). It was used to evaluate the pH sensitivity of microspheres in this paper. All measurements of DLS were performed at 20 °C if no specific comment.

Results and discussion

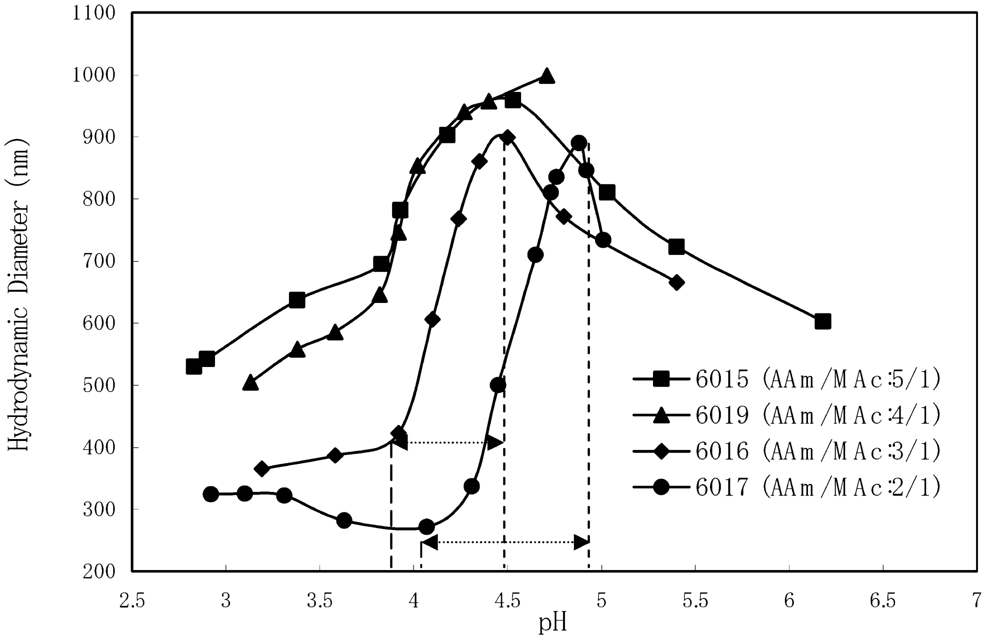
Ratios of AAm/MAc

At the level of 2 wt% solid content, the cross-linking degree (CLD) of 5 mol% was firstly formulated with AAm/MAc= 5/1 (Supplementary material Table 1, Run6014). The microspheres were prepared, but they were swollen too much to be measurable. Even at pH as low as ca. 2, the aqueous solution was transparent. Because DLS was established on the light scattering of domains, the above result indicated that the domain confined by hydrogel network was not established in a microsphere. According to the mechanism of microsphere formation [10–12, 16, 17], the minimonomer droplets firstly formed due to the decrease of solubility of AAm–MAc complexes with the increase of temperature. It implies that, at lower solid content as much as 2 wt%, the amount of cross-linking agent, MB is larger, which was not taken in the minimonomer droplets, especially in the case of high ratio of AAm/MAc. Therefore, the cross-linked degree of 5 mol% was too low. A 10-mol% CLD was chosen for the following experiments.

On the other hand, when AAm/MAc=1/1 (Run6018), the microspheres were also prepared, but a problem arose that they agglomerated during the process of separation from ethanol, and more notably, the agglomerates could not be dispersed in water. In ethanol, the microspheres may be swollen due to high content of MAc segments, i.e., they were soft. The pH-sensitive results of other samples are shown in Fig. 1.

Hydrogel microsphere is a networked structure of polymer chains cross-linked to each other and surrounded by an aqueous solution. Many factors affected the equilibrium of swelling/deswelling of hydrogel microspheres. At present, there is no quantitative approach applicable to discuss the behavior of hydrogel swelling; instead, two models are popularly used to qualitatively describe the swelling equilibrium, i.e., thermodynamics model developed by Siegel [18] and triphasic mechanoelectrochemical model postulated by Lai et al. [19]. The thermodynamic model considered three sources contributing to the total free energy of hydrogel–solvent system, namely, hydrogel–solvent mixing, deformation of polymer networks, and osmotic pressure of mobile ions, while the mechanoelectrochemical model combined the physicochemical theory for ionic and polyionic solutions expressed in terms of their chemical potentials which gradients were considered as the driving forces for their movements. Comparatively, the mechanoelectrochemical model tended to introduce the mechanical factors into the model with an assumption that the solid network deformation was small. These situations were not conformal to those of hydrogel microsphere. In the thermodynamic model, the surface tension, an inevitable factor in a microsphere system, was not considered. However, by adding a domain effect [20] included in the factor of osmotic pressure, this model was applicable. Accordingly, we considered that the factors of hydrogel–solvent mixing, deformation of polymer network, and osmotic pressure of mobile ions dominated the behavior of microsphere swelling. Therefore, segments of poly-MAc played the dominant role in the free energy of hydrogel–solvent mixing in terms of the hydrophobic/hydrophilic

Fig. 1 Effects of comonomer composition on pH–volume transition



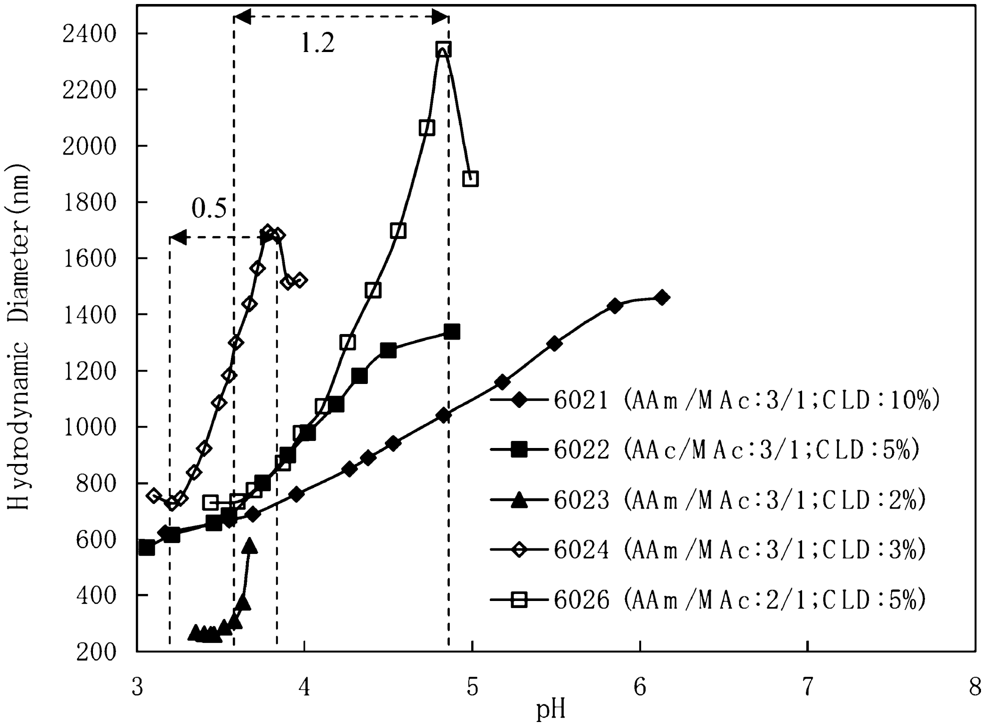
interaction. Associated poly-MAc segments were hydrophobic which unfavored to the hydrogel–solvent mixing, whereas dissociated poly-MAc segments drastically decreased the free energy of mixing due to their intensive hydrophilicity. Due to this characteristic of poly-MAc, it should be appropriate that the interaction of poly-MAc segments was a kind of physical cross-link that the switch of noncross-link/cross-link was completely dependent to the dissociation of MAc. Of course, the dissociated degree of poly-MAc was dependent to the deformation degree of polymer network and osmotic pressure of OH^- and Na^+ charged. Meanwhile, deformation degree was determined by the chain length between two cross-linking points, while the osmotic pressure was determined by the densities of cross-linking points and poly-MAc segments within the domain. In fact, a domain with high concentration of MAc units naturally produced a high osmotic pressure stemming from the electric shielding of counterions.

As shown in Fig. 2, a noticeable characteristic was that the hydrodynamic diameters of microspheres increased with the increase of AAm composition in the microspheres irrespective of pH. One of the reasons was, as commented above, that at high content of AAm, the less cross-linking agent MB was taken in the microspheres. The next mechanistic reason was that, at the first step of microsphere formation, the nuclei mainly by cross-linking agents and the coupling of the nuclei constitute a network structure [11, 12]. At high content of AAm, such a network structure contained more poly-AAm segments than those at high content of MAc. These two factors determined the lower cross-linked degree as well as lower osmotic pressure of domains inside of microspheres with higher content of AAm. Therefore, due to the lower osmotic pressure, even at lower pH, the microspheres with high content of AAm expanded readily and without apparent sharp transition of hydrodynamic diameter in the whole region of pH change.

Segments of poly-MAc contributed to the factors of hydrogel–solvent mixing and osmotic pressure. Mechanistically [11, 12], in the firstly formed network structure, the composition of poly-MAc segments was dominant due to its preferential copolymerization reactivity ratio. The bound poly-MAc segments in the network, as discussed in the thermodynamic models, contributing to the free energy of hydrogel–solvent mixing in virtue, could be regarded as a kind of physical cross-link. Therefore, as shown in Fig. 1, at lower pH, the hydrodynamic diameter of microspheres with higher content of MAc (Run6016, Run6018) almost kept unchanged due to higher cross-linked degree until at a pH where the osmotic pressure was overcome. The osmotic pressure increased with the increase of MAc content, thereby the onset of sharp change of diameter moving to higher pH as the content of MAc increased. When the osmotic pressure was overcome, it was imaginable that both the favorable free energy of hydrogel–solvent mixing and decross-linking of bound poly-MAc segments in the domains made the diameter of microspheres drastically increase with the increase of pH.

Another noticeable characteristic was that, as shown in Fig. 1, all the hydrodynamic diameters drastically decreased after passing a maximum value. The reason was unclear so

Fig. 2 pH–volume transition of microspheres with different cross-linked degree



far. A possible explanation was that the scattering domains degraded at higher pH.

The monotonic variation of hydrodynamic diameter vs pH is pursued for our purpose, namely, in the proper range of ion strength, the hydrodynamic diameters just depended on the value of pH regardless of the process of pH adjustment (for the concision, the figures are combined in Fig. 3). This property provides a tool to exactly control the microspheres. It was confirmed that the hydrodynamic diameters were dependent to the pH value irrespective of the pH-adjusting process.

As shown in Fig. 1, the more MAc was added, the larger or sharper change of volume was observed; additionally, both the onset of sharp change and the maximum value of diameter moved to the higher pH. pK_a of MAc was usually introduced to evaluate the pH sensitivity of hydrogel [21, 22]. pK_a of free MAc is 4.7, but that of poly-MAc was reported to be between 6 and 7 [21, 22]. However, no apparent relationship between these pK_a values and sharp transitions was found in this work. In fact, according to the thermodynamic model of hydrogel, the pK_a values of MAc obtained from free MAc or poly-MAc were not applicable for the segments of poly-MAc bound in the cross-linked domains. The osmotic pressure that mobile ions, i.e., OH^- and Na^+, evaded into the cross-linked domains was the most important factor to determine the pH value where the pH–volume transition happened. This will be discussed later.

Finally, it should be remarked that, in this paper and our successive papers, all the pH values refer to the final pH values of a solution where microspheres have been dispersed. As we know, the dispersed microspheres will change the original pH of a solution, especially in the solution used for DLS measurements where at least 0.5 wt% of microsphere powder was needed, which is high enough to obviously change the original pH of solutions. In these cases, if the original pH was used to evaluate the pH sensitivity of microspheres, the results likely quite different from ours were concluded. Buffer solution was usually employed to evaluate the pH sensitivity of hydrogel [21, 22], but the additional effects of other ions on the pH sensitivity were inevitable. In fact, RCOOH and RCOONa are a pair of components composing of buffer solution. Corresponding to a series of original pH values, when the microspheres were dispersed, the final pH values of solutions virtually are the same or change little compared with the original ones, especially in the cases of microspheres with high MAc composition. Therefore, it is possibly reasonable to draw a different conclusion.

We considered that, in the practical application, it is unexpected that the pH of original system is obviously changed due to the addition of pH-sensitive microspheres. The pH-sensitive microspheres should sharply sense the change of environmental pH. Therefore, the final pH of a solution, i.e., the pH of a solution where microspheres had been dispersed, was the realistic factor to evaluate the pH–volume sensitivity of pH-sensitive microspheres. Of course, more NaOH solution was required to change the final pH value in the case of microspheres with high content of MAc.

In this study, there is a problem—that is how to evaluate the sharpness of the change, namely, where is the onset of change? Because there is no any strict standard, we

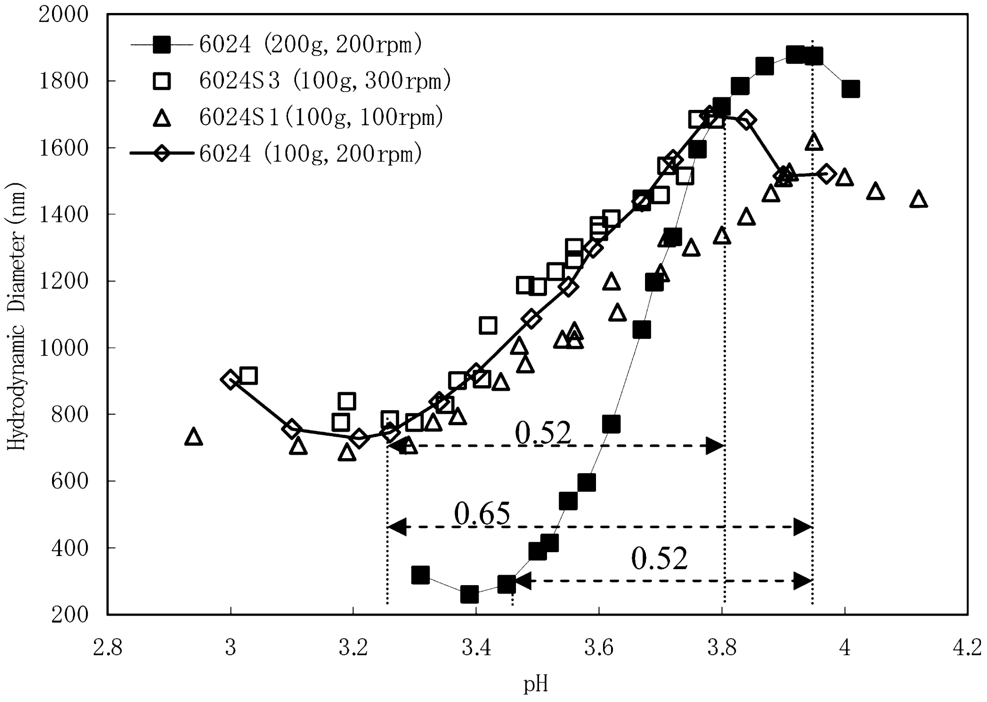


Fig. 3 pH–volume transition of microspheres prepared at different agitation rate

considered that the sample of Run6016 was the best of them for the application, which had a relatively definite onset and a narrower range of pH (ca. 0.5) compared with those of Run6017 (ca. 0.8).

Solid content and cross-linking degree

In this section, we report the effects of total initial concentration of monomers or solid content on the pH sensitivity of microspheres. According to the results reported above, the recipes of AAm/MAc=3/1 and 2/1 were selected for the investigations. The recipes are shown in Table 1, and the results are shown in Fig. 2.

A most noticeable characteristic of hydrodynamic diameters shown in Fig. 2 is that, at lower pH, i.e. at the nonswollen state, all the hydrodynamic diameters were generally bigger than those of microspheres prepared at 2 wt% solid content regardless to the cross-linked degree and ratios of AAm/MAc, whereas their SEM diameters (shown in supplementary material Table 1) told little difference. For example, Run6016 and Run6021 had the same level of cross-linked degree as well as ratio of AAm/MAc. Their SEM diameters were almost equal; however, the hydrodynamic diameter of Run6021 at pH 3.5 (Fig. 2) was almost two times as big as that of Run6016 (Fig. 1). This result is considered to be resulted from the different molecular weight of polymer prepared at different initial concentration of monomers. We will show in the later papers that the higher concentration of monomers may give the higher molecular weight of polymer.

The structure of microspheres prepared at different solid content was different. Evidence was that, with the same CLD, 10%, the microspheres show a sharp diameter transition at 2 wt% (Fig. 1, Run6016); however, a broad diameter transition was observed at 5 wt% (Fig. 2, Run6021). Moreover, in the case of 2 wt% solid content, when CLD was equal to or lower than 5%, the microspheres were too overswollen to be measurable in the aqueous solution. However, in the case of 5 wt%, as shown in Fig. 2, the lower limitation of CLD changed—for instance, when AAm/MAc=3/1, CLD could be as low as 3%—while as for AAm/MAc=2/1, CLD could be as low as 5% (Run6026). The number of domains wherein the MAc segments are bound is larger in a microsphere prepared at 5 wt% compared with that prepared at 2 wt%. Hence, to prepare the microspheres with sharp transition with 5 wt%, CLD must decrease. It was verified that the pH–volume transition became sharper with the decrease of CLD (Fig. 2, Run6021–6025). Another result observed in Fig. 2 is that the onset of sharp transition moved to higher pH with the increase of cross-linked degree. It gives further evidence proving that the osmotic pressure was a key factor for the pH–volume transition. Run6026 had a recipe of AAm/MAc =2/1; therefore, the onset of pH–volume transition moved to pH 3.6, and its pH–volume transition became broader due to its higher cross-linked degree (CLD= 5 mol%) compared with that of Run6024 (AAm/MAc= 3/1) (pH 3.2).

Agitation rate and other polymerization parameters

It has been reported [9–17] that the operation parameters of polymerization such as agitation rate, total volume of reaction mixture, solvent, and temperature affected the size and its distribution, composition of polymer, as well as morphology of microspheres. Therefore, it is possible that the operation parameters of polymerization should affect the pH sensitivity of microspheres. Figure 3 shows the pH–volume transition of microspheres prepared at different conditions. It should be pointed out that the data of 6024S1 and 6024S3 were randomly measured, namely, that the pH adjustment was randomly made. The good reproducibility of pH and diameter proved that the diameter was monotonically dependent to pH irrespective of pH-adjusting paths. As we discussed above, Run6024 having sharp pH–volume transition was prepared at the conditions of 100 g total reaction mixture and 200 rpm agitation rate. The other conditions hence were applied for the preparation of microspheres, i.e., 100 and 300 rpm with the total amount of reaction mixture, 100 g, and 200 rpm with total amount of reaction mixture, 200 g. It should be remarked that, prepared under these conditions, no obvious variation was found in the size and its distribution as well as the morphology of microspheres. However, as shown in

Table 1 Characteristics of microspheres with sharp pH–volume transition

Run	AAm/MAc (mol/mol)	CLD (mol%)	Solid (wt%)	pH_{onset}	pH_{max}	ΔpH	ΔD (nm)	D_{max}/D_{onset}	$\Delta D/\Delta pH$
6016	3/1	10	2	3.9	4.4	0.5	599	2.25	1,198
6017	2/1	10	2	4.1	4.9	0.8	618	3.27	773
6024	3/1	3	5	3.3	3.8	0.5	950	2.27	1,900
6026	2/1	5	5	3.6	4.8	1.2	1,600	3.19	1,333

pH and hydrodynamic diameters were measured at 20 °C ($\Delta pH = pH_{max} - pH_{onset}$; $\Delta D = D_{max} - D_{onset}$).

CLD Cross-linking degree, *D* hydrodynamic diameter, D_{onset} the artificial onset diameter, D_{max} the biggest diameter

Fig. 3, the pH–volume transition of microspheres shows some differences. For example, with the total amount of reaction mixture, 100 g, the pH–volume transition of microspheres prepared at 300 and 200 rpm, respectively, had no difference. The two curves overlapped with the sharp pH–volume transition range of 0.52, whereas, at 100 rpm, the pH–volume transition range of microspheres was 0.65 and the maximum hydrodynamic diameter was smaller than those at 200 and 300 rpm. Microspheres prepared with the total amount of reaction mixture, 200 g, and 200 rpm gave bigger maximum diameter, bigger difference of diameter, and higher onset of pH, or in other words, the microspheres prepared with 200 g have sharper pH–volume transition than those prepared with 100 g although they had the same range of pH transitions, 0.52.

In fact, the effects of agitation rate on the properties of microspheres have not been clarified by far although many mechanisms for the microspheres formation have been postulated. Nevertheless, because the initiator, DMAIB, was applied in the polymerizations, which dominantly partitioned in the continuous phase [16, 17], the capture of entities generated in the continuous phase into growing microspheres played an important role in the formation of microspheres. It was considered as the main factor affecting the pH–volume transition of microspheres.

As a summary of discussion so far, we listed the characteristics of microspheres with sharp pH–volume transition in Table 1. Run6016 and Run6024 were equally considered to be the microspheres with sharpest pH–volume although their $\Delta D/\Delta$pH were different. Namely, their volumes expanded or shrank almost 12 times ($D_{\max}/D_{\text{onset}}$=2.25~2.27) as much as their original volume within such a narrow range of pH variation as much as about 0.5. The value of ΔD was another interesting parameter. As shown in Table 1, the ΔD values of microspheres prepared at 5 wt% were fairly bigger that those prepared at 2 wt%. For example, the ΔD of Run6026 was 1600 nm, but the ΔD of Run6017 was 618 nm. Obviously, their difference reflected the effects of cross-linked degree inside of microsphere, or in other words, the deformable degree of cross-linked polymer network in a microsphere. The lower cross-linked degree there was, the larger degree the cross-linked polymer network was deformable. On the other hand, the data of pH_{onset} and $\text{pH}_{\max}$ indicated that the range of pH where the pH–volume transition of microspheres was sharp was selectable from 3.3 to 4.9.

Conclusions

Targeting to prepare the P(AAm/MAc/MB) microspheres with sharp pH–volume transition, we investigated the effects of main factors: composition of copolymer, cross-linked degree, and initial total concentration or solid content of comonomers. Thermodynamic model was better to explain the pH sensitivity of hydrogel microspheres. The osmotic pressure required for OH^- ions invading domains rich in MAc segments and the deformation of cross-linked polymer network were considered as the two dominant factors influencing the characteristics of pH–volume transition. High content of MAc and high cross-linked degree increased the osmotic pressure, thereby moving the onset pH value of sharp pH–volume transition to higher pH. Meanwhile, association/disassociation of poly-MAc segments in the domains contributed to the free energy of hydrogel–solvent mixing by means of hydrophobic/hydrophilic interactions as well as the osmotic pressure in terms of counterion-shielding effects within locally concentrated poly-MAc domains. Therefore, at high content of MAc, when pH was high enough to overcome the osmotic pressure, the dissociated poly-MAc segments simultaneously decreased the osmotic pressure and free energy of hydrogel–solvent mixing, thereby the sharp and large volume transition was expectable. Additionally, the structure of microsphere was different as it was prepared at different solid content. At higher solid content, the more cross-linking agent MB was taken in the network structure forming at the early stage of microspheres formation, which resulted in larger number and smaller domains in a microsphere. Based on these results, the microspheres were prepared that the volumes expanded or shrank almost 12 times ($D_{\max}/D_{\text{onset}}$=2.25~2.27) as much as their original volume within such a narrow range of pH variation as much as about 0.5.

Acknowledgments This project was funded by the Hekel Co. Ltd (2001.6–2003.3) and was accomplished in the Hekel Research Center of Advanced Technology, Institute of Molecule Engineering, Kinki University in Kyushu, Japan.

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