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ORIGINAL CONTRIBUTION

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

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Abstract The forming process and characteristics of monodispersed hydrogel microspheres of poly(acrylamide–methacrylic acid) with sharp pH–volume transition were studied. pH-/ion-sensitive and thermosensitive behaviors of microspheres sampled at various stage of polymerization were evaluated by using the dynamic light scattering. It was observed that the sharpness of pH–volume transition increased with the increase in monomer conversion. Both thermo- and ion-sensitive behaviors were affected by pH. At pH 4.3, the hydrodynamic diameter of microspheres monotonically and slightly decreased with the increase in temperature, whereas at pH 3.5 and 3.8, the curves of thermo–volume transition were similar to those of pH–volume transition with a maximum temperature at 25 and 20 °C, respectively. Increasing the [$CaCl_2$] was to decrease the hydrodynamic diameters of microspheres, irrespective of pH. However, a region at lower [NaCl] was found, where the diameter increased with the increase in [NaCl]. Moreover, the range of diameter increasing extended to higher [NaCl] as pH increased.

Keywords Hydrogel microsphere
Poly(acrylamide–methacrylic acid) · pH-sensitive ·
Thermosensitive · Ion-sensitive

Introduction

Hydrogels are three-dimensional hydrophilic polymer networks that can be swollen to many times their mass and volume in aqueous environments [1, 2]. Polyelectrolyte hydrogel is known to exhibit additional swelling in response to environmental stimuli, such as pH [3], polarity of the solvent [4], ionic strength or electric field [2, 5], and temperature [6]. Hydrogel microspheres of poly(acrylamide [AAm]/methacrylic acid [MAc]) are typical polyelectrolyte hydrogel. Their preparation methods and pH-sensitivity have been widely studied [7–13, Ni et al., submitted for publication]; however, systematical investigation of microspheres responding to other stimuli such as temperature and ionic strength has not been carried out yet. Compared with the rods, blocks, or fibers of hydrogel, the microspheres have smaller sizes, thereby the entire response of hydrogel to stimuli is evaluated readily, which is advantageous to elucidate the relationship of structure and response. For example, the studies on pH–volume transition of poly (AAm/MAc) microspheres disclosed the structure of polymer network inside of a microsphere [11–13, Ni et al., submitted for publication]. It provided strong evidence to support the mechanism of microspheres by polymerization [7, 8, 14–16]. Therefore, it is necessary to further investigate the response of microspheres to other stimuli.

In the previous paper, we reported on the preparation of hydrogel microspheres with sharp pH–volume transition (Ni et al., submitted for publication). As a series of work, in this paper, we will continuously address the topics on the

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characteristics of microspheres with sharp pH–volume transition prepared in the previous paper, such as their thermosensitivity and sensitivity to other ions besides pH.

Experimental

Materials

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

Methods

The general methods to prepare the microspheres have been described in the previous paper (Ni et al., submitted for publication). Only one recipe was selected in this paper, i.e., AAm/Mac = 3/1 (mol/mol), cross-linking agent MB, 3 mol% based on the total amount of comonomer, corresponding to the recipe of microspheres with sharp pH–volume transition. The total initial concentration or solid content was 5 wt.%. In the case of sampling during the polymerization, a total amount of 700 g reaction mixture was selected, which else a total amount of 100 g reaction mixture was used. The samples for conversion measurements were treated as following: approximately 3 g dispersion was extracted from the reaction system with a syringe and then immediately put in a −30 °C refrigerator to quench the polymerization (in a 10 ml screw bottle). The samples were stored in the refrigerator until they were weighted at room temperature. It took about 30 min for each sample to recover the temperature from −30 °C to room temperature before weighting. The samples were then treated twice at room temperature with cycles of centrifuge (13,000 rpm/5 min)-draining serum-dispersion, and then dried in the vacuum oven at room temperature for 24 h.

Characterization

The general characterization methods and equipments were introduced in the previous paper (Ni et al., submitted for publication). Here, to avoid confusion, we remarked again that all the pH values mentioned in this paper were the pH values of dispersion measured after the pH was adjusted, not the initial pH of solution used to adjust the pH of dispersion. Therefore, the effect of dissociation of microspheres on the pH of solution was included in the pH values given in this paper. With respect to the effects of ionic strength on the hydrodynamic diameter of microspheres, the investigations were carried out as following. The aqueous solutions of NaCl (or $CaCl_2$) with various concentrations such as 0.01, 0.1, and 1 N were preliminarily prepared without further calibration. The ionic strength in the dispersion was calculated in accordance with the volumes of dispersion and salt solution added. As for the effects of temperature on the pH-sensitivity of microspheres, the cells containing the dispersion solution were settled in the chamber, which the temperature was controllable for 30 min before starting the operation of dynamic light scattering (DLS) measurements.

Results and discussion

Structure and pH-sensitivity during the microspheres formation

Hydrogel microspheres form by the polymerization with minimonomer droplets composed of AAm (MB)-MAc complexes because of the decrease in complex solubility in EtOH with the increase in temperature [7, 8, 14]. Structure development of hydrogel microspheres at each step of conversion suggests that the polymerization proceeded in two steps [12, 13]. First, formation of nuclei takes place mainly by cross-linking agents and coupling of nuclei to form the network structure. Then, the remaining minimonomer droplets are incorporated onto the microsphere surface and further cross-linking with free double bonds in microsphere takes place, thereby to form different domains. MAc is more reactive than AAm [10–13], hence the nuclei rich in poly-MAc segments is expectable. Such an image of structure development should reflect in the development of pH-sensitivity during the polymerization. Therefore, we selected the recipe of microspheres with sharp pH–volume transition as the research object. The variation curves of conversion and diameters vs. polymerization time were shown in Fig. 1, respectively, meanwhile, some scanning electron microscopy (SEM) photos of microspheres forming at different stage of conversion were given in Fig. 2. As shown in Fig. 2, the microspheres were ellipsoidal spheres from the very beginning of polymerization. Therefore, two diameters were given simultaneously in Fig. 1. The kinetic of polymerization was generally similar to those else reported in [10–13]. The conversion rapidly increased until 6 h, approximately 70 wt.%, and then turned to slowly increased within the last 6 h. Both longer diameter and shorter diameter increased simultaneously until the fourth hour, at which time point the shorter diameter leveled off, but the longer diameter obviously increased.

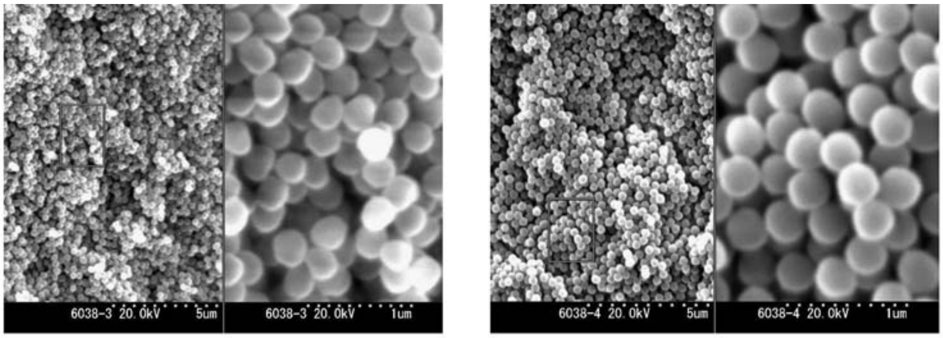

Fig. 1 Conversion and average SEM diameters vs. polymerization time

a) Polymerization time: 2h, Conversion: 43%, b) Polymerization time: 3h, Conversion: 60%

The pH-sensitivity of these microspheres is shown in Fig. 3. A noticeable characteristic of pH–volume transition is that the microspheres formed at the beginning stage (0.5–3 h), the pH–volume transition was not reversible. For example, the intrinsic hydrodynamic diameter of microspheres obtained at 0.5 h was 725 nm corresponding to the intrinsic pH 4.4. In the previous paper (Ni et al., submitted for publication), we reported that the hydrodynamic diameter of microspheres monotonically changed with the change of pH, regardless to the process of pH-adjustment. However, as shown in Fig. 3 (0.5 h), when pH increased from the intrinsic pH 4.4 to 4.8 and then decreased passing through pH 4.4, the diameter became 650 nm. It implies that the microspheres lost some domains during the pH adjustment. The microspheres obtained at 1 h showed the similar pH-sensitive behavior, but for the microspheres obtained at 3 h, the hydrodynamic diameter was not measurable because of the low intension of scattering light during the process that pH was adjusted up to pH 4.50 and then down to pH 4.18. It was interesting that the diameter at its intrinsic pH 4.38 kept on constant, at least, for a day. This result further disclosed the formation process of the network in the microsphere, namely, that the network firstly formed at the beginning of polymerization, and then the successively formed domains fulfilled in the spaces of network. The domains grew with the propagation of polymerization, which in turn enlarge the mesh size of the network. As a result, it decreased the osmotic pressure, thereby the pH–volume became sharper, and the onset pH moved to lower pH. However, because the cross-link between the network and new domains was not completed, the new domains were lost while the network expanded at high pH. Anyway, it was a turning point because the microspheres obtained at fourth, and 12th hour, respectively, showed good reversibility of pH–volume transition as we reported (Ni et al., submitted for publication), except for that obtained at 6 h. It is remarkable that, in the case of 6 h, as shown in Fig. 3 (6 h), the hydrodynamic diameters decreased to almost half of the intrinsic diameter after pH was adjusted. Reminding of the irregular microspheres shown in Fig. 2c), we got a good hint that the lost part of microsphere in Fig. 2c) was a newly incorporated minimonomer droplet. The domain forming by the polymerization in the newly incorporated minimonomer droplet possibly lost in higher pH because of uncompleted cross-linking reactions with the host microsphere. It indicated that the post-6-h polymerization was very important to the structure development of microsphere because the completion of cross-link network was dependent to the reactions of free double bonds between the domains in the microsphere, although as shown in Fig. 1, almost 85% monomers had converted into polymer.

On the other hand, the pH–volume transition became sharper as the conversion increased, except for the curve of 0.5 h, meanwhile, the onset of pH at which the sharp pH–volume transition started moved to the lower pH with the propagation of polymerization. The microspheres obtained at 0.5 h showed a sharp pH–volume transition, resulting from the uncompleted structure of the network.

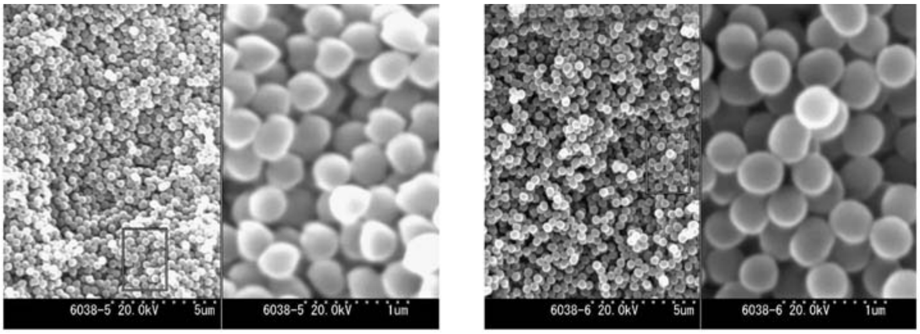

Fig. 2 Typical morphologies of microspheres

c) Polymerization time: 4h, Converison: 68.8%, d) Polymerization time: 6h, Conversion: 84.4%

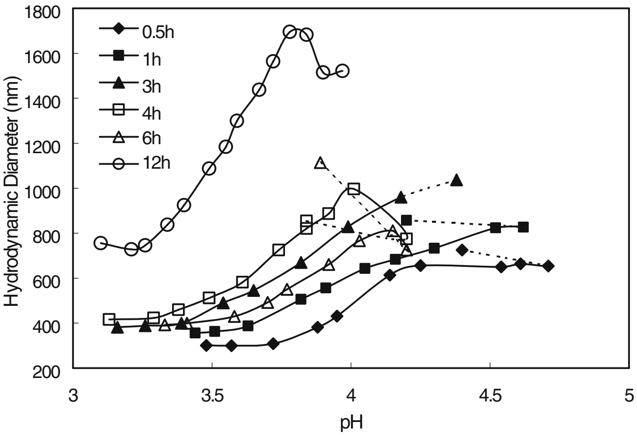

Fig. 3 pH-sensitivity of microspheres at different conversions

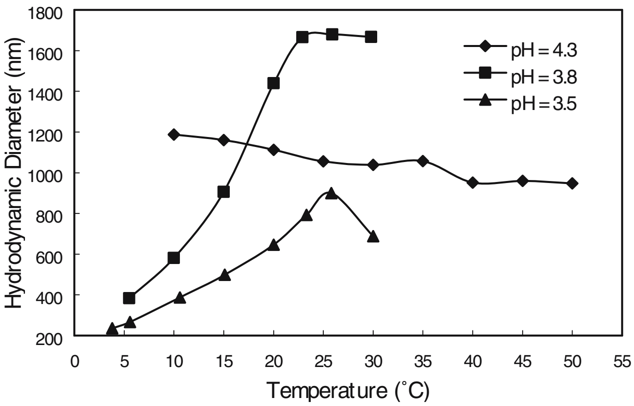

Fig. 4 Thermo-sensitivity of microspheres at different pH

Thermosensitivity of microspheres with sharp pH–volume transition

The phase transition responding to temperature change is the common characteristic of hydrogel. In principle, hydrogel has a volume phase transition temperature (VPTT) corresponding to the critical variation of hydrophilicity–hydrophobicity transition of hydrogel, i.e., it dissolves below VPTT because of the hydrophilicity, whereas above VPTT, this phase separates from the aqueous phase. VPTT was determined by the structure and composition of the polymer [17–19]. For example, poly(*N*-isoprapylacrylamide) hydrogel has a VPTT close to 32 °C, and moreover, it was reported that VPTT increased with the increase in pH [20]. As for poly-AAm, the VPTT was reported to be about 100 °C [21]. It implies that no phase transition is accessible at ambient temperature. However, Kawaguchi et al. [12] has reported that the hydrogel microsphere of poly(AAm/MAc) was found to contract continuously by heating, even at low pH. The microsphere with a lower-content MB exhibited more pronounced heat-contraction. Therefore, the thermosensitivity of microspheres likely attributed to the poly-MAc segments.

In this paper, as a representative of microspheres with sharp pH–volume transition, we investigate the thermosensitivity of microspheres (AAm/MAc, 3/1 mol/mol; MB, 3 mol %). The curves of thermosensitivity are shown in Figs. 4 and 5, respectively. The photos of aqueous solution at different temperatures shown in Fig. 5 clearly indicated that the temperature did affect the diameters of hydrogel particles. It was observed that, as shown in Fig. 4, the thermo–volume transition changed at different pH, i.e., the pH lower, equal, and higher than (to) the pH corresponding to the maximum diameter (Hereafter, briefly, we call it the maximum pH; Ni et al., submitted for publication). The microspheres in aqueous solution actually contracted; thus, the turbidity of solution increased when the temperature increased, but it was the phenomenon at pH that was lower or equal to the maximum pH. As shown in Fig. 4, at pH 3.5 lower than the maximum pH 3.8, the hydrodynamic diameter increased until attaining a maximum at 25 °C, then abruptly decreased as temperature increased. At pH 3.8, the diameter increased and leveled off at 20 °C, whereas at pH 4.3 higher than the maximum pH 3.8, the diameter slightly decreased as the temperature increased. It was hardly to explain solely by employing the concept of dissociation of poly-MAc segments in microsphere, because the dissociation of MAc groups depended on the temperature monotonically, namely, that the dissociation degree increased with the increase in temperature.

The thermodynamic model of hydrogel [22] may be applicable to these cases. Hydrogel is a network of cross-linked polymer chains swollen by water. The swollen degree can be qualitatively estimated, employing three factors in accordance with thermodynamic model, namely, free energy of polymer–water mixing, deformation of network, and osmotic pressure resulted from mobile ions. In principle, the free energy of polymer–water mixing is inversely proportional to temperature, whereas the osmotic pressure commonly increases with the increase in temperature. An exception is the case that chemical reaction takes place; it may change. Both the frozen state and overactive state of polymer chains are not favorable to the deformation of the network. Therefore, at pH 4.3, the microspheres were overswollen. It implies that the polymer chains are strained greatly so as to satisfy the biggest deformation of the network where most of MAc have dissociated, meanwhile,

Fig. 5 Thermo-sensitivity of microspheres sampled at different conversions

a) 15°C b) 3°C

the osmotic pressure has been lowered much or even to be negligible. Under such a circumstance, the increase in temperature consequentially resulted in the contraction of microspheres. At the other pHs, the action of temperature reflects the comprehensive effects of these three factors. Therefore, the microspheres show the phase transition similar to pH–volume transition. As we discussed in the previous paper, the effect of increasing pH was to decrease the osmotic pressure as well as the free energy of poly-MAc segments–water mixing.

Analogous to the studies of pH–volume transition, the thermosensitivity of microspheres sampled at different polymerization was also investigated. As shown in Fig. 6, the thermosensitive properties of microspheres had the similar curves of diameter vs. pH to those of their pH–volume transitions. The thermo–volume transition became sharp as the propagation of polymerization. At the beginning of polymerization (0.5 h, 1 h in Fig. 6), the diameter of microspheres increased slightly with the increase in temperature and leveled off at higher temperature. From the third hour, the microspheres show an abrupt decrease in diameter at higher temperature, moreover, the maximum temperature decreased as the polymerization propagated. For example, the maximum temperatures of microspheres sampled at the third, fourth, and sixth hour are 30, 25, and 20 °C, respectively. It indicated that the great change of microsphere structure took place after the polymerization performed for 1 h. On the other hand, the similarity of thermosensitivity with pH-sensitivity of microspheres evidenced the correction of our analysis on the structure formation of microspheres during the polymerization.

Effects of ion strength on the hydrodynamic diameters of microspheres

When salt is added into polyelectrolyte hydrogel, two factors must be considered in general, i.e., the osmotic pressure of mobile ions and the electric shielding effect of counterions [4, 23, 24]. Osmotic process of mobile ions in hydrogel microsphere is an equilibrium process, namely, that one ion osmoses and accumulates concentration in a microsphere simultaneously means the increase in tendency that the ion inversely osmoses the microsphere. In a comprehensive item, NaOH used for pH adjustment is a kind of salt. The difference from the other salts is that OH^- can combine with H^+ to form an extremely dissoluble compound, water. This characteristic implies that the osmotic equilibrium drastically moves to side favoring the osmosis of Na^+. The osmosis of OH^- leads to the drastic decrease in H^+ or increase in fixed anions in the polyelectrolyte hydrogel, which in turn accelerate the osmosis of Na^+. Meanwhile, the decrease in total number of OH^- in the solution further discharges the inverse osmosis of Na^+ in hydrogel; thus, the osmotic process of mobile Na^+ and OH^- is an inter-accelerating process. Therefore, besides the limitation of the network, the hindrance to Na^+ osmosis in pH adjustment may be simply resulted from the electric field shielding of Na^+, especially at the loci where there is a high concentration of MAc segments. Comparatively, for other salts, the osmotic equilibrium is seriously dependent to the property and concentration of ions. Effect of electric field shielding is resulted from the electrostatic interactions between the fixed anions and mobile cations. The electric field of cations around the anions weakens the repulsive interactions between anions themselves. When the effect of electric field shielding is intensive enough, depending on the property and concentration of cations, polyelectrolyte hydrogel will collapse.

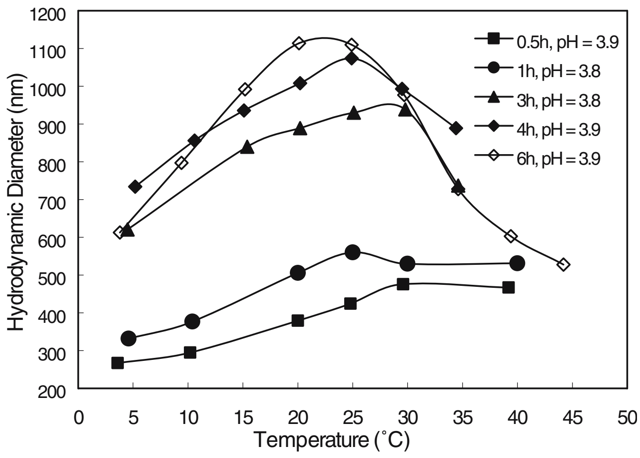

Fig. 6 Photos of turbid Run6024s solution at different temperatures

In this paper, the salts, NaCl and $CaCl_2$, were selected to investigate the characteristics of ion-sensitivity of microspheres with sharp pH–volume transition. Run6024 at three pH values were selected as the hydrogel, and the results were shown in Figs. 7 and 8. At almost the same pH, as shown in Fig. 7, compared with the monotonic decrease in diameter with the increase in [$CaCl_2$], it is remarkable that a range of [NaCl] was observed where the hydrodynamic diameter increased with the increase in [NaCl]. To confirm this special characteristic of NaCl effect, the investigations were carried out at the maximum pH 3.93 and the lower pH 3.7. As shown in Fig. 8, the same phenomenon was also found, although the region where the diameter increase became apparently narrower and the increment of diameter became smaller at the lower pH. The pH always decreased with the increase in salt content in the solution, irrespective to the types of salts and pH. It indicated that the fixed H^+ in the associated MAc was released as soon as the salts were added. The more cations added, the more H^+ was released from the hydrogel microspheres. As we know, one Ca^{2+} ion can release two H^+, but stoichiometrically, it is able to

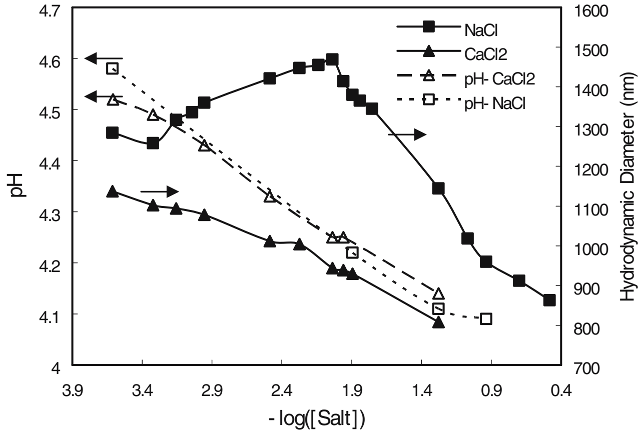

Fig. 7 Na^+- and Ca^{2+}-sensitivity of microspheres at pH 4.6

intensively interact with a pair of COO^- anions simultaneously. In such a circumstance, no matter whether a pair of COO^- ions is intra- or intermolecular anions, the interactions will contract the poly-MAc segments, or in other words, the repulsive interaction of fixed COO^- is strongly shielded because of the existence of Ca^{2+} cations. This was the reason, we considered, that the hydrodynamic diameter monotonically decreased as the [$CaCl_2$] increased in the solution.

Apparently, there were two osmotic equilibriums of cations established in a swollen microsphere because the anions of COO^- were fixed in the polymer chains, i.e., H^+ and Na^+. When Cl^- anions were added, they also established an osmotic equilibrium in the microsphere. Meanwhile, the newly added Na^+ in the solution moved the osmotic equilibrium of Na^+ to the direction that favor the exchange of Na^+–H^+ in the microsphere. These two factors attributed to the increase in diameter at lower [NaCl]. Furthermore, at pH 4.6, as we discussed in the context, the microspheres had been overswollen. It implies that the poly-MAc segments have been sufficiently separated by the aqueous solution. The bigger space between the poly-MAc segments allowed more Na^+ to accumulate and interchange with the resident COOH groups on the polymer chains until the local concentration of Na^+ was high enough to shield the repulsive interaction of COO^-. It is the reason that, as shown in Figs. 7 and 8, the diameter continuously increased to higher concentration of NaCl at pH 4.6, whereas the diameter increased at lower [NaCl] with a narrow range of [NaCl] at pH 3.7 and 3.93, respectively. Of course, we cannot expect that NaCl is able to substitute NaOH, because the osmotic equilibrium of H^+ exists in the system. It suppresses the exchange of Na^+–H^+ with COOH groups in the domains of microsphere; thus, the excessive Na^+ provokes the effect of electric field shielding and finally collapses the swelling of microsphere.

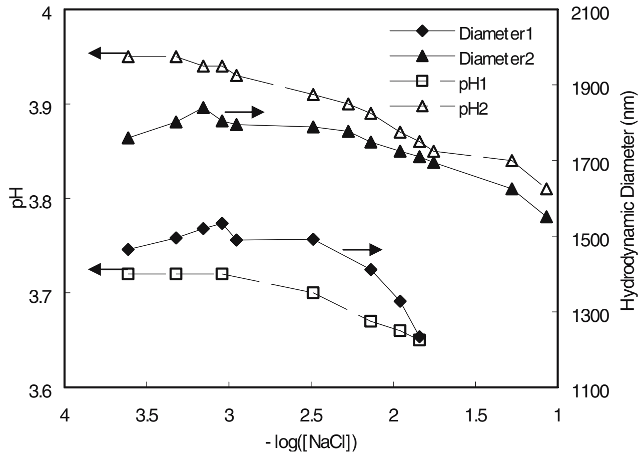

Fig. 8 Na^+-sensitivity of microspheres at pH 3.7 and 4.9

Conclusions

The forming process and characteristics of monodispersed hydrogel microspheres of poly(AAm-MAc) with sharp pH–volume transition were studied. The pH-, ion-, and thermosensitive behaviors of microspheres sampled at various stages of polymerization were evaluated by using the DLS. It was observed that the sharpness of pH–volume transition increased with the increase in monomer conversion. The structure of microspheres formed at two steps, i.e., the star-shaped nuclei-forming step and then incorporating minimonomer droplets remained on the surface of the nuclei to react with the free double-bonds in the nuclei. Therefore, at lower conversion, the pH–volume transition was board because of higher cross-linked degree, and some domains were lost during the process of pH adjustment because of the uncompleted cross-linking reaction. Such a structure formation process also reflected in the thermo- and ion-sensitive behaviors. However, both thermo- and ion-sensitive behaviors were affected by pH. At pH 3.8, the microspheres sampled at different monomer conversion showed the curves of thermo–volume transition similar to those of their pH–volume transition. The thermo–volume transition became sharper, and the maximum temperature generally moved to lower temperature as the polymerization propagated. For the mature microspheres (95.8% conversion), at pH 4.3, above the maximum pH 3.8, the hydrodynamic diameter of microspheres monotonically and slightly decreased with the increase in temperature, whereas at pH 3.5, the diameter increased to a maximum temperature, 25 °C, and then drastically decreased. At pH 3.8, the diameter increased to a maximum temperature of 20 °C and then leveled off as the temperature increased. Thermodynamic factors such as osmotic pressure, free energy of poly-MAc segments–water mixing, and deformation of network were the main reasons attributing to the thermo–volume transition. Increasing [$CaCl_2$] was to decrease the hydrodynamic diameters of microspheres, irrespective of pH, because of the intensive effect of electric field shielding. However, a region at the lower [NaCl] was found where the diameter increased with the increase in [NaCl]. Moreover, the range of the diameter-increasing region extended to a higher [NaCl] as pH increased. Osmotic equilibriums of ions and their dependence to the concentration of ions attributed to the increase in diameter in the region of [NaCl].

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References

1. Ilmain F, Tanaka T, Kokufuta E (1991) Nature 349:400
2. Li L, Hsieh Y-L (2005) Nanotechnology 16:2852
3. Kim SJ, Spinks GM, Prosser S, Whitten PG, Wallace GG, Kim SI (2006) Nature Materials 5:48
4. English AE, Tanaka T, Edelman ER (1997) J Chem Phys 107:1645
5. Osada Y, Hasebe M (1985) Chem Lett 9:1285
6. Yashida R et al (1995) Nature 374:240
7. Ni H-M, Kawaguchi H (2004) J Polym Sci A Polym Chem 42:2823
8. Ni H-M, Kawaguchi H (2004) J Polym Sci A Polym Chem 42:2833
9. Kawaguchi H (2000) Prog Polym Sci 25:1171
10. Matsuoka H, Fujimoto K, Kawaguchi H (1999) Polym J 31:1139
11. Matsuoka H, Fujimoto K, Kawaguchi H (1998) Polym Gels Netw 6:319
12. Nakazawa Y, Kamijo K, Fujimoto H, Kawaguchi Y, Yuguchi H, Urakawa H, Kajiwara K (1996) Angew Makromol Chem 240:187
13. Kamijo Y, Fujimoto K, Kawaguchi H, Yuguchi Y, Urakawa H, Kijiwara K (1996) Polym J 28:309
14. Ni H-M, Du Y-Z, Ma G-H, Nagai M, Omi S (2001) Macromolecules 34(19):6577
15. Ni H-M, Ma G-H, Nagai M, Omi S (2001) J Appl Polym Sci 82:2692
16. Ni H-M, Ma G-H, Nagai M, Omi S (2001) J Appl Polym Sci 82:2679
17. Kawaguchi H, Hoshino F, Otsuka Y (1986) Makromol Chem Rapid Commun 7:109
18. Hoshino F, Fujimoto T, Kawaguchi H, Otsuka Y (1987) Polym J 19:241
19. Kwaguchi H, Kawahara M, Yaguchi N, Hoshino F, Ohtsuka Y (1988) Polym J 20:903
20. Kuckling D, Adler H-JP, Arndt K-F, Ling L, Habicher WD (2000) Macromol Chem Phys 201:273
21. Tanaka T, Ishiwata S, Ishimoto C (1973) Phys Rev Lett 38:771
22. Siegel RA (1990) Pulsed and self-regulated drug delivery. CRC, Boca Raton, FL, p 129
23. Higgs PG, Joanny J-F (1991) J Chem Phys 94:1543
24. Kudaibergenov SE, Sigitov VB (1999) Langmuir 15:4230