Release Behavior of Freeze-Dried Alginate Beads Containing Poly(*N*-isopropylacrylamide) Copolymers

Jae-Hyung Choi, Hyeon Yong Lee, Jin-Chul Kim

School of Biotechnology and Bioengineering and Institute of Bioscience and Biotechnology, Kangwon National University, 192-1, Hyoja 2 Dong, Chunchon, Kangwon-Do 200-701, Korea

Received 14 February 2008; accepted 30 April 2008
DOI 10.1002/app.28620
Published online 13 June 2008 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: Beads composed of alginate, poly(*N*-isopropylacrylamide) (PNIPAM), the copolymers of *N*-isopropylacrylamide and methacrylic acid (P(NIPAM-*co*-MAA)), and the copolymers of *N*-isopropylacrylamide, methacrylic acid, and octadecyl acrylate (P(NIPAM-*co*-MAA-*co*-ODA)), were prepared by dropping the polymer solutions into $CaCl_2$ solution. The beads were freeze-dried and the release of blue dextran entrapped in the beads was observed in distilled water with time and pH. The degree of release was in the order of alginate bead < alginate/PNIPAM bead ≈ alginate/P(NIPAM-*co*-MAA) bead < alginate/P(NIPAM-*co*-MAA-*co*-ODA) bead. On the other hand, swelling ratios reached steady state within 20 min, and the values were 200–800 depending on the bead composition. The degree of swelling showed the same order as that of release. Among the beads, only alginate/P(NIPAM-*co*-MAA-*co*-ODA) bead exhibited pH-dependent release. At acidic condition, inter- and intraelectrostatic repulsion is weak and P(NIPAM-*co*-MAA-*co*-ODA) could readily be assembled into an aggregate due to the prevailing hydrophobic interaction of ODA. Thus, it could block the pore of bead matrix, leading to a suppressed release. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 110: 117–123, 2008

Key words: alginate; *N*-isopropylacrylamide; methacrylic acid; octadecyl acrylate; pH-dependent release

INTRODUCTION

Extensive researches have been focused on stimuli-sensitive drug carriers, which respond to environmental stimuli, such as pH, ionic strength, temperature, and electromagnetic radiation.[1–12] Among them, pH- or temperature-sensitive carriers are the most commonly studied ones.[2,3] Poly(*N*-isopropylacrylamide) (PNIPAM) has been utilized as a thermo-sensitive modulator in developing temperatures-sensitive carriers. Temperature-sensitive liposomes were prepared by modifying the surface of liposomes with hydrophobically-modified PNIPAM.[5–10] Water-soluble active ingredients entrapped in liposomes release by the thermal contraction of the polymer. Copolymers containing methacrylic acid (MAA) were used as a pH-sensitive modulator to control the release from liposomes.[11–13] The copolymers exhibit different conformations depending on pH, because MAA has a titrable group, carboxylic acid. Carboxyl groups are unionized under acid conditions and accordingly the copolymer takes random coils. In alkali conditions, carboxyl group is ionized and the chains of polymer would stretch out due to intramolecular electrostatic forces. Based on these characteristics, pH-sensitive drug delivery systems such as self assembly,[14] microparticle,[15,16] liposome,[12,13,17] and nanosphere[18] have been developed. On the other hand, alginate has been extensively used in preparing drug carrier due to its biocompatibility, good morphological and mechanical properties.[13,14] The alginate is the salt forms of alginic acid and it is obtained from brown marine algae.[15,16] It has the property of gelation in an aqueous solution with aid of divalent cations such as Ca^{2+} and Mg^{2+}.[17] Electrostatic interactions between carboxylate groups of the alginate and Ca^{2+} lead to the formation of mechanically-stable networks.[18] In our previous study, PNIPAM was grafted to alginate, and the beads of alginate-*g*-PNIPAM were prepared in an aqueous solution of Ca^{2+}. The main idea is that the main chain, alginate, would form a porous rigid matrix and the side chain, PNIPAM, might be a temperature-sensitive valve for the pores of the beads. The releases from the beads were suppressed below the lower critical solution temperature, whereas above that temperature the release became extensive.[19]

In this study, poly(*N*-isopropylacrylamide) (PNIPAM), the copolymer of *N*-isopropylacrylamide and methacrylic acid (P(NIPAM-*co*-MAA)), and the copolymer of *N*-isopropylacrylamide, methacrylic acid, and octadecylacrylate (P(NIPAM-*co*-MAA-*co*-ODA)) were prepared by a free radical reaction.

Correspondence to: J.-C. Kim (jinkim@kangwon.ac.kr).
Contract grant sponsor: Korea Forest Service, Republic of Korea.

MAA was copolymerized as a pH-sensitive unit and ODA was copolymerized as a hydrophobic one. Four kinds of beads, namely bead composed of alginate (alginate bead), bead composed of alginate and PNIPAM (alginate/PNIPAM bead), bead comprising alginate and P(NIPAM-*co*-MAA) (alginate/P(NIPAM-*co*-MAA) bead), and bead made up of alginate and P(NIPAM-*co*-MAA-*co*-ODA) (alginate/P(NIPAM-*co*-MAA-*co*-ODA) bead), were prepared by dropping the corresponding aqueous solutions into an aqueous solution of Ca^{2+}. The main idea is that alginate would form a porous rigid matrix, and the additives, NIPAM copolymers, might be a pH-sensitive valve for the pores of the beads. The releases from the beads were observed under acidic and alkali conditions. In parallel, the degrees of swelling of beads were also investigated. The pH-sensitive bead would be applicable to oral vaccination. Because it could suppress the release in acidic stomach and it could promote the release in neutral intestine.

EXPERIMENTAL

Materials

Sodium alginate and blue dextran were purchased from Sigma Chemical (Milwaukee, WI). Octadecylacrylate (ODA) was purchased from Adrich Chemical (St. Louis, MO). *N*-isopropylacrylamide (NIPAM) and methacrylic acid (MAA) was purchased from TCI (Tokyo, Japan). Concentrated phosphate buffer solution (10× PBS, pH 7.2) was obtained from BIO-RAD (Hercules, CA). Water was doubly distilled in a Milli-Q water purification system (Millipore, Billerica, MA) until the resistivity was 18 *M*Ω/cm. All other reagents were in analytical grade.

Methods

Synthesis of NIPAM copolymers

NIPAM copolymers were prepared by a free radical reaction.[20] NIPAM (9.9 mmol), MAA (1.49 mmol), ODA (0 mmol or 0.1 mmol), and azobisisobutyronitrile (0.05 mmol) were dissolved in 20 mmol of freshly distilled dioxane. The content of MAA corresponds to 10%, based on the total mass of the monomers. The content of ODA corresponds to 1%. The solution was degassed by bubbling N_2 for 1 h and then heated to 65°C for 12 h. The copolymers were precipitated upon the addition of diethylether. For purification, the precipitated polymers were dissolved in dioxane and reprecipitated with diethylether.

^{1}H NMR

^{1}H NMR spectra of PNIPAM, P(NIPAM-*co*-MAA) and P(NIPAM-*co*-MAA-*co*-ODA) were taken on a Varian VXR-500S spectrometer using $CDCl_3$ as a solvent.

Preparation of alginate/NIPAM copolymer beads containing blue dextran

Blue dextran (100 mg), a model drug, was dissolved in 25 g of aqueous solution containing alginate (2%) and NIPAM copolymer (1.0%). The solution was dropped into 200 mL of $CaCl_2$ aqueous solution (100 m*M*) and then the beads were stirred for 1 h. To remove unentrapped dextran, the beads were washed two times with distilled water and they were freeze-dried in a freeze-dryer (FD5508, Ilshin Lab) or air-dried at 40°C in an oven.

Scanning electron microscopy

Air-dried bead and freeze-dried one were cross-sectioned using a blade and they were mounted on metal stubs with double-sided tape, sputtered with gold, and viewed in a scanning electron microscope (Jeol JSM-840A).

Release of blue dextran from alginate/NIPAM copolymer beads

To investigate the pH-dependent release from alginate/NIPAM copolymer beads, the releases were observed at pH 3.5, pH 5.2, and pH 8.4. About 0.5 g of freeze-dried beads was put into 30 mL of distilled water, preadjusted to a specific pH and contained in a 50-mL-beaker, and it was stirred at 100 rpm on a magnetic stirrer. About 0.2 mL of the release mediums was taken at predetermined time intervals and the amount of blue dextran released was determined by measuring the absorbance of the medium at 630 nm on a UV spectrophotometer (BECKMAN DU-7500). Every time the release medium was taken away, distilled water of the same pH, 0.2 mL, was added to compensate for the amount of the release medium taken for the measurements. To determine the total amount of blue dextran entrapped in the beads, 20 mg of the beads was completely dissolved in 50 mL of phosphate buffer solution (PBS, pH 7.2) by stirring for 24 h. After filtering the solution using a syringe filter, the amount of blue dextran entrapped in the beads was determined at 630 nm. The % of release is defined as the percentage of the released amount on the basis of the total amount entrapped in the beads.

Determination of amount of NIPAM copolymers leached out of beads

The amount of NIPAM copolymer, P(NIPAM-*co*-MAA) and P(NIPAM-*co*-MAA-*co*-ODA), would be leached out of beads during the release of blue dextran. It was determined by titrating MAA residue. Freeze-dried beads free of blue dextran, 0.5 g, was

put into 30 mL of distilled water, which was preadjusted to pH 3.5, pH 5.5, and pH 8.5 and contained in a 50-mL-beaker. They were stirred at 100 rpm on a magnetic stirrer for 6 h. About 20 mL of the release medium was taken and then phenolphthalein solution of 20 μL (1%), an indicator, was added to the medium. The medium were titrated with KOH solution (0.01*M*). The concentration of MAA residue in the medium was determined using a calibration curve, and the mass of NIPAM copolymers leached out was calculated using the composition of copolymers.

Swelling ratio of alginate/NIPAM copolymer beads

The swelling property of alginate/NIPAM copolymer beads was investigated with time and pH. Freeze-dried beads free of blue dextran, 0.5 g, was put into 30 mL of distilled water, which was preadjusted to pH 3.5, pH 5.5, and pH 8.5 and contained in a 50-mL-beaker. They were stirred at 100 rpm on a magnetic stirrer for 6 h. The weight was measured after removing the excess free water with a filter paper. The swelling ratio is defined as follows.

$$\% \text{ Swelling ratio} = \left(\frac{\text{wetweight} - \text{dryweight}}{\text{dryweight}}\right) \times 100$$

RESULTS AND DISCUSSION

^{1}H NMR

In the spectrum of PNIPAM, the peak around 1.1 ppm comes from the methyl proton of isopropyl groups, the peak around 3.67 ppm is due to the methylene proton of vinyl groups, and the peak around 4.0 comes from C-2 proton of the isopropyl group. The spectrum of P(NIPAM-*co*-MAA) was similar to that of homo PNIPAM except that a weak and broad peak was found around 11.65 ppm. This peak arises from the carboxylic proton of MAA. Accordingly, it is believed that MAA was copolymerized with NIPAM. On the other hand, the spectrum of P(NIPAM-*co*-MAA-*co*-ODA) was similar to that of P(NIPAM-*co*-MAA) except that peaks around 0.85 and 1.25 ppm were observed. Since the peak of 0.85 ppm are due to the terminal methyl proton of ODA and the peak of 1.25 ppm are attributed to the methylene proton of ODA, it is concluded that ODA was successfully copolymerized with NIPAM and MAA. It was reported that the molar rations of the NIPAM residues to MAA ones in copolymers were almost the same as the feed ratios.[21] Hence, it could be assumed that the feed compositions are the same as those of the produced copolymers.

Release of blue dextran from alginate/NIPAM copolymer beads

Figure 1(A) shows the releases from alginate beads containing no NIPAM polymers at three different pHs (pH 3.4, pH 5.6, and pH 8.6). The time-dependent degrees of the releases were almost the same regardless of pH. The initial rates of release were high and the degrees of release were $\sim$ 17% for the first 10 min. Thereafter, the rates were slackened down in a saturated manner and the degrees of release in 6 h were $\sim$ 32%. The initial amount of release, 17%, might come from the outer layer of the alginate beads, and the suppressed release in the later stage is possibly because blue dextran entrapped in the inner part of the beads takes time to diffuse out of the alginate matrix. Alginate is the salt form of alginic acid and it has no titrable group. Accordingly, the matrix of alginate is insensitive to pH change and this would account for the pH-insensitive release.

Figure 1(B) shows the release from alginate/PNIPAM beads. The release patterns were similar to those of alginate beads and the degree of release was independent on pH. But the degree of release was about 45% in 6 h and the value was somewhat higher than that of the alginate bead. Since alginate is crosslinked by Ca^{2+}, the swelling of the alginate beads would be restricted. In case of alginate/PNIPAM beads, PNIPAM would be physically entangled with the network of alginate. Accordingly, the crosslinking density would be less than that of alginate beads free of PNIPAM, and the swelling would be more favorable than the swelling of alginate beads. This may explain the higher degree of release. Figure 1(C) shows the release from alginate/P(NIPAM-*co*-MAA) beads. The degrees of release were $\sim$ 50% in 6 h, and the value was a little higher than that of the alginate/PNIPAM bead. Since MAA is a kind of hydrophilic monomer, P(NIPAM-*co*-MAA) is more hydrophilic than PNIPAM. Hence, alginate/P(NIPAM-*co*-MAA) bead could imbibe more water than alginate/PNIPAM bead. That may be responsible for the higher degree of release from alginate/P(NIPAM-*co*-MAA) beads. P(NIPAM-*co*-MAA) has carboxylic groups, but whatever was the pH, the degree of release was almost the same. According to a previous report, the solution of P(NIPAM-*co*-MAA) was insensitive to pH in terms of turbidity change.[20] This indicates that the pH-induced conformational change of P(NIPAM-*co*-MAA) was too small to change the turbidity and, accordingly, it would not be enough to control the degree of release through the porous matrix of the beads. Figure 1(D) shows the release from alginate/P(NIPAM-*co*-MAA-*co*-ODA) beads. Like the other beads, blue dextran released in a saturation manner. Unlike the other beads, however, the degree of release was dependent on pH. At lower pHs (pH 3.6 and pH 5.2), the

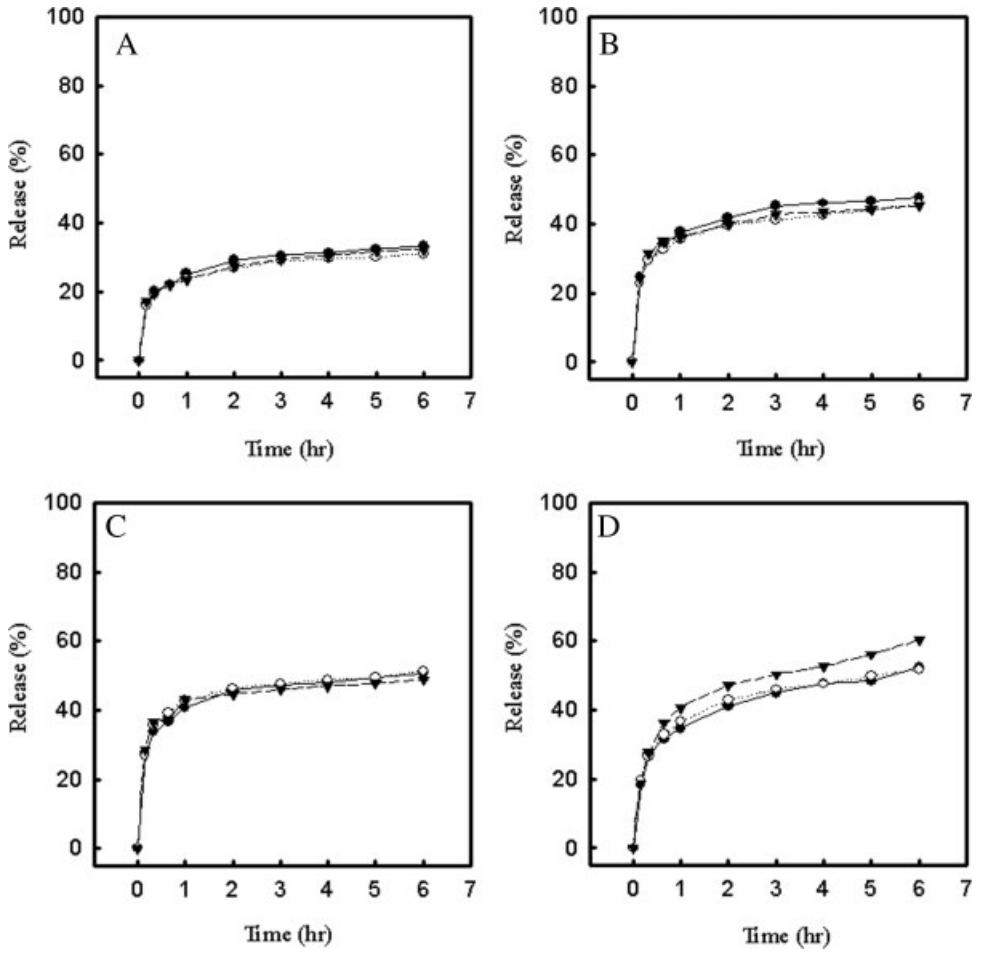

Figure 1 Releases of blue dextran from alginate bead (A), alginate/PNIPAM bead (B), alginate/P(NIPAM-*co*-MAA) bead (C), and alginate/P(NIPAM-*co*-MAA-*co*-ODA) bead (D) at three different pHs, pH 3.5 (●), pH 5.5 (○), pH 8.5 (▼).

degree of release was 52% in 6 h. At higher pH (pH 8.5), the degree of release was 60% in 6 h. Below the p*K* value of carboxylic acid, most of carboxylic acids of the copolymer are unionized and, in turn, little electrostatic inter- and intramolecular repulsion force occurs. In this circumstance, the chain of the copolymer is likely to collapse or to be assembled into an aggregate with aid of the hydrophobic interaction of ODA. In fact, according to the result of pH-dependent transmittance, the solution of P(NIPAM-*co*-MAA-*co*-ODA) became turbid at acidic conditions.[20] It indicates that polymeric aggregates were formed. The aggregates may block pores of the bead matrix, leading to the lower level of release. On the other hand, above the p*K* value of carboxylic acid, most of carboxylic acids of the copolymer are ionized and, in turn, electrostatic inter- and intramolecular repulsion force occurs. In this circumstance, although the copolymer could be assembled due to the hydrophobic interaction of ODA, the number of chain constituting an aggregate and the size might be reduced due to the electrostatic repulsion force. In fact, according to the result of pH-dependent transmittance, the solution of P(NIPAM-*co*-MAA-*co*-ODA) became transparent at neutral and alkali pHs.[20] It means that polymer does not form a large aggregate. As a result, the pore of bead matrix would be less blocked and thus the diffusion of blue dextran through the pores could be less restricted than in case of acid conditions, resulting in the higher level of release.

Determination of amount of NIPAM copolymers leached out of beads

To determine the amount of NIPAM copolymers leached out of beads, MAA contents in NIPAM copolymers was to be determined. A calibration curve for the titration of MAA was $V = 476\ C + 0.895$ with $R^2 = 0.9995$, where V is the volume (mL) of KOH solution (0.01*M*) required for titrating MMA solution and C is the concentration of MAA in the titrated solution. The contents of MAA in P(NIPAM-*co*-MAA) and P(NIPAM-*co*-MAA-*co*-ODA) were 9.44 and 9.51%, respectively. Based on the composition of the polymer, the amount of NIPAM copolymers leached out of bead could be determined by titrating MAA residue in the

TABLE I
Amount of NIPAM Copolymers Leached Out of Beads for 6 h

	P(NIPAM-*co*-MAA)	P(NIPAM-*co*-MAA-*co*-ODA)
pH 3.4	0.99%	0.89%
pH 5.3	0.95%	1.03%
pH 8.5	1.18%	1.17%

release medium. Table I shows the results. Regardless of pH of medium, the amount leached out was around 1%, whatever the beads were. The NIPAM copolymers are water-soluble at room temperature. Nevertheless, the leached amount was quite small. This means that the copolymers could be entangled with crosslinked alginate matrix. In this circumstance, the copolymers would hardly escape from the matrix. Therefore, it is believed that the leaching out have little effect on the degree of blue dextran release.

Swelling ratio of alginate/PNIPAM copolymer beads

Figure 2(A) shows the swelling ratio of alginate beads with time and pH. Swelling ratio reached steady state within 20 min and the value was around 200. Air-dried beads swelled slowly over 3 h.[19] According to the result of SEM, the pores of freeze-dried bead are much larger than those of air-dried ones (Fig. 3). Therefore, the uptake of water by freeze-dried bead was completed earlier. This fast rate of swelling may account for the fast initial rate of release in Figure 1(A), since the rate of release strongly depends on the rate of swelling. The swelling ratio was almost invariable with respect to pH. This is possibly because there is no titrable group in alginate. Figure 2(B) shows the swelling ratio of alginate/PNIPAM beads. Like alginate beads, the swelling was completed within 20 min. But the swelling ratio was 530–600 and the value was much higher than that of alginate bead. In PNIPAM/alginate bead, alginate would be physically entangled with PNIPAM and the crosslinking of alginate may be hindered by PNIPAM, leading to less crosslinking density. This may explain why the swelling ratio is higher when PNIPAM is included in the bead. Another possible reason is that the bead including PNIPAM could imbibe more water than alginate bead because PNIPAM is hydrophilic polymer at

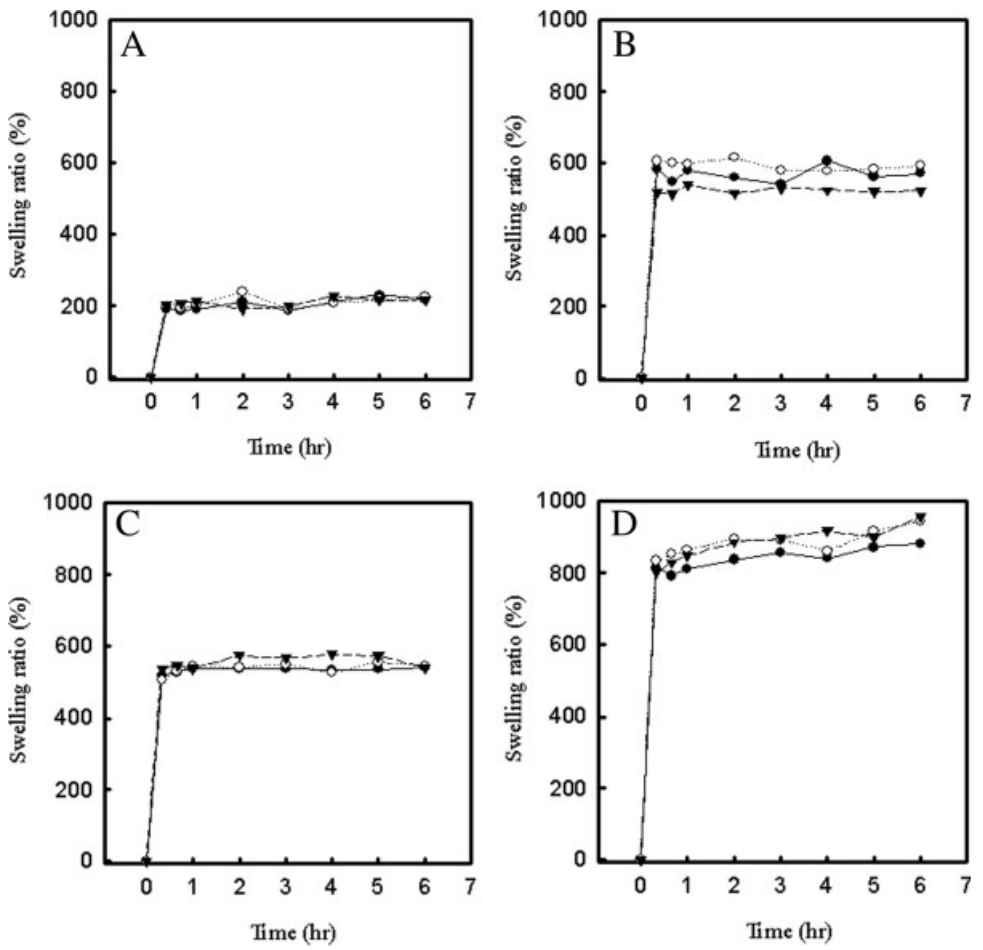

Figure 2 Swelling ratio of alginate bead (A), alginate/PNIPAM bead (B), alginate/P(NIPAM-*co*-MAA) bead (C), and alginate/P(NIPAM-*co*-MAA-*co*-ODA) bead (D) at three different pHs, pH 3.5 (●), pH 5.5 (○), pH 8.5 (▼).

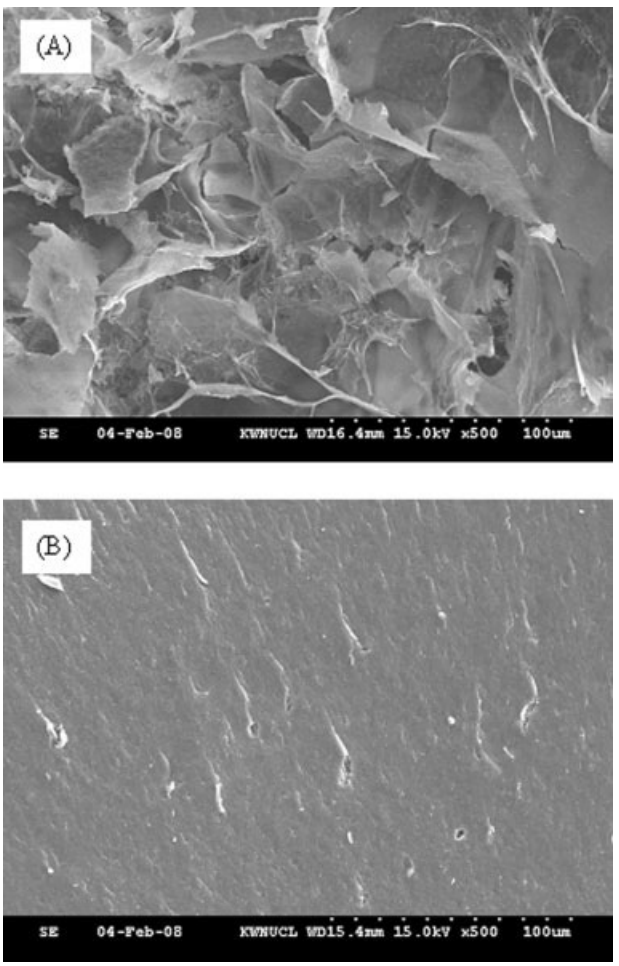

Figure 3 Cross sections of air-dried alginate bead (A) and freeze-dried alginate bead (B) observed on scanning electron microscope. Magnification was ×500.

room temperature. Figure 2(C) shows the swelling ratio of alginate/P(NIPAM-*co*-MAA) bead. The swelling ratio was not significantly different from that of alginate/PNIPAM bead and the value was almost constant with respect to pH. As described previously, the solution of P(NIPAM-*co*-MAA) was insensitive to pH in terms of turbidity change.[20] It indicates that, without a hydrophobic moiety such as ODA, polymers are hardly assembled or aggregated in an aqueous phase whatever pH is. This could be one of the reasons why swelling ratio is invariable with pH. Figure 2(D) shows the swelling ratio of alginate/P(NIPAM-*co*-MAA-*co*-ODA) bead. The swelling ratios were greater than 800 and the values were much higher than those of the other beads. Because of the hydrophobic interaction of ODA, P(NIPAM-*co*-MAA-*co*-ODA) could form assemblies or aggregates, at both acidic and alkali condition.[20] The assembled polymers may hinder the formation of crosslinking more effectively than unassociated polymers (e.g., copolymers containing no ODA). As a result, higher swelling could be obtained with alginate/P(NIPAM-*co*-MAA-*co*-ODA) bead. On contrary, one of the main factors affecting the degree of swelling is crosslinking density. Once alginate has been crosslinked by multivalent ions, the linkage is stable and robust with respect to pH change.[19] That is why the degree of swelling is almost constant with pH. Whereas, P(NIPAM-*co*-MAA-*co*-ODA) within the matrix could form variable size of aggregates depending on pH, leading to pH-dependent release of alginate/P(NIPAM-*co*-MAA-*co*-ODA) bead [Fig. 1(D)].

In general, higher swelling ratio results in higher degree of release, since swelling ratio is a measure of mesh size of the bead matrix. In this study, the order of swelling ratio (alginate/P(NIPAM-*co*-MAA-*co*-ODA) bead $>$ alginate/P(NIPAM-*co*-MAA) bead $\approx$ alginate/PNIPAM bead $>$ Alginate bead) was the same as the order of the degree of release.

CONCLUSIONS

Four kinds of beads, namely alginate/P(NIPAM-*co*-MAA-*co*-ODA) bead, alginate/P(NIPAM-*co*-MAA) bead, alginate/PNIPAM bead, and alginate bead, were prepared. The order of % release was alginate/P(NIPAM-*co*-MAA-*co*-ODA) bead $>$ alginate/P(NIPAM-*co*-MAA) bead $\approx$ alginate/PNIPAM bead $>$ alginate bead. The degree of release was closely related to the swelling ratio. Among the beads, only alginate/P(NIPAM-*co*-MAA-*co*-ODA) bead exhibited pH-dependent release. The bead showed a suppressed release at acidic conditions, since the hydrophobic interaction of the copolymer prevails the electrostatic repulsion at acidic condition, possibly resulting in an aggregate.

References

1. Ju, H. K.; Kim, S. Y.; Lee, Y. M. Polymer 2001, 42, 6851.
2. Peppas, N. A.; Bures, P.; Leobandung, W.; Ichikawa, W. Eur J Pharm Biopharm 2000, 50, 27.
3. Hirasa, O.; Ito, S.; Yamauchi, A.; Fujishige, S.; Ichijo, H. Polymer Gels, Fundamentals and Biomedical Application; Plenum: New York, 1991; p 247.
4. Ju, H. K.; Kim, S. Y.; Kim, S. J.; Lee, Y. M. J App Polym Sci 2002, 83, 1128.
5. Yatvin, M. B.; Weinstein, J. N.; Dennis, W. H.; Blumenthal, R. Science 1978, 202, 1290.
6. Weinstein, J. N.; Magin, R. L.; Cysyl, R. L.; Zaharko, D. S. Cancer Res 1980, 40, 1388.
7. Hayashi, H.; Kono, K.; Takagishi, T. Biochim Biophys Acta 1996, 1280, 127.
8. Kim, J. C.; Bae, S. K.; Kim, J. D. J Biochem 1997, 121, 15.
9. Kono, K.; Nakai, R.; Morimoto, K.; Takagishi, T. Biochim Biophys Acta 1999, 1416, 239.
10. Kono, K.; Henmi, A.; Yamashita, H.; Hayashi, H.; Takagishi, T. J Controlled Release 1999, 59, 63.

11. Kim, J. C.; Kim, M. S.; Kim, J. D. Korean J Chem Eng 1999, 16, 28.
12. Kim, J. C.; Kim, J. D. Colloid Surf B: Biointerf 2002, 24, 45.
13. Yessine, M. A.; Lafleur, M.; Meier, C.; Petereit, H. U.; Leroux, J. C. Biochim Biophy Acta: Biomembranes 2003, 1613, 28.
14. Mao, B. W.; Gan, L. H.; Gan, Y. Y.; Tam, K. C.; Tan, O. K. Polymer 2005, 46, 10045.
15. Kumar, A.; Lahiri, S. S.; Singh, H. Int J Pharm 2006, 323, 117.
16. Sipahigil, O.; Gürsoy, A.; Çakalağaoğlu, F.; Okar, İmer. Int J Pharm 2006, 311, 130.
17. Roux, E.; Passirani, C.; Scheffold, S.; Benoit, J. P.; Leroux, J. C. J Controlled Release 2004, 94, 447.
18. Lugo, M. T.; García, M.; Record, R.; Peppas, N. A. J Controlled Release 2002, 80, 197.
19. Kim, M. H.; Kim, J. C.; Lee, H. Y.; Kim, J. D.; Yang, J. H. Colloid Surf B: Biointerfaces 2005, 46, 57.
20. Choi, J. H.; Lee, H. Y.; Kim, J. C. J Appl Polym Sci 2008, DOI 10.1002.
21. Diez-Pena, E.; Quijada-Garrido, I.; Barrales-Rienda, J. M. Polymer 2002, 43, 4341.