Preparations and Temperature- and pH-Dependent Release Property of Ethylcellulose Microcapsules Containing *N*-Isopropylacrylamide Copolymer

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ABSTRACT: Microcapsules composed of poly(*N*-isopropylacrylamide-*co*-methacrylic acid) [P(NIPAM-*co*-MAA)] cores and ethylcellulose matrix were prepared by an emulsification and spray-drying method. First, water-in-oil (W/O) emulsions were prepared using P(NIPAM-*co*-MAA) solution in distilled water (3%) as a water phase, and ethylcellulose solution in dichloromethane (8%) as an oil phase. The emulsion was spray-dried around 50°C to evaporate dichloromethane, and then the resulting particle was air-dried to remove residual water. Blue dextran loaded in the cores of microcapsules readily released below lower critical solution temperature (LCST) but the release was suppressed above the phase transition temperature. It is believed that the NIPAM copolymer acts as a thermal trap for blue dextran when temperature is above LCST. In addition, the microcapsules were also pH-sensitive in terms of release, which could be explained by the pH-dependent contraction and expansion of the copolymer. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 118: 421–427, 2010

Key words: *N*-isopropylacrylamide; methacrylic acid; water-in-oil emulsion; spray drying; temperature/pH-dependent release

INTRODUCTION

Poly(*N*-isopropylacrylamide) (PNIPAM) is well known as a thermosensitive polymer, and it has been extensively studied as a thermal sensitizer for the development of temperature–sensitive drug carriers. The thermal property of PNIPAM is characterized by lower critical solution temperature (LCST). The polymer is water-soluble below LCST, and it becomes hydrophobic and water-insoluble if the temperature increases across LCST.[1–5] In terms of polymeric chain motion, a thermal contraction of the chain occurs upon increasing temperature across LCST. To control LCST, various kinds of hydrophilic and lipophilic monomers were copolymerized with NIPAM. It was reported that the hydrophilic monomers such as *N*,*N*-dimethylacrylamide and acrylamide increase LCST, and the hydrophobic monomers including butyl methacrylate and methyl methacrylate decrease the phase transition temperature. Several types of drug carriers have taken advantage of the thermal property for their temperature-sensitive releases.[6–15] Surface-modified liposomes with NIPAM copolymers exhibited an extensive release around LCST, because the thermal contraction imposes a stress on the liposomal membrane.[16] Thermosensitive monoolein cubic phase was designed by including NIPAM copolymers in the water channels, where the polymers were supposed to act as a thermal valve.[17] And thermosensitive alginate beads were developed by including NIPAM copolymers in the matrix of the beads either by a covalent attachment[18] or by a physical entanglement.[19] In addition, thermosensitive microcapsules were proposed by embedding nano-sized PNIPAM particles in the wall of the microcapsules.[20] According to the results, the release was accelerated at a temperature above LCST, where PNIPAM particles undergo a thermal contraction leading to the formation of voids in the wall of the microcapsule. On the other hand, when NIPAM copolymers had ionizable groups such as carboxylic group and amino group, some carriers exhibited pH-sensitive release as well as temperature-sensitive release.[4] Like examples of thermosensitive carriers presented earlier, most of them exhibited a positively thermosensitive release. That is, release was promoted above LCST, and it was suppressed below the temperature.

In this study, novel microcapsules were prepared for a negatively thermosensitive release by spray-

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drying a water-in-oil emulsion. A negatively thermosensitive release is that the release is accelerated at a lower temperature. The copolymer of NIPAM and methacrylic acid (MAA), and blue dextran (a dye) were codissolved in the water phase of the emulsion, and ethylcellulose was dissolved in the oil phase. Because the water phase of the emulsion contained both PNIPAM copolymer and blue dextrans, and the oil phase included ethylcellulose, the microcapsules prepared by a spray-drying method will have the domains of PNIPAM copolymer mixed with blue dextran within the matrix of ethylcellulose. Since both PNIPAM and blue dextran are polymers, there would be physical entanglement between two kinds of polymers. The physical entanglement would be pronounced above LCST due to the thermal contraction of PNIPAM, and it could be slacken down below the temperature due to the expansion of PNIPAM. As a result, the release could be suppressed above LCST, and it will be promoted below LCST. The stability spray drying of emulsion was evaluated in terms of the phase separation and the change in droplet size. The effect of spray-drying temperature on the shape of microcapsules was investigated. Finally, temperature-dependent releases from the microcapsules were observed along with pH-dependent release.

EXPERIMENTAL

Materials

Ethylcellulose 10cP (5% w/w in 80/20 toluene/ethanol), methacrylic acid (MAA), Hydrion^{TM} buffer, sorbitan sesquioleate (Arlacel 83), sorbitan monooleate (Span 80), blue dextran (MW 2,000,000), and L-α-phosphatidyl choline were purchased from Sigma-Aldrich Inc. *N*-isopropylacrylamide (NIPAM) was obtained from Tokyo Kasei Kogyo Co. (Japan). α,α′-Azobis(isobutyronitrile) (AIBN) was provided by Junsei Chemical Co. (Japan). All other reagents were in analytical grade.

Preparation and characterization of NIPAM copolymer Copolymer of NIPAM and MAA [P(NIPAM-*co*-MAA)] were prepared by a free radical reaction.[4] The weight ratio of NIPAM to MAA monomer was 85/15. The FTIR spectrum was taken in KBr pellet using Perkin Elmer Fourier Transformed Infrared spectrophotometer instrument (EXCALIBER Series). The molecular weight was measured by a gel permeation chromatography (Waters, Elstree, UK).[21] The content of MAA in the copolymer was determined by titrating MAA residue.[19] To investigate the effect of pH on the temperature-induced phase transition of the copolymer, the turbidity of copolymer solution in distilled water (pH3.0, pH5.0, pH7.0, and pH 9.0) was measured at 600 nm on a UV spectrophotometer (JENWAY 6505), equipped with a temperature controller (JENWAY Peltier Controller).

Preparation of emulsion

Stabile water-in-oil (W/O) emulsions are required for the preparation of microcapsules. A few conditions for the preparation of W/O emulsion were adopted to obtain stable W/O emulsion. P(NIPAM-*co*-MAA) solution in distilled water (3%) and ethylcellulose in dichloromethane (8%) were used as a water phase and an oil phase, respectively. The volumetric ratio of water to oil phase was 1 : 4. As emulsifiers, Arlacel 83, Span 80, phosphatidylcholine were employed. The emulsifier was dissolved in the oil phase so that the concentration is either 0.25 or 0.5%. While homogenizing the oil phase at 12,000 rpm using a homo mixer (DIAX 900, Germany), water phase was put to the oil phase over 5 min, and the homogenization was continued for further 25 min. The resulting phase systems were stood at room temperature, and the stabilities were observed in terms of the change in droplet size and the phase separation.

Preparation of microcapsules

Microcapsules of P(NIPAM-*co*-MAA) were prepared by an emulsification and spray-drying method. The copolymer and blue dextran were codissolved in distilled water so that the concentrations are 3.0 and 8.0%. In parallel, ethylcellulose was dissolved in dichloromethane so that the concentration is 8.0%. While homogenizing 80 ml of the oil phase at 12,000 rpm, 20 ml of the water phase was put to the oil phase over 5 min, and the homogenization was continued for further 25 min. And then the resulting W/O emulsion system was spray-dried in a spray dryer (BUCHI B-290, Switzerland). The inlet temperature was either 50°C or 120°C, aspiration was 100%, and pumping was 15%. When inlet temperature was 50°C, the resulting microcapsules were further dried in an oven to remove residual water.

Field emission scanning electron microscopy

Spray-dried microcapsules and spray-dried and subsequently air-dried microcapsules were laid on metal stubs, coated with gold and observed in a Field Emission scanning electron microscope (S-4300/HITACHI).

Release from microcapsules

When the effect of temperature on the release from microcapsules was observed, 0.3 g of dry microcapsules was put in 50 ml of Hydrion buffer (pH 5.0),

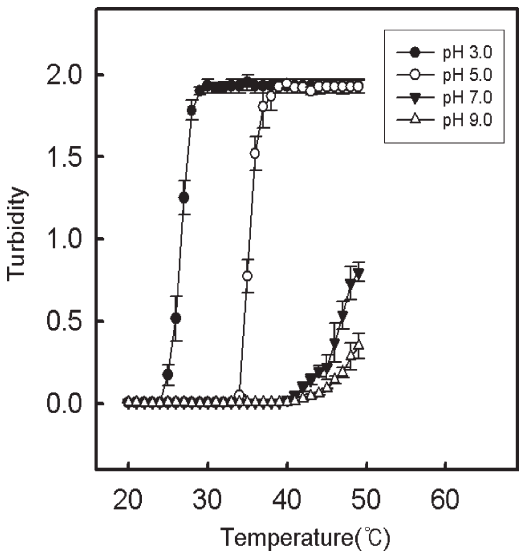

Figure 1 Change in turbidity of P(NIAPM-*co*-MAA) solution (2%) in distilled water with temperature and pH.

thermostated at 20°C, 30°C, 40°C, or 45°C, and then the suspension was stirred using a magnetic bar. The suspension of 2 ml was taken at predetermined time intervals, and it was filtered using a syringe filter (0.45 μm). The amount of blue dextran released was determined by measuring the absorbance of the filtrate at 630 nm. When the effect of pH on the release was investigated, the microcapsules was put in Hydrion buffer (pH 3.0, pH 5.0, pH 7.0, or pH 9.0), thermostated at 20°C or 40°C. The compositions of Hydrion buffer were sulphamic acid (20–30%)/potassium biphthalate (70–80%) for pH 3.0, potassium biphthalate (75–85%)/sodium phosphate dibasic (15–25%) for pH 5.0, potassium phosphate monobasic (30–40%)/sodium phosphate dibasic (60–70%) for pH 7.0, and sodium carbonate (20–30%)/sodium bicarbonate (70–80%) for pH 9.0.

RESULTS AND DISCUSSION

Characterization of NIPAM copolymer

The weight ratio of NIPAM to MAA residues was 84.2/15.8. In FTIR spectrum, the peak of carboxylic carbonyl group of MAA residues was found around 1706 cm^{-1}, and the peak of amide carbonyl group of NIPAM residues was observed around 1641 cm^{-1}. The number average molecular weight (M_n) and the weight average molecular weight (M_w) were 6751 and 14,214, respectively. Figure 1 shows the effect of pH on the temperature-induced phase transition of P(NIPAM-*co*-MAA). At pH 3.0, the turbidity of the copolymer solution started to increase around 25°C. PNIPAM and their copolymers have their own lower critical solution temperatures (LCSTs). Below that temperature, the polymers are soluble in an aqueous phase, and the chains are in an extended configuration. Above that temperature, the polymers are insoluble, and the chains are in a contracted configuration. Accordingly, the turbidity of the solution increases when the temperature increases across LCST. At pH 5.0, the turbidity started to increase around 34°C. At pH 7.0 and 9.0, the turbidity started to increase around 41 and 43°C, respectively, but there were no sharp increase. The thermal contraction of the copolymer upon increasing temperature occurs due to the dehydration and the subsequent hydrophobic intramolecular interaction.[22] At acidic conditions, carboxylic acids are in a unionized form, and there is little intramolecular electrostatic repulsion force. As pH increases, carboxylic acids are likely to be ionized, and there would be significant intramolecular electrostatic repulsion force, preventing the contraction of the polymer chains. This may account for why the phase transition temperature increased with increasing pH.

Stability of emulsion

The formation and the stability of emulsion were summarized in Table I. The W/O emulsion could be obtained with the emulsifiers of Arlacel 83 and phosphatidylcholine, but not with Span 80. Emulsions prepared using Arlacel 83 at the concentrations of 0.25 and 0.5% exhibited phase separations 6 and 10 hr, respectively, after the emulsifications. On the other hand, the phase separation of emulsion prepared using phosphatidylcholine occured 2.5 hr after the emulsification. Therefore, it is concluded that the emulsion prepared using Arlacel 83 at the concentration of 0.5% is the most stable in terms of phase separation. Figure 2 show the photos of the emulsion prepared using Arlacel 83 immediately and 3 hr after the preparation of the emulsion. The size of water droplets ranged from 1 to 3 μm immediately after the preparation of the emulsion, and the no significant change in the size was observed at the time

TABLE I
Formations and Stabilities of W/O Emulsions

Emulsifier	Arlacel 83	Arlacel 83	Span 80	Span 80	Phosphatidylcholine
Emulsifier concentration	0.5%	0.25%	0.5%	0.25%	0.5%
Emulsification	Successful	Successful	Failed	Failed	Successful
Phase separation	10 hr later	6 hr later	Not determined	Not determined	2.5 hr later

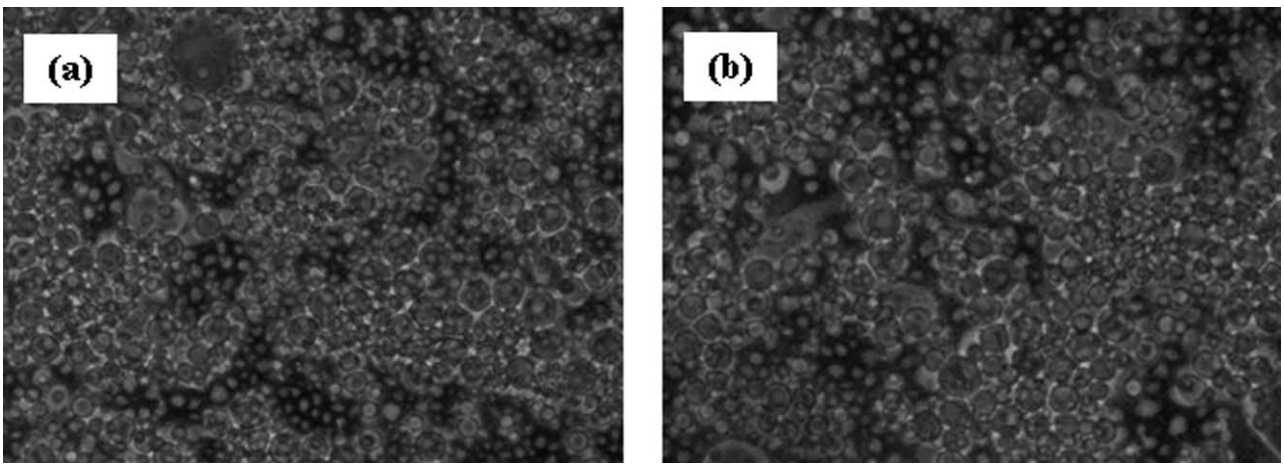

Figure 2 The photos of the emulsion prepared using Arlacel 83 immediately (panel a) and 3 h (panel b) after the preparation (magnification, ×400).

elapse of 3 h. According to the above results, Arlacel 83 was included in the oil phase so that the concentration is 0.5% when W/O emulsion was prepared to produce microcapsules. To keep the size of water droplets constant, the spray drying of the W/O emulsion was completed within 3 hr.

Field emission scanning electron microscopy

Figure 3(a) shows the FESEM photos of particles obtained by spray-drying W/O emulsion with the inlet temperature of 120°C. Sphere-like particles were observed together with irregular debris. When the emulsion is micronized into droplets by the nozzle of a spray dryer, the resulting droplets still will be W/O emulsion. That is, smaller droplets of water phase are included in the continuous oil phase of a micronized droplet. If the droplets of W/O emulsion are subject to the temperature of 120°C, volatile dichloromethane, which is the solvent of continuous oil phase, will first evaporate and then water, which is the solvent of dispersed water phase, will do. In this circumstance, ethylcellulose matrix of continuous phase could first be formed, and the water phase would be enclosed by the matrix. And then the water evaporation would develop a high pressure inside the ethylcellulose matrix, leading to the burst of the spherical particles leaving debris. To avoid the burst of the particles, the emulsion was spray-dried with the inlet temperature of 50°C, and the result is shown in Figure 3(b). Sphere or flat ball-like particle was observed but there was no trace of debris. The inlet temperature is above the boiling point of dichloromethane, 40°C, and it is far below the boiling point of water. Accordingly, only dichloromethane would evaporate leaving dry ethylcellulose matrix and the water droplets containing P(NIPAM-*co*-MAA) would be enclosed by the matrix. To evaporate water, the particles shown in Figure 3(b) were air-dried in an over, and the FESEM photo of dry particles was represented in Figure 3(c). The shape of particles after evaporating water [Fig. 3(c)] was almost the same as that of particles before removing water [Fig. 3(b)], and no debris was observed. In case, the spray-dried wet particles were dried for the evaporation of water in an oven (40°C), water will be removed gradually through the preformed ethylcellulose matrix, and the vapor pressure evolved within the matrix would be insignificant. As a result, there would no burst of the particles, giving a rise to no debris after removing water in an oven. The two step drying procedure, the spray-drying for the formation of outer matrix and then an air-drying for the removal of water in inner core, was adopted for the preparation of microcapsules, which were subsequently used for the further experiment.

Size distributions

Figure 4 shows the size distribution of microcapsules. Small portion of the population was either less than 1 micron or greater than 100 microns, and most of particles were in the range of few to tens of microns. The size range is a typical one obtained by a spray-drying method,[23] and it was in a good agreement with the size observed on FESEM photos.

Release from microcapsules

Figure 5 shows the temperature-dependent release profiles from microcapsules at pH 5.0. The release patterns resemble the first order release, where a fast release is followed by a slow release. A fast release was observed for the first 20 min and a slow release for the last 40 min. Thereafter, no significant release was found. At 20°C, the degree of release for 60 min was about 80%. When the temperature increased to 40°C, the release was suppressed, and

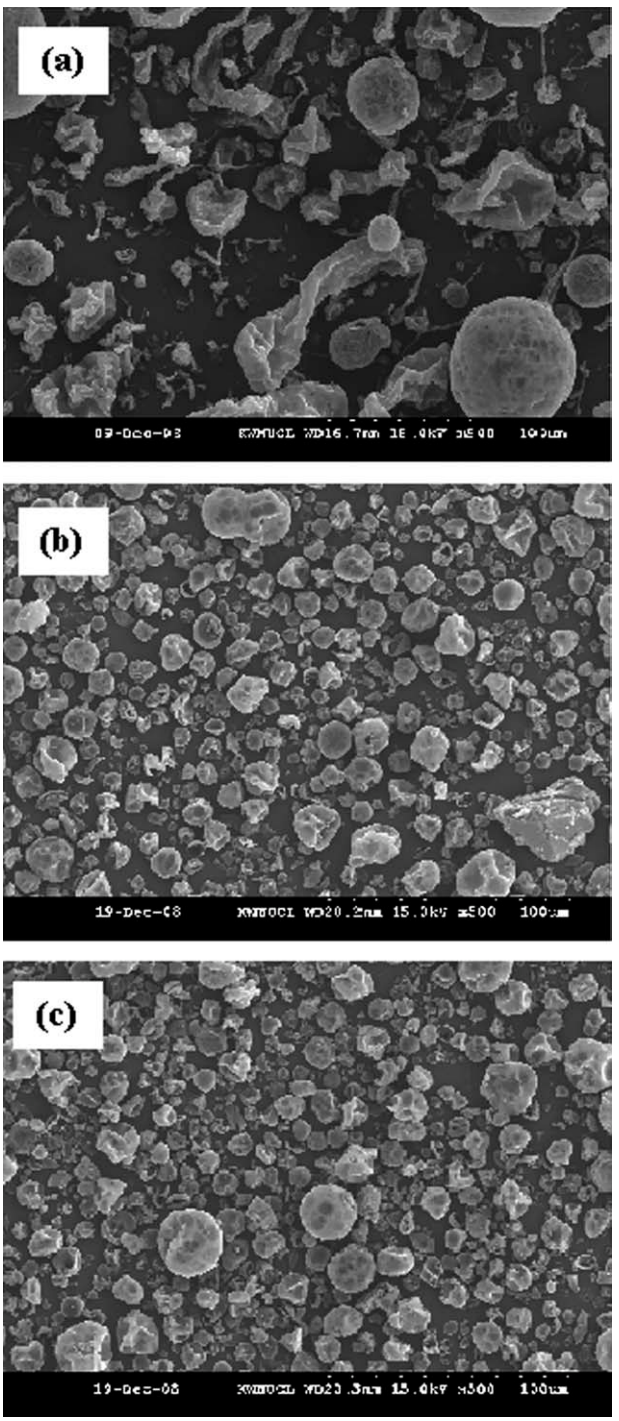

Figure 3 FESEM photos of particles obtained by spray-drying W/O emulsion with the inlet temperature of either 120°C (panel a) or 50°C (panel b). The particles shown in panel b were air-dried in an oven, and the photo of dry particles was represented in panel c.

the degree of release for the same period was about 67%. When the temperature further increased to 45°C, the release was more suppressed, and the degree of release was 55%. The microcapsules were prepared by spray-drying W/O emulsion and then air-drying the particles. Since PNIPAM copolymer

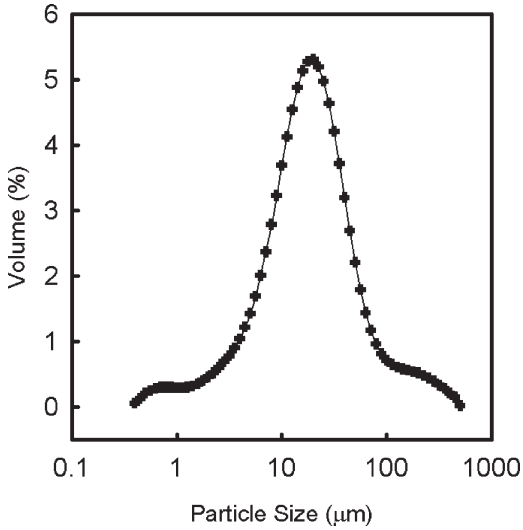

Figure 4 Size distribution of microcapsules composed of P(NIPAM-*co*-MAA) cores and ethylcellulose matrix.

and blue dextran were codissolved in the dispersed water phase of the emulsion, they would coexist in the dispersed domains (cores) of dry microcapsules. Therefore, the interaction of blue dextran with PNIPAM copolymer would be a major factor determining the release of the dye. Below LCST, PNIPAM copolymer is soluble in water so there would be no interaction between the copolymer and blue dextran. This is the case of 20°C, since LCST of the copolymer was about 35°C at pH 5.0 [Fig. (1)]. Above LCST, PNIPAM copolymer is hydrophobic so the polymer chains will aggregate into small particles in the dispersed domain of the microcapsules due to the inter/intra molecular hydrophobic interactions. In this circumstance, blue dextran could be entrapped in PNIPAM copolymer aggregates by the entanglements of the two kinds of polymers. This

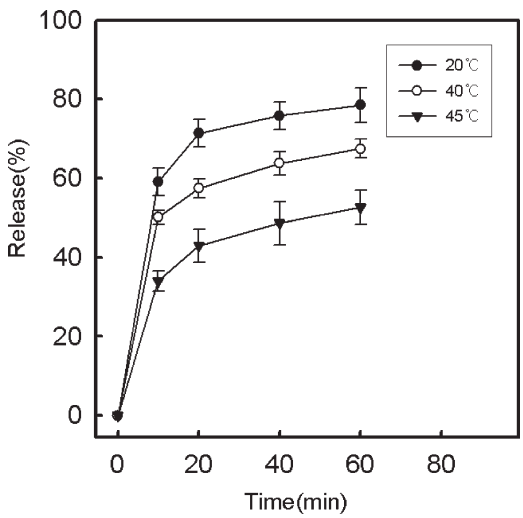

Figure 5 Temperature-dependent release profiles from microcapsules at pH 5.0.

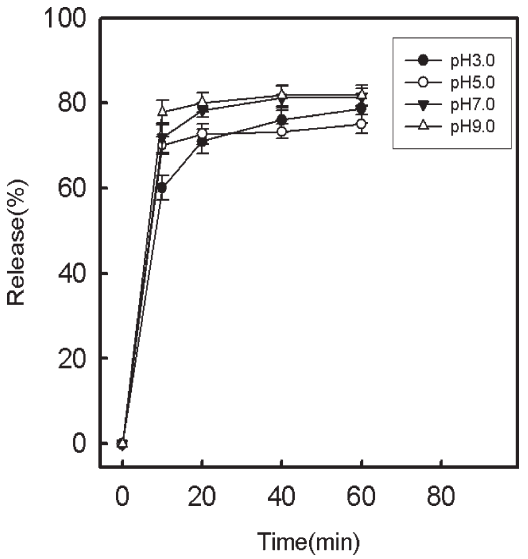

Figure 6 pH-dependent release profiles from microcapsules at 20°C.

would account for why the release was suppressed at the temperatures (40°C and 45°C) above LCST. The matrix of ethylcellulose acts as a container for PNIPAM domains, and it would maintain the domains even below LCST by confining them. And the mass transfer through the ethylcellulose matrix would be controlled by diffusion, so the release from PNIPAM domain will be retarded by the matrix. Without ethylcellulose matrix, the release below LCST will be completed in a few seconds because PNIPAM domain will be disintegrated upon dissolution in bulk aqueous phase below the temperature. H. Ichikawa and Y. Fukumori prepared core/shell type of thermosensitive microcapsule by an air suspension coating technique.[20] The core was water-soluble drug particle, and the shell was ethylcellulose matrix in which nano-sized PNIPAM gel was embedded. With this system, a positively thermosensitive release was observed. Below LCST, PNIPAM particles shrink, voids are created, and the release is promoted. Above LCST, the particles swell, voids disappear, and the release is suppressed. Accordingly, the PNIPAM particles act as a thermal valve in their research. On the contrary, a negatively thermosensitive release was obtained in this study. Since NIPAM copolymer is in a close contact with blue dextran in the dispersed domains, the polymer chains would trap the dye above LCST, and they would release the dye below LCST. Hence, NIPAM copolymer could act as a thermal trap instead of a thermal valve. This may account for why a negatively thermosensitive release was obtained in this study.

Figure 6 shows the pH-dependent release profiles from microcapsules at 20°C. As in the temperature-dependent release profiles, the release increased in a saturation manner for 60 min. The initial rate of release and the degree of release for 60 min were somewhat higher under neutral and alkali conditions (pH 7.0 and 9.0) than those observed under acidic conditions (pH 3.0 and 5.0). The conformation of P(NIPAM-*co*-MAA) chain will be dominated by two major forces. One is an electrostatic intramolecular repulsion by the MAA residues. The other is a hydrophobic intramolecular interaction by the NIPAM segments. The dramatic change of the chain conformation is caused by the hydrophobic interaction.[24] The temperature of 20°C, where the data in Figure 6 was obtained, is below LCSTs whether pH of the medium is 3.0, 5.0, 7.0, or 9.0 (Fig. 1). It means that, upon pH change from 3.0 to 9.0 at 20°C, the copolymer will not undergo a hydrophobic interaction and a phase transition. Therefore, when pH was varied from 3.0 to 9.0 at 20°C, the chain conformation would be dominated not by a hydrophobic interaction but by an intramolecular electrostatic repulsion. P(NIPAM-*co*-MAA) tends to take an stretched form under neutral and alkali conditions due to an intramolecular electrostatic repulsion between ionized MAA residues. As a result, the release would hardly be suppressed by the stretched copolymer. On the contrary, the copolymer chain is likely to take a random coil under acidic conditions due to the lack of an intramolecular electrostatic repulsion. Accordingly, the release would be suppressed by the coiled copolymer. The electrostatic intramolecular repulsion is believed to explain small differences in the pH-dependent release at 20°C.

Figure 7 shows the pH-dependent release profiles from microcapsules at 40°C. The release for 60 min was about 45% at pH 3.0. When the pH of release medium increased to 5.0, 7.0, and 9.0, the releases

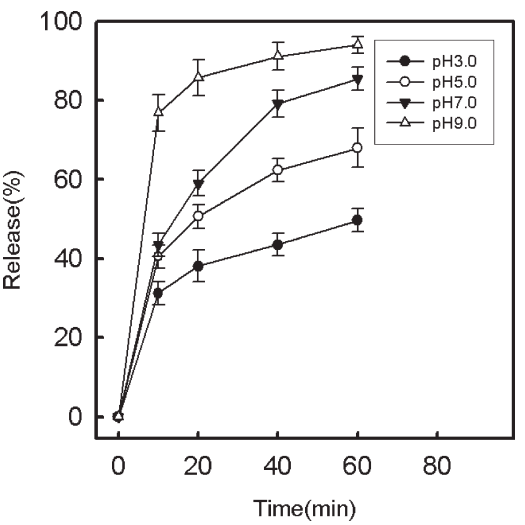

Figure 7 pH-dependent release profiles from microcapsules at 40°C.

for the same period were about 64%, 85%, 95%, respectively. When pH was 3.0, the temperature of 40°C is far above the LCST (Fig. 1). Accordingly, the copolymer would be hydrophobic. In addition, there would be no significant electrostatic repulsion at pH 3.0. Thus, the copolymer is likely to take a collapsed and contacted form, and it would trap blue dextran, leading to a suppressed release. When pH was 9.0, the temperature of 40°C is far below the LCST (Fig. 1). Therefore, the copolymer would be hydrophilic. In addition, there would be a significant electrostatic repulsion at pH 9.0. As a consequence, the copolymer will take an expanded form, and it tends to free blue dextran, resulting in a promoted release. The intramolecular hydrophobic forces together with electrostatic forces would be responsible for marked differences in the pH-dependent release at 40°C.

Whatever the pH and the temperature were, significant portion of dextran (20–35%) hasn't been released in our conditions. Both of PNIPAM copolymers and blue dextran are polymers so they may interact with each other by physical entanglement even in the conditions where the PNIPAM copolymers take an expanded configuration. In addition, the release of blue dextran is due to the diffusion through ethylcellulose membrane but not due to the disintegration of the microcapsules. When the dextran diffused out of the microcapsules, the dye would accumulate in the release medium since the release system was not under sink conditions. Accordingly, the equilibrium between the inside and the outside of the microcapsules will be reached with time elapse, and a significant portion of the dye would be retained in the microcapsules.

The microcapsules developed in this study released their contents in a controlled manner depending on pH and temperature. The release was suppressed in acidic conditions, and it was accelerated in neutral and alkali conditions (Fig. 7). Accordingly, it could be applied, in principle, to oral vaccinations and oral insulin delivery if P(NIPAM-*co*-MAA) were biocompatible. Protein drugs are denatured in gastric condition (strong acidic and digestive) but it could be protected from the harsh condition when encapsulated in the microcapsules. On the other hand, the microcapsules released their contents in a negatively thermosensitive manner (Fig. 5). If pesticides were encapsulated in the microcapsules, the availability to harmful insects could be much enhanced. Some harmful insects show high activity after sunset,[25] and they would be exposed to much more pesticides during night when temperature is relatively low. Whatever the application are, the duration of release is required to be prolonged for the practical use of the microcapsules. One of the methods would be to increase the concentration of ethylcellulose in the oil phase of emulsions so that the matrix of microcapsules is more compact.

CONCLUSIONS

Microcapsules composed of P(NIPAM-*co*-MAA) cores and ethylcellulose matrix were prepared by an emulsification and spray-drying method. Blue dextran loaded in the cores released in a negatively controlled manner. That is, it readily released below LCST but the release was suppressed above the phase transition temperature. It is believed that NIPAM copolymer acts as a thermal trap for blue dextran. In addition, microcapsules were also pH-sensitive in terms of release. The pH-sensitive release could be explained by the pH-dependent contraction and expansion of NIPAM copolymer.

References

1. Cheng, C. J.; Chu, L. Y.; Zhang, J.; Zhou, M.-Y.; Xie, R. Desalination 2008, 234, 184.
2. Yamashita, K.; Nishimura, T.; Nango, M. Polym Adv Technol 2003, 14, 189.
3. Duracher, D.; Elaõèssari, A.; Pichot, C. Colloid Polym Sci 1999, 277, 905.
4. Zhang, J.; Chu, L.-Y.; Li, Y.-K.; Lee, Y. M. Polymer 2007, 48, 1718.
5. Yin, W.; Chen, M.; Lu, T.; Akashi, M.; Huang, X. Eur Polym J 2006, 42, 2523.
6. Kim, J.-C.; Bae, S. K.; Kim, J.-D. J Biochem 1997, 121, 15.
7. Kim, J.-C.; Kim, J.-D. Colloid Surf B 2002, 24, 45.
8. Kono, K.; Henmia, A.; Yamashita, H.; Hayashi, H.; Takagishi, T. J Control Release 1999, 59, 63.
9. Crowther, H. M.; Vincent, B. Colloid Polym Sci 1998, 276, 46.
10. Kawaguchi, H.; Fujimoto, K.; Mizuhara, Y. Colloid Polym Sci 1992, 270, 53.
11. Shiroya, T.; Tamura, N.; Yasui, M.; Fujimoto, K.; Kawaguchi, H. Colloid Surf B 1995, 4, 267.
12. Gao, H.; Yang, W.; Min, K.; Zha, L.; Wang, C.; Fu, S. Polymer 2005, 46, 1087.
13. Park, J.-H.; Lee, Y.-H.; Oh, S.-G. Macromol Chem Phys 2007, 208, 2419.
14. Shen, Z.-Y.; Ma, G.-H.; Dobashi, T.; Maki, Y.; Su, Z.-G. Int J Pharm 2008, 346, 133.
15. Eve, R.-G.; Leroux, J.-C. Eur J Pharm Biopharm 2004, 58, 409.
16. Kono, K.; Nakai, R.; Morimoto, K.; Takagishi, T. Biochim Biophys Acta 1999, 1416, 239.
17. Choi, J. H.; Lee, H. Y.; Kim, J.-C.; Kim, Y. C. J Ind Eng Chem 2007, 13, 380.
18. Kim, M. H.; Kim, J.-C.; Lee, H. Y.; Kim, J. D.; Yang, J. H. Colloid Surf B 2005, 46, 57.
19. Choi, J.-H.; Lee, H. Y.; Kim, J.-C. J Appl Polym Sci 2008, 110, 117.
20. Hideki, I.; Fukumori, Y. J Control Release 2000, 63, 107.
21. Jo, S.-M.; Kim, J.-C. Colloid Polym Sci 2009, 287, 379.
22. Erbil, C.; Sarac, A. S. Eur Polym J 2002, 38, 1305.
23. Thies, C. In Microencapsulation; Benita, S., Ed.; CRC Press, New York, 1996; Chapter 1.
24. Kratz, K.; Hellweg, T.; Eimer, W. Colloid Surf A 2000, 170, 137.
25. Bonnemaison, L. Annu Rev Entromol 1965, 10, 233.