Polym. Bull. (2011) 67:67–76
DOI 10.1007/s00289-010-0403-x

ORIGINAL PAPER

Preparation and characterization of pH-sensitive hydrogel microparticles as a biological on–off switch

Eunmi Lee · Bumsang Kim

Received: 31 January 2010 / Revised: 26 September 2010 / Accepted: 5 November 2010 /
Published online: 16 November 2010

Abstract The pH-responsive swelling and release behaviors of anionic P(MAA-*co*-EGMA) hydrogel microparticles having various MAA and EG contents were investigated as a biological on–off switch for the design of an intelligent drug delivery system triggered by external pH changes. When DC was used as a dispersion stabilizer, well-dispersed hydrogel microparticles having an average diameter of approximately 4 μm were obtained. There was a drastic change of the equilibrium weight swelling ratio of P(MAA-*co*-EGMA) hydrogels at a pH of around 5, which is the pK_a of PMAA. When the MAA content in the hydrogel increased, the swelling ratio increased at a pH above 5 due to the more electrostatic repulsion between the charged groups of MAA. The P(MAA-*co*-EGMA) hydrogel microparticles showed a pH-responsive release behavior. At low pH (pH 4.0) small amounts of Rh-B were released while at high pH (pH 6.0) relatively large amounts of Rh-B were released from the hydrogels. The difference in the released amount of Rh-B from the hydrogels between pH 4.0 and 6.0 decreased when the MAA content in the hydrogels decreased, which means that the pH-responsive release behavior of the P(MAA-*co*-EGMA) hydrogel microparticles is closely related to the pH-responsive swelling property of the hydrogel.

Keywords pH-responsive · Hydrogel microparticles · Anionic hydrogels · Biological on–off switch · Intelligent drug delivery system

E. Lee · B. Kim (✉)
Department of Chemical Engineering, Hongik University, 72-1, Sangsu-dong, Mapo-gu, Seoul 121-791, Korea
e-mail: bskim@hongik.ac.kr

Introduction

Hydrogels are three-dimensional polymer networks that are capable of absorbing large amount of water or aqueous solvent, yet are insoluble because of the presence of cross-links, entanglements, or crystalline regions [1]. When a hydrogel is brought into contact with a compatible penetrant like water, the penetrant enters the hydrogel, and bringing the hydrogel network into a swollen state. If a solute is incorporated into the hydrogel, the solute is released [2–6]. In addition, due to the presence of certain functional groups along the polymer chains, hydrogels are often sensitive to environmental conditions, such as pH, temperature, ionic strength, electric field, or chemical compounds, resulting in drastic changes in their swelling ratio. By using the swelling behavior in response to the external environment, the release of solutes loaded within hydrogels can be controlled according to a specific site or time. Recently there have been many efforts to make use of anionic hydrogels for intelligent drug delivery systems. Anionic hydrogels contain ionizable groups that become ionized as the pH of the external swelling medium increases over the pK_a of the hydrogels. The pK_a is the negative logarithm of K_a, the acid dissociation constant, and at a pH above the pK_a of the hydrogel, the anionic hydrogel networks swell abruptly. Thus, if a solute is incorporated into the anionic hydrogel, at a pH above the pK_a the solute is released.

In the design of an intelligent controlled drug release device it is important to make a biological on–off switch that can control the release of the solute, such as a drug or biologically active material, depending on the external stimuli. For example, for the development of an oral drug delivery system for therapeutic proteins, the therapeutic proteins should not be released in the stomach but be released in the small intestine. In this system a biological on–off switch triggered by an external pH change is required since there is a significant difference in pH between the stomach (pH $\sim$ 2) and the intestine (pH $\sim$ 6) [7–11].

Anionic hydrogels containing poly(methacrylic acid) (PMAA) or poly(acrylic acid) (PAA) can form polyelectrolyte or hydrogen-bonded complexes that are strongly dependent on environmental pH and ionic strength [12–16]. In this study, we describe a preparation of the polymer hydrogel microparticles with methacrylic acid (MAA) and poly(ethylene glycol) methacrylate (PEGMA), henceforth designated as P(MAA-*co*-EGMA) hydrogel microparticles, and evaluate the feasibility of the P(MAA-*co*-EGMA) hydrogel microparticles as a biological on–off switch for an intelligent drug delivery system triggered by an external pH change. The effect of hydrogel composition of MAA and EG on pH-sensitive swelling and release behavior was investigated.

Experimental

Materials

Methacrylic acid (MAA), poly(ethylene glycol) methacrylate (PEGMA, molecular weight 360), poly(ethylene glycol) dimethacrylate (PEGDMA, molecular weight

330), and silicon oil were purchased from Sigma-Aldrich (USA). 1-Hydroxycyclohexyl phenyl ketone (otherwise known as Irgacure® 184) was obtained from Ciba Specialty Chemicals (USA). Dimethicone copolyol (DC) was obtained from Nabion (Korea). Rhodamine B (Rh-B) as a model solute was purchased from Junsei (Japan).

Synthesis of P(MAA-*co*-EGMA) hydrogel microparticles

The P(MAA-*co*-EGMA) hydrogel microparticles were synthesized via dispersion photopolymerization. Monomers with feed compositions (molar ratio) of 1:1, 0.8:1, 0.6:1, 0.4:1, and 0.2:1 MAA:EG were mixed. In each set of monomer mixtures, PEGDMA was added as a cross-linker in an amount of 0.75 mol% of total monomers. Irgacure® 184, as a UV light sensitive initiator, was added in an amount of 0.5 wt% of total monomers and these mixtures were then diluted with deionized water to 25% by weight of total monomers. The mixture was purged with nitrogen for 10 min to remove dissolved oxygen that would act as an inhibitor of the reaction and then added to 30 mL of silicon oil to which various amounts of DC was added. DC was used as a dispersion stabilizer. The hydrophilic mixture was dispersed in the silicon oil by an ultrasonic processor (VCX750, Sonics & Materials) for 2 min. The dispersion solution was irradiated with UV light (intensity 1000 mW/cm^2) for 300 s for the polymerization. Synthesized particles were then separated from oil by several repeated cycles of washing with deionized water, centrifugation and sonication. The washed particles were lyophilized using a freeze-dryer (Ecospin 3180C, Biotron) for 24 h at maximum vacuum. The distribution and size of the synthesized microparticles were observed using microscope (Olympus BX51), scanning electron microscopy (SEM, Jeol 6300), and dynamic light scattering (DLS, Malvern Nano S90).

Swelling studies of P(MAA-*co*-EGMA) hydrogel microparticles

To determine the pH-responsive swelling behavior of the P(MAA-*co*-EGMA) hydrogel microparticles, the freeze-dried microparticles were weighed and then placed in phosphate-citrate buffer solutions with pH values in the range from 2.0 to 8.0. The ionic strength of each buffer solution was adjusted to 0.5 M by the addition of potassium chloride. After swelling, the microparticles were removed from the buffer solution by centrifugation and weighed. The swelling of the microparticles was expressed as the weight swelling ratio, q, defined as the ratio of the weight of the swollen microparticles to the weight of the dried microparticles. The equilibrium weight swelling ratio was obtained when the weight of swollen microparticles reached a constant value ($\pm 1\%$).

Rh-B incorporation and release studies

A concentration of 0.01 mg/mL of Rh-B stock solution was prepared and the absorbance of the solution was measured in an UV–visible spectrophotometer (Varian, Cary 100) at a wavelength of 554 nm. Incorporation of Rh-B was

accomplished by soaking the hydrogel microparticles in 20 mL of Rh-B stock solution for 24 h. At specific time points, 0.5 mL samples were withdrawn from the solution and the absorbance measured to determine the Rh-B loading efficiency, calculated as the ratio of the amount of Rh-B incorporated into the hydrogel to the amount of Rh-B in the stock solution. After 24 h, the Rh-B-loaded hydrogel microparticles were separated from the solution by centrifugation and then used for the release experiments. To release Rh-B from the particles, 0.05 g of Rh-B-loaded hydrogel microparticles were placed in 25 mL of buffer solutions with pH values of 4.0 and 6.0. At specific time points, 0.5 mL of sample was withdrawn from the solution and the absorbance was measured. The concentration of incorporated and released Rh-B was obtained from the calibration curve of Rh-B concentrations versus their absorbance.

Results and discussion

P(MAA-*co*-EGMA) hydrogel microparticle synthesis

P(MAA-*co*-EGMA) hydrogel microparticles were synthesized via dispersion photopolymerization of an aqueous monomer mixture in a continuous phase of silicon oil. The formation of P(MAA-*co*-EGMA) hydrogel microparticles was based on UV-initiated free-radical polymerization of the methacrylate end groups of MAA, PEGMA, and PEGDMA. Since PEGDMA was used as a cross-linking agent, highly cross-linked polymer networks were formed. Without a stabilizer, there was an agglomeration of hydrogel particles after the polymerization. Thus, DC was used as a dispersion stabilizer since DC is a polyoxyethylene-based surfactant and has ethylene glycol units as hydrophilic parts of the molecule, which have a high structural similarity to ethylene glycol in the monomers and the cross-linker that were used in this study. The structure of DC is illustrated in Fig. 1. Figure 2 shows optical microscope images of P(MAA-*co*-EGMA) hydrogel microparticles synthesized with and without DC as a dispersion stabilizer. As expected, when a stabilizer was used, a well-dispersed hydrogel particle suspension was obtained (Fig. 2b). In order to investigate the effect of the amount of stabilizer on the particle preparation, the P(MAA-*co*-EGMA) hydrogel microparticles were synthesized using various concentrations of DC. The average size of the particles was approximately 4 μm, which was measured by DLS, and there was no significant difference in particle size depending on the DC concentration. However, when a small amount (0.5 wt%) of DC was used, dispersion of the particles was not good so a concentration of 2.0 wt% was finally chosen to obtain a good dispersion of the particles. The SEM image of P(MAA-*co*-EGMA) hydrogel microparticles synthesized with DC 2.0 wt% is shown in Fig. 3.

pH-sensitive swelling behavior of P(MAA-co-EGMA) hydrogel microparticles

In general, the pH-sensitive swelling behavior of anionic hydrogels results from the ionization or deionization of functional groups in response to external pH changes.

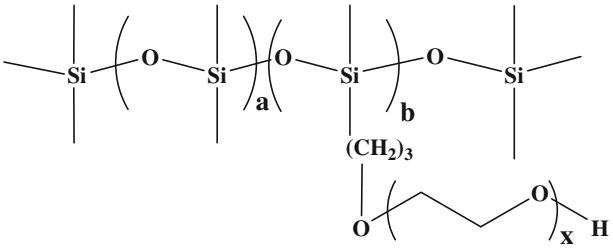

Fig. 1 Structure of dimethicone copolyol (DC) as a dispersion stabilizer

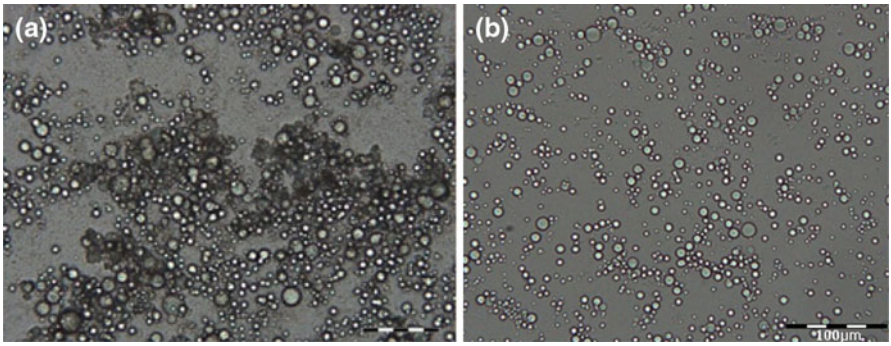

Fig. 2 Optical microscope images of P(MAA-*co*-EGMA) hydrogel microparticles synthesized without and with DC as a dispersion stabilizer: **a** without DC and **b** with DC 2.0 wt%. *Scale bar* is 100 μm

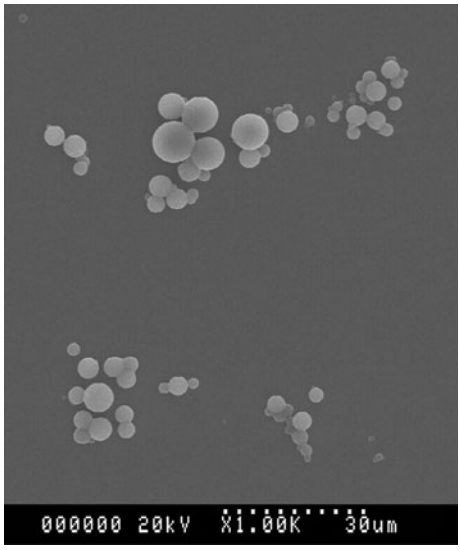

Fig. 3 SEM image of P(MAA-*co*-EGMA) hydrogel microparticles synthesized with DC 2.0 wt%. *Scale bar* is 30 μm

Figure 4 shows the equilibrium weight swelling ratios of P(MAA-*co*-EGMA) hydrogel microparticles having various MAA and EG contents as a function of pH in the range from 2.0 to 8.0. There was a drastic change in the equilibrium weight

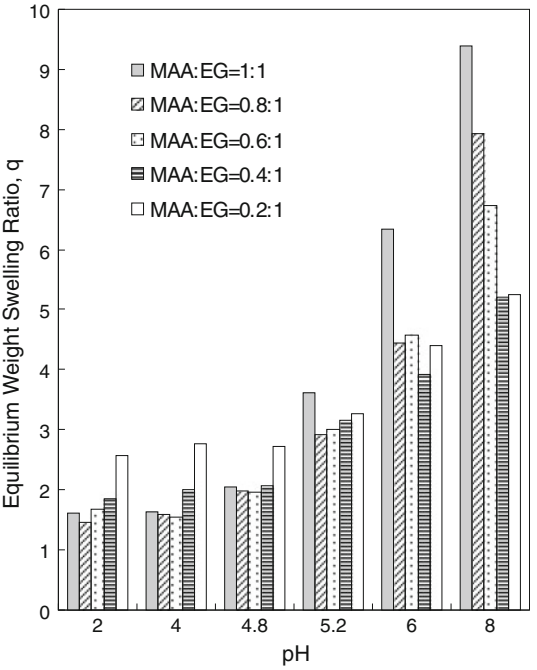

Fig. 4 Equilibrium weight swelling ratio of P(MAA-*co*-EGMA) hydrogel particles having various MAA and EG contents: MAA:EG = 1:1 (*medium shaded square*), MAA:EG = 0.8:1 (*square with upper right to lower left fill*), MAA:EG = 0.6:1 (*dotted square*), MAA:EG = 0.4:1 (*square with horizontal fill*), and MAA:EG = 0.2:1 (*empty square*) ($N = 3$)

swelling ratio of P(MAA-*co*-EGMA) hydrogels at a pH of around 5, which is the pK_a of PMAA. At a pH below 5, the hydrogels were in a relatively collapsed state but at a pH higher than 5, the hydrogels swelled to a high degree. In addition, as the MAA content in the hydrogel increased, the swelling ratio increased at a pH above 5. The reason for this was that at a pH higher than the pK_a of the hydrogels, the hydrogels that had more MAA content could produce more ionized carboxylic acid groups, which resulted in a greater electrostatic repulsion between the charged groups, leading to the high swelling ratio. This sharp transition between the swollen and collapsed states at a specific pH indicates that the P(MAA-*co*-EGMA) hydrogel microparticles can be used as an on–off switch with which the release of the solute from the hydrogels can be controlled by an external pH change and the pK_a of the hydrogels. In order to quantify the pH-sensitivity of the P(MAA-*co*-EGMA) hydrogel microparticles, the ratios of the equilibrium weight swelling ratio at pH 6 to the equilibrium weight swelling ratio at pH 4 depending on the MAA and EG contents of the hydrogel were calculated and are summarized in Table 1. The difference in swelling ratio between pH 4.0 and 6.0 decreased as the MAA content decreased.

Loading and pH-sensitive release behavior of P(MAA-*co*-EGMA) hydrogel microparticles

In the Rh-B loading experiments, as the hydrogel particles in a Rh-B stock solution absorbed water, the Rh-B was transported with water in the hydrogels due to the

Table 1 Ratio of equilibrium weight swelling ratios at pH 6 (q_{pH6}) to pH 4 (q_{pH4}) of P(MAA-*co*-EGMA) hydrogel microparticles having various MAA and EG contents ($N = 3$)

MAA:EG	q_{pH6}/q_{pH4}
1:1	3.9 (±0.60)
0.8:1	3.1 (±0.67)
0.6:1	2.9 (±0.21)
0.4:1	2.0 (±0.50)
0.2:1	1.5 (±0.03)

concentration gradient of Rh-B between the outside and the inside of the hydrogel. The loading efficiencies of P(MAA-*co*-EGMA) hydrogel microparticles having various MAA and EG contents as a function of time are shown in Fig. 5. For the P(MAA-*co*-EGMA) hydrogel microparticles with different MAA:EG contents, more than 83% of Rh-B was incorporated into the hydrogels except for P(MAA-*co*-EGMA) particles with a 0.2:1 ratio of MAA:EG. This indicates that the incorporation of Rh-B into the hydrogel was affected by both the degree of swelling of the hydrogel and the electrostatic interaction between the hydrogel and Rh-B. At pH 7.0, where the loading experiments were carried out, the hydrogel networks had negative charges due to the ionization of the carboxylic acid groups of MAA while the Rh-B had positive charges [17]. Therefore, there was the electrostatic attraction between negatively charged hydrogel networks and positively charged Rh-B, leading to the high loading efficiency. However, The P(MAA-*co*-EGMA) hydrogel having a 0.2:1 ratio of MAA:EG had a relatively small amount of negative charges because of the small MAA content, which produced the least

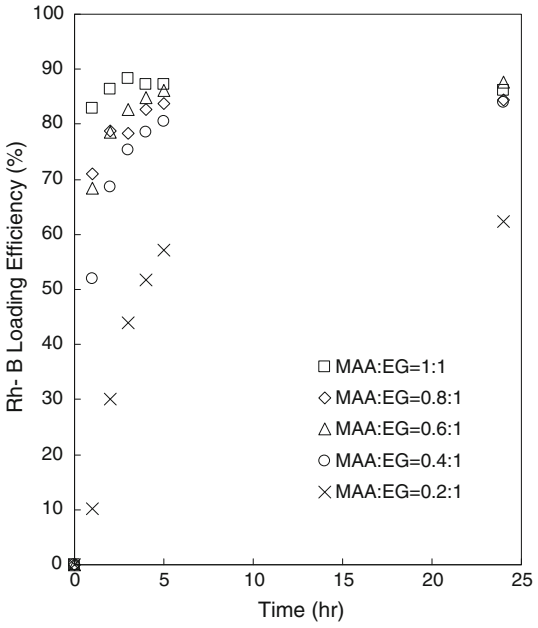

Fig. 5 Rh-B loading efficiency of P(MAA-*co*-EGMA) hydrogels having various MAA and EG contents as a function of time: MAA:EG = 1:1 (*square*), MAA:EG = 0.8:1 (*diamond*), MAA:EG = 0.6:1 (*triangle*), MAA:EG = 0.4:1 (*circle*), and MAA:EG = 0.2:1 (*times*) ($N = 3$)

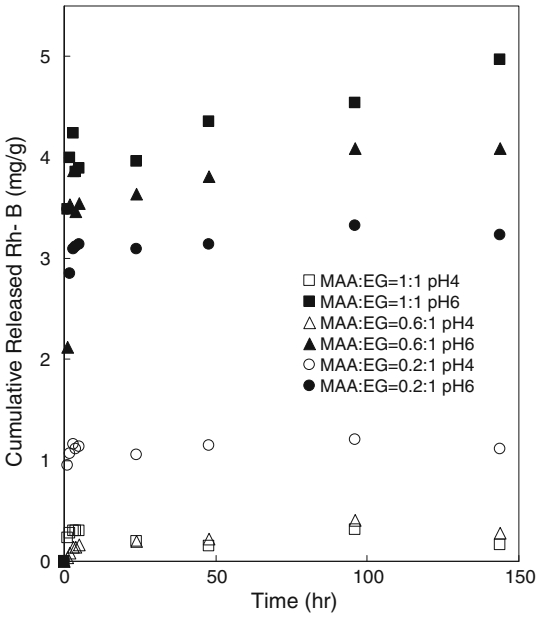

Fig. 6 Cumulative amount of Rh-B released from P(MAA-*co*-EGMA) hydrogel microparticles having various MAA and EG content as a function of time ($N = 3$)

negative charges resulting in the lowest swelling ratio at pH 7.0, shown in Fig. 4. Thus, it exhibited the lowest loading efficiency, about 62%.

To investigate the pH-sensitive release behavior of P(MAA-*co*-EGMA) hydrogel microparticles, the Rh-B-loaded hydrogel particles were placed in pH 4.0 and 6.0 buffer solutions. The cumulative amount of Rh-B released from the particles as a function of time is presented in Fig. 6. The P(MAA-*co*-EGMA) hydrogel microparticles showed a pH-sensitive release behavior, i.e., at low pH (pH 4.0), small amounts of Rh-B were released from the particles while at high pH (pH 6.0), relatively large amounts of Rh-B were released from the particles. In addition, there was a significant difference in the released amount of Rh-B from the hydrogels between pH 4.0 and 6.0 according to the MAA content in the hydrogel. In order to quantify the pH-responsive release behavior of the P(MAA-*co*-EGMA) hydrogel microparticles according to the MAA content in the hydrogel, the ratios of the average cumulative amount of the released Rh-B at pH 4.0 to that at pH 6.0 for 144 h were calculated and are listed in Table 2. The difference in the released amount of Rh-B between pH 4.0 and 6.0 decreased when the MAA content in the hydrogel decreased. This behavior resulted from the pH-responsive swelling behavior of the hydrogel. For instance, the P(MAA-*co*-EGMA) particles having a 0.2:1 ratio of MAA:EG showed the least difference in swelling ratio between pH 4 and 6, and a similar behavior was observed in the amount of released Rh-B between pH 4 and 6, which means that the pH-responsive release behavior of the P(MAA-*co*-EGMA) hydrogel microparticles are closely related to the pH-responsive swelling property of the hydrogel. These results indicate that the P(MAA-*co*-EGMA) hydrogel microparticles can be used as a biological on–off switch for an intelligent drug delivery system triggered by an external pH change in the body. In addition,

Table 2 Average cumulative amount (M_R) of released Rh-B at pH 4.0 and 6.0 for 144 h ($N = 3$)

MAA:EG	M_R (mg/g)		$M_{R6.0}/M_{R4.0}$
	pH 4.0	pH 6.0	
1:1	0.16 (±0.11)	4.97 (± 0.07)	31.1
0.8:1	0.22 (±0.10)	4.97 (±0.07)	22.6
0.6:1	0.28 (±0.09)	4.09 (±0.13)	14.6
0.4:1	0.56 (±0.29)	4.57 (±0.11)	8.2
0.2:1	1.11 (±0.59)	3.23 (±2.17)	2.9

the P(MAA-*co*-EGMA) hydrogel microparticles did not release Rh-B for about 6 days at low pH (pH 4.0), which means that the P(MAA-*co*-EGMA) hydrogel particles can keep the solute, such as drugs or biologically active materials, inside the hydrogels for a long period and release the solute from the hydrogels in response to an increase in the external pH above the pK_a of the hydrogel.

Conclusions

pH-Sensitive P(MAA-*co*-EGMA) hydrogel microparticles having various MAA and EG contents were prepared by free-radical photopolymerization. When DC was used as a dispersion stabilizer, well-dispersed hydrogel particles were obtained. There was a drastic change in the equilibrium weight swelling ratio of P(MAA-*co*-EGMA) hydrogel microparticles at a pH of around 5, which is the pK_a of PMAA, i.e., low swelling ratios at a pH less than 5 but high swelling ratios at a pH greater than 5. In addition, as the MAA content in the hydrogel increased, the swelling ratio increased at a pH above 5. The P(MAA-*co*-EGMA) hydrogel microparticles showed a pH-responsive release behavior. At low pH (pH 4.0) small amounts of Rh-B were released while at high pH (pH 6.0) relatively large amounts of Rh-B were released from the hydrogels. The difference in the released amount of Rh-B from the hydrogels between pH 4.0 and pH 6.0 decreased when the MAA content in the hydrogel decreased, which was very similar to the pH–sensitive swelling behavior of the hydrogel. These results indicate that the P(MAA-*co*-EGMA) hydrogel microparticles can be used as a biological on–off switch with which the release of solutes from the hydrogel can be controlled by an external pH change.

Acknowledgments This work was supported by 2010 Hongik University Research Fund and the Grant of Small and Medium Business Administration, Republic of Korea (No. S1065960).

References

1. Peppas NA, Bures P, Leobandung W, Ichikawa H (2000) Hydrogels in pharmaceutical formulations. Eur J Pharm Biopharm 50:27–46
2. Bell CL, Peppas NA (1996) Water, solute and protein diffusion in physiologically responsive hydrogels of poly (methacrylic acid-g-ethylene glycol). J Control Release 39:1203–1218

3. Soppirnath KS, Aminabhavi TM (2002) Water transport and drug release study from cross-linked polyacrylamide grafted guar hydrogel microspheres for the controlled release application. Eur J Pharm Biopharm 53:87–98
4. Tada D, Tanabe T, Tachobana A, Yamauchi K (2005) Drug release from hydrogel containing albumin as crosslinker. J Biosci Bioeng 100:551–555
5. Scott RA, Peppas NA (1999) Highly crosslinked PEG-containing copolymers for sustained solute delivery. Biomaterials 20:1371–1380
6. Kim C, Lim S (2002) Recent progress in drug delivery systems for anticancer agents. Arch Pharm Res 25:229–239
7. Kim B, Flamme KL, Peppas NA (2003) Dynamic swelling behavior of pH-sensitive anionic hydrogels used for protein delivery. J Appl Polym Sci 89:1606–1613
8. Peppas NA (2004) Devices based on intelligent biopolymers for oral protein delivery. Int J Pharm 277:11–17
9. Kim B, Peppas NA (2003) In vitro release behavior and stability of insulin in complexation hydrogels as oral drug delivery carriers. Int J Pharm 266:29–37
10. Morishita M, Goto T, Peppas NA, Joseph JI, Torjman MC, Munsick C, Nakamura K, Yamagata T, Takayama K, Lowman AM (2004) Mucosal insulin delivery systems based on complexation polymer hydrogels: effect of particle size on insulin enteral absorption. J Control Release 97:115–124
11. Foss AC, Goto T, Morishita M, Peppas NA (2004) Development of acrylic-based copolymers for oral insulin delivery. Eur J Pharm Biopharm 57:163–169
12. Kim KC, Lee SJ (1989) pH-dependent swelling properties of methacrylic acid copolymer hydrogels. J Pharm Soc Kor 33:372–376
13. Donini C, Robinson DN, Colombo P, Giordano F, Peppas NA (2002) Preparation of poly(methacrylic acid-g-poly(ethylene glycol)) nanospheres from methacrylic monomers for pharmaceutical applications. Int J Pharm 245:83–91
14. He H, Li L, Lee LJ (2006) Photopolymerization and structure formation of methacrylic acid based hydrogels in water/ethanol mixture. Polymer 47:1612–1619
15. Kim B, Peppas NA (2003) Analysis of molecular interactions in poly(methacrylic acid-*g*-ethylene glycol) hydrogels. Polymer 44:3701–3707
16. Robinson DN, Peppas NA (2002) Preparation and characterization of pH-responsive poly(methacrylic acid-*g*-ethylene glycol) nanospheres. Macromolecules 35:3668–3674
17. Mchedlov-Petrossyan NO, Vodolazkaya NA, Doroshenko AO (2003) Ionic equilibria of fluorophores in organized solutions: the influence of micellar microenvironment on protolytic and photophysical properties of rhodamine B. J Fluoresc 13:235–248