Front. Mater. Sci. China 2009, 3(4): 374–379
DOI 10.1007/s11706-009-0069-1

RESEARCH ARTICLE

Synthesis and characterization of a pH/temperature responsive glycine-mediated hydrogel for drug release

Kui-lin DENG (✉), Peng-fei ZHANG, Xiao-bo REN, Hai-bin ZHONG, Yu-bo GOU, Li-rong DONG, Qian LI

College of Chemistry & Environmental Science, Hebei University, Baoding 071002, China

Abstract In this work, a pH/temperature responsive hydrogel (PMEA) from *N*-acryloylglycine methyl ester (NAGME), *N*-acryloylglycine ethyl ester (NAGEE), and acrylic acid (AAc) was synthesized by free radical polymerization. The swelling behaviors and drug release properties of hydrogels were systematically investigated at different temperature, pH, and AAc content. It was found that the hydrogel PMEA demonstrated pH and temperature responsive nature. The caffeine-release behaviors showed that only 49.1% caffeine was released from PMEA in pH 2.70 phosphate buffer solution (PBS) after 500 minutes, whereas more than 93.9% caffeine was gradually diffused into the medium in pH 7.49 PBS over the same time interval. In addition, the caffeine release was much higher at 37°C than that at 14°C in deionized water. As seen from the results, the PMEA seems to be a potential drug carrier with pH-temperature responsiveness.

Keywords pH/temperature-responsive, *N*-acryloyl glycinate, drug release, polymer gels

1 Introduction

The intelligent hydrogels, as hydrophilic and crosslinked polymer networks, can change their volumes but not dissolve in response to the stimuli in the surroundings. The stimuli contain some physical and chemical changes such as temperature [1,2], pH [3,4], and different concentration and kinds of ions in the solution [5,6]. Among these, pH- and thermo- responsive polymers have drawn much attention, because the two factors are important environments in the human body [7]. In recent years, these polymeric hydrogels have been extensively investigated as the potential carriers for the drug controlled-release [1,2,7–9]. For instance, 5-fluorouracil release rate from the thermo-sensitive poly(*N*-isopropylacrylamide) (PNIPAm) hydrogel was found to be effectively regulated by temperature changes between 10°C and 37°C [2]. Also, Shi et al. reported that the cumulative release ratio of indomethacin was higher at 37°C than that at 25°C and increased slightly with increasing PNIPAm content [7].

Because of the good balance between hydrophilic and hydrophobic interactions in the polymers, poly(*N*-isopropylacrylamide) (PNIPAm) showed well-defined lower critical solution temperature (LCST) in water at around 32°C [10,11]. As a result, PNIPAm was widely selected as candidate to prepare the temperature sensitive hydrogel. For the pH-responsive hydrogels, either acidic ($-COOH$) or basic ($-NH_2$) pendent groups are contained in the network. The pH/temperature responsive hydrogels were synthesized by incorporating the pH-responsive and temperature-sensitive components [12–14]. For example, Liu et al. investigated fast responsive thermo- and pH-sensitive poly[(*N,N*-diethylacrylamide)-*co*-(acrylic acid)] hydrogels [12]. In order to get the both temperature and pH sensitive copolymeric hydrogels, itaconic acid was used to modify the thermo-sensitive polymer of *N*-Isopropylacrylamide [13].

As a drug carrier in the biochemical field, safety and nontoxicity to body should be considered in synthesizing pH or thermo-sensitive copolymers. Compared to PNIPAm, the polymers derived from *N*-acryloylglycine methyl ester (NAGME) and *N*-acryloylglycine ethyl ester (NAGEE) have the similar structure. Also, due to the presence of labile ester groups, these polymers have high degradability. Similar to poly[bis(glycine ethyl ester) phosphazene] [15], the presence of natural amino acids moieties in the polymer chain may enhance the susceptibility to biocompatibility. Therefore, the polymers from NAGME and NAGEE can be used as materials for preparing the pH or thermo-sensitive polymer hydrogels.

It has been reported that the LCST of the homo-polymer

Received September 5, 2009; accepted September 25, 2009

E-mail: dkl369@hbu.edu.cn

derived from NAGME and NAGEE were 64°C and 14°C, respectively [16]. The copolymer hydrogel with LCST in the physiological temperature range can be obtained by adjusting the ratio of NAGME to NAGEE in the polymerization. To our knowledge, there is no report on the synthesizing the copolymer from NAGME, NAGEE, and AAc. The purpose of this study is to prepare a pH- and thermo-responsive polymer hydrogel from NAGME, NAGEE, and AAc. We investigated the effects of pH, temperature, inorganic salts, and AAc contents on the swelling behaviors of copolymer hydrogel. In addition to this, the caffeine release from polymeric hydrogels was evaluated as a function of pH value and temperature. It was found that caffeine release showed a considerable dependence on the pH value and temperature change. The pH/thermo-sensitive polymer hydrogel was expected to be a potential pH/temperature controlled drug release carrier in the biochemical fields.

2 Experimental

2.1 Materials

Acrylic acid (AAc, Analytical purity) from Tianjin Chemical Company was distilled under reduced pressure prior to use. *N*-acryloylglycine methyl ester (NAGME) and *N*-acryloylglycine ethyl ester (NAGEE) were synthesized according to the reported procedure [16]. Ammonium persulfate (APS) and sodium bisulfite (SBS) from Tianjin Chemical Company, China, were of analytical purity grade and used as received. *N*-methylenebisacrylamide (NMBA) from Tianjin Huadong Chemical Factory was used as a crosslinker. All solvents and other chemicals used were of analytical grade.

2.2 Preparation of hydrogel PMEA

The pH/temperature responsive hydrogels (PMEA) were prepared through the free-radical copolymerization of NAGME, NAGEE, and AAc in the aqueous solution. The synthetic scheme for PMEA is illustrated in Fig. 1. In the polymerization, NAGME (0.143 g, 0.001 mol), NAGEE (0.157 g, 0.001 mol), deionized water (2 mL), and AAc (3%, 5%, and 10%, based on total weight of NAGME, NAGEE, and AAc) were added into a glass polymerization tube. NMBA (3%) and APS/SBS (4%), based on total weight of NAGME and NAGEE, were then introduced into the polymerization tube. Dry nitrogen was bubbled into the solution for 30 min to remove dissolved oxygen from the tube. Then, the polymerization was carried out at 20°C for 24 h. After

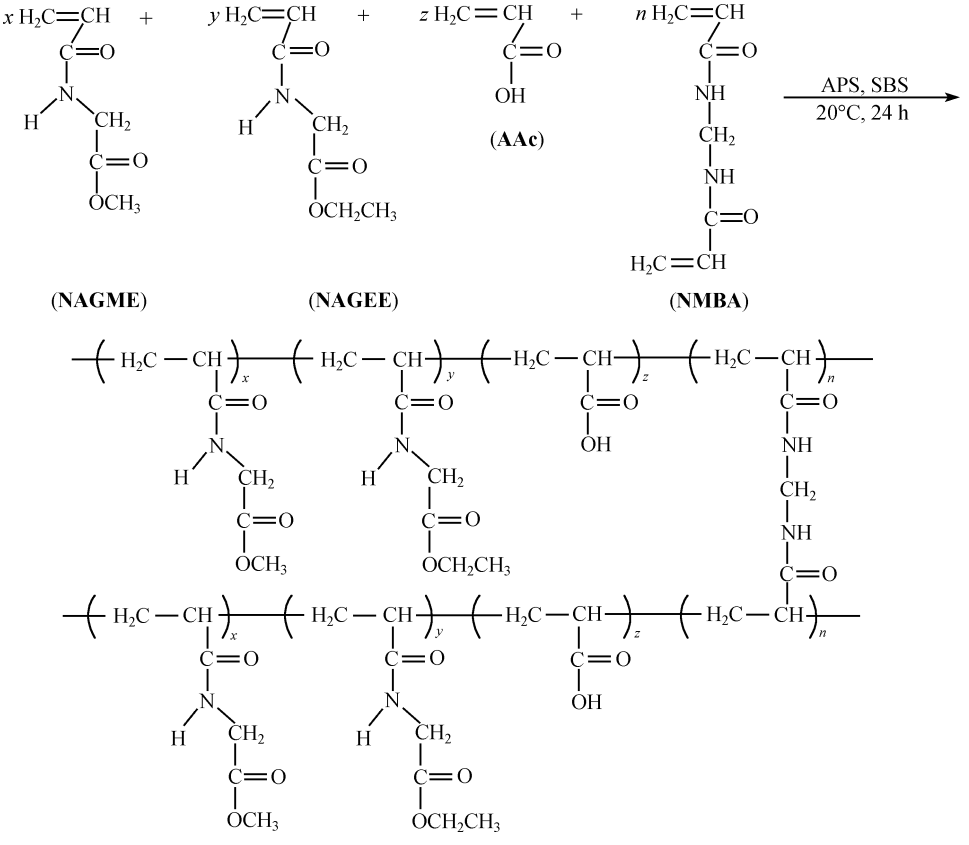

Fig. 1 Synthetic scheme for copolymer hydrogel PMEA

polymerization, the copolymer hydrogel was immersed in deionized water for one day to remove the unreacted monomers.

2.3 Measurement of swelling ratio

The swelling behaviors of PMEA were investigated in deionized water, phosphate buffer solution, and different ionic strength medium. The hydrogels PMEA were immersed in the swelling medium. At given time intervals, the hydrogels were removed from the medium, and the weight of the swollen hydrogel was determined after removing the surface water through blotting with filter paper. The equilibrium swelling ratio (SR) was calculated by the following equation:

$$\mathrm{SR} = \frac{W_\mathrm{d} - W_\mathrm{o}}{W_\mathrm{o}} \quad (1)$$

Here, the SR was considered to be the equilibrium value when the swollen hydrogels reached a constant weight. In Eq. (1), W_o and W_d were the weights of PMEA before and after swelling, respectively.

2.4 Drug loading and release experiments

According to the reported procedure [17], the caffeine-loaded hydrogel was prepared by copolymerization of NAGME, NAGEE, and AAc. In the polymerization, NAGME (0.143 g, 0.001 mol), NAGEE (0.157 g, 0.001 mol), and AAc (5 wt.%, based on the total monomer weight) were dissolved in 2 mL deionized water in a glass tube. NMBA (3 wt.%, based on the total weight of monomers) was controlled in the process of polymerization. The model drug, caffeine, was fixed at 5% with respect to the total weight of monomers. The dried nitrogen was bubbled into the polymerization system for 30 min to remove the oxygen dissolved in the reaction mixture. APS and SBS as a redox initiator (4 wt.%) was quickly added to the mixture. The mixture was then kept at 20°C for 24 h. After completing the polymerization, the caffeine-loaded hydrogels were dried at 50°C under vacuum to constant weight and stored at 20°C before use.

The caffeine release studies were performed by placing caffeine-loaded hydrogel (about 40 mg) separately in 40 mL release solutions (pH 2.70, 7.49 PBS and deionized water). The mixture was stirred at 100 rpm with a magnetic stirrer. At specified time intervals, 4 mL sample solution was withdrawn from the release medium, and 4 mL fresh solution was added into the release medium to maintain the unchanged solution volume. The absorbance of sample was measured by UV spectrophotometer at 272 nm wavelength. Finally, the weight of the released caffeine was calculated with the standard calibration curve.

3 Results and discussion

3.1 Swelling behaviors of PMEA

3.1.1 Sensitivity of hydrogel PMEA to temperature

It has been reported that when the molar ratio of NAGME to NAGEE was fixed at 1:1; the LCST of the corresponding copolymer is 29.5°C [16]. As a result, the ratio of NAGME to NAGEE was also fixed in order to get desired hydrogel with phase transition temperature in the range of the physiological temperature.

Figure 2 has shown the swelling behaviors of hydrogel PMEA with different acrylic acid content (3 wt.%, 5 wt.%, and 10 wt.%) in deionized water at different temperature. As seen in Fig. 2, all the hydrogels have shown sensitivity to the temperature change. The hydrogel PMEA had almost the same volume phase transition temperature between 25°C and 40°C, which is close to the physiological temperature. On one hand, the increasing of AAc content resulted in an increase of the swelling ratio (SR). For example, when the temperature was controlled at 20°C, the swelling ratio of PMEA with 3%, 5%, and 10% AAc were 17.5, 19.4, and 21.3, respectively. With increasing AAc content, the hydrophilicity of PMEA hydrogel was increased greatly, leading to higher swelling ratio. On the other hand, the range of phase transition temperature became wider with increasing AAc content in PMEA. Additionally, all the hydrogels exhibited high swelling ratio at lower temperature, but they deswelled at higher temperature. This phenomenon may be due to the following fact: at low temperature, the H-bonding interactions among polymer chains and water molecules are dominant, bringing about higher swelling ratio. When the temperature is raised above the phase transition temperature, the hydrophobic interactions among the polymer chains become dominant and break the delicate balance between H-bonding and hydrophobic interactions. The increased hydrophobic interactions in PMEA consequently

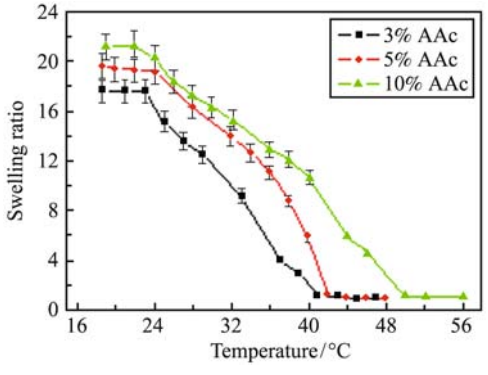

Fig. 2 Dependence of swelling ratio on the temperature (medium: deionized water; pH: 6.5)

caused the polymer chains to collapse and decrease the swelling ratio dramatically [18,19].

3.1.2 Sensitivity of hydrogel PMEA to pH

Due to the considerable dependence of the swelling ratio to the ion strength, it was very necessary to maintain the same ion strength in investigating pH-swelling ratio relation. In this study, all the ion strengths in the phosphate buffer solution were adjusted to $I = 0.60$. Figure 3 has illustrated the swelling behaviors for PMEA in the solutions with different pH value at 20°C. With increasing pH value, the equilibrium swelling ratios of the hydrogel PMEA increased. Specially, it was necessary to mentioned that the prepared PMEA were very sensitive to the two pH changing range including 4–6 and above 8. When pH values in the solution were increased to 6 from 4 and to 10 from 8, the swelling ratio of PMEA with 5% AAc were upgraded to 11.06 from 5.90 and to 17.9 from 11.4, respectively.

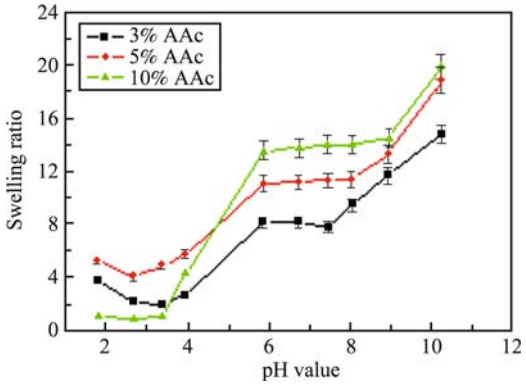

Fig. 3 Dependence of the swelling ratio on pH value in the medium (medium: PBS; temperature: 20°C)

In addition, the swelling ratio also exhibited a remarkable dependence on the AAc content. In general, the higher AAc content in PMEA leads to great swelling ratio. For instance, the swelling ratio for PMEA reached 7.8, 11.3, and 14.0 in pH = 7.4 PBS, as the AAc content were controlled at 3%, 5%, and 10%. The higher the AAc content in PMEA is, the stronger the hydrophilicity of polymer chains is, especially in the basic solution. Therefore, the higher hydrophilicity for PMEA with the high AAc content consequently induced to the free outspread of polymer chain in hydrogel, resulting in a high swelling ratio [20,21].

3.1.3 Sensitivity of hydrogel PMEA to salts

Considering the practical application in the drug release system, the investigation on swelling behaviors of PMEA in salt solution seems to be more significant. The reason is that the swelling behaviors of hydrogel are also an important factor in drug releasing. As shown in Fig. 4, the effect of different inorganic salts on the swelling ratio of PMEA was studied at room temperature. It was found that the swelling behaviors showed a dependence on the kinds of the inorganic salt and its ion strength, especially in the lower ion strength range. For KI, KBr, and KCl, the swelling ratio of PMEA decreased dramatically with increasing salt concentration. For example, when KCl concentrations were fixed at 0.005, 0.01, 0.025, and 0.05 mol/L, the swelling ratios for PMEA with 3% NMBA and 5% AAc was 15.54, 15.34, 11.69, and 11.09, respectively. In carboxymethyl cellulose and polyacrylic acid hydrogel [22], and PNEPAM [23] system, the similar results that the swelling ratio decreased with increasing the salt concentration were also found. Additionally, the swelling ratio was significantly sensitive to the kinds of inorganic salts. At the same salt concentration, KCl induced the highest decrease of swelling ratio among the three inorganic salts. KCl belongs to a “salt-out” agent, which factually decreases the H-bonding (hydrophilic interaction) between water molecules and polymeric chains [24]. This influence necessarily caused a decrease of LCST, that is, the earlier collapse of polymer chains occurred, implying lower swelling ratio.

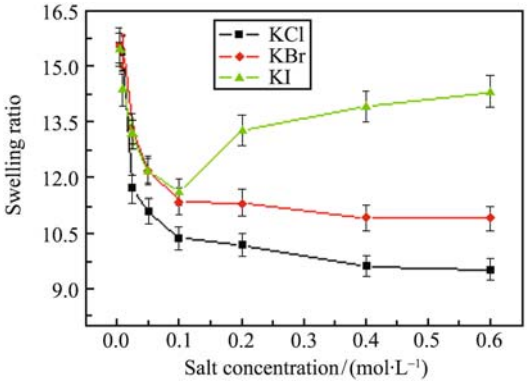

Fig. 4 Dependence of the swelling ratio on ion strength in the medium (temperature: 20°C; pH: 6.5; NMBA: 3%)

3.2 Caffeine release from PMEA

3.2.1 Effect of temperature on caffeine release from PMEA

The effect of temperature on caffeine release from PMEA with 5% AAc was performed in deionized water. As shown in Fig. 5, the higher caffeine release rate at 37°C was found with comparison to that at 14°C. The same changing tendency of drug release with temperature was also observed in chitosan/PNIPAm hydrogel [25] and calcium alginate/PNIPAm semi-IPN beads systems [7]. This releasing behavior could be ascribed to the two main reasons. First, the drug release is a kind of molecular

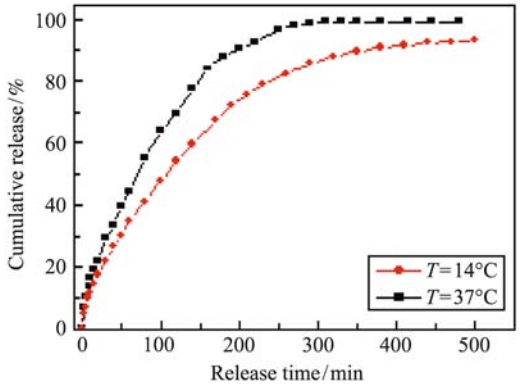

Fig. 5 Effects of temperature on caffeine release behavior (deionized water; NMBA: 3%; AAc: 5%)

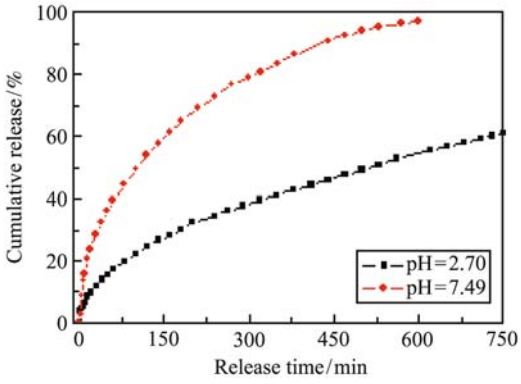

Fig. 6 Effects of pH value on caffeine release behavior (NMBA: 3%; AAc: 5%; 20°C)

diffusion process. The drug release was consequently prevented by H-bonding interactions between drug molecule and polymeric chains in hydrogel. In this case, the H-bonding interactions of $-C=O$, $-N=$ groups in caffeine molecules was formed with ester and amides groups on polymer chains. Second, it is well known that H-bonding interactions weakened or disappeared as the temperature increased. Compared with at 14°C, the weaker H-bonding interaction at 37°C accelerates the diffusion of caffeine from PMEA to the medium. As a result, caffeine-release rate at 37°C is faster than that at 14°C in deionized water.

In addition, as Fig. 2 predicted, the phase transition temperature of PMEA was about 25°C–40°C. Below its LCST, the polymer chains in hydrogel network were solvated by water and outspreaded to some extent, whereas the polymer chains collapsed/aggregated above the LCST. The porous size in PMEA for drug release was enlarged via the effective collapse of the polymer chains at higher temperature. Therefore, more caffeine diffused out from the PMEA hydrogel at 37°C over the same time interval.

3.2.2 Effect of pH value on caffeine release from PMEA

Figure 6 has shown the caffeine release profiles from PMEA with 5% AAc in two different pH solutions at room temperature. It was found that the caffeine release was controlled via adjusting the pH value in the environment. That is, the hydrogel was sensitive to pH value in the process of caffeine release. It could be seen that only 49.1% of caffeine was released from the hydrogel PMEA within 500 min at pH = 2.70 solution, whereas 93.9% caffeine diffused into the medium at pH = 7.49 medium. The similar results were also reported in the alginate/poly (*N*-isopropylacrylamide) semi-IPN beads [7] and pectin-based superabsorbent hydrogel systems [26]. The reason for this caffeine-release behavior is probably related to the discrepancy of swelling behaviors for PMEA. As shown in Fig. 3, the swelling ratio of PMEA was 4.13 and 11.34 when the pH value was fixed at 2.70 and 7.49 in the medium. The smaller swelling ratio at lower pH means that there is smaller aperture for drug caffeine release in the medium. The smaller aperture, implying higher hindrance in drug releasing, resulted in slow caffeine release. In addition, in the acidic solution, caffeine may form a kind of organic salt in the swelling process because caffeine is an organic basic compound. The salt form of caffeine diffuses slowly as compared to the pure caffeine molecules, which leads to slower release of caffeine at acidic medium.

Also, another reason is probably related to the ionization of carboxyl groups on the AAc repeating unit. In a pH 2.70 solution, the ionization of carboxyl groups is low, so the drug release is lower because of the stronger H-bonding between carboxyl group and caffeine molecules. However, at pH 7.49 medium, the higher ionization degree of carboxyl groups in PMEA induced weaker H-bonding interaction between carboxyl group and caffeine, leading to faster release of caffeine.

4 Conclusions

In this study, the glycine-mediated hydrogels PMEA were prepared by free-radical copolymerization among NAGME, NAGEE, and AAc in order to get pH/temperature sensitive drug release carrier. The swelling measurements of PMEA clearly demonstrated the responsive nature of pH and temperature. It was found that PMEA hydrogel was very sensitive to the pH change, especially in the range of 4–6 and over 8. The release behaviors of caffeine from the PMEA were evaluated as a function of pH and temperature. Compared with the acidic release medium (pH = 2.70), a remarkable increase in caffeine release was observed as the pH of PBS was controlled at 7.49. The release rate was faster at 37°C than that at 14°C due to the occurrence of phase transition of the polymer chains. These results indicated that PMEA hydrogels are expected to be used as an effective pH/

temperature-sensitive drug delivery carrier in the biomedical field.

Acknowledgements The authors are grateful for financial support from the Hebei Natural Science Foundation of China (B2008000573).

References

1. Fundueanu G, Constantin M, Ascenzi P. Preparation and characterization of pH- and temperature-sensitive pullulan microspheres for controlled release of drugs. Biomaterials, 2008, 29(18): 2767–2775
2. Zhang X Z, Zhuo R X, Cui J Z, et al. A novel thermo-responsive drug delivery system with positive controlled release. International Journal of Pharmaceutics, 2002, 235(1–2): 43–50
3. Dai S, Ravi P, Tam K C, et al. Novel pH-responsive amphiphilic diblock copolymers with reversible micellization properties. Langmuir, 2003, 19(12): 5175–5177
4. Kim J-H, Lee T R. Thermo- and pH-responsive hydrogel-coated gold nanoparticles. Chemistry of Materials, 2004, 16(19): 3647–3651
5. Ricka J, Tanaka T. Swelling of ionic gels: quantitative performance of the donnan theory. Macromoleules, 1984, 17(12): 2916–2921
6. Mahdavinia G R, Pourjavadi A, Hosseinzadeh H, et al. Modified chitosan 4. Superabsorbent hydrogels from poly(acrylic acid-co-acrylamide) grafted chitosan with salt- and pH-responsiveness properties. European Polymer Journal, 2004, 40 (7): 1399–1407
7. Shi J, Alves N M, Mano J F. Drug release of pH/temperature-responsive calcium alginate/poly(*N*-isopropylacrylamide) semi-IPN beads. Macromolecular Bioscience, 2006, 6(5): 358–363
8. Bae Y H, Okano T, Hsu R, et al. Thermo-sensitive polymers as on-off switches for drug release. Die Makromolekulare Chemie, Rapid Communications, 1987, 8(10): 481–485
9. Schmaljohann D. Thermo- and pH-responsive polymers in drug delivery. Advanced Drug Delivery Reviews, 2006, 58(15): 1655–1670
10. Xiao X C. Effect of the initiator on thermosensitive rate of poly(*N*-isopropylacrylamide) hydrogels. eXPRESS Polymer Letters, 2007, 1(4): 232–235
11. Prevot M., Déjugnat C, Möhwald H, et al. Behavior of temperature-sensitive PNIPAM confined in polyelectrolyte capsules. ChemPhysChem, 2006, 7(12): 2497–2502
12. Liu H L, Liu M Z, Jin S P, et al. Synthesis and characterization of fast responsive thermo- and pH-sensitive poly[(*N,N*-diethylacrylamide)-*co*-(acrylic acid)] hydrogels. Polymer International, 2008, 57(10): 1165–1173
13. Taşdelen B, Kayaman-Apohan N, Güven O, et al. Investigation of drug release from thermo- and pH-sensitive poly(*N*-isopropylacrylamide/itaconic acid) copolymeric hydrogels. Polymers for Advanced Technologies, 2004, 15(9): 528–532
14. Shtanko N I, Lequieu W, Goethals E J, et al. pH- and thermo-responsive properties of poly(*N*-vinylcaprolactam-*co*-acrylic acid) copolymers. Polymer International, 2003, 52(10): 1605–1610
15. Qiu L Y, Zhu K J. Novel blends of poly[bis(glycine ethyl ester) phosphazene] and polyesters or polyanhydrides: compatibility and degradation characteristics *in vitro*. Polymer International, 2000, 49 (11): 1283–1288
16. Deng K L, Zhong H B, Zheng X Y, et al. Synthesis, characterization, and drug release behaviors of a novel thermo-sensitive poly(*N*-acryloylglycinates). Polymers for Advanced Technologies, DOI: 10.1002/pat.1471
17. Arun A, Reddy B S R. *In vitro* drug release studies from the polymeric hydrogels based on HEA and HPMA using 4-{(E)-[(3Z)-3-(4-(acryloyloxy)benzylidene)-2-hexylidene] methyl} phenyl acrylate as a crosslinker. Biomaterials, 2005, 26(10): 1185–1193
18. Liu H L, Bian F L, Liu M Z, et al. Synthesis of Poly(*N,N*-diethylacrylamide-*co*-acrylic acid) hydrogels with fast response rate in NaCl medium. Journal of Applied Polymer Science, 2008, 109 (5): 3037–3043
19. Kuckling D, Richter A, Arndt K-F. Temperature and pH-dependent swelling behavior of poly(*N*-isopropylacrylamide) copolymer hydrogels and their use in flow control. Macromolecular Materials and Engineering, 2003, 288(2): 144–151
20. Turan E, Demirci S, Caykara T. Thermo- and pH-induced phase transitions and network parameters of poly(*N*-isopropylacrylamide-*co*-2-acrylamido-2-methyl-propanosulfonic acid) hydrogels. Journal of Polymer Science Part B: Polymer Physics, 2008, 46(16): 1713–1724
21. Li S F, Liu X L. Synthesis, characterization, and evaluation of enzymatically degradable poly(*N*-isopropylacrylamide-*co*-acrylic acid) hydrogels for colon-specific drug delivery. Polymers for Advanced Technologies, 2008, 19(11): 1536–1542
22. Bajpai A K, Mishra A. Ionizable interpenetrating polymer networks of carboxymethyl cellulose and polyacrylic acid: evaluation of water uptake. Journal of Applied Polymer Science, 2004, 93(5): 2054–2065
23. Şölener M, Uğuzdoğan E, Nurbaş M, et al. A novel thermo-responsive hydrogel matrix based on poly(*N*-ethoxypropylacrylamide). Polymer Bulletin, 2006, 57(3): 341–349
24. Nishio Y, Chiba R, Miyashita Y, et al. Salt addition effects on mesophase structure and optical properties of aqueous hydroxypropyl cellulose solutions. Polymer Journal, 2002, 34(3): 149–157
25. Guo B-L, Gao Q-Y. Preparation and properties of a pH/temperature-responsive carboxymethyl chitosan/poly(*N*-isopropylacrylamide) semi-IPN hydrogel for oral delivery of drugs. Carbohydrate Research, 2007, 342(16): 2416–2422
26. Pourjavadi A, Barzegar S. Synthesis and evaluation of pH and thermosensitive pectin-based superabsorbent hydrogel for oral drug delivery systems. Starch-Stärke, 2009, 61(3–4): 161–172