Drug Release Behaviors of a Novel pH/Temperature-Responsive Chitosan-Poly(*N*-acryloylglycinate) Hydrogel

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ABSTRACT: A pH/temperature and degradable-responsive hydrogel (PSMEA) was prepared from chitosan (CS), *N*-acryloylglycine methyl ester (NAGME), *N*-acryloylglycine ethyl ester (NAGEE), acrylic acid (AA), and *N*-methylenebisacrylamide (NMBA). The swelling properties of PSMEA were systematically investigated at different temperatures, pH, and CS contents. It was found that the PSMEA demonstrated obvious pH and temperature-responsive natures. The caffeine-release behaviors showed that only 42.9% caffeine was released from PSEMA in pH 2.1 phosphate buffer solution (PBS) after 360 min, whereas more than 71.5% caffeine was gradually diffused into pH 7.4 PBS over the same time interval. In addition, the caffeine release was much higher at 37.0°C than that at 14.0°C in PBS medium. The apparent degradability of PSMEA was also observed in the pH 7.4 PBS at 37.0°C through the chemical cleavage of CS. As seen from the results, PSEMA seems to be a potential application in the drug-delivery system controlled by the external pH value and temperature. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 120: 3297–3303, 2011

Key words: drug-delivery systems; hydrogels; swelling

INTRODUCTION

In the recent years, there has been a great deal of researching interest in the development of stimulus-sensitive polymeric hydrogels. The hydrogels are defined as the three-dimensional networks of hydrophilic polymers that are not soluble but swell in water. Furthermore, these hydrogels can respond to external stimuli and simultaneously exhibit some abrupt changes in the physical or chemical nature of hydrogel network.[1,2] Among the sensitive hydrogels, pH or temperature-responsive hydrogels have been extensively investigated in the biomedical fields due to the easy controlling and applications both *in vitro* and *in vivo* conditions.[3–5]

To prepare a stimulus-sensitive polymeric hydrogel, many investigations have been made to combine some biopolymers such as starch, alginate, and chitosan (CS) with thermosensitive polymers. CS including its derivatives, as a widely-studied biopolymer, is one of the most abundant naturally occurring polysaccharide. Recently, it has attracted much interest in the biomedical fields such as drug-release carriers and tissue engineering because of its excellent biodegradability, biocompatibility, and antimicrobial activity.[6–8] The pH sensitivity of hydrogel is usually originated from the introduction of carboxyl (—COOH) or amine ($—NH_2$) groups into macromolecular chains. As a result, acrylic acid (AA), methacrylic acid, vinyl pyridine, aminoethyl acrylylate, and so on are often selected as the comonomers or modifier in the preparation. Among the thermosensitive polymers, poly(*N*-isopropylacrylamide) (PNIPAm) is one of the most important temperature-sensitive polymers. PNIPAm exhibits the lower critical solution temperature (LCST) around 32°C close to human body temperature.[9–11] For example, methylene blue release rate from the pH/temperature sensitive poly(*N*-isopropylacrylamide/itaconic acid) copolymeric hydrogels was found to be effective regulated by temperature change between 25 and 37°C.[9] Also, a pH/temperature-responsive carboxymethyl CS/poly (*N*-isopropylacrylamide) semi-IPN hydrogel was prepared using as oral delivery of drugs, and the release of coenzyme A was much higher in pH = 7.4 than in pH = 2.1 phosphate-buffered saline (PBS).[10]

Recently, we have synthesized a series of novel thermosensitive polymers containing poly(*N*-acryloylglycinates methyl ester), poly(*N*-acryloylalanine methyl ester), poly(*N*-methyl acryloylglycine methyl ester), and so on.[12–14] Theoretically, the introduction of glycine and alanine moeity into the macromolecular chains can improve its biocompatibility and degradation of the titled polymer.[15,16] In fact, aiming to obtain the thermosensitive amphiphilic copolymer with good biocompatibility, glycine ethyl ester has been selected to modify the copolymer of PNIPAm

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and polyphosphazene.[15] Based on glycine, a novel biodegradable pH/thermal-responsive poly(*N*-acryloylglycine)-CS hydrogel was also prepared to get a crosslinker in controlled drug-delivery studies.[16] To our knowledge, there is a little work on the combination of poly(*N*-acryloylglycinates) and CS aiming to get a pH and temperature-responsive drug-delivery carrier.

On the basis of our previous work on thermosensitive poly(*N*-acryloylglycine methyl ester) poly (NAGME) and poly(*N*-acryloylglycine ethyl ester) poly(NAGEE), we prepared a new hydrogel from CS, NAGME, NAGEE, AA, and *N*-methylenebisacrylamide (NMBA) using as a new drug-delivery system. Herein, CS was chosen as a component due to its biodegradability and biocompatibility. NMBA was used as a crosslinker in the preparation of PSMEA. The copolymer of NAGME/NAGEE, AA, and CS endows the prepared PSMEA with temperature and pH-sensitive nature, respectively. The swelling behaviors of PSMEA were investigated as function of pH, temperature, content of CS, and AA. In addition, caffeine, as a drug model, was loaded in PSMEA. The experiment showed that caffeine release behaviors depended on temperature, pH (simulated to gastric and intestinal fluid), and AA in this study.

EXPERIMENTAL

Materials

AA from Tianjin Chemical Company was refined by reduced-pressure distillation before use. NAGME and NAGEE were synthesized according to the reported procedure.[12] Ammonium persulfate (APS) and sodium bisulfite (SBS) from Tianjin Chemical Company, China, were of analytical purity grade and used as received. NMBA as a crosslinker was provided by Tianjin Huadong Chemical Factory. CS from a shrimp shell was purchased from Yuhuan Ocean Biochemical (Zhejiang, China), degree of deacetylation was 90%, and M_v was 8.0×10^5. The PBS with different pH values was made on base of the stand methods. The other chemicals were used without any further purification.

Synthesis of *N*-acryloylglycinates ethyl ester

Acryloyl chloride (5.4 mL) was synthesized by reacting AA with benzoyl chloride according to the reported procedure.[17] Ethyl glycinate hydrochloride (8.37 g) prepared with glycine and ethanol[18] was added to dichloromethane in a flask. Then, triethylamine (35.0 mL) was added dropwise to the mixture in an ice bath about 1.0 h and after that acryloyl chloride was added over 2.0 h at the same condition. The mixture was allowed to unceasingly stir at the same temperature for 3.0 h. The reaction mixture was filtrated, and the filtered liquid was evaporated to give a crude NAGEE. The pure NAGEE was obtained by column chromatography using ethyl ether as an eluent. NAGME was also prepared according to a similar procedure.

Preparation of the hydrogel PSMEA from CS, AA, NAGME, and NAGEE

A series of PSMEAs with different composition were prepared as shown in Table I. In a typical polymerization, AA was first added into 2-mL deionized water in a tube, and then CS was added into AA aqueous solution and dissolved completely under stirring after about 30 min. At the same time, NAGME, NAGEE, NMBA, and APS were dissolved in 3.50-mL deionized water. Then, the two solutions were mixed in a test tube. The test tube was pretreated with standard cycles of evacuation with nitrogen gas to remove oxygen. SBS (based on total weight of NAGME, NAGEE, CS, and AA) dissolved in 0.5-mL deionized water was introduced into the polymerization tube with syringe. Afterward, the polymerization was carried out for 24 h at room temperature. After the polymerization, the copolymer hydrogel, which was cut into small pieces, was immersed in deionized water for 1 day to remove the unreacted monomers. The PSMEAs were dried in a vacuum oven for 2 days at 40.0°C to a constant weight. And, it was found that the weight of dry hydrogel was almost equal to the weight of the materials (NAGME, NAGEE, AA, CS, and NMBA). The synthetic route for hydrogdel PSMEA from

TABLE I
The Component of the Hydrogel Sample

Component	Sample code				
	PSMEA-1	PSMEA-2	PSMEA-3	PSMEA-4	PSMEA-5
CS (g)	0.060	0.120	0.160	0.060	0.060
AA (mL)	0.06	0.06	0.06	0.03	0.10
NAGME–NAGEE (g)	0.400	0.400	0.400	0.400	0.400
NMBA (g)	0.026	0.029	0.031	0.025	0.028
APS (g)	0.011	0.012	0.012	0.011	0.011
H_2O (mL)	6.00	6.00	6.00	6.00	6.00

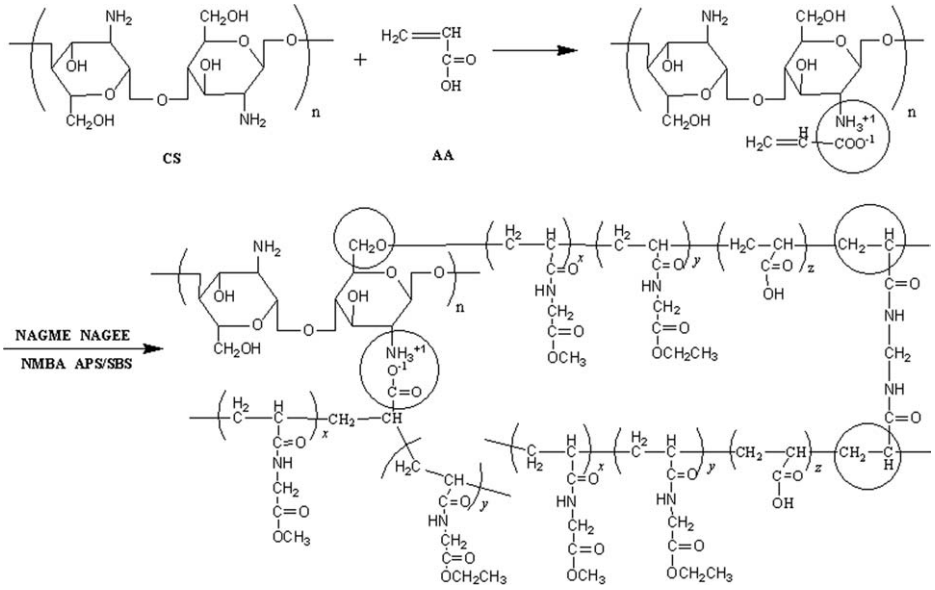

Figure 1 The synthetic route for PSMEA from NAGME, NAGEE, AA, and CS.

NAGME, NAGEE, AA, and CS in their aqueous solution is illustrated in Figure 1.

Swelling studies of PSMEA

The swelling ratio (SR) of PSMEA was determined by immersing the dried hydrogel in deionized water or PBSs with different pH values. After reaching the swelling equilibrium, they were taken out from the aqueous solution and weighed after the removal of surface water with a filter paper. The SR was calculated using the following equation:

$$\mathrm{SR} = (W_S - W_d)/W_d$$

where W_d and W_s represent the weights of hydrogels before and after swelling, respectively.

Drug loading and drug-release experiments

The drug-loaded hydrogel was prepared according to the similar technique in the abovementioned hydrogel synthesis. In the preparation, caffeine was introduced with the ratio of 3.0% (w/w) relative to the total weight of NAGME, NAGEE, CS, and AA. After completing the polymerization, the caffeine-loaded hydrogels were dried at 40.0°C under vacuum to constant weight and stored in the dryer before use.

The caffeine release studies were performed by immersing caffeine-loaded hydrogel (about 40.0 mg) in 40.0 mL PBS in a separate beaker. At a given time interval, 3.0-mL release solution was taken out for determining. The caffeine released from caffeine-loaded hydrogel was measured by the absorption at 272 nm using a UV spectrometer. The detailed calculation of caffeine concentration was referred to the calibration curve constructed from a series of caffeine solution with standard concentration. In this study, the determined caffeine solution was poured out, and the beaker was added with 3.0-mL fresh release medium to keep the unchanged volume of release system.

Degradation observation

In this study, the degradation of PSMEA in pH = 7.4 PBS was performed according to the reported literature.[19–20] PSMEA was prepared in a small test tube, and then 5.0 mL PBS (pH = 7.4) was added into the tube. The test tube was maintained at 37.0°C for the degradation. After a given time interval, the PBS was poured out, and the hydrogel was photographed with a digital camera. The test tube was then supplied with fresh PBS for the subsequent degradation.

Characterization

FTIR spectra of PSMEA and CS were recorded using KBr pellets on a Vector 22 FTIR instrument. The concentration of released caffeine was determined by monitoring the optical transmittance at 272 nm using a Shimadzu UV-120-02 spectrophotometer.

RESULTS AND DISCUSSION

FTIR characterization

The synthetic route for PSMEA from NAGME, NAGEE, AA, NMBA, and CS in their aqueous solution is illustrated in Figure 1. It is necessary to state

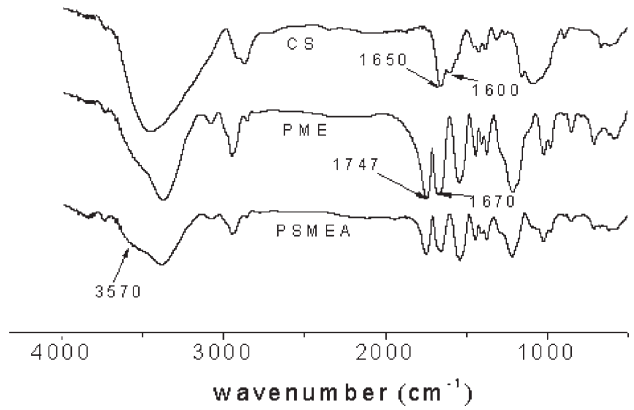

Figure 2 FTIR spectra of CS, PME, and hydrogel PSMEA.

that CS was dissolved in AA solution via the interaction between —COOH from AA and —NH_2 from CS. Namely, AA acts as the role of acetic acid or HCl. As a result, poly(NAGME-NAGEE-AA) was grafted onto the CS chains by the acid-base interaction between AA and CS. Also, the radical transition also produced some grafting sites on CS chain using for the grafting polymerization of monomers. Two kinds of grafting were involved in this study. In addition, the polymerization of NMBA as a crosslinker in the system played a key role in the formation of PSMEA.

The FTIR spectra of CS, copolymer of NAGME-NAGEE (PME), and PSMEA were shown in Figure 2. For the FTIR spectrum of CS, the characteristic signals of CS at 1650 cm^{-1} and 1600 cm^{-1} for the C=O stretching (amide) and N—H bending were observed, respectively. In the spectrum of PME, the absorption at 1747 cm^{-1} and 1670 cm^{-1} were assigned to the carbonyl groups for ester and amide group. In the spectrum of PSMEA, the peak at 1750 cm^{-1} standing for PME was found, and the absorption at 1100 cm^{-1} presenting CS was also observed. In addition, A strengthened peak appears at 3200–3600 cm^{-1} was recorded, which is assigned to —OH, —NH_2 amides groups from CS and PME. All the above spectral data indicated that we have synthesized the titled PSMEA.

Swelling behaviors of hydrogel PSMEA

Temperature sensitivity of hydrogel PSMEA

When the weight ratio of NAGME and NAGEE was fixed at 2:1 in the polymerization, the LCST of the corresponding copolymer was 35.0°C.[12] In this study, the ratio of NAGME to NAGEE was also controlled to get desired hydrogel PSMEA with phase-transition temperature near to the body temperature.

Figure 3 has shown the swelling behaviors of PSMEA in deionized water with different CS content (PSMEA-1, PSMEA-2, and PSMEA-3) at different temperatures. As seen from Figure 3, all PSMEAs have shown sensitivity to the external temperature between about 24.0 and 50.0°C. On one hand, all the hydrogels exhibited high-SR at lower temperature, but they shrunk at higher temperature. This behavior may be attributed to the following fact: PSMEA chain contains a hydrophilic group (—NHCO) and some hydrophobic groups (—CH_2—CH_3, —CH_3 and —CH_2—CH_2—), and the hydrophilic group in the polymer structure will form an intermolecular stronger H-bond with water molecules at low temperature. When the temperature was increased, the intermolecular H-bonds weakened, even disappeared. At the same time, the hydrophobic interaction from PSMEA network also increased, leading to decreasing of the SR.[12] On the other hand, the increase of CS content resulted in a decrease of the SR. For instance, when the temperature was controlled at 18.0°C, the SR of PSMEA-1, PSMEA-2, and PSMEA-3 was 11.9, 9.4, and 7.3, respectively. Compared to PME, with an increase of CS content, the hydrophilicity of PSMEA hydrogel was decreased to some extent, resulting in the lower SR.

pH-sensitivity of hydrogel PSMEA

Because of the existence of inorganic ions, it was very essential to maintain the same ion strength in PBS as investigating the relationship between SR and pH. In our study, the ion strength in all PBS was adjusted to $\mathbf{I} = 0.60$. Figure 4 has illustrated the swelling behaviors of PSMEA in the different pH PBS at 20.0°C. With increasing pH value, the equilibrium SRs of PSMEA increased. It can be seen that the prepared PSMEA was very sensitive to the two pH changing range including 2.0–6.0 and above 9.66. When pH was increased to 5.6 from 3.3 and to 11.3 from 7.4, the SR of PSMEA-1 was upgraded to 30.4 from 14.1 and to 30.9 from 28.5, respectively.

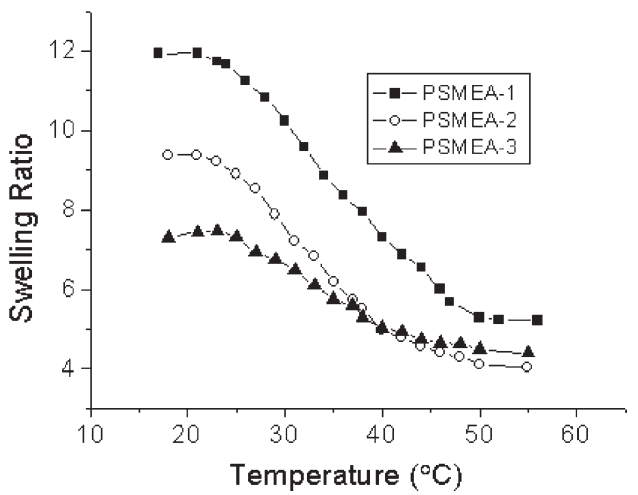

Figure 3 The effect of temperature on swelling behaviors of PSMEA.

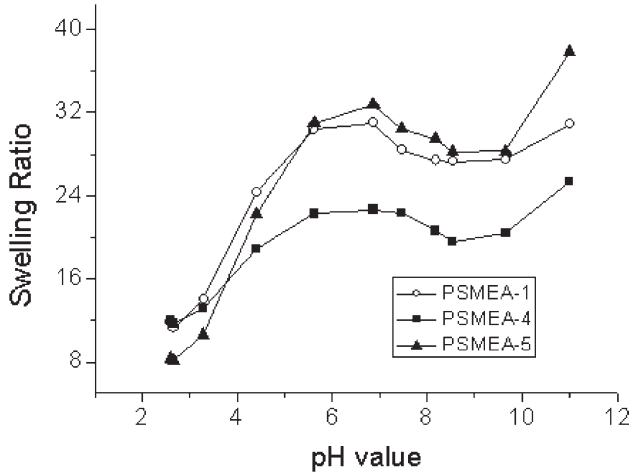

Figure 4 The effect of pH on swelling behaviors of PSMEA at 20.0°C.

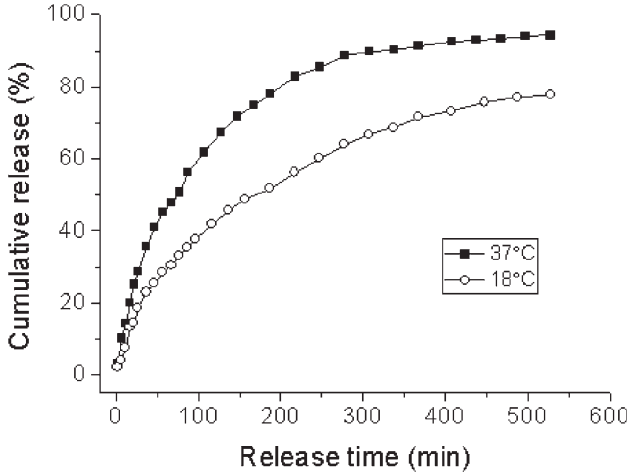

Figure 5 Effect of temperature on caffeine release from PSMEA-1 at pH 7.4.

Additionally, the SR also exhibited a remarkable dependence on the AA content. In general, the higher AA content in PSMEA led to a higher SR, especially in the basic solution. For example, the SRs for PSMEA-5, PSMEA-1, and PSMEA-4 reached 30.5, 28.4, and 22.3 in pH = 7.4 PBS. Because the pK_a is about 4.3 for AA, the carboxyl groups from AA turned into —COO^- form in the pH = 7.4 PBS. The higher repulsion among —COO^- groups induced to the outspread of polymer chain in PSMEA, resulting in a higher SR.[21,22]

Caffeine release behaviors from PSMEA

Effect of temperature on caffeine release

Figure 5 has showed the caffeine release profiles from PSMEA-1 in pH = 7.4 PBS at different temperatures. Seen from Figure 5, the higher release rate of caffeine was found at 37.0°C, while the lower release rate was observed at 18.0°C. The same tendency of drug release with temperature was also reported in other drug-release system composed of CS/PNIPAm hydrogel[10] and calcium alginate/PNIPAm semi-IPN beads.[23] Two important reasons seem to interpret the release behaviors of caffeine in PSMEA system. First, PME chains are water solvated and randomly distributed below the LCST, but collapsed or aggregated above the LCST.[12] The porous size in PME network for drug release was enlarged via the effective collapse of the polymer chains at higher temperature, resulting in the faster release of drug.[12,24,25] Second, the H-bond interactions were formed by caffeine molecules with —OH, —NHCO, and —NH_2 groups in PSMEA. It is well known that H-bond becomes weaker or disappears at higher temperature. With rising the temperature of the release solution, the weakened H-bonding of caffeine molecules with PSMEA network accelerated the release of caffeine. As a result, the release rate in medium at 37.0°C was found to be much faster than that at 18.0°C.

Effect of pH on caffeine release

Figure 6 has shown the release profiles of caffeine from PSMEA-1 in pH 2.1, 7.4 and 10.0 PBS. There is an initial burst release within 10 min for the three buffer solutions and followed by an almost constant release of caffeine from PSMEA-1. The prime burst release is related to the release of caffeine molecules loaded in the surface of PSMEA-1. In addition, the amount of caffeine release is much higher in alkaline solution than that in acidic solution. It could be seen that only 42.9% of caffeine was released from PSMEA-1 within 360 min in pH = 2.1 PBS, whereas 71.5% caffeine was diffused into pH = 7.4 PBS. The similar results were also reported in the alginate/poly(*N*-isopropylamide) semi-IPN beads[23]

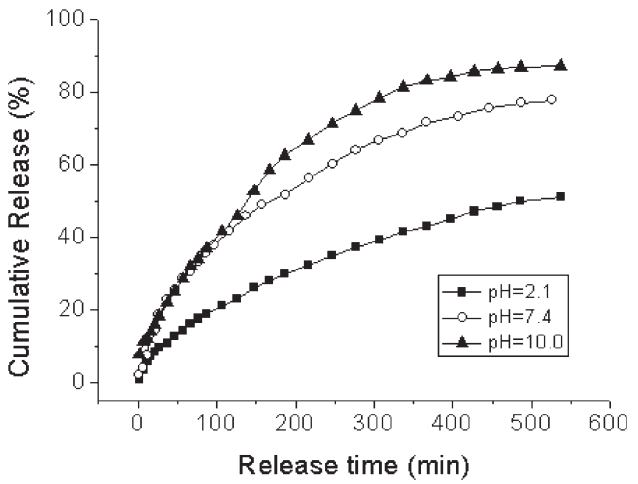

Figure 6 Effect of pH value on caffeine release from PSMEA-1.

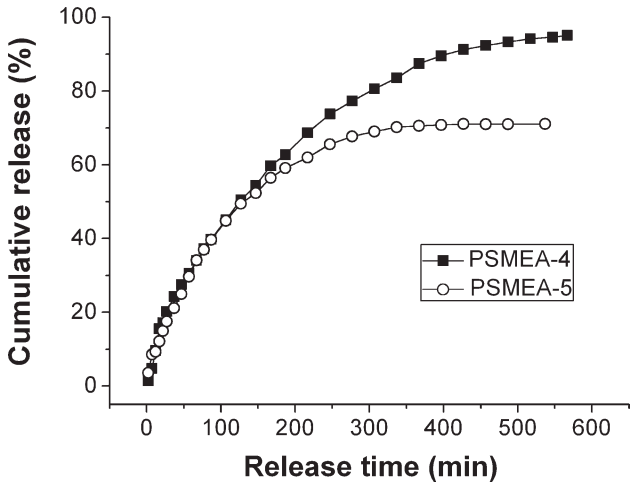

Figure 7 Effect of AA content on release of caffeine in pH 7.4 PBS.

and pectin-based superabsorbent hydrogel system.[26] The drug release in the hydrogel is affected by the swelling behaviors of PSMEA and interaction between drug molecules and the polymer network. According to Figure 4, the SR of PSMEA at acidic solution is lower than that in the alkaline solution. The higher SR of PSMEA created larger surface areas and porous aperture for the drug release. Consequently, the bigger aperture led to a high-caffeine release because of the lower hindrance in drug releasing. In addition, in the acidic medium, caffeine may form a kind of organic salt in the swelling process, because caffeine is an organic basic compound. The salt of caffeine spreads slowly when compared with the pure caffeine molecules, which also bring about a slower delivery of caffeine in acidic medium.[27] Another reason is probably related to the ionization of carboxyl groups from AA repeating unit. In pH = 2.1 PBS, the ionization of carboxyl groups is low, so the stronger H-bonds between caffeine and carboxyl groups led to the lower drug release in polymeric network.

Effect of AA content on caffeine release

Figure 7 has shown the drug release of PSMEA with different AA content in pH = 7.4 PBS at room temperature. Different from Figures 5 and 6, the caffeine release from PSMEA-4 and PSMEA-5 showed two distinct phases. In the first release phase, the similar release behaviors were found before PSMEAs reached to swelling equilibrium over 200 min. This was mainly ascribed to the same external release conditions including pH and temperature, which are very crucial to the drug-release behaviors. According to Figure 4, the SR of PSMEA-5 (higher AA content) was much higher than that of PSMEA-4 in pH 7.4 PBS. The caffeine release from PSMEA-5 should be faster than that in PSMEA-4 due to its larger pores. In the second release phase, however, in comparison with PSMEA-4, a decreasing tendency of caffeine release from PSMEA-5 was observed after 200 min. In this study, caffeine is an organic base, and a large amount of carboxyl groups are appended to the PSMEA network. Compared to the influence of pore size, the stronger interaction of caffeine with carboxyl groups from PSMEA maybe played more significant role in the drug release. As a result, the resistance of caffeine diffusion in PSMEA-5 was higher than that in PSMEA-4, leading to the slower release from PSMEA-5.

Degradation of PSME

To illuminate visually the degradation behavior of PSMEA, the height change of cylinder-shaped hydrogel in the tube was determined as time prolonged in pH = 7.4 PBS. Figure 8 showed the time-dependent height change of PSMEA-1 contained 11.5% CS, 11.5% AA, 77% NAGME/NAGEE, and 5.0% NMBA. The hydrogel first slightly swells and then begins to dissolve slowly in the next more 20 days. The time-dependent decrease of PSMEA height in the PBS can be ascribed to the following facts. The chemical cleavage from CS macromolecules chains led to slow

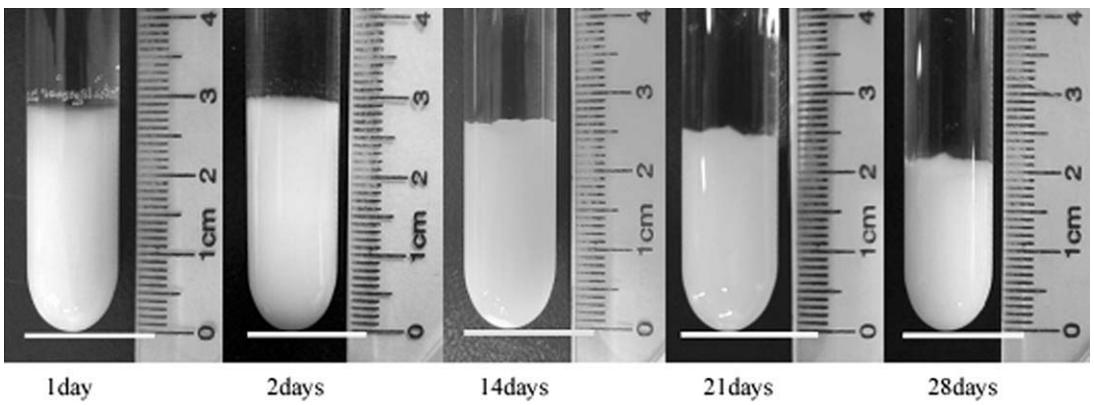

Figure 8 The degradation of PSMEA-1 in pH 7.4 PBS at 37.0°C.

erosion of the hydrogel bulk from the surface.[28–31] Apparently, the introduction of biopolymer, CS makes for the effective degradation of PSMEA.

CONCLUSIONS

A pH/temperature-sensitive drug-release system based on the PSMEA from CS, poly(*N*-acryloylglycinates), and AA was synthesized. The equilibrium-swelling measurements of PSMEA clearly demonstrated the independent pH- and temperature-responsive nature of the materials. The drug-release behavior of caffeine from PSMEA was evaluated as a function of pH, temperature, and the content of AA and CS. More than 71.5% caffeine was released into pH 7.4 PBS after 360 min, whereas only 42.9% caffeine was diffused out in pH 2.1 PBS over the same time interval. Additionally, the caffeine release rate at 37.0°C was much faster than that at 18.0°C due to the sensitivity to temperature originating from poly(*N*-acryloylglycinates). The obvious degradation of PSMEA was also observed in the pH = 7.4 PBS. The experimental results indicated that CS/poly(*N*-acryloylglycinates) hydrogel have the potential application in an effective pH/temperature-controlled drug-delivery system in the biomedical fields.

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