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Effect of gelatin on the drug release behaviors for the organic hybrid gels based on *N*-isopropylacrylamide and gelatin

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Abstract A series of organic hybrid gels were prepared based on poly(*N*-isopropylacrylamide), poly (NIPAAm), and gelatin. The hybrid gels were crosslinked through a two-step process with genipin or glutaraldehyde. The swelling behavior and physical properties of the gels were investigated in the previous report. In this study, we loaded sulfanilamide, caffeine, vitamin B12, phenol red, and neutral red in the hybrid gels. The effects of gelatin on the drug release profile were demonstrated. The ionicity of hybrid gels strongly influenced the release of phenol red (anionic) and neutral red (cationic). However, the releases of sulfanilamide, caffeine and vitamin B12 were not influenced by the ionicity of hybrid gel. The drug released from the gels crosslinked with genipin was significantly smaller than that released from the gels crosslinked with glutaraldehyde.

Introduction

Hydrogels are three-dimensional hydrophilic polymers, which swell but do not dissolve when brought into contact with water. They sometimes undergo a volume phase change in response to a change in surrounding conditions, such as temperature [1, 2], pH [3], ionic strength [4], and electric field [5, 6].

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Thermosensitive hydrogels such as Poly(NIPAAm) hydrogel, collapses at temperature above the critical gel transition temperature (CGTT). The volume change occurs within a very narrow temperature range. Those hydrogels can be used in many applications such as drug delivery system and enzyme activity control [7–14].

Gelatin is obtained by thermal denaturation or physical and chemical denaturation of collagen, which is the most abundant protein in the connective tissues, such as skin, tendon, and bone [15–17]. Gelatin is an important biomedical material used for the manufacture of hard and soft capsules, microspheres, wound dressing and adsorbent pad for surgical use [15, 16, 18]. The mechanism of gelation and the properties of gelatin gels have been extensively investigated [19–21]. Gelatin readily undergoes chemical crosslinking due to its large number of functional side groups. The crosslinking of gelatin is usually performed with bifunctional reagents, such as glutaraldehyde and diisocyanates. Carbodiimides, polyepoxy compounds, and acyl azide methods were also reported [22–24].

Glutaraldehyde (GA) stabilizes collagenous materials effectively. The mechanism of crosslinking of collagen-based biomaterials with glutaraldehyde can be found in the literature [25, 26]. Genipin (GP) is a natural crosslinking agent, which can be obtained from an iridoid glucoside, geniposide, and is abundant in gardenia fruits. The mechanism of amino-group-containing compounds crosslinked with genipin has been discussed in the literature [27, 28].

Hydrogel drug delivery systems have attracted significant attention recently. The drug entrapped in hydrogels can be released into an aqueous medium by a controlled way. Changing the crosslinking density of

the hydrogel and controlling the swelling ratio make hydrogels particularly suitable as drug carriers in the controlled release of pharmaceuticals [29]. The permeability and release rate of drugs are influenced by the type of releasing agent and the water content in hydrogels [30].

The swelling behavior and physical properties of a series of the organic hybrid hydrogels based on NIPAAm and gelatin were investigated systemically and reported in the previous study [31]. The results showed that the swelling ratio decreased with an increase of the gelatin content in these hybrid gels. Besides, the gels crosslinked with GP have lower swelling ratios than those gels crosslinked with GA. Because the drug release behavior of the hybrid gels is related to their swelling ratio, crosslinking density, and drug type, we will investigate the role of gelatin in the present gels, and its effect on drugs with different molecular size and charges.

Experimental

Materials

N-isopropylacrylamide (NIPAAm) (Wako Pure Chemical Co. Ltd. Osaka, Japan) was recrystallized in n-hexane before use in order to remove an inhibitor. Ammonium persulfate (APS) (Wako Pure Chemical Co. Ltd.), as an initiator, was purified by recrystallization. The crosslinking agent, *N*,*N*′-methylene bisacrylamide (NMBA) (SIGMA Chemical Co. St. Louis, MO), the accelerator, *N*,*N*,*N*′,*N*′-tetramethylethylene diamine (TEMED) (Fluka chemical Co. Buchs, Switzerland), and the model drugs, sulfanilamide (SIGMA Chemical Co.), caffeine (Fluka), vitamin B_{12} (SIGMA Chemical Co.), phenol red (Tokyo Kasei Kogyo Co. Tokyo, Japan) and neutral red (Fluka) were used as received. Gelatin purchased from Sigma Chemical co, was a polymer purified from porcine skin, and classified as type A, 300 Bloom, with isoelectric point (IEP) around pH 7–9, and a molecular weight range of 50–100 kDa. Glutaraldehyde (GA) (Wako Pure Chemical Co. Ltd.) and Genipin (GP) (Challenge Bioproducts, Taiwan) as gelatin crosslinking agents were used as received.

Preparation of hydrogels

NIPAAm and gelatin with various ratios and 4 mol% of NMBA, which was based on total monomer content, were dissolved in 10 mL of deionized water. About 1 mol% of APS and 1 mol% of TEMED as redox initiator were added to this solution. The mixture was immediately injected into the space between two glass plates. The thickness of the gel membrane was adjusted with a silicone rubber spacer between the two glass plates. Polymerization was carried out at 25 °C for 1 day. After gelation was completed, the gel membrane was further crosslinked with 20 mL of 1 wt% glutaraldehyde or genipin solutions, which was prepared in phosphate buffer solution of pH 7.4, for 24 h at room temperature. At the end of crosslinking reaction, the gel membrane was cut into disks with 10 mm in diameter, and then immersed in an excess of deionized water for 3 days to remove the unreacted monomer. The hybrid gels were dried at 40 °C for 3 days, and then dried in a 25 °C vacuum oven for 1 day. The sample codes, compositions and equilibrium-swelling ratios of the gels are listed in Table 1.

Measurement of swelling ratio

The pre-weighed dried gels (W_d) were immersed in an excess amount of deionized water at 25 °C until swelling equilibrium was attained. Each gel was then removed from the water bath, tapped with filter paper to remove excess surface water, and weighed the wet weight (W_w). The swelling ratio (SR) was calculated from the following equation:

$$SR = \left(\frac{W_w - W_d}{W_d}\right) \quad (1)$$

Zeta-potential analysis

The fine powder of dried hybrid gels (30 mg) was immersed in 20 mL of deionized water for 12 h and then made them suspended in colloidal solution by homogenizer (Polytron, PT3100, Littau, Switzerland). The zeta potentials of the hydrogels were measured by Zeta-meter microscope 3.0+ (Staunton, VA).

Drug release experiment

The solutes used in drug release were sulfanilamide (Mw = 172), caffeine (Mw = 194), vitamin B12 (Mw = 1355), phenol red (Mw = 354), and neutral red (Mw = 288). The concentration of all the drug solutions is 300 ppm. Drugs were loaded by immersing dry gel in drug solution and equilibrated at 25 °C for 1 day. The drug release experiments were carried out by transferring previously incubated-drug gels into 10 mL of deionized water at 37 °C. The gels were repeatedly removed and transferred into 10 mL of

Table 1 Feed compositions and equilibrium-swelling ratio of the poly(NIPAAm)/gelatin hybrid gels

Sample codes	NIPAAm (M)	Gelatin (g) (wt%)	NMBA mol (%)	Equilibrium swelling ratio at 25 °C (g/g)
N	1	0	4	12.0
GP5	1	0.056 (5)	4	6.27
GP10	1	0.113 (10)	4	4.55
GP20	1	0.226 (20)	4	3.32
GP30	1	0.339 (30)	4	3.01
GP40	1	0.452 (40)	4	2.81
GA5	1	0.056 (5)	4	6.41
GA10	1	0.113 (10)	4	4.90
GA20	1	0.226 (20)	4	4.02
GA30	1	0.339 (30)	4	3.40
GA40	1	0.452 (40)	4	3.13

fresh deionized water at each fixed time interval. The released drugs were analyzed by ultraviolet spectrophotometer (JASCO V530, Tokyo Japan). The absorbance of sulfanilamide at 258 nm, caffeine at 272 nm, vitamin B12 at 360 nm, phenol red at 430 nm, and neutral red at 275 nm, were recorded.

Results and discussion

Characterization of the poly(NIPAAm)/gelatin hybrid gels

The properties of poly(NIPAAm)/gelatin hydrogels with various feed compositions are shown in Table 1. N represents the poly(NIPAAm) hydrogel, GPx and GAx represent the content of x wt% gelatin dispersed in the network of poly(NIPAAm) hydrogel. The gels in GPx and GAx are crosslinked with 1 wt% genipin and glutaraldehyde, respectively. The results of swelling experiment showed that the equilibrium swelling ratios decreased with increase in gelatin content. Suggesting that the incorporation of gelatin into poly(NIPAAm) hydrogel matrix and further crosslinked with GP or GA result in the denser gel network. Our previous report [31] also showed that the crosslinking densities of the present gels increased with increase gelatin content. Comparing the swelling ratios of GP series gels are lower than those for GA series gels, we found the GA series gels have higher swelling ratios. The heterocyclic structure of GP may cause the gels relaxing more difficult than those gels crosslinked with linear GA.

Drug release behavior of the poly(NIPAAm)/gelatin hybrid gels

The model drugs used in drug release studies include nonionic drug, sulfanilamide, caffeine and vitamin B12, anionic drug, phenol red, and cationic drug, neutral red. The amounts of drugs loaded into the hybrid gels are shown in Table 2.

The loaded amount of sulfanilamide (577 ppm), caffeine (415 ppm), and vitamin B12 (288 ppm) for N gel decreased with increase in molecular size of the drug. But, when adding 5 wt% gelatin into NIPAAm gel, the loaded amount of sulfanilamide for GP5 (GA5) decreased to 455 ppm (429 ppm); the loaded amount of caffeine for GP5 (GA5) increased to 690 ppm (698 ppm). This opposite result implicitly indicated that the affinity of caffeine toward the GP or GA gel is stronger than that of sulfanilamide under the same loading conditions. However, when adding more gelatins into NIPAAm gel, the loaded amount of caffeine for GP series gels decreased with increase in gelatin content, while the loaded amount of sulfanilamide increased with an increase of gelatin content in the hybrid gels. The loaded amount of vitamin B12 is not significantly affected by the gelatin content. Table 2 also showed that the loading amounts of sulfanilamide and vitamin B12 for GA series are larger than those for GP series gels with higher gelatin content. However, the loaded amount of caffeine was similar to those series gels. In comparison with sulfanilamide, caffeine and vitamin B12, we can find that the loaded amount of vitamin B12 was smaller than sulfanilamide and caffeine, but the release ratio of vitamin B12 was larger than sulfanilamide and caffeine in the gels and decreased with increase in the gelatin content. The results suggested that vitamin B12, with the largest molecular size, could not infiltrate the smaller pore into the inside gel, and most of vitamin B12 adsorbed onto the surface layer of the gel. So vitamin B12 can easily release out of the gel.

For anionic phenol red, the loaded amount increased with increase in gelatin content of the gel, but for cationic neutral red, the loaded amount decreased with increase in gelatin content of the gel. From above

Table 2 The amount of drug loaded and the maximum release ratio of poly(NIPAAm)/gelatin hybrid gels

Model drugs		N	GP5	GP10	GP20	GP30	GA5	GA10	GA20	GA30
Sulfanilamide	Loaded amount (ppm drug/g gel)	577	455	496	569	614	429	587	632	826
(Mw = 172)	At 1440 min Release ratio (%)	82.7	64.6	56.5	47.8	45.1	72.9	61.8	51.3	47.5
Caffeine	Loaded amount (ppm drug/g gel)	415	690	604	569	598	698	594	558	601
(Mw = 194)	At 1440 min Release ratio (%)	76.3	59.1	43.9	39.7	32.9	68.1	57.8	47.5	37.7
Vitamin B12	Loaded amount (ppm drug/g gel)	288	313	277	287	345	358	339	298	348
(Mw = 1355)	At 1440 min Release ratio (%)	90.2	79.2	57.1	38.3	31.2	81.7	78.7	67.0	50.1
Phenol Red (–)	Loaded amount (ppm drug/g gel)	1223	1474	1553	2021	2118	1485	1871	2402	2912
(Mw = 354)	At 1440 min Release ratio (%)	82.6	46.2	40.2	36.4	35.1	54.7	43.8	39.5	37.6
Neutral Red (+)	Loaded amount (ppm drug/g gel)	654	617	601	580	556	640	634	616	577
(Mw = 288)	At 1440 min Release ratio (%)	93.1	31.6	41.1	43.7	49.4	54.8	60.6	65.7	72.5

results, we can find that the amount of drug loaded depends on the molecular size, the charge nature, the affinity of the drug toward the gel, the swelling ratio, the crosslinking density, and the charge of the gel.

Effect of the molecular size of drug on the release behavior

The fractional release profile of sulfanilamide, caffeine, and vitamin B12 for poly(NIPAAm)/gelatin cross-linked with GP or GA hybrid gels at 37 °C in deionized water are shown in Figs. 1–6, respectively. The results in Figs. 1 and 2 exhibited that the fractional release of sulfanilamide in the hybrid gels decreased with an increase of gelatin content. That is, N > GP5 > GP10 > GP20 > GP30; N > GA5 > GA10 > GA20 > GA30. Similar results were observed from the release of caffeine and vitamin B12 shown in Figs. 3–6.

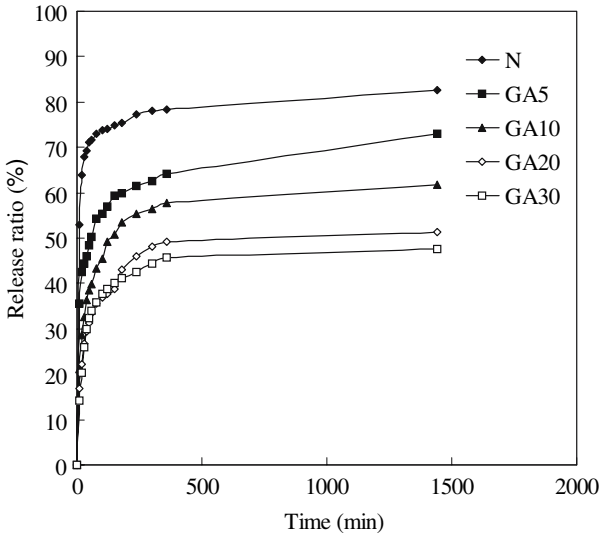

Fig. 2 Sulfanilamide release profile during loading at 25 °C and releasing at 37 °C for the GA series gels in deionized water

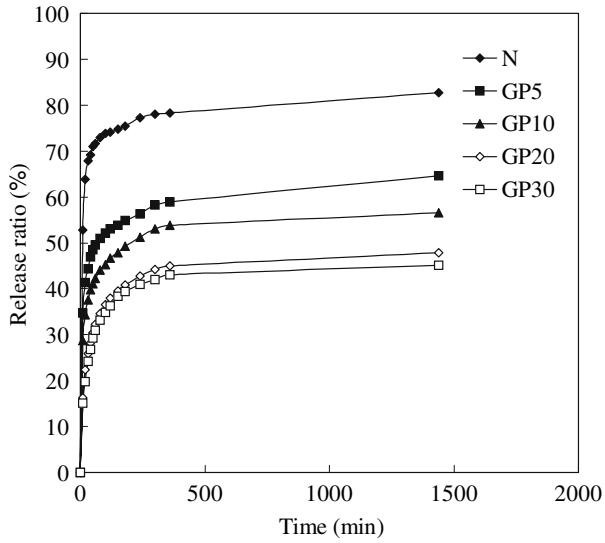

Fig. 1 Sulfanilamide release profile during loading at 25 °C and releasing at 37 °C for the GP series gels in deionized water

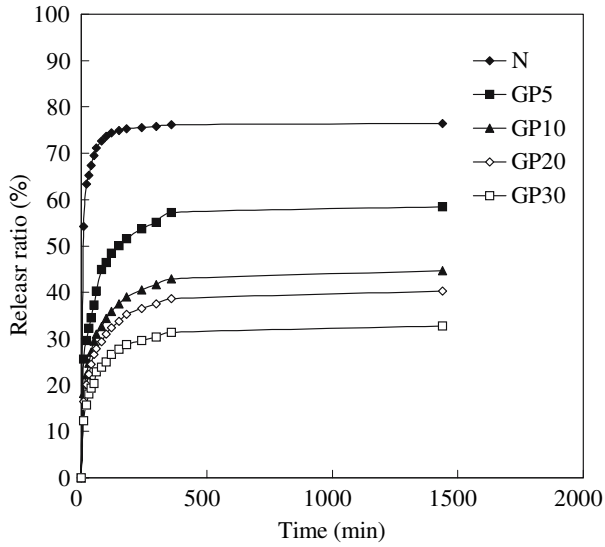

Fig. 3 Caffeine release profile during loading at 25 °C and releasing at 37 °C for the GP series gels in deionized water

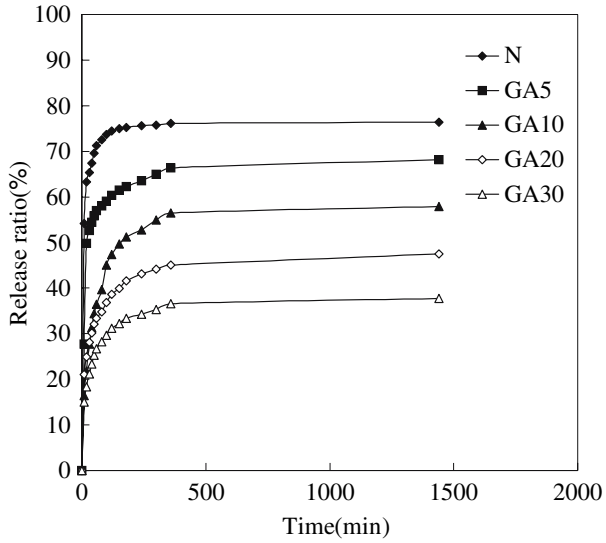

Fig. 4 Caffeine release profile during loading at 25 °C and releasing at 37 °C for the GA series gels in deionized water

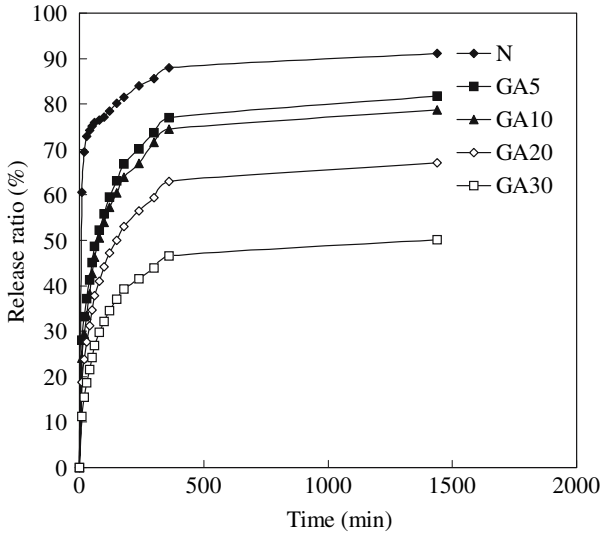

Fig. 6 Vitamin B12 release profile during loading at 25 °C and releasing at 37 °C for the GA series gels in deionized water

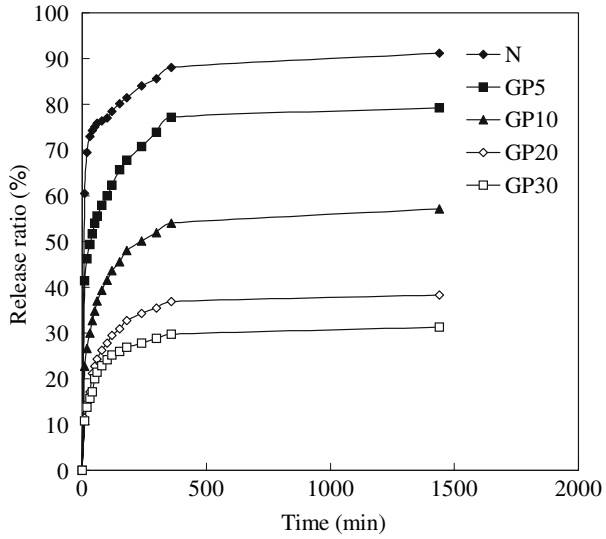

Fig. 5 Vitamin B12 release profile during loading at 25 °C and releasing at 37 °C for the GP series gels in deionized water

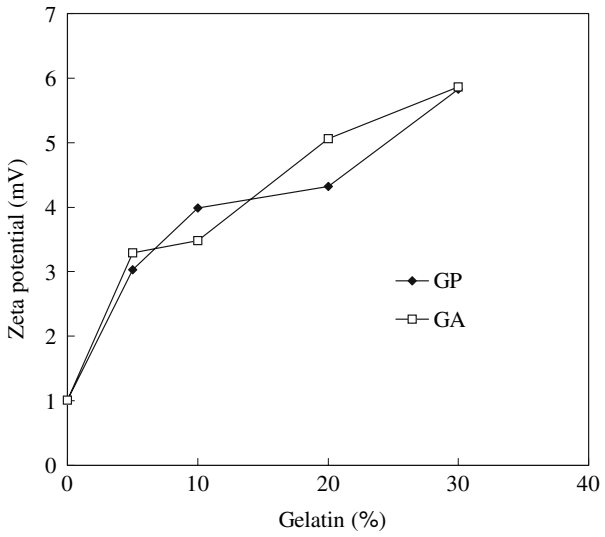

Fig. 7 Effect of gelatin contents on the zeta potential for the poly(NIPAAm)/gelatin hybrid gels in deionized water

Because of chain expansion of the hybrid gels in deionized water, the gels can absorb a larger amount of unbound water and allow a lot of solutes transport. Therefore, the fractional release of sulfanilamide, caffeine, and vitamin B12 in N gel is the highest due to the largest swelling ratio. Incorporating gelatin into poly(NIPAAm) hydrogel matrix and further crosslinked with genipin (GP) or glutaraldehyde (GA) made the gel network denser. Hence, the fractional release of drug decreased with an increase of the content of gelatin in these hybrid gels. The difference of maximum fractional release between N gel and GP30 (GA30) gel for sulfanilamide, caffeine, and vitamin B12 are 37.6% (35.2%), 43.4% (38.6%), and 59.0% (40.1%), respectively. The results explicitly indicated that most drugs could not be released from the hybrid gels, especially for vitamin B12. This implicitly indicated the key role of molecular size on the drug release profile. Besides, the gels crosslinked with GP have lower loaded amount and release ratio than those gels crosslinked with GA (also see Table 2), which is due to the heterocyclic structure of GP. Comparing these two series gels, the GP series gels appeared to be more difficult to load and release drugs than those gels crosslinked with GA.

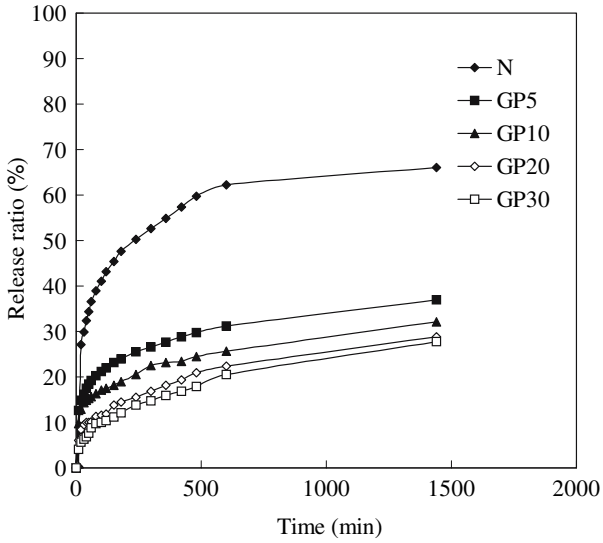

Fig. 8 Phenol red release profile during loading at 25 °C and releasing at 37 °C for the GP series gels in deionized water

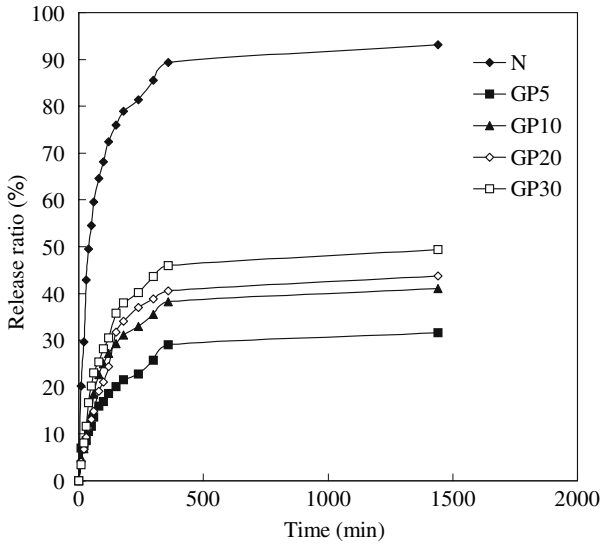

Fig. 10 Neutral red release profile during loading at 25 °C and releasing at 37 °C for the GP series gels in deionized water

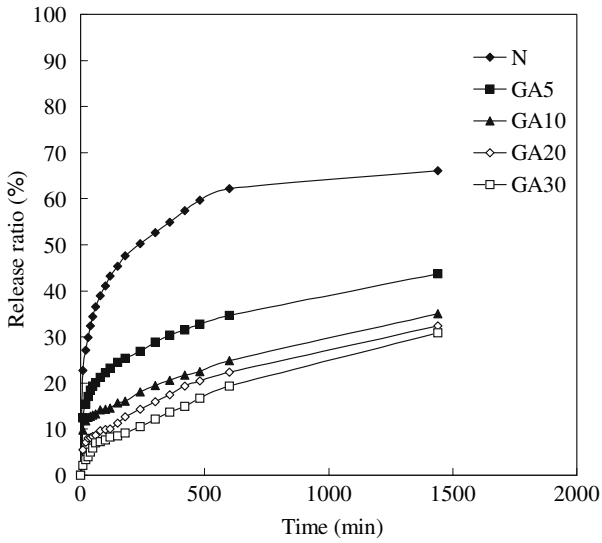

Fig. 9 Phenol red release profile during loading at 25 °C and releasing at 37 °C for the GA series gels in deionized water

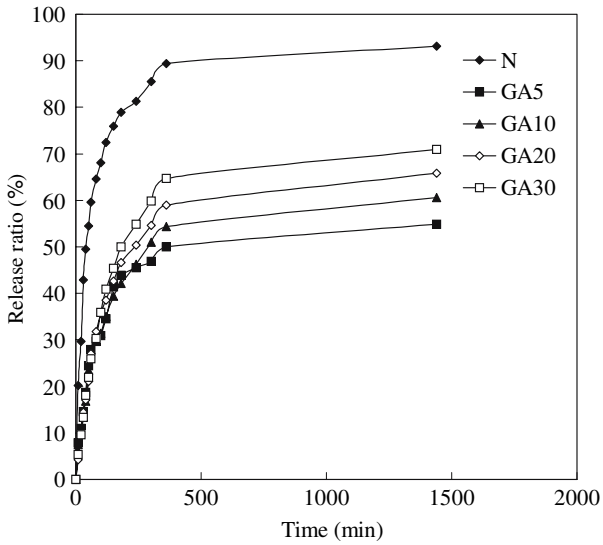

Fig. 11 Neutral red release profile during loading at 25 °C and releasing at 37 °C for the GA series gels in deionized water

Charge effect of drug on the release behavior

Gelatin is an amphoteric polyelectrolyte with an isoelectric point (IEP) at pH = 7–9. Gelatin abounds with primary amines and Carboxyl groups in its molecular chains. The type A gelatin was dissolved in deionized water and exhibited weak acid (pH = 5.5) in this study. At this pH, more primary amines on the gelatin were protonated, resulting in the net positive charges on the gels. The zeta potential of the gel colloid was measured to confirm this presumption. The result shown in Fig. 7 indicated that the zeta potential of the gels increased with the increase of gelatin content in the gel, suggesting more gelatin in the gel leading to higher cationic charge of the gel.

The fractional releases of anionic phenol red in deionized water from GP and GA series hybrid gels were studied at 37 °C. The results are shown in Figs. 8 and 9, respectively. When the charges of the drug and the hybrid gel are opposite, there is the electrostatic attraction force. Therefore, the phenol red strongly binds to the cationic gels (GP and GA series) and is difficult to release from the gel accompanied with

unbound water. So the loaded-amount of phenol red in the gel was the largest and increased with the increase of gelatin content. The fractional release of phenol red is lower and decreases with the increase of gelatin content (see Table 2). The electrostatic force between phenol red and N gel is smaller than that of cationic gels, so the fractional release of phenol red from N gel is higher.

The fractional releases of cationic neutral red from cationic GP and GA series hybrid gels are shown in Figs. 10 and 11, respectively. When the charges of the drug and hydrogel are the same, the drug release ratio of gels is higher. This is due to the charge repulsion exists between the drug solutes and gels. It is difficult to load the solute into the hydrogel. However, the loaded solute is easy to release from the gels. Hence, the release ratios are higher and increase with the increase of gelatin content; the loaded-amount is smaller and decreases with an increase of gelatin content (see Table 2). Besides, loaded-amount and release ratios for the GP gels are lower than those for GA gels, resulting from the difference of crosslinked structure of hybrid gels. From the above results, the drug releases of the hybrid gels were significantly affected by the charge of drug and gel as well as the molecular size of drug. Similar results were also observed from our previous reports [32, 33].

Conclusions

A series of NIPAAm/gelatin hybrid gels were prepared. The amount of drug loaded in these gels is dependent on the molecular size, and the charge nature of the drug, and the affinity between the drug and the gel. The swelling ratio, crosslinking density, and the charge of the gel also contributed in the amount of drug loaded. The drug release profile from the NIPAAm/gelatin hybrid gels is mainly determined by the ionicity of hybrid gels and drug types. The fractional release of nonionic drugs, such as sulfanilamide, caffeine and vitamin B12, in the hybrid gels was not affected by the ionicity of hydrogels. However, the swelling ratio of the hybrid gels and molecular size of the drug influence their release profile. The anionic solute (phenol red) strongly interacted with cationic hybrid gel, so the fractional release of phenol red in cationic gels is very low. On the other hand, the cationic solute, neutral red, only adsorbed on the skin layer of the cationic hydrogel due to the charge repulsion and released quickly. Therefore, the fractional release is the highest for the combination cationic hydrogel and cationic drug.

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