pH-Responsive Semi-Interpenetrating Networks Hydrogels of Poly(acrylic acid-acrylamide-methacrylate) and Amylose. I. Synthesis and Characterization

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ABSTRACT: The semi-interpenetrating networks gels of hydrophobically-modified poly(acrylic acid-acrylamide-methacrylate) and amylose were prepared using 4,4′-bis(methacryloylamino) azobenzene as a crosslinker. Investigation of mechanical strength of gels showed that the equilibrium elastic modulus G changed from 12.4 to 32.9 g/cm^2 with increasing the contents of amylose in the case of low content of crosslinker. The swelling behavior of gels indicated that the aggregation of the hydrophobic groups of hydrogels could be destroyed by expanded amylose and the electrostatic repulsion between the charged $-COO^-$ groups because of the ionization of the carboxylic groups in pH 7.4 buffer solutions. The hydrophobicity and flexibility of the side chain, the entanglement extent of the amylose, and the ionization of carboxyl groups in the gels greatly affected the swelling rates and swelling ratios. Investigations of swelling/deswelling periodically in pH 2.2 and 7.4 media indicated that the semi-IPN gel was more pH-sensitive than the nonsemi-IPN gel. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 106: 3792–3799, 2007

Key words: semi-IPN hydrogel; swelling behavior; diffusion

INTRODUCTION

Protein or peptide drugs like insulin cannot be administered through the oral route because it could be digested by gastric and pancreatic enzymes in the stomach and small intestine. Therefore, many researches suggested that the colon could be the best site for delivery of proteins and drugs because of the longer resident time and the lower digestive, enzymatic activities in the colon.[1]

Many approaches used for the drugs targeting to the colon such as formation of a prodrug, time-dependent systems, pH-sensitive coating, pressure-dependent systems, and micro-flora activated systems were well developed in the past two decades.[2] Because the pH-sensitive hydogels respond to different pH media, there is a potential application for the colon site-specific delivery of protein and peptide drugs along the gastrointestinal (GI) tract.[3] However, site-specific drug delivery to the colon cannot be achieved only by using pH-sensitive hydrogels because the pH values in the small intestine and large intestine are almost the same.[4] It is well known that there is a special micro-flora in the colon, which can produce some enzymes. On the basis of the unique physiological characteristics of the colon, some attempts have been made in the drug delivery systems for the colon site such as pH-dependent hydrogels crosslinked by azocompounds,[5–7] β-cyclodextrins crosslinked by terphthaloylchloride,[8] guar gum-grafted acrylamide crosslinked by glutaraldehyde,[9] dextran hydrogels,[10] konjac glucomannan-grafted polyacrylate hydrogels,[11] and methacrylated inulin hydrogels.[12] All of these drug delivery systems were designed based on the fact that the polymer crosslinked by an azo compound or polysaccharides could be degraded by the colon enzymes.[2] To further improve selective degradation in the colonic environment, Maris and coworkers reported degradable inulin hydrogels (MA-IN) containing both pH-sensitive acidic monomers and 4,4′-bis(methacryloylamino) azobenzene (BMAAB) as a crosslinker.[13] The results showed that the incorporation of BMAAB in MA-IN hydogels caused a decrease of hydrophilicity and an increase of crosslinking density. Therefore, it was hard to find an optimal balance between high swelling to allow degradation in the colon and low swelling to prevent release of the drug in the stomach. Recently, Bajpai and coworkers reported a semi-interpenetrating networks (semi-IPN) hydrogel of poly(acrylic acid) and starch.[14] They proposed a combined mechanism involving enzymatic degradation and pH-dependent swelling.

In our earlier work,[15–17] we prepared hydrogels containing pH-sensitive monomers and an enzymatically

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degradable azocompound crosslinker as well as hydrophobic *n*-alkyl methacrylate. The advantages of these hydrogels include controllable swelling and degradation by the hydrophobic side chains and density of crosslinking. In fact, a low content of crosslinker is often used to obtain high degradation. However, the lower content of crosslinker also leads to a decrease of the hydrogels strength. We note that Ghandehari and coworkers also reported that it was hard to degrade hydrogels with highly covalent crosslinking.[18] Yao and coworkers reported chitosan semi-IPN hydrogels crosslinked with glutaraldehyde and studied their swelling kinetics.[19] Kim and coworkers also reported preparation and properties of the semi-IPN composed of β-chitin and PEG macromer.[20]

In the present work, we prepared pH-responsive semi-IPN gels of poly(acrylic acid-acrylamide-methacrylate) crosslinked by an azo compound and amylose. The structures, mechanical properties, and swelling behavior of the gels were characterized. The aim of this work is to develop a colon site-specific delivery system, which combines the advantages of pH-sensitive hydrogels containing an azo compound crosslinker and amylose semi-IPN both enzymatically degradable by colonic enzymes.[21] The influential factors on the swelling behavior such as the hydrophobicity and flexibility of the side chain, the entanglement extent of the amylose and the ionization of carboxyl groups in different pH media are discussed.

EXPERIMENTAL

Materials

Acrylic acid (AA), methyl methacrylate (MMA), ethyl methacrylate (EMA), and butyl methacrylate (BMA) were purchased from Tianjin Chemical Group, China and were distilled before use. Amylose, acrylamide (AM) and all other chemicals were of analytical reagent grade and used without further purification. 4,4′-Bis (methacryloylamino)azobenzene (BMAAB) was prepared according the method described earlier.[11] Yield ratio of BMAAB: 30%, mp 268–269°C, ^{1}H NMR (Mercury VX-300, Varian, $CDCl_3$): δ2.10 (s, 6H, —CH_3), δ5.52, 5.83 (d, 4H, —CH_3C=CH_2), δ7.64 (s, 2H, —NH—), δ7.72–7.75, 7.81–7.84 (m, 8H, Ar—H).

Preparation of semi-IPN hydrogels

The hydrogels were prepared through aqueous free radical polymerization of AA, AM, *n*-alkyl MA (MMA, EMA, BMA) in the presence of amylose, according to the method described earlier.[22] The feed composition for the preparation of gels is listed in Table I. Briefly, the designed amount of AA, AM, *n*-alkyl MA, and BMAAB were dissolved in dimethyl sulfoxide (DMSO). Then the designed amounts of an aqueous solution containing 1.1% amylose and 2,2′-azobis(isobutyronitrie) (AIBN) were added to the mixture solutions. This solution was bubbled with nitrogen to discharge oxygen for about 30 min. The copolymerization was carried out at 80°C for 48 h. The solid copolymer slab was cut into circular disks using punches. The samples were immersed in absolute ethanol for 3 days, then in 60% ethanol/deionized water for 6 days, and gradually transferred into deionized water to remove the unreacted monomers and solvent. Finally, the samples were dried in vacuum at 50°C to a constant weight and stored for further use. The samples are designated as *nx-y*, where *x* is the carbon number of the *n*-alkyl group and *y* denotes the amount of amylose (ml). The measurements of the FTIR spectra (EQUINOX55, Bruker, KBr pellet) indicate that the absorption band at 1007 cm^{-1} which is the characteristic absorption band of C—O groups of primary alcohol in amylase also appears in the spectra of the semi-IPN hydrogel. This provides evidence that amylose macromolecules are incorporated into the polymer network structure.

Mechanical strength of the semi-IPN gels

The elastic modulus in compression *G* was determined using a bench comparator described earlier.[15] The measurements were performed at 37°C with the swollen sample immersed in pH 7.4 buffer solutions. The equilibrium height of the sample was measured after applying a given weight. Then the sample was

TABLE I
Feed Composition of Semi-IPN Hydrogels[a]

Sample	AA (mol %)	AM (mol %)	*N*-alkyl MA (mol %)	BMAAB (mol %)	AIBN (mg)	DMSO (g)	amylose[a] (ml)
*n*0-1	59.9	40	0	0.1	30	7	1
*n*1-1	49.9	40	10(methyl)	0.1	30	7	1
*n*2-1	49.9	40	10(ethyl)	0.1	30	7	1
*n*4-0	49.9	40	10(*N*-butyl)	0.1	30	7	0
*n*4-1	49.9	40	10(*N*-butyl)	0.1	30	7	1
*n*4-2	49.9	40	10(*N*-butyl)	0.1	30	7	2
*n*4-3	49.9	40	10(*N*-butyl)	0.1	30	7	3

[a] The concentration of amylose in deionized water was 1.1% (w/v).

allowed to relax to the original height, and a new weight was applied. The maximum deformation was limited within 20% of its original height. For each gel, six to eight different values of weight and height were obtained and the elastic modulus was calculated as an average of the three determinations. The equilibrium elastic modulus G (in pascals) was calculated by the following equation:[23]

$$f/A = -G(\lambda - \lambda^{-2}) \quad (1)$$

Herein, f/A is the applied stress, f the force applied (pascals), A the surface area of the swollen sample, $\lambda = h/h_0$, and h is the height of sample when no force is applied. A plot of f/A versus $(\lambda - \lambda^{-2})$ gave a straight line with a slope equal to the elastic modulus G. The effective crosslinking density (ρ_x) can then be calculated from the equilibrium elastic modulus and the equilibrium swelling ratio (SR) of gels as follows:[23]

$$\rho_x = G(\mathrm{SR})^{1/3}/RT \quad (2)$$

where, R is the gas constant and T is the absolute temperature (310 K).

Morphology of semi-IPN xerogels

The equilibrium swollen gels were frozen at −80°C for 12 h in a refrigerator, then freeze-dried and fractured. The fractured specimens were covered with gold vapor. The morphology of the fractured surface of the xerogels were observed by field emission scanning electron microscopy (Sirion 200, FEI) with an acceleration voltage of 10 kV.

Swelling studies

The dry hydrogel was immersed in the swelling medium at 37°C. At regular time intervals, the gels were removed from the medium, the weight of the swollen hydrogels was determined after the removal of the surface water through blotting with filter paper. The equilibrium swelling ratio was calculated by the following equation:

$$\mathrm{SR} = (W_s - W_d)/W_d \quad (3)$$

When the swollen hydrogels reached a constant weigh, the SR was considered to be in equilibrium. W_d and W_s were the weight of xerogel and equilibrium swollen gel, respectively.

The following equation was used to determine the nature of the swelling process:

$$M_t/M_\infty = kt^a \quad (4)$$

where, M_t and M_∞ are the amounts of equilibrium water uptake at time t and the maximum water uptake, respectively and k is a proportional constant and exponent n describes the type of diffusion mechanism.[24] A plot of ln M_t/M_∞ versus ln t was used to calculate n and k from the slope and intercept. This equation is applicable to the initial stages of swelling and only yields straight lines up to nearly 60% of the maximum amount of absorption water.

RESULTS AND DISCUSSION

Mechanical strength of the semi-IPN hydrogels

Figure 1 shows the stress–strain relationship for the semi-IPN hydrogels. The equilibrium elastic modulus G was obtained from the slope of the curves according the eq. (1). The G values of the semi-IPN hydrogels were higher than that of the nonsemi-IPN hydrogel n4-0 and increased with an increasing content of amylose. The crosslinking densities (ρ_x) of gel n4-0, n4-1, n4-2, and n4-3 were 1.68, 2.18, 2.87, and 3.49 (10^{-6} mol/cm^3), respectively, calculated from eq. (2). Obviously, it can be seen that the physical crosslinking density plays an important role in defining the equilibrium elastic modulus G. With the increase of the physical crosslinking density, the equilibrium elastic modulus G increased and the gels became tougher. This is mainly attributed to more amylose macromolecules being trapped in the gel networks because of an increasing of the amylose content. On the other hand, a large content of amylose makes it hard to prepare homogeneous solutions of the gels.

Field emission scanning electron microscopy (FE-SEM) images of the hydrogels

Figure 2 shows FE-SEM images of cross sections of the nonsemi-IPN gel n4-0 (images A) and semi-IPN gel n4-1(images B). As shown in Figure 2, both gels are

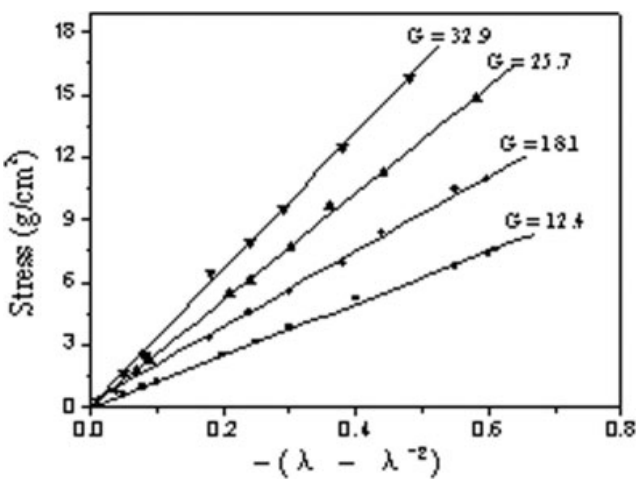

Figure 1 Stress-strain relationships for hydrogels n4-0 (■), n4-1 (●), n4-2 (▲), and n4-3 (▼).

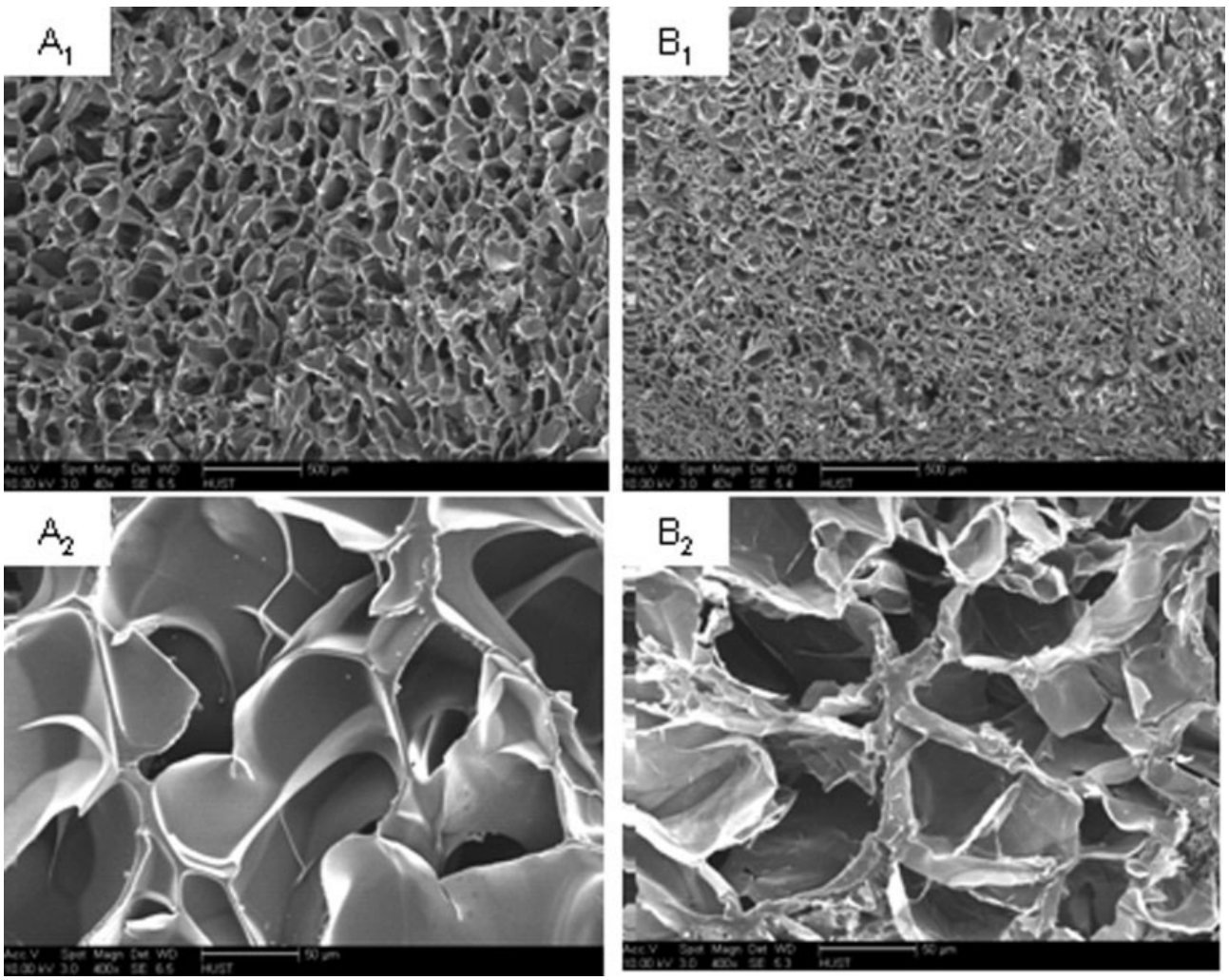

Figure 2 FE-SEM images of hydrogel *n*4-0 (A1, A2) and semi-IPN hydrogel *n*4-1 (B1, B2). The magnification for A1, A2 and B1, B2 was ×40 and ×400, respectively.

porous, with the pore size of semi-IPN gel *n*4-1 being smaller than that of the nonsemi-IPN gel *n*4-0. In addition, the pore walls of semi-IPN gel *n*4-1 were thicker than those of the gel *n*4-0. These features indicate that the strength of the gel can be increased through the formation of polymer-amylose semi-IPN structure, although the concentration of azo compound cross-linker is very low (0.1 mol %). This result accords well with the elastic modulus discussed earlier. As shown in images A2 and B2, the fractured surface of the semi-IPN gel *n*4-1 was cruder than that of gel *n*4-0. It is well known that amylose can absorb water and expand because of its hydrophilicity. When hydrophilic amylose was introduced into the gel system, the hydrophobic microdomains may be destroyed. Hydrophilic amylose and hydrophobic microdomains are incompatible and this may lead to a certain extent of phase separation. However, the gel system can be steady owing to interactions of the —OH of amylose and C=O of acrylate in polymer chains. Zhang and coworkers also reported that a large content of starch could weaken the aggregation of the hydrophobic groups of hydrogels.[25]

The equilibrium swelling ratio (SR) and swelling kinetics of semi-IPN gels

In general, the swelling behavior of polyelectrolyte networks is determined by a balance of three primary forces: (1) the Gibbs energy of mixing of the network chains and solvent, (2) the osmotic pressure within the network resulting from the mobile counter ions surrounding the fixed charged groups (ion osmotic pressure), and (3) the elastic retractile force of the network (network swelling pressure).[26] The semi-IPN hydrogels studies include carboxyl groups in the polymer chain and *n*-alkyl methacrylate ester groups of various chain lengths and amylose. Therefore, their swelling behavior should be determined by the balance of the three forces.

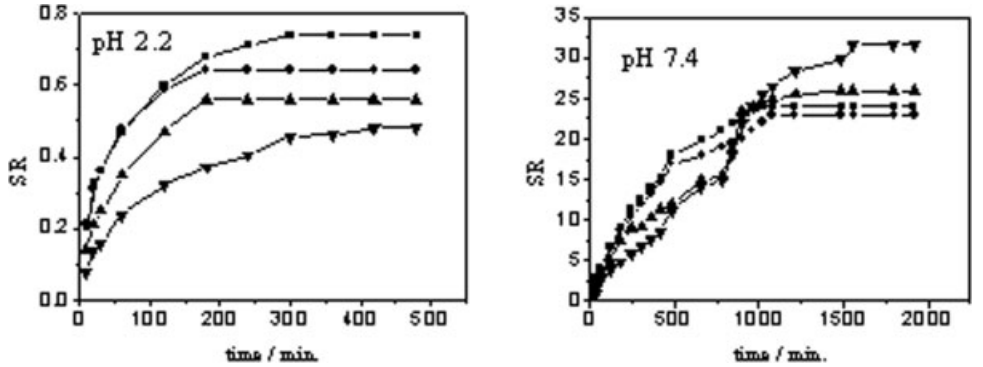

Figure 3 Swelling kinetics of hydrogels at pH 2.2 and 7.4 buffer solutions at 37°C. *n*0-1 (■), *n*1-1 (●), *n*2-1 (▲), and *n*4-1 (▼).

The effect of the side chain length of *n*-alkyl MA on the swelling kinetics and SR is shown in Figure 3. Obviously, the swelling rates decrease with an increase of the side chain length of *n*-alkyl MA in both acidic and alkaline media. In pH 2.2 media, the SR decreases with an increase of the side chain length and all values of SR are less than 0.8. In pH 7.4 media, a similar trend of swelling behavior was observed. However, all values of equilibrium SR are more than 20.

A possible explanation is: in the pH 2.2 media, gels are in a collapsed status and amylose is tightly encapsulated in the gel because of the existence of hydrophobic interaction and hydrogen bonding between the carboxylic and amide groups of the polymer chains. As a result, the swelling rates and SR are low which is in accord with other reports.[15–17] On the contrary, in the pH 7.4 media, ionization of the carboxyl groups led to an increase of the hydrophilicity and osmotic pressure of the networks. Therefore, expansion of amylose and electrostatic repulsion between the charged —COO^- groups destroyed the hydrophobic microdomains formed by the hydrophobic interactions of the side chains.[27] As a result, the values of SR were much higher than those in pH 2.2 media. As Philippova and coworkers suggested, the hydrophobic aggregates existing in an uncharged gel may be destroyed by the electrostatic repulsion between the charged —COO^- groups during the gel ionization of the gel.[28] In the present semi-IPN gels, the content of hydrophobic monomer is 10% (feed composition). Apparently, the aggregation of the hydrophobic groups is easily destroyed by the presence of amylose and the electrostatic repulsions among the charged —COO^- groups because of the ionization of the carboxylic groups. This pH-dependent swelling behavior is schematically depicted in Figure 4. This characteristic is significant for extensive degradation of the gels in the colon.

Effects of the amylose content on the swelling kinetics of semi-IPN hydrogels

To use these semi-IPN hydrogels as the drug carriers for colon-specific delivery, it is important to investigate the swelling behavior of these semi-IPN hydrogels under varied pH conditions because of the different pH in the human stomach (pH 1–3) and the colon (pH 7–8).[18] Generally, the transit time of drugs in both the stomach and small intestine is about 3 h, and the resident time in the colon is ~ 20–25 h.[4] Therefore, the

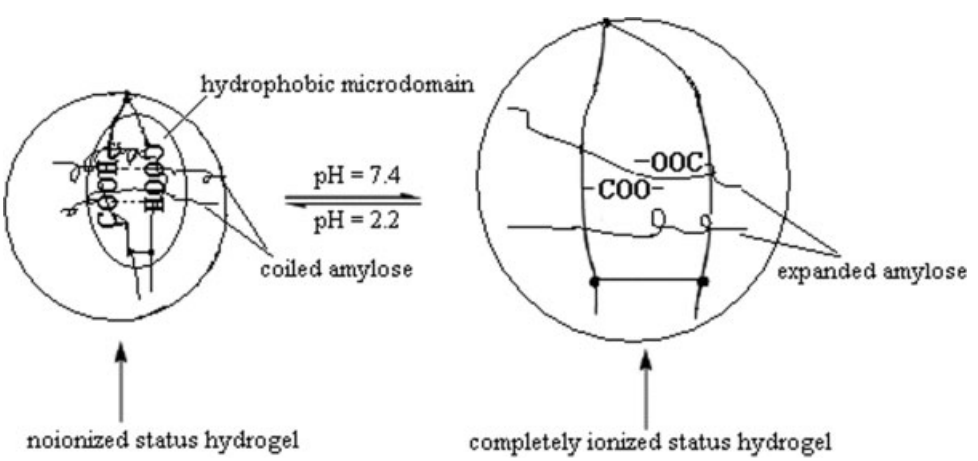

Figure 4 Schematic representation of pH-dependent swelling of semi-IPN hydrogel.

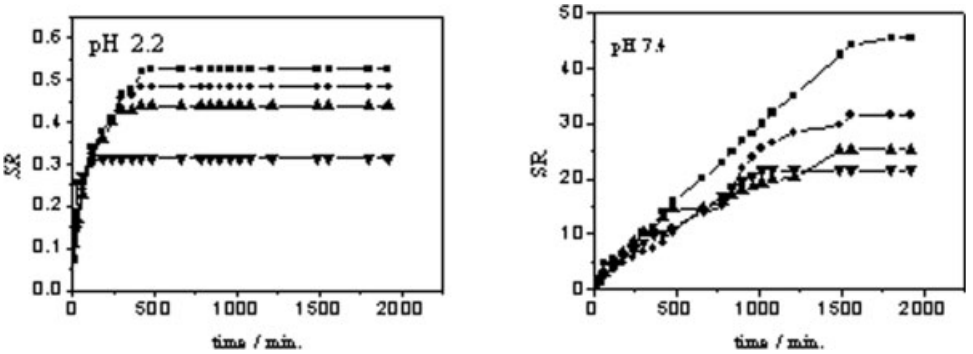

Figure 5 Swelling kinetics of hydrogels in pH 2.2 and 7.4 buffer solution at 37°C. *n*4-0 (■), *n*4-1 (●), *n*4-2 (▲), and *n*4-3 (▼).

pH-dependent swelling experiments were carried out based on a simulation of this transition process. Figure 5 shows the swelling kinetics of *n*4-0, *n*4-1, *n*4-2, and *n*4-3 gels in pH 2.2 and pH 7.4 media. The trend of the swelling kinetics is similar to that of the gels with varied hydrophobic side chains (Fig. 3). However, in acid medium, the time for the equilibrium swelling of gels decreased in the following order:

$$n4\text{-}0\ (8\,\mathrm{h}) > n4\text{-}1\ (6\,\mathrm{h}) > n4\text{-}2\ (5\,\mathrm{h}) > n4\text{-}3\ (3\,\mathrm{h})$$

In alkaline medium, a similar order was found:

$$n4\text{-}0\ (30\,\mathrm{h}) > n4\text{-}1\ (25\,\mathrm{h}) > n4\text{-}2\ (24\,\mathrm{h}) > n4\text{-}3\ (16\,\mathrm{h})$$

In other words, the time for the equilibrium swelling of the gels depends on the content of amylose. In addition, an increase of amylase content leads to more chains being entangled and a break of the hydrophobic micro domains in equilibrium. In this case, the values of SR decrease.

The change of the SR of four hydrogels as a function of the pH values of buffer solutions are shown in Figure 6. The SR of the gels shows an abrupt increase in the range of pH 4–6. The extent of the change decreased in the following order:

$$n4\text{-}0 > n4\text{-}1 > n4\text{-}2 > n4\text{-}3$$

This result may be attributed to the ionization of carboxyl groups. In the range of pH of 2.2–4, the pH dependence is not significant because of the carboxyl groups being largely ionized. With an increase of the pH, a complete ionization of carboxyl groups causes a transition of the network segments from the compact coils to extended coils. As a result, the SR of the gels exhibits an abrupt change in the range of pH 4–5. This characteristic is valuable for keeping drugs from releasing in the stomach. In summary, the hydrophobicity and flexibility of the side chains, entanglements of the amylose groups, and the ionization of carboxyl groups greatly affect the swelling rates and SR of the semi-IPN gels.

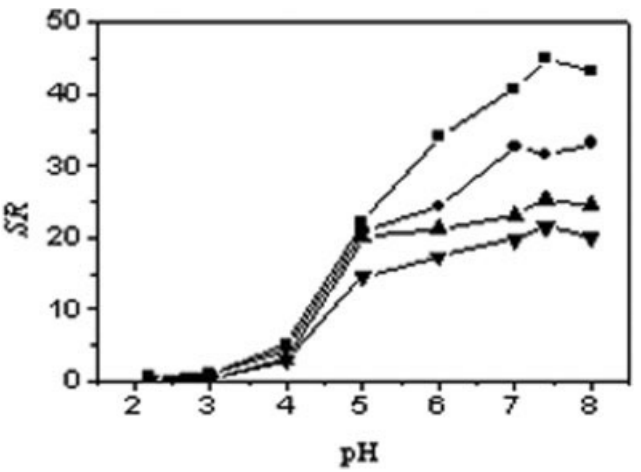

Figure 6 Plot of the SR versus pH values of the buffered solutions at 37°C. *n*4-0 (■), *n*4-1(●), *n*4-2 (▲), and *n*4-3 (▼).

Water diffusion process at different pH values in buffer solutions

According to swelling kinetics eq. (4), a plot of ln M_t/M_∞ versus ln t was used to calculate the values of n and k from the slope and intercept. These values are listed in Table II. The exponent n expresses the diffusion mechanism of water. For cylindrical hydrogels, when n is 0.5, it signifies a Fickian diffusion mechanism.[24] When n is 1, it signifies that macromolecular relaxation is predominant and controls water diffusion in the swelling process. When n is between 0.5 and 1.0, the diffusion mechanism is non-Fickian, where both water diffusion and macromolecular relaxation control the swelling process.

TABLE II
Diffusion Exponents *n* of the Gels

pH	*n*4-0	*n*4-1	*n*4-2	*n*4-3
2.2	0.53 ± 0.01	0.50 ± 0.03	0.45 ± 0.03	0.47 ± 0.02
7.4	0.75 ± 0.02	0.69 ± 0.02	0.74 ± 0.01	0.71 ± 0.03

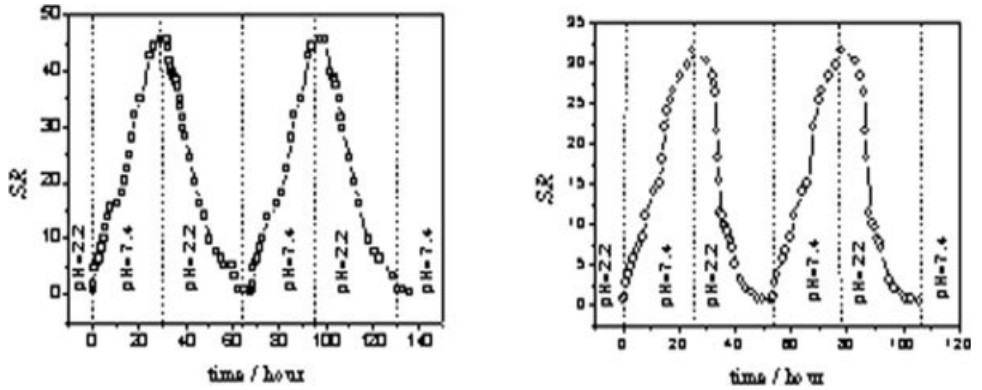

Figure 7 Swelling/deswelling of gel *n*4-0 (left) and *n*4-1 (right) as a function of times at pH 2.2 and 7.4 media, respectively.

Table II shows that the *n* values at pH 2.2 are ~ 0.5, which indicates Fickian diffusion. However, the *n* values at pH 7.4 indicate a non-Fickian transport.[24] In other words, the *n* values increase with a pH increase of the external medium. Apparently, the occurrence of the non-Fickian mechanism is due to the ionization of side chain carboxyl groups of the gels, when the pH values of media are higher than the pK_a of the polyacrylic acid (~ 4.28, as shown in Ref. 29). In this case, ionization of carboxyl groups causes electrostatic repulsion and affects the relaxation of the macromolecular chains.

Swelling-deswelling studies

For a protein- or peptide-loaded polymeric hydrogel as a site-specific drug delivery agent along the gastrointestinal tract, there are two basic considerations: (a) protein or peptide drugs should not be released from hydrogels, i.e., the gels are in the collapsed status in the stomach where the pH is ~ 2.2. In addition, the gels should undergo full swelling and show maximum release in the colon where the pH is ~ 7.4. (b) The mechanical strength and structural integrity of the drug-loaded hydrogels should be maintained in above process. Figure 7 shows periodic swelling-deswelling of gel *n*4-0 and *n*4-1 in buffer solutions of pH 2.2 and 7.4.

For nonsemi-IPN gel *n*4-0, the swelling and deswelling process took about 30 h and 35 h, respectively. However, for semi-IPN gel *n*4-1, these times were about 25 h and 28 h, respectively. A possible explanation for this difference is that the presence of amylose changes porous structures of gels as discussed earlier on their FE-SEM images. Also the presence of amylose enhances the relaxation of the macromolecule chains. In this case, semi-IPN gel *n*4-1 shows a more pH-sensitive behavior. Moreover, it was found that the shape of the semi-IPN hydrogel *n*4-1 was remained well when compared with that of gel *n*4-0 after swelling/deswelling cycles. It implies that the semi-IPN hydrogel showing good strength would resist the vermicular stress of the gastrointestinal tract and is able to keep drug in the gels before delivery to the colon.

CONCLUSIONS

The favorable strength of pH-responsive hydrogels containing amylose could be attributed to the polymer-amylose semi-IPN structure, although the content of crosslinker is low (0.1 mol %). The swelling behavior of the semi-IPN gels indicated that the aggregation of the hydrophobic groups of the gels can be broken by the chain entanglements of amylose and the electrostatic repulsion between the charged COO^- groups due to the ionization of the carboxylic groups at pH 7.4. The time for the equilibrium swelling of semi-IPN gels depends on the content of amylose. Water diffusion from gels in acidic or alkaline media was controlled by different mechanisms. In the swelling simulation of the gastrointestinal tract and colon, the semi-IPN hydrogels exhibited excellent pH sensitivity.

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