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Synthesis and characteristics of pH-sensitive semi-interpenetrating polymer network hydrogels based on konjac glucomannan and poly(aspartic acid) for in vitro drug delivery

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ABSTRACT

A novel pH-sensitive semi-interpenetrating polymer network (semi-IPN) hydrogels were prepared by using konjac glucomannan (KGM) and poly(aspartic acid) (PAsp) with trisodium trimetaphosphate (STMP) as the cross-linking agent. The effects of component ratio, cross-linking density (STMP concentration), pH and ionic strength on the swelling properties of hydrogels were investigated. The structure of the semi-IPN hydrogels were characterized by fourier transform infrared spectroscopy (FT-IR), surface area analyzer and scanning electron microscopy (SEM). The equilibrium swelling characteristics were investigated at 37 °C in buffer solutions of pH 2.2 and 7.4 as simulated gastric and intestinal fluids, respectively. At pH 2.2, the release amount of 5-Fluorouracil (5-FU) incorporated into the hydrogels was about 23% within 180 min, while this value approached to 95% at pH 7.4. These results showed that the semi-IPN hydrogels could be a suitable polymeric carrier for site-specific drug delivery in the intestine.

1. Introduction

Hydrogels are crosslinked, three-dimensional, hydrophilic polymer networks, which swell, but do not dissolve, when brought into contact with water (Hoffman, 2002; Kim, Shin, Lee, & Kim, 2003). Since the introduction of hydrogels as soft contact lenses in the 1960s (Wichterle & Lim, 1960), their use has increased tremendously and nowadays they are favored in a broad range of pharmaceutical and biomedical applications (Fedorovich et al., 2007; Peppas, 1997; Van Tomme & Hennink, 2007). Hydrogels have been studied with a particular emphasis on their reversible volume changes in response to external stimuli, such as pH, solvent composition, temperature, ionic concentration, and an electric field (Basan, Gümüşderelíoğlu, & Orbey, 2002; Kim, Park, Kim, Shin, & Kim, 2002; Lee, Kim, & Lee, 2000). Especially, pH-sensitive hydrogels have been extensively investigated due to their potential applications in controlled release systems (Kost, Horbett, Ratner, & Singh, 1985).

There is a considerable interest in developing controlled drug delivery systems using natural polymers, such as their non-toxicity, biodegradability and biocompatibility (Heidrick, Pippitt, Morgan, & Thurnau, 1994). KGM is a high-molecular weight, water-soluble, non-ionic, natural polysaccharide isolated from the tubers of the amorphophallus konjac plants and the main crop in mountainous areas of China (Yu, Huang, & Xiao, 2006). It is mainly composed of a high-molecular weight glucomannan in which mannose and glucose units in a ratio of 1.6:1 are connected by β-(1,4) linkages (PéCrols et al.,1997). Due to its biodegradability, biocompatibility, not degradable by digestive enzymes in upper gastrointestinal tract but degradable by β-mannanase (He, Zhang, & Huang, 2001) or other β-glycosidases abundant in colon, KGM shows promise in controlled release systems and serves as biomedical materials (Gonzalez et al., 2004; Liu, Hu, & Zhuo, 2004; Liu, Shao, & Lü, 2006; Wen, Cao, Yin, Wang, & Zhao, 2009).

As opposed to many synthetic materials, for poly(amino acid)-based materials the degradation products are small-molecules nutrients, which are excreted or utilized by physiological processes in the body (Gyenes, Torma, Gyarmati, & Zrínyi, 2008). Poly(aspartic acid) (PAsp) has free carboxylic acid groups or amino groups and is a polymer based on natural amino acids (Zhao, Kang, & Tan, 2006). PAsp has fully biodegradable, water-soluble properties and toxicological suitability such as lack of toxicity, antigenicity, immunogenicity and has become an attractive candidate for drug carriers (Matsuyama, Kokufuta, Kusumi, & Harada, 1980).

Semi-IPN is "a way of blending two polymers where only one is crosslinked in the presence of another to produce a mixture of fine morphology (Čulin et al., 2005; Jin, Liu, Zhang, Chen, & Niu, 2006)". Semi-IPN hydrogels can be easily prepared using semi-IPN technique and a combination of some excellent properties such as various sensitivity and good mechanical strength from these two polymer networks (Zhao et al., 2006).

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The aim of this study was to develop a novel pH-sensitive semi-IPN hydrogel-based controlled release system for drug delivery. This hydrogel was composed of a water-soluble KGM and PAsp blended with STMP as cross-linking agent to form a semi-interpenetrating polymeric network (semi-IPN). STMP is a nontoxic cross-linker, which is used legally to cross-link food grade starches in the United States (Kasemsuwan, Bailey, & Jane, 1998).

This report describes the preparation and the physico-chemical characterization of a series of phosphated the konjac glucomannan/poly (aspartic acid) hydrogels. The effects of component ratio, cross-linking density (STMP concentration), pH and different concentrations of NaCl on the hydrogels swelling properties were investigated. Additionally, release profiles of a model drug 5-Fluorouracil (5-FU) from test hydrogels were studied in simulated gastric and intestinal pH media.

2. Experiment

2.1. Materials

Konjac glucomannan (KGM) was purchased from Engineering Center of Chongqing Universities of Konjac Resources Research, Chongqing, China. The content of glucomannan is above 99%. Poly(aspartic acid) (PAsp, with MW 140,000) was purchased from Tianxiang Chemical Co., Jiangsu, China, which was food grade. Trisodium trimetaphosphate (STMP), potassium acid phthalate, hydrochloride, potassium dihydrogen phosphate, and sodium hydroxide were purchased from Taixing Chemical Co., chongqing, China. 5-Fluorouracil (5-FU) was purchased from Sigma, St. Louis, MO, USA. All the reagents were of analytical grades unless otherwise mentioned in the text.

2.2. Preparation of PAsp/KGM semi-IPN hydrogels

A weighed amount of KGM was fed slowly into a rapidly stirred NaOH solution (pH 11) then allowed to hydrate at least 2 h under constant stirring in a 250 mL round-bottomed flask at room temperature. The varying amounts of STMP, and PAsp were added to the reactor according to Table 1. The polymerization was then allowed to proceed at 50 °C for 6 h. The obtained hydrogels were rinsed several times with distilled water to remove the unreacted STMP, KGM and other soluble agents, and cut into discs (10 mm OD). Then the discs were further dried to constant weight and stored until further used.

2.3. Swelling measurements

The swelling ratio (*SR*) of a hydrogel was measured after it was swollen to a desired state at 37 °C. It was carefully taken out from the solution, wiped with a filter paper for the removal of the free water on the surface, and then weighed. *SR* (g/g) of a sample was calculated as follows (Eq. (1)):

$$SR = (W_t - W_d)/W_d \quad (1)$$

where W_d and W_t are the weights of dry and wet samples at time t, respectively. When a hydrogel reaches its swelling equilibrium state under a fixed condition, its swelling ratio is called equilibrium swelling ratio (*ESR*). All measurements were replicated five times for each sample. Hydrogels used in this study were freeze-dried.

Table 1
Feed composition for the preparation of PAsp/KGM semi-IPN hydrogels.

Sample code	Weight ratio (%) PAsp/KGM	Molar ratio STMP/KGM[a]
KP01	20	0.9
KP02	40	0.9
KP03	60	0.9
KP04	80	0.9
KP05	100	0.9
KP06	60	0.3
KP07	60	0.6
KP08	60	0.9
KP09	60	1.2
KP10	60	1.5

[a] KGM is calculated based on monosaccharide unit.

2.4. The measurement of pH-sensitive properties of PAsp/KGM semi-IPN hydrogels

Buffer solutions of different pH values (2, 4, 6, 8; ionic strength = 0.1 M) were used to study the pH-sensitivity of hydrogel. The pH values were precisely checked by a pH-meter (Leici/E-201-C, accuracy ± 0.01). Then, 0.50 g of dried sample was used for the swelling measurements in buffers (pH 2, potassium acid phthalate/hydrochloride buffer; pH 4 and 6, potassium acid phthalate/sodium hydroxide buffer; pH 8, potassium dihydrogen phosphate/sodium hydroxide buffer) at 37 °C according to the above method described in the Section 2.3.

2.5. Ionic strength-sensitivity of hydrogels

The effect of ionic strength on swelling ratio was investigated in NaCl solution with concentrations ranging from 0.005 to 0.1 mol/L at 37 °C. About 0.50 g of dried sample was used for measuring the swelling ratio of sample according to the above method described in the Section 2.3.

2.6. In vitro cumulative release studies

The drug-loaded and released property was evaluated by using 5-FU as drug target. The dry hydrogels were equilibrated in 0.02 g 5-FU/mL of the solutions for 24 h at room temperature to load drug into the hydrogels.

To study the release profiles for the drug-loaded PAsp/KGM hydrogels, dried test samples were immersed in a phosphate buffer solutions of pH 2.2 and 7.4 (ionic strength = 0.1 M) at 37 °C in a shaking water bath incubator. After a given time, a 5 mL of the solution released was withdrawn and at the same time a 5 mL of fresh solution was added to maintain a fixed volume of release medium. The amount of 5-FU released was analyzed at 266 nm by an ultraviolet spectrophotometer (UV-2550, Shimadzu, Tokyo, Japan).

2.7. Fourier transform infrared (FT-IR) spectral measurements

The chemical structures of the hydrogel were investigated by using fourier transform infrared spectroscopy (FT-IR). The samples were dried overnight under a vacuum condition, until constant weight. The dried hydrogels were analyzed in KBr discs by a Nicolet (Madison, WI, USA) 170SX fourier transform infrared spectrometer in the region of 4000–500 cm^{-1}.

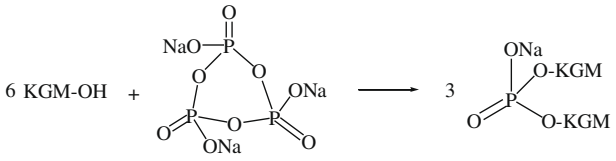
2.8. The average pore size, morphology of the semi-IPN hydrogels

The average pore size measurements were made for the freeze-dried hydrogel with different extent of cross-linking powders using a surface area analyzer (Quantachrome Autosorb-1, USA). The morphology of freeze-dried hydrogels was determined using a scanning electron microscope (Hitachi S-4800, Japan). Hydrogel samples were sputtered with gold and scanned at an accelerating voltage of 20 kV.

3. Results and discussion

3.1. FT-IR spectrum of PAsp/KGM semi-IPN hydrogel

Fig. 1 shows the FT-IR spectrum of PAsp (a), KGM (b), and the semi-IPN hydrogel (c). In the case of PAsp (a), the carboxylate group symmetric stretching vibrations is indicated by a band observed at 1402 cm^{-1}. Three bands observed at 1697, 1532 and 1257 cm^{-1} are due to amide-I, amide-II and amide-III, respectively (Zhao et al., 2006). KGM (b) shows the characteristic absorption peaks of the mannose appeared at 874 and 808 cm^{-1} (Yu et al., 2006). In the case of the semi-IPN hydrogel, all the peaks appeared both in PAsp and KGM were observed. In addition, the new peaks were observed at 1094 and 1026 cm^{-1} indicating the P–O–C stretchings vibration of phosphate ester group (Liu, Fan, Wang, & He, 2007). These bands confirm the formation of cross-link between KGM chains by STMP. These indicate that PAsp and KGM were incorporated into the gel system and the semi-IPN hydrogel network structure was formed. The crosslinked polymer is formed from KGM hydroxyl groups and STMP as follows:



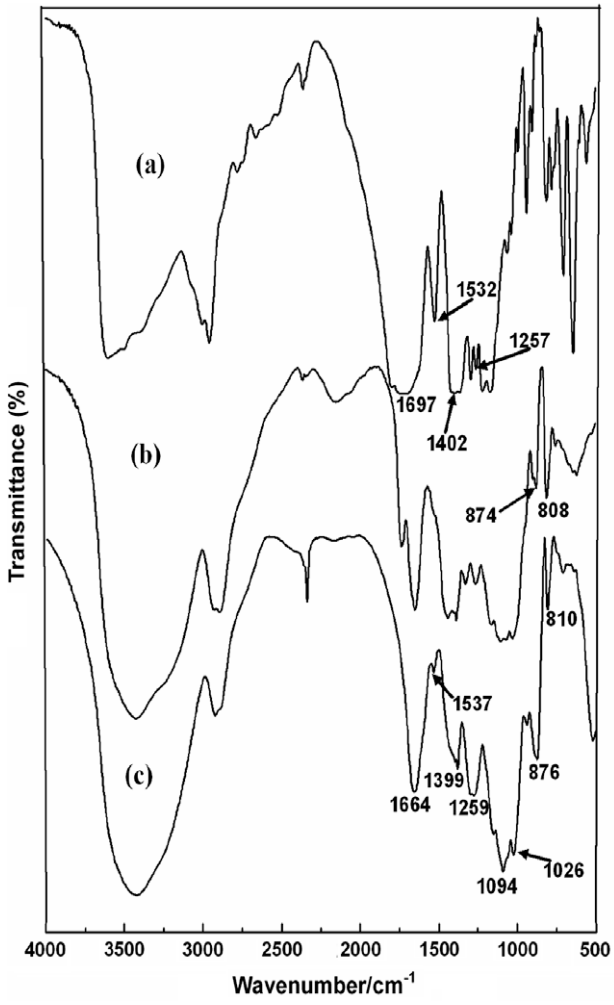


Fig. 1. FT-IR spectrum of (a) PAsp, (b) KGM, (c) semi-IPN hydrogel.

3.2. The average pore size, morphology of the semi-IPN hydrogels

The cross-linker content strongly affects the pore size of the semi-IPN hydrogels. In order to investigate the influence of cross-linker content on the pore structure of the samples, the average pore size of hydrogels was analyzed. Fig. 2(a) shows the average pore diameter of semi-IPN hydrogels with different STMP concentration. The sample (KP08) shows a maximum in the average pore diameter. The average pore diameter of the PAsp/KGM semi-IPNs is dependent on the content of STMP. As the content of STMP in the hydrogels increase, the average pore diameter of hydrogels increase. This may be attributed to the fact that the crosslinked reaction with STMP caused KGM to lose its non-ionic nature, with the increase of the STMP concentration within the hydrogels, the negative charge repulsive force increase, thus resulting in an increase in the average pore diameter of hydrogels. However, with continuous increase in the STMP concentration, the contribution of the cross-linking density becomes predominant. Therefore, the average pore diameter of hydrogels decreases with further increase in the STMP concentration.

To further investigate the influence of cross-linker content on the pore structure of the samples, the SEM images of a selection of the samples (KP07, KP08 and KP09) were presented in the Fig. 2(b). The results showed the changes of the cross-section morphology with different STMP concentration of semi-IPN hydrogels, which are in agreement with results of the influence of cross-linker content on the average pore diameter of semi-IPN hydrogels.

3.3. Effect of PAsp content

The weight ratio of PAsp and KGM is varied from 20% to 100% to study the effect of PAsp content on the swelling ratios of the semi-IPN hydrogels (Fig. 3). With increasing the amount of PAsp, the swelling ratios of the semi-IPN hydrogels are found to increase. This behavior is caused by hydrophilic properties of the PAsp, which is derived from hydrophilic groups (–COOH, –CONH– and $–CONH_2$) in its structure. The swelling ratios of the semi-IPN hydrogels increase from 17.9 to 38.9 with increasing PAsp content. The higher the PAsp content in the semi-IPN hydrogels the higher the content of hydrophilic groups (–COOH, –CONH– and $–CONH_2$). On the other hand, the hydrogels swell more significantly due to a large swelling force created by the electrostatic repulsion between

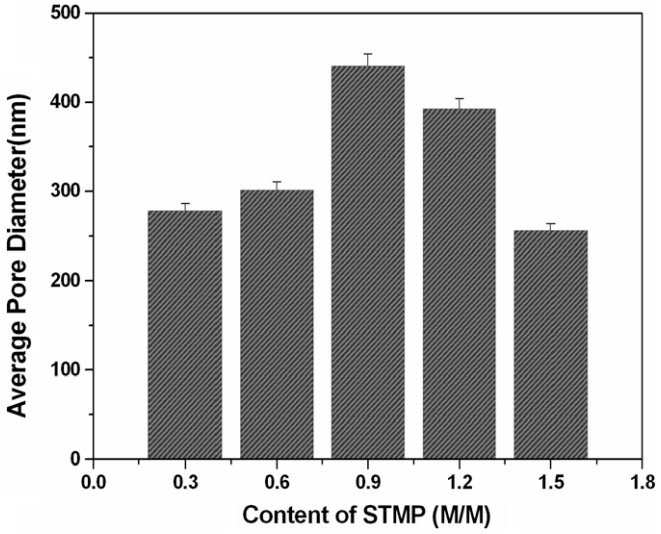


Fig. 2a. (a) Influence of the amount of STMP on the average pore diameter of semi-IPN hydrogels.

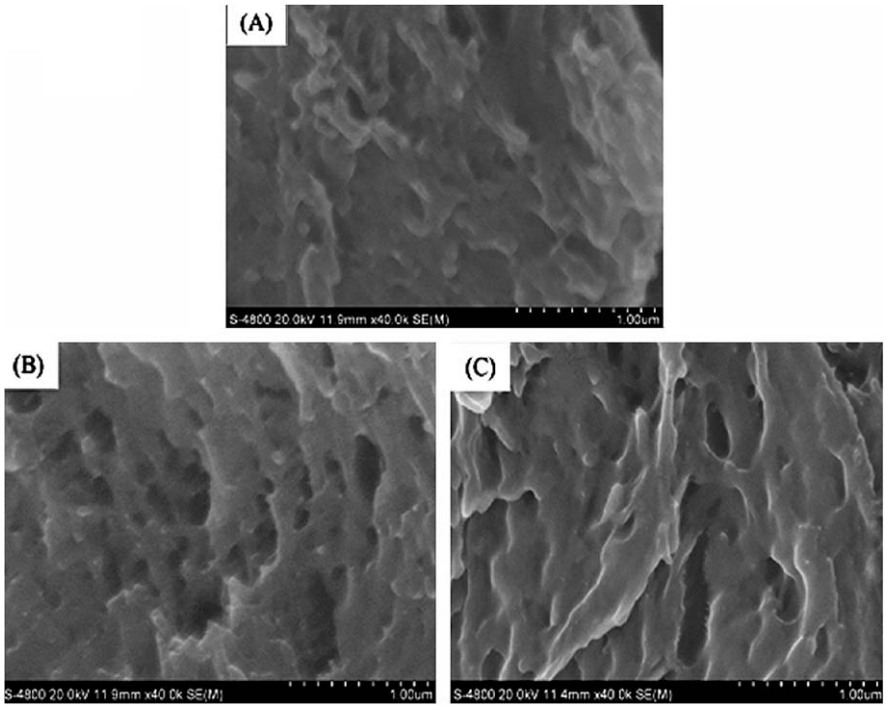


Fig. 2b. SEM photograph of cross-section of the semi-IPN hydrogels: (A) KP07, (B) KP08, and (C) KP09.

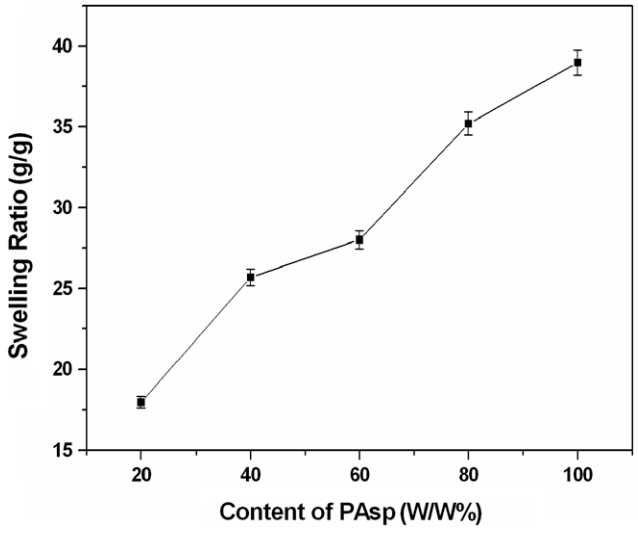


Fig. 3. Influence of PAsp on the swelling ratio of the hydrogels in distilled water at 37 °C.

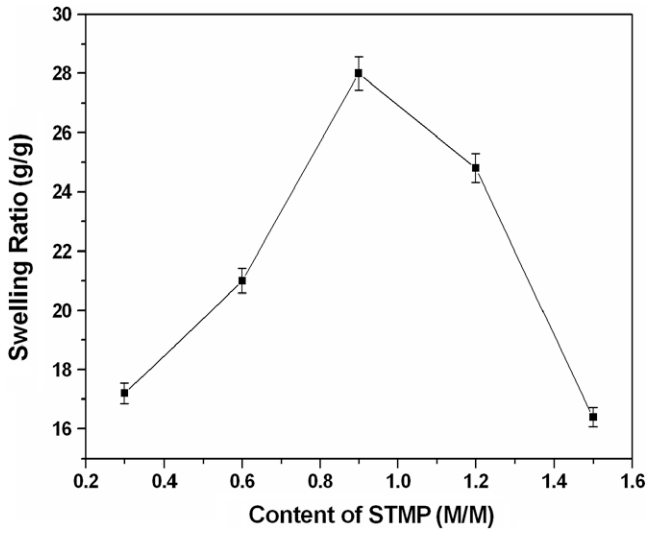


Fig. 4. Influence of the amount of STMP on swelling degree of hydrogels in distilled water at 37 °C.

ionic groups (–COO^-) in the semi-IPN hydrogels. (Chena et al., 2004). Similar behavior has been reported in poly(aspartic acid)/poly(acrylic acid) semi-IPN hydrogels (Zhao et al., 2006).

3.4. Effect of cross-linker

The molar ratio of STMP and KGM is varied from 0.3 to 1.5 to study the effect of STMP content on the swelling ratios of the semi-IPN hydrogels (Fig. 4). The hydrogel KP08 has the maximum swelling ratio among the semi-IPN hydrogels. Before the amount of STMP increases to this maximum swelling, the swelling of hydrogel is mainly influenced by negative charge repulsions. After the maximum swelling the contribution of the cross-linking density becomes predominant (Dulong et al., 2004). With larger quantity of STMP, the cross-linker is sufficient to reduce the macromolecular chain mobility and the swelling ratios of the semi-IPN hydrogels are lower. The above results are consistent with the influence of cross-linker content on the average pore size and the morphology of semi-IPN hydrogels. Analogous viewpoints have been reported (Gliko-Kabir, Yagen, Penhasi, & Rubinstein, 2000).

3.5. Effect of swelling medium pH

To investigate the swelling behavior of the PAsp/KGM semi-IPN at various pH levels, the hydrogel samples are swollen in different buffer solutions of pH 2, 4, 6 and 8 with an ionic strength of 0.1 M at 37 °C. As shown in Fig. 5(a), the swelling ratios of the semi-IPN

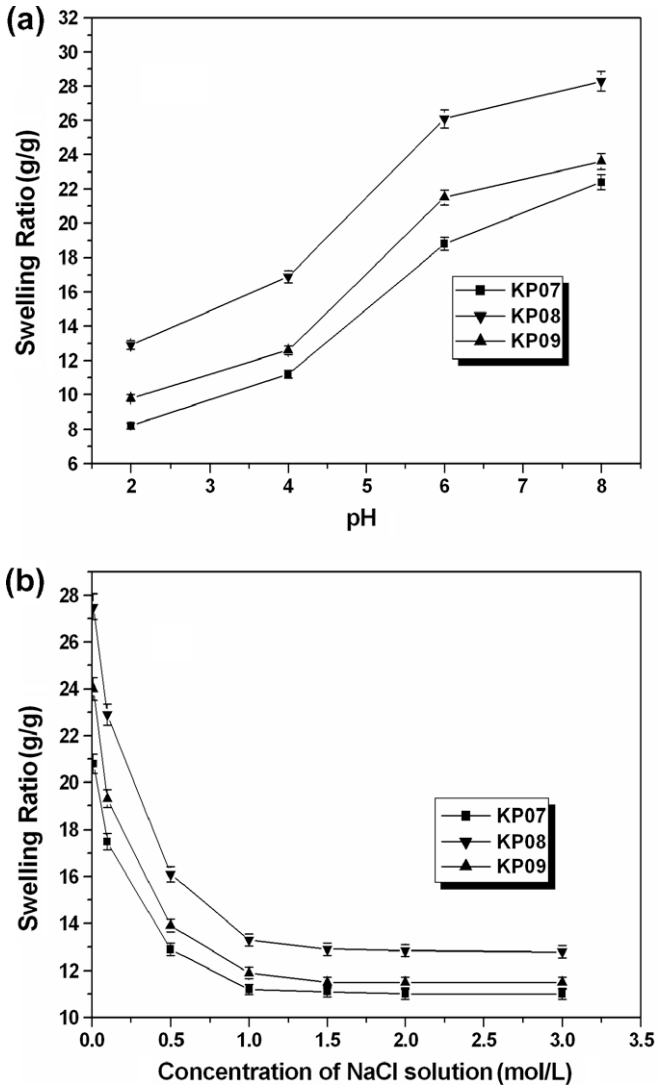


Fig. 5. Swelling ratios of the PAsp/KGM semi-IPN hydrogels as a function of pH value (a) and ionic strength (b) at 37 °C.

hydrogels increase with increases pH values. This is mainly attributed to the carboxyl group of PAsp in the semi-IPN hydrogel, in which the pK_a of PAsp is about 2.09–3.86 for the amino acid monomer. As the solution becomes less acidic, the ionization of the carboxylic acid groups occurs, resulting in electrostatic repulsions between the ionized groups, which cause swelling degree of the hydrogels to reach to a relatively larger value accordingly (Dong & Hoffman, 1991; Gil & Hudson, 2004; Kikuchi & Okano, 2002; Liu & Fan, 2002; Liu, Liu, & Zhuo, 2006; Liu et al., 2006; Mullarney, Seery, & Weiss, 2006; Park & Bae, 1999; Philippova, Hourdet, Audebert, & Hokhlov, 1997; Tonge & Tighe, 2001; Torres-Lugo & Peppas, 1999; Zhang & Peppas, 2000). It would be a desirable characteristic for a pH-sensitive controlled release system because of the acidic degree of the stomach (pH about 2.2) and intestines (pH about 7.4).

3.6. Effect of ionic strength on swelling ratio

The effect of the salt concentration in aqueous solution on the equilibrium swelling is also studied for the semi-IPN hydrogels. The swelling degree of the semi-IPN hydrogels in saline solutions is appreciably reduced when compared with the values measured in deionized water. This well-known phenomenon, commonly observed in the swelling of ionic hydrogels (Flory, 1953), often results from a charge screening effect of the additional cations. Fig. 5(b) illustrates the relationship between swelling and saline concentration at 37 °C. It shows that the changes of the NaCl concentration higher than about 1 mol/L has no appreciable influence on the equilibrium swelling ratio of the PAsp/KGM semi-IPN hydrogels (Mahdavinia, Pourjavadi, Hosseinzadeh, & Zohuriaan, 2004; Zhao, Su, Fang, & Tan, 2005).

3.7. Swelling kinetics

To use these semi-IPN hydrogels as 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) (Ghandehari, Kopečková, & Kopecek, 1997). The swelling kinetics of different semi-IPN hydrogels are investigated at both pH 2.2 and 7.4 phosphate buffer solutions at 37 °C, to simulate the stomach and colon conditions, and the results are shown in Fig. 6. It can be seen that swelling increase with time and level off around 3 h at pH 2.2, and after 7 h at pH 7.4. In addition, the swelling ratio of all the samples at pH 7.4 are higher compared with those of the same semi-IPN hydrogels at pH 2.2, implying

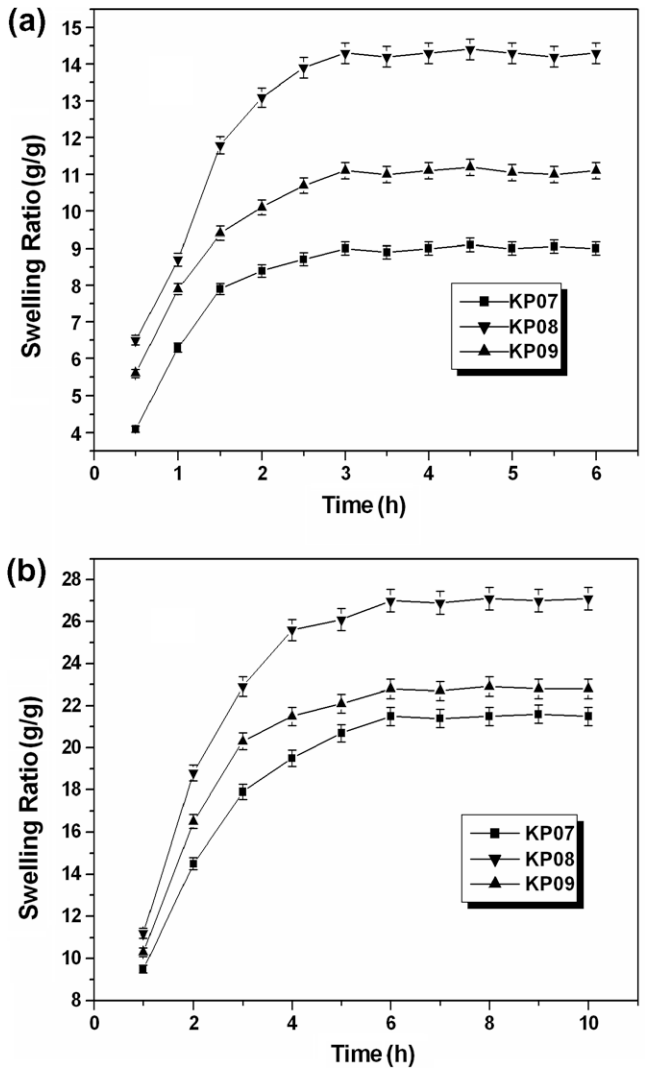


Fig. 6. Swelling kinetics for semi-IPN hydrogels (KP07, KP08 and KP09) in pH 2.2 (a) and pH 7.4 (b) buffer solutions at 37 °C.

their swelling property is highly dependent on pH values. At low pH (pH 2.2), the swelling ratio of the PAsp/KGM semi-IPN hydrogel is limited due to formation of intermolecular hydrogen bonds between PAsp (–COOH and –NH) and KGM (–OH). At pH 7.4, the carboxylic acid groups on the PAsp/KGM semi-IPN hydrogel are dissociated ($–COO^-$). In this case, the hydrogels swell more significantly due to a large swelling force created by the electrostatic repulsion between the dissociated acid groups (Chena et al., 2004).

3.8. In vitro release study

Fig. 7 shows the cumulative release profiles of 5-FU from KP07 in phosphate buffers of pH 2.2 and 7.4 (ionic strength = 0.1 M) at 37 °C as a function of time. The percentage cumulative release (R) of 5-FU from the semi-IPN hydrogel is calculated using (Eq.(2))

$$R = \frac{M_t}{M_0} \times 100\% \quad (2)$$

where M_t is the amount of drug released at time t and M_0 is the initial loaded drug amount.

From Fig. 7 it can be seen that drug release is pH dependent. The percentage of 5-FU released from the semi-IPN hydrogel is 23% within 3 h in simulated gastric fluid (pH 2.2) and the equilibrium release in simulated intestinal fluid (pH 7.4) reaches nearly 95% of the initial drug content within 7 h. The extent of release increases as the hydrogel swelling increases due to the increase in pH, which leads to ionization of the carboxyl groups. In addition, this result is also in good agreement with the influence of the pH values on swelling of hydrogels as discussed in the Section 3.5. Namely, at pH 7.4, the relatively high swelling degrees of the semi-IPN hydrogel result in higher release rates (Yu & Xiao, 2008).

In order to precisely understand the release mechanism, the drug released from the semi-IPN hydrogel is fitted to following equations proposed by Ritger and Peppas (1987) (Eq. (3)).

$$\frac{M_t}{M_\infty} = kt^n \quad (3)$$

where M_t/M_∞ is the fraction of drug released at time t and k is a constant related to the properties of the drug delivery system and n is the diffusion exponent, which determines the release mechanism. From the plot of ln M_t/M_∞ versus ln t, the drug diffusion parameter, n, is calculated. When $n < 0.5$, the release is dominated by Fickian diffusion (Lynch & Dawson, 2004); when $0.5 < n < 1$, the release follows non-Fickian diffusion; and $n = 1$ there is continuous zero-order release, where the system will be relaxation controlled (Lynch & Dawson, 2004; Mullarney et al., 2006). The values of the exponent of release n calculated according to above method are found to be 0.46 and 0.75 in the medium of pH 2.2 and 7.4, respectively. The values clearly indicate that the hydrogel follows Fickan diffusion controlled release mechanism in pH 2.2, while in pH 7.4 follows non-Fickan diffusion controlled mechanism (Ritger & Peppas, 1987).

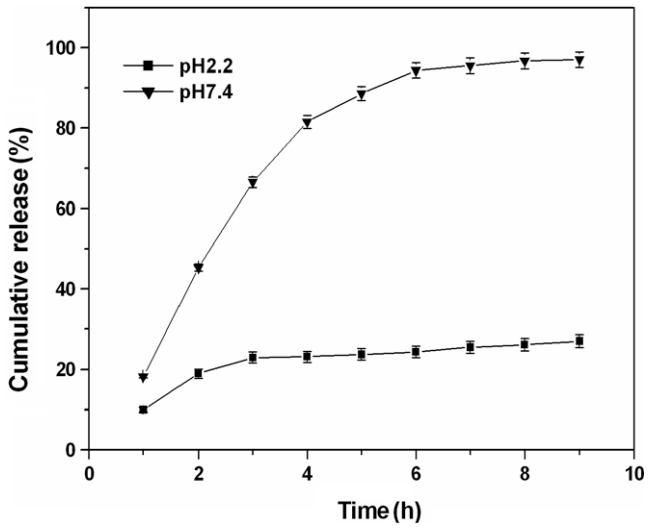


Fig. 7. 5-FU released profiles for KP07 in pH 2.2 and in pH 7.4 buffer solutions at 37 °C.

4. Conclusions

A novel pH-sensitive drug delivery system based on konjac glucomannan and poly(aspartic acid) was proposed. The semi-interpenetrating polymer network (semi-IPN) hydrogels were prepared by using konjac glucomannan and poly(aspartic acid) with trisodium trimetaphosphate as the cross-linking agent. The molar content of cross-linker and poly(aspartic acid) has a significant influence on swelling ratio of the obtained hydrogels. The studies on the swelling behavior of hydrogels reveal their sensitive response to environmental pH values change. The results of in vitro 5-FU release indicate that the release is controlled by swelling of the hydrogel. In addition, the released amount of drug from the semi-IPN hydrogel is higher at pH 7.4 than at pH 2.2. This phenomenon can be explained on the basis of higher degree of swelling due to ionization of carboxylic groups in the network at pH 7.4. All the results indicate that this semi-IPN hydrogel may serve as a potential device for the delivery of drugs in which the primary target is the upper small intestine.

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