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Novel biodegradable hydrogels based on pachyman and its derivatives for drug delivery

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ABSTRACT

Two kinds of hydrogels were synthesized based on pachyman and its hydroxyethyl derivatives (hydroxyethyl pachyman, HEP) by the crosslinking reactions with confunctional crosslinker agent epichlorohydrin (ECH). Hydrogels with different crosslinking ratio were obtained by varying the content of the crosslinker and the polymer. The structure and morphology of hydrogels were characterized and the pH-dependent swelling of hydrogels was confirmed to be strongly influenced by the polymer properties, structure and the crosslinker contents. In the swelling assays, the hydrogels based on pachyman exhibited significant pH sensitivity, while the hydrogels based on hydroxyethyl pachyman tended to have notable swelling capability. In the drug release study, two drugs salicylic acid and bovine serum albumin (BSA) were chosen as model drugs. The results indicated that both two kinds of hydrogels showed better drug sustained release behavior for protein drug BSA than salicylic acid. In addition, evaluated by two model equations, the drug transport mechanism showed anomalous in both two kinds of hydrogels. Importantly, this study offers an entirely new window of developing hydrogels based on this natural polysaccharide, which has great potential for using as a novel sustained release carrier for protein drugs.

1. Introduction

Hydrogels are a class of materials, which contain large volumes of water in their swollen three-dimensional structure without dissolution (Bae and Kim, 1993; Peppas and Sahlin, 1996). Throughout the years, due to the similarity between this highly hydrated three-dimensional network and hydrated body tissues as well as highly biocompatible property (Zhang et al., 2004; Inoue et al., 1997; De Groot et al., 2001), hydrogels have been gained increased relevance including drug delivery systems (Drury and Mooney, 2003), scaffold materials to organize cells into a three-dimensional architecture (Matsuda and Magoshi, 2002; Berger et al., 2004), tissue replacements (Kane et al., 1996), wound dressings (Ishihara et al., 2002; Chen et al., 2003) and immobilization of proteins and cells, and in several chemical applications as well (Hennink and Van Nostrum, 2002; Hoffman, 2002; Peppas et al., 2000). In particular, hydrogels are of special interest in controlled release applications for the delivery of various low molecular weight drugs and biotech therapeutics such as proteins, peptides and oligonucleotides (Salmaso et al., 2007; Mahato, 2006; Crommelin et al., 2003; Cleland et al., 2001), because of the ease that the drugs in hydrogels are dispersed in the matrix, and the high degree of control achieved by selecting the physical and chemical properties of the polymer network (Chen et al., 2004; Risbud et al., 2000). Since the clinical use of hydrogels is increasing, a variety of synthetic or natural polymeric hydrogels have been employed as the controlled release systems for drug delivery (Carvalho et al., 2007; Mi et al., 2002). Nowadays, much effort has been devoted to developing protein delivery systems for many pharmaceutically active proteins. Unfortunately, parenteral administration of proteins is still the major problem in protein drugs (Qiu et al., 2003). The most challenging task in the development of protein pharmaceuticals is to deal with physical and chemical instabilities of proteins (George and Abraham, 2007). Protein drugs exhibit an extremely short plasma half-life, due to their denaturation or degradation occurring in the gastrointestinal tract (Sytkowski et al., 1998), which is the reason that protein pharmaceuticals are administered traditionally through injection rather than taken orally like most small chemical drugs (Wang, 1999). In order to achieve the successful oral delivery of protein drugs, the most important thing is to protect protein drugs from the harsh environment of gastric media in the stomach before they were absorbed in the intestine, for the reason that the natural pH environment of GI tract variation of the acidic condition (pH ~1.2) in the stomach and slightly alkaline condition in the intestine (pH ~7.4) (Shargel and Yu, 1999.).

Recently, pH-sensitive hydrogels, as promising biomaterials in the design of oral delivery of peptide or protein drugs, have been attracting increasing attentions. This kind of hydrogels with regular swelling ability by pH change can protect peptide or protein drugs from enzymatic hydrolysis in the upper gastrointestinal tract

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and can enhance drug release at colon by means of extensive gel swelling and degradation. Until now, a variety of synthetic or natural polymers with acidic or basic pendant groups have been fabricated as the pH-sensitive controlled release systems for getting the desired controlled release of protein drugs (George and Abraham, 2007; Vandamme et al., 2002). Among them, natural polymers which possess properties such as non-toxicity, non-immunogenicity and biodegradability have been commonly used in recent years.

Pachyman, a fungus polysaccharide, is a naturally occurring linear polysaccharide produced by a sclerotium of *Poria cocos* (Chihara et al., 1970; Wang et al., 2004a,b), which is one of the most important herbs in China and many other Asian countries. It is composed by 1,3-β-linked D-glucose units, which is in similar structure character like starch and cellulose. Up to now, pachyman is confirmed to have many pharmaceutical values, for it is well known for its diuretic (Narui et al., 1980), mitogenic, complement activating (Yamada et al., 1992), anti-inflammatory (Schinella et al., 2002) and immunoactive properties (Wang et al., 1995). Moreover, many derivatives of pachyman also have been prepared and developed for many pharmaceutical and biomedical applications. In our previous investigations, a novel kind of amphiphilic conjugates were fabricated based on carboxymethylated derivative of pachyman (CMP) which displayed very satisfactory properties that may have great potential for using as the delivery vehicles for anti-tumor drugs (Hu et al., 2008). In addition, Xiao et al. studied the properties of CMP and hydroxypropyl pachyman (HPP) and explored them as tablet disintegrants. From their experiment, many desirable results were obtained (Xiao et al., 2007).

In the view of the chemical structure, pachyman possesses active functional groups (i.e., –OH group) which can be chemically modified to form hydrogels via crosslinking. In this paper, based on polysaccharide and its hydroxyethylated derivatives (HEPs), we fabricated two kinds of novel hydrogels (i.e., ECH crosslinked pachyman hydrogels, EPCS and ECH crosslinked hydroxyethyl pachyman hydrogels, EHEP) by using a simple crosslinking reaction. Various hydrogels with different crosslinking degree were obtained and their physicochemical properties were characterized such as the effective crosslinking density, selling ratio and morphology. Meanwhile, two model drugs, salicylic acid and bovine serum albumin (BSA), were chosen and tested to pursue their applicability in this controlled drug delivery systems.

2. Materials and methods

2.1. Materials

Pachyman, the average molecular weight of 2.2×10^5, extracted with 0.5 M NaOH aqueous solution from the sclerotium of *P. cocos* was used (Wang et al., 2004a,b). Epichlorohydrin, salicylic acid and 2-chlorethanol were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). BSA was purchased from Sigma Chemical Company (St. Louis, MO, USA). All other chemical reagents were analytical grade and obtained from commercial sources.

2.2. Synthesis of hydroxyethylated pachyman (HEP)

Pachyman was hydroxyethylated according to the method previously reported (Wang et al., 2004a,b). Briefly, 1.2 g of native pachyman was dissolved in 100 ml of 1.5 mol/l NaOH/0.5 mol/l urea aqueous solution in an ice bath with stirring for 3 h. Then 16.0 ml 2-chlorethanol was slowly added to the solution with stirring. The solution was stirring at room temperature for 5 h and then at 60 °C for 2 h to obtain the hydroxyethylated derivatives. After the solution was cooled to room temperature, 0.5 M HCl was added to adjust pH to 7.0 and then the solution was dialyzed at 4 °C by a regenerated cellulose tube (Mw cut-off 8000) against distilled water for 7 days. The resulting solution was concentrated by rotary evaporator at reduced pressure below 40 °C. Finally, the HEP solution was lyophilized by using a lyophilizer to give the product. HEP was identified by using Fourier transform infrared spectrophotometer (FTIR, Spectrum One, Perkin Elmer, USA), and ^{1}H NMR and ^{13}C NMR (D_2O) (Bruker DPX spectrometer, 400 MHz).

2.3. Preparation of pachyman hydrogels (EPCS) and HEP hydrogels (EHEP)

Synthesis of EPCS and EHEP hydrogels were carried out by intermolecular side-chain reaction of hydroxyl groups of polymers with confunctional crosslinking agent, epichlorohydrin, in alkaline solutions. In a typical experiment, 0.8 g of native pachyman or HEP were dissolved in 20 ml of 2% (w/v) NaOH at room temperature for 2 h to obtain transparent solution. Different amounts of epichlorohydrin were added to the pachyman or HEP solution and allowed to crosslink under constant magnetic blending at room temperature. The molar ratios of ECH to every glucose unit of polysaccharide or HEP in four different hydrogels formed were 0.5:1, 0.75:1, 1:1, 2:1, respectively. We defined the crosslinker feeding ratio as the mole multiple number of introduced ECH in relation to the mole amount of glucose unit of pachyman and HEP. After stirred at room temperature for 30 min, the crosslinking reaction continued at 50 °C for another 3 h. Then, the hydrogel obtained was taken out from the bottle and cut into disks of 6-mm in diameter and 3-mm in thickness and placed in distilled water for 3 days in order to get rid of the impurities (salts and/or crosslinkers). The different ratios of above prepared samples were coded as 0.5EPCS, 0.75EPCS, 1EPCS, 2EPCS for ECH crosslinked pachyman hydrogels, and 0.5EHEP, 0.75EHEP, 1EHEP, 2EHEP for ECH crosslinked HEP hydrogels, respectively. Washed hydrogels were dried in a vacuum oven under ambient temperature until no weight loss could be detected and then stored for further use. Table 1 lists the ratios of reactants and conditions used for preparing each of the hydrogel samples.

2.4. Characterization of the gel by FTIR

The FTIR analysis was done to identify the atomic structure of the hydrogels. The vacuum-dried samples were mixed with KBr powder and pressed into tablets under vacuum. The spectra were collected on a Fourier transform infrared spectrophotometer (FTIR, Spectrum One, Perkin Elmer, USA) at the wavelength range of 400–4000 cm^{-1} with a resolution of 4 cm^{-1}.

2.5. Morphological study of EPCS and EHEP

Scanning electron microscopy was performed on hydrogels (freeze-dried to maintain the porous structure without any collapse) to obtain information on the pore structure of hydrogels. The sufficiently swollen hydrogels were removed from the solution, quickly frozen in liquid nitrogen, and then freeze-dried under vacuum at room temperature for 3 days to remove the imbibed water completely until the samples became completely dry prior to morphological observation. The freeze-dried hydrogels were fractured carefully to reveal the interior and mounted onto the base plate and coated with gold. The interior morphology of the swollen pachyman and HEP hydrogels at room temperature were observed using a scanning electron microscope (SEM, JEOL JSM-6700F, Japan).

2.6. Swelling studies of EPCS and EHEP

The swelling characteristics of test hydrogels were determined by immersing dried test samples to swell in aqueous solutions with different pH value or temperature in sealed containers. At specific time intervals, the hydrogels were taken out. When the excess

Table 1
Reaction conditions for preparation of pachyman and HEP hydrogels.

Sample code	Volume of alkaline solution, ml	Polymer concentration, % (w/v)	Feed mole ratio, ECH/glucose unit	Volume of ECH, ml
0.5EPCS	20	4[a]	0.5:1	0.19
0.75EPCS	20	4[a]	0.75:1	0.29
1EPCS	20	4[a]	1:1	0.38
2EPCS	20	4[a]	2:1	0.78
0.5EHEP	20	4[b]	0.5:1	0.17
0.75EHEP	20	4[b]	0.75:1	0.26
1EHEP	20	4[b]	1:1	0.34
2EHEP	20	4[b]	2:1	0.68

[a] Polymer solution of pachyman.
[b] Polymer solution of HEP.

water was removed carefully and rapidly with filter paper from the hydrogels surface, the hydrogels were weighed on a sensitive balance immediately. The media of different pH at 25 °C for the swelling studies were 0.1N HCl (pH 1.2), phosphate buffer (pH 7.4), or 0.1 M NaOH (pH 12.5). For studying the swelling behavior of the gels in media of different temperature in phosphate buffer (pH 7.4), preweighed hydrogel samples were immersed in phosphate buffer at 25, 37 or 45 °C, respectively.

The swelling ratios (SRs) of test samples were calculated from the following expression:

$$\mathrm{SR} = \frac{W_s - W_d}{W_d}$$

where W_d and W_s are the weight of the dry and swollen hydrogels, respectively.

The above procedure lasted until no weight increase was observed for at least three continuous measurements. Constant weight indicated that the gel had reached equilibrium swelling state. Equilibrium water content (EWC) of the hydrogels was calculated using the following equation:

$$\mathrm{EWC} = \frac{W_s - W_d}{W_d}$$

where W_s and W_d represent of the weight of swollen and dry state samples, respectively.

2.7. Measurement of in vitro drug release

Since BSA was hydrolyzed in the basic condition during the preparation of hydrogels, BSA was loaded into hydrogels by absorption after the hydrogels extraction in distilled water for 7 days. The extracted and dried pachyman and HEP hydrogel disks were weighted and then immersed into a 10% (w/w) BSA solution in distilled water for 72 h. Preliminary tests showed that 3 days was the minimum time to ensure complete swelling of gel and maximum loading of active substance. The swollen hydrogels were taken out and dried in a vacuum oven for about 3 days at room temperature until a constant weight was obtained. Each weighed disk was put into 50 ml of pH 7.4 phosphate buffer shaken at 37 °C in a rotary water bath shaker at 100 rpm. At a certain interval, the 2 ml solution was removed and replaced with a fresh phosphate buffer. The amount of released BSA was determined spectrophotometrically by a UV spectrophotometer (UV–VIS Spectrophotometer; Lambda 35, Perkin Elmer, Norwalk, USA) at 280 nm for released BSA. The percentage of cumulative amount of released BSA was determined from standard calibration curves and plotted against time. All experiments were repeated in three times.

In the other experiment, salicylic acid was dissolved in distilled water to produce 10 mM solution. Dried gel disks were soaked in each drug solution at 4 °C for 2 days and room temperature for 2 days. Then, the swollen hydrogels were dried in a vacuum oven at room temperature for about 3 days. Drug-loaded hydrogel disks were immersed in 50 ml of pH 7.4 phosphate buffer and shaken at 37 °C in a rotary water bath shaker at 100 rpm. At a certain interval, the 2 ml solution was removed and replaced with a fresh phosphate buffer. The amount of drug released into the medium was quantified by measuring the absorbance of the drug at 296 nm. The salicylic acid release studies were carried out in triplicate.

The drug loading capacity of the studied hydrogels is defined as the ratio of the amount of loaded drug to weight of dry hydrogels.

The drug release results were presented in terms of the cumulative release as a function of time:

$$\text{cumulative amount released (wt.\%)} = \frac{M_t}{M_\infty} \times 100\%$$

where M_t is the amount of BSA or salicylic acid released from the hydrogels at time t and M_∞ is the amount loaded in the hydrogels.

3. Results and discussion

3.1. Hydrogel synthesis

Pachyman is a special natural polysaccharide which is insoluble in water but good soluble in basic solution. Based on this property, pachyman can be easily prepared to hydrogel by ECH because ECH is a convenient base-catalyzed crosslinking agent which has been widely used for the crosslinking of carbohydrates in polysaccharide chemistry (Denizli et al., 2004; Miguel et al., 1999; Delval et al., 2005; Lee et al., 2004).

In other aspect, in order to improve the solubility of pachyman in aqueous solution, a water-soluble pachyman derivative HEP was synthesized (Fig. 1). It is worthy to mention that in the preparation process, we found accidentally that if we decreased the temperature of the HEP solution to 4 °C, the solution turned to semifluid condition. Based on its special properties, we hypothesized that HEP had the potential to produce to hydrogels. Therefore, we prepared the other kind of EHEP hydrogel besides of pachyman hydrogel.

For the preparing procedure of HEP, it was carried out easily by using 2-chlorethanol as reaction agent as reported (Fig. 1). The hydroxyethyl substitution of pachyman was confirmed by IR and ^{13}C NMR spectrum (data is not given) according to the data reported in the literature for HEP (Wang et al., 2004a). The substitution degree (DS) of HEP sample determined by elemental analysis was 0.67 units of hydroxyethyl group per glucose.

Both the hydrogels of EPCS and EHEP were prepared by using epichlorohydrin as a crosslink agent. This bifunctional agent was easy to react in base solution with the hydroxyl group in the polysaccharide molecules. In the first stage of the reaction between polymer and epichlorohydrin, the free chlorohydrin fragments were formed in the side chain of linear macromolecules, which occurred with the opening of epoxy rings. Then in the presence of NaOH, the chlorohydrin fragments formed could be easily transformed to an epoxy function by dehydrochlorinating. This

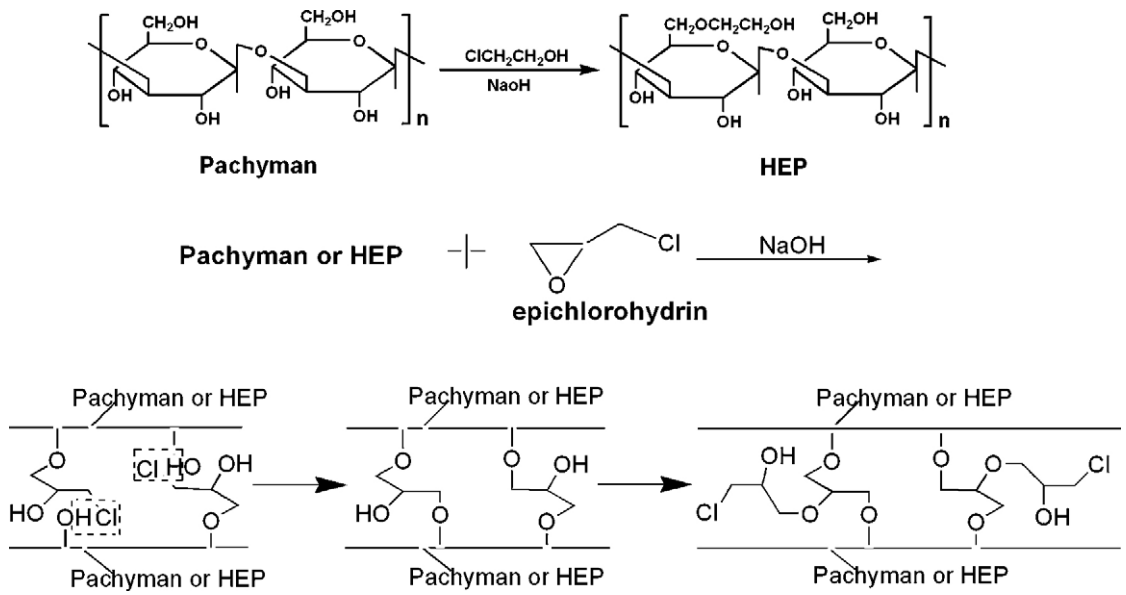

Fig. 1. Synthesis schemes of pachyman hydrogels and HEP hydrogels.

dehydrochlorination reaction between two macromolecules containing OH- and Cl-substitute was realized by providing formation of a crosslinking structure (Güner et al., 2001).

The crosslink reaction between the polymer and ECH took place easily, if we firstly dissolved the polysaccharide and HEP samples in the alkaline solution. In addition, we have studied the effect of crosslinking time and temperature on the gelation extent of the hydrogel. The repeated experiments indicated that 3 h and 50 °C were enough for the complete gelation. Prolonging the time and increasing the temperature scarcely improved the crosslinking degree and the profile of the hydrogels. However, before adding the ECH into the polymer solution, it was very important and necessary to remove the air bubble from the polymer solution.

Adopting the above method, by varying molar ratio of ECH to polymer (shown as mole ratio of ECH/glucose units), four kinds of EPCSs and four kinds of EHEPs with different reticulation degrees were prepared, as shown in Table 1.

3.2. IR characterization

FTIR is a very effective tool for studying the molecular structure of polymers. Fig. 2 presented the FTIR spectra of pachyman and its hydrogel product from ECH. Although all the FTIR spectra exhibited broad peaks around 3000–4000 cm^{-1} corresponding to O–H stretching vibration modes and they seemed similarly in whole, they were indeed different in detail. In the IR spectra of EPCS, the peak at 2800–3000 cm^{-1} signed to C–H stretching and 1158 cm^{-1} corresponding to the C–O–C stretching displayed an obviously increase in their intensity compared with that of pachyman, which indicated the crosslinking reaction was occurred between the hydroxyl groups of pachyman and crosslinking agent ECH.

The FTIR spectra of pachyman, HEP and EHEP were shown in Fig. 3. Compared with the spectra of HEP, the peaks at 2920, 2870 cm^{-1} (C–H stretching) and 1122 cm^{-1} (C–O–C stretching) was remarkable increasing in EHEP, which was able to confirm that crosslinking occurs through the side-chain reactions of HEP's hydroxyl groups with epoxy ring and Cl group of ECH.

3.3. Morphological studies

The hydrogels were fully transparent. The SEM images with different magnification were obtained to characterize the microstructure of the freeze-dried 1EPCS and 1EHEP gels and presented in Fig. 4. According to cross-section SEM images, it should

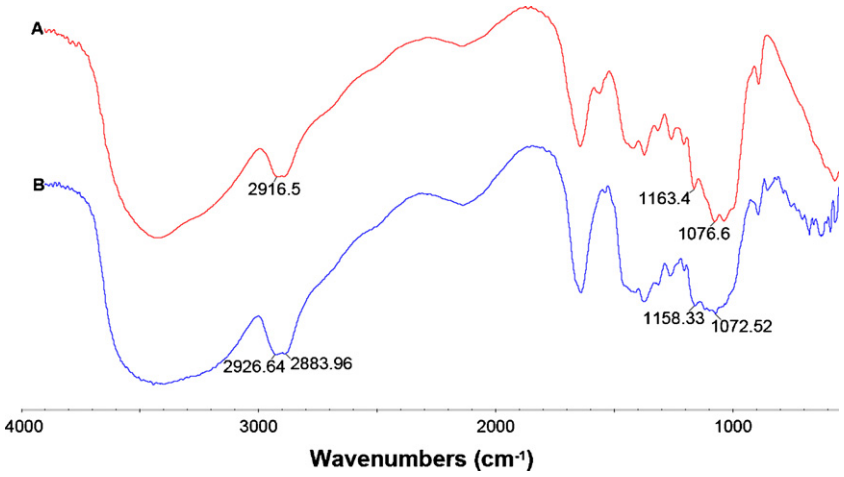

Fig. 2. FTIR spectra of (A) pachyman, and (B) EPCS hydrogel.

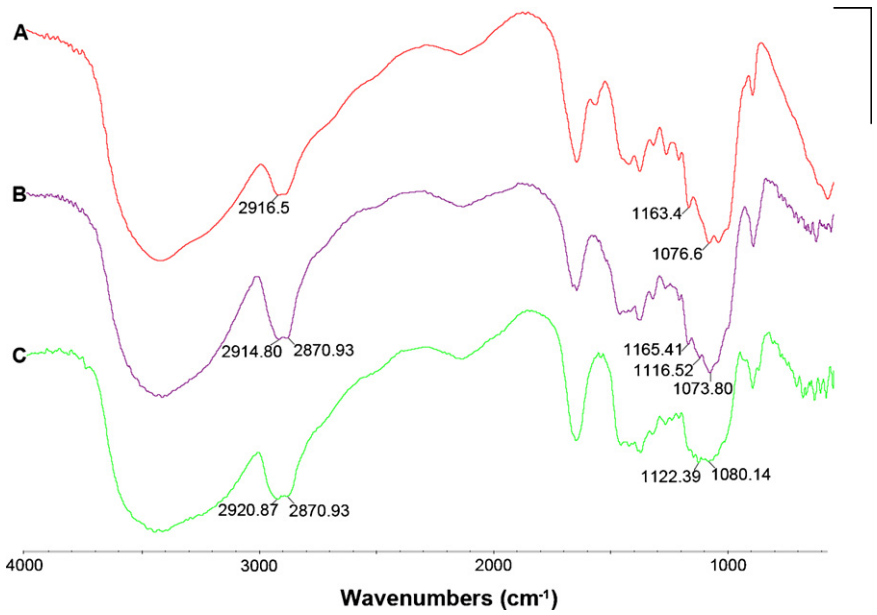

Fig. 3. FTIR spectra of (A) pachyman, (B) HEP, and (C) EHEP hydrogel.

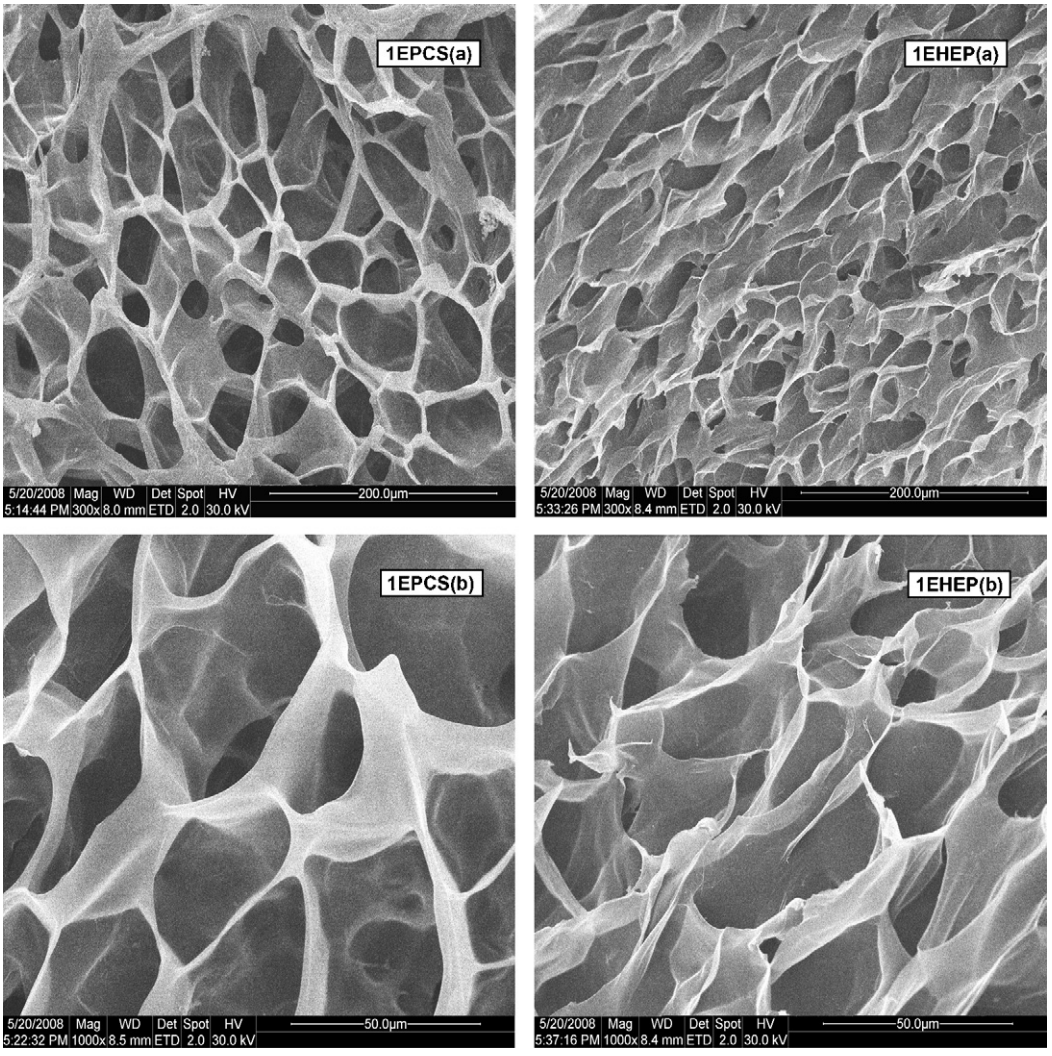

Fig. 4. SEM images of 1EPCS hydrogels and 1EHEP hydrogels with different magnification.

be highlighted that these hydrogels had a continuous and porous structure which was the result of crystal formation by freeze-drying step, resembling other natural macromolecular hydrogels system structure. These porous structures could be detected by the pores whose diameters were in the range of 30–50 μm. The interconnection between pores could be assigned to the network formed by the crosslinking with epichlorohydrin. Obviously, the internal morphology of 1EPCS was significantly different from that of 1EHEP. It seemed that the network structure profiles of 1EHEP were more unregular than that of 1EPCS. On the contrary, the structures of 1EPCS were more compact and rigid. But the image of 1EHEP appeared to be torn in structure, that is maybe the reason why the 1EHEP hydrogels swelling faster than 1EPCS samples.

3.4. Swelling characteristics

The swelling characteristics of hydrogels have a significant influence on the diffusion behavior of small molecules through the gels. In order to get insights into the water transport process through pachyman or HEP hydrogels, the effects of chemical crosslinking ratio and the swelling medium with different pH and temperature on the swelling behavior were studied. The swelling behaviors and kinetics of hydrogels based on pachyman and its derivatives related to the variation of pH and temperature have not been reported yet. In this study, the swelling behaviors of eight hydrogels with different crosslinking degree were deeply studied.

3.4.1. Effect of the amount of crosslinker on the swelling properties of the gel

It is well known that the crosslinking degree is one of the important factors for determining the water absorbency of the hydrogels. The dried pachyman and HEP hydrogel samples with different crosslinking ratio were allowed to hydrate in phosphate buffer (pH 7.4) at 25 °C and the time kinetic curves were illustrated in Fig. 5a and b. It could be clearly seen that swelling capabilities of all hydrogels crosslinked with distinct molar ratio of ECH. The swell equilibrium of EPCS and EHEP hydrogels were almost reached after 50 h of the test. It was noted that the degree of swelling ratio decreased sharply with increasing crosslinking ratio at the same pH medium no matter in EPCSs or in EHEPs. The higher degree of crosslinking, which enhanced the crosslinking density and compactness of the hydrogel network, would hinder the permeation of solvent and result in the lower SR. Therefore, the lowest EWC value ~1.36 and ~1.43 were presented by 2EPCS and 2EHEP hydrogels, respectively.

Compared from EPCSs and EHEPs hydrogels with the same crosslinking ratio, we could obtain that EHEP hydrogels showed notably higher swelling abilities than that of EPCS hydrogels. The equilibrium swelling ratio of 0.5EPCS, 0.75EPCS, 1EPCS and 2EPCS were 14.68, 14.51, 13.81 and 1.36, respectively. And the equilibrium swelling ratio of 0.5EHEP, 0.75EHEP, 1EHEP and 2EHEP were 40.26, 37.30, 17.78 and 1.43, respectively. The reason for this result may be due to the hydroxyethyl fabrication of pachyman, which could further improve the hydrophilic properties of pachyman. Therefore, the HEP hydrogels exhibited better swelling ratio than EPCS samples.

3.4.2. Effect of the temperature on the swelling properties of the gel

Time-dependent swelling behaviors of the hydrogels in phosphate buffer (pH 7.4) at 25, 37, and 45 °C have been plotted in Fig. 6. As shown in figures, there were not notably changes on the swelling ratio of the hydrogel samples no matter in 1EPCS or in 1EHEP. It seems that the temperature has little effect on the swelling behavior of the hydrogels. The similar result was also obtained from other hydrogel samples with different crosslinking ratio (The data is not given.). We can see that when the temperature kept at 25, 37 and 45 °C, the equilibrium swelling ratio were 13.81, 13.67 and 14.01 in 1EPCS and 17.78, 17.80 and 17.51 in 1EHEP, respectively.

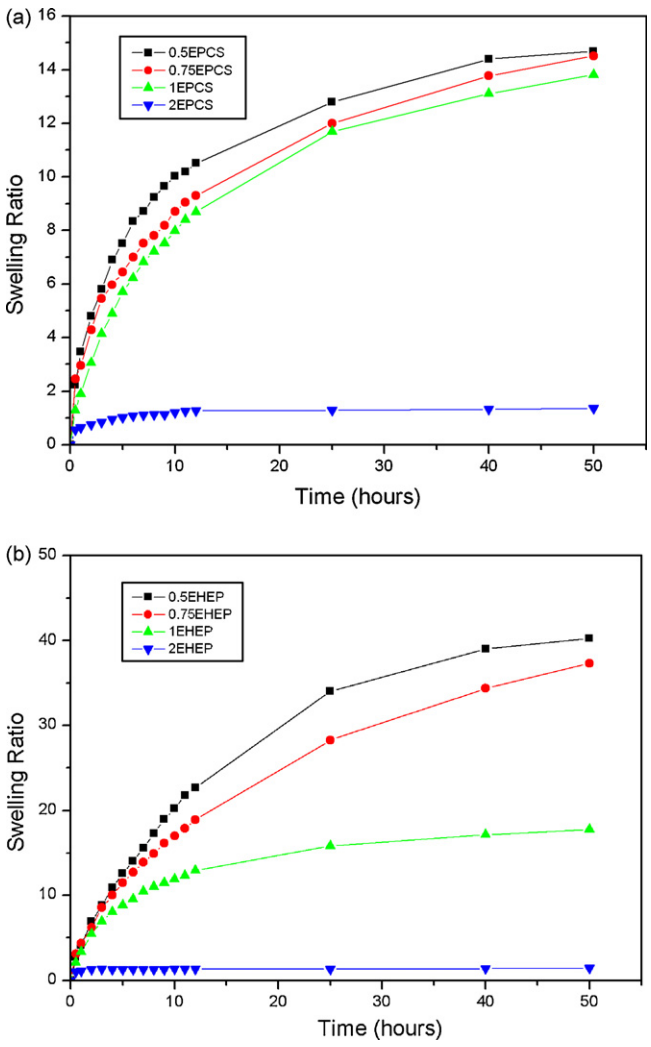

Fig. 5. Swelling behavior of (a) EPCSs hydrogels and (b) EHEPs hydrogels with different crosslinking ratios at pH 7.4 and at 25 °C.

3.4.3. Effect of pH on the swelling properties of the gel

Swelling properties of the hydrogels with different crosslinking ratios were studied in media of different pH value. Time-dependent swelling behavior of the hydrogels in acidic (0.1N HCl, pH 1.2), phosphate buffer (pH 7.4) and in alkaline (0.1 M NaOH, pH 12.5) media at 25 °C were plotted in Figs. 7–9. The same swelling process was kept for all samples. As seen here, these hydrogels swelled rapidly in water at first and then gradually steadied up to the equilibrium value in approximately 50 h or more. The steady swelling ability of the crosslinked hydrogels indicated negligible mass loss ratio with soaking time in the different medium. Moreover, as shown in Figs. 7a, 8a and 9a, the swelling behavior of the EPCS with various degrees of crosslinking in different pH buffers showed obviously pH sensitivity. It was noted that for the same crosslinking ratio of EPCS hydrogels under different pH buffer medium, the SR value increased with the increase in pH, especially in basic medium. Probably it can be explained on the basis of the good solubility of pachyman in basic solution, thus the EPCS hydrogels prepared from them also showed an alkaline soluble property to some extent. Therefore, in pH 12.5, all the EPCS hydrogels exhibited their biggest swelling ratios than in other pH values.

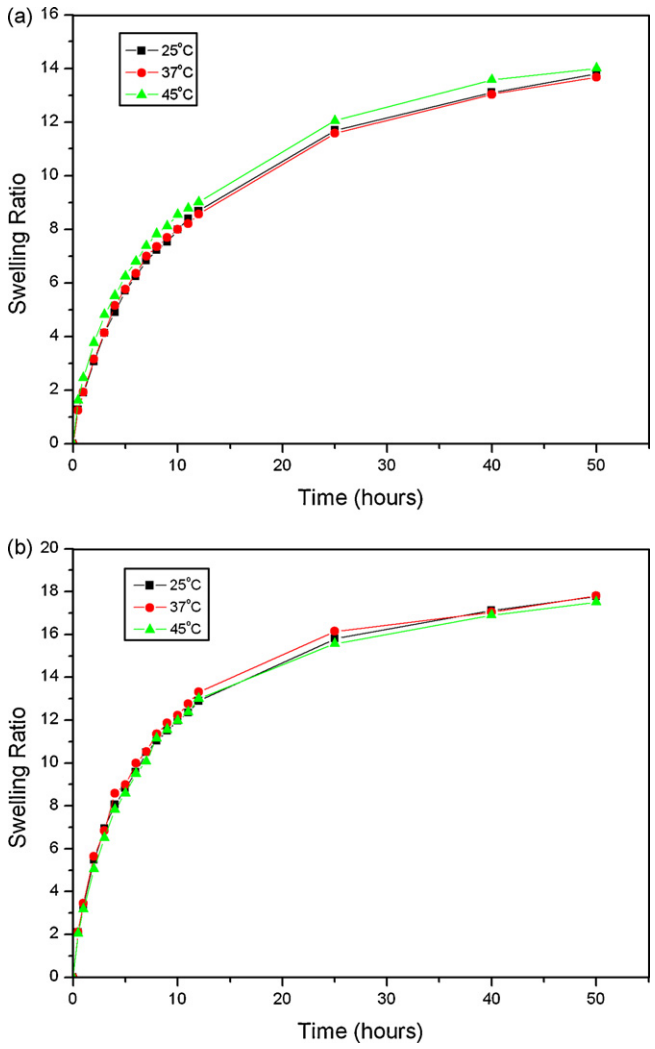

Fig. 6. Swelling behavior of (a) 1EPCS hydrogels and (b) 1EHEP hydrogels at pH 7.4 and at different temperatures.

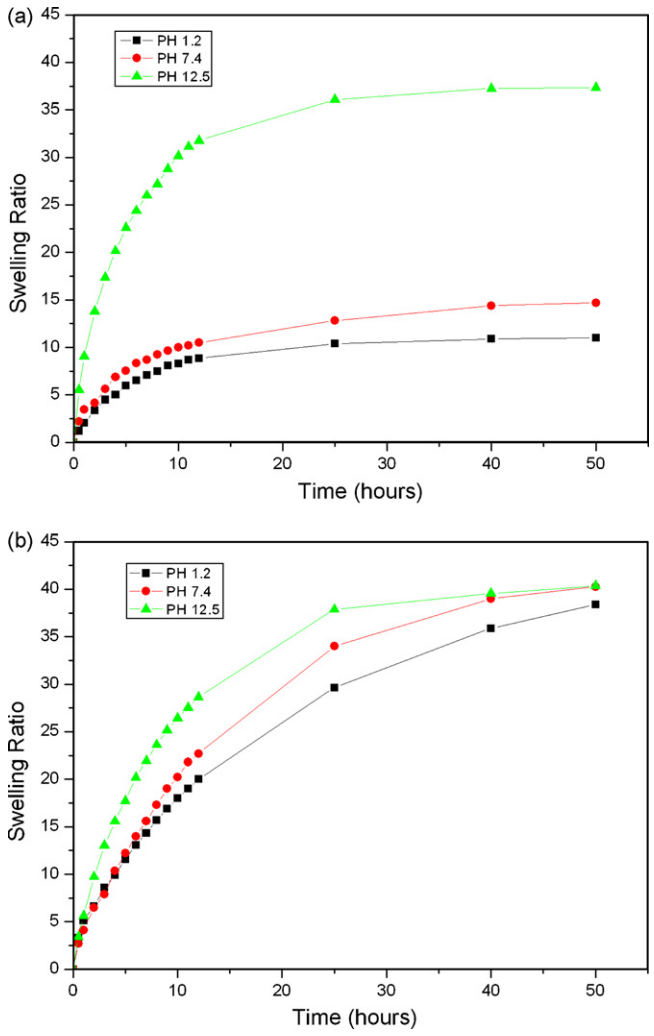

Fig. 7. Swelling behavior of (a) 0.5EPCS hydrogels and (b) 0.5EHEP hydrogels at different pHs at 25 °C.

In addition, compared with EHEP samples, the EPCS hydrogels showed much better pH sensitive properties. As for the same crosslinker ratio, the EPCS hydrogels reached their biggest swelling ratio in basic solution. But for the EHEP samples, although the swelling ratio increased with the pH value increasing, the change was not significant. For example, the swelling ratio of 1EPCS in the medium with pH value of 1.2, 7.4, and 12.5 were about 11.40, 13.81 and 19.00, respectively. However, for the 1EHEP hydrogels at the same condition, the swelling ratios were 17.30, 17.78 and 17.96.

We also studied the swelling ability of the samples with different crosslinking ratio affected by different pH conditions. It is worthy to mention that the EPCS samples with low crosslinking ratio showed better pH sensitivity than the highly crosslinking ones. That is to say, the pH-dependent swelling of hydrogels was strongly influenced by crosslinker content. For 0.5EPCS hydrogels, the swelling ratio in alkaline solution was 37.35, which showed about 3.4 times higher than what was in the acidic solution. But for 1EPCS hydrogels, in alkaline solution the swelling ratio was just 1.7 times higher than in the acidic solution. As for the EHEP hydrogels, this phenomenon was not very obvious, although like EPCS hydrogels, the EHEP ones displayed much faster swelling speed in alkaline medium than in other pH values. In fact, for the 0.5EHEP, the equilibrium swelling ratio was 40.36 in alkaline medium, but 38.40 in acidic solution. For the 1EHEP, the swelling ratios in alkaline and acidic solution were 17.96 and 17.30, respectively.

However, although the EPCS hydrogels displayed swelling property with significant pH sensitivity, their swelling ratios were not so much higher as EHEP hydrogels. Due to the excellent hydrophilicity of the rigidity backbone of hydroxyethyl pachyman and their macroporous structure, all of the EHEP hydrogels displayed good water uptake ability than EPCS with the same crosslinking ratio no matter in the acid solution or alkaline medium.

3.5. Drug incorporation and in vitro release

By taking into account that at pH 7.4, a higher usage volume of epichlorohydrin showed a greater extent of chemical crosslinking of the polymer chains that greatly restricts the mobility of the polymer chains. In this study, we decided to use the hydrogels 1EPCS and 1EHEP which were crosslinked with just onefold mole amount of ECH to glucose units for the study of release profiles of two model drugs, salicylic acid and BSA.

Both two drugs were loaded into hydrogels via a swelling method. Drug release from the hydrogel was observed by drying the drug-loaded hydrogel and subsequently immersing it in PBS solution (pH 7.4) at room temperature.

When salicylic acid was used as small molecular model drug, salicylic acid from 1EPCS hydrogel showed release tendencies similar to that of salicylic acid from the 1EHEP hydrogel (Fig. 10a). The salicylic acid release from the EPCS and EHEP at pH 7.4, sep-

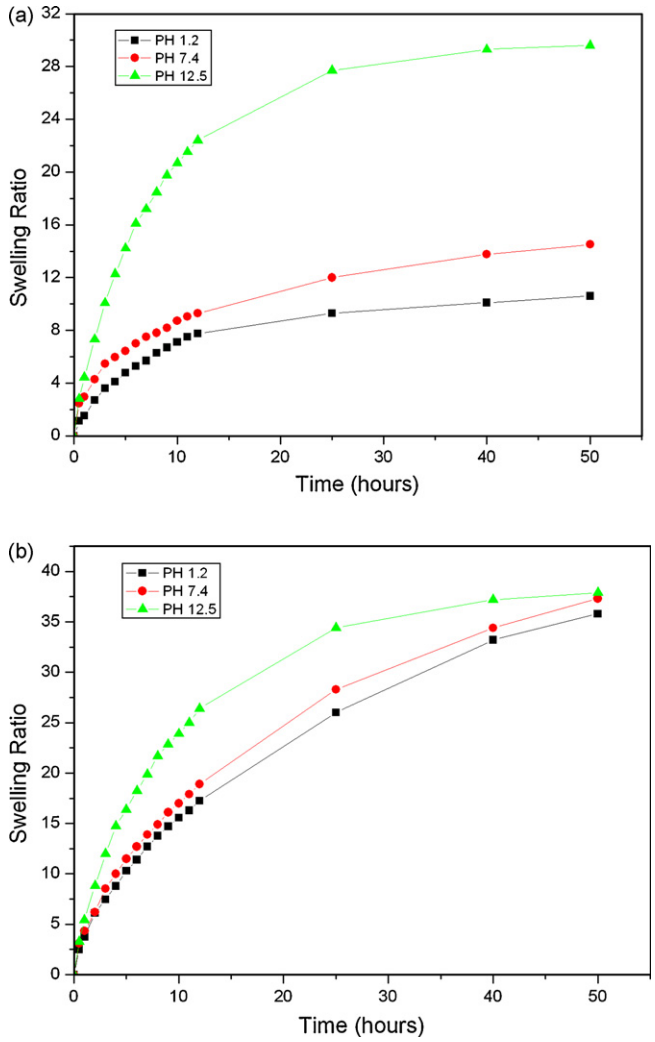

Fig. 8. Swelling behavior of (a) 0.75EPCS hydrogels and (b) 0.75EHEP hydrogels at different pHs at 25 °C.

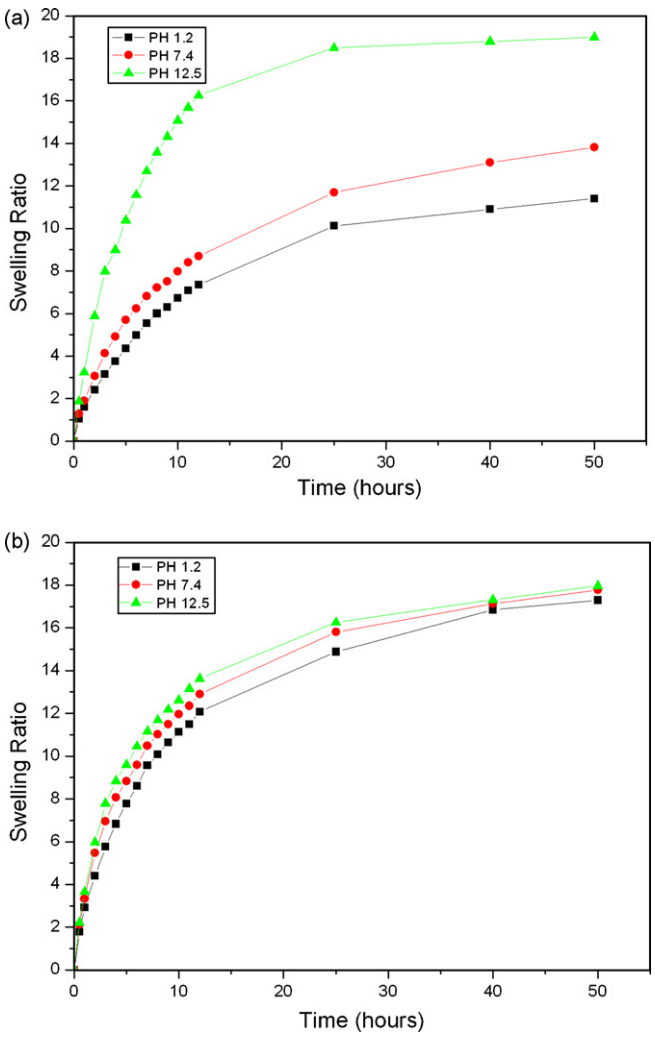

Fig. 9. Swelling behavior of (a) 1EPCS hydrogels and (b) 1EHEP hydrogels at different pHs at 25 °C.

arately gave a maximum release of 94.4% and 98.3%, respectively in 6 h. The main difference of these two kinds of hydrogels was that the 1EPCS showed a slightly lower release rate than 1EHEP, which was probably caused by the regular and compact inner structure of EPCS. Moreover, both of the release profiles were characterized by an "initial burst" of drug during the first few hours, which may be attributed to coeffect of several factors, such as the polymer/drug interactions (surface adsorption), morphology and porous structure of hydrogel materials.

Fig. 10b shows the BSA release profiles from the 1EPCS and 1EHEP hydrogels at pH 7.4. As shown, the release behavior of BSA from these hydrogels was significantly different from that of salicylic acid release profile. The "initial burst" effect of BSA from the hydrogels was not very obvious. This may be related to the inner structure of 1EPCS and 1EHEP. As shown in the SEM images, the pore size of 1EPCS and 1EHEP were very large, which were very suitable for carrying large amounts of large molecular drugs. The loading amount ratio of 1EPCS and 1EHEP were 75.9% and 166.6%, respectively. Apparently, we also found that the rate of drug release in 1EPCS hydrogel was much slower than that in 1EHEP hydrogel, which was probably related to swelling behavior of the 1EPCS hydrogel in the PBS solution. Due to the compact network structure in 1EPCS, a lower level of swelling was found and hence a slower rate of BSA released, i.e., more sustained release were observed for 1EPCS than 1EHEP. It also indicated that EPCS hydrogels showed much better sustained BSA release ability than EHEP hydrogels. This qualitative correlation between swelling ratio and drug released from hydrogels was first suggested by Kim et al. in the study of doxorubicin release from dextran/methacrylate hydrogels (Kim and Chu, 2000). After a 30-h release at pH 7.4, the cumulative BSA release from 1EHEP reached at approximately 81%, and 58% for 1EPCS hydrogel. And only 31% of loaded drug was released from 1EPCS in first 10 h of the experiment.

Moreover, both the protein loading and releasing profile elucidated that the EPCS and EHEP hydrogels had obviously more protein loading and more sustained release capability than what for the small drug salicylic acid. The exact reason for this phenomenon may be related to the inner structure of the hydrogels, both the properties of the drug and polymer and the intermolecular interactions between the drug and the hydrogels, etc.

3.6. Evaluation of drug transport mechanisms

In order to study the drug transport mechanism from both of the two kinds of hydrogels, the experimental data was further analyzed according to the following Ritger–Peppas (Ritger and Peppas, 1987) model (Eq. (1)) and Peppas–Sahlin (Peppas and Sahlin, 1989) model (Eq. (2)).

$$\frac{M_t}{M_\infty} = k_1 t^n \quad (1)$$

Table 2
Comparison of drug release parameters from 1EPCS and 1EHEP hydrogels using two different modeling equations.

Sample code	Ritger–Peppas model				Peppas–Sahlin model			
	k_1	n	SSR × 10^2	AIC	k_1	k_2	SSR × 10^2	AIC
Salicylic acid								
1EPCS	0.07998	0.5148	4.890	−20.14	0.07035	−0.00120	1.166	−31.61
1EHEP	0.08274	0.5622	7.631	−14.01	0.08613	−0.00184	0.725	−30.49
BSA								
1EPCS	0.0070	0.5874	0.096	−100.18	0.01051	0.0006718	0.185	−90.39
1EHEP	0.0151	0.5246	0.818	−53.67	0.01737	0.0001759	0.944	−51.95

$$\frac{M_t}{M_\infty} = k_1 t^{1/2} + k_2 t \quad (2)$$

In the above equations, t is the release time, M_t/M_∞ is the fraction of drug released at time t, k_1 is a kinetic constant, and n is the diffusional exponent that can be related to the drug transport mechanism. The value of n is 0.5, indicating that the drug release in the systems follows the Fickian diffusion; if the value of n is 1, case II transport occurs; when the n value is between 0.5 and 1, anomalous transport is observed. In Eq. (2), the first term of this equation represents the contribution of Fickian diffusion and the second term refers to the non-Fickian behavior (polymer chain relaxation) contribution on the overall release mechanism.

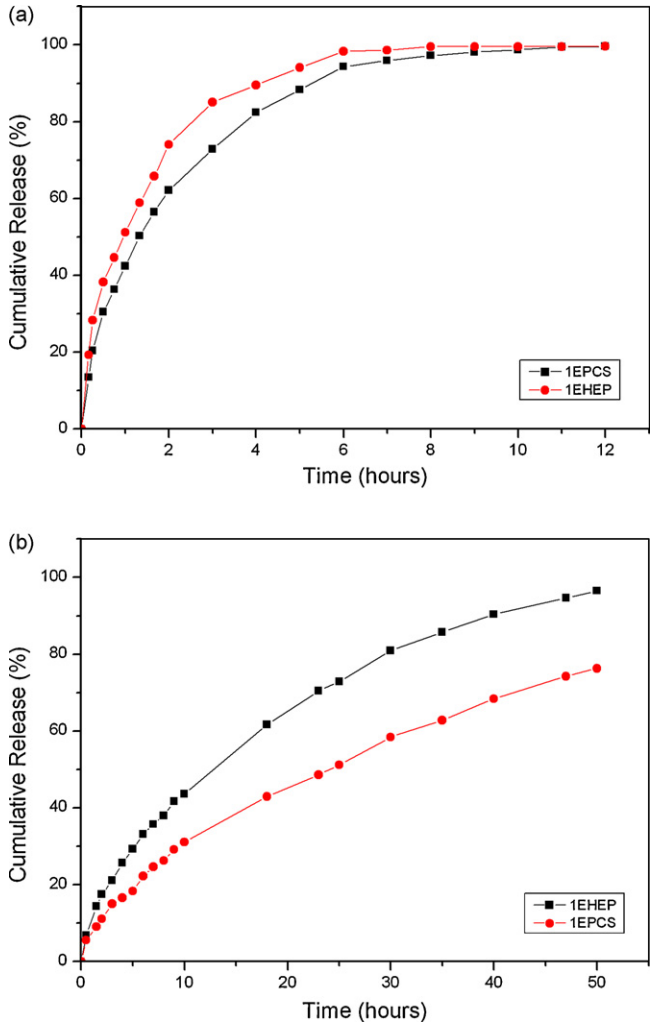

Fig. 10. Salicylic acid (a) and BSA (b) release profiles from 1EPCS hydrogels and 1EHEP hydrogels at pH 7.4.

These mathematical models are valid only for the first 60% of the drug release. The Akaike information criterion (AIC) was applied to distinguish the most appropriate models in this paper. The AIC can be defined as

$$\mathrm{AIC} = N(\ln \mathrm{SSR}) + 2p$$

where N is the number of experimental data points, SSR is the sum of the squared residuals, and p is the number of parameters. The model what shows the smallest AIC value statistically describes the drug release mechanism best (Yamoaka et al., 1978). Various parameters and SSR, AIC values of 1EPCS and 1EHEP hydrogels for both two model drugs salicylic acid and BSA's release study were shown in Table 2.

As the values of AIC shown in Table 2, both the Ritger–Peppas model (Eq. (1)), and the Peppas–Sahlin model (Eq. (2)) statistically best described the experimental data obtained from protein drug BSA release research, no mater in 1EPCS or 1EHEP hydrogels. Both of the two models account for the effects of Fickian diffusion and polymer chain relaxation on the overall drug release mechanism. That is to say, the BSA transport mechanism of these hydrogels observed is anomalous.

When the salicylic acid release experiments were conducted in both two kinds of hydrogels, their resulting data obtained from the Peppas–Sahlin models (Eq. (2)) fit better to the Ritger–Peppas model (Eq. (1)). The AIC values indicated that the former model was more appropriate to describe the release data than the latter. Comparing these data, we also obtained that both of the two models were more suitable for the BSA's transport mechanism study conducted in 1EPCS and 1EHEP hydrogels than for salicylic acid, because the AIC values obtained from BSA's release were lower.

The value of the exponent n in Eq. (1) was calculated as an indicator of the drug transport mechanism. As Table 2 shows, values of 0.5148 and 0.5622, respectively, were obtained from 1EPCS and 1EHEP hydrogels for salicylic acid's release, and 0.5874 and 0.5246 for BSA's release, respectively. All these n values were between 0.5 and 1, indicating that anomalous drug transport was observed in all these cases. The result is another indication that the drug transport mechanism from these hyrogels appears to be anomalous, and both the Fickian diffusion and the polymer chain relaxation process can influence the drug release.

4. Conclusions

Hydrogel is one of the promising and versatile materials with enormous possibilities and potentials. In particular, controlled release system is capable of delivering drugs at constant rate over an extended period of time. This research, based on pachyman generating two kinds of crosslinked hydrogel networks assembly on reaction with epichlorohydrin, provided a simple method for preparing hydrogels from pachyman and its derivatives. On one hand, these hydrogels displayed remarkable swelling capacity and solution stability in wide range of pH value, which can be utilized in drug delivery application or specific food applications demanding of pH-resistance. On the other hand, both two hydrogels exhibited

notably sustained release behavior for BSA, which indicated that hydrogels based on pachyman and HEP may have great potentials for protein drugs. Therefore, this report opens up another interesting perspective to develop this kind of natural polysaccharide with emerging new applications, which may have great potential for using in the pharmaceutical or biochemical field.

Acknowledgments

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