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Preparation and Performance Characteristics of Poly(γ-glutamic acid)/Poly(vinyl alcohol) Hydrogels

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Poly(γ-glutamic acid) (PGA) and poly(vinyl alcohol) (PVA) are polymers of great importance because of their many appealing characteristics specifically for various pharmaceutical and biomedical applications. Physically cross-linked hydrogel membranes composed of different amounts of poly(γ-glutamic acid) and poly(vinyl alcohol) were prepared by applying freeze-thawing method. This freeze-thawing cycle was repeated for three consecutive cycles. Physicochemical properties of PGA-PVA membrane gel such as feeding ratio, swelling, water retention, enzymatic biodegradability and drug release were investigated. The influence of pH on the equilibrium swelling ratios of the hydrogels was investigated too. A higher poly(γ-glutamic acid) content resulted in lower equilibrium swelling ratios. However, the water resistance and retention were improved. The biodegradation rate of the stimuli-sensitive hydrogels in the presence of enzyme was directly proportional to the poly(γ-glutamic acid) content. Lysozyme was chosen as a model drug and loaded into the hydrogels. The *in vitro* drug release experiment was carried out at different pH values and the release data suggested that both the pH and poly(γ-glutamic acid) content played important roles in the drug release behaviours of the hydrogels.

Keywords: Poly(γ-glutamic acid), Poly(vinyl alcohol), Hydrogel.

INTRODUCTION

Recent interest in stimuli-sensitive materials has promoted numerous efforts in preparing intelligent hydrogels[1-5]. The "smart" hydrogels have various potential applications in biomedical materials field, especially in the controlled drug delivery system[6-7]. There are many kinds of stimuli-responsive hydrogels that can response to the external changes in environmental conditions such as temperature, pH, photo and electric field[8-12]. Among all of the above-mentioned stimuli, temperature and pH are the most common physical and chemical ones used in biotechnological and biomedical applications, respectively[13]. Hydrogels containing carboxylic groups exhibit pH-sensitive swelling-deswelling behaviours and are widely used in controlled drug delivery systems[14,15]. However, most of the synthetic pH-sensitive polymers are not biodegradable, which becomes a serious limitation in some applications. Therefore, more attention has been focused on the development of biodegradable and pH-sensitive hydrogels based on polypeptides and natural macromolecules[16-18]. As compared to natural macromolecules, polypeptides have more regular structures and their molecular weights can be controlled more precisely[19]. Poly(γ-glutamic acid) (PGA) and its derivatives are the most widely studied pH-responsive polypeptides due to their biocompatibility and biodegradability.

Poly(vinyl alcohol) (PVA), Fig. 1a) is a semi-crystalline polymer with high crystalline content and clear melting behaviour (T_m, 230 °C) as well as glass transition (T_g, 80 °C). Poly(vinyl alcohol) shows excellent chemical resistance and biocompatibility. Poly(vinyl alcohol) is a water soluble polymer, non-toxic and can be used in the production of paper, clothes, glues, paints, pharmaceutical products, building materials, ceramics, *etc.*[20-22]. Poly(vinyl alcohol) has been reported to be substantially biodegradable in several test systems after a lag time for microbial acclimation. Almost 100 % degradation of 30-day BOD with a poly(vinyl alcohol)-acclimated culture can be reached and thus it has been widely used in various biomedical applications[23].

In order to obtain pH-sensitive hydrogels of non-toxic, biocompatible, biodegradable and modifiable, in this paper, we prepare five types of PGA-PVA hybrid matrices. The as-prepared materials exhibited different pH response capabilities based on the variation of hydrogels compositions. The degradation properties of the hydrogels were also assessed. In addition, preliminary drug release studies were studied using lysozyme as the model protein drug. The pH depending release

behaviours indicated the promising application of these materials as controlled drug delivery vehicles.

EXPERIMENTAL

Poly(vinyl alcohol) (MW about 70 kD) was provided by Shanghai Institute of Organic Chemistry Academia Sinica. γ-Poly(γ-glutamic acid) was prepared in our laboratory. Papain was purchased from Sinopharm Chemical Reagent Co. Lid. Lysozyme (Mw = 14000) was obtained from Amresco.

Preparation of matrices: The method of preparing the pH-responsive hydrogel is shown in Table-1. Typically, poly(γ-glutamic acid) and poly(vinyl alcohol) (total amount of 100 mg) at different weight ratios, *e.g*. PGA:PVA = 3:7, 4:6, 5:5, 6:4 and 7:3 (w/w), were dissolved in 5 mL deionized water at 80 °C by stirring to form homogeneous solutions. Each mixture was mixed and then centrifuged for 5 min at 3000 rpm. Proper amounts of this mixture were poured in standard dishes, followed by freezing at -20 °C for 18 h and thawing for 6 h at 25 °C for three continuous cycles. The yield was calculated from the dry weight of the gelling product and the feeding weight. The matrices were dried again in the oven at 50 °C for 24 h and then stored at 4 °C for further analyses. Hydrogels were denoted as Gel 3/7, 4/6, 5/5, 6/4 and 7/3 corresponding to their initial PGA/PVA feeding ratios of 3:7, 4:6, 5:5, 6:4 and 7:3 (w/w).

TABLE-1
FEEDING RATIO OF HYDROGEL SAMPLES

Sample	PGA (mg)	PVA (mg)	Yield (%)
Gel (3/7)	30	70	78.6
Gel (4/6)	40	60	72.5
Gel (5/5)	50	50	62.5
Gel (6/4)	60	40	74.5
Gel (7/3)	70	30	67.8

Swelling of hydrogel: To measure the swelling ratio of the hydrogels, the dried sample was immersed in various solutions with certain pH for 2 days. The buffer solution was replaced frequently throughout the swelling process to insure complete equilibration at the desired pH. The swelling ratio (SR) (ram per gram) of the hydrogels was calculated from the following equation:

$$SR = (W_t - W_0)/W_0 \quad (1)$$

***in vitro* Enzymatic degradation of hydrogels:** Biodegradation of hydrogels was carried out in a small vial containing a small piece of dry hydrogel sample and PBS buffer (pH = 7.4, 0.01 M) with papain at a concentration of **1** mg/mL. The mixture was then incubated at 37 °C with constant shaking (100 rpm). At different time interval, the samples were taken out and rinsed thoroughly with deionized water; then they were lyophilized to determine the dry weights of the hydrogels. The solution was replaced once a day in order to maintain enzymatic activity. The percentage of weight loss [W_l (%)] was calculated based on the following equation:

$$W_l = (W_0 - W_d)/W_0 \times 100 \quad (2)$$

where W_0 is the original weight of the dried gel sample before immersion and W_d is the weight of the dried sample after degradation at predetermined days.

Drug release studies: A hydrophilic model drug, lysozyme, was loaded into the hydrogel by a swelling-diffusion method. The drug solution (2 mg/mL) was prepared in a 0.01 M phosphated buffer at pH 7.4. Then a dried and weighted hydrogel was placed in 20 mL of the drug solution and allowed to swell for 3 days at 4 °C. After swelling equilibrium, the hydrogel was taken out and rinsed thoroughly with 0.01 M PBS (pH = 7.4). The left drug solution and the PBS used to rinse the drug-loaded hydrogel were collected together and diluted to 50 mL in a volumetric flask. Then the amount of lysozyme left in the loading medium was determined by a UV-6000PC spectrophotometer at a wavelength of 280 nm.

For the drug release studies, the drug-loaded hydrogels were immersed in 10 mL of 0.01 M PBS (pH = 7.4) or 0.01 M citrate-buffered saline (pH = 4). The samples were incubated at 37 °C with constant shaking (100 rpm). At selected time intervals, 1 mL of the release medium was withdrawn and replaced with 1 mL of the fresh solvent. The amount of released lysozyme was quantified by the UV/visible spectrophotometer.

RESULTS AND DISCUSSION

Characterization of prepared hydrogel: The FTIR spectra of Gel 5/5 are shown in Fig. 1. In the FTIR spectrum of hydrogel, the absorption peak of carboxyl at 1647 cm^{-1} and O-H stretching vibration and bending vibration at 3294 and 1462 cm^{-1} from poly(vinyl alcohol) of hydrogel were observed. The result clearly confirmed that the biodegradable pH sensitive hydrogel was prepared.

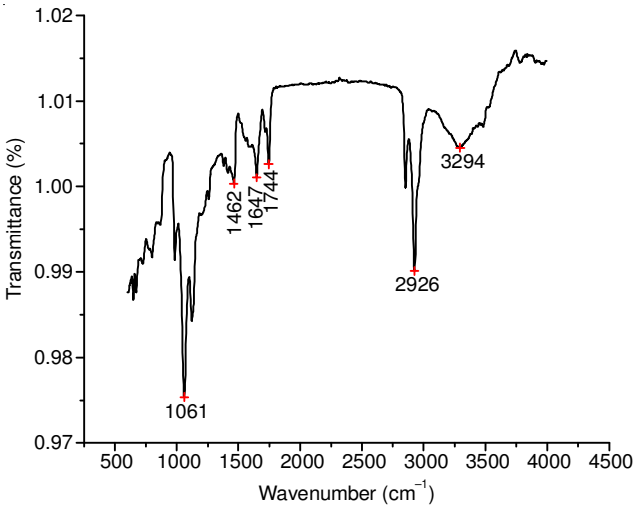

Fig. 1. FTIR spectra of hydrogel

Swelling behaviours: The swelling behaviours of Gel 3/7, 4/6, 5/5, 6/4 and 7/3 were measured at different pH (Fig. 2). As expected, it was clearly found the PGA/PVA hydrogels revealed the pH-sensitive property. As Gel 5/5 for example, the swelling ratio was 2.74, 3.96 and 3.66 in the pH 4.0,7.4 and 9.0 mediums, respectively. It was probably due to the effect of carboxylic group of poly(γ-glutamic acid) poly-peptide (pK_a pH 4.8). Therefore, the PGA/PVA hydrogels made possible to be controlled drug release carrier. Combined with Fig. 2,3, it was found that the larger the poly(γ-glutamic acid) amount, the lower the swelling rate would be. However, the water retention was enhanced from 88.6 for Gel3/7 to 95.8 for Gel 7/3. This suggested the structures of the hydrogel was too tight to expand or diffuse water, due to the H-bone crosslinking

effect. Fig. 4 shows the swelling rate of hydrogels at pH 7.4. It was found that all hydrogels exhibited a fast swelling and reached equilibrium within 20 min.

The pH-responsive swelling-deswelling property of hydrogels was demonstrated by Gel 5/5 between pH 4 and 7.4 at 37 °C. As shown in Fig. 5, the swelling-deswelling process was repeatable as pH changes across the cycles. It was found that the deswelling rate was faster than the swelling rate in each cycle. This behaviour made it possible to control drug release by a feed-back mechanism[24].

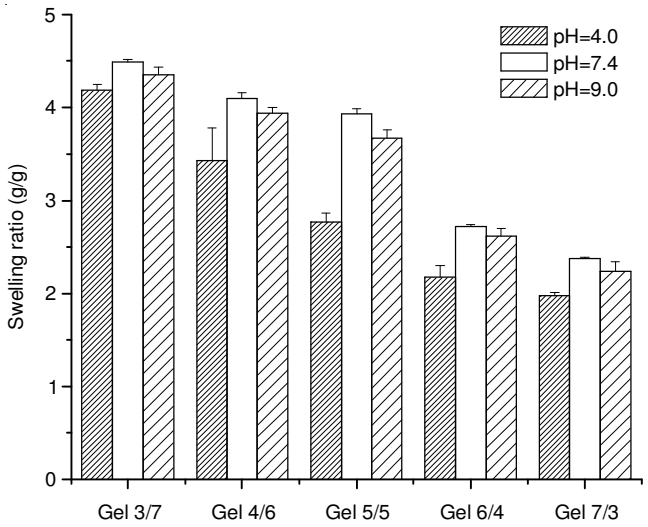

Fig. 2. Swelling ratio (SR) of hydrogel samples in buffers with different pH values at 37 °C

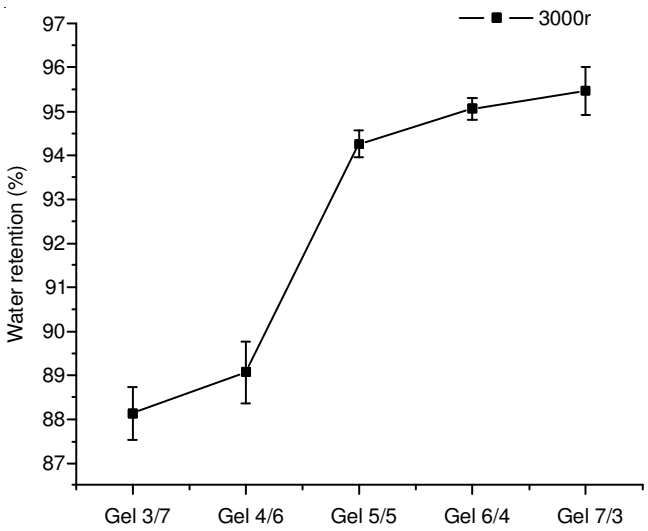

Fig. 3. Water retention (WR) of hydrogel samples

***in vitro* Enzymatic biodegradability of hydrogels:** Poly(γ-glutamic acid) is one of the most studied synthetic polypeptide and has been proved to be biodegradable *in vitro* and *in vivo*. Papain is endopeptidase and it is an analogue of cathepsin. So the presence of papain can accelerate the brokenness of poly(γ-glutamic acid) main chains (amide bonds) of network. The enzymatic degradation behaviour of hydrogels in the presence of papain is shown in Table-2. In our experiments, all the hydrogels showed various extents of weight loss.

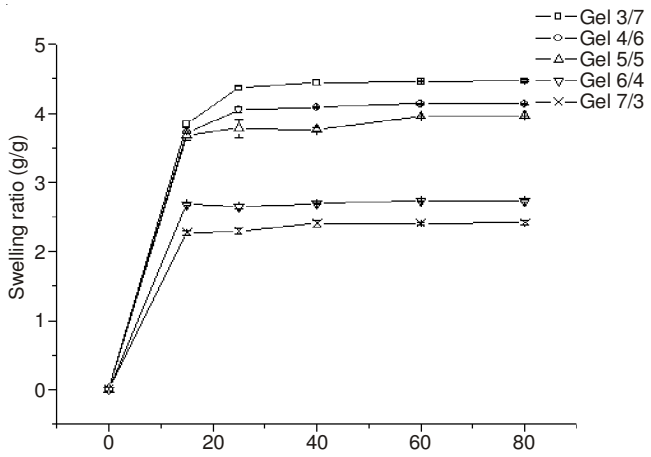

Fig. 4. Swelling kinetics of hydrogel samples in the buffer of pH 7.4

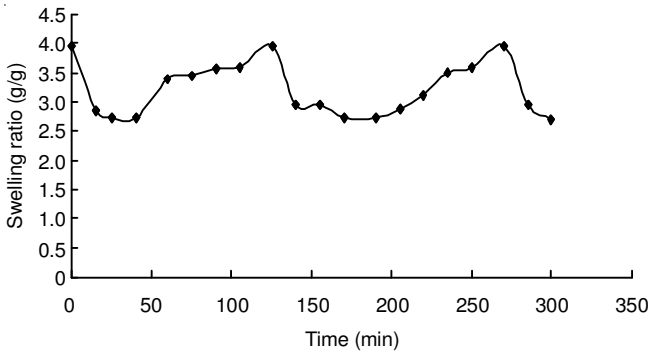

Fig. 5. Reversible swelling-deswelling behaviours of Gel 5/5 between pH 4 and pH 7.4

TABLE-2
in vitro DEGRADATION RATE OF γ-POLY-GLUTAMIC ACID HYDROGEL

Sample	7 h	11 h	3 d
	Weightlessness rate (%)		
Gel3/7	29.57 ± 6.00	29.57 ± 6.00	33.08 ± 6.00
Gel4/6	45.28 ± 2.00	45.28 ± 2.00	45.28 ± 0.39
Gel5/5	53.43 ± 9.07	53.43 ± 9.07	53.43 ± 3.43
Gel6/4	60.54 ± 0.57	60.54 ± 0.57	60.54 ± 0.82
Gel7/3	63.75 ± 8.66	65.00 ± 8.00	65.00 ± 0.52

It was found that there was obvious disintegration after 7 h of enzymatic degradation for Gel7/3. However, there was a little weight loss for Gel3/7. The degradation rate decreased with the order of Gel 7/3 > Gel 6/4> Gel 5/5 > Gel 4/6 > Gel 3/7. After 3 days, weightlessness rate reached the maximum. As the hydrogel degrading, the soluble part including poly(γ-glutamic acid) and its degraded residue were released and diffused into the buffer solution. So the weight of the remaining crosslinked network decreased.

***in vitro* release of lysozyme:** *in vitro* Lysozyme release profiles of hydrogels at 37 °C were investigated in pH 7.4 and 4 buffers, as shown in Fig. 6A and B, respectively. It was found that there were burst releases during the initial stage at pH 7.4. This relative constant rapid release at the initial stage was attributed to the release of drug that located at the hydrogel surface. When the hydrogel was placed into the buffer solution, the drug at the hydrogel surface could be dissolved immediately, leading to the burst release. After the initial burst, the hydrogels served as diffusion barriers and the drugs were

mainly released by the diffusion mechanism. As shown in Fig. 6A, the release rate decreases in the order of Gel 3/7 > Gel 4/6 > Gel 5/5 > Gel 6/4 > Gel 7/3, which is consistent with the crosslinking density change of the hydrogels. After about 1 day, all hydrogels exhibited no or very slow release of lysozyme. That was probably attributed to the interactions between the remained lysozyme and the hydrogel network, which prevented the release of the drugs. The low drug concentration preserved in the hydrogels, which leads to a low drug concentration gradient and weak release driving force, may be the another reason.

Poly(γ-glutamic acid) and its derivatives are most widely studied polypeptides for their pH-sensitive property, so it is necessary to examine the lysozyme release profile under an acidic condition. Fig. 6B shows the *in vitro* cumulative release of lysozyme from the hydrogels at pH 4. It was found that the initial burst was suppressed compared with that at pH 7.4. The release profiles at pH 4 were quite different from those at pH 7.4. All hydrogels exhibited lower amounts of the cumulative releases at pH 4 than those at pH 7.4 at corresponding release time. As a polypeptide bearing carboxylic side groups, poly(γ-glutamic acid) was protonated under an acidic condition. Therefore, the diffusion of drugs was restricted by the constrained network at pH 4.

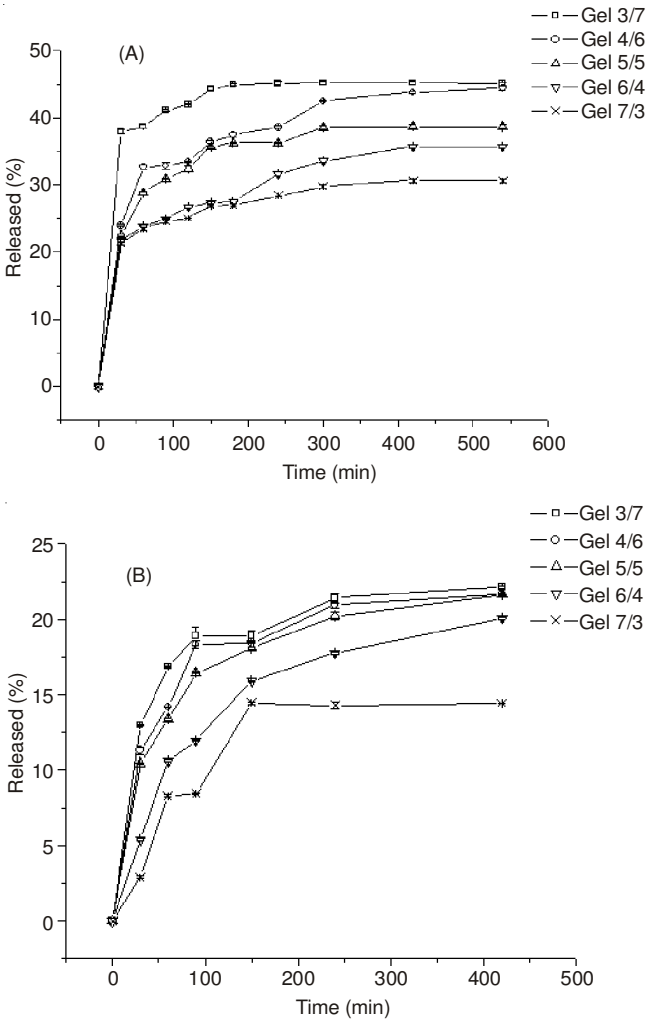

Fig. 6. Cumulative release of lysozyme from the hydrogels at pH 7.4 (A) and pH 4 (B), 37 °C as a function of time

Conclusion

In summary, the PGA-PVA hydrogel membranes have been developed using a physical crosslinking method. The swelling ratios of hydrogels decreased with the poly(γ-glutamic acid) content increase, however the water retention increased. And these hydrogels had pH-responsive swelling-deswelling property. In the presence of enzyme, all hydrogels exhibited biodegradability and the degradation rate could be controlled by changing the composition ratio. The *in vitro* release of lysozyme from the hydrogels at pH 7.4 demonstrated that the hydrogel with a lower crosslinking density had a faster release rate. The drug release rate at pH 4 was slower than that at pH 7.4 due to the protonation of the poly(γ-glutamic acid) part at an acidic condition. The results display the possibility of using this pH sensitive hydrogel as an intelligent drug delivery carrier.

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