Degradation Behavior of Hydrogel Based on Crosslinked Poly(aspartic acid)

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ABSTRACT: The degradation behavior of hydrogel based on poly(aspartic acid) (PASP) which crosslinked by 1, 6-hexamethylene diamine (HD) was studied in α-chymotrypsin solution. The degradation behaviors of PASP under different crosslinking levels were evaluated as a function of biodegradation time by monitoring the changes of crosslinking density, molecular weight, rheological behavior, interior morphology, mass loss, and swelling ratio. It was found that the degradation behaviors relied heavily on the crosslinking density which calculated from the Flory-Rehner equation. A pseudo first-kinetic equation was proposed to model the mass loss behavior and the degradation rate constant was calculated under different crosslinking levels. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 117: 178–185, 2010

Key words: crosslinking density; degradation; hydrogel; poly(aspartic acid)

INTRODUCTION

Hydrogels are water-swollen polymeric materials that maintain the distinct three-dimensional structure.[1,2] Biodegradable hydrogels have the same hydrophilic properties like traditional hydrogels, but they contain bonds within the network structure that can be cleaved either hydrolytically or enzymatically.[3–5] While the degradation is a complex process, which involves profound changes in polymer characteristics, such as solution viscosity, molecular weight and mass loss. Therefore, knowing the method and the hydrolytic kinetics process of the three-dimensional network are extremely important for quantifying the hydrogel degradation and evaluating the performance of a degradable system for given applications.

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Biodegradable hydrogels have been proposed as environmental compatible materials in recent years.[6–8] Some commonly used hydrogels such as crosslinked poly(acrylic acids), partial hydrolysis products of starch-acrylonitrile, and starch-acrylic acid graft copolymers are poor in degradability, their monomers remain in water or soil and thereafter cause potential environmental concerns. Therefore, hydrogels prepared from natural materials have received increasing attentions. The biocompatibility and biodegradation of poly(amino acid)s make them ideal candidates for many applications in detergent, cosmetics, biomedicine, and horticultural fertilizer.[9–11] Poly(aspartic acid) (PASP) belonging to the family of chemical synthetic poly(amino acid)s with free carboxylic groups on chain, is a biodegradable water soluble polymer.[12,13] It is synthesized by thermal polycondensation of aspartic acid (ASP) and ammonium salts of maleic acid to form poly(succinimide) (PSI).[14] Generally, there are two steps within chemical synthesis of PASP hydrogel (crosslinking and hydrolysis) and the properties of hydrogel could be controlled by changing concentration of crosslinking agent, crosslinking reaction time, and dry process. Recently, Tabata et al. reported two PASP degradation bacteria isolated from *Pedobacter sp.* and *Sphingomonas sp.* that could degrade the PASP into different molecular weights (5,000–150,000 Da and below 5,000 Da, respectively), and the branched unit structure and molecular weight affected its biodegradation.[15] The PASP was initially hydrolyzed to aspartic acid oligomer *via* endotype degradation and then the oligomer was hydrolyzed to aspartic acid

monomer *via* exodegradation.[9,15] However, the application of bacteria degradation of crosslinked PASP hydrogel is currently lacking. So the aim of this study was to evaluate the mass loss and molecular weight decrease as a function of degradation time. The changes of swelling ratio and mechanical strength were interpreted in terms of crosslinking density by the Flory–Rehner equation and the surface morphology of PASP hydrogel was also observed.

MATERIALS AND METHOD

Materials

L-aspartic acid, *N*,*N*-dimethylformamide (DMF) and 1, 6-hexamethylene diamine (HD) (Beijing chemical reagent company, China) were used without any further purification. The α-chymotrypsin purchased from Sigma Co. was chosen as enzyme to hydrolyze the amide bond.

Preparation of psi and PASP hydrogel

L-aspartic acid (2 mol) and 85 wt % phosphoric acid (1 mol) were mixed at room temperature and then the mixture was heated to 210°C for 4.5 h in a kneader under the vacuum of 0.09 MPa. The product was washed with water until neutral and then dried at 85°C under the vacuum of 0.09 MPa.

The precursor polymer, 0.97 g of PSI (0.01 mol of succinimide monomer unit) was dissolved in 28 mL DMF with a 250 mL beaker under magnetic stirring, and 8 mL deionized water as a dispersant was added into the beaker. The mixture containing PSI, DMF, and deionized water was stirred for 0.5 h, and then 0.0005 mol 1, 6-hexamethylene diamine used as crosslinking agent was added into the beaker. The crosslinking reaction was carried out for 1 h at 40°C. The imide rings of the crosslinked polymer were hydrolyzed with 27 wt % NaOH at 40°C until pH 9. Then 100 mL ethanol was added and the precipitate was dried by vacuum drying at 40°C for 48 h. Thereby, the crosslinked PASP resin was obtained (Fig. 1).[16]

Gel strength

The gel strength of PASP hydrogel was measured by the falling ball method.[17] In brief, a cylindrical measuring tube closed by rubber plugs at both ends was 2.5 cm in diameter, 350 cm in length. There was an iron spike in one of the rubber plugs, and a permanent magnet on the iron spike. The PASP resin (0.5 g) was placed into a 250 mesh tea bag and it was immersed in excess amount of the deionized water for 24 h. Then the tea bag was pulled out and

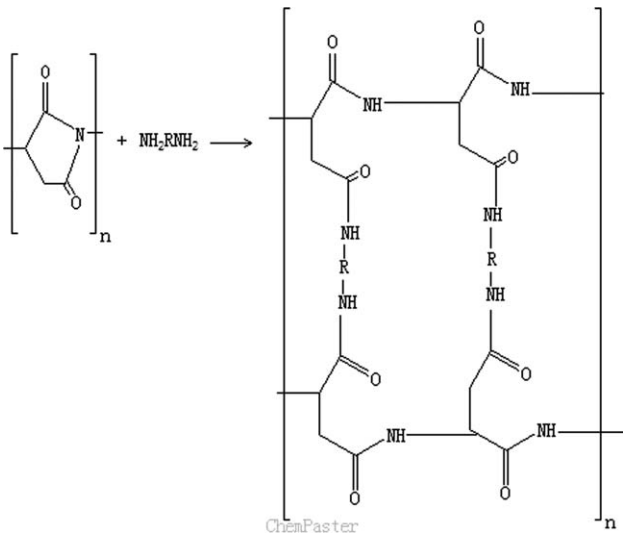

Figure 1 Preparation of PASP.

the sufficiently swollen PASP hydrogel was filled in a vertical cylindrical measuring tube, and then the rubber plug with an iron spike and a permanent magnet was closed. There was a steel ball (diameter 1 cm) at the far end of iron spike and the ball went into free fall when the permanent magnet was taken away. The gel strength of PASP hydrogel was correlated to the time that the ball needed to traverse a definite distance of 25 cm in the measuring tube. The test result was given as gel strength of PASP hydrogel in term of second.

Swelling measurement and determination of crosslinking density

The PASP hydrogel was placed in water at 37°C and weighted after the excess water on the hydrogel surface was removed. The swelling ratio (Q) was defined as the reciprocal of volume fraction of the polymer (v_2) and it was calculated by the following equation:

$$Q = v_2^{-1} = [1/\rho_p[(Q_m/\rho_s) + (1/\rho_p)]^{-1}]^{-1} \quad (1)$$

where ρ_p was the density of polymer (1.20 g/cm^3), ρ_s was the density of water (0.9933 g/cm^3 at 37°C) and Q_m was the swelling ratio, defined as the weight ratio of swollen and dried gel.[5]

According to the Flory–Rehner equation, the crosslinking density (M_c) of hydrogel could be calculated when the hydrogel attained swelling equilibrium state,[7,18,19]

$$M_c = \frac{-[In(1 - v_2) + v_2 + \chi_{12}v_2^2]}{(v_2^{1/3} - 2v_2/f_A)} \quad (2)$$

where χ_{12} was the polymer-solvent interaction parameter, v_2 was the volume fraction of polymer in the swollen gel, f_A was the fraction of ionic units in the polymer ($f_A = 2$ in the current study), and M_c(mol/cm^3) was the molecular weight of polymer chain between two successive crosslinks. The value of χ_{12} could be measured by the Flory–Huggins equation:

$$\frac{\Delta\mu_m}{\mathrm{RT}} = In(1 - v_2) + v_2 + \chi_{12}v_2^2 \quad (3)$$

where $\Delta\mu_m$ was the standard chemical potential.

Enzymatic degradation of PASP hydrogel

The degradation of PASP hydrogel was carried out in pH 7.4 phosphate buffer saline (PBS) solution with or without α-chymotrypsin (0.05 mg/ml). The hydrogel (20 g) was put into a 100 mL flask containing 50 mL PBS buffer at 37°C with reciprocal shaking (100 rpm). The ionic strength of buffer was adjusted to 0.01 mol/L by NaCl and the buffer solution was replaced each time after the weight of the gel was measured. Every 10 h, the sample was taken out and washed by deionized water, its final mass was obtained after complete dry in the vacuum oven at 60°C for 48 h. During the degradation process, the changes of swelling ratio, mass loss, and gel strength were measured. The mass loss ratio was calculated by the following equation:

$$\text{mass loss} = (\mathrm{W}_i - \mathrm{W}_t)/\mathrm{W}_i \quad (4)$$

where W_i was the original weight of resin before immersion and W_t was the dry sample weight after t time incubation.

GPC analysis of degraded fragment

The molecular weight of fragment degraded from PASP hydrogel was measured by gel permeation chromatography (GPC) with a column Superose 12 (Pharmacia Biotech Corp., 1 cm×30 cm) at UV 206 nm (LKB 2238, SIIBROMMA Corp). The fragment was filtered by 0.45 μm polypropylene filter and diluted to 5 mg/ml in phosphate buffer (NaH_2PO_4 0.01 mol/L and Na_2HPO_4 0.03 mol/L, pH 7). The degassed phosphate buffer was used as mobile phase at flow of 0.4 mL/min. The column was calibrated by dextran standard sample which was obtained from Pharmacia Biotech Corp.

Viscosity measurement

The PASP hydrogel viscous was measured on a stress-controlled rheometer (DSR, Piscataway). The steady stress viscosity measurement was tested by using a couette geometry with inside bob, outside cup radii, and bob length of 14.75 mm, 16.00 mm, and 44.25 mm, respectively.

Interior morphology observation

The morphology of degraded PASP hydrogel was observed by scanning electron microscope (SEM) (Hitachi S-4700). Cryofixation technology was applied to observe the swollen hydrogel interstructure. Briefly, the sample was taken from buffer solution and washed by deionized water, and then the water on surface was removed. Then the sample was immediately immersed into the liquid nitrogen to maintain its swollen state. Subsequently, the sample was freeze-dried for 48 h at −45°C. Finally, the dry sample was taken out and the surface was coated with gold for SEM observation.

RESULTS AND DISCUSSION

Degradation behavior

Polymer degradation is a process that causes by kinds of external factors, such as thermal, photo, oxygen, humidity, radiation, and enzyme, and leads to material's chemical and physical properties changes. There are two categories of degradation process: surface erosion and bulk erosion. If the rate that water diffusion into a sample is slower than the hydrolysis process, then water is consumed on the surface before it can diffuse into the bulk of the sample. This category is called surface-erosion. While bulk-erosion occurs when a sample has strong hydrophilic property and the rate that water diffusion into the sample is much faster than the hydrolysis process.[8] It is generally considered that: (1) the reaction between the amino and carboxyl is fast and the amide linkage is labile to hydrolysis and (2) the crosslinked PASP hydrogel has a high swelling ratio. These facts suggest the degradation of PASP in aqueous belongs to the bulk-erosion process. Upon exposure to water, the PASP hydrogel attained equilibrium state that the change in free energy was a minimum or the chemical potential was equal between the inside and outside of the hydrogel. Because of the hydrolysis of amide bonds, the molecular weight between two successive crosslinks increased which led to the increase in swelling ratio.

The degradation behavior of crosslinked PASP hydrogel was studied by sample mass loss as a function of degradation time and the curve was shown in Figure 2. Because of α-chymotrypsin cleaved peptides at the carboxyl side of tyrosine, tryptophan, and phenylanaline, the control experiments were also conducted to evaluate the activity of the

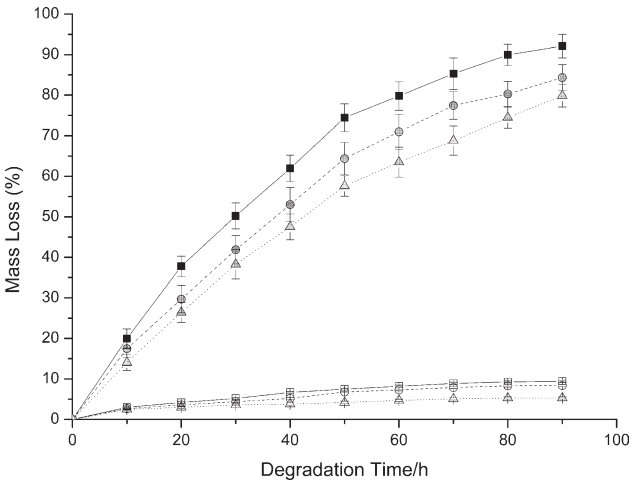

Figure 2 Mass loss of PASP hydrogel at different levels of HD as a function of degradation time at 37°C (α-chymotrypsin = 0.05 g/mL, average ± SD, $n = 3$). ■ 0.0313 mol/L of HD in enzyme solution; □ 0.0313mol/L of HD in PBS solution; ● 0.0352 mol/L of HD in enzyme solution; ○ 0.0352 mol/L of HD in PBS solution; ▲ 0.0391 mol/L of HD in enzyme solution; △ 0.0391 mol/L of HD in PBS solution.

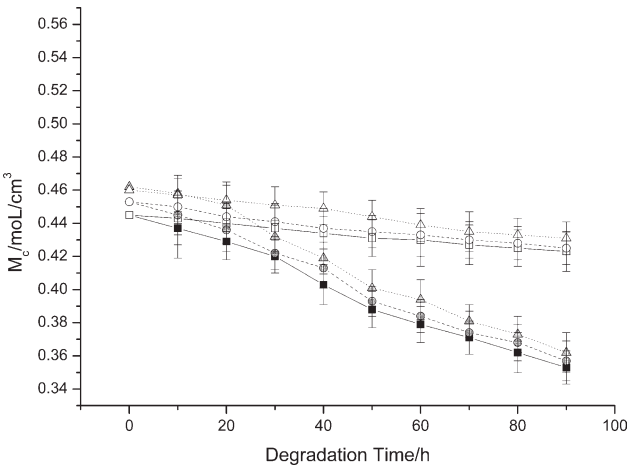

Figure 3 Changes of PASP crosslinking density at different levels of HD as a function of degradation time at 37°C (α-chymotrypsin = 0.05 g/mL, average ± SD, $n = 3$). ■ 0.0313 mol/L of HD in enzyme solution; □ 0.0313 mol/L of HD in PBS solution; ● 0.0352 mol/L of HD in enzyme solution; ○ 0.0352 mol/L of HD in PBS solution; ▲ 0.0391 mol/L of HD in enzyme solution; △ 0.0391 mol/L of HD in PBS solution.

enzyme (experiments conducted in the absence of the enzyme). The mass loss occurred gradually after the sample was placed in α-chymotrypsin buffer solution. The higher concentration of HD in PASP, the slower degradation rate was. The mass loss of PASP hydrogel crosslinked by 0.0313 mol/L and 0.0352 mol/L HD was 92.12 and 84.36% after 90 h, respectively, while the mass loss was 79.86% at HD level of 0.0391 mol/L. It is well known that high concentration of crosslinking agent leads to the high crosslinking density. The high concentration of crosslinking agent could form dense surface of the hydrogel and it is effective to retard the degradation process occurring in networks.[20]

As aforementioned that polymer degrades through chemical, physical, and enzyme mechanisms, while the enzyme degradation via break of crosslinks is the focus of this study. The change of crosslinking density of PASP during degradation process was measured (Fig. 3) and it showed that the crosslinking density decreased continuously with degradation time increasing. Because the property of gel strength was important to many applications, the change of gel strength was also measured (Fig. 4). The gel strength of PASP hydrogel at low level of HD decreased much faster than that the sample crosslinked by high level of HD.

Model of bulk-erosion process

The three-dimensional networks in the PASP hydrogel were crosslinked by bifunctional crosslinking agent HD, which had an amino group on the each end of backbone chains. The degradation of PASP hydrogel occurred by cleavage of amide bonds within the crosslinks. Initially, the buffer solution was clear and transparent, then the solution became turbid gradually as the degradation time increased. The increase in turbidity of system was an indication of PASP fragments dissolution. The result of such degradation process included the building block-PASP copolymer segment releasing from bulk polymer. It was assumed that all macromers fully

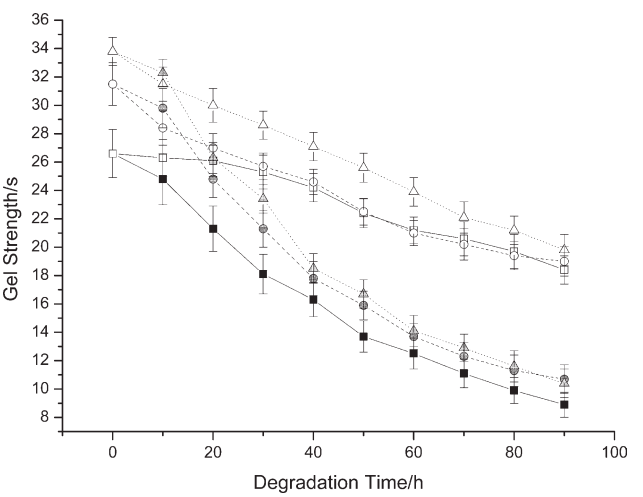

Figure 4 Changes of gel strength at different levels of HD as a function of degradation time at 37°C (α-chymotrypsin = 0.05 g/mL, average ± SD, $n = 3$). ■ 0.0313 mol/L of HD in enzyme solution; □ 0.0313mol/L of HD in PBS solution; ● 0.0352 mol/L of HD in enzyme solution; ○ 0.0352 mol/L of HD in PBS solution; ▲0.0391 mol/L of HD in enzyme solution ▲0.0391 mol/L of HD in PBS solution.

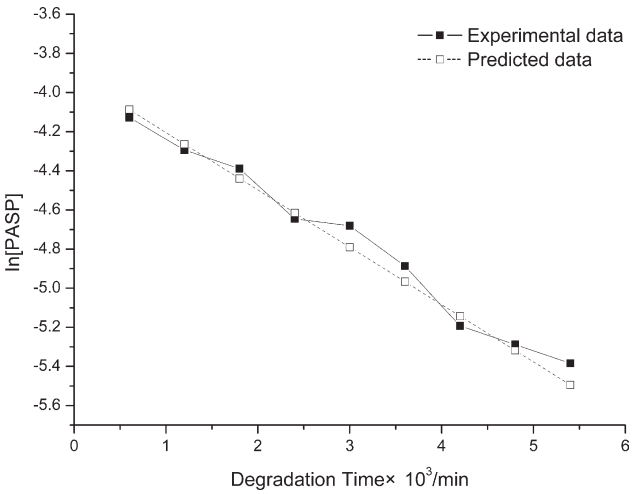

Figure 5 Experimental measurement and model prediction of PASP concentration as a function of degradation time at 37°C (crosslinking agent concentration = 0.0391 mol/L, α-chymotrypsin = 0.05 g/mL, average ± SD, $n = 3$).

reacted during network formation and the degradable unit was hydrolyzed homogeneously. The degradation products diffused out of the well swollen hydrogel was assumed to occur faster than the degradation rate. So once the connecting linkages were broken, the mass of erosion product immediately appeared as mass loss from the bulk polymer and no longer contributed to the network mass. The individual PASP unit within the crosslinked gel was assumed to be hydrolyzed according to the following pseudo first-kinetic equation:

$$\frac{d[\mathrm{PASP}]}{dt} = -k[\mathrm{PASP}] \tag{5}$$

where [*PASP*] was the concentration of degradable PASP unit within the network, and k was the apparent rate constant. The first order of model was based on following assumptions: (1) the constant pH and ionic strength of medium during the degradation process and (2) the constant solvent concentration because of the high swelling ratio of PASP hydrogel. Integrating and solving for [PASP] in eq. (5) led to an exponentially decreasing PASP unit concentration versus time:

$$[\mathrm{PASP}] = [\mathrm{PASP}]_o e^{-kt} \tag{6}$$

where $[PASP]_o$ was the initial concentration of PASP unit within the hydrogel, and t was the degradation time. The effect of degradation time on PASP concentration was shown in Figure 5 and all parameters of the model by different crosslinkage levels were given in Table I. The deviation of predicted data from experimental data was probably due to the diffusion rate changed during the degradation and the detail reasons needed further investigations. It could be seen that the value of apparent rate constant k decreased with concentration of crosslinking agent increasing: the higher concentration of HD led to the denser network of hydrogel and it retarded the entry of enzyme and retarded the hydrolysis process.

TABLE I
Degradation Rate Constant for Crosslinked PASP Hydrogels with Different Concentration of crosslinking Agent from eq. (6) and (7) (α-chymotrypsin Solution = 0.05 mg/mL)

Concentration of HD (moL/L)	0.0313	0.0352	0.0391
k ($\times 10^{-4}$min^{-1})	3.15	3.07	2.93
r^2	0.9746	0.9733	0.9707
k' ($\times 10^{-7}$min^{-1})	3.11	2.97	2.75
r^2	0.9796	0.9695	0.9841

As the degradation occurred, the crosslinkages were cleaved homogeneously in the entire hydrogel. When one of these crosslinkages was hydrolyzed, the surrounding networks were broken. This ongoing cleavage of crosslinkages gradually decreased the crosslinking density of the overall hydrogel and this cleavage of crosslinks in the network led to the physical and mechanical properties changes of gel. The degradable linkages of crosslinked macromers were cleaved, which reducing the average number of crosslinks with time and eventually causing mass loss.

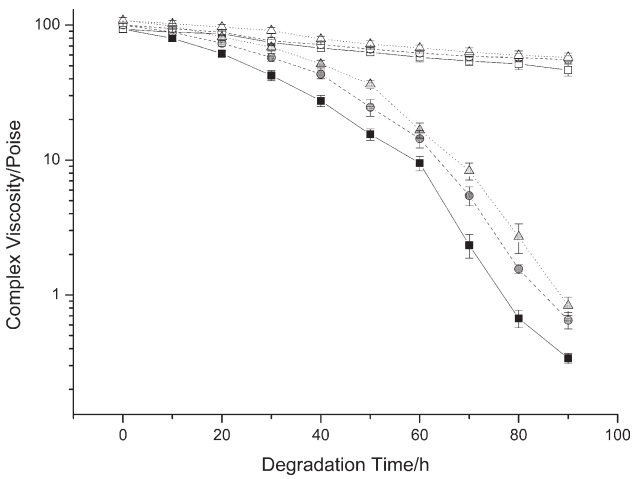

Figure 6 Viscosity (constant angular frequency of 1 rad/s) of crosslinked PASP hydrogel as a function of degradation time at 37°C.The reduction of viscosity showed two distinct phases: initially fast decrease and slow rate subsequently (α-chymotrypsin = 0.05 g/mL, average ± SD, $n = 3$). ■ 0.0313 mol/L of HD in enzyme solution; □ 0.0313mol/L of HD in PBS solution; ● 0.0352 mol/L of HD in enzyme solution; ○ 0.0352 mol/L of HD in PBS solution; ▲ 0.0391 mol/L of HD in enzyme solution; △ 0.0391 mol/L of HD in PBS solution.

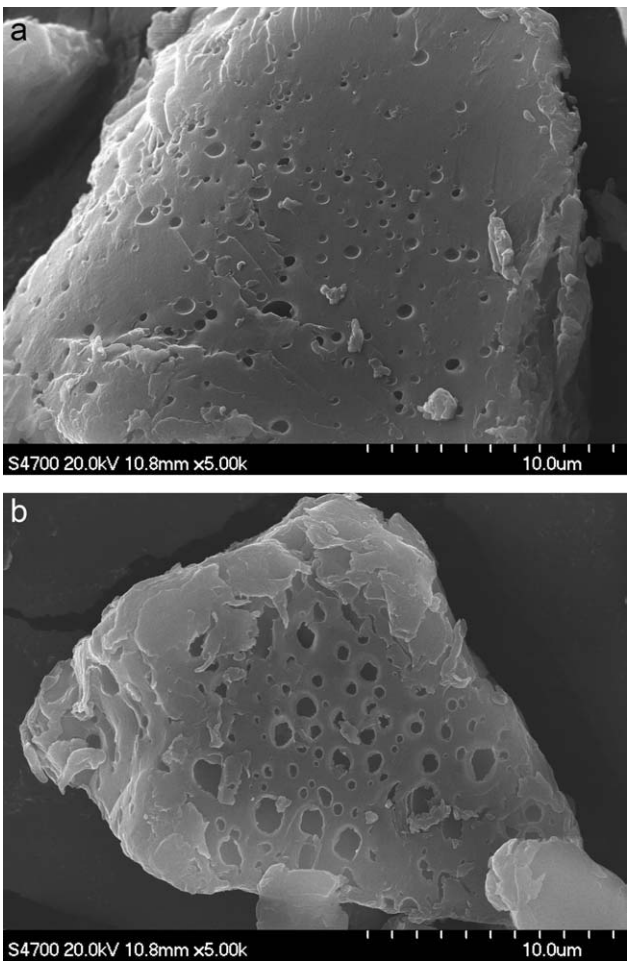

Figure 7 SEM images of PASP hydrogel exposed to α-chymotrypsin solution degradation (crosslinking agent concentration = 0.0391 mol/L, α-chymotrypsin = 0.01 mg/mL): (a)10 h and (b)60 h.

Viscosity changes for degradation

It is well known that the viscosity of polymer solution is dependent on the concentration of polymer, molecular weight, interaction between polymer and solvent. The cleavage of crosslinks inside networks led to the changes in mechanical and physical properties of the hydrogel. The viscosity of PASP hydrogel reduced significantly within the 10–50 h, and the trend became gradually leveled off thereafter. The Figure 6 showed a typical hydrogel viscosity versus degradation time at 37°C. A reduction of viscosity indicated the crosslinkage broke gradually and crosslinked PASP hydrogel was hydrolyzed into water soluble molecule. This apparent transformation from hydrogel to solution took approximately 80 h.

SEM observation

The interior morphological changes of PASP hydrogel were shown in Figure 7. As the degradation proceeded, the network size became large and the edge of pore was found irregular. This observation was in agreement with the results discussed in mass loss section.

Molecular weight changes for PASP hydrogel degradation

The typical molecular weight changes for PASP hydrogel, degraded in PBS solution and α-chymotrypsin solution at 37°C were shown in Figure 8. At initially 20 h, a significant reduction in molecular weight was observed during the process of hydrolysis and then the degradation rate decreased gradually afterward. Because of the PASP hydrogel was a kind of crosslinking structure and it belonged to water-insoluble material, so the GPC detectable degradation products came from the bulk chains of PASP: once the crosslinking points were hydrolyzed, the water-soluble linear PASP chains diffused from inside of hydrogel into buffer solution and therefore detected by GPC. At initial stage, the degradation mainly occurred at the crosslinking points, so the high molecular weight fragments could be detected because the precursor of PASP hydrogel was PSI with molecular weight of 199,950 Da. With degradation processed, the degradation region transferred to the amide bonds within linear PASP chains which connected single aspartic acid, so the long PASP chains were degraded into short fragments, and the low molecular weight products showed up therefore. According to other earlier reports,[21,22] a first-order kinetic equation was used to describe polymer degradation:

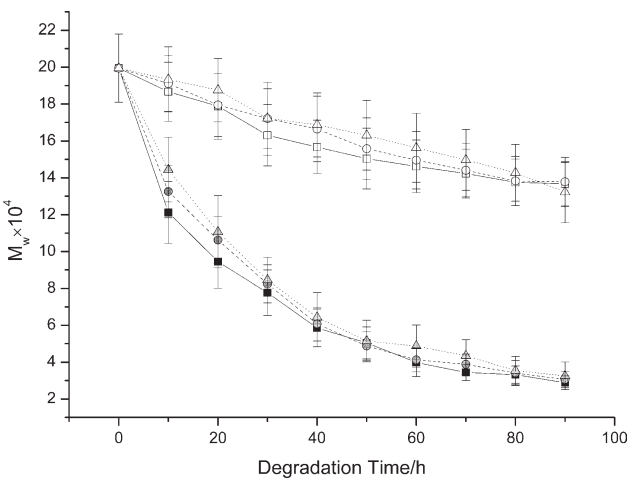

Figure 8 Weight-average monomer molecule weight as a function of degradation time at 37°C (α-chymotrypsin = 0.05 g/mL, average ± SD, $n = 3$). ■ 0.0313 mol/L of HD in enzyme solution; □ 0.0313mol/L of HD in PBS solution; ● 0.0352 mol/L of HD in enzyme solution; ○ 0.0352 mol/L of HD in PBS solution; ▲ 0.0391 mol/L of HD in enzyme solution; △ 0.0391 mol/L of HD in PBS solution.

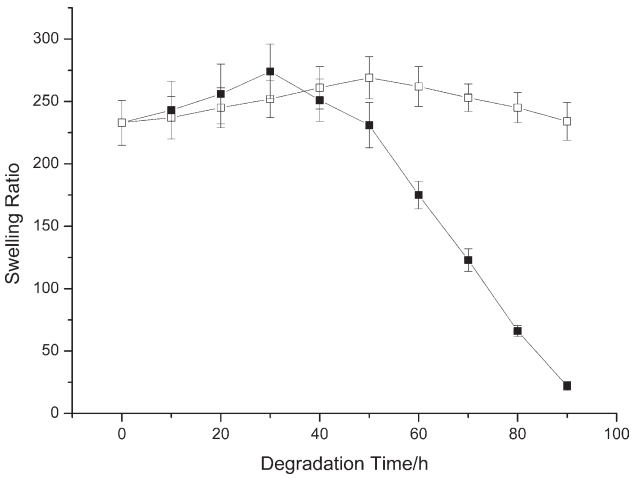

Figure 9 Swelling ratio of PASP hydrogel as a function of degradation time at 37°C (crosslinking agent concentration = 0.0391 mol/L, α-chymotrypsin = 0.05 g/mL, average ± SD, n=3). ■ in enzyme solution, □ in PBS.

$$\frac{1}{M_t} = \frac{1}{M_o} + k't \tag{7}$$

where k', M_t and M_o was the rate constant, molecular weight of polymer at time of t and zero, respectively. So the rate constant k' could be determined by the linear relation between inverse value of molecular weight and degradation time. This linear relation between inverse value of molecular weight and degradation time was considered as an evidence for first-order kinetic in many systems. The relation plotted as a function of degradation time for enzyme hydrolysis was summarized in Table I.

Mechanism of degradation

One of the focuses of this study was to clarify the process of degradation. Here we measured the changes of molecular weight and crosslinking density as possible reasons of the degradation, i.e., the polymer chain degradation rate and swelling ratio during the degradation. According to the monomer molecular weight changes followed by the GPC in Figure 8, the reduction rate of molecular weight was fast initially and then became slowly at subsequent time. In addition, the swelling ratio, viscosity, and crosslinking density showed the corresponding trends. First, the hydrolysis reaction initially occurred at the crosslinkages, which played the role to connect the polymer chains and formed the three-dimensional networks, so the viscosity and crosslinking density changed accordingly. Because of the scission of crosslinkage, the backbone of polymer could not maintain the long-range connectivity, so the degradation of networks significantly increased chain mobility, the gel strength and crosslinking density reduced fast initially. However, the monomer of PASP had a relative high molecular weight (M_w = 199,950 Da), the viscosity of hydrogel was less dominate controlled by the crosslinking density. Therefore, a small decrease of crosslinking density may not lead to a significant loss of viscosity before the mass loss was noted. As for the swelling ratio change, it showed three distinct regimes: an initial small increase, then a small decrease at intermediate time, and finally an accelerated reduction in the following degradation time (Fig. 9). One of the major factors that affected the swelling ratio was the hydrogel network size, too big or small size could not contain enough solvent molecules. Initially, the scission of crosslinkages led to the increase of molecule weight between crosslinking points, thus the network became relative bigger and the swelling ratio increased; however, as the degree of scission increased further, the network size became too large, the rate of water molecules entered into the networks was slower than the rate that came out, so the swelling ratio reduced gradually. Finally, with the disappearance of three-dimensional networks, the polymer became fully water soluble and the swelling ratio reduced at an accelerative rate.

CONCLUSIONS

Enzyme degradation of poly(aspartic acid) hydrogel was studied and the degradation process belonged to bulk-erosion process. The changes of crosslinking density, mass lost were depended on the concentration of crosslinking agent. The model of bulk-erosion process was characterized by a first-order kinetic equation and it fitted to the experimental results well. According to viscosity, molecular weight, and swelling measurements, it was assumed that the degradation initially occurred at crosslinking points, then the amide bonds were hydrolyzed, which caused the three dimensional networks collapsed.

References

1. Soppimath, K. S.; Aminabhavi, T. M.; Dave, A. M.; Kumbar, S. G.; Rudzinski, W. E. Drug Dev Ind Pharm 2002, 28, 957.
2. Rudzinski, W. E.; Dave, A. M.; Viashnav, U. H.; Kumbar, S. G.; Kulkarni, A. R.; Aminabhavi, T. M. Des Monomers Polym 2002, 5, 39.
3. Van Dijk-Wolthuis, W. N. E.; Hoogeboom, J. A. M.; Van Steenbergen, M. J.; Tsang, S. K. Y.; Hennink, W. E. Macromolecules 1997, 30, 4639.
4. Franssen, O.; Van Ooijen, R. D.; De Boer, D.; Maes, R. A. A.; Hennink, W. E. Macromolecules 1999, 32, 2896.
5. Lee, K. Y.; Bouhadir, K. H.; Mooney, D. J. Macromolecules 2000, 33, 97.
6. Guo, K.; Chu, C. C. Biomaterials 2007, 28, 3284.
7. Shah, N. M.; Pool, M. D.; Metters, A. T. Biomacromolecules 2006, 7, 3171.

8. Metters, A. T.; Bowman, C. N.; Anseth, K. S. J Phys Chem B 2000, 104, 7043.
9. Hiraishi, T.; Kajiyama, M.; Tabata, K.; Yamato, I.; Doi, Y. Biomacromolecules 2003, 4, 80.
10. Obst, M.; Steinbuchel, A. Biomacromolecules 2004, 5, 1166.
11. Shinoda, S.; Asou, Y.; Suetsugu, A.; Tanaka, K. Macromol Biosci 2003, 3, 34.
12. Yang, J.; Wang, F.; Tan, T. W. J Appl Polym Sci 2009, 111, 1557.
13. Gyenes, T.; Torma, V.; Gyarmati, B.; Zrínyi, M. Acta Biomater 2008, 4, 733.
14. Tomida, M.; Yabe, M.; Arakawa, Y.; Kunioka, M. Polymer 1997, 33, 2791.
15. Tabata, K.; Abe, H.; Doi, Y. Biomacromolecules 2000, 1, 157.
16. Tan, T. W.; Fang, L.; Cao, H. China Pat. ZL01141663.7, 2003.
17. Fang, L.; Yang, J.; Tan, T. W. J Sol-Gel Sci Techn 2006, 40, 89.
18. Flory, P. J.; Rehner, J. J Chem Phys 1943, 11, 521.
19. Aithal, U. S.; Aminabhavi, T. M.; Cassidy, P. E. J Membr Sci 1990, 50, 225.
20. Nie, H. R.; Liu, M. Z.; Zhan, F. L.; Guo, M. Y. Carbohydr Polym 2004, 58, 185.
21. Tayal, A.; Pai, V. B.; Khan, S. A. Macromolecules 1999, 32, 5567.
22. Tayal, A.; Kelly, R. M.; Khan, S. A. Macromolecules 1999, 32, 294.