Preparation of Biodegradable Crosslinking Agents and Application in PVP Hydrogel

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ABSTRACT: Firstly, biodegradable crosslinking agents (BCA) were synthesized by ring-opening polymerization reaction of lactide, four kinds of which with different molecular weights were got by means of changing the ratio of DL-lactide(LA) and glycerol(GL). Then a series of poly(*N*-vinyl pyrrolidone) (PVP) hydrogels were prepared successfully by radical polymerization of BCA and *N*-vinyl pyrrolidone(NVP). Both the ratio of NVP/BCA and the molecular weight of BCA were used to control the performance of PVP hydrogels, which were measured in terms of the ratio of swelling, contact angle, mechanical properties, and biodegradability in vitro. This study showed that increasing both the ratio of NVP/BCA and the molecular weight of BCA resulted in a low crosslinking density of the hydrogels. The crosslinking density played an important role in determining the properties of biodegradable PVP hydrogels. Both the ratio of NVP/BCA and the molecular weight of BCA contributed to high ratio of swelling. A smaller amount of crosslinking agent caused a lower contact angle, while the molecular weight of BCA had little effect on it. In terms of mechanics of hydrogels on both dry and wet conditions, tensile modulus decreased along with decreasing BCA, while the extension at break increased at the beginning and decreased at the end. In the end, measured by mass loss, biodegradability in vitro of hydrogels had two stages: an initial stage with approximately constant loss of mass (stage 1) followed by a stage with rapid mass loss (stage 2). Both increased content and molecular weight of BCA improved the degradation rate of the hydrogels. J Appl Polym Sci 101: 1515–1521, 2006

Key words: biomaterials; biodegradable crosslinking agent; PVP hydrogel; swelling; mechanical properties

INTRODUCTION

Hydrogel is a polymeric network which can absorb and retain large amounts of water. In a polymeric network, hydrophilic groups or domains are hydrated to create a hydrogel structure in an aqueous environment. Crosslinking agents have to be introduced to avoid dissolution of the hydrophilic polymeric chains or segments into the aqueous phase. A great variety of methods of crosslinking have already been used to prepare hydrogels.[1]

Because of its excellent performance, hydrogel has been applied in many fields, including biosensors, bioreactors, bioseparators, tissue engineering, and drug delivery.[2–7] Among them, hydrogel has shown great promise as a scaffold material for tissue engineering.[8–11] Hydrogel, with a high water content and tissue-like elasticity, is easily formed in situ during implantation and is then encapsulated by cells as it is crosslinked with tissue surfaces. Furthermore, its relative biocompatibility makes it a prime candidate for tissue engineering applications.

Currently, many synthetic biocompatible polymers, such as poly(vinyl alcohol) (PVA),[12,13] poly (DL-lactide) (PLA),[14] and poly(2-hydroxyethyl methacrylate) (pHAMA),[15] as well as natural polymers such as gelatin, alginate, and peptide,[16] have been introduced into hydrogel. As a hydrosoluble polymer, PVP has already been used for the synthesis of hydrogel for many years.

For its well-known biocompatibility, PVP has many biomedical applications such as a blood plasma extender, and a carrier for drug delivery.[17–21] Because of its special molecular structure, PVP has many outstanding properties. First of all, it is soluble in water and also in most organic solvents. Second, the structure of pyrrolidone provides it with similar properties to those of a protein. For example, it can be precipitated by most protein precipitators. Third, it has little immunogenicity and antigenicity, which is the foundation of its wide application in fields related to human health.[22] In addition to these, it has a favorable chelating ability and is used in the drug and food field as a chelating agent. Finally, PVP has positive biocom-

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patibility and hydrophilic properties, which have been proposed as an effective and interesting tissue engineering matrix. However, high water-solubility prevents PVP from being shaped in vivo, so that PVP is used mainly in the form of a crosslinked network. On the other hand, hydrogel, after it is crosslinked, becomes nonbiodegradable, which limits its application in the field of tissue engineering.

Since it is advantageous in many biomedical applications for hydrogel to be biodegradable under physiological conditions, labile bonds are frequently introduced into gels. Two typical approaches are used to obtain degradable hydrogels.[23] First, the backbone of the gelling polymer is designed to be degradable by hydrolysis and/or enzymatic action. For example, many polymers, including aliphatic polyester and collagen, can undergo main chain scission by hydrolysis and enzymatic action respectively. A second attractive approach is to introduce biodegradable crosslinking agents into systems composed of nondegradable polymer.[24–27]

In this study, a series of biodegradable crosslinking agents were synthesized, in which glycerol provided multi-arm initiating sites, and the segments of lactide copolymer offered labile hydrolysable bonds, and vinyl acted as functional groups for crosslinking. Then PVP hydrogels were prepared by radical polymerization of BCA and NVP. Finally, the effect of crosslinking agents on the performance of hydrogels was investigated.

EXPERIMENTAL

Materials

LA was synthesized and recrystallized twice with dried acetic ether and then dried for 24 h in vacuum at 60°C before use. GL was obtained from Guangdong Reagent Inc. (China), purified by reduced pressure distillation and dried by molecule sieve to remove any residual moisture. Triethylamine, acryloyl chloride, NVP, and stannous octoate (ACROS, USA) were all purified by reduced pressure distillation. Azoisobutyronitrile (AIBN), purchased from Tianjin Reagent Inc. (China), was purified by recrystallization with dried ethanol and then dried for 24 h in vacuum at room temperature before used. Other chemicals such as dichloromethane and acetone were purchased from domestic chemical distributors, and were of analytical grade.

Synthesis of biodegradable crosslinking agents

A series of biodegradable triacrylate crosslinking agents were synthesized as described previously by Sawhney et al.[24,28] Briefly, different ratios of LA to GL were put into the ampoules that were treated by silication before used, and then stannous octoate was added. After purging three times with dried nitrogen, the ampoules were sealed in vacuum. The ampoules were heated up to 140°C for 24 h. Then the ampoules were broken and the products were dissolved in dichloromethane, followed by microfiltering. The solution was precipitated out of cooled hexane and the precipitate was dried for 24 h in vacuum at 50°C to get the copolymer of LA and GL, the molecular weight of which was measured by GPC (WATERS). A certain amount of copolymer and triethylamine was dissolved in dichloromethane and stirred, and then acryloyl chloride was added slowly. The reaction continued about 6 h in an ice–water bath and 24 h at the room temperature. After removal of the precipitate of triethylamine hydrochloric acid by microfiltering, the solution was precipitated by cooled diethyl ether and filtered. The precipitate was dried for 24 h in vacuum at room temperature to obtain the biodegradable crosslinking agents.

Preparation of crosslinked PVP hydrogels

Different ratios of biodegradable crosslinking agents to NVP were dissolved in dichloromethane and 0.5% (w/w) AIBN was added, followed by thorough stirring. The solution was cast onto a mold and after the reaction of heating up with infrared lamp the hydrogel films were obtained. The films were extracted with acetone in Suo's distiller for 3 h to remove NVP monomers and PVP homopolymers, then dried for 24 h in vacuum at room temperature for further measurements.

Testing of swelling

The measurement of swelling was carried out in a PBS at a pH 7.4. The hydrogel films, categorized by weight, were cut into round shapes with a diameter of 1 cm and a thickness of 1 mm, and then equilibrated in 15 mL of PBS at 37°C for 24 h. The sample was weighed after removal of excessive surface liquid using an absorbant paper. Swelling ratio Q was calculated as followed:

$$Q\% = (W_S - W_0)/W_0 \times 100$$

where W_S is the weight of swollen films and W_0 is the weight of dry films. For each sample, measuring the swelling involved three trials and the average of three values was reported.

Testing of contact angle

The static contact angle of water was used to monitor the change of wettability of the surface of hydrogel films. Briefly, a 5-μL drop of distilled H_2O was placed

on the surface and static contact angle was measured using a CAM-PLUS goniometer (TANTEC, Germany) at 25°C with 60% relative humidity. For each reported contact angle value, five measurements on different areas of the surface were obtained and the average taken.

Testing of mechanical properties

The tensile test has been widely accepted and used successfully for characterization of hydrogel films. For testing, the hydrogel films were cut into strips with dimensions of 5 cm in length, 1 cm in width and 0.1 cm in thickness. Before mechanical testing, some of the films were hydrated in 0.1*M* phosphate buffered saline (PBS; pH 7.4), while others were tested directly. An Instron machine (AG-5000G, Shimadzu, Japan) with a 1-kN load cell was used for tensile mechanical test. The crosshead speed was set at 1 mm/min and the load was run until the film was broken. Tensile modulus was calculated with the slope of the initial linear portion of the stress–strain curve. The extension at break was determined as followed:

$$\text{Extension at break\%} = (L - L_0)/L_0 \times 100$$

where L is the length of the hydrogel films at break and L_0 is the length of original films. For each test, five times of measurements were obtained on different hydrogel films and the average taken.

Biodegradation in vitro

Two series of PVP hydrogels were used for the degradability research. One series was made from the different ratios of NVP to BCA with one fixed crosslinking agent BCA-6. The other series were made from different kinds of crosslinking agents with a fixed ratio of NVP to BCA of 3. The initial dry PVP hydrogel films were placed in weighing bottles, each containing 20 mL of 0.2*M* PBS. The samples were shaken at 37°C up to 8 weeks. PBS was changed every week. At the end of each week, the samples were taken from PBS and air-dried overnight and further vacuum-dried for 24 h at room temperature till they attain constant weight. The weights of these samples were then recorded respectively. After weighing, the dry hydrogel films were placed into PBS again as before for further mass loss measurement. The following equation was used to determine the mass loss ratio:

$$\text{Mass loss ratio \%} = (W_0 - W)/W_0 \times 100$$

where W and W_0 are the weights of the samples after degradation and the initial weights of the samples, respectively. The effect of the amount and the molecular weight of crosslinking agents on mass loss was determined using the same procedure. In each situation, measurements of samples were performed, and the average of the five values was reported.

TABLE I
Molecular Weights of Biodegradable Crosslinking Agents

Types (BCA-*m*)[a]	LA/GL[b]	M_n (calc)[c]	M_n (GPC)	M_w/M_n (GPC)
BCA-4	12	1769	1375	1.3083
BCA-5	15	2222	1808	1.3618
BCA-6	18	2648	2116	1.3467
BCA-7	21	3074	2494	1.3768

[a] m is the number of repeating units of lactide on each arm of glycerol.
[b] LA/GL stands for the molar ratio of LA to GL.
[c] M_n (calc) is calculated from 144 × LA/GL + MW (glycerol).

RESULTS AND DISCUSSION

Synthesis of biodegradable crosslinking agents

The biodegradable crosslinking agents were synthesized by a ring-opening polymerization reaction of lactide using glycerol as an initiator and stannous octoate as a catalyst, followed by functionalization of the hydroxyl end group with acryloyl chloride in the presence of triethylamine. Generally, in the ring-opening polymerization with stannous octoate as catalyst, the polymerization mechanism was neither a cationic, nor anionic, nor pseudoanionic mechanism. It is theorized that it is a "complexation mechanism" or "coordinate-insertion mechanism."[29] It is at the hydroxy site where species are combined with the catalyst and polymerization initiated. In this research, it is glycerol's three hydroxyl groups, which can be called coinitiators, that offered three polymerizing sites and finally formed a three-arm polymer. Many hydrogels have been synthesized using di-, tri-, and multi-arm copolymers obtained from copolymerization of lactide in the presence of polybasic alcohol.[24,28] They show a wide range of interesting properties depending on the relative chain length of PLA segments. Usually, molecular weight of the copolymer can be adjusted by changing the ratio of lactide to —OH groups. In this research, four ratios of LA to GL were studied, (12, 15, 18, and 21) to get three-arm copolymers with different molecular weights. As shown in Table I, molecular mass increased with the increasing LA/GL ratio. Compared with the theoretic molecular weight, the results from GPC suggested that initiation of polymerization of lactide occurred via —OH group of glycerol. The copolymers could easily be functionalized by acryloyl chloride by esterification. This reaction was an exothermic reaction and must be carried out in ice–

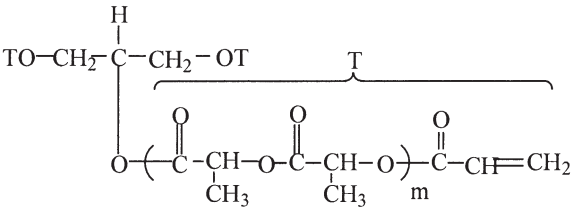

Figure 1. The structure of biodegradable crosslinking agents.

water bath. Acryloyl chloride rapidly reacts with even a small amount of water, so the reaction system must be anhydrous. This was a useful method to introduce terminal double bonds and ester groups in each arm of the triarm BCA macromolecule, the structure of which was shown in Figure 1.

Testing of swelling

In this study, a series of PVP hydrogels were prepared successfully by radical polymerization of BCA and NVP with AIBN as an initiator. The swelling behavior of crosslinked hydrogel depended on the nature of both the polymer and the solvent. Mass swelling of the crosslinked polymer in solvent is the most important parameter normally examined in swelling studies. As we know, there are many factors concerning solvent, which can affect the swelling behavior of hydrogel, including pH,[30] temperature,[31] ionic strength,[32] and others.[33] In this study, the equilibrium swelling behavior of PVP hydrogel was investigated using water as a solvent with the pH and temperature fixed. In addition, the effect of the types of BCA and various mass ratios of BCA to NVP on swelling behavior was the point of interest in this study. Equilibrium swelling curves of PVP hydrogels were shown in Figure 2. It can be seen that the swelling capability of PVP hydrogels increased with the increasing mass ratios of NVP/BCA after a certain period of time. Another phenomenon was that the swelling of PVP hydrogels

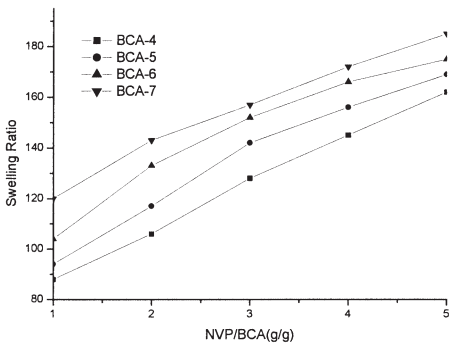

Figure 2. The effect of the ratio of NVP/BCA (g/g) on swelling ratio

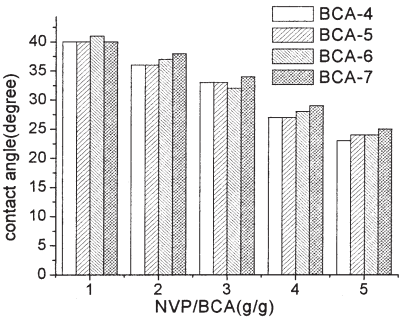

Figure 3. The effect of the ratio of NVP/BCA (g/g) on contact angle.

depended on the type of BCA. The condition of fixed mass ratio of NVP/BCA, increased the molecular weight of BCA and decreased crosslinking density, which resulted in a higher equilibrium swelling ratio.

Testing of contact angle

Contact angle, formed on the three-phase line of solid/liquid/gas system, provides a simple and inexpensive technique in a laboratory situation for describing the wetting behavior of biomaterials. The measuring condition of biomaterials is usually fixed with pure water or PBS as liquid phase and air as gaseous phase. Besides the nature of biomaterials and liquid, there are several factors affecting the value of contact angle, such as contact time, drop size, and surrounding humidity. Figure 3 shows that the values of contact angle of PVP hydrogels decreased with the increasing mass ratios of NVP/BCA. Namely, with the increasing content of NVP, the wettability of hydrogels increased. Another phenomenon observed from Figure 3 was that the molecular weight of BCA had little effect on contact angle of PVP hydrogel, unlike the observation with swelling. Swelling is a bulk property of hydrogel and depends on not only the component of hydrogel but also the structure, in particular, the crosslinking density. However, contact angle was a measurement of the surface properties. It strongly relied on the composition of the surface. Though molecular weight of BCA could affect crosslinking density, it had little effect on the composition once the mass ratio of BCA to NVP was fixed.

Testing of mechanics

The tensile test was adopted to examine mechanical property of hydrogels prepared using BCA-4. The tensile modulus and the extension at break were showed in Figure 4 and Figure 5. In crosslinked materials, such as hygrogels, crosslinking density plays a key role in affecting not only the swelling of materials but also their mechanical performance. Figure 4 shows that the

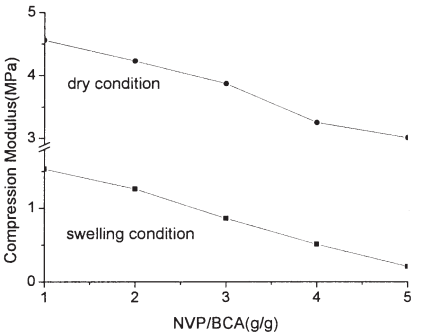

Figure 4. The effect of the ratio of NVP/BCA (g/g) on tensile modulus.

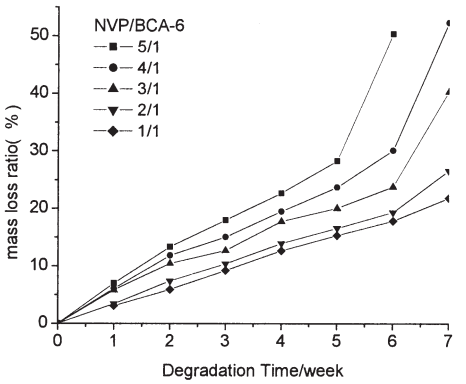

Figure 6. The effect of the content of crosslinking agent on mass loss ratio.

tensile modulus decreases with the increasing ratio of NVP to BCA, namely with the decreasing of the crosslinking density. The curves of the extension at break are plotted in Figure 5. A maximum tensile strength was observed with respect to the ratio of NVP to BCA within the range of ratios that were studied. The crosslinking density had two kinds of effects on the extension at break. That is to say, low crosslinking density meant weak tension of hydrogel. However, when it was too high, the hydrogel proved to be rigid and brittle. The extension at break was lower with low or extremely high crosslinking density.

Biodegradation in vitro

With mass loss as function of degradation time, the biodegradation in vitro of PVP hydrogels was shown in Figure 6 and Figure 7. In both figures, a two-stage mass loss curve was observed: an initial approximate constant mass loss (stage 1) followed by a rapid mass loss (stage 2). After stage 2, the integrity of the hydrogels was so greatly diminished, that the degradation could not be measured any further. Figure 6 is the profile of the mass loss ratio of the hydrogels with different ratios of NVP to BCA-6. Although the initial approximately constant mass losses were observed for all PVP hydrogels irrespective of ratios of NVP to BCA-6, the degradation rates of different hydrogels were different. For example, when the ratio of NVP to BCA-6 was 5, the degradation rate was the highest. When the ratio was 1, the degradation rate was the lowest. Another result observed from Figure 6 was that the beginning time of the stage 2 was different. For instance, when the ratio was 5, the beginning time of the stage 2 was in the fifth week. However, when the ratio was 1, the constant mass loss was kept for 7 weeks. It was easy to explain the phenomena. With the ratio of NVP to BCA-6 decreased, hydrophobic/biodegradable PLA chains in crosslinked hydrogel increased. More importantly, the crosslinking density became bigger. Both factors made the degradation rate lower and the collapse time of hydrogels longer.

The profile of the degradation of hydrogels with different molecular weights of BCA but fixed ratio of NVP to BCA is shown in Figure 7. The tendency of the four curves is the same as that shown in Figure 6. The duration of stage 1 became shorter as the molecular weight of BCA increased, e.g., 7, 6, 6, and 5 weeks for BCA-4, BCA-5, BCA-6, and BCA-7, respectively. At the same time, the degradation rate became higher

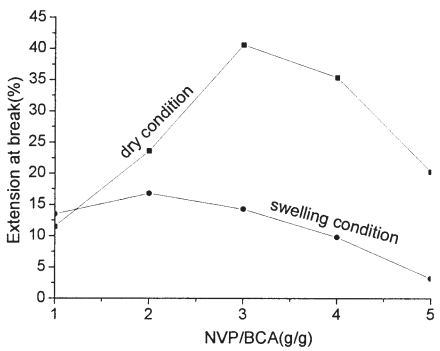

Figure 5. The effect of the ratio of NVP/BCA (g/g) on the extension at break.

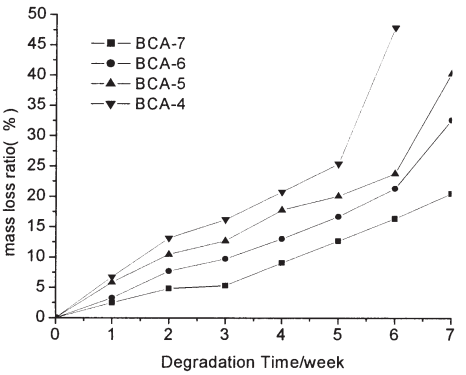

Figure 7. The effect of the molecular weight of the crosslinking agent on mass loss ratio.

along with the increasing molecular weight of BCA. In these four types of hydrogels, the mass ratio of NVP to BCA was the same, and the content of hydrophobic PLA chains was the same as well. The crosslinking density became lower as the molecular weight of BCA increased, which resulted in the same change as that in Figure 7.

Many researchers have proven that crosslinking structure plays an important role in determining the macroscopical properties of hydrogel during degradation.[26] The hydrolysis of crosslinked PVP hydrogel was based on the ester groups, the hydrolysis dynamics of which was similar to that of linear polymer molecules, such as PLA. However, because of the crosslinking structure of the PVP hydrogel, its macroscopical degradation was different to that of linear polymer molecules.[34] Figures 6 and 7 could easily be explained in view of the crosslinking structure of PVP hydrogel. PVP chains were crosslinked into a polymeric network by means of connecting the hydrolyzable and degradable PLA segments on their both sides. Along with the degradation of PLA segment on both sides of PVP chains, these PVP chains would be released at a relatively constant rate by first order kinetic ways. From the aspect of hydrolysis dynamics, the concentration of ester groups and further the degradation rate of hydrogel decreased along with time. And the net structure began to be destroyed along with the degradation process, so the PVP chains could be released even by the breaking of PLA on only one side of each PVP chain, which counteracted the decrease of the degradation rate. Macroscopically, an overall constant mass loss ratio was kept as illustrated by stage 1 of the mass loss curves. Later, further degradation of PLA segments destroyed the crosslinking structure completely and tridimensional net structure was unbound into linear polymer chains that soon dissolved into the surrounding solvent, which led to the rapid collapse of the whole polymeric network as shown in stage 2 of the mass loss curves.

From above results, the degradation rate and degradation time of hydrogels can easily be adjusted by controlling the content and molecular weight of degradable crosslinking agents.

CONCLUSIONS

In this study, the molecular weight of crosslinking agent was controlled successfully by changing the ratio of LA to GL. Further, PVP hydrogels were prepared by radical polymerization of above biodegradable crosslinking agents and NVP using AIBN as initiator. The crosslinking density, which was adapted by both the ratio of NVP to BCA and the molecular weight of the crosslinking agent, played an important role in determining the properties of PVP hydrogel. Along with the increasing ratio of NVP to BCA, the ratio of swelling increased and the value of contact angle decreased. In addition, the molecular weight of BCA had a significant effect on the former but little on the latter. The test of mechanics on dry and wet conditions showed that the tensile modulus of hydrogels decreased along with the increasing ratio of NVP to BCA, while the extension at break increased first and decreased in the end. In conclusion, biodegradability in vitro included two stages: the first with constant mass loss ratio and the second with rapid one. The content and molecular weight of degradable crosslinking agents could adjust the degradation rate and time of hydrogels.

In short, by changing the content and molecular weight of crosslinking agent, various ideal performances of hydrogel can be gained. In view of its biodegradability and swelling ability, biodegradable PVP hydrogel has a great potential utility in the field of tissue engineering and drug delivery carrier. More importantly, the synthesis of biodegradable crosslinking agents provides a new means to obtain the biodegradable hydrogel.

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