Biodegradable Polyesterurethane Networks for Controlled Release of Aspirin

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ABSTRACT: In this article, poly(*D,L*-lactide-*co*-glycolide)urethane (PULG) networks were prepared from hydroxyl telechelic star-shaped oligo(*D,L*-lactide-*co*-glycolide) coupled with 1,6-diisocyanate-2,2,4-trimethylhexane and 1,6-diisocyanate-2,4,4-trimethylhexane or isophorone diisocyanate. The release of model drug aspirin (ASP) from biodegradable polyesterurethane networks was studied in phosphate buffered saline pH = 7.0 at 37°C. PULG networks turned from transparent to opaque after ASP loading. PULG networks with lower crosslinking density always resulted in higher drug loaded content. The results of differential scanning calorimetry and scanning electron microscope measurements demonstrated that ASP was uniformly distributed in the networks. The drug release courses of ASP from PULG networks in phosphate buffered saline pH = 7.0 at 37°C could be divided into three stages. Firstly, ASP release was at approximately uniform rate from PULG networks; Secondly, the release rate obviously increased due to the degradation of the PULG networks; Thirdly, the release rate decreased gradually because most of the ASP had diffused out of the PULG networks. The crosslinking density of polyesterurethane networks also affected both degradation of the polymer networks and drug release rate. The *in vitro* release test revealed that ASP accelerated the degradation process of PULG, which exhibited a typical erosion-controlled release mechanism. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 116: 861–867, 2010

Key words: aspirin; degradation; network; polyesterurethane; release

INTRODUCTION

Poly(lactide-*co*-glycolide) (PLGA) is a biocompatible polymer that degrades to nontoxic products, such as glycolic acid and lactic acid.[1–3] Drug delivery systems (DDS) based on PLGA have been widely investigated in the past decades. Many factors influenced the release and degradation of the PLGA release systems. These factors included polymer composition, molecular weight, interaction of polymer and drugs, properties of drugs, plasticizer, or additives.[1,4,5] It was found that increasing glycolide content in PLGA promoted the release of drugs from the matrix. Acidic drugs in the matrix accelerated the degradation of polymers and then enhanced the rate of the drug release.[1] Basic drugs could accelerate drug release at early stages. Meanwhile, basic drugs reacted with —COOH that generated from PLGA during the degradation process. As mentioned earlier, the two conflicting effects dominated drug release, which resulted in much longer release course.[5,6] Up to present, these studies were focused on high molecular weight PLGA[7] or oligomers[1,3,6,8] and microparticles.[9–11] These release systems always had initial burst in the first few days, which limited their potential applications.

In this article, poly(*D,L*-lactide-*co*-glycolide)urethane (PULG) networks with a wide range of properties were obtained via crosslinking the star PLGA oligomers with different arm lengths. These PULG networks have many advantages, such as biodegradability, biocompatibility, and shape-memory property. They drew increasing attention as promising intelligent materials with potential applications in minimally invasive surgery. For example, they could be used as self-expanding stent to treat vascular diseases. Biodegradable thermo-sensitive surgical sutures degraded to nontoxic products in the body, and another surgery to removing them was

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unnecessary.[12] Drugs were usually loaded in stent to prevent restenosis. Aspirin (ASP, acetylsalicylic acid) was widely used as antithrombotic agent in the treatment of cardiovascular disease. PLGA could control the release of ASP from 9 to 13 days depending on the PLGA composition.[1] Herein, ASP was loaded in PULG networks prepared from PLGA to prolong drug sustained release course. The crosslinking structure of PULG networks might increase the homogeneous degradation without initial burst.

The results showed that ASP loaded PULG networks behaved a smooth release stage in the first 8 days and the resulted release profiles fitted line well, an accelerating release in the following 5 days subsequently, and then a descending release at the end stage. The release exhibited a typical erosion-controlled mechanism for ASP loaded networks. ASP loaded PULG networks system may have potential application as matrix for rapid and large amount of ASP delivery to prevent thrombus or vascular stricture.

MATERIALS AND GENERAL METHODS

Materials

D,L-Lactide (LA) and glycolide (GA) were synthesized by the literature method.[13] 1,6-Diisocyanate-2,2,4-trimethylhexane and 1,6-diisocyanate-2,4,4-trimethylhexane (TMDI), isophorone diisocyanate (IPDI), ASP, stannous octoate (95%, $Sn(oct)_2$), and dibutyl tin oxide (DBTO) were obtained from Sigma Chemical Co. Acetonitrile was of high performance liquid chromatography grade (HPLC) reagent from Meker Co., Germany. All other chemicals used were analytical grade reagents from Tianjin Chemical Reagents Co. and used as received.

Synthesis of four-armed PLGA

The four-armed oligomers PLGA were synthesized and the values of M_n and GA content (GA wt %) of resultant PLGA were calculated according to literature.[12,14] To evaluate the effect of crosslinking density on ASP release behavior, oligomers with different arm lengths were synthesized and controlled by molar ratio of monomers and initiator in feed. Take PLGA10 as an example, typically, 17.000 g of LA, 3.000 g of GA, and 0.272 g of pentaerythritol were added to an exhaustively flamed dry reactor with machine stirring and nitrogen-purged set. After the pentaerythritol was completely dissolved in the system, DBTO or $Sn(oct)_2$ (0.2 wt %) was added, and the polymerization was carried out in bulk at 130°C for 2 days. Then the resultant PLGA was dissolved in dichloromethane, precipitated in *n*-hexane, washed with *n*-hexane three times, dried under vacuum (4 mmHg) at room temperature to constant weight. The chemical composition and molecular weight of obtained oligomers were summarized in Table I.

TABLE I
Chemical Compositions and Molecular Weight of PLGA Oligomers

Sample ID	GA content in feed (GA wt %)[a]	M_n[a]	GA content in oligomers (GA wt %)[b]	M_n[b]
PLGA 3	15.0	3000	15.6	2750
PLGA 5	15.0	5000	15.8	4500
PLGA 10	15.0	10000	15.4	8800

[a] Calculated by GA, LA, and initiator in feed.
[b] Calculated by ^{1}H NMR of the oligomers.

PLGA: yield 90–94%. ^{1}H NMR ($CDCl_3$, δ, ppm): 1.58–1.63 (3H, CH_3), 4.16–4.22 (8H, C-$(CH_2)_4$), 4.26–4.34 (2H, GA-CH_2-OH), 4.35–4.40 (1H, LA-CH-OH), 4.78–4.85 (2H, GA-CH_2) 5.16–5.30 (1H, LA-CH). M_n and GA content were calculated according to ^{1}H NMR results.[12]

Synthesis of poly(lactide-*co*-glycolide)urethane networks (PULG)

The PULG networks were prepared using TMDI or IPDI as couple agent and PLGA as polyester segment. Appropriate PLGA was firstly dissolved in dichloromethane 10 wt %/vol %, and then calculated amount of TMDI or IPDI according to —NCO/—OH = 1.05 (mol/mol) and $Sn(oct)_2$ (0.5 wt % based on PLGA) as catalyst were added. The solution was transferred to teflon molds, which were placed in desiccators with phosphorus pentoxide to avoid moisture. After dichloromethane was evaporated under N_2 stream, the films were transferred to oven at 80°C for 2 days. The polyesterurethane networks were obtained with approximately 0.3 mm thickness.

Preparation of drug loaded films

The PULG networks were processed into disk-shape specimens with the diameter of 10.0 mm. Firstly, PULG networks were placed in anhydrous diethyl ether for 2 h, and then extracted in dichloromethane for 24 h at room temperature to remove unreacted PLGA oligomers. Secondly, they were dried to constant weight to obtain the dry weight (m_i). The dried films were swollen in saturated ASP dichloromethane solution in dark for 24 h. Finally, the films were removed from the solution and dried to constant. The ASP loaded films turned from transparent to opaque. The weight of drug loaded in films (m_d) was determined by HPLC methods and the amount of drug loaded content was calculated according to the following equation.

$$\text{Drug loading content } \% = \frac{m_d}{m_i} \times 100\% \qquad (1)$$

where m_i and m_d were the weight of dried films and the weight of drug loaded in films, respectively.

Characterization of polymer networks

^{1}H NMR spectra were recorded at room temperature with a Bruker spectrometer operating at 300 MHz using $CDCl_3$ as solvent. Chemical shifts (δ) were given in ppm using tetramethylsilane as an internal reference. Differential scanning calorimetry (DSC) measurements were carried out on a Perkin-Elmer system with a heating or cooling rate of 10°C/min range from −100 to 150°C under constant N_2 stream (30 mL/min). About 5 mg of PULG was used for each measurement, and an empty aluminum pan as reference. The glass transition temperature (T_g) and the melting temperature of ASP were determined according to the second heating run. Scanning electron microscopy (SEM) (Philips XL30) was used to characterize dispersed state of ASP in PULG networks.

Swelling degree (Q), water absorption, and weight loss

PULG networks containing ASP were immersed in dichloromethane at room temperature for 24 h and the values for Q were calculated by following equation:

$$Q\,\% = \frac{m_s}{m_i} \times 100\% \quad (2)$$

where m_s was the weight of swelling samples.

Samples were weighted to obtain the wet weight (m_w) after release at appropriate time. Then the samples were dried to constant dry weight (m_t). The measurements were repeated five times to obtain an average value for each sample. Water absorption and weight loss were calculated according to the equations below:

$$\text{Water absorption}\,\% = \frac{m_w - m_t}{m_t} \times 100\% \quad (3)$$

$$\text{Weight loss}\,\% = \frac{m_i - m_t}{m_i} \times 100\% \quad (4)$$

where m_w and m_t were the weight of samples in wet and dry state after t day's release of ASP, respectively.

In vitro release experiments

Drug release experiments were carried out in 5.0 mL phosphate buffered saline (PBS, Na_2HPO_4 0.100 mol/L, and NaH_2PO_4 0.063 mol/L, pH = 7.0) solution in a 25 mL glass vial and placed in a shaker bath (37 ± 0.5°C, 100 rpm). The buffer solution was refreshed every day and filtered with a 0.45 μm cellulose filter to remove indiscerptible particles. The released amounts of drugs were determined by HPLC method as described later. The measurements were also repeated for five times to obtain an average value for each sample.

ASP and its main hydrolysate salicylic acid (SA) as standard samples were determined by HPLC methods simultaneously. Standard stock solution of ASP (1 mg/mL) was prepared by dissolving 50 mg ASP in 50 mL mobile phase. The stock solution was stored in brown glass bottle and kept at 4°C for a maximum of 1 month. The stock solution was diluted with the mobile phase to obtain the appropriate concentrations for calibration curve. Generally, ASP and SA were separated with mobile phase consisting of acetonitrile/water/phosphoric acid (40/60/0.2), using C_{18} (diamond), 5 mm × 250 mm × 4.6 μm column in a column oven set at 35°C and at a flow rate of 1.0 mL/min with UV detection at 228 nm for ASP and SA. The retention times were 5.2 min for ASP and 7.1 min for SA, respectively.[15]

$$\text{Cumulative amount ASP release}\,\% = \frac{M_{\text{ASP}} + M_{\text{SA}}}{M_\infty} \times 100\% \quad (5)$$

where M_{ASP}, M_{SA} were the molar amount of ASP and SA released at time t, respectively, and M_∞ was the drugs loaded molar amount in the PULG films, which was determined by HPLC methods.[1]

ASP release data analysis

The mechanism of ASP release from PULG network films was studied by fitting the data to the following equation.

$$\frac{(M_{\text{ASP}} + M_{\text{SA}})}{M_\infty} = K \times t \quad (6)$$

where $(M_{\text{ASP}} + M_{\text{SA}})/M_\infty$ was the molar fraction of drug released at time t, and K was a constant, respectively.

RESULTS AND DISCUSSION

Synthesis and properties of PLGA and PULG networks

The four-armed oligomers PLGA were synthesized via ring-opening polymerization of LA, GA, and pentaerythritol as initiator with DBTO or $Sn(oct)_2$ as catalyst. The values of M_n and GA content (GA wt %) of PLGA were calculated according to literature.[12,14] It could be seen from Table I that the average-number molecular weight (M_n) and the composition of resultant PLGA were close to values calculated by feeds. The PULG networks were prepared by polyaddition of the four-armed oligomers

TABLE II
ASP Loaded Content and Degree of Swelling *Q* of PULG Networks Used in Release Experiments

Sample ID[a]	Oligomer	Crosslinking agent	Degree of swelling *Q* (%)	Loading content (%)
TPULG3	PLGA 3	TMDI	400 ± 20	3.1
TPULG5	PLGA 5	TMDI	850 ± 60	5.5
TPULG10	PLGA 10	TMDI	1380 ± 70	8.4
IPULG5	PLGA 5	IPDI	940 ± 80	4.0
IPULG10	PLGA 10	IPDI	1190 ± 110	6.3

[a] TPULG and IPULG were denoted as polyesterurethanes prepared from PLGA coupled with TMDI or IPDI, respectively. The numbers after them were corresponding to that of oligomers PLGA.

PLGA with IPDI or TMDI. The polyurethane network formation was monitored by the decline of the isocyanate signal at 2250–2275 cm^{-1} in the IR-spectra of the reaction mixtures. The water uptake and weigh loss of all PULG networks without drugs were lower than 4.5 and 1.2% in the first 20 days, respectively.

Drug loaded content and dispersed state

Table II showed that ASP was efficiently incorporated in PULG networks through swelling method. The ASP loaded content in PULG films varied from 3.1 to 8.4 wt % by variation of network parameters. The crosslinking density of the PULG networks was determined indirectly by swelling degree in dichloromethane solvent. It could be adjusted by varying the arm length of PLGA or crosslinking reagent. PULG prepared from PLGA oligomers with higher molecular weight resulted in lower crosslinking density, higher *Q* values of networks in dichloromethane, and hence higher drug loaded content. IPULG networks always had lower *Q* values and drug loaded content than that of TPULG networks prepared by similar PLGA oligomers.

DSC and SEM measurements could be used to determine whether the drugs incorporated into the networks were in amorphous state or in crystalline dispersed state.[16] From DSC result in the second run in Figure 1, ASP loaded PULG network did not show a melting peak around the melting temperature of ASP (134–136°C). In SEM image, crystalline ASP domain could not be found in PULG networks (Fig. 2). These results confirmed that ASP was uniformly dispersed in PULG networks. The COOH group of ASP could form hydrogen bonding with urethane groups of PULG networks. The hydrogen bonding is favorable for ASP load and its homogeneous dispersion.

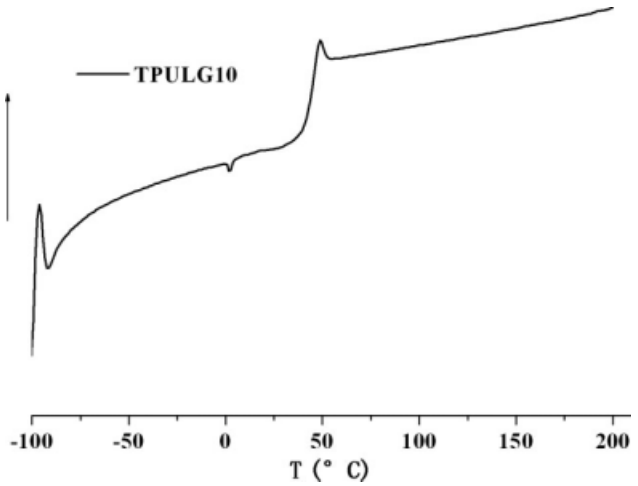

Figure 1 DSC curve of ASP loaded TPULG10 network.

ASP release from PULG networks

Figure 3 showed the release of ASP from different PULG networks during the *in vitro* release over 20 days at 37°C. The burst-effect was not observed at the beginning of release course. It is due to the crosslink structure of the PULG networks, which could decrease the biodegradation of the polyurethanes. The profiles of ASP release from PULG networks showed a typical reverse "S" type curve and the whole release course could be divided into three stages. There was a smooth stage in the first 8 days and then an accelerated release followed in the next 5 days with about 90% ASP having been released. Finally, the release rate descended quickly till approximate completion of the release. ASP release from TPULG networks always had higher release rate than that from IPULG networks prepared by similar PLGA oligomers. IPDI has a ring structure and high steric hindrance, which resulted in lower

Figure 2 SEM micrograph of TPULG10 loading with ASP before release experiment beginning.

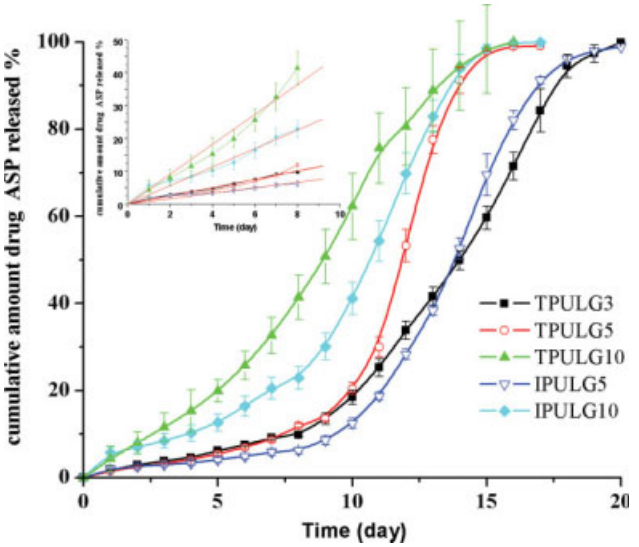

Figure 3 Cumulative amount curves of ASP from different PULG networks during *in vitro* release test ($n = 5$). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

chain mobility, lower drug load amount and release rate.

Water uptake and degradation of PULG networks

Figure 4 demonstrated water uptake of ASP loaded PULG networks during the release *in vitro*. Clearly, ASP loaded PULG networks slowly absorbed water from PBS solution. During the first 10 days, water uptake increased gradually. And then water uptake of ASP loaded PULG networks increased rapidly. Water uptake of TPULG10 increased from 10 to 50% when the release time increased from 1 day to 10 days, whereas it increased dramatically up to 580% at the 15th day. Both ASP loaded TPULG networks and IPULG networks exhibited the similar tendency.

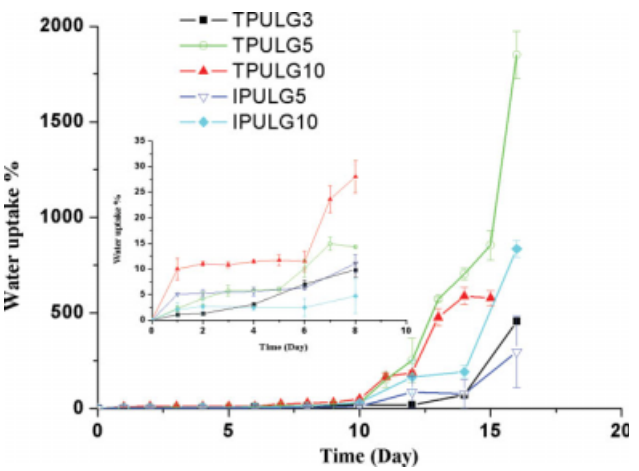

Figure 4 Water uptake of ASP loaded PULG networks during *in vitro* release test ($n = 3$). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

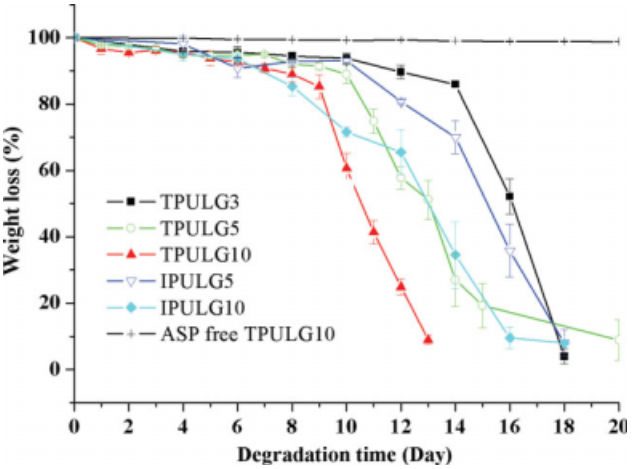

Figure 5 Weight loss of ASP loaded PULG networks during *in vitro* release ($n = 3$). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

In the case of bulk degradation of polymers,[17] water penetration into the polymer networks occurred before polymer degradation. Figure 5 illustrated the degradation course of drug loaded PULG networks *in vitro* release. All ASP loaded PULG networks showed similar tendency. In the first 10 days, the weight of PULG networks decreased slowly for the crosslinking structure of polymer networks. Following the induction period, PULG networks entered into fast weight loss process stage (Fig. 5). The crosslinking density of PULG networks had an effect on both the ASP release and degradation of the networks. The higher crosslinking density resulted in lower degradation rate of networks and also lower release rate of ASP.

PLGA degraded in PBS followed a bulk degradation mechanism, in which acid or base accelerated the degradation process.[18] The model drug of ASP has moderately water solubility (3 mg/mL), acidic nature and its p*K*a is 3.49. Loading ASP in PULG networks caused the material to be higher hydrophilic. Increasing hydrophilicity of matrix accelerated the degradation of polymer networks. The internal micro-environmental pH of drug loaded films decreased due to the dissociation of ASP after water absorption. The labile ester bands —COO— near hydrogen ions began to cleave under acid-catalysis, which accelerated the degradation of PULG.[1] But the release profiles of ASP were quite different from naproxen (p*K*a = 4.15), which released from PLGA networks, probably because the acidity of ASP was stronger than that of naproxen.[5]

In the first stage of ASP release, the diffusion rate of ASP from PULG networks into PBS solution decreased

gradually after the release of ASP in superficial PULG networks region. If the PULG did not erode, the release should be followed the square root of time kinetics as Higuchi's model.[19] While, the release profiles fitted line well at the first 8 days in Figure 3, which indicated the combined effect of degradation and diffusion as the controlling factor for ASP release from PULG networks in this period. Table III summarized data fitting results of ASP release from PULG networks. Clearly, ASP released quickly from low crosslinking density PULG networks, which had high K value.

In the second stage, along with the degradation of PULG networks, more water was absorbed into the PULG networks and the polymer chains became more flexible. The absorbed water in polymer networks acted as an accelerating agent, which enhanced the release of the drug. The drug diffused easily out of the networks with the help of absorbed water. The hydrophilic degradation products diffused quickly from PULG networks into PBS solution in this period, which also speeded up the weight loss of PULG. PULG degraded into oligomers and, finally, glycolic acid and lactic acid.

In the third stage, the drug release rate decreased rapidly because the concentration of ASP in PULG networks was low. PULG networks turned to be ductile films and most of drugs had been released out of the PULG networks. The whole release process was in agreement with erosion-controlled kinetics.

Thermal properties of PULG networks

The thermal properties of PULG networks after immersion in PBS solution were measured by DSC method. T_gs were obtained upon second heating course. TPULG5 samples exhibited only one glass transition, but no melting peaks and the results were shown in Figure 6. The PULG was presumed to be amorphous and did not crystallize during degradation. It could be seen from Figure 6 that T_g of the degraded TPULG5 decreased with the increase of immersion time with exception of 2 day.[20] T_g of TPULG5 decreased from initial 45.8 to 17.3°C within 7 days immersion in PBS solution. The decreasing of polymer chain entanglement and crosslinking degree of the TPULG5 resulted in high mobility of polymer chains of TPULG5 in bulk during release in PBS solution. T_g of the degraded TPULG3 and TPULG10 showed a decreasing tendency similarly to that of the degraded TPULG5. DSC results demonstrated that the degradation mainly took place in bulk.

TABLE III
Kinetic Values Calculated from Data Fitting of ASP Release

Sample ID	K (min^{-1})	R^2
TPULG3	0.01253	0.9963
TPULG5	0.01267	0.9887
TPULG10	0.04554	0.9864
IPULG5	0.00832	0.9822
IPULG10	0.02818	0.9861

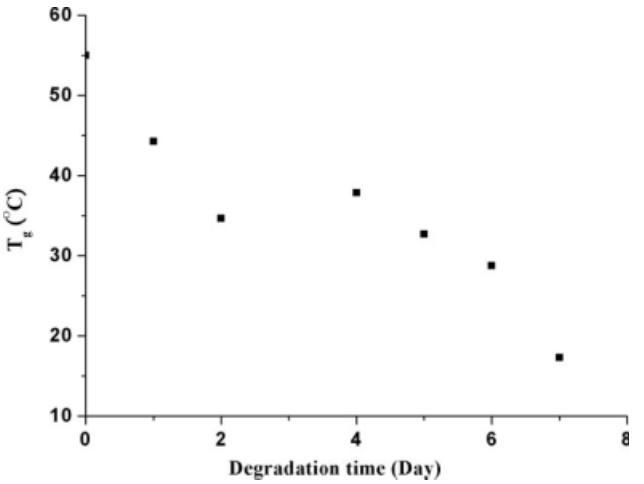

Figure 6 T_g of ASP loaded TPULG5 network after immersion in PBS solution.

The weigh loss of the free ASP loaded TPULG network in PBS was less than 1.2% in first 20 days. The acid-catalyzed degradation process of ASP loaded networks was dramatically faster than that of drug free networks in PBS solution. The course of ASP continued releasing from different molecular weights copolymers PLGA was reported to be about 9–13 days.[1] Whereas, the sustained drug release course from PULG networks was about 15–20 days depending on crosslink density, which is longer than that from PLGA oligomer.

CONCLUSION

ASP could be effectively incorporated into PULG networks by swelling methods. ASP loaded PULG networks release test *in vitro* showed a smooth stage in the first 8 days, an accelerating release in the following 5 days and a descending release at end stage. The crosslinking density of PULG networks affected the release rate of ASP significantly. The higher crosslinking density resulted in the slower release rate of ASP and degradation rate of the networks. The release behavior from PULG networks agreed with erosion-controlled mechanism. As these materials combine shape-memory capability, drug release and degradability in terms of a multi-functionality they have a high application potential for biomedical applications, such as smart implants or medical devices. PULG networks are promising for developing site-specific controlled DDS in the treatment of cardiovascular disease.

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