

# The Synthesis and Characterization of Poly(Ethylene Glycol) Grafted on Pullulan

Yonghua Jiao, Yu Fu, Zhenhua Jiang

College of Chemistry, Jilin University, Changchun, 130023, China

Received 14 October 2002; accepted 20 April 2003

**ABSTRACT:** The pullulans grafted with poly(ethylene glycol) with different degrees of substitution (DS: 0.02–0.2) are synthesized by reaction of pullulan and poly(ethylene glycol) terminated with carboxylic acid. The structure of the resulting modified pullulans is characterized with  $^1\text{H-NMR}$  and FTIR spectra. The DS of the products increase with the amount of the poly(ethylene glycol) added. The appearances

of the products change considerably and the solubility in organic solvent is enhanced visibly. It was found that pullulan derivative is biodegradable. © 2003 Wiley Periodicals, Inc. *J Appl Polym Sci* 91: 1217–1221, 2004

**Key words:** pullulan; poly(ethylene glycol); NMR spectroscopy; FTIR spectroscopy; chemical modification

## INTRODUCTION

Pullulan, a water-soluble extracellular neutral glucan first described in 1959,<sup>1,2</sup> is a biodegradable polysaccharide synthesized by the fungus *Aureobasidium pullulans*. It consists of a linear polymer of maltotriosyl repeating unit linked via  $\alpha$ -1,6-glycosidic bonds, as shown in Figure 1. Pullulan is a promising material in environmental aspects because it shows good biocompatibility and enzymatic degradation behavior, which produces nontoxic substance, and is produced from plant-based starch or sugars. Moreover, pullulan can be cast from aqueous media, so it could be used to replace non-degradable films. Therefore, it has recently received attention for its potential uses in the pharmaceutical, food, and cosmetics fields. However, similar to most polysaccharides, pullulan is difficult to process by conventional melt processing techniques without the use of processing aids and is insoluble in common organic solvents.

Chemical modification (for example, graft copolymerization) of natural polymers is one valuable method used to improve the properties of a polymer and has been extensively used in the graft modification of gelatin, cellulose, and starch. The chemical modification of pullulan has also attracted attention. Donabedian et al. reported the acetylated pullulan prepared by the reaction of pullulan with acetic anhydride and pyridine<sup>3</sup>; moreover, pullulan 6-hydroxyhexanoates and pullulan 6-dilactates were prepared

by the reaction of pullulan with  $\epsilon$ -caprolactone and L-lactide.<sup>4</sup> Sunamoto et al. have reported the synthesis and properties of palmitoyl<sup>5</sup> and cholesteryl<sup>6,7</sup> modified pullulan derivatives and their use as host for protein guests.<sup>8,9</sup> Shibata et al. synthesized the modified pullulan derivatives with urethane group in the side chain by the reaction of pullulan with isocyanate compounds and studied their properties, especially the thermoplasticity of the modified derivatives.<sup>10</sup> Chemical modification of pullulan such as oxidation,<sup>11</sup> carbonation,<sup>12</sup> etherification,<sup>13</sup> and succinylation<sup>14</sup> for uses as prodrug carrier, have been reported.

Poly(ethylene glycol) (PEG) presents outstanding physicochemical and biological properties including hydrophilicity, solubility in water and in organic solvents, lack of toxicity,<sup>15</sup> and absence of antigenicity and immunogenicity,<sup>16</sup> which allowed PEG to be used for many biomedical and biotechnological applications. As far as we know, none has reported on the pullulan grafted with PEG. In the present study, we synthesized modified pullulan derivatives by the reaction of pullulan with PEG terminated with carboxylic acid and investigated their properties.

## EXPERIMENTAL

### Measurements

$^1\text{H-NMR}$  spectra were recorded on a Bruker Avance (500 MHz) spectrometer by using the tetramethylsilane (TMS) proton signal as an internal standard with dimethylsulfoxide (DMSO) or  $\text{D}_2\text{O}$  solution. Fourier transform infrared (FTIR) spectra were recorded on a Bruker IFS 66 V instrument. FTIR spectra were recorded for the products as films cast directly on  $\text{CaF}_2$  plates. The above measurement proceeded at room

Correspondence to: Z. H. Jiang, College of Chemistry, Jilin University, Jiefang Road 119, Changchun, 130023, P. R. China (jiangzh@public.cc.jl.cn).

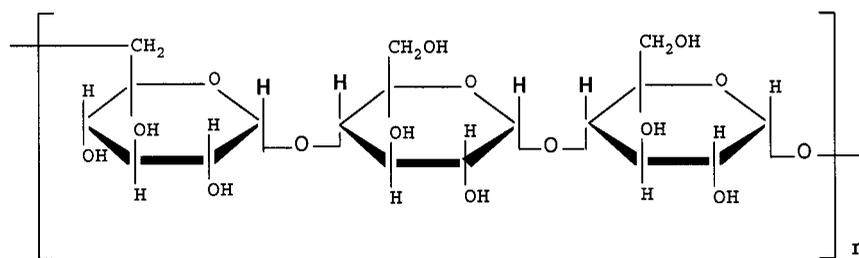


Figure 1 The structure of pullulan.

temperature. Weight-average molecular weight of the polymers were estimated by GPC [column, Shodex KB-805; eluant, water; detector, refractive index (RI); standard, pullulan] at a flow rate of 0.8 mL/min.

### Materials

Pullulan, purchased from Fluka Chemical Co. ( $M_w = 118,400$  g/mol,  $M_n = 58,880$  g/mol), was dried under vacuum at  $60^\circ\text{C}$  for 24 h before use. The analytical grade DMSO was dried over  $\text{CaH}_2$  for 2 days and distilled before use. PEG methyl ether was purchased from Aldrich Chemical Co., Inc. (average  $M_n = 350$  g/mol). Acetone was dried over  $\text{P}_2\text{O}_5$  for 24 h and was freshly distilled before use. 4-Dimethylaminopyridine (from ACROS) was used without further purification.

### Synthesis of PEG terminated with carboxylic acid group

To a solution of 4.3 mL PEG (0.0133 mol) in 50 mL dry acetone was added 3 g succinic anhydride (0.03 mol) and 4.12 mL triethylamine. The resulting solution refluxed for 12 h. After evaporation under reduced pressure, the product was solved into purified water and extracted three times by dichloromethane. The dichloromethane solution was dried by anhydrous  $\text{Na}_2\text{SO}_4$  and decolorized two times by active carbon. By removing the solvent under vacuum, the product, a pale yellow sticky liquid, was obtained.

Yield: 3.6 g (60%).  $^1\text{H-NMR}$  in  $\text{DMSO-d}_6$ : 3.24 ppm (s, 3H); 3.43 ppm (t, 2H,  $J = 5.0$ ); 3.51 ppm (d, 24H,  $J = 5.0$ ); 4.12 ppm (t, 2H,  $J = 5.0$ ); 2.50 ppm (m, 4H); 12.20 (s, 1H).

### Coupling of pullulan with PEG

One gram of pullulan (6.17 mmol) and 0.02 g  $N,N$ -dimethylaminopyridine were dissolved in 10 mL dry DMSO. Separately, 1.386 g PEG terminated with carboxylic acid (3.08 mmol) was dissolved in 10 mL dry DMSO together with 0.634 g  $N,N'$ -dicyclohexylcarbodiimide (3.08 mmol); the mixture was stirred for 30 min at room temperature and then added to the above pullulan solution. The reaction was run for 2 days at

room temperature. Then the polymer was precipitated two times by dropping the reaction solution into excess ethyl alcohol and washed with warm alcohol. Finally, the precipitation was dried under vacuum. By varying the amount of PEG added, a range of pullulan grafts with different amounts of substitution were obtained, and characterized by  $^1\text{H-NMR}$  in  $\text{D}_2\text{O}$  and DMSO.

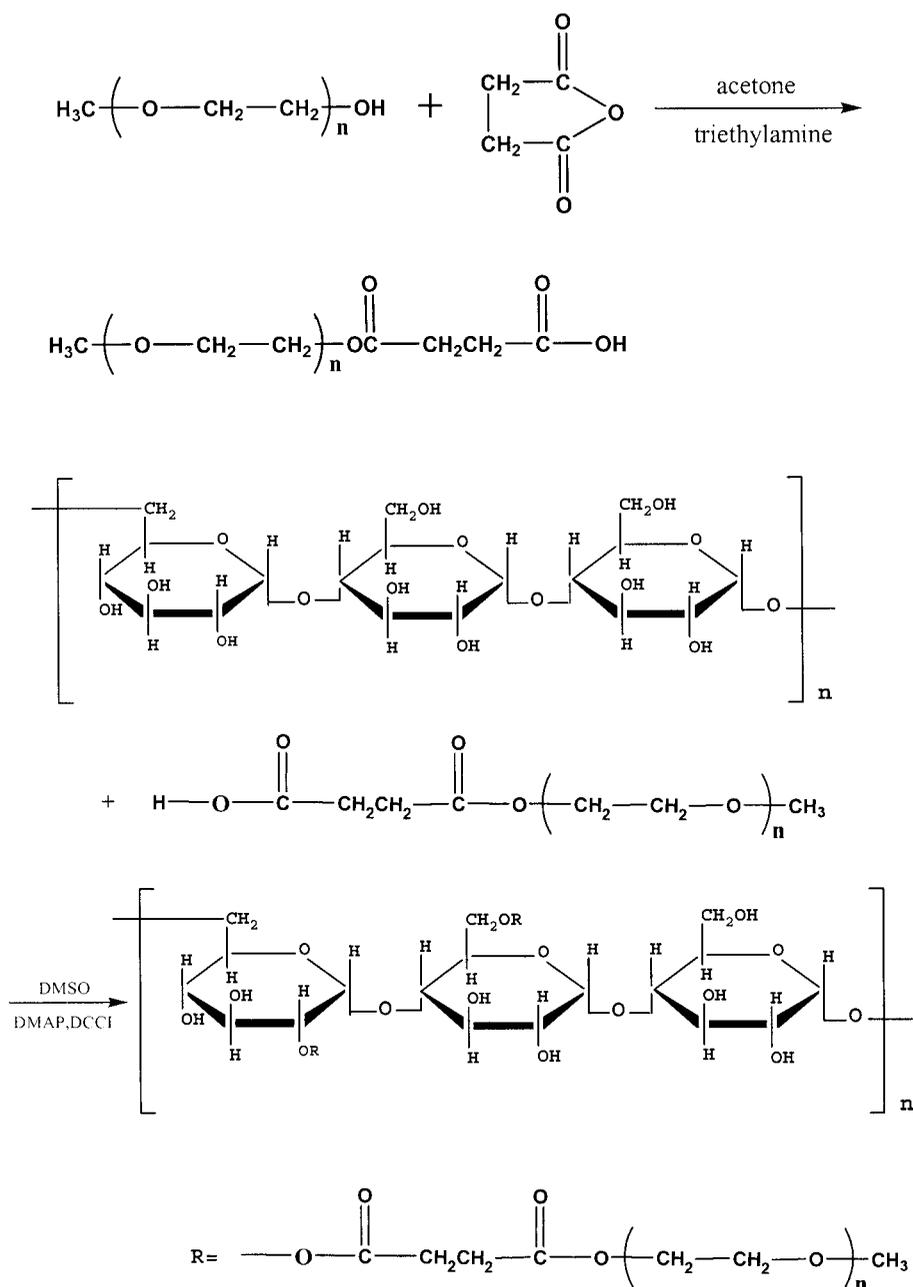
### Hydrolytic degradation of P-PEG-1

Hydrolytic degradation of P-PEG-1 was conducted<sup>17</sup> in a phosphate-buffer solution of  $\text{pH} = 7.0$  at  $37^\circ\text{C}$  for various periods of time. After hydrolysis, the product was precipitated from water and dried at  $60^\circ\text{C}$  *in vacuo*. The degree of degradation was estimated from weight loss (%) of the product.

## RESULTS AND DISCUSSION

A series of pullulan derivatives with different substituted degrees was prepared by esterifying pullulan with carboxylic acid terminated PEG. The synthesis route is shown as Scheme 1. The manner in which PEG is added to pullulan can be described in terms of degree of substitution (DS). The DS designates the average number of hydroxyl groups on the anhydroglucose ring that have been reacted with PEG. Table I shows the feed molar ratio of PEG and glucose ring of pullulan and the DS values of the products. The DS values of the products were determined by high-resolution  $^1\text{H-NMR}$  spectroscopy in  $\text{D}_2\text{O}$ <sup>18,19</sup>; it is obtained by comparing the integration value at 5.2–5.4 ppm (2H) assigned to H-4 with that at 4.2 ppm assigned to the modified PEG methylene (2H) protons. The table shows that DS values of the products increase with the amount of PEG added. When the feed ratio increases from 0.5 to 1, the DS value increases greatly; however, when the ratio increases from 2 to 3, the DS changes much less. It reveals that the DS of the pullulan derivatives approaches saturation with the increase of the amount of PEG added.

$^1\text{H-NMR}$  measurement was employed to verify the structure of the products. The  $^1\text{H-NMR}$  data of the



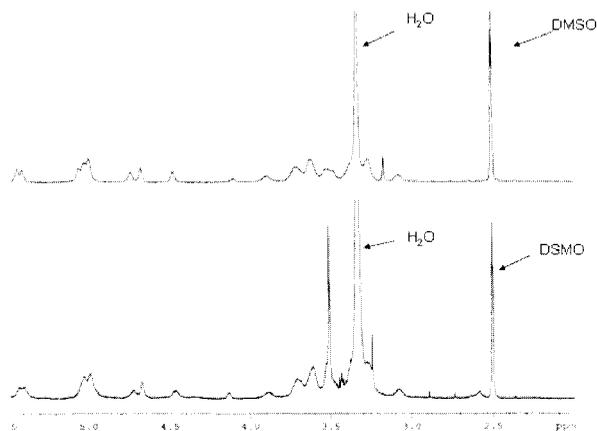
Scheme 1

esterified PEG show a single peak of the active proton of carboxylic acid group at 12.20 ppm; however, the peak disappears in the  $^1\text{H-NMR}$  spectrum of P-PEG-1

in. DMSO- $d_6$  solution<sup>20</sup> (Fig. 2). Meanwhile, in  $^1\text{H-NMR}$  spectrum of P-PEG-1, the two peaks at 4.13 and 2.50 ppm corresponding to the protons of methylene

TABLE I  
Reaction Condition for Pullulan Esterification and Appearance of the Products

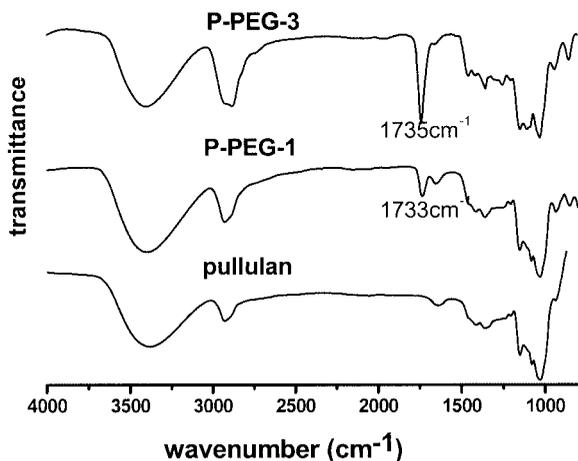
Sample code	Molar ratio PEG/glucose ring of pullulan	DS	Appearance	$M_w$
P-PEG-1	0.5	0.02	White flake	$1.28 \times 10^5$
P-PEG-2	1.0	0.12	White sticky mass	$1.63 \times 10^5$
P-PEG-3	2.0	0.16	Colorless sticky mass	$1.81 \times 10^5$
P-PEG-4	3.0	0.20	Colorless sticky mass	$1.98 \times 10^5$



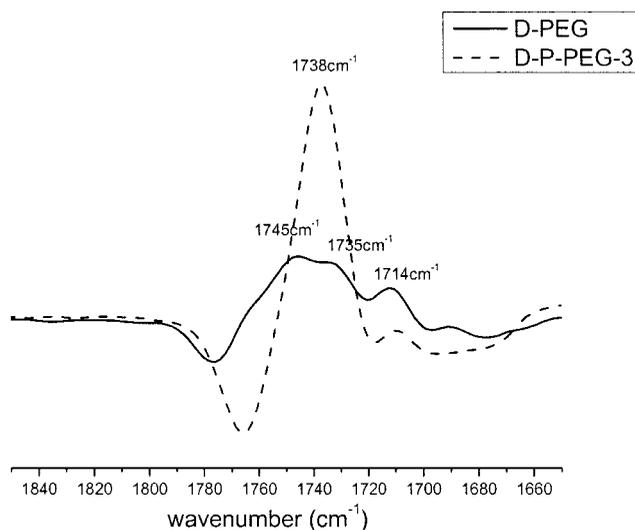
**Figure 2**  $^1\text{H-NMR}$  spectra of pullulan (top) and P-PEG-1 (bottom).

groups of esterified PEG are observed. Additionally, the peaks between 4.46 and 5.56 ppm attributed to protons of hydroxyl in pullulan remained in the  $^1\text{H-NMR}$  spectra of pullulan derivatives and the integration value of those peaks decreases. Taking into account the relative reactivity of different hydroxyl groups in anhydroglucose units, no apparent differences were observed in  $^1\text{H-NMR}$  spectra of pullulan derivatives. All the above results indicate that the esterified PEG has been grafted into the pullulan by the esterification of the  $-\text{COOH}$  group of PEG with the hydroxyl group of pullulan.

Comparison of the FTIR spectra of the native and modified pullulan confirms the esterification. As shown in Figure 3, in FTIR spectrum of P-PEG-1, the absorption peak appearing at  $1733\text{ cm}^{-1}$  is assigned to the stretching vibration of the ester carbonyl group. To compare the peaks of carbonyl groups in the PEG terminated with  $\text{COOH}$  and the products, the second derivative plots of FTIR spectra of P-PEG-3 and PEG



**Figure 3** FTIR spectra of pullulan and its derivatives.



**Figure 4** The second derivative plot of FTIR spectra of PEG-terminated carboxylic acid group and P-PEG-3.

terminated with  $\text{COOH}$  are shown in Figure 4. Three peaks at  $1745$ ,  $1714$ , and  $1735\text{ cm}^{-1}$  are found in that of PEG terminated with  $\text{COOH}$ . The two peaks at  $1745$  and  $1714\text{ cm}^{-1}$  are attributed to carbonyl group of carboxylic acid group in free and dimer states, respectively. The peak at  $1735\text{ cm}^{-1}$  is assigned to ester carbonyl group. However, there is only a single peak at  $1738\text{ cm}^{-1}$  assigned to stretching vibration of ester carbonyl groups in P-PEG-3. At the same time, the most characteristic band for a polysaccharide in the range of  $970\text{--}1200\text{ cm}^{-1}$  with three peaks is observed in the spectra of both pullulan and its derivatives.<sup>21</sup> These confirm the  $^1\text{H-NMR}$  result that PEG has been grafted onto pullulan successfully. Another characteristic band of pullulan is the one at  $3000\text{--}3700\text{ cm}^{-1}$  assigned to hydroxyl bond stretching vibration.<sup>21</sup> It is found that this peak's maximum is shifted toward higher wave numbers. This change is possibly caused by a decrease in the concentration of hydrogen-bonded hydroxyls for they are converted into ester groups during the reaction. With increasing DS, the intensities of the ester carbonyl group at  $1733\text{ cm}^{-1}$  and the methylene group appearing at  $2800\text{--}2950\text{ cm}^{-1}$  compared to that of the hydroxyl group absorption band increase accordingly. However, the intensity of the hydroxyl group compared to that at  $970\text{--}1200\text{ cm}^{-1}$  decreases slightly. The reason may be that the degree of substitution is small; a similar result was also found by Nordmeier et al.<sup>22</sup>

Compared to native pullulan, the modified pullulan shows much difference in physical properties. The appearances of the products are different from the native pullulan. The native pullulan is a white flake; however, the derivatives with low DS have the form of a white flake and those with higher DS become a colorless sticky mass, as shown in Table I. In addition

TABLE II  
Solubility of the Pullulan Derivatives

Sample	Solvent				
	CHCl <sub>3</sub>	THF	CH <sub>3</sub> COCH <sub>3</sub>	CH <sub>3</sub> OH	H <sub>2</sub> O
P-PEG-1	Insoluble	Insoluble	Insoluble	Insoluble	Soluble
P-PEG-2	Insoluble	Insoluble	Insoluble	Slightly soluble	Soluble
P-PEG-3	Insoluble	Slightly soluble	Slightly soluble	Soluble	Soluble
P-PEG-4	Insoluble	Slightly soluble	Slightly soluble	Soluble	Soluble

to the appearances of the polymers, solubility is also changed because of the graft reaction. In contrast to pullulan, which is soluble only in H<sub>2</sub>O and DMSO, some products become soluble in CH<sub>3</sub>OH and some become slightly soluble in acetone and THF, as shown in Table II. The hydrolysis behavior of P-PEG-1 was investigated in KH<sub>2</sub>PO<sub>4</sub>/Na<sub>2</sub>HPO<sub>4</sub> buffer solution (pH = 7.0) at 37°C (Fig. 5). It was found that weight loss (%) of modified pullulan (DS = 0.02) reached 47% after hydrolysis for 12 days. So the pullulan derivative with PEG as side chain is biodegradable.

PEG has good solubility in organic solvent; pullulan is soluble in water, so these polymer products may self-associate into micelles in selected organic solvents. These polymer micelles are promising biodegradable materials for biomedical application because of their unique size, architecture, and biocompatibility with blood, body fluids, and tissue.

### CONCLUSION

We have successfully synthesized the graft product of pullulan by the reaction of pullulan and PEG-terminated carboxylic acid groups and investigated their properties. The analysis of <sup>1</sup>H-NMR and FTIR spectra

supports our above views. The appearances of these modified derivatives have changed considerably. Furthermore, unlike the native material, which is soluble only in DMSO and H<sub>2</sub>O, the solubility of the grafted polysaccharides in organic solvent increases greatly. As a result of improvement of solubility, these polymers will probably be easy to process. The polymer is also biodegradable. Consequently, PEG grafted onto pullulan is a possible promising biodegradable polymer material in food and pharmaceutical application.

### References

- Bender, H.; Lehmann, J.; Wallenfels, K. *Biochem Biophys Acta* 1959, 36, 309.
- Wallinfells, K.; Bender, H.; Keilich, G.; Bechtler, G. *Angew Chem* 1961, 73, 245.
- Donabedian, D.; Gross, R.; McCarthy, S. *Polym Prepr (Am Chem Soc, Mater Sci Eng)* 1992, 67, 301.
- Donabedian, D.; McCarthy, S. *Macromolecules* 1998, 31, 1032.
- Tokada, M.; Yuzurike, T.; Katayama, K.; Iwamoto, K.; Sunamoto, J. *Biochem Biophys Acta* 1984, 802, 237.
- Akiyoshi, K.; Yamaguchi, S.; Sunamoto, J. *Chem Lett* 1991, 1263.
- Akiyoshi, K.; Deguchi, S.; Moriguchi, N.; Yamaguchi, S.; Sunamoto, J. *Macromolecules* 1993, 26, 3062.
- Nishikawa, T.; Akiyoshi, K.; Sunamoto, J. *Macromolecules* 1994, 27, 7654.
- Akiyoshi, K.; Nagai, K.; Nishikawa, T.; Sunamoto, J. *Chem Lett* 1992, 1727.
- Shibata, M.; Asahina, M.; Teramoto, N.; Yosomiya, R. *Polymer* 2001, 42, 59.
- Bruneel, D.; Schacht, E. *Polymer* 1993, 34, 2628.
- Bruneel, D.; Schacht, E. *Polymer* 1993, 34, 2633.
- Mahner, C.; Dieter, M.; Nordmeier, E. *Carbohydr Res* 2001, 331, 203.
- Bruneel, D.; Schacht, E. *Polymer* 1994, 35, 2656.
- Herold, D. A.; Keil, K.; Bruns, D. E. *Biochem Pharmacol* 1989, 38, 73.
- Richter, A. W.; Akerblom, E. *Int Arch Allergy Appl Immunol* 1983, 70, 124.
- Ohya, Y.; Maruhashi, S.; Ouchi, T. *Macromolecules* 1998, 31, 4662.
- McIntyre, D.; Vogel, H. *Starch* 1993, 45, 406.
- Glinel, K.; Sauvage, J.; Oulyadi, H.; Huguet, J. *Carbohydr Res* 2000, 328, 343.
- Bruneel, D.; Schacht, E.; Andre De Bruyn J. *Carbohydr Chem* 1993, 12, 769.
- Aburto, J.; Alric, I.; Thiebaud, S.; Borredon, E.; Bikiaris, D.; Prinos, J.; Panayiotou, C. *J Appl Polym Sci* 1999, 74, 1440.
- Mahner, C.; Lechner, M. D.; Nordmeier, E. *Carbonhydr Res* 2001, 331, 203.

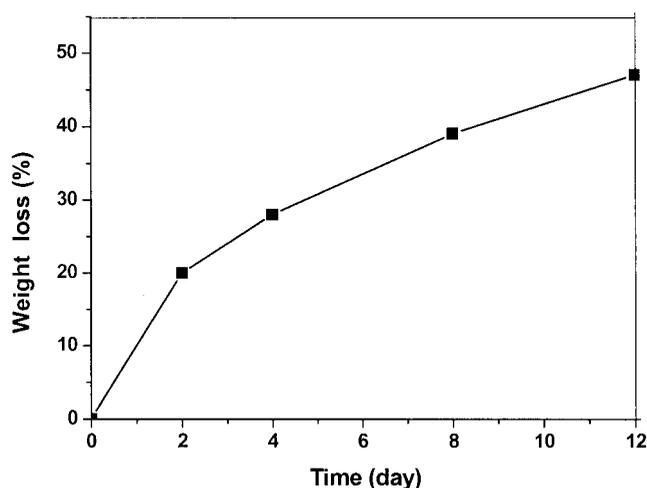


Figure 5 Degradation behavior of P-PEG-1 in KH<sub>2</sub>PO<sub>4</sub>/Na<sub>2</sub>HPO<sub>4</sub> buffer (pH = 7.0) at 37°C.