http://dx.doi.org/10.5012/bkcs.2012.33.12.4122

The Synthesis of Cellulose-graft-poly (L-lactide) by Ring-opening Polymerization and the Study of Its Degradability

Lin Dai, Shu Xiao, Yue Shen, Baichuan Qinshu, and Jing He*

Institute of Materials Science and Technology, Beijing Forestry University, 10083, Beijing, China
**E-mail: hejing2008@sina.com*
Received June 14, 2012, Accepted September 27, 2012

Cellulose-graft-poly (L-lactide) (cellulose-*g*-PLLA) was successfully prepared *via* ring-opening polymerization (ROP) by using 4-dimethylaminopyridine (DMAP) as an organic catalyst in an ionic liquid 1-allyl-3-methylimidazolium chloride (AmimCl). The structure and morphology of the polymer was characterized by nuclear magnetic resonance (NMR) and transmission electron microscope (TEM). From wide-angle X-ray powder diffraction (WAXD) and degradation test (by acid, alkaline, PBS and enzyme solution), changes in the crystalline structure as a result of degradation was also investigated. The results indicated that materials which have low degree of crystallinity showing higher degradability, however, in acid liquor, enzyme solution, alkaline liquor and PBS system, the degradation rate of the polymer decreased by the above sequence. Moreover, with the further increase of graft degree of this material, its degradation degree decreased.

Key Words : Cellulose, Poly(L-lactide) (PLLA), Ring-opening polymerization (ROP), Ionic liquid, Degradability

Introduction

With the rising attention to "environmentally friendly" concepts, cellulose and its derivatives with synthetic degradable polymers have been actively investigated to produce degradable polymers.[1-4] As the most abundant renewable natural biomass, cellulose offered a favourable combination of low cost, biodegradation, the ease of fiber surface modification, good mechanical properties, low density, and recyclability comparing with its inorganic counterparts.[5,6] However, there were also some challenges to the chemical modification of cellulose because of its high molecular weight, high crystallinity, rigidity of backbone chain and insolubility. Green solvents, for example, ionic liquids 1-*n*-butyl-3-methylimidazolium chloride (BmimCl)[7] and 1-allyl-3-methylimidazolium chloride AmimCl[8] had received great attention recently as reaction media of cellulose.

Graft polymerization could be one of the most promising and effective ways to increase the utility of cellulose by incorporating different polymer ingredients at a molecular structural level.[9] And ROP was an effective method of synthesizing aliphatic polyesters. These could lead to the control of general material properties, including degradability.[10-12] PLLA was an authentic biopolymer because it was usually derived from agricultural products and its monomer can be produced by microbial fermentation. PLLA was completely nontoxic and fully compostable, reverting through biological action to their basic constituents – carbon dioxide and water.[13] Owing to the excellent degradability, PLLA and its polymers were especially concerned for their applications as biomedical materials, such as degradable sutures, drug delivery systems, and temporary scaffolds for tissue.[14-17] Several articles were available on the physical and chemical properties of modified cellulose and the relationship with degradation.[18-21]

Miyamoto *et al.*[22] examined tissue biocompatibility of cellulose and its derivatives in two *in vivo* tests. The *in vivo* absorbance in living tissue was found to depend on the degree of crystallinity and the chemical structure of the sample. Mayumi *et al.*[23] and Dong *et al.* synthesized cellulose-*g*-PLLA polymers by ring-opening graft polymerization of L-lactide onto cellulose backbone with tin(II) 2-ethylhexanoate ($Sn(oct)_2$) catalyst in a *N,N*-dimethylacetamide (DMAc)/LiCl and an ionic liquid AmimCl, respectively. Teramoto and Nishio had also summarized their work on cellulose acetate-based graft polymers.[24-26] It was found in experiments that the growth rate of spherulites in crystallized polymers was much lower than that of PLLA in crystallization. So, it was assumed that if the cellulose was grafted with an amount of PLLA, low crystallinity cellulose-*g*-PLLA polymers might be obtained, which may contribute to the performance of degradation.

Thus, this study aimed to synthesize the novel and well-defined graft polymers based on the cellulose backbone with L-lactide (L-LA) in an ionic liquid AmimCl, using DMAP as an organic catalyst. NMR and TEM were employed in analyzing the samples. Moreover, the study of degradability which would be the fundament for the drug release was emphasized. Different degradation rates of polymers were measured by hydrolyzed and enzymatic hydrolysis. This study was expected to provide basic information for researches on amphiphilic and environmentally friendly cellulose-*g*-PLLA polymers.

Experimental

Materials. Microcrystalline cellulose (MCC) with a degree

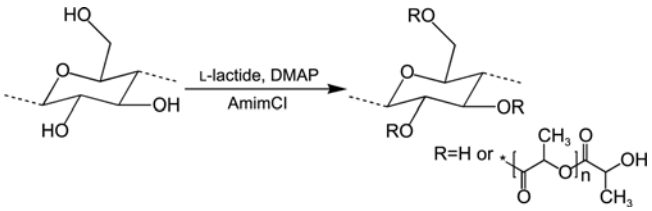
of polymerization (DP) of 225 and *N*-methylimidazole of 99% concentration were provided by the J&K Chemical Reagent Co., Ltd, China. Allyl chloride of 98% concentration was purchased from Acros Organis, USA. AmimCl was synthesized according to the literature.[27] L-LA with a purity of 98% was purchased from A Johnson Matthey Co., Great Britain. DMAP with a purity of 99.5% was provided by Haili Chemical Industry Co., Ltd. The lipase L3126 from porcine pancreas used in hydrolysis test from porcine pancreas was provided by Beijing Solarbio Science & Technology Co., Ltd.

Preparation of Cellulose-*g*-PLLA. Based on the previous experiments,[28-30] a typical polymerization procedure was employed as follows. 4% (w/w) microcrystalline cellulose/AmimCl solution was firstly prepared by mechanical stirring at 80 °C under nitrogen atmosphere for 1 h in a previously dried Schlenk tube. Then L-LA, DMAP were added into the tube. After dissolution, the tube was degassed for three times in vacuum/N_2 of 1 h cycle. Then the reaction was kept at 80 °C under nitrogen atmosphere with vigorous stirring for 12 h. After cooling to room temperature, the resultant polymer was precipitated with deionized water, and then the polymer was dissolved in toluene to obtain a purified graft polymer. The purified polymer was dried in a vacuum oven at 60 °C until reaching a constant weight.

Hydrolyzed by Acid, Alkali and PBS. Specimens were put in vials filled with pH 7.40 phosphate-buffered saline solution (PBS), pH 2.0 acid liquor and pH 14.0 alkaline liquor respectively, seal saved and placed in a thermostat for various periods at 37 °C. Cleaning and weighing after a period of time.

Evaluation of Degradability.

Enzymatic Hydrolysis. Specimens (250 mg) and acetate buffer (25 mL; PBS, pH 7.40) were placed in a conical flask with 25 mg lipase. After degradation, the mixture was allowed to proceed in an incubator at 37 °C for a specified time, and then enzyme solution was diluted into 200 times and was detected by spectrophotometer at 210 nm. The concentration of lactic acid in solution was obtained based on the standard curve: c (g/L) = A/0.6298, where A was the UV absorbance at 210 nm. The weight loss of the polymer was calculated from the formula: weight loss% = (c × 5000)/ 250 × 100, where c was the concentration of lactic acid in solution.



Scheme 1. Synthesis of cellulose-*g*-PLLA in AmimCl.

Measurements. ^{1}H NMR spectra of cellulose-*g*-PLLA was recorded on a Bruker AV400-MHz NMR spectrometer. DMSO-d_6 was used as the solvent with a drop of trifluoroacetic acid-d to shift active hydrogen to lower field area, and tetramethysilane (TMS) as an internal standard.

WAXD was performed by XRD-6000 X-ray diffractometer (Shimadzu, Japan) using Ni-filtered Cu Kα radiation (40 kV, 30 mA) with 4°/min scanning rate at room temperature. Diffraction intensity was measured in a range of 2θ = 5-40°.

A drop of sample dissolved in DMSO 0.01% (w/v) was placed on a copper grid with formvar film and dried before measurement by JEM-100CXa TEM at an acceleration voltage of 100 kV.

Ultraviolet analysis was carried out on UV2000 spectrophotometer (UNICO, China).

Results and Discussion

Polymerization of Ring-opening. The homogeneous ROP reaction of LA with DMAP catalyst was shown in Scheme 1. Hedrick *et al.* reported that DMAP could be used as a kind of highly efficient catalyst for the controlled synthesis of PLLA.[31] Table 1 showed the research results obtained by using various feed ratios of L-LA/MCC. Based on previous experiments,[27-30] the polymerization temperature was set at 80 °C. It can be seen that the grafting content of PLLA in polymers increased with the increase of weight ratio of LA and DMAP to cellulose in feed. One possible reason was that L-LA reacted with DMAP to form intermediate. This intermediate can attack alcohol hydroxyl groups of cellulose easier than L-LA. Although there were many PLLA branches on the cellulose chain, with the amount of lactic acid increasing, the gain of each PLLA branch was limited. As

Table 1. Results and Reaction Conditions of the Graft Polymerization of PLLA on Cellulose in AmimCl

No.	MCC (g)	AmimCl/MCC (wt/wt)	L-LA/MCC (mol/mol)	Temp (°C)	-OH/DMAP (mol %)	Reaction time (h)	DP_{PLLA}	MS_{PLLA}
1	0.6	4%	6/1	80	0.5	11	2.55	3.95
2	0.6	4%	8/1	80	0.5	11	2.99	4.08
3	0.6	4%	10/1	80	0.5	11	3.27	4.13
4	0.6	4%	6/1	80	1.0	11	2.84	4.00
5	0.6	4%	8/1	80	1.0	11	3.28	4.15
6	0.6	4%	10/1	80	1.0	11	3.62	4.35
7	0.6	4%	6/1	80	1.5	11	3.15	4.10
8	0.6	4%	8/1	80	1.5	11	3.61	4.37
9	0.6	4%	10/1	80	1.5	11	3.98	4.45

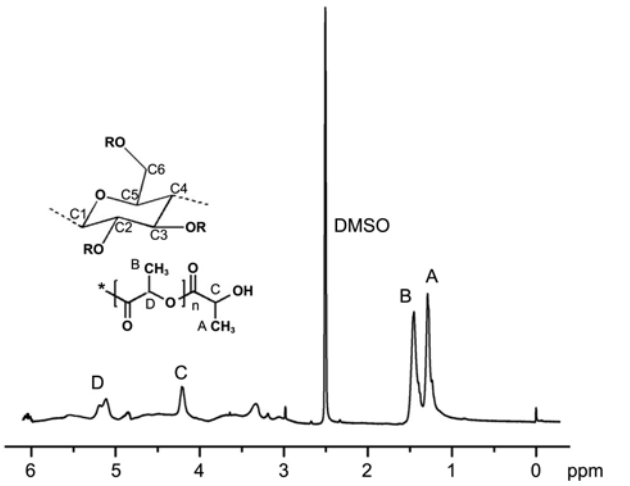


Figure 1. ^{1}H NMR spectrum of cellulose-*g*-PLLA (DP_{PLLA} = 3.15) in DMSO-d_6.

can be seen from Table 1, the highest DP value of cellulose-*g*-PLLA polymer was 3.98. These values were much higher than those reported in the DMAc/LiCl system[32] and those in AmimCl with DMAP as a catalyst.[33]

^{1}H NMR Analysis of the Cellulose-*g*-PLLA. ^{1}H NMR spectrum of cellulose-*g*-PLLA (DP_{PLLA} = 3.15) was shown in Figure 1. In the spectrum, an area from the terminal methyl protons of lactyls was labelled A, an area from terminal methyl protons of lactyls in PLLA side-chains was labeled B, an area from terminal methine protons of lactyls in PLLA side-chains was labelled C, a resonance peak area derived from internal methine protons of lactyls in PLLA side-chains was designated as D. The degree of lactyl substitution (DS) was defined as an average number of hydroxyls substituted for lactyls per anhydroglucose residue of cellulose and the molar substitution (MS) was defined as the average number of introduced lactyl units per anhydroglucose residue of cellulose. The average degree of polymerization of the PLLA-side chain (DP_{PLLA}) which was equal to the molar amounts of combined LA per glucopyranoside unit of cellulose-*g*-PLLA was estimated directly by ^{1}H NMR analysis according to the following equations:

$$MS_{PLLA} = \frac{\text{lactyl units}}{\text{anhydroglucose units}} = \frac{IA_{(a+b)}/3}{[IA_c - IA_{(a+b)}/3]/7} \quad (1)$$

$$DS_{PLLA} = \frac{\text{terminal lactyl units}}{\text{anhydroglucose units}} = \frac{IA_b/3}{[IA_c - IA_{(a+b)}/3]/7} \quad (2)$$

$$DP_{PLLA} = \frac{MS}{DS} = \frac{IA_{(a+b)}/3}{IA_b/3} = \frac{IA_a}{IA_b} + 1 \quad (3)$$

in which, molar substitutionthe IA_a and IA_b were a resonance peak area derived from internal methine protons of lactyls in PLLA side-chains and an area from terminal methine protons of lactyls in PLLA side-chains, respectively; and the IAc was the area of all protons of anhydroglucose unit.

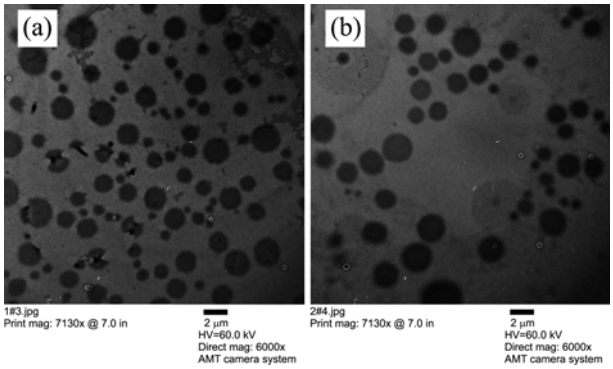


Figure 2. TEM micropicture of cellulose-*g*-PLLA for Sample-7 (DP_{PLLA} = 3.15) (a) and Sample-8 (DP_{PLLA} = 3.61) (b).

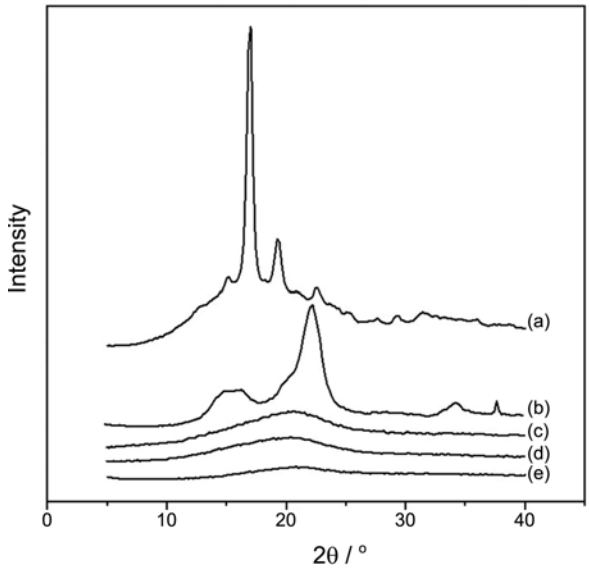


Figure 3. WAXD spectra of PLLA (a), cellulose (b), and cellulose-*g*-PLLA (DP_{PLLA} = 3.98) (c), (DP_{PLLA} = 3.61) (d), (DP_{PLLA} = 3.15) (e).

Morphology Characterization of Cellulose-*g*-PLLA. Micelles morphology was investigated by TEM. It can be seen from Figure 2 that the cellulose-*g*-PLLA polymer mainly formed spherical micelles in DMSO and their diameters did not increase obviously with the increase of the DP.

Crystalline Structure Analysis of Cellulose-*g*-PLLA. The crystalline structure of PLLA, cellulose, and cellulose-g-PLLA were examined by WAXD measurement (Figure 3). PLLA showed the strongest diffraction peak at 2θ = 17°, whereas cellulose showed the strongest diffraction peak at 2θ = 22.4°. However, neither the crystallization peak of PLLA nor that of cellulose was observed on cellulose-*g*-PLLA polymers, only a dispersive broad peak around 2θ = 20.6° (20.4°, 20.8°) was obtained, which indicating that the cellulose becoming amorphous when being grafted by PLLA. In a study made by Teramoto *et al.*,[34] it showed that CDA-*g*-PLLA polymers had a crystalline diffraction pattern, which could be caused by the relatively long PLLA side-chains.

Hydrolyzed by Acid and Alkali. Acid solution was not compared because samples were degraded rapidly in acid

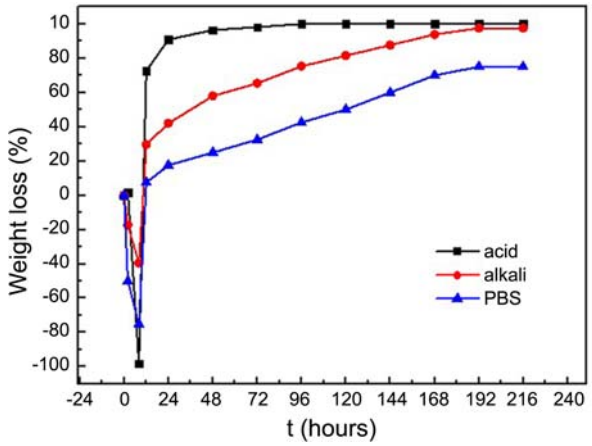


Figure 4. Influence of degradable fluid on the degradability of Sample-7 (DP_{PLLA} = 3.15).

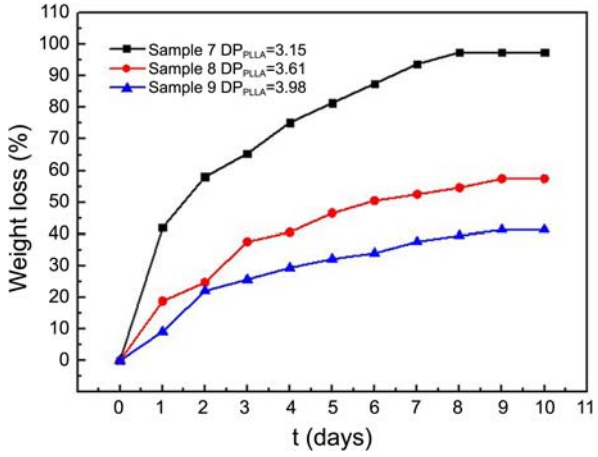


Figure 5. Influence of L-LA: cellulose ratio on the degradabilities of cellulose-*g*-PLLA in alkali liquor.

(Figure 4). As shown in Figure 4, Sample-7 (DP_{PLLA} = 3.15) was degraded in different fluids (in acid liquor, alkaline liquor and PBS system), and the degradation rate of the polymer decreased in sequence. Obviously, cellulose-*g*-PLLA swelled firstly and then dissolved and hydrolyzed into solutions. The weight loss of polymers appeared as negative numbers because of the swelling phenomenon.

The weight loss of the samples in alkali liquor with different DP_{PLLA} were shown in Figure 5. The weight loss decreased with the increase of the DP of the polymers which might due to polyester-like macromolecule rapidly decaying. But different crystalline structures had different degradation rates. Solid PLLA and microcrystalline cellulose were both partially crystalline polymers, whose crystalline region was packed very compactly. However, after the graft polymerization, cellulose-*g*-PLLA exhibited lower crystallinity owing to the PLLA branches. Lower crystallinity, which could easily stimulate the biological and chemical reagents, was conducive to the degradation of materials. So, cellulose-*g*-PLLA had good degradability. These results were in accordance well with the WAXD results.

Both PLLA and cellulose could be degraded in alkali liquor. And PLLA was able to hydrolyze through de-esterification which was easier to degrade in alkali liquor than in acid conditions. But cellulose was more stable in alkali than in acid due to the internal glucosidic bond. Degradation reaction of cellulose in alkaline could create new reducing end base and decrease the degree of polymerization and fiber strength.

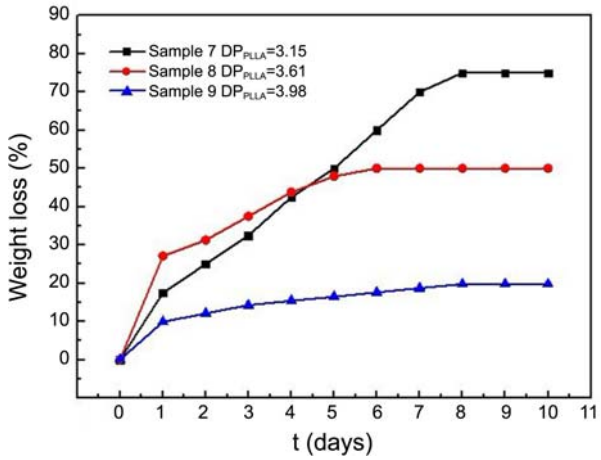


Figure 6. Influence of L-LA: cellulose ratio on the degradabilities of cellulose-*g*-PLLA in PBS.

Biodegradation Studies.

PBS Hydrolysis. Hydrolysis had great effects on biodegradation of polymers in enzyme solution, therefore the hydrolysis of polymers should be researched firstly. Figure 6 showed that the weight loss decreased with the increase of the DP of the polymers. These results were in accordance well with those of hydrolyzed in alkali. Hydrolysis of PLLA was the main reaction of cellulose-*g*-PLLA in PBS. The good water affinity of cellulose allowed water penetrating into the polymer molecules and breaking ester bond inside, leaving low molecular mass polymers. Moreover, the hydrolysis process can be promoted by the increase of the concentration of terminal carboxyl group, with which PLLA was finally resolved into carboxylic acid and alcohol.

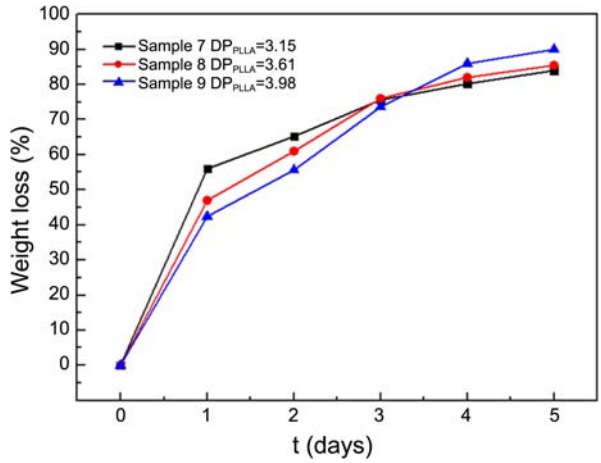


Figure 7. Influence of L-LA: cellulose ratio on the degradability of cellulose-g-PLLA in enzyme solution.

The PBS solution entered into the amorphous regions and the crystalline regions in sequence. The hydrolytic proceeded more preferentially in amorphous regions than in crystalline regions. Biodegradation process of PLLA was also indirect.[35] Firstly, PLLA was hydrolyzed by cutting the unstable bonds and hydrolysising into oligomers. Secondly, it could be further degraded by enzymes into lactic acid. So the performance of polymer biodegradation was greatly affected by its hydrolysis ability.

Enzymatic Hydrolysis. Cellulose-*g*-PLLA was hydrolyzed by lipase to produce lactic acid, which can produce strong absorption in 210 nm wavelength. UV absorption represented the dissolved amount of aqueous lactic acid in a solution; that was, PLLA materials in enzyme solution were degraded to lactic acid, and the concentration of it, was quantified by the analysis of UV absorption. Figure 7 showed that the weight loss of Sample 9, Sample 8 and Sample 7 finally decreasing in sequence. Meanwhile, it could be also found that the weight loss increased by the extension of the processing of the lipase, but three days later weight loss rose more slowly towards balance. These results strongly proved that this polymer could be hydrolyzed by lipase and when the ester bonds of the branched chains were hydrolyzed to certain degree, even if the lipase processing time was extended, the reaction in the fluid of lactic acid would not be more dramatic and the weight loss curve tended towards balance.

The correlation was examined between actual degradability data and properties of samples which were presumed to affect degradability. Based on the results from correlation analysis, the degree of polymerization appeared to be the most influential factor for the degradability. Crystallinity was exhibited to be negatively correlated. And in acid liquor, enzyme solution, alkaline liquor and PBS system, the degradation rate of the polymer decreased in above sequence. The amphiphilic cellulose-*g*-PLLA polymer was hydrolyzed firstly and then further degraded by enzymes.

Conclusions

This work presented here demonstrated that the degradable material cellulose-*g*-PLLA polymer could be prepared successfully *via* ROP of cellulose and L-LA under homogeneous conditions in AmimCl. Samples were observed by NMR, WAXD and UV measurements to investigate the cellulose-*g*-PLLA graft polymers structure and thermal properties. Moreover, the study of degradability was emphasized. Cellulose-*g*-PLLA had good degradability in different conditions. The degradability of this material decreased a little with the increase of its DP. Therefore, the material was expected to be environmentally friendly. Finally, the microstructure of polymer was investigated by TEM, which laid the foundation for future researches.

Acknowledgments. This study was financially supported by the Major State Basic Research Projects of China (973-2010CB732204), which is gratefully acknowledged.

References and Notes

1. Braganca, F. C.; Rosa, D. S. *Polym. Ad. Technol.* **2003**, *14*, 669.
2. Wang, D. S.; Xuan, Y. N.; Huang, Y.; Shen, J. R. *J. Appl. Polym. Sci.* **2003**, *89*, 85.
3. Lee, S. H.; Yoshioka, M.; Shiraishi, N. *J. Appl. Polym. Sci.* **2001**, *81*, 243.
4. Teramoto, Y.; Ama, S.; Higeshiro, T.; Nishio, T. *Macromol. Chem. Phys.* **2004**, *205*, 1904.
5. Goñi, I.; Ferrero, M. C.; Jiménez-Castellanos, R. M.; Gurruchaga, M. *Drug Dev. Ind. Pharm.* **2002**, *28*, 1101.
6. Trejo-O'reilly, J. A.; Cavaillé, J. Y.; Paillet, M.; Gandini, A.; Herrera-Franco, P. *Polym. Compos.* **2000**, *21*, 65.
7. Heinze, T.; Schwikal, K.; Barthel, S. *Macromol. Biosci.* **2005**, *5*, 520.
8. Wu, J.; Zhang, J.; Zhang, H.; He, J. S.; Ren, Q.; Guo, M. L. *Biomacromolecules* **2004**, *5*, 266.
9. Tetamoto, Y.; Yoshioka, M.; Shiraishi, N.; Nishio, Y. *J. Appl. Polym. Sci.* **2002**, *84*, 2621.
10. Hatakeyama, H.; Yoshida, T.; Hatakeyama, T. *J. Therm. Anal. Calorim.* **2000**, *59*, 157.
11. Lee, S. H.; Yoshioka, M.; Shiraishi, N. *J. Appl. Polym. Sci.* **2001**, *77*, 2908.
12. Videki, B.; Klebert, S.; Pukanszky, B. *Eur. Polym. J.* **2005**, *41*, 1699.
13. Schlechter, M. *Biodegradable Polymer*, 2001; Communications Co., Inc., Norwalk.
14. Brostrom, J.; Boss, A.; Chronakis, I. S. *Biomacromolecules* **2004**, *5*, 1124.
15. Wang, F.; Bronich, T. K.; Kabanov, A. V.; Rauh, R. D.; Roovers, J. *Bioconjugate Chem.* **2005**, *16*, 397.
16. Ho, M. H.; Hou, L. T.; Tu, C. Y.; Hsieh, H. J.; Lai, J. Y.; Chen, W. J.; Wang, D. M. *Macromol. Biosci.* **2006**, *6*, 90.
17. Meng, F. L.; Zheng, S. X.; Zhang, W. A.; Li, H. Q.; Liang, Q. *Macromolecules* **2006**, *39*, 711.
18. Shen, D. W.; Huang, Y. *Polymer.* **2004**, *45*, 7091.
19. Lonnberg, H.; Zhou, Q.; Brumer, H., 3rd; Teeri, T. T., Malmstrom, E.; Hult, A. *Biomacromolecules* **2006**, *7*, 2178.
20. Teramoto, Y.; Nishio, Y. *Biomacromolecules* **2004**, *5*, 407.
21. Vlèek, P.; Janata, M.; Látalová, P.; Krí, J.; Eåadová, E.; Toman, L. *Polymer.* **2006**, *47*, 2587.
22. Miyamoto, T.; Takahashi, S.; Ito, H.; Inagaki, H.; Nioshiki, Y. *J. Biomed. Mater. Res.* **1989**, *23*, 125.
23. Mayumi, A.; Kitaoka, T.; Wariishi, H. *J. Appl. Polym. Sci.* **2006**, *102*, 4358.
24. Teramoto, Y.; Nishio, Y. *Cellul. Commun.* **2004**, *11*, 115.
25. Teramoto, Y.; Nishio, Y. *Biomacromolecules* **2004**, *5*, 397.
26. Teramoto, Y.; Nishio, Y. *Biomacromolecules* **2004**, *5*, 407.
27. Xiao, S.; Xin, T. T.; He, J. *For. Stud. China* **2012**, *13*, 245.
28. Xiao, S.; Yuan, T. Q.; Cao, H. B.; Dai, L.; Shen, Y. *Bioresources* **2012**, *7*, 1748.
29. Xiao, S.; Dai, L.; He, J. *Adv. Mater. Res.* **2012**, *476-478*, 1897.
30. Xin, T. T.; Yuan, T. Q.; Xiao, S.; He, J. *Bioresources* **2011**, *6*, 2941.
31. Nederberg, F.; Connor, E. F.; Möller, M.; Glauser, T.; Hedrick, J. L. *Angew Chem, Int. Ed.* **2001**, *40*, 2712.
32. Mayumi, A.; Kitaoka, T.; Wariishi, H. *J. Appl. Polym. Sci.* **2006**, *102*, 4358.
33. Dong, H. Q.; Xu, Q.; Li, Y. Y.; Mo, S. B.; Cai, S. J.; Liu, L. J. *Colloid Surface B* **2008**, *66*, 26.
34. Teramoto, Y.; Nishio, Y. *Polymer* **2003**, *44*, 2701.
35. Vert, M.; Mauduit, J.; Li, S. M. *Biomaterials* **1994**, *15*, 1209.