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Synthesis and characterization of chitosan-graft-polycaprolactone copolymers

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Abstract

The graft copolymers of chitosan with polycaprolactone (PCL) were prepared through a protection-graft-deprotection route using phthaloylchitosan as intermediate. PCL macromonomers terminated with isocyanate groups reacted with hydroxyl groups of phthaloyl-protected chitosan regioselectively, and then phthaloyl groups were deprotected to give the free amino groups. The graft reaction was carried out in homogeneous system and yielded copolymers with high grafting content due to solubilization. FTIR, NMR and XRD were detected to characterize the resultant chitosan-graft-PCL copolymers.

Keywords: Chitosan; Polycaprolactone; Graft copolymers

1. Introduction

Chitosan is the fully or partially deacetylated product of chitin, the second most abundant natural resource next to cellulose. Recently, much attention has been paid to utilize chitosan in biomedical applications, for example, as a wound dressing, skin grafting template, hemostatic agent, hemodialysis membrane and drug delivery vehicle, etc. [1–4]. Despite these high potential of chitosan, its insolubility in common organic solvents and non-thermal plasticity have delayed its utilization and basic research. Of the possible chemical modifications of this rigid aminopolysaccharide, grafting with synthetic polymers has been explored as an interesting alternative method to develop novel hybrid materials, such as chitosan-graft-polystyrene, chitosan-graft-poly(methyl methacrylate), chitosan-graft-poly(ethylene glycol) and so on [5–8].

Polycaprolactone (PCL), which is biodegradable, has been frequently explored as implantable carriers for drug delivery systems and as surgical repair materials because of its excellent mechanical strength, biocompatibility and non-toxicity. Therefore, it is promising to combine chitosan with PCL to produce a new bio-synthetic polymer hybrid applicable for a variety of purposes. But it is difficult to achieve for the solubility difference between chitosan and PCL. Though some blends of PCL with chitosan have been prepared by mechanically mixing a chloroform solution of PCL with an aqueous acetic acid solution of chitosan [9], or by mixing them using 1,1,1,3,3,3-hexafluoro-2-propanol [10], there are few reports about chemical combination of chitosan and PCL.

Recently, Detchprohm et al. [11] reported synthesis of chitin-graft-oligo(ε-caprolactone) via ring-opening

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graft polymerization of ε-caprolactone catalyzed by tin (II) 2-ethylhexanoate. The oligo(ε-caprolactone) side chains were grown from chitosan at amino group in the presence of water as a swelling agent. However, when we consider the unique structure of chitosan, it is important to maintain the aminosaccharide unit for various specific functions, including biological activities and cationic polymer properties.

In this paper, we developed a method to connect PCL macromonomers with chitosan regioselectively at the hydroxyl group via phthaloyl-protected chitosan. Phthaloylchitosan not only enabled the grafting reaction to be carried out in a homogeneous system but also protected the abundant amino groups in the grafting procedure.

2. Experimental

2.1. Materials and reagents

Chitosan (degree of deacetylation = 100%, determined by ^{1}H NMR spectra and Elemental analyses) was purchased from Yuhuan Ocean Biochemical Co. Ltd. (Zhejiang, China). Two polycaprolactones (M_n 1250 and 2000) terminated with hydroxyl groups were purchased from SOLVAY, England. 2,4-Tolylene diisocyanate (TDI), phthalic anhydride and hydrazine monohydrate were supplied by the First Reagent Factory of Shanghai (China). The chitosan and PCL were dried in vacuum oven at 50 °C for 24 h prior to use. Dimethylformamide (DMF) was distilled under reduced pressure from calcium hydride and stored over molecular sieves (4 Å). All other commercially available chemicals were used as received without further purification.

2.2. Phthaloylation of chitosan

Chitosan was heated with excess phthalic anhydride in dried DMF to give phthaloylchitosan (PHCS) according to the previously reported procedure [12,13]. It was obtained as a yellow powdery material and the degree of substitution (DS) of phthaloyl groups was determined by elemental analysis.

2.3. Preparation of graft copolymers

Step 1: *Preparation of TDI-terminated prepolymers (PCL–NCO)*. The prepolymers were prepared by dropping PCL solution into TDI in the NCO/OH ratio of 2:1 (mol/mol). The reactions were performed under N_2 at 60 °C, using DMF as solvent. At the end of the reaction, in order to produce PCL–NCO prepolymers with only one active isocyanate group, an amount of isopropanol with the same mol as TDI was added to envelop NCO at one end.

Step 2: *Graft copolymerization*. Vacuum dried PHCS (1 g) was dissolved in anhydrous DMF (10 ml), then the solution containing an amount of prepolymers and the catalyst was added. The reaction was continued at 80 °C for 3 h under stirring. The obtained product was separated by filtration and the unreacted PCL–NCO was removed by Soxhlet extraction with acetone for 24 h.

2.4. Deprotection of the graft product

The obtained phthaloyl-protected graft copolymer (1 g) was stirred in 10 ml of DMF and heated to 100 °C under nitrogen. Hydrazine monohydrate was added and the reaction was continued for 1 h to deprotect the phthaloyl group. The yellow solution was allowed to cool to room temperature in precipitate. Then the precipitate was collected, washed thoroughly with ethanol and dried.

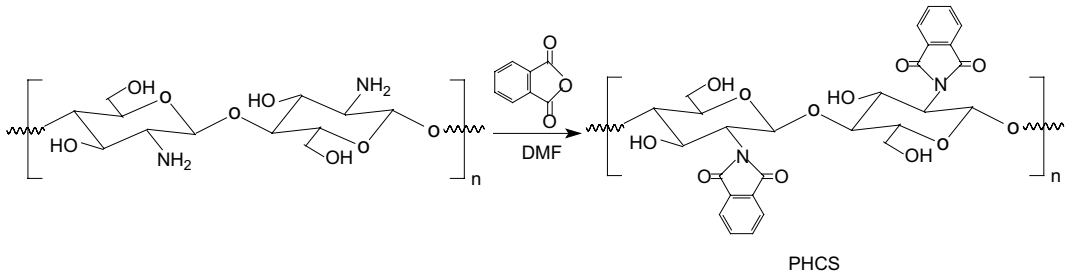
2.5. Measurements

All infrared spectra were obtained from samples in KBr pellets using a Bruker EQUINOX 55 FT-IR spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were taken by a Bruker AV 300 spectrometer operating at 300.13 MHz and 100.6 MHz, respectively, in D_2O/CF_3COOD 95:5 v/v. Elemental analyses were performed with an Elementar Vario EL-III elemental analyzer. X-ray powder diffraction diagrams were recorded with a Japan D/Max-rA X-ray diffractometer using graphite-monochromatized CuKα radiation (λ = 1.54178 Å).

3. Results and discussion

3.1. Preparation of phthaloylchitosan

In this work, we tried to graft hydrophobic polycaprolactone onto hydrophilic chitosan, and to make grafting occur mainly at hydroxyl groups while amino groups remained free. To achieve this goal, phthaloylchitosan was prepared firstly. Phthaloylchitosan, in which phthaloyl group could be deprotected easily to regenerate the free amino group, was prepared by reacting chitosan with phthalic anhydride, as shown in Scheme 1. It has been utilized as a versatile key intermediate for some regioselective chemical modifications of chitosan [14,15]. On the other hand, the introduction of bulky phthaloyl groups prevented the formation of intra- and inter- hydrogen bonds of chitosan and consequently improved its solubility in organic solvents, such as DMF and DMSO [12,13]. This made it possible to carry out grafting of PCL onto chitosan in homogeneous system, and the more important consequence is to mix chitosan together with PCL at the molecular level. Therefore, the



Scheme 1.

N-phthaloyl group would be indispensable for both protection and solubilization.

Fig. 1 clarified that phthaloychitosan showed the phthalimido characteristic peaks at 1712 cm^{-1} and 1777 cm^{-1} referring to the carbonyl anhydride and at 721 cm^{-1} belonging to the aromatic ring. The DS value of PHCS prepared here was found to be about 1.05, calculated from the elemental analysis data.

3.2. "Grafting onto" procedure and removal of protecting group

The whole grafting procedure was shown in Scheme 2. The graft reaction started from the reaction of PCL macromonomers with excess TDI to produce PCL–NCO prepolymers. Then the PCL chains were grafted onto chitosan due to the reaction between OH groups from phthaloychitosan and the terminated NCO groups from prepolymers in DMF homogeneous solution. As indicated in FTIR spectrum (Fig. 1), the new peak at 1538 cm^{-1} confirmed the formation of amide ester linkage (–OCONH–). And the evidence of stronger absorbance at 2800–3000 cm^{-1} for ^{v}C–H (of CH_2) implied significantly the successful introduction of the PCL chains. Nevertheless, the strong characteristic absorptions due to phthalimido group at 1712 cm^{-1} and 1777 cm^{-1} made it not easy to discern the characteristic peaks around 1735 cm^{-1} belonging to ester groups of PCL branches before removing the phthaloyl groups. Although the addition of PCL and isopropanol to NCO was rather slow, prepolymer chains with OCN–PCL–NCO, R–OCONH–PCL–NCO and R–OCONH–PCL–NHOCOR formed randomly. The diurethane could be removed by the extracting procedure, but diisocyanate polymers will inevitably produce some crosslinked copolymers.

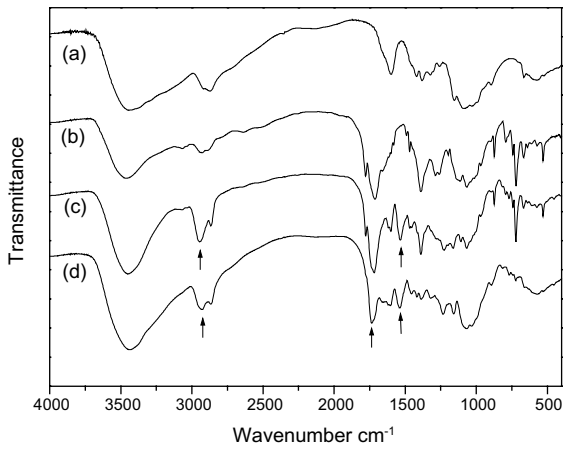
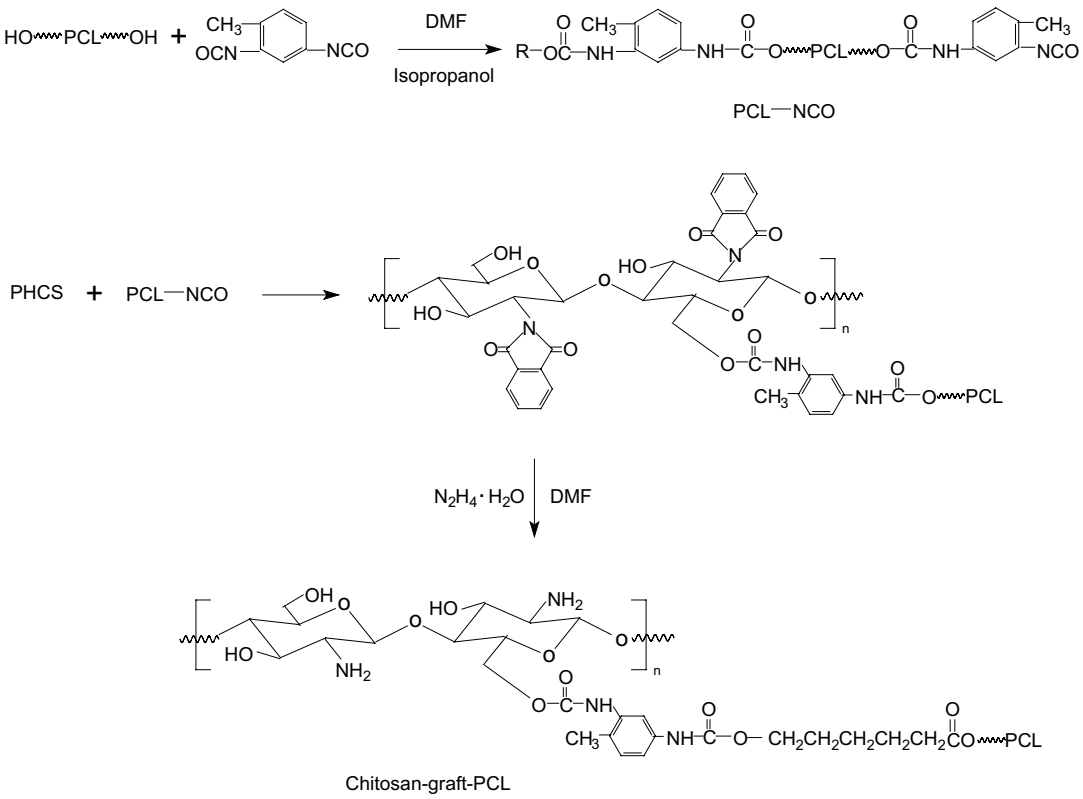


Fig. 1. FT-IR spectra of chitosan (a), PHCS (b), PHCS-graft-PCL (c) and the final chitosan-graft-PCL (d).

Then the deprotection of *N*-phthaloyl groups was carried out by incubation with hydrazine to bring active amino groups back to chitosan. Thus, we obtained the final chitosan-graft-PCL copolymers having hydrophobic PCL side chains and free amino groups. However, the deprotection of phthalimido groups by hydrazine may compete with the reduction of carbamate bonds. The optimum conditions were determined in DMF solution at 100 °C under nitrogen for 1 h to selectively deprotect the phthalimido groups. In this condition, the removal of phthalimide was prior to the reduction of carbamate bonds, probably because a number of PCL chains were packed together and it was difficult for hydrazine to penetrate this hydrophobic environment, as reported in Ref. [12].

3.3. Grafting content of PCL in chitosan-graft-PCL copolymers

The results of samples from different grafting reaction trials with varying the feed amounts of PCL macromonomers are listed in Table 1. As anticipation, the grafting content of PCL enhanced with the increase of PCL amount in the feed. But it was rather low even when PCL/PHCS ratio increased up to 5:1 (w/w). It was probable because the "grafting-onto" method used here connected the PCL macromonomer chains directly with the linear chitosan backbone at a particular site,



Scheme 2.

Table 1
Graft copolymerization conditions and results

Sample no.[a]	PHCS:PCL (g/g)	Catalyst[b]	Grafting[c] (%)
1	1:1	/	0
2	1:3	/	9.7
3	1:5	/	21.2
4	1:1	√	30.1
5	1:3	√	81.5
6	1:5	√	127
7	1:3	√	47.3

[a] All samples from PCL-1250 except no. 7 from PCL-2000.
[b] Tin (II) dibutyl dilaurate, 0.2 mol% to –NCO.
[c] Weight of PCL/weight of chitosan, determined by ^{1}H NMR.

and a few of attached long PCL chains would bring steric hindrance and hinder more PCL chains added. Nevertheless, the grafting efficiency was much improved by adding tin (II) dibutyl dilaurate as catalyst, and the grafting content could reach beyond 100% with PCL-1250.

3.4. Fourier transform infrared analysis of chitosan-graft-PCL copolymer

The FTIR spectrum of the final grafted product, chitosan-graft-PCL, was shown in Fig. 1. Compared to the spectrum of the product before deprotection, the peaks at 1777 cm^{-1}, 1712 cm^{-1} (carbonyl anhydride) and at 721 cm^{-1} (phenyl ring) had almost disappeared, while the peaks at 1540 cm^{-1} (amide ester) and 2800–3000 cm^{-1} (methylene groups) still existed. Moreover, an ester carbonyl stretching band was observed at 1735 cm^{-1}, which belonged to PCL. It proved that the PCL chains were grafted onto chitosan successfully and not removed by hydrazine.

In order to clarify the grafting reaction, difference spectra, as shown in Fig. 2, were obtained by subtracting the original chitosan contribution from the spectra of the graft copolymers, with fixing absorbance at 895 cm^{-1} (asymmetrical pyranose ring stretching). It was quite apparent that the subtraction of the absorptions due to chitosan revealed the presence of newly C–H, C=O and amide ester bonds of the added PCL side chain. And the relative absorbance increased with increasing grafting content of PCL.

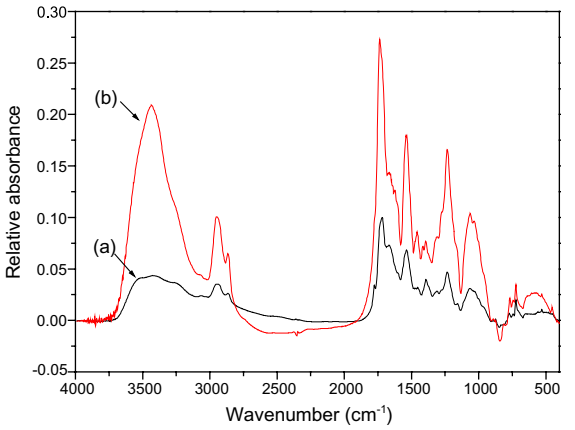


Fig. 2. FT-IR different spectra of chitosan-graft-PCL prepared in sample 4 (a) and 5 (b), subtracted the original chitosan contribution.

3.5. NMR analysis of chitosan-graft-PCL copolymer

The NMR spectra also confirmed the grafting of PCL chain onto chitosan. In Fig. 3, ^{1}H NMR spectrum of the final chitosan-graft-PCL copolymers, the strong signals due to the α-, β-, γ-, δ-, and ε-methylene protons to the carbonyl group of the PCL side chain were detected at 2.2 ppm, 1.4 ppm, 1.2 ppm, 1.5 ppm and 3.5 ppm, respectively, besides the broad peaks of chitosan backbone hydrogens (at 2.8–4.0 ppm). What's more, the ^{1}H NMR data suggested that some incomplete deprotection occurred since there were weak aromatic phthalimido peaks at 7.0–8.0 ppm associated with phenyl ring of TDI.

On the basis of the above results, the grafting contents of PCL part to chitosan in the copolymers were determined with the signal intensities for the CL unit and the chitosan unit. The signal of H-2 proton from chitosan at 2.8 ppm is clearly differentiated from the β-, γ- and δ-protons of the CL unit that appear at around 1.2–1.5 ppm. So the grafting contents of PCL branches could be calculated as follows:

$$\text{PCL grafting content}\% = \frac{114 \times I_{1.2\text{–}1.5\ \text{ppm}}}{161 \times 6I_{2.8\ \text{ppm}}} \times 100,$$

where 114 and 161 are the molecular weights of CL and chitosan unit, respectively.

Chemical structures of chitosan-graft-PCL were further investigated by the corresponding ^{13}C NMR spectrum (Fig. 4). Obviously the six peaks with equivalent intensity between 55–76 ppm belonged to the chitosan backbone (C_1, C_4, C_5, C_3, C_6, and C_2 from lower field to higher). Several strong peaks appeared around 24–34 ppm and at 61 ppm, indicating the presence of hydrocarbon chains of PCL, and at 179 ppm for the ester linkages. The peak observed at 165 ppm corresponded to carbonyl carbon of carbamate group. And it also showed aromatic groups with peaks around 126–134 ppm. All these evidence affirmed the structure of chitosan-graft-PCL copolymers.

3.6. X-ray diffraction analysis

The X-ray powder diffraction patterns of chitosan and chitosan-graft-PCL copolymers were detected as illustrated in Fig. 5. It was known that the PCL homopolymers was easy to crystallize [10,11]. Compared with the original chitosan, chitosan-graft-PCL showed a weaker and broader peak in the $2\theta = 15$–$25°$ region, which demonstrated that the conjugation of PCL with

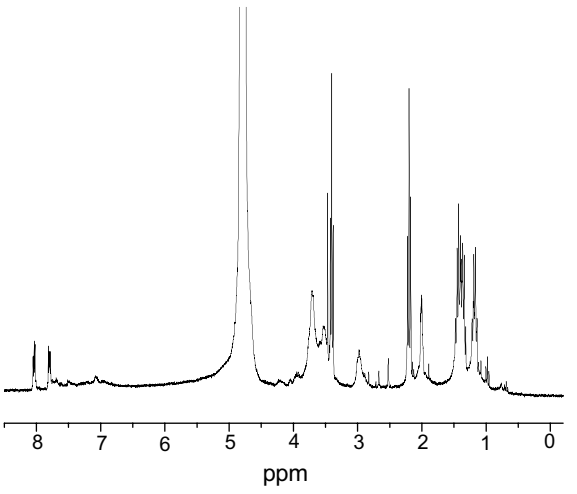


Fig. 3. ^{1}H NMR spectrum of chitosan-graft-PCL.

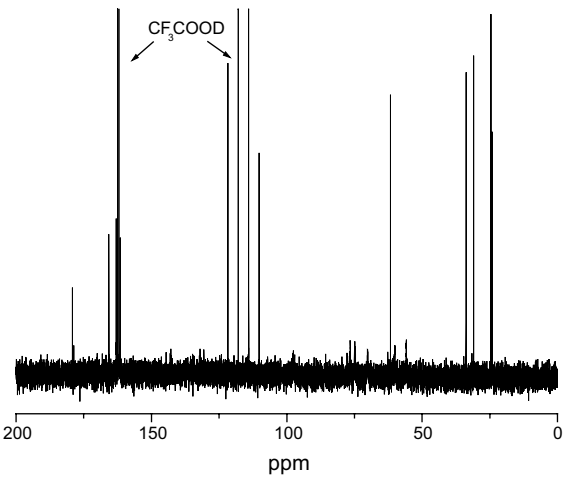


Fig. 4. ^{13}C NMR spectrum of chitosan-graft-PCL.

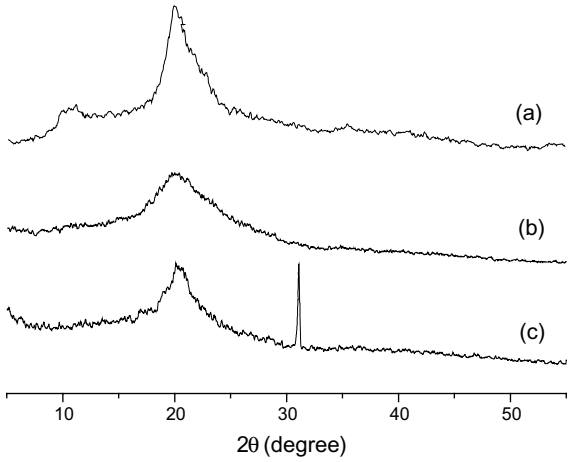


Fig. 5. X-ray diffraction of chitosan (a) and chitosan-graft-PCL obtained in sample 4 (b) and sample 5 (c).

chitosan suppressed the crystallization of both chitosan and PCL to some extent. It was suggested that chitosan and PCL chains were mixed well at a molecular level. However, when the PCL grafting content increased up to 81% in the copolymer, a new sharp signal appeared at $2\theta = 31°$. It is probably due to the phase coarsening of PCL branches influenced by the stiff chitosan chain.

4. Conclusion

Chitosan-graft-PCL copolymers were readily synthesized through a new protection-graft-deprotection route using phthaloylchitosan as intermediate. The graft copolymerization was carried out in homogenous system. It is a potential method to combine chitosan with the hydrophobic synthetic polymers. In these chitosan-graft-PCL copolymers, the PCL branches were grafted regioselectively at the hydroxyl groups of chitosan while the free amino groups remained. Thus the copolymer was expected as an amphoteric nature/synthetic hybrid material to have considerable importance in many fields, because of their biocompatibility, biodegradability and functionality. Further work on the thermal properties, mechanical properties and biodegradation behaviour is in progress on these chitosan-graft-PCL copolymers.

Acknowledgments

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References

[1] Vasudev SC, Chandy T, Sharma CP. Biomaterials 1997;18:375–81.
[2] Park IK, Kim TH, Park YH, Shin BA, Choi ES, Chowdhury EH, et al. J Control Release 2001;76:349–62.
[3] Agnihotri SA, Aminabhavi TM. J Control Release 2004;96:245–59.
[4] Kumar MR. React Funct Polym 2000;46:1–27.
[5] Jenkins DW, Hudson SM. Chem Rev 2001;101:3245–73.
[6] Don TM, Hsu SC, Chiu WY. J Polym Sci: Polym Chem Ed 2001;39:1646–55.
[7] Silva SS, Menezes SMC, Garcia RB. Eur Polym J 2003;39:1515–9.
[8] Don TM, King CF, Chiu WY. J Appl Polym Sci 2002;86:3057–63.
[9] Olabarrieta I, Forsstron D, Gedde UW, Hedenqvist MS. Polymer 2001;42:4401–8.
[10] Senda T, He Y, Inoue Y. Polym Int 2001;51:33–9.
[11] Detchprohm S, Aoi K, Okada M. Macromol Chem Phys 2001;202:3560–70.
[12] Nishimura S, Kohgo O, Kurita K. Macromolecules 1991;24:4745–8.
[13] Kurita K, Ikeda H, Yoshida Y, Shimojoh M, Harata M. Biomacromolecules 2002;3:1–4.
[14] Kurita K, Kojima T, Nishiyama Y, Shimojoh M. Macromolecules 2000;33:4711–6.
[15] Yoksan R, Akashi M, Biramontri S, Chirachaanchai S. Biomacromolecules 2001;2:1038–44.