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Synthesis and characterization of diblock and triblock copolymer by enzymatic ring-opening polymerization of ε-caprolactone and ATRP of styrene

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Summary

Diblock and triblock copolymer of ε-caprolactone(ε-CL) and styrene(St) were obtained by the combination of two different polymerization process, namely enzymatic ring-opening polymerization(ROP) and atom transfer radical polymerization(ATRP) methods. Mono-/di- hydroxyl terminated macromolecules were prepared by enzymatic ROP of ε-CL in the presence of Novozyme-435 and methanol/ethylene glycol as biocatalyst and initiator, respectively, and subsequently converted to bromine ended polycaprolactone(PCL) by the esterification of the resulting macromolecules with α-bromopropionyl bromide. The mono- and difunctional macroinitiators were employed in ATRP of styrene using CuCl/2, 2´-bipyridine(bpy) as the catalyst system. The GPC and ^{1}H-NMR analysis indicated a controlled/living radical polymerization which resulted in the formation of block copolymers with narrow polydispersities.

Introduction

The desire to control polymer properties through the synthesis of block copolymers is a continuing theme throughout polymer chemistry, due to the fact that their special structure yields unusual physical properties. Block copolymers have attracted considerable attention in many fields such as surfactants, adhesive, thermoplastic elastomers, dispersant, ect [1-2]. Different techniques for the preparation of copolymer have been developed, in which the difunctional initiator carrying two different radical forming sites that allow the synthesis of block copolymer from two dissimilar monomers has been used，for example, Andreas Heise et al [3] first combined enzymatic ROP of **ε**-CL and ATRP of St initiated by the difunctional initiator to prepare block copolymer(PSt-*b*-PCL); however, block copolymers prepared in such a way are ill-defined, generally homopolymer formation and a tedious synthesis process of the initiator are unavoidable, hence the block polymerization initiated with macroinitiator formed by transformation of homopolymer end groups is an elegant method [4-5], which allows to combine various polymerization mechanisms to obtain the materials with novel properties.

Enzymatic ring-opening polymerization of lactones [6-7] (i.e**. ε**-caprolactone [8-9]) is of major interest owing to the special properties, such as biodegradability,

biocompatibility, no toxicity and good mechanical property, in addition enzymatic ROP using environmentally benign process is a promising alternative technique to the chemical ROP [10] employing organometallic catalyst. At the same time, as a living/controlled radical polymerization ATRP [11] is the most versatile and popular due to the mild reaction conditions and being suitable for most of the common monomers [12-18], therefore it enables the preparation of many novel well-defined polymeric materials.

In this paper, it was reported that the biocatalyst Novozyme-435 catalyzed ROP of **ε**-CL initiated by methanol and ethylene glycol. A subsequent reaction of the homopolymer polycaprolactone chain end—the hydroxyl group with α-bromopropionyl bromide permitted subsequent block-ATRP of St, which resulted in di- and triblock copolymer(PCL-*b*-PSt and PSt-*b*-PCL-*b*-PSt) combining biodegradable PCL and well-defined PSt segment. The block copolymer must be an interesting material due to its potential application [1-2]. The resulting copolymers were determined by means of ^{1}H-NMR and GPC.

Experimental

Materials

Candida antarctica lipase B(CALB), Novozyme-435, (7000PLU/g), an immobilized enzyme, was a gift from Novo Nordisk A/S. **ε**–caprolactone was obtained from Aldrich Chemical Company and distilled over CaH_2 under vacuum. CuCl (Beijing chemical Co.) was purified by precipitation from acetic acid to remove Cu^{2+}, filtrated and washed with ethanol and then dried. 2, 2′-bipyridine (Beijing chemical Co.) and α-bromopropionyl bromide (Fluka) were used without further purification. Triethylamine (Beijing chemical Co.) was refluxed for 12h in the presence of CaH_2 and distilled under vacuum. Toluene (Tianjin chemical Co.) and Dichloromethane (Beijing chemical Co.) were dried with CaH_2 and distilled. Methanol (Beijing chemical Co.) and ethylene glycol (Tianjin chemical Co.) were distilled over sodium. Styrene (Beijing chemical Co.) was distilled over CaH_2 under vacuum before use.

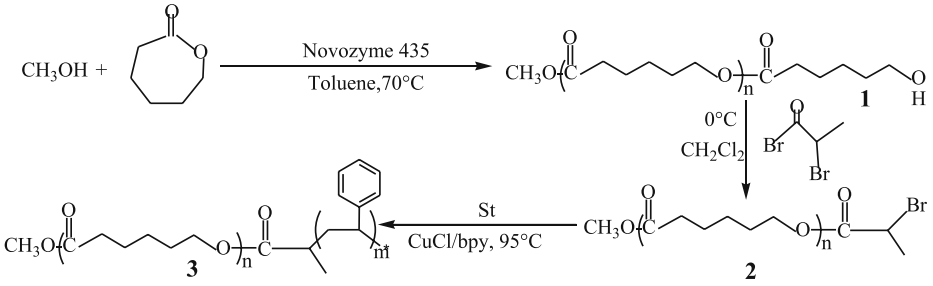
Instruments

^{1}H-NMR spectra was recorded on a Bruker ARX-500 spectrometer with $CDCl_3$ as solvent at 500 MHz. Chemical shifts (in parts per million, ppm) were reported downfield from 0.00 ppm using trimethylsilane (TMS) as internal standard. Gel permeation chromatography (GPC) was carried out at room temperature using a Waters 410 GPC. THF was used as the eluent at a flow rate of 1 mL/min. Molecular weights were calculated using polystyrene standards.

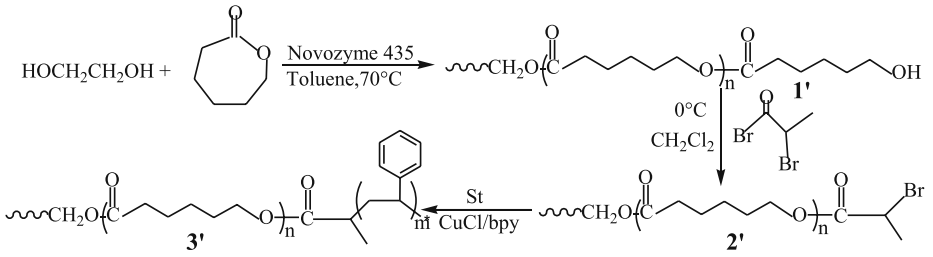
Synthesis of PCL

Novozyme-435 (0.108g, 5% w/w of the monomer weight), dried over P_2O_5 in a vacuum desiccator (0.1mmHg, 25℃, 24h), was transferred into oven-dried 50 mL vial under an argon atmosphere which was coppered with rubber septa and sealed with Teflon tape. The monomer ε–Caprolactone (2 mL), the initiator methanol (0.025 mL) or ethylene glycol (0.035 mL) and the solvent toluene (4.3mL, twice w/v of the monomer) were added via syringe under argon into the reaction vial. The vial was then placed into a constant temperature (70℃) oil bath with magnetic stirring for 4h.

Reactions were terminated by pouring into excess cold chloroform and removing the enzyme by filtration. The enzyme was washed several times with hot chloroform. The filtrate was in large part removed by rotary evaporation. PCL was precipitated in methanol and then dried in a vacuum oven.



Scheme 1. The synthesis of AB-type diblock copolymer.



Scheme 2. The synthesis of ABA-type triblock copolymer.

Synthesis of macroinitiator

The resulting PCL was added into a bath of 1 mL of triethylamine in 5 mL of dry dichloromethane and then cooled in an ice bath (0℃). After stirring for 5 min, 5 mL dichloromethane solution containing 0.6 ml of α-bromopropionyl bromide was added dropwise to it over a period of 0.5h. The reaction was carried out at 0℃ for 2 h and then at room temperature for 22 h. The color of the solution changed from white to yellow. The white Precipitate was filtrated and then the filtrate was in large part removed by rotary evaporation and precipitated in methanol.

ATRP of St from macroinitiator

All the different solids (0.019g CuCl, 0.091g bpy, 0.4g macroinitiator) were introduced into a toasted flask. A cycle of vacuum-argon was repeated three times to remove the oxygen, the liquids (1 mL toluene, 0.687 mL St) were then added via a syringe under argon. The flask was then immersed into 95℃ oil bath with sufficient stirring. After 4 hours the reaction was terminated using ice bath. The reaction mixture passed through an alumina column and precipitated in methanol. The resulting polymer was dried in a vacuum oven.

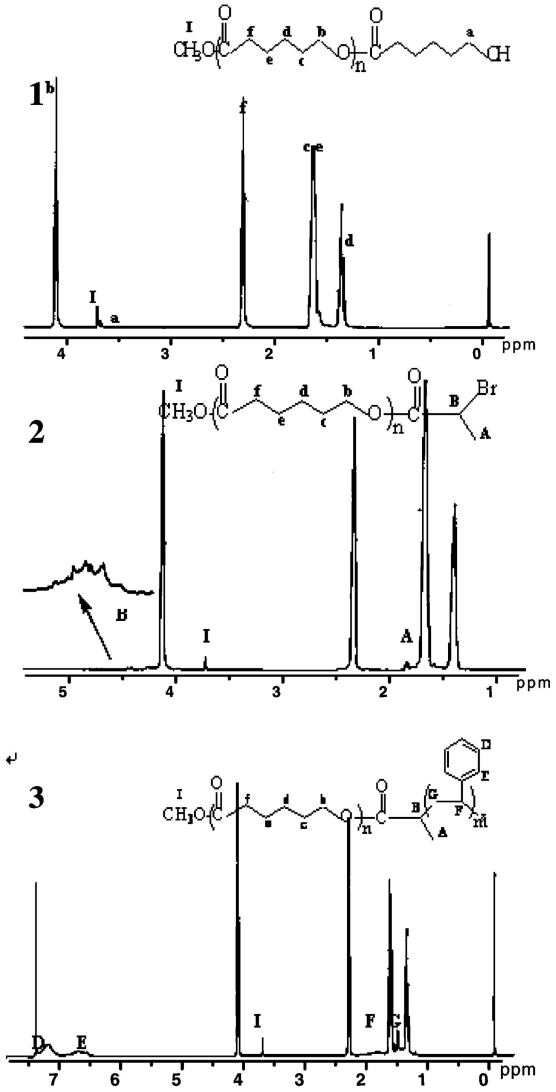


Figure 1. ^{1}H-NMR ($CDCl_3$) spectra of CH_3OH-initiated PCL **1** ($5.0*10^3$g/mol, $M_{n.nmr}=(5I_{4.05}/2I_{3.6\text{-}3.7})*M_{\varepsilon\text{-}CL}$), macroinitiator **2** and AB-type poly(styrene-*b*-caprolactone) **3** ($7.9*10^3$ g/mol, $M_{n.nmr}=(I_{6.8\text{-}7.2}/I_{3.67})*M_{St}+(5I_{4.05}/2I_{3.6\text{-}3.7})*M_{\varepsilon\text{-}CL}$). The molecular weights ($M_{n.nmr}$) were calculated from the ^{1}H-NMR integrated peak areas **I** of peak **n** ($\mathbf{I_n}$), $\mathbf{M_Z}$ represented the molecular weight of **Z**.

Results and discussion

Synthesis of AB-type diblock copolymer

Enzymatic ROP of lactones initiated by methanol had been reported [19], in which lipase PPL (porcine pancreatic lipase) and lipase PS 30 (pseudomonas ceracia lipase) were used as the biocatalyst, however, in rather a long reaction time (about several

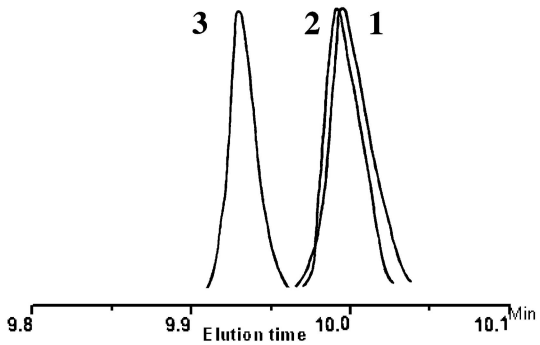


Figure 2. GPC traces of enzymatically polymerized PCL **1** (Mn=5.9*10^3g/mol, polydispersity=1.35), macroinitiator **2** (6.3*10^3g/mol, 1.22) and AB type diblock copolymer **3** (8.07*10^3g/mol, 1.20). The molecular weight and polydispersities were determined by GPC calibrated with polystyrene.

weeks), when most of the monomers were consumed, only low molecular weight oligomers with high polydispersities were obtained. Based on the above reason, to obtain high molecular weight polymer with low polydispersities we chose the more active biocatalyst Novozyme-435 (lipase CALB immobilized on an acrylic resin) to carry out lipase catalyzed ROP of **ε**-CL at 70℃ for 4h in toluene (twice w/v of monomer) where methanol was used as the initiator.

The ^{1}H-NMR spectrum (Figure 1.1) of CH_3OH-initiated PCL **1** (Scheme 1) was carefully examined to determine whether the initiator was attached to PCL chains. The multiplet signals, centered at 1.4, 1.6, 2.3, and 4.1 ppm, corresponded to PCL chain protons, at the same time, the triplet signal **a** at 3.65 ppm corresponded to the methylene protons of the terminal hydroxyl groups. The characteristic signals **I** of the initiator segment (CH_3O-) at the end of the chain could be pointed out at 3.67 ppm, which clarified methanol initiated lipase-catalyzed ROP of **ε**-CL.

Since water was an effective initiator in the enzymatic ROP, there was the possibility of competitive initiation by water and the hydroxyl group of the initiator methanol. Therefore it was important to thoroughly dry the reaction components in order to minimize the water initiation. If a fraction of the PCL chains were initiated by water, it would result in terminal carboxyl acid groups, thus, the methylene protons linked to it should appear in the region of about 2.4 ppm, the absence of any resonance at 2.4ppm in Figure 1.1 suggested that the initiation of the PCL chains was carried out quantitatively by methanol, and the amount of water-initiated PCL could reduce to less than 2%(the limitation of detection by NMR analysis), which was further supported by the absence of a C^{13} signal at 177 ppm corresponding to the carbon atom of the terminal carboxylic acid. Moreover, the unimodal and symmetrical shape of the trace obtained at GPC (Figure 2) also proved this result. Combining GPC analysis, it was clear that the molecular weights of **1** (5000g/mol) calculated from the ^{1}H-NMR spectrum **(**Figure 1.1) was lower than those (5900g/mol) obtained by GPC. The discrepancy could be mainly attributed to the GPC measurement, during which polystyrene was used for calibration.

The $M_{n.nmr}$ determined by ^{1}H-NMR was higher than the expected molecular weight ($M_{n.th}$) of PCL **1** (Table 1), which was possibly caused by the low efficiency of initiation (about 50%) of the initiator methanol. The possible reason was the partial volatilization of the initiator at the initial stage of the reaction at 70℃.

The macroinitiator **2** (Scheme 1) for ATRP was derived from the subsequent esterification reaction between terminal OH group of the resulting PCL **1** and α-bromopropionyl bromide. In the process triethylamine was used as the catalyst and absorbed HBr from the solution to generate a precipitate of quaternary ammonium halide $(CH_3CH_2)_3NH^+Br^-$, which benefited the esterification.

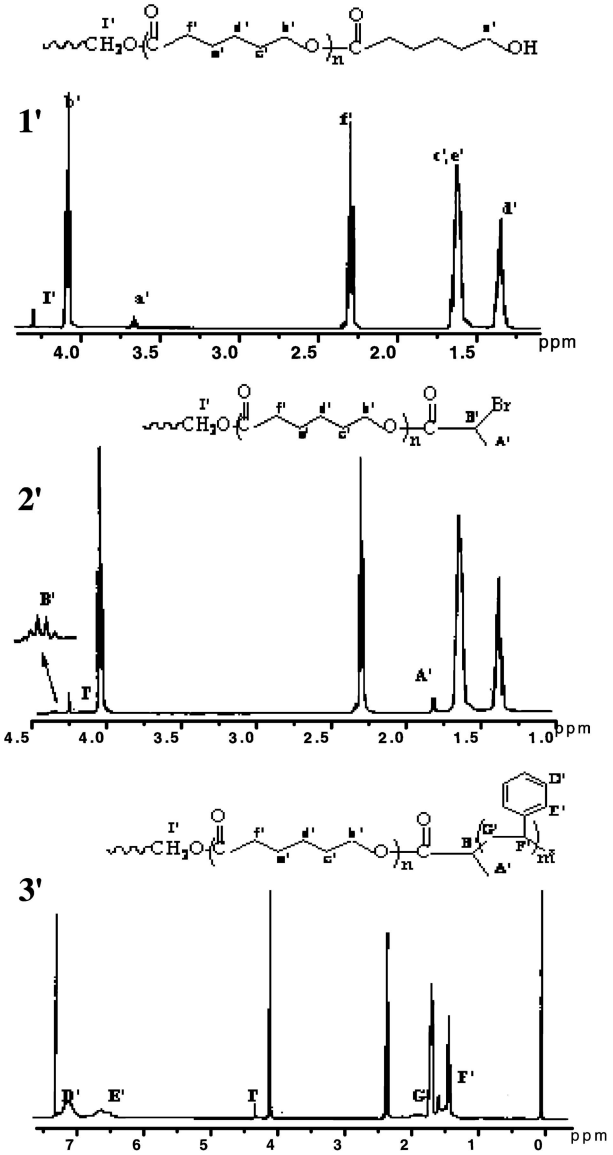


Figure 3. ^{1}H-NMR ($CDCl_3$) spectra of ethylene glycol-initiated PCL **1′**($5.2*10^3$g/mol, $M_{n.nmr}=(I_{4.02}/I_{4.28})*M_{\varepsilon\text{-}CL}$), difunctional macroinitiator **2′** and ABA-type poly (styrene-*b*-caprolactone-*b*-styrene) **3′**($1.1*10^4$g/mol, $M_{n.nmr}=(2I_{6.8\text{-}7.2}/3I_{4.28})*M_{St}+(I_{4.02}/I_{4.28})*M_{\varepsilon\text{-}CL}$). The molecular weights ($M_{n.nmr}$) were calculated from the H^1-NMR integrated peak areas **I** of peak **n** ($\mathbf{I_n}$), $\mathbf{M_Z}$ represented the molecular weight of **Z**.

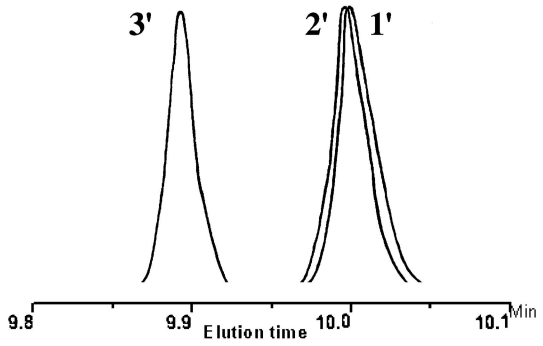


Figure 4. GPC traces of enzymatically polymerized PCL **1′**(Mn=6.2*10^3g/mol, polydispersity=1.25), macroinitiator **2′**(6.4*10^3g/mol, 1.14) and ABA type triblock copolymer **3′**(1.05*10^4g/mol, 1.13). The molecular weight and polydispersities were determined by GPC calibrated with polystyrene.

Due to the ester formation of the adjacent hydroxyl function, the methylene protons **a** experienced a shift from 3.65ppm to 4.05ppm, moreover, the signals **B** and **A** at 4.36ppm and 1.80ppm assigned to the >CH- protons and the CH_3- protons closed to the active bromide, respectively, were also able to be detected (Figure 1.2), which indicated that the α-bromoester group was attached to the PCL chain end. The existence of the signal **I** revealed that the esterification didn't interfere with the terminal CH_3O- group. Based on the above data, it was obvious that the macroinitiator had been prepared. Moreover, according to the exclusive disappearance of the signal **a**, it was concluded that the degree of end functionalization of the PCL 1 was more than 98%.

As shown in Figure 2, it was interesting to note that the polydispersity after the esterification reaction was usually lower than those of the starting PCL, on the contrary number average molecular weight (Mn) was slightly higher. This could be due to inevitable fractionation of macroinitiator during the course of precipitation after polymerization.

The ATRP of styrene from macroinitiator **2** was carried out at 95℃, using CuCl/bpy as the catalyst system and toluene as the solvent. After 4h, 8.0% monomer conversion was reached and the final copolymer **3** (Scheme 1) with Mn=8070 and polydispersities of 1.20 was obtained (Figure 2). The conversion was determined gravimetrically; the GPC traces of the starting PCL **1,** macroinitiator **2** and the final block copolymer **3** were present in Figure 2. It was clear that the ATRP of styrene using macroinitiator **2** resulted in an increase in molecular weight and a decrease in polydispersities. The unimodal and symmetrical shape on the GPC plot of the block copolymer suggested the absence of a homopolymer composed of either styrene or **ε**–Caprolactone and the complete initiation of the macroinitiator during the ATRP process.

From the ^{1}H-NMR spectra of the AB type diblock copolymer **3** (Figure 1.3), we could see that besides the dominant CH_3OH-initiated PCL signals of the macroinitiator **2**, the occurrence of the signal at 6.5-7.0ppm corresponding to aromatic protons **D** and **E** of the PSt block revealed the formation of the block copolymer **3** (PCL-*b*-PSt), moreover, the number average molecular weights (8070g/mol) determined by GPC were in good agreement with the theoretical ones (7650g/mol) calculated (Table 1).

The unimodal and symmetrical shape of the trace obtained at GPC and the structure determined by ^{1}H-NMR spectra proved the formation of the AB type diblock copolymer.

Synthesis of ABA-type triblock copolymer

To obtain triblock copolymer by the above method, the difunctional initiator such as ethylene glycol ($HOCH_2CH_2OH$) must be used at the initial step of enzymatic ROP, thus the hydroxyl group would occupy at both end of the resulting linear PCL chains **1′**. By the esterification reaction with α-bromopropionyl bromide and subsequent ATRP, difunctional macroinitiator **2′**and ABA-type triblock copolymer**s 3′** (Scheme 2) were obtained. In the ^{1}H-NMR spectra (Figure 3), except that the new signal located at about 4.28ppm corresponding to the methylene protons **I'** of the initiator segment took place of the signal of the methyl protons **I** (Figure 1), the variance of the signals in Figure 3 were the same as those during the course of the synthesis of diblock copolymer, which indicated that the structure of the ABA-type triblock copolymer was in agreement with that we expected. At the same time, it was concluded from the NMR spectrum that less than 2% of the amount of the PCL chains was initiated by water (Table 1).

The number average molecular weight (6200g/mol) of **1′** obtained by GPC analysis (Figure 4) was higher than that (5200g/mol) calculated from the ^{1}H-NMR spectra. Moreover, the low efficiency of initiation (about 45%) of the initiator ethylene glycol was also obtained. The degree of end functionalization of PCL **1′** was more than 98% due to the exclusive disappearance of the signal **a′** (figure 3).

Table 1. The results of PCL. Macroinitiator and block copolymer.

PCL	[Mo/Io]	mol% carboxyl terminal chains [a]	monomer conv. [b]	Mn.th [c]	Mn.nmr [a]	f [d]	Mn.GPC [e]	Mw/Mn [e]
1	30/1	<2%	73%	2600	5000	52%	5900	1.35
1'	30/1	<2%	65%	2300	5200	45%	6200	1.25
Macro-initiator		mol%the degree of end functionalization [a]					Mn.GPC [e]	Mw/Mn [e]
2		>98%					6300	1.22
2'		>98%					6400	1.14
copolymer	[Mo/Io]		monomer conv. [b]	Mn.th [c]	Mn.nmr [a]	CL/St [f]	Mn.GPC [e]	Mw/Mn [e]
3	150/1		8%	7650	7900	50/20	8070	1.20
3'	150/1		20%	9520	11260	55/48	10555	1.13

a. determined by ^{1}H-NMR analysis; **b**. the conversion was determined gravimetrically; **c**. the theoretical molecular weights ($M_{n.th}$) calculated from the ratio of the monomer to the initiator [Mo]/ [Io] and the monomer conversion. **d**. the efficiency of initiator, f = $M_{n.th}/M_{n.nmr}$; **e**. determined by GPC measurements; **f**. the degree of polymerization of PCL:PSt calculated from the ^{1}H-NMR spectra.

Polydispersities of difunctional macroinitiator **2′** were lower than that of the PCL **1′**after the esterification, in subsequent ATRP of St the peak pertaining to **2′** was shifted to higher elution volumes, which revealed a new well-defined PSt block was formed from the macroinitiator **2′** by ATRP. In addition, there was good accordance between Mn(10555g/mol) of **3′** by GPC analysis and the theoretical molecular weights (9520g/mol) (Table 1), which suggested that during ATRP no homopolymer PSt and PCL appeared, this was also proved by the unimodal and symmetrical shape of **3′** at GPC.

Conclusions

By combining enzymatic ROP of **ε**-CL and ATRP of St, the synthesis of block copolymers had been demonstrated. The mono- and difunctional macroinitiators were prepared by lipase catalyzed ring-opening polymerization of **ε**-CL in the presence of methanol/ethylene glycol as the initiating system followed by the esterification of the resulting polymer with α-bromopropionyl bromide. The ^{1}H-NMR spectrum and the unimodal and symmetrical shape of the trace obtained at GPC proved that the di- and triblock copolymer were obtained. The good correlation between determined molecular weight of the block copolymer and the calculated one indicated a controlled /living radical polymerization. On the side, experiments aiming at the kinetic study of ATRP from macroinitiator are currently under way.

References

1. Noshay A, McGrath JE（1977）Block copolymer. In: Overview and critical survey. New York，Academic Press
2. Goodman I（1982）Developments in block copolymer. London，Applied science
3. Meyer U, Palmans A, loontjens T, Heise A（2002）Macromolecules 35:2873
4. Jankova K, Kops J, Chen X, Gao B, Batsberg W（1998）Polymer Bulletin 41:639
5. Zheng GD, Stover HDH（2003）Macromolecules 36:7439
6. Kobayashi S, Uyama H, Kimura S（2001）Chem Rev 101: 3793
7. Gross RA, Kumar A, Kalra B（2001）Chem Rev 101: 2097
8. Mei Y, Kumar A, Gross R（2003）Macromolecules 36:5530
9. He F, Li S, Vert M, Zhou R（2003）Polymer 44: 5145
10. Cretu A, Gattin R, Brachais L, Barbier-Baudry D（2004）Polymer Degradation and Stability 83:399
11. Matyjaszewski K, Xia J（2001）Chem Rev 101: 2921
12. Pascual S, Coutin B（1999）Macromolecules 32: 1432
13. Matyjaszewski K, Patten TE（1997）J. Am. Chem. Soc 119: 674
14. Liou S, Rademacher JT（2000）Macromolecules 33: 4295
15. Kajiwarei A, Matyjaszewski K (1999) Polymer 31: 70
16. Woodworth BE, Metzmer Z, Matyjaszewski K (1998) Macromolecules 31:7999
17. Xiao DQ, Wirth MJ (2002) Macromolecules 35:2919
18. Yeodorescu M, Matyjaszewski K (1999) Macromolecules 32:4826
19. Nobes GAR, Kazlauskas RJ, Marchessault RH (1996) Macromolecules 29:4829