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Synthesis and characterization of diblock copolymer by enzymatic ring-opening polymerization and ATRP from a novel bifunctional initiator

Ke Sha, Dongshuang Li, Shuwei Wang, Lu Qin and Jingyuan Wang (✉)

Department Of Chemistry, Jilin University, ChangChun 130021, P. R. China
E-mail: jingyuan@jlu.edu.cn

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Summary

A new method is reported for synthesizing AB-type diblock copolymer polycaprolactone-block-polystyrene (PCL-*b*-PSt) from a novel bifunctional initiator 2.2.2-trichloroethanol (TCE) by combining two different polymerization techniques: enzymatic ring-opening polymerization (ROP) and atom transfer radical polymerization (ATRP). Trichloromethyl terminated PCL was prepared by enzymatic ROP of ε-caprolactone (**ε**-CL) in the presence of Novozyme-435 and TCE as biocatalyst and initiator, respectively, and subsequently employed in ATRP of styrene (St) using CuCl/2, 2´-bipyridine (bpy) as the catalyst system. The GPC and NMR analysis indicated the formation of the diblock copolymer including the biodegradable PCL block and the well-defined PSt block.

Introduction

Recently enzymatic polymerization in vitro has become an area of increasing research activity as a new environmental friendly methodology for material synthesis. In contrast to "chemical" organometallic catalysts, the "green" biocatalyst enzyme is a promising alternative due to the special properties, such as nontoxicity, recyclability, high enantio-, regio- and chemoselectivities and so on.[1-2] For example, Lipase B from Candida Antarctica (CALB) immobilized on an acrylic macroporous resins (Novozyme 435) has been proven to be an effective biocatalyst in homopolymer syntheses via both ring-opening polymerization of lactones (i.e. ε-CL) [3-4] and polycondensation polymerization [5] between dicarboxylic acids and diols, as well as further used in the preparation of block copolymers [6-7] and hyperbranched copolymers [8]. At one time, as an important living/controlled radical polymerization, ATRP has been considered as the most powerful and versatile polymerization method due to the mild reaction conditions and the suitability for most of the functional (meth)acrylate and styrenic monomers [9]. Since its discovery in 1995 [10], ATRP has enabled the preparation of various well-defined functional macromolecules (homopolymer and copolymer, ect).

Much attention has been likewise focused on the development of block copolymers for their use as surfactants, adhesives, thermoplastic elastomers and dispersants [11], based on the reason that their special structures can bring on the

unique polymer properties of novel materials. Multitudinous techniques for the preparation of block copolymers have been developed, among which there is a successful method, that is, oligomer is transformed into macroinitiator by chemical modification of the terminal groups and then used to initiate the polymerization of other monomers. Our group has successfully made use of this technique to synthesize di/triblock copolymer PCL-b-PSt/PSt-b-PCL-b-PSt by combining enzymatic ROP of ε-CL and ATRP of St [12]. However, the synthesis process in such a manner is fussy and must needs an intermediate transformation step, hence another method has been more widely employed in the preparation of block copolymers, which utilizes the bifunctional initiator carrying two different radical forming sites to combine various polymerization mechanisms, e. g. Andreas Heise et al incorporated enzymatic ROP with ATRP from a bifunctional initiator to obtain diblock copolymer [13-15]. Nevertheless during the course of the polymerization a tedious synthesis process of the bifunctional initiator including a primary alcohol and a α-bromoester group is unavoidable, which necessarily restricts its application in fundamental and industrial fields of polymer science. To overcome this disadvantage, our group tried to make use of 2,2,2-trichilroethanol, an industrial material, as a novel bifunctional initiator to combine enzymatic ROP of ε-CL and ATRP of St without an intermediate transformation process.

In this paper, it was reported that TCE quantitatively initiated Novozyme 435 catalyzed enzymatic ROP of ε-CL. Trichloromethyl-terminated PCL macroinitiator prepared in this way permitted subsequent block-ATRP of St, which resulted in the diblock copolymer PCL-*b*-PSt. The resulting copolymers were determined by means of NMR and GPC.

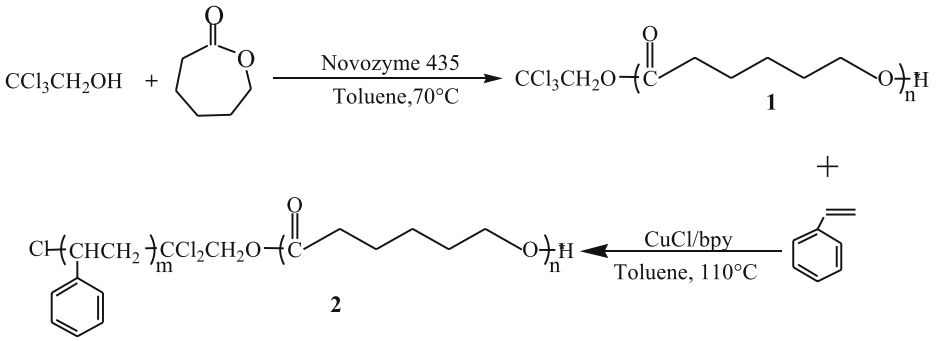
Experimental

Materials

Novozyme-435 (7000PLU/g) was a gift from Novo Nordisk A/S and employed without further purification. Styrene and **ε**–caprolactone were obtained from Aldrich Chemical Co. and distilled over calcium hydride (CaH_2) under vacuum before use. 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.) was used without further purification. Toluene (Tianjin chemical Co.) was dried with CaH_2 and distilled. 2,2,2-Trichloroethanol was purchased from Aldrich chemical Co. and stored over freshly activated type 4 molecular sieves. All the reagents used in this study were of analytic grade.

Instruments

The monomer conversion was determined gravimetrically. Measurements of nuclear magnetic resonance (NMR) spectra were conducted on a Bruker ARX-500 NMR spectrometer with $CDCl_3$ as solvent, operating at 500 and 125 MHz for the corresponding ^1H and ^{13}C nuclei. Chemical shifts (in parts per million, ppm) were reported downfield from 0.00 ppm using trimethylsilane (TMS) as internal standard. Molecular weights and molecular weight distributions were measured on a Waters 410 Gel permeation chromatography (GPC) apparatus equipped with a 10-μm Styragel HT6E column (300mm×7.8mm) using linear polystyrene standards. THF was used as the eluent at a flow rate of 1 mL/min.



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

Synthesis of trichloromethyl terminated PCL

Predetermined amounts of Novozyme-435 (0.108g, 5% w/w of the monomer weight), vacuum dried in a desiccator with phosphorus pentoxide as desiccant (0.1mmHg, 25℃, 24h), was transferred into oven-dried 50 ml reaction vial under an inert atmosphere of dry argon, and the vial was immediately stoppered with a rubber septum and sealed with Teflon tape. The reagents ε–CL (2 ml), TCE (0.090 ml) and solvent toluene (4.3mL, twice v/w of the monomer weight) were added via gastight syringe under argon into the reaction vial. The vial was then placed into a constant temperature (70℃) oil bath with magnetic stirring for a predetermined time period. The reaction was terminated by pouring the reactants into excess cold chloroform and the enzyme was removed via filtration. The filtrate was concentrated with rotary evaporation to obtain the crude polymer and further precipitated in methanol.

ATRP of St from -CCl_3 terminated PCL

Solid reagents CuCl (0.024g) and bpy (0.112g) were added into a toasted flask containing macroinitiator PCL (0.168g). The reaction flask was sealed and immersed in ice water/NaCl mixture at about -10℃ and degassed by vacuum-argon for three times to remove the oxygen. Monomer styrene (2.1ml) and solvent toluene (2.1ml) degassed by inert dry argon were introduced into the flask via a syringe under argon. After the PCL macroinitiator was completely dissolved, the reaction flask was heated at 110℃ under sufficient stirring for a predetermined time. Finally the polymerization reaction was terminated in an ice bath. The catalyst was removed by passing the polymer solution through a short alumina column. The crude polymer was precipitated in methanol and dried in a vacuum oven.

Results and discussion

Novel bifunctional initiator TCE contains a single primary alcohol group to initiate enzymatic ROP and an activated trichloromethyl (–CCl_3) group, an effective initiating group for ATRP [16-17], which replaces the α-bromoester group in previous reports [13-15]. As shown in scheme 1, the –CCl_3 terminated PCL **1**, prepared by

TCE-initiated enzymatic ROP of ε-CL, was used to initiate the ATRP of St to yield the diblock copolymers PCL-*b*-PSt **2** in two consecutive polymerization without an intermediate transformation step. Enzymatic ROPs of various cyclic lactones initiated by different kinds of initiators (i.e. H_2O, alcohol, amine) have been extensively investigated [1-2], however, it is not yet reported that TCE initiates the enzymatic ROP of lactones, so the feasibility of TCE as the initiator in enzymatic ROP must be validated first.

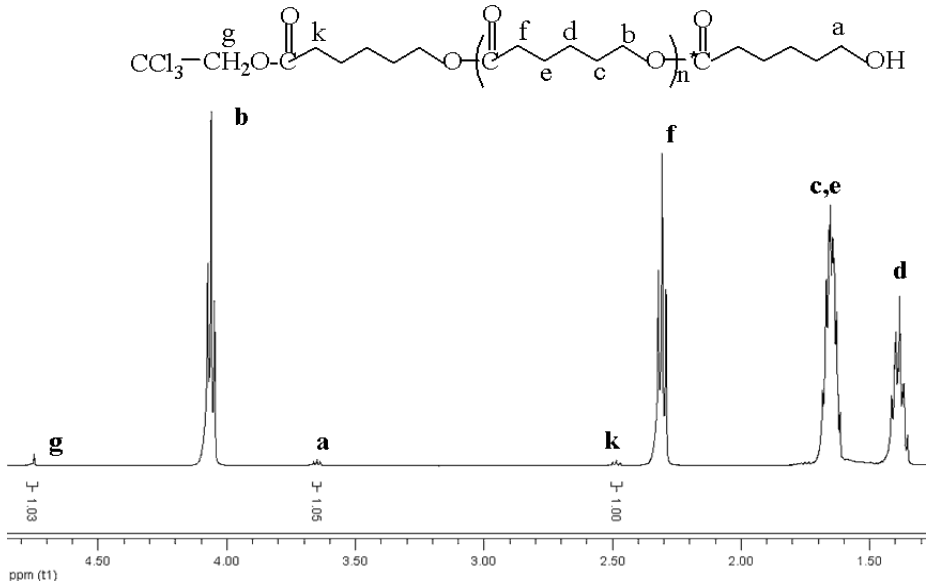


Figure 1. The ^{1}H-NMR of the trichloromethyl-terminated PCL **1** ($8.5*10^3$g/mol, Mn.nmr = $(I_{4.05}/I_{3.65})*M_{\varepsilon\text{-CL}}+M_{TCE}$). The molecular weight (Mn.nmr) is calculated from the ^{1}H-NMR integrated peak area I of peak n (I_n), M_Z represents the molecular weight of Z.

Since water is an effective initiator for enzymatic ROP, there is the possibility of competitive initiation between water and initiator TCE. Hence it is necessary to dry the reagents (especially the biocatalyst Novozyme 435) thoroughly in order to minimize the water initiation.

Figure 1 shows the ^{1}H-NMR spectrum of the resulting TCE-initiated PCL **1**. Besides the multiplet signals assigned to the PCL main chain protons, the triplet signal **a** at 3.65 ppm corresponds to the methylene protons attached to the terminal hydroxyl group. It is paramount that the characteristic signal **g** of the initiator segment (CCl_3-CH_2-O-) at the end of the PCL chains could be pointed out clearly at 4.75ppm, which clarifies enough that TCE initiates successfully enzymatic ROP of ε-CL.

At the same time, as water-initiated enzymatic ROP will result in PCL end-functionalized with a carboxylic acid instead of the ATRP initiator, the absence of any resonance at about 2.35ppm corresponding to the methylene protons linking to the terminal carboxyl acid and the identical integrated areas of peaks **a** and **g**, suggests that the initiation of the PCL chain is carried out quantitatively by TCE and the mole percentage of water-initiated PCL without the trichloromethyl end group can be

reduced to less than 2% (the limitation of detection by NMR analysis), which is further supported with the absence of a ^{13}C-NMR signal at 177ppm corresponding to the carbon atom of the terminal carboxylic acid. Combining GPC analysis (Figure 2), the fact that the resulting macroinitiator PCL **1** shows a unimodal and symmetrical trace also proves this conclusion.

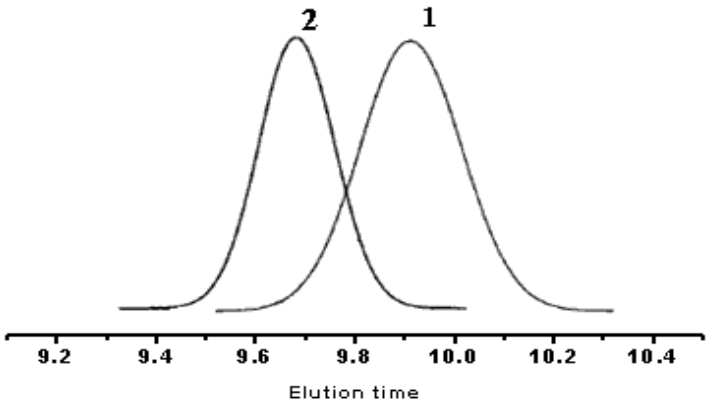


Figure 2. GPC traces of TCE-initiated PCL **1** (Mn=1.07*10^{4}g/mol, polydispersity=1.36) and AB-type diblock copolymers PCL-*b*-PSt **2** (Mn=1.70*10^{4}g/mol, polydispersity=1.28). Values determined by GPC calibrated with PSt.

It is clear that the calculated molecular weight (Mn.nmr) of PCL **1** (8500g/mol) based on the ^{1}H-NMR spectrum is observed to be about 20% lower than that (10700g/mol) determined by GPC. The discrepancy most likely results from the GPC analysis, in which polystyrene is used for calibration. At one time, Mn.nmr of PCL **1** is found to be very much greater than the expected theoretical value (Mn.th=2050g/mol), which may be attributed to the low efficiency of initiation (about 24%) due to the speedy volatilization of initiator TCE at the initial stage of ROP at 70℃ (see Table 1).

Halogenated alkanes, such as R-CCl_3/R-CBr_3 derivatives, have been employed successfully as initiating species in the ATRP of styrenic and (meth)acrylates monomers [16-18]. Thus, our group carried out the ATRP of St from the –CCl_3 terminated PCL **1** using CuCl/2,2´-bipyridine (bpy) as the catalyst system and toluene as the solvent, respectively, at 110℃ according to scheme 1. After 11 hours, 5.0% monomer conversion was reached and the final copolymer **2** with Mn=17033 and polydispersity of 1.28 was obtained.

As shown in Figure 2, it is observed that the ATRP of St from macroinitiator **1** results in an increase in molecular weight and a slight decrease in polydispersity, which could be due to inevitable fractionation of polymer during precipitation of the crude reaction product; at the same time, the unimodal and symmetrical shape of the peaks on the GPC plots of the diblock copolymers **2** suggests the absence of a homopolymer composed of St or ε-CL.

Figure 3 represents the ^{1}H-NMR spectrum of the diblock copolymer PCL-*b*-PSt, in addition to the dominant PCL signals, the occurrence of the signals at 6.3-7.3ppm corresponding to aromatic protons **D** and **E** of the PSt block shows further that new

well-defined PSt segments are connected with PCL **1**. Besides, as shown in Table 1 there is good accordance between the number average molecular weights (Mn,=17033g/mol) of the diblock copolymers **2** and their theoretical molecular weights (Mn.th=16400g/mol), which leads to the same conclusion that during ATRP no homopolymer PSt or PCL appears.

The unimodal and symmetrical shape of the trace obtained at GPC and the structure determined by NMR spectra proved the formation of the AB-type diblock copolymer PCL-*b*-PSt.

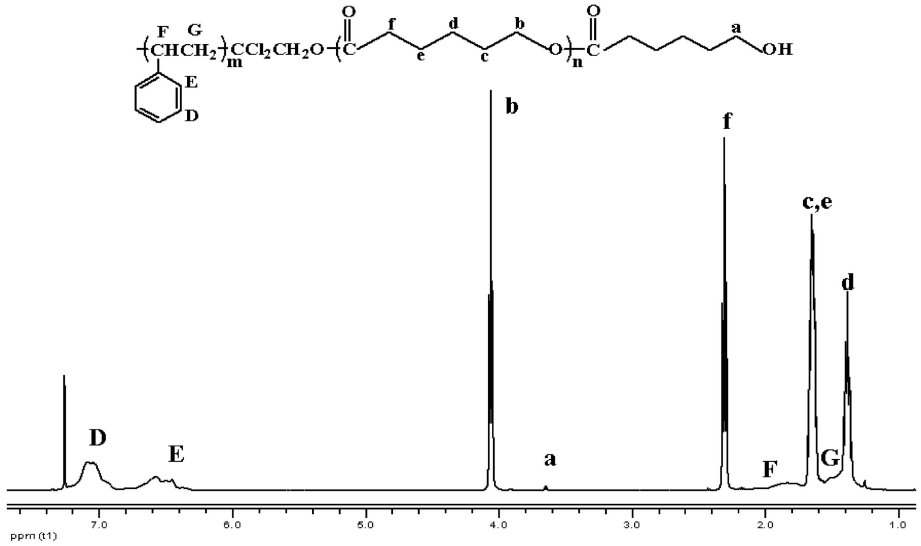


Figure 3. The ^{1}H-NMR spectrum of the AB-type diblock copolymer PCL-*b*-PSt **2** (Mn.nmr=1.65*10^{4}g/mol, Mn.nmr=($I_{6.2\text{-}6.8}/I_{3.65}$)*$M_{St}$+Mn.macroinitiator). The molecular weights (Mn.nmr) were calculated from the ^{1}H-NMR integrated peak areas **I** of peak **n** (**In**), $\mathbf{M_Z}$ represents the molecular weight of **Z**.

Table 1. The results of PCL Macroinitiator **1** and diblock copolymer **2**.

PCL	[Mo]/[Io]	mol% carboxyl terminal chains [a]	monomer conv. [b]	Mn.th [c]	Mn.nmr [a]	EI [d]	Mn.GPC [e]	Mw/Mn [e]
1	**20/1**	**<2%**	**83%**	**2050g/mol**	**8500g/mol**	**24%**	**10700g/mol**	**1.36**
copolymer	**[Mo]/[Io]**	**time**	**monomer conv. [b]**	**Mn.th [c]**	**Mn.nmr [a]**	**CL/St [f]**	**Mn.GPC [e]**	**Mw/Mn [e]**
2	**1100/1**	**11h**	**5.0%**	**16400g/mol**	**16500g/mol**	**76/56**	**17033g/mol**	**1.28**

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, EI = $M_{n.th}/M_{n.nmr}$; **e**. determined by GPC measurements; **f**. the degree of polymerization of PCL:PSt calculated from the ^{1}H-NMR spectra.

Conclusions

The methodology of producing the $-CCl_3$ terminated PCL by enzymatic ROP of ε-CL initiated with a novel bifunctional initiator TCE has been proven to be a useful strategy for the synthesis of ATRP macroinitiator for subsequent preparation of diblock copolymers. The $-CCl_3$ terminated macroinitiators were prepared by Novozyme-435 catalyzed ring-opening polymerization of **ε**-CL in the presence of TCE as the initiating system. The ^{1}H-NMR spectrum and the unimodal and symmetrical shape of the trace obtained at GPC proved the preparation of the diblock copolymer PCL-*b*-PSt. The good correlation between determined molecular weight of the block copolymer and the calculated one indicated a controlled /living radical polymerization. Besides the study reported here, our group is currently investigating whether this novel bifunctional initiator TCE is employed in a single-step, simultaneous, one-pot synthesis of diblock copolymer by the combination of enzymatic ROP and ATRP.

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