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The chemoenzymatic synthesis of AB-type diblock copolymers from a novel bifunctional initiator

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Abstract

A novel bifunctional initiator 2,2,2-trichloroethanol (TCE) is used for the chemoenzymatic synthesis of AB-type diblock copolymer polycaprolactone-*block*-polystyrene (PCL-*b*-PSt) by combination of two fundamentally different synthetic techniques: enzymatic ring-opening polymerization (ROP) of ε-caprolactone (ε-CL) and atom transfer radical polymerization (ATRP) of styrene (St). The kinetic study on the TCE-initiated enzymatic ROP of ε-CL in the presence of the biocatalyst Novozyme-435 was investigated. By optimization of the reaction conditions, TCE quantitatively initiated enzymatic ROP of ε-CL. Trichloromethyl-terminated PCL macromolecules prepared in this way were subsequently employed as macroinitiators in the ATRP of St using CuCl/2,2′-bipyridine as the catalyst system to afford well-defined AB-type diblock copolymers PCL-*b*-PSt. The kinetic analysis of ATRP indicated a 'living'/controlled radical polymerization. The polymeric nanospheres were prepared by the precipitation method from two resulting PCL–PSt diblock copolymers with different content ratio of PSt to PCL. It was determined by DLS and AFM that two different diameter nanospheres had been obtained.
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Keywords: Atom transfer radical polymerization; Bifunctional initiator; Enzymatic ring-opening polymerization

1. Introduction

In recent years, biocatalytic approaches in polymer science have become an area of increasing research activity as a new environmental friendly methodology. 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, Novozyme 435 (Lipase B from Candida Antarctica immobilized on an acrylic macroporous resins) has been proven to be an effective biocatalyst in polymer syntheses via both ring-opening polymerization of lactones (i.e. ε-CL) [3] and condensation polymerization between dicarboxylic acids and diols [4], as well as further used in the preparation of block copolymers [5] and hyperbranched copolymers [6]. Enzymatic ROP of ε-CL catalyzed by Novozyme 435 has been extensively studied and the resulting PCL is one of the most important environmentally biodegradable materials [7]. However, the full exploitation of biocatalysis in polymer synthesis will require the development of mutually compatible chemo- and biocatalytic methods.

Based on the above reason, the integration of biocatalytic polymerization method (i.e. enzymatic ROP) and living/controlled radical polymerization method [8] (i.e. ATRP [9,10]) has been attempted to synthesize block copolymers, 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 second polymerization of other monomers [11]. Our group has successfully made use of this technique to synthesize di/triblock copolymer PCL-*b*-PSt/PSt-*b*-PCL-*b*-PSt including the biodegradable PCL blocks and the well-defined PSt blocks by combining enzymatic ROP of ε-CL and ATRP of St [12]. However, the synthesis process in such a manner is fussy and need 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. for the first time incorporated enzymatic ROP with ATRP initiated by

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a dual initiator to obtain the PCL–PSt diblock copolymer [13]. Nevertheless during the course of polymerization a tedious synthesis process of the bifunctional initiator including a primary alcohol and a tertiary bromide 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-trichloroethanol, an industrial material, as a novel bifunctional initiator to combine enzymatic ROP of ε-CL and ATRP of St. Moreover, the good compatibility of ATRP and ROP mediated by organometallic compounds from a dual initiator has already reported in the literature [14].

In this paper, we report that a novel bifunctional initiator TCE is successfully employed in the synthesis of the PCL–PSt diblock copolymers by combining enzymatic ROP and ATRP. The $-CCl_3$ terminated PCL macroinitiator, resulted from TCE-initiated enzymatic ROP of ε-CL catalyzed by Novozyme 435, permits subsequent *block*-ATRP of St. The composition of the resulting block copolymer was confirmed by NMR, IR and GPC analysis. In addition, self-assembly of the diblock copolymer PCL-*b*-PSt into polymeric nanospheres in aqueous media was also studied by virtue of DLS and tapping mode AFM.

2. Experimental section

2.1. 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 (bpy, 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.

2.2. Analytical methods

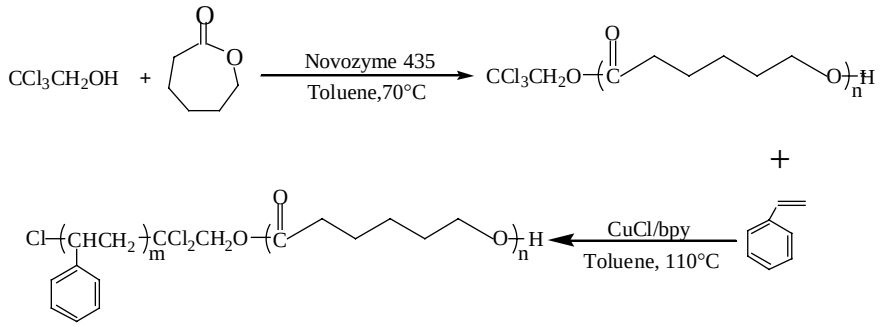
The monomer conversion was determined gravimetrically. Measurements of nuclear magnetic resonance (NMR) spectra were conducted on a Bruker ARX-500 NMR spectrometer with $CDCl_3$ as solvent, operating at 500 and 125 MHz for the corresponding ^1H and ^{13}C nuclei. Chemical shifts (in parts per million, 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 (300 mm×7.8 mm) using linear polystyrene standards. THF was used as the eluent at a flow rate of 1 mL/min. The infrared spectra (IR) of polymers was recorded on a NICOLET Impact 410 at room temperature. Dried samples (20 mg) were mixed with 100 mg of dry KBr and pressed into disk (100 kg cm^{-2}). Mean diameter and size distribution of the prepared PCL-*b*-PSt nanospheres were determined by the dynamic light scattering (DLS) method using a Brookheaven BI9000AT system (Brookheaven Instruments Corporation, USA). Each measurement was carried out in triplicate at 25 °C at a scattering angle of 90°. The atomic force microscopy (AFM) observations of the surface were carried out with the commercial instrument (Digital Instrument, Nanoscope IIIa, Multimode). All the tapping mode images were taken at room temperature in air with the microfabricated rectangle crystal silicon cantilevers (Nanosensor). The topography images were obtained at a resonance frequency of approximate 365 kHz for the probe oscillation.

2.3. TCE-initiated enzymatic ROP of ε-CL

Predetermined amounts of Novozyme-435 (0.108 g, 5% w/w of the monomer weight), vacuum dried in a desiccator with phosphorus pentoxide as desiccant (0.1 mmHg, 25 °C, 24 h), 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-caprolactone (2.156 g, 1.89×10^{-2} mol), 2,2,2-trichloroethanol (0.14 g, 9.4×10^{-4} mol) and solvent toluene (4.3 mL, 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 °C) oil bath with magnetic stirring for a predetermined time period. Aliquots were removed from the reaction mixture at selected time intervals to monitor the reaction progress. 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.

2.4. ATRP of St from $-CCl_3$ terminated PCL

Solid reagents CuCl (0.006 g, 6×10^{-5} mol) and bpy (0.028 g, 1.8×10^{-4} mol) were added into a toasted flask containing macroinitiator PCL (0.06 g, 6×10^{-6} mol). The reaction flask was sealed and immersed in ice water/NaCl mixture at about −10 °C and degassed by vacuum-argon for three times to remove the oxygen. Monomer styrene (0.686 g, 6.6×10^{-3} mol) and solvent toluene (0.76 mL) degassed by inert dry argon were introduced into the flask via a syringe under argon. After PCL macroinitiator was completely dissolved, the reaction flask was heated at 110 °C under sufficient stirring for a predetermined time. Aliquots were removed from the reaction mixture at selected time intervals to monitor the reaction progress. Finally, the polymerization reaction was terminated in an ice bath. The polymer solution was poured into methanol.



Scheme 1. Synthesis of AB-type diblock copolymer PCL-*b*-PSt.

The solid was collected after filtration and dried in a vacuum oven overnight.

2.5. Formation of nanospheres

The diblock copolymers PCL-*b*-PSt (1 mg) were first dissolved in 1 mL of distilled tetrahydrofuran (THF). The blue tint solution appeared when water (50 mL) was then added into the above solution, indicating the formation of nanospheres. After the mixture was sonicated for about 1 h at room temperature. About 1 μL drop of the nanosphere solvent was put onto the surface of the silicon wafer with a burette. The silicon wafer covered with nanospheres was placed into a desiccator to remove the solvent (H_2O), and then analyzed by means of DLS and AFM.

3. Results and discussion

Bifunctional initiator TCE contains a single primary alcohol group to initiate enzymatic ROP and an activated trichloromethyl group, an effective initiating group for ATRP [15,16], which replaces the α-bromoester group employed in previous reports [13]. Thus, the $-CCl_3$ terminated PCL, 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 without an intermediate transformation step (Scheme 1). Enzymatic ROPs of various cyclic lactones initiated by different kinds of initiators (e.g. H_2O, alcohol and amine) have been extensively investigated [1,2], however, it is not yet reported that TCE initiates enzymatic ROP of lactones, so the feasibility of TCE as the initiator in enzymatic ROP must be validated first. Wherein we chose the most active biocatalyst Novozyme 435 to carry out TCE-initiated ROP of ε-CL at 70 °C in toluene (twice v/w of the monomer weight).

Since, water is an effective initiator for enzymatic ROP, there is the possibility of competitive initiation between water and the hydroxyl group of initiator TCE. Hence, it is necessary to dry the reagents thoroughly in order to minimize water initiation. In addition to the anhydrous conditions, the optimization of reaction time is also equally important, because Lipase can also catalyze transesterification reaction. Based on this reason, our group carried out the kinetic study of enzymatic ROP of ε-CL initiated with TCE.

Fig. 1 shows the linearity between the semi logarithmic plot $\ln([M]_0/[M]_t)$ versus the reaction time indicating that the polymerization is first-order with respect to monomer. A plot of the GPC-determined number average molecular weight (M_n), theoretical molecular weight ($M_{n\ th}$) and polydispersity index (M_w/M_n) versus the monomer conversion is shown in Fig. 2. It is obvious that M_n increases linearly with conversion as the reaction progress, however, M_n starts deviating from the linear relationship and then reaches a plateau value (about 12,000 g/mol) when the conversion exceeds about 80%. The polyester polydispersity varies little over the polymerization giving relatively low values, less than 1.55. In addition, the theoretical molecular weights ($M_{n\ th}$) are much lower than the experimental values (M_n), which may be attributed to low efficiency of initiation (Table 1) due to the speedy volatilization of initiator TCE at the initial stage of ROP at 70 °C. The conclusions obtained from Figs. 1 and 2 indicate that Novozyme 435-catalyzed ROP of ε-CL initiated with TCE approaches to 'controlled' polymerization.

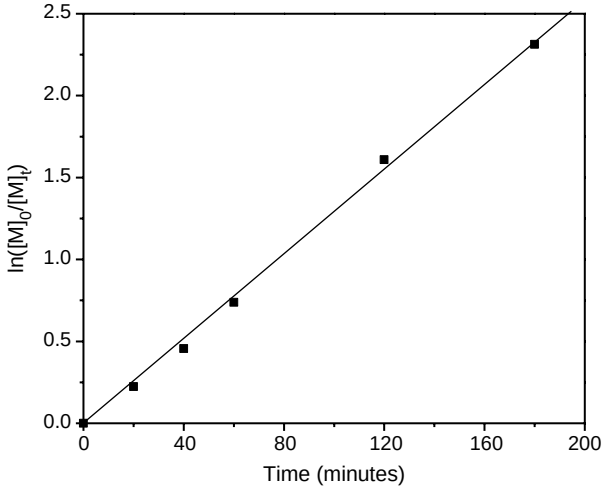


Fig. 1. $\ln([M]_0/[M]_t)$ as a function of time for enzymatic ROP of ε-CL initiated by TCE. $[M]_0$ and $[M]_t$ represent the initial monomer concentration and the monomer concentration after time *t*, respectively. The initial composition of the reaction mixture is given in Section 2.

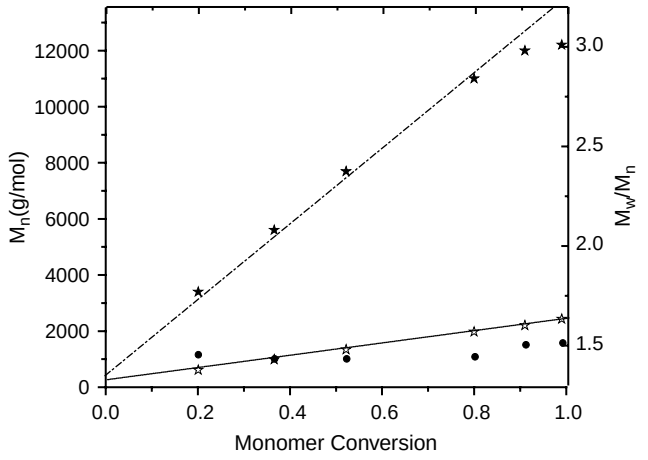


Fig. 2. Evolution of M_n (★), $M_{n\ th}$ (☆) and polydispersity index (M_w/M_n, ●) with monomer conversion in enzymatic ROP of ε-CL initiated by TCE. The theoretical molecular weights ($M_{n\ th}$) were calculated from Eq. (1) (Table 1). The initial composition of reaction mixture is given in Section 2.

Gross et al. firstly reported that butylamine initiated ROP of ε-CL by porcine pancreatic lipase catalysis shared many features of living polymerization [17]. However, only low M_n values were obtained at this system, the effect of chain transfer on enzymatic ROP was not observed. Thus, they made suggestions that this effect perhaps be investigated by analyzing the results from higher M_n products, which was validated by our research results. It is believed that the results from Fig. 2 are mainly ascribed to the nature of lipase, whose catalysis enables the simultaneous existence of chain propagation reactions and polymer degradation reactions. In the initial course (0–120 min) of the reaction, chain propagation reaction is dominant relative to transesterification reaction, therefore, the former values masks the latter, which results in the linearity of M_n as a function of conversion. When most of the monomer is consumed at high conversion (>80%, 120 min later), enzymatic transesterification reaction (including hydrolysis resulting from the fact that water reacts with the intrachain esters) may occur at a greater rate due to gradually increasing of polyester molecular weight. As a result, when the monomer conversion progresses to about 80%, M_n deviates from the linearity. It is concluded that at high conversion (after 120 min), enzymatic transesterification reaction can lead to a decrease in the mole fraction of the trichloromethyl end group and an increase in carboxylic acid chain end, so we choose 120 min as the optimal reaction time of TCE-initiated enzymatic ROP of ε-CL.

TCE has been approved an effective ATRP initiator [15]. Thus, the resulting PCL **1** (Table 1) must be precipitated using methanol/chloroform to remove residual TCE prior to ATRP. Based on comparison with GPC analysis before and after purification, it is found that M_n remains invariable, but a lower polydispersity (from 1.43 to 1.36). The ^{1}H NMR spectrum (Fig. 3) of PCL **1** is carefully examined to determine whether initiator TCE is successfully attached to PCL chains or not. The multiplet signals, centered at 1.4, 1.6, 2.3 and 4.1 ppm, are assigned to the PCL main chain protons **d**, **c** **e**, **f** and **b**, respectively. The triplet signal **a** at 3.65 ppm corresponds to the methylene protons attached to the terminal hydroxyl group. It is of importance 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 4.75 ppm, moreover, the ratio of integrated areas of peaks **a** and **g** is close to 1.00, which clarifies enough >98% of polymer terminals are occupied by the initiator segments.

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 in the region of about 2.35 ppm, corresponding to the methylene protons linking to terminal carboxyl acid, suggests that the mole percentage of water-initiated PCL without 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 177 ppm corresponding to the carbon atom of the

Table 1
Results of macroinitiator PCL **1** and block copolymers PCL-*b*-PSt **2**, **3**

PCL	$[M]_0/[I]_0$	Mol% carboxyl terminal chains[a]	Monomer conv.[b]	$M_{n\ th}$[c]	$M_{n\ nmr}$[a]	EI[d]	$M_{n\ GPC}$[e]	M_w/M_n[e]
1	20/1	<2%	83%	2050	8500	24%	10,700	1.36
Copolymer	$[M]_0/[I]_0$	Time (h)	Monomer conv.[b] (%)	$M_{n\ th}$[c]	$M_{n\ nmr}$[a]	CL/St[f]	$M_{n\ GPC}$[e]	M_w/M_n[e]
2	1100/1	6	4.9	16,100	14,100	76/33	14,200	1.33
3	1100/1	11	8.7	20,400	16,500	76/56	17,033	1.30

[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 initial monomer concentration to the initial initiator concentration $[M]_0/[I]_0$ and the monomer conversion.

$$M_{n\ th} = ([M]_0/[I]_0)M_{monomer}\text{con.-\%} + M_{n\ (macro)initiator} \quad (1)$$

[d] EI represents 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.

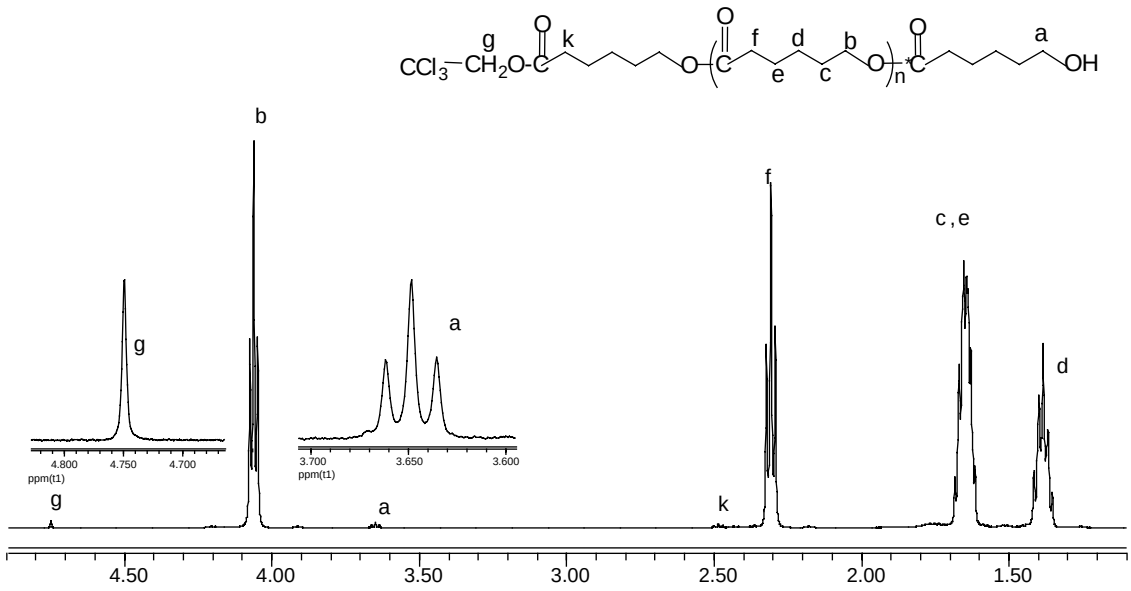


Fig. 3. The ^{1}H NMR spectrum of the –CCl_3 terminated PCL (8.5×10^3 g/mol, $M_{n\ nmr}=(I_{4.05}/I_{3.65})M_{\varepsilon\text{-CL}}+M_{TCE}$), $M_{n\ nmr}$ is calculated from the ^{1}H NMR integrated peak area **I** of peak at 4.05 and 3.65 ppm, $M_{\varepsilon\text{-CL}}$ and M_{TCE} represents the molecular weight of ε-CL and TCE, respectively.

terminal carboxylic acid. Combining GPC analysis (Fig. 4), the fact that the resulting –CCl_3 terminated PCL shows a unimodal and symmetrical trace in the GPC also proves this conclusion. It is clear that the calculated molecular weight ($M_{n\ nmr}$) of PCL **1** (8500 g/mol) based on the ^{1}H NMR spectrum is observed to be about 20% lower than that (10,700 g/mol) determined by GPC. The discrepancy most likely results from the GPC analysis, in which polystyrene is used for calibration.

Halogenated alkanes, such as R–CCl_3/R–CBr_3 derivatives, have been employed successfully as initiating species in the ATRP of styrene and (meth)acrylates [14–16]. No evidence suggesting double or triple initiation at the CCl_3 terminus was observed in previous studies of –CCl_3 macroinitiator, it is implicitly accepted that the terminal groups are single initiator units, as proved by unimodal and symmetrical GPC traces of the resulting block copolymers [15]. Thus, our group carried out the ATRP of St from the –CCl_3 terminated PCL **1** using CuCl/bpy as the catalyst system and toluene as the solvent, respectively, at 110 °C according to Scheme 1.

Illustrated in Fig. 5 is the variation in the molecular weight (M_n and $M_{n\ th}$) and polydispersity (M_w/M_n) as a function of monomer conversion for ATRP with its initial composition in Section 2. M_n increases linearly with conversion while the polydispersity index varies only a few degrees, from 1.36 to 1.25. Lower polydispersity may be due to the inevitable fractionation of copolymer during the course of precipitation by pouring copolymer solution into methanol to remove ATRP catalysts. The $M_{n\ th}$ values are higher than the experimental, resulting from the GPC technique underestimating M_n because the PCL segments decrease hydrodynamic volumes compared to PSt homopolymer. The linear relationship implies that the –CCl_3 terminated PCL does indeed initiate the controlled radical polymerization of St.

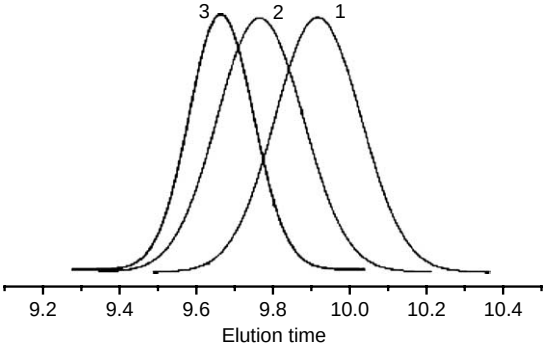


Fig. 4. GPC traces of TCE-initiated PCL **1** ($M_n=1.07\times10^4$ g/mol, polydispersity = 1.36), AB-type diblock copolymers PCL-*b*-PSt **2** (1.42×10^4 g/mol, 1.33) and PCL-*b*-PSt **3** (1.70×10^4 g/mol, 1.30). The molecular weight and polydispersity were determined by GPC calibrated with polystyrene.

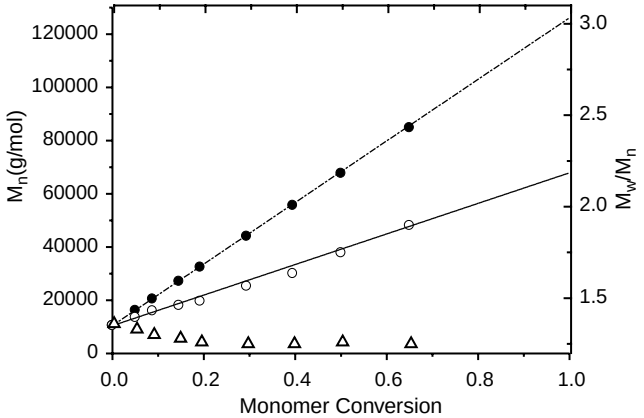


Fig. 5. Dependence of M_n (○), $M_{n\ th}$ (●) and polydispersity index (△) on monomer conversion for ATRP of styrene using PCL macroinitiator.

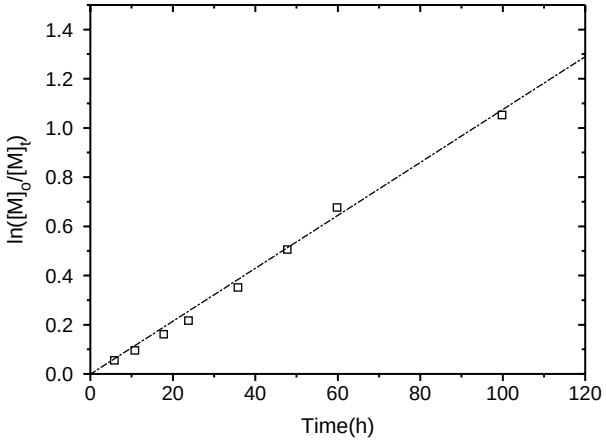


Fig. 6. Relationship between $\ln([M]_0/[M]_t)$ and polymerization time for ATRP of St using PCL macroinitiator.

Fig. 6 plots the time dependence of $\ln([M]_0/[M]_t)$. The linear relationship indicates that the polymerization is first-order with respect to monomer and that the concentration of active species remains constant throughout the reaction. The kinetic behavior of ATRP indicates that polymerization of St is a 'living'/controlled radical process.

It is obvious that the ATRP of St from macroinitiator **1** results in an increase in molecular weight and a slight decrease in polydispersity. Our goal is to synthesize diblock copolymers with relatively short PSt block lengths for the preparation of nanospheres; hence ATRP is terminated prior to high conversion. Because of the relative low activity of the $-CCl_3$ group, ATRP of St from it is much slower in comparison with that of St initiated by the α-bromoester group. For example, as shown in Table 1 it takes 11 h at 110 °C to reach only 8.7% monomer conversion. As shown in Fig. 4, the unimodal and symmetrical shape of the peaks on the GPC plots of the diblock copolymers **2**, **3** (Table 1) suggests the absence of a homopolymer composed of St or ε-CL. In the case, the unwanted PSt homopolymer results from subsequent ATRP of unreacted initiator TCE during enzymatic ROP, which can be effectively removed during the precipitation step of macroinitiator **1**, furthermore, water-initiated PCL homopolymer can also be reduced to a minimum by carefully drying of the starting reagents as mentioned above. Therefore, according to the GPC analysis, it is concluded that the ATRP initiation of St exclusively occurred from macroinitiator **1**.

From the ^{1}H NMR spectrum of the diblock copolymer PCL-*b*-PSt (Fig. 7), it is observed that in addition to the dominant PCL signals, the occurrence of the signals at 6.3–7.3 ppm corresponding to aromatic protons **D** and **E** of the PSt blocks shows further that new well-defined PSt segments are connected with PCL **1**, furthermore, the increase in the integral aromatic peak area with reaction time indicates the progressive incorporation of St into the copolymer.

Fig. 8 shows the respective IR of the PCL macroinitiator and the diblock copolymer PCL-*b*-PSt. For PCL macroinitiator, the characteristic absorption bands appear in the wavenumber region of 1740 cm^{-1} assigned to the ester carbonyl group of the PCL main chains. After ATRP of St from PCL macroinitiator, besides the PCL peaks the most readily quantifiable changes in the IR spectrum used as the indicative of successful ATRP initiation are the occurrence of the new

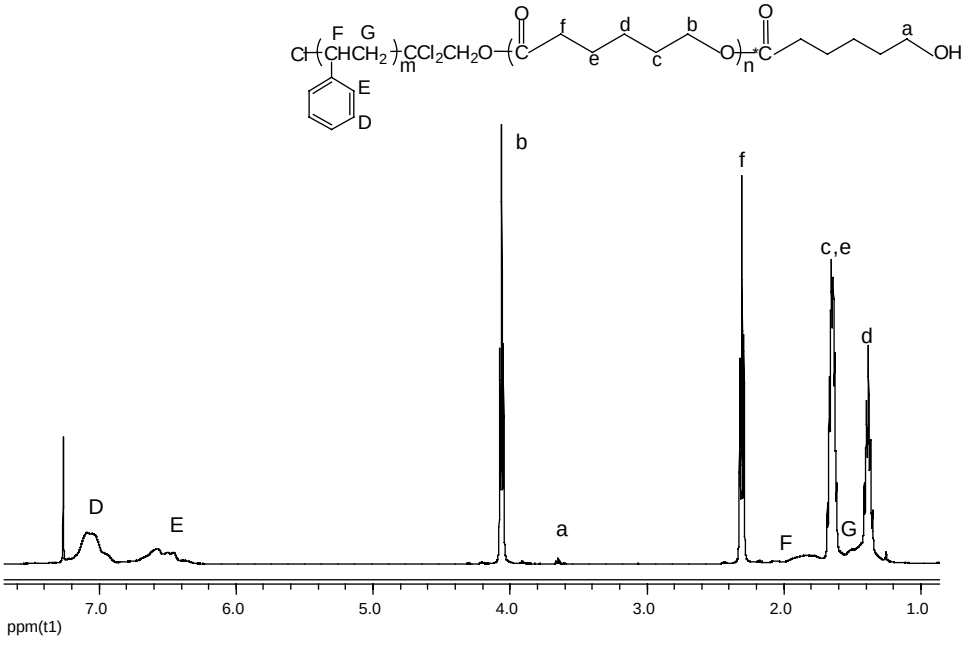


Fig. 7. The ^{1}H NMR spectrum of the AB-type diblock copolymer PCL-*b*-PSt ($M_{n\ nmr2}$ = 14,100 g/mol and $M_{n\ nmr3}$ = 16,500 g/mol). $M_{n\ nmr} = (I_{6.2-6.8}/I_{3.65})M_{St} + M_{n\ (macro)initiator}$. The molecular weight ($M_{n\ nmr}$) is calculated from the ^{1}H NMR integrated peak area **I** of peak at 6.2−6.8 and 3.65 ppm, M_{St} and $M_{n\ (macro)initiator}$ represents the molecular weight of St and macroinitiator, respectively.

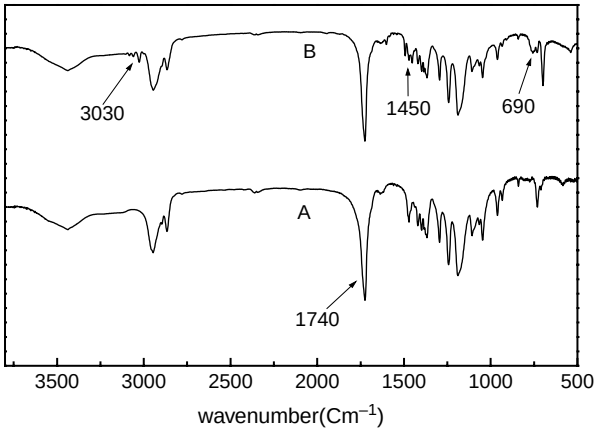


Fig. 8. IR spectra of $-CCl_3$ terminated macroinitiator PCL (A) and its diblock copolymer PCL-*b*-PSt (B).

Table 2
AFM diameters and hydrodynamic diameters of polymeric nanosphere

Copolymer[a]	AFM diameter (nm)	Hydrodynamic diameter (nm)	Dispersity from DLS
2	60	280	0.11
3	90	350	0.13

The preparation of the polymeric nanosphere is given in Section 2.
[a] The composition of the diblock copolymers is shown in Table 1.

absorption bands at the wavenumbers of about 3030, 1450 and 690 cm^{-1} ascribed to the ring vibration of the aromatic group of PSt. The variance of the IR spectroscopic results confirms the formation of the PSt blocks, in agreement with the structure of AB-type diblock copolymers as expected.

The well-defined PCL–PSt diblock copolymer has a hard PSt block and a soft PCL block, they can self-assemble in aqueous solution to form polymeric nanospheres through molecular interaction. Formation of PCL-*b*-PSt nanospheres in aqueous solution was judged from the bluish tinge of such a solution. In H_2O/THF with 2% THF, the average hydrodynamic diameters for the diblock copolymers **2** and **3** nanospheres were determined by DLS to be 280 and 350 nm, respectively (Table 2).

AFM can be used to take measurements of size and size distribution in the solid state, which eliminates perturbation caused by solvent swelling. By taking cross section through the three-dimensional AFM height image, it is observed in Fig. 9 that the polymeric nanospheres with monodispersed size are distributed over the silicon surface. The influence of the diblock copolymer composition upon nanosphere dimensions is also investigated. Alteration of the relative content ratio of PSt to PCL for diblock copolymer has a dramatic effect on the size of nanospheres. Increase of the PSt/PCL ratio will result in increased polymer chain aggregation numbers in the copolymer nanospheres to make its diameter augment. For example, diblock copolymer **3** has the same PCL length as diblock copolymer **2** but a longer PSt block, as a result, larger average particle diameter (about 90 nm) for diblock copolymer **3** is obtained by tapping mode AFM in comparison to that (about 60 nm) for diblock copolymer **2** under the same conditions of nanosphere preparation due to the increase of PSt percentages. It is obvious that these hydrodynamic diameters from DLS are larger than the AFM diameters as shown in Table 2, because DLS and AFM measure the diameters of nanospheres in the solvent-swollen and dry states, respectively.

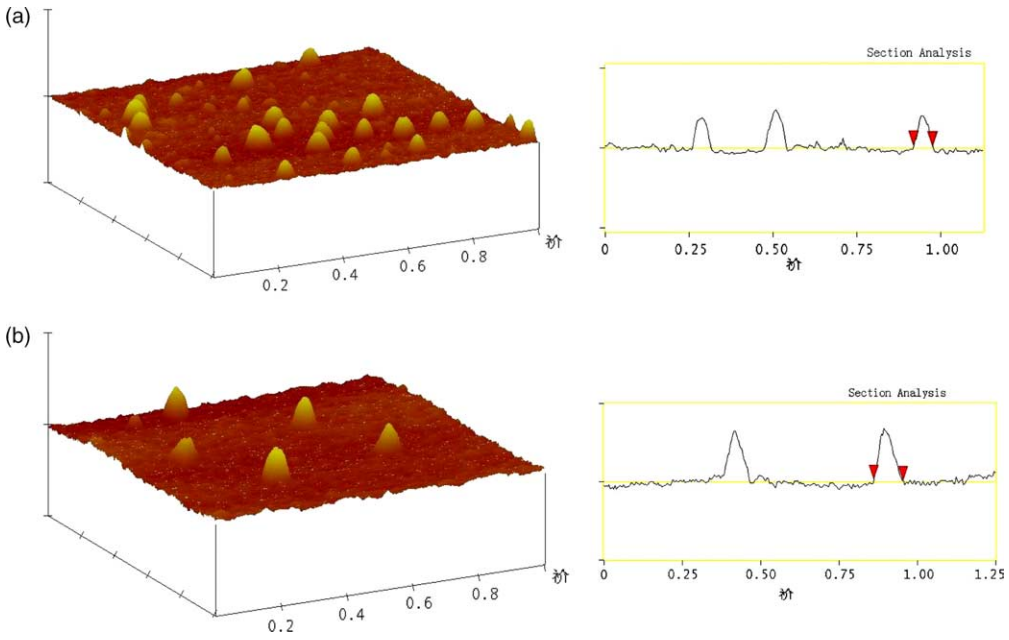


Fig. 9. Three-dimensional height images and the cross section of nanospheres formed by self-assembly of the PCL–PSt diblock copolymers **2** (a) and **3** (b) on the silicon surface.

4. 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 optimal reaction time is chosen according to the kinetic investigation of enzymatic ROP under anhydrous conditions in order that at least 98% of PCL chains are terminated with the $-CCl_3$ groups. The resulting $-CCl_3$ terminated PCL macroinitiator effectively initiates ATRP of St to obtain the diblock copolymers PCL-*b*-PSt without an intermediate transformation step. The unimodal and symmetrical shape of the trace on GPC and the structure determined by means of NMR and IR testify the successful synthesis of the diblock copolymers. It is concluded from DLS and AFM that the polymeric nanospheres prepared by the self-assembly of the PCL–PSt diblock copolymers have a mean diameter and a spherical shape. Otherwise, the experiments aiming at cascade copolymerization from TCE by one-pot approach are in progress.

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