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Biocatalytic Synthesis of Multi-block Copolymer Composed of Poly(tetrahydrofuran) and Poly(ethylene oxide)†

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Multi-block copolymers consisting of poly(tetrahydrofuran) (PTHF) as the hydrophobic part and poly(ethylene oxide) (PEO) as the hydrophilic part were synthesized using lipase-catalyzed polymerization. The self-assembly micelle of the synthesized copolymer in the presence of water was investigated using fluorescence spectroscopy and dynamic light scattering. Fluorescence spectroscopy measurements suggested that the copolymers were associated in water to form polymeric micelles, and the critical micelle concentrations (CMC) value of the block copolymers was 0.0005–0.005 mg/ml.

Keywords enzyme polymerization, polymeric micelle, amphiphilic polymer

Introduction

Due to their relevance to biological systems and industrial applications, self-association phenomena for hydrophobically modified water-soluble polymers in aqueous system are of current scientific and technological interest (1–5). PEO-based copolymers have been widely studied and used for making nonionic macromolecular surfactants, as well as amphiphilic polymers. These types of polymers find widespread industrial applications in dispersion stablitization, foaming, emulsification and lubrication (6, 7). One of the most promising applications of PEO-based copolymers is for drug delivery systems (DDS) (1). PEO-based copolymers with ester linkage have many advantages over other existing systems: they are nontoxtic, biocompatible (8), biodegradable (9, 10), and additionally they are soluble and inexpensive, and easily tailored into a variety of copolymers. Recently, some of the research activity has been focused on PEO-PPO copolymers, but little attention was paid to the PEO-PTHF copolymer (11–13).

†Dedicated to the memory of Professor Sukant K.Tripathy

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Herein, we focus on the reaction between PTHF and PEG of various chain length catalyzed by Novozyme-435, an immobilized lipase from Candida Antarctica. Compared to conventional chemical synthesis, the enzyme catalyzed polymerization provides several advantages, such as: (i) catalysis under mild reaction conditions with regard to temperature, pressure, and pH, which often lead to energy efficiency and environmentally friendly reaction condition; (ii) high enantio-, regio-, and chemoselectivities; (iii) Toxic heavy metal catalyst can be avoided and in some cases, reactions can be carried out without the use of solvents (14–16). Therefore, the enzymatic polymerizations can be regarded as "green polymer chemistry"

In this work, we will focus on the synthesis of amphiphilic PTHF-PEG block copolymer and their self-assembly properties in the presence of water.

Experimental

Materials

Novozyme-435, an immobilized enzyme, was purchased from Sigma Co. All other chemicals and solvents were of analytical grade and used as received unless otherwise noted. Poly(tetrahydrofuran) (Mn = 1,000), diethyl malonate, poly(ethylene glycol)-600, PEG-900, PEG-1500, PEG-3400 were purchased from Aldrich Chemical Co.

Characterization

Gel permeation chromatography (GPC) was used to determine the molecular weights and the molecular weights distributions of the samples.

^{1}H-NMR was recorded on a 200 MHz (Bruker ACF200) spectrometer using $CDCl_3$ as a solvent unless otherwise stated. Infrared spectra were recorded on a Perkin-Elmer 1760 FTIR spectrometer. A thin film was cast on a KBr disk from the copolymer solution in chloroform.

Synthesis of Monomer 3

PTHF-1000 (**1**, 1.0 mmol) and diethyl malonate (**2**, 10 mmol) were placed in a round-bottom flask, and to this mixture was added the enzyme (10 wt% of monomers). The flask was then placed in a constant temperature oil bath maintained at 60°C under vacuum. The reaction was allowed to proceed for 24 h, then, the reaction was quenched by adding chloroform and filtering off the enzyme. The filtrate was evaporated at 100°C under vacuum, and the unreacted monomer **2** was removed. Monomer **3** was obtained as a viscous liquid.

Polymerization

An equimolar amount of monomer **3** and monomer **4** (PEG-600, PEG-900, PEG-1500 or PEG-3400) were placed in a round-bottom flask, and to this mixture was added the enzyme (10 wt% of monomers). The flask was then placed in a constant temperature oil bath maintained at 60–70°C under vacuum. The reaction was allowed to proceed for 48–72 h; after that the reaction was quenched by adding chloroform and filtering off the enzyme. The filtrate was concentrated and dialyzed using a membrane (MWCO 3000). Polymer **5** was obtained as a semisolid after dialysis.

Sample Preparation for CMC and Light Scattering Experiments

Stock solutions were prepared by dissolving the multi-block copolymers in distilled water, filtered through a 0.2 μm membrane filter. A series of polymer solutions with different concentration was prepared by dilution. After filtering, the samples were allowed to stand overnight at room temperature to equilibrate.

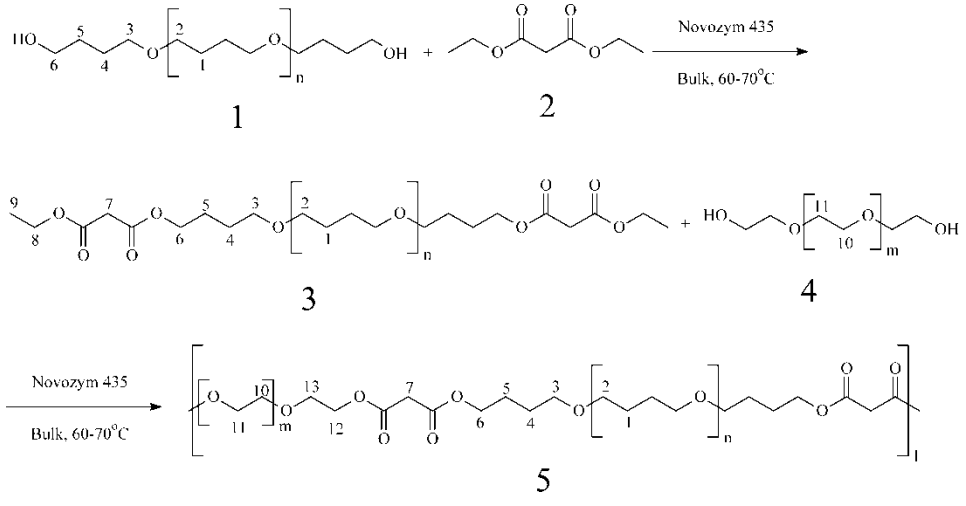
Determination of Critical Micelle Concentration (CMC)

Fluorescence measurements were performed using a luminescence spectrometer LS55 (Perkin-Elmer Instrument.) with a thermostated cell. Pyrene was used as a fluorescence probe. 1 ml of pyrene solution in THF (1.2×10^{-3} M) was added to 1 L distilled water. THF was removed by a rotavapor at 30°C for 3 h, and the resulting pyrene solution in water (1.2×10^{-6} M) was used for a fluorescence probe. The pyrene concentration in a block copolymer solution was 6.0×10^{-7} M. For the measurement of the pyrene fluorescence spectra, scan speed and scan accumulation number were set at 100 nm/min and 2, respectively.

Results and Discussion

Synthesis of PEG-PTHF Block Copolymer

The condensation reaction of monomer **3** and PEG-600 (or PEG-900, PEG-1500 and PEG-3400) catalyzed by novozyme-435 under solventless condition gave polyester **5** with 82% yield (Scheme 1). Figure 1 shows ^{1}H-NMR spectra of monomer 3 and polymer **5**. Comparison of the ^{1}H-NMR spectra of monomer **3** and polymer **5**, the resonance peak at $\delta = 1.21$ ppm disappeared after reaction. The chemical shift of C-8 proton and C-6 proton in monomer 3 are very close and their peaks are overlapped because the C-8 proton and C-6 proton have a similar chemical environment. After reaction, the $C^9H_3C^8H_2$- group is replaced by -O $C^{13}H_3C^{12}H_2$- group, so the chemical shift of C-12



Scheme 1. Biocatalytic synthesis of PEG-PTHF block copolymer.

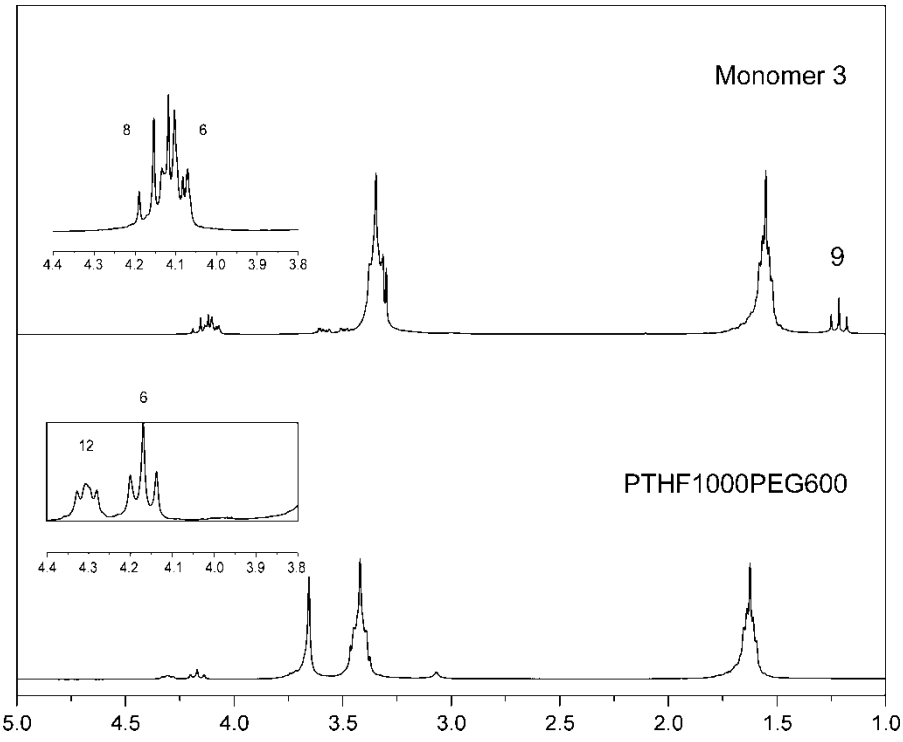


Figure 1. ^{1}H-NMR spectrum for monomer 3 and polymer 5.

proton moves to downfield. In the ^{1}H-NMR spectrum of polymer 5, the C-12 proton peak and the C-6 proton peak are completely separated and this confirmed the trans-esterification between the CH_2OH group of monomer **4** and the ethyl ester group of monomer **3**.

FT-IR

The FTIR spectra of PTHF1000, PTHF1000-PEG3400 and PTHF1000-PEG600 are shown in Figure 2. As a result of the reaction, the characteristic peaks arising from the PTHF-PEG copolymer can be easily identified if the spectrum for PTHF-PEG copolymer is compared with the spectrum of PTHF. The vibration band at 3470 cm^{-1}, which is attributed to the OH stretching band, nearly disappeared after the enzyme polymerization. The vibration band at ca. 1736 cm^{-1} is attributed to the stretching of C═O band, and this band is incorporated into the copolymer chain in the first step of the polymerization (Scheme 1), and the intensity of this vibration peak increases as the length of PEG segment decreases.

Fluorescence Experiment (17–20)

The fluorescence spectra of the PEG1500-PTHF1000 multi-block copolymer at different concentrations in the presence of 6×10^{-7} M pyrene are shown in Figure 3. The polymer solution is excited at 310 nm. At low pyrene concentration (6×10^{-7} M), there is no

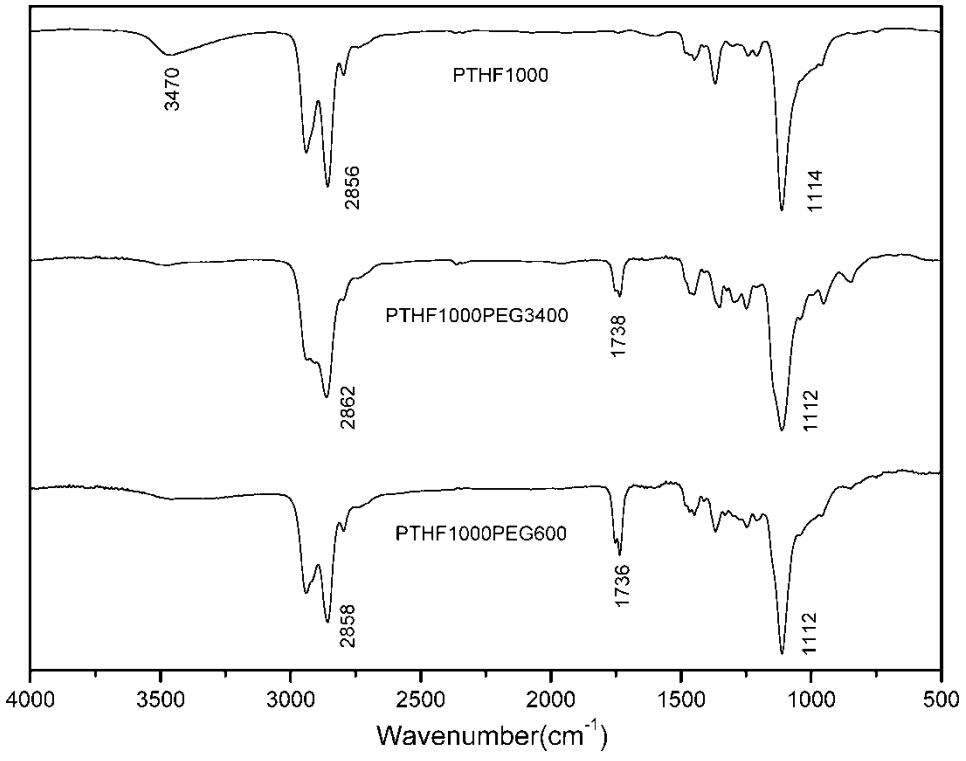


Figure 2. FTIR spectra of PTHF, PTHF-PEG600 and PTHF-PEG3400.

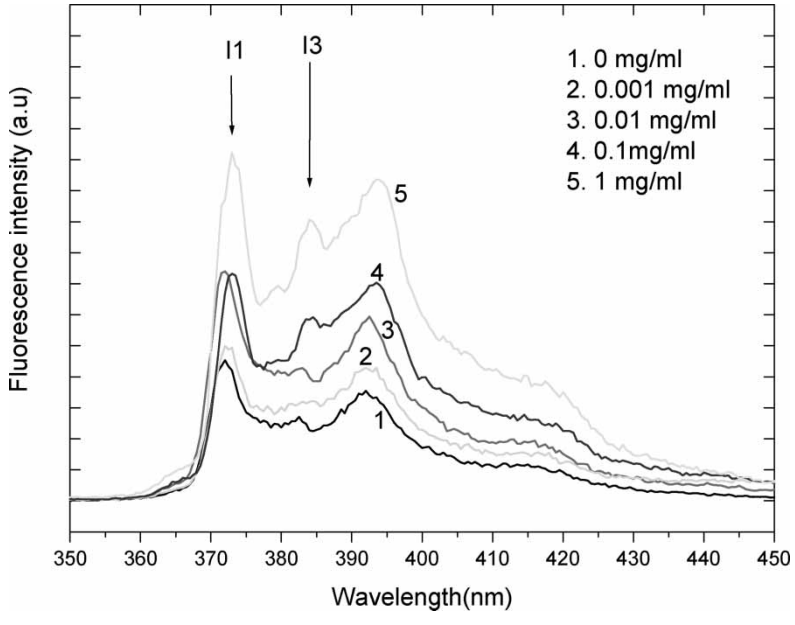


Figure 3. Fluorescence spectra of pyrene ($6 \times 10\text{-}7$ M) in the presence of increasing of concentrations of PTHF1000-PEG1500 copolymer sample.

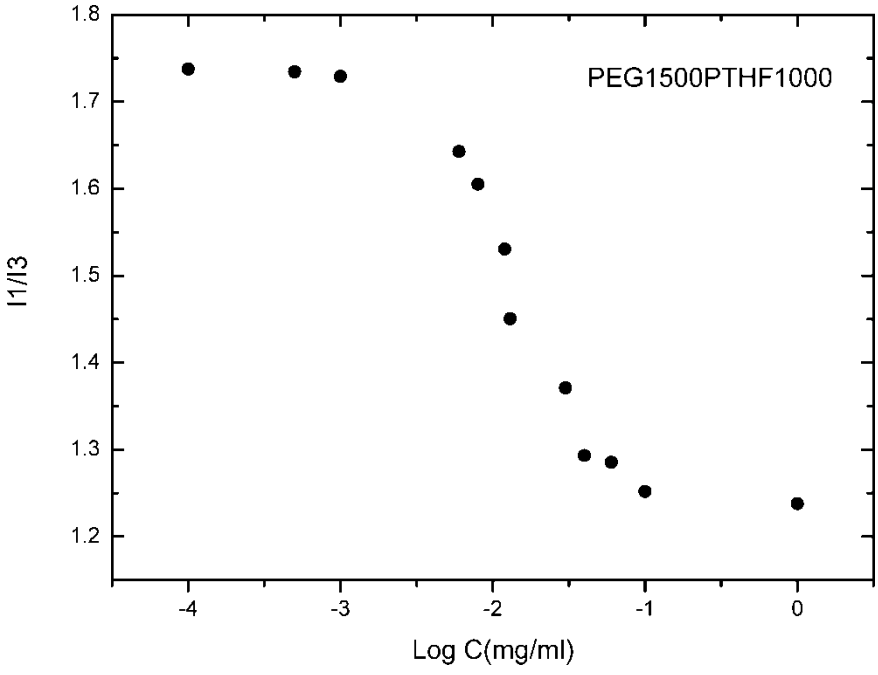


Figure 4. Plots of the ratio of the intensities (I1/I3) of the vibrational bands in the pyrene fluorescence spectrum as a function of polymer concentration for the sample shown in Figure 3.

significant fluorescence intensity at around 480 nm arising from the excimer, and the effect of excimer formation on the monomeric fluorescence of pyrene is negligible. One of the noteworthy features is the small change in the fluorescence intensity ratio of the first and third vibronic bands, I1/I3. The plot in Figure 4 shows the relation between I1/I3 peak height ratios and the polymer solution concentration. Below CMC, there are no micelles present and the I1/I3 ratio is around 1.73. However, as the polymer solution concentration increases above the CMC, the pyrene is solubilized in the hydrophobic interior of the micelles as illustrated by the decreased I1/I3 ratio. So the sharp decrease in the I1/I3 ratio indicates the onset of micellization at this concentration.

The micelle size was measured using Microtrac UPA150 dynamic light scattering equipment. Table 1 shows the summary of CMC and micelle size of block copolymer with various MW. From the data in Table 1, the CMC and micelle size of the copolymer increase with the increasing of the Mw of PEG block segment.

Table 1
CMC and micelle size of block polymer

	Mn	CMC	Micelle size
PTHF1000PEG600	13,600	0.0005 mg/ml	15.6 nm
PTHF1000PEG900	16,300	0.0017 mg/ml	17.1 nm
PTHF1000PEG1500	15,000	0.005 mg/ml	19.9 nm
PTHF1000PEG3400	18,400	0.011 mg/ml	22.1 nm

Conclusions

PTHF-PEG multi-block copolymers were synthesized through linking two monomers with hydroxyl groups using two-step enzyme-catalyzed polymerization. Structure of the copolymer was characterized by ^{1}H-NMR and FT-IR. The copolymers were self-associated in water to form polymeric micelles. The CMC value and micelle size increase with the increasing of PEG segment length.

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