

ABA TRIBLOCK COPOLYMERS WITH PENDANT HYDROXYL GROUPS PREPARED BY CONTROLLED CATIONIC POLYMERIZATION AND THEIR USE AS DELIVERY CARRIER FOR PACLITAXEL*

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Abstract To improve the hydrophilicity of poly(styrene-*b*-isobutylene-*b*-styrene) (SIBS), this study focuses on the synthesis of novel functional ABA triblock copolymer thermoplastic elastomers (TPEs) with polyisobutylene (PIB) as rubbery segments. The precursor poly{(styrene-*co*-4-[2-(*tert*-butyldimethylsiloxy) ethyl]styrene)-*b*-isobutylene-*b*-(styrene-*co*-4-[2-(*tert*-butyldimethylsiloxy)ethyl]styrene)}(P(St-*co*-TBDMES)-PIB-P(St-*co*-TBDMES)) triblock copolymer was first synthesized by living sequential cationic copolymerization of isobutylene (IB) with styrene (St) and 4-[2-(*tert*-butyldimethylsiloxy)ethyl]styrene (TBDMES) using 1,4-di(2-chloro-2-propyl)benzene (DiCumCl)/titanium tetrachloride (TiCl₄)/2,6-di-*tert*-butylpyridine (DtBP) as the initiating system. Then, P(St-*co*-TBDMES)-PIB-P(St-*co*-TBDMES) was hydrolyzed in the presence of tetra-butylammonium fluoride to yield poly{[styrene-*co*-4-(2-hydroxyethyl)styrene]-*b*-isobutylene-*b*-[styrene-*co*-4-(2-hydroxyethyl)styrene]} (P(St-*co*-HOES)-PIB-P(St-*co*-HOES)) with pendant hydroxyl groups. P(St-*co*-HOES)-PIB-P(St-*co*-HOES) used as the paclitaxel carrier was also investigated in this study. Comparing with SIBS, P(St-*co*-HOES)-PIB-P(St-*co*-HOES) has exhibited better compatibility with paclitaxel and higher release rate.

Keywords: Living cationic polymerization; Hydrophilicity; Miscibility; Paclitaxel.

INTRODUCTION

Biomaterials used in soft tissue replacement and reconstruction depend on their chemical structures^[1]. Polyurethane biomaterials are characterized by excellent mechanical and fatigue properties. However, they are subject to biodegradation, specifically, the *in vivo* hydrolysis of polyurethanes may lead to potentially toxic diamines^[2, 3]. Silicone rubbers are classified as “thermoset rubber”. They are based on poly(dimethylsiloxane), carrying functional groups for cross-linking (vulcanization) with peroxides or platinum. However, reinforced medical grade silicone rubber remains weak in comparison with other reinforced rubbers. PIB-PS has been introduced as a new soft biomaterials with mechanical properties bridging the range between polyurethane and silicone rubber^[4]. Moreover, PIB-PS exhibits superior oxidative, chemical and thermal stabilities due to saturated structure.

With the development of living cationic polymerization of isobutylene (IB) and styrenic monomers^[5–7], the synthesis of ABA triblock copolymer thermoplastic elastomers (TPEs) with polyisobutylene (PIB) as rubbery middle segments and styrenic polymers as plastic outer segments became possible. These ABA triblock copolymers with 10 wt%–40 wt% PS display thermoplastic elastomeric (TPE) properties, that is, they behave

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like crosslinked rubbers at room temperature, whereas melt at temperatures above the T_g of the glassy PS blocks and therefore can be processed like plastics. Because of the nanometer scale of the PS domains, these microphase-separated materials are macroscopically transparent. Transparency and processability are important considerations in medical-device manufacturing. In addition, PIB-PS TPEs have very low permeability, outstanding aging resistance, high damping and excellent flex-fatigue properties as compared with polybutadiene- or polyisoprene- based block copolymer TPEs^[8]. Puskas *et al.* have proved that SIBS has good biocompatibility, with no adverse biological effects^[9]. SIBS has been employed as the drug carrier matrix for the TAXUS Express Paclitaxel-Eluting Coronary Stent system^[4] and approved as a coating on drug eluting cardiovascular stents by FDA^[10].

Paclitaxel (PTx) is a very good drug preventing cardiovascular events and has been applied in coronary artery restenosis treatment^[11]. PTx contains several sites suitable for hydrogen bonding, including ester, amide and hydroxyl functionalites. However, hydrophilic paclitaxel are incompatible with hydrophobic PIB-based TPEs. Faust and co-workers recently reported on the synthesis of poly(*p*-hydroxystyrene-*b*-isobutylene-*b*-*p*-hydroxystyrene) (PHOS-*b*-PIB-*b*-PHOS), which have good compatibility with paclitaxel by introduction of polar groups^[4]. The main objective of present study is to synthesize a novel hydroxyl functional PIB-based TPEs by living sequential cationic copolymerization. The miscibility of PTx with PIB-based TPEs and release rate of PTx were also discussed in this paper.

EXPERIMENTAL

Materials

Titanium tetrachloride (TiCl₄) (Beijing Chemical Co., 99.9%), 1,1-diphenylethylene (DPE) (Alfa, 98%), 2,6-di-*tert*-butylpyridine (DtBP) (Alfa, 97%) were used. 1,4-Di(2-chloro-2-propyl)benzene (DiCumCl) was prepared from the 1,4-di(2-hydroxy-2-propyl)benzene (reactive with dichloro-sulfoxide at ice water temperature under nitrogen atmosphere in methylene chloride (MeCl₂)), the resulting DiCumCl was recrystallized three times with hexane at low temperature^[12]. The preparation of 4-[2-(*tert*-butyldimethylsiloxy)ethyl]styrene (TBDMES) has been described^[13]. Styrene (St, Yanshan Petrochemical Co., 99.9%) was freshly vacuum distilled from calcium hydride before use. Methyl chloride (MeCl, Yanshan Petrochemical Co., 99.99%) and isobutylene (IB, Yanshan Petrochemical Co., 99.99%) were dried in the gaseous state by passing them through in-line gas-purifier columns packed with CaSO₄/drierite. They were condensed in the cold bath of a glovebox prior to polymerization. Methylcyclohexane (MeChx, Aldrich, anhydrous grade), anhydrous methanol (MeOH, 99.8%) and *tetra*-butylammonium fluoride (Alfa, 1.0 mol/L solution in tetrahydrofuran) were used as received. Anhydrous crystalline paclitaxel (PTx, Beijing Norzer Pharmaceutical Co. Ltd, 99.5%) was used as received.

Synthesis of P(St-co-TBDMES)-PIB-P(St-co-TBDMES)

All polymerizations were carried out under a dry nitrogen atmosphere in a stainless steel glove-box using the CH₃Cl/MeChx (50/50, *V/V*) solvent mixtures. IB was first polymerized in the DiCumCl/TiCl₄/DtBP/−80°C initiating system for 90 min and then DPE stock solution was added. After 60 min, St/TBDMES mixture solution (70/30, *W/W*) was added. The polymerization was terminated by prechilled methanol after 3.5 h. After the evaporation of volatiles, the polymer was dried in a vacuum oven at 30°C to a constant weight.

Hydrolysis

P(St-co-TBDMES)-PIB-P(St-co-TBDMES) (1.0 g) was dissolved in 100 mL THF solvent, *tetra*-butylammonium fluoride (5 mL, 1.0 mol/L in THF) was added, and the solution was stirred at 45°C. After 24 h, the solvent was cooled and precipitated into 300 mL water. The polymer was filtered off, dried in a vacuum and extracted with 30 mL hexane at room temperature. After the extraction, the polymer was dried again in a vacuum.

Measurements

Molecular weights and molecular weight distribution (MWD) were measured with a size-exclusion chromatography system equipped with a model 510 HPLC pump, a model 410 differential refractometer, a model 441 UV/Visible detector, an online multiangle light scattering (MALLS) detector (Minidawn, Wyatt

Technology Inc.) and four ultra-styragel GPC columns connected in the following series: 500, 10^3 , 10^4 and 10^5 . Samples were eluted in tetrahydrofuran (THF) at a flow rate of 1.0 mL/min at room temperature. The dn/dc values of triblock copolymers were calculated by assuming 100% recovery of the injected mass.

$^1\text{H-NMR}$ was used for the structural elucidation of block copolymer composition. $^1\text{H-NMR}$ spectra were obtained on a Bruker 400 MHz spectrometer using 5 mm tubes with sample concentrations of 50–100 g/L in CDCl_3 .

Thermal transitions of the copolymers were studied using a TA DSC Q2000 analysis system and analyzed with TA Universal Analysis software. The samples were heated to 200°C , quenched to -90°C , and reheated to 200°C at a rate of 10 K/min.

Thermogravimetric analysis of the copolymers was studied using a TA TGA Q500 analysis system and analyzed with TA Universal Analysis software. The samples were heated to 500°C at a rate of 10 K/min.

Phase separation structures of the polymers were measured using an AFM analysis system (SPM-9500J3, Shimadzu Co., Japan) and then analyzed using a digital image processing software. Thin films of the block copolymer and the drug (PTx)-copolymer were prepared by spin coating 5 wt% solutions in toluene onto silicon wafers using a headway resist spinner at 6000 r/min for 30 s. Analyses were performed at 25°C .

The hydrophilic property was evaluated by measuring the contact angle of a water droplet on the films under an ambient condition of $5\text{--}12^\circ\text{C}$ in air atmosphere. Contact angle measurements were made in air using the sessile-drop method. A droplet was injected on the surface using 1 μL micro-injector. Assuming that the geometry of the sessile drop is a spherical section, the contact angle could be estimated by measuring the spreading diameter of the contact circle using a reading microscope.

The drug release of the drug/copolymer ($0.5\text{ cm} \times 0.5\text{ cm} \times 0.3\text{ mm}$, about 5 mg) was performed in 30 mL of the release media (phosphate buffer solution (PBS), pH 7.4) at 37°C . At predetermined time intervals, 3 mL samples were withdrawn and replaced with an equal volume of fresh medium. The concentration of PTx in the samples was measured by UV spectrometer (UV-2450, Shimadzu Co., Japan) scans at 210 nm wavelength absorbance, in accordance with the PTx standard equation. The accumulated release of PTx is calculated from the Eq. (1):

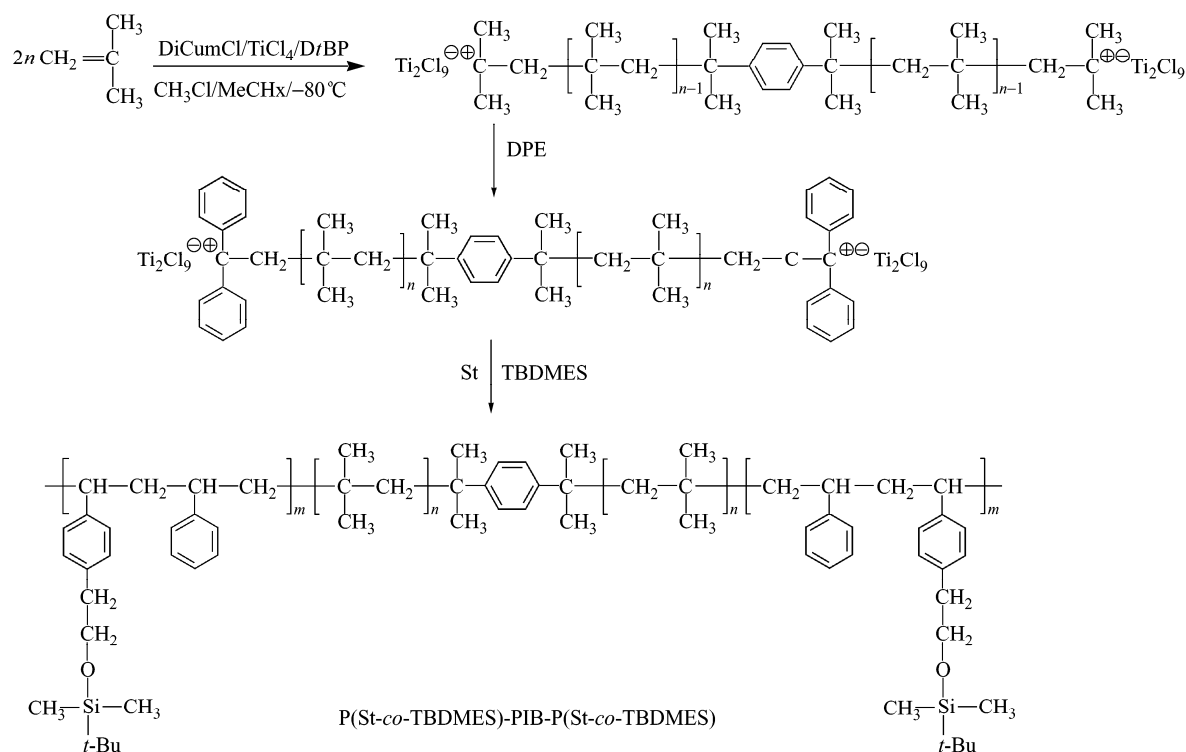
$$A_t = \frac{V_d \sum_{i=1}^{n-1} c_i + V_0 c_n}{m_{\text{PTx}}} \quad (1)$$

where A_t is accumulated release, %; V_d is the refresh volume of PBS at 0, 3 and 5 mL; V_0 is the volume of release media at 30 mL, c_i is the concentration of PTx in the i -th replacement, $\mu\text{g/mL}$; m_{PTx} is the quantity of PTx with sample, μg ; and n is the number of PBS replacement.

RESULTS AND DISCUSSION

Synthesis of the P(St-co-TBDMES)-PIB-P(St-co-TBDMES) Block Copolymer

The method for synthesizing the cationic monomer TBDMES is reported by Wu *et al.*^[13]. The hydroxyl group in 4-(2-hydroxyethyl)styrene must be protected before cationic polymerization, because the hydroxyl group often tends to enter into irreversible termination and chain transfer reactions. Scheme 1 outlines the overall synthetic strategy for the preparation of P(St-co-TBDMES)-PIB-P(St-co-TBDMES) triblock copolymers. First, IB was polymerized by the $\text{DiCumCl/TiCl}_4/\text{DIBP}$ initiating system to yield living PIB ($M_n = 47600$, $\text{MWD} = 1.04$). After 90 min, the capping reaction was carried out by reacting living PIB with DPE. Upon the addition of DPE to living PIB, a red color slowly developed, indicating the generation of stable diphenylcarbeniumions. Finally, the St/TBDMES mixture solution in MeCHx was added. Figure 1 shows the SEC (RI) traces of the PIB segment and the P(St-co-TBDMES)-PIB-P(St-co-TBDMES) triblock copolymer. The shift in the peak of the triblock copolymers towards higher molecular weights (lower retention times) relative to the PIB segment indicates significant St-co-TBDMES incorporation.



Scheme 1 Synthesis of P(St-co-TBDMES)-PIB-P(St-co-TBDMES)

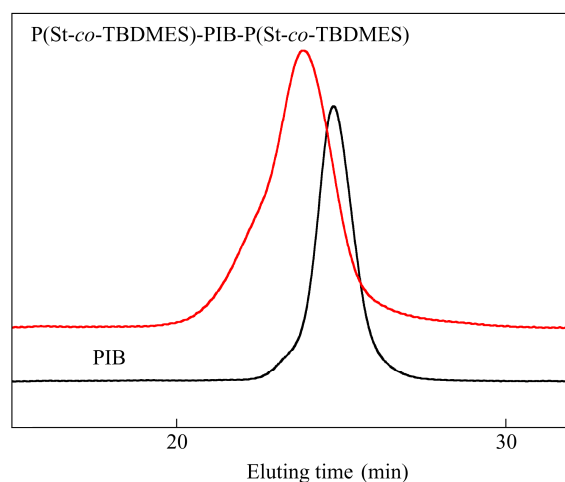


Fig. 1 SEC (RI) traces of PIB and P(St-co-TBDMES)-PIB-P(St-co-TBDMES)

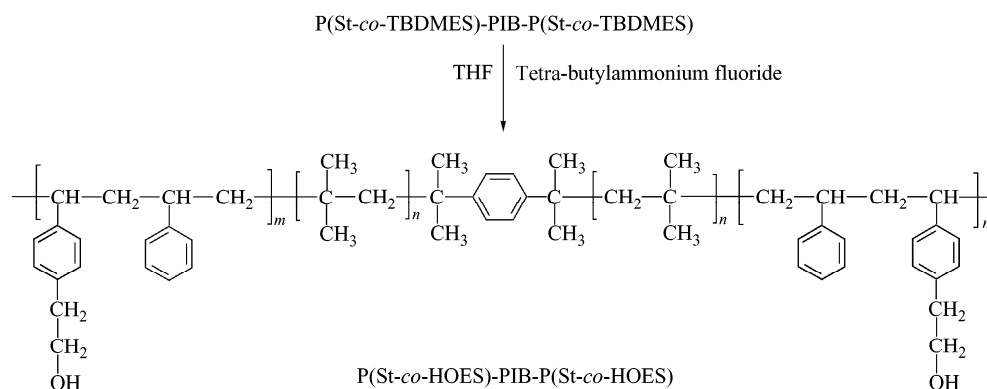
PIB: $M_n = 47600$, MWD = 1.04; P(St-co-TBDMES)-PIB-P(St-co-TBDMES): $M_n = 91800$, MWD = 1.27;

[IB] = 1.5 mol/L, [St/TBDMES] = 0.65 mol/L, [St]:[TBDMES] = 70:30 (W/W), [DiCumCl] = 2×10^{-3} mol/L, [DtBP] = 6×10^{-3} mol/L, [TiCl₄] = 4.8×10^{-2} mol/L, [DPE] = 8×10^{-3} mol/L

Hydrolysis of P(St-co-TBDMES)-PIB-P(St-co-TBDMES) Block Copolymer

Tetra-butylammonium fluoride was used for the deprotection of the P(St-co-TBDMES)-PIB-P(St-co-TBDMES) triblock copolymer. This is because tetra-butylammonium fluoride is an effective reagent for the cleavage of *tert*-butyldimethylsilyl ether. The hydrolysis of *tert*-butyldimethylsilyl ether was examined in the presence of tetra-butylammonium fluoride in THF solvents to yield P(St-co-HOES)-PIB-P(St-co-HOES) (Scheme 2). The

characteristic results of P(St-co-TBDMES)-PIB-P(St-co-TBDMES) and the hydrolyzed product are summarized in Table 1. After hydrolysis, the M_n values of P(St-co-HOES)-PIB-P(St-co-HOES) show acceptable agreement with the value calculated with the assumption of complete P(St-co-TBDMES) segment hydrolysis in the precursor triblock copolymers. Furthermore, the molecular weight distributions remain narrow. These results indicate that the polymeric backbone remains intact after hydrolysis.



Scheme 2 Hydrolysis of P(St-co-TBDMES)-PIB-P(St-co-TBDMES)

Table 1. Characteristics of the PIB, the P(St-co-TBDMES)-PIB-P(St-co-TBDMES) and the hydrolyzed P(HOES-co-St)-PIB-P(St-co-HOES)^a

Sample	PIB segment		P(St-co-TBDMES)-PIB-P(St-co-TBDMES)			P(St-co-HOES)-PIB-P(St-co-HOES)			
	M_{nSEC}	MWD	M_{nSEC}	MWD	PIB wt% ¹ H-NMR	M_{ntho}	M_{nSEC}	MWD	PIB wt% ¹ H-NMR
A	47600	1.04	91800	1.27	69.5	88500	89900	1.25	75.4
B	42700	1.03	79900	1.34	72.5	77900	78500	1.32	80.6
C	42700	1.03	78600	1.32	75.4	76000	76800	1.30	83.1

^a Conditions: PIB polymerization, [IB] = 1.5 mol/L, [DtBP] = 6×10^{-3} mol/L, [TiCl₄] = 4.8×10^{-2} mol/L; Polymerization time: 90 min;

Copolymerization: [St/TBDMES] = 0.65 mol/L, [St]:[TBDMES] = 70:30 (*W/W*), [DPE] = 8×10^{-3} mol/L;

Solvent: CH₃Cl/MeCH_x (50/50, *V/V*); Temperature: -80°C; Polymerization time: 3.5 h

¹H-NMR Spectroscopy of the P(St-co-TBDMES)-PIB-P(St-co-TBDMES) and P(St-co-HOES)-PIB-P(St-co-HOES) Triblock Copolymers

Figure 3(I) shows a typical ¹H-NMR spectrum of a P(St-co-TBDMES)-PIB-P(St-co-TBDMES). In addition to the large absorption of main-chain methyl (b) and methylene (c) of PIB units, a small peak at $\delta = 0.08$ corresponding to dimethyl group (a) of the silyl functionality is detected in the spectrum. Obviously, these dimethyl groups of the silyl functionality completely disappear after hydrolysis to P(St-co-HOES)-PIB-P(St-co-HOES) in Fig. 3(II), indicating a complete conversion.

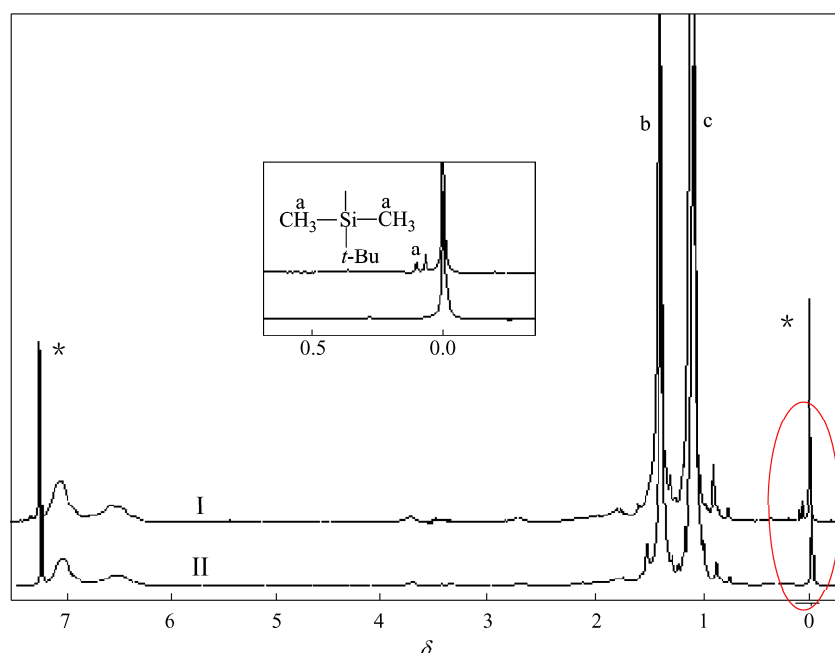


Fig. 3 ^1H -NMR spectra of P(St-co-TBDMES)-PIB-P(St-co-TBDMES) (I) and P(St-co-HOES)-PIB-P(St-co-HOES) (II)

Thermal Characterization of the P(St-co-TBDMES)-PIB-P(St-co-TBDMES) and P(St-co-HOES)-PIB-P(St-co-HOES) Block Copolymers

P(St-co-TBDMES)-PIB-P(St-co-TBDMES) and P(St-co-HOES)-PIB-P(St-co-HOES) triblock copolymers show two glass transitions, where the PIB inner block phase shows the lower and similar T_g , whereas the outer blocks show different T_g (Fig. 4). The outer block of P(St-co-TBDMES)-PIB-P(St-co-TBDMES) exhibits a lower glass transition temperature (T_g ca. 65.06°C) than that of SIBS (T_g ca. 77.28°C). This result can be attributed to the presence of *tert*-butyldimethylsilyl ether moieties. Whereas, the hydrolysis product P(St-co-HOES)-PIB-P(St-co-HOES) shows a higher glass transition temperature (T_g ca. 102.56°C) than that of SIBS. Hydroxyl bond polarity may lead to a higher glass transition temperature. It is interesting that the thermal stability of the P(St-co-HOES)-PIB-P(St-co-HOES) has not declined by the introduction of pendant hydroxyl groups as comparing with SIBS (Fig. 5).

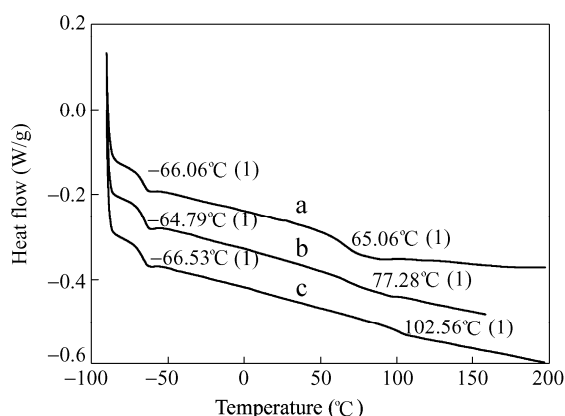


Fig. 4 DSC thermograms of P(St-co-TBDMES)-PIB-P(St-co-TBDMES) (a), SIBS (b) and P(St-co-HOES)-PIB-P(St-co-HOES) (c)

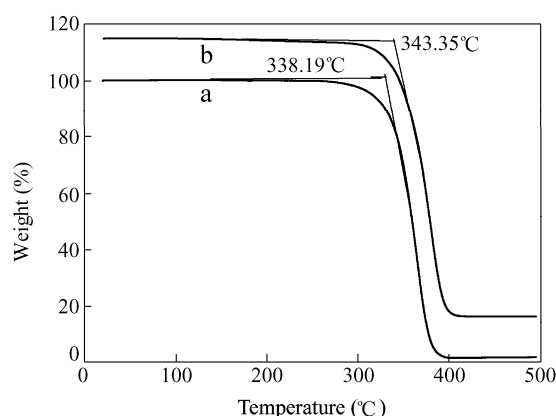


Fig. 5 TG thermograms of SIBS (a) and (St-co-HOES)-PIB-P(St-co-HOES) (b)

Hydrophillic Properties

In order to further confirm chemical modification of triblock copolymer, we investigated the water contact angles of SIBS, P(St-co-TBDMES)-PIB-P(St-co-TBDMES) and P(St-co-HOES)-PIB-P(St-co-HOES). As shown in Table 2, these triblock copolymers have similar M_n and content of PS outer block. Obviously, P(St-co-HOES)-PIB-P(St-co-HOES) exhibits minimum water contact angle due to introduction of pendant hydroxyl groups.

Table 2. Characteristics of the P(St-co-TBDMES)-PIB-P(St-co-TBDMES), SIBS and the hydrolyzed P(St-co-HOES)-PIB-P(St-co-HOES)

Sample	SIBS	P(St-co-TBDMES)-PIB-P(St-co-TBDMES)	P(St-co-HOES)-PIB-P(St-co-HOES)
M_{nSEC}	94500	91800	89900
PIB wt% (^1H -NMR)	67.2	69.5	75.4
Contact angle ($^\circ$)	75.0	78.5	58.0

P(St-co-HOES)-PIB-P(St-co-HOES) as a Carrier for Paclitaxel

TAXUSTM is a coronary drug-eluting stent system utilizing a formulation consisting of cellular-target drug PTx and SIBS. This paper focuses on study morphology and intermolecular interaction of PTx in the PTx-P(St-co-HOES)-PIB-P(St-co-HOES) system. Figure 6(a) shows a typical AFM image of PTx-SIBS system, the PTx is not miscible in the hydrophobic SIBS and crystallizes in SIBS surface. However, the AFM image of PTx-P(St-co-HOES)-PIB-P(St-co-HOES) system is homogeneous (Fig. 6b). Therefore, we propose that pendant hydroxyl groups help to improve the compatibility of P(St-co-HOES)-PIB-P(St-co-HOES) with PTx.

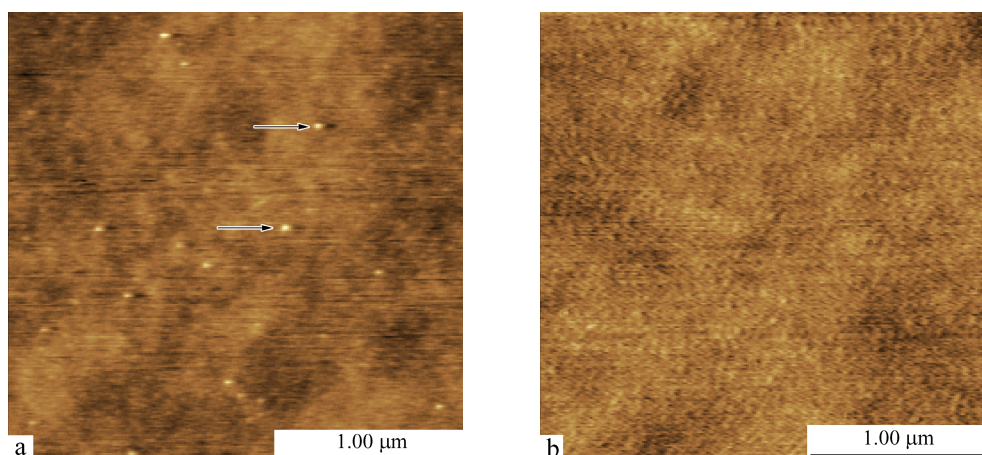


Fig. 6 AFM images of SIBS (a) and P(St-co-HOES)-PIB-P(St-co-HOES) (b) containing 25% paclitaxel by weight

Figure 7 shows the DSC thermograms of PTx-SIBS and PTx-P(St-co-HOES)-PIB-P(St-co-HOES) systems. Miscibility between the drug and either segment of a block copolymer can be demonstrated by a shift in the specific block glass transition toward the PTx T_g (150°C)^[14]. The melting point and decomposition temperature of the PTx are similar and difficult to resolve. For this reason, peaks above 200°C were not evaluated. As shown in Fig. 7 curve a, in addition to two T_g s corresponding to PIB and PS segments, there is a separate T_g present at approximately 150°C. This T_g indicates the presence of discrete particles of drug, which is phase separated from the SIBS copolymer. As shown in Fig. 7 curve b, a shift in the T_g of the hard block of P(St-co-HOES)-PIB-P(St-co-HOES) to a temperature between the PTx T_g and the P(St-co-HOES) T_g suggests some degree of miscibility. Furthermore, the difference in the miscibility of PTx with P(St-co-HOES) and PS may be explained by the hydrogen bonding interaction between P(St-co-HOES)-PIB-P(St-co-HOES) and PTx.

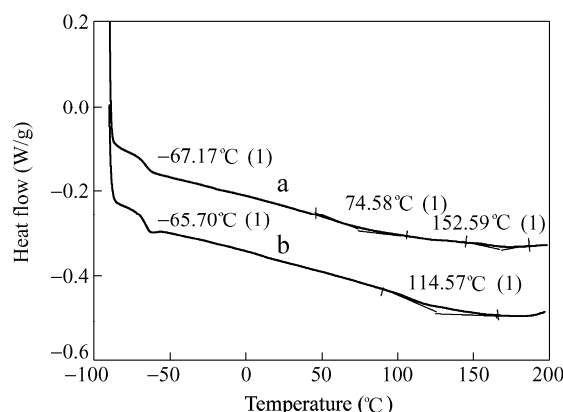


Fig. 7 DSC thermograms of SIBS and P(St-co-HOES)-PIB-P(St-co-HOES) films containing 25% PTx

Figure 8 shows the cumulative PTx release profiles of SIBS (a) and P(St-co-HOES)-PIB-P(St-co-HOES) (b) triblock copolymers. PTx was continuously released from the triblock copolymer in the aqueous medium (PBS). The release was characterized by an initial burst of PTx that occurred from 2 days to 7 days, followed by a slower sustained release after 8 days. The drug release rate of the P(St-co-HOES)-PIB-P(St-co-HOES) triblock copolymer is also greater than that of SIBS. Thus, the increase in the hydrophilicity of the P(St-co-HOES)-PIB-P(St-co-HOES) triblock copolymer allows the drug to rapidly diffuse into PBS.

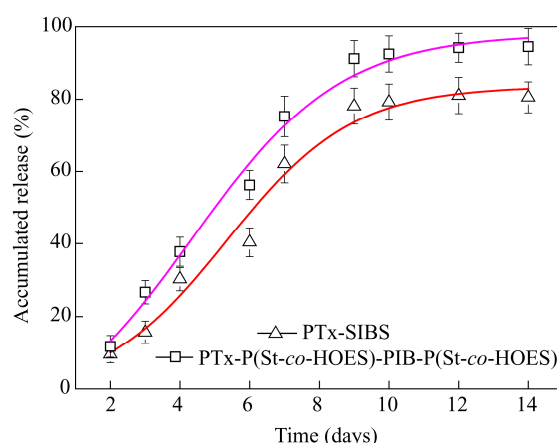


Fig. 8 Release of paclitaxel of samples containing 25 wt% PTx and 75 wt% of either SIBS or P(St-co-HOES)-PIB-P(St-co-HOES)

CONCLUSIONS

The synthesis of a well-defined P(St-co-TBDMES)-PIB-P(St-co-TBDMES) triblock copolymer was accomplished through a DiCumCl/TiCl₄/DtBP initiating system at -80°C using living cationic polymerization. P(St-co-TBDMES)-PIB-P(St-co-TBDMES) was hydrolyzed by *tetra*-butylammonium fluoride in THF solvents to yield P(St-co-HOES)-PIB-P(St-co-HOES). ¹H-NMR results demonstrated that the hydrolysis of P(St-co-TBDMES)-PIB-P(St-co-TBDMES) was complete, leaving the polymeric backbone intact. The triblock copolymer also exhibits typical block copolymer phase separation characterized by DSC. The P(St-co-HOES) segment exhibits a higher T_g than the P(St-co-TBDMES) segment, which can be attributed to the presence of polarity in the hydroxyl group. Compared with SIBS, the P(St-co-HOES)-PIB-P(St-co-HOES) shows higher hydrophilic properties. PTx is more miscible with the P(St-co-HOES)-PIB-P(St-co-HOES) triblock copolymer compared with SIBS. Due to the hydrophilic nature of the St-co-HOES endblocks, the release rate of PTx from P(St-co-HOES)-PIB-P(St-co-HOES) copolymers increases.

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