Synthesis of Poly[(R,S)-3-hydroxybutyrate-*block*-ethylene glycol-*block*-(R,S)-3-hydroxybutyrate] via Anionic ROP

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Summary: New synthetic pathway of a-PHB/PEG/a-PHB triblock amphiphilic copolymers is presented. The copolymers are obtained via racemic BL polymerization initiated with respective PEG macroinitiators. The structure of resulting block copolymers has been proved by SEC and NMR spectroscopy.

Keywords: anionic polymerization; block copolymer; PEG; PHB

Introduction

Poly(3-hydroxybutyrate) is the representative of poly(hydroxyalkanoates) family. The natural one (PHB) is produced and stored by many prokaryotic organisms as carbon and energy source.[1,2] It is consisted of (R) 3-hydroxybutyrate units so the polymer is high crystalline rigid material. The atactic analogue of PHB (a-PHB) is a totally amorphous biomimetic polymer that can be easily obtained via polymerization of (R,S)-β-butyrolactone (BL).[3–5] Both isomers are biocompatible and bioresorbable,[6–8] however, their drawback for eventual applications in regenerative medicine is poor hydrophilicity. One of the best known hydrophilic polymers is poly(ethylene glycol) (PEG), commonly approved for medical applications.[9] Thus, the simple physical modification may be a blending of those hydrophobic and hydrophilic materials. Despite of very good miscibility of PEG with PHB[10] one can expect elution of PEG macromolecules in aqueous media. Therefore, PHB(a-PHB)/PEG copolymers were synthesized previously.[11,12] However, Chen *et al.* used toxic tin compounds as initiators and did not received quantitative conversion of BL while Zhao *et al*. obtained multiblock copolymers but no control on the distribution of PEG and PHB blocks was achieved. The aim of presented work is to synthesize copolymers of a-PHB and PEG with strictly defined structure via anionic polymerization of BL initiated with PEG macroinitiators instead of highly toxic tin compound.

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Experimental

Materials

Poly(ethylene glycol) (PEG) $M_n = 1000$, poly(ethylene glycol) bis(carboxymethyl) ether Mn = 600 (both from Aldrich) were dried with toluene and than under vacuum at 40–80 °C to constant weight. Succinic anhydride (Aldrich) was recrystallized from dry chloroform. β-butyrolactone (BL) (Aldrich) was dried distilled over CaH_2 (56 °C, 9 mmHg). Tetrabutylammonium hydroxide (Bu_4NOH) 1 M solution in methanol (Aldrich) and Dowex 50 W × 2 cation exchange resin in acidic form (Aldrich) were used as received.

Macroinitiator Preparation

The succinic anhydride (3.786 g; 37.86 mmol) has been added into glass reactor containing the solution of PEG (18.912 g, $M_n = 1000$) in dry toluene (ca. 100 cm^{-3}) and heated at 85 °C for 72 h. The reaction course was monitored using ^1H and ^{13}C NMR spectroscopy. After reaction completion (quantita-

tive esterification of PEG by anhydride) toluene was stripped-off and the product was dried under vacuum at 50 °C.

Esterified PEG has been titrated potentiometrically in solution of dry ethanol (10 w/v) with 0.1 M solution of Bu_4NOH in methanol. Solvents were stripped-off under vacuum and macroinitiator was dried azeotropically with benzene.

Polymerization

The BL anionic polymerizations have been initiated with prepared macroinitiators. They have been carried out in a solution of THF ($[BL]_0 = 1$ mol/dm^{-3}). All the polymerization experiments have been conducted at room temperature. Progress of the reaction have been measured by FTIR spectroscopy basing on the carbonyl carbon signals of BL and the poly(3-hydroxybutyrate) (PHB) at 1815 cm^{-1} and 1735 cm^{-1}, respectively. When the polymerization have been completed polymer is acidified with cation exchange resin, precipitated in dry diethyl ether and dried under vacuum. Final products have been characterized with ^{1}H NMR and GPC techniques.

Measurements

FTIR spectra were recorded with FTS 40A Bio-Rad spectrometer. ^{1}H and ^{13}C NMR spectra were recorded at room temperature in $CDCl_3$ with tetramethylsilane (TMS) as internal standard using a Varian VCR-300. Number-average molecular weights and molecular weight distributions (M_w/M_n) values of polymers were determined by SEC experiments conducted in $CHCl_3$ at 40 °C at a flow rate of 1 ml/min., equipped with two styragel columns Mixed-C connected in series and a Shodex SE 61 refractive index detector. Polystyrene standards with narrow molecular weight distributions have been used to generate a calibration curve. Electrospray mass spectrometry analyses were performed using an LCQ ion trap mass spectrometer (Finnigan, San Jose, CA, USA). Samples were dissolved in methanol. The polymer solution was introduced to the ESI source by continuous infusion by means of the instrument syringe pump at a rate of 3 (L min^{-1}. The LCQ ESI source was operated at 4.5 kV and the capillary heater was set to 200 °C.

Results and Discussion

The anionic ring opening polymerization of (R,S)-BL has been chosen as a method of synthesis of PEG/PHB block copolymers. Thus, the first step in described synthesis

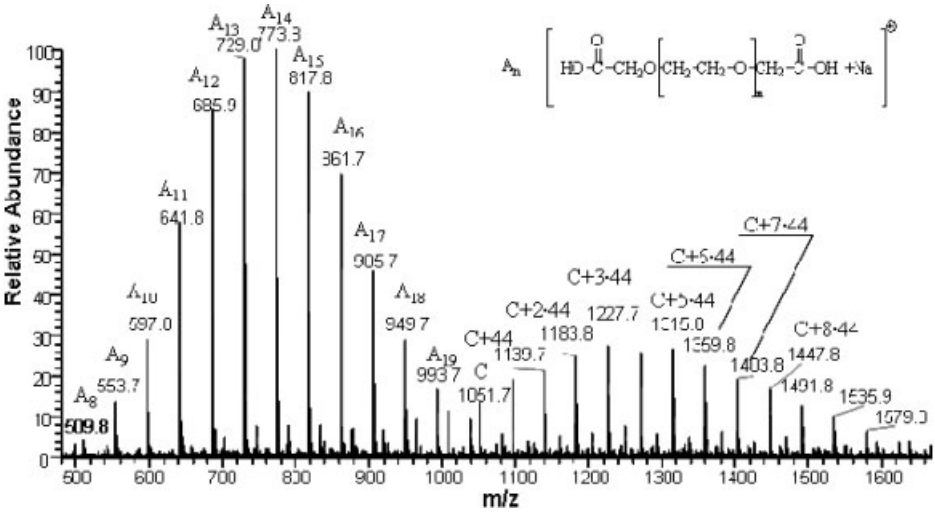
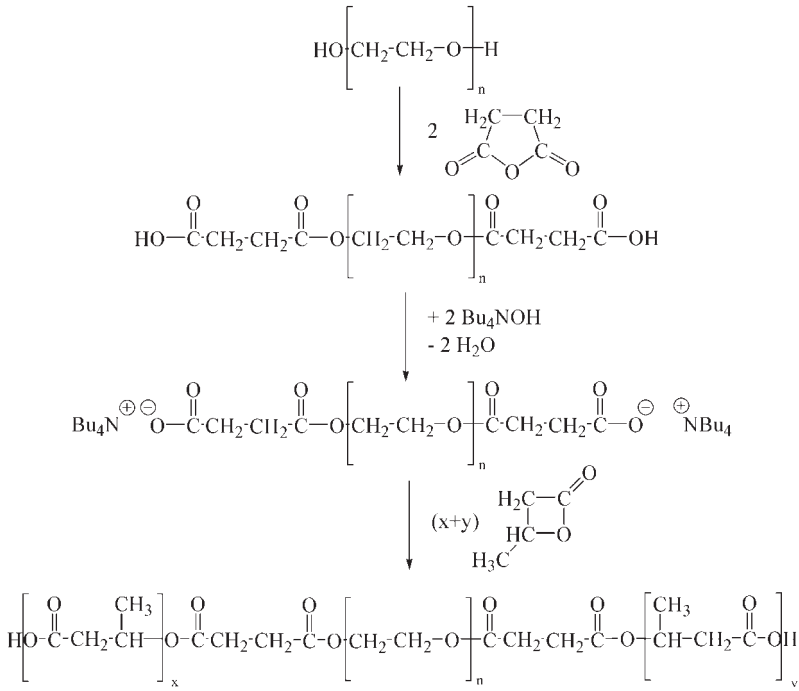


Figure 1.
ESI-MS mass spectrum in positive ion mode of PEG-$(COOH)_2$ ($M_n = 600$).



Scheme 1.

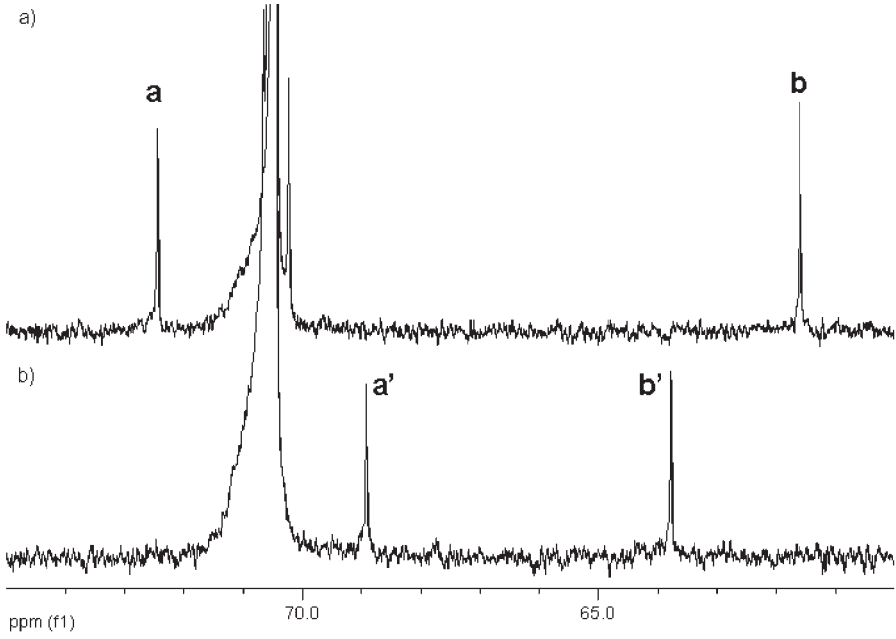


Figure 2.
^{13}C NMR spectrum (PEG methylene group expanded region) of a) PEG $M_n = 1000$ (**a** – **C**H_2CH_2OH, **b** – **C**H_2OH) and b) respective PEG bis(succinate), $M_n = 1200$ (**a′** - **C**$H_2CH_2OC(O)$, **b′** - **C**$H_2OC(O)$).

pathway is to obtain PEG macroinitiator functionalized with two carboxylate end-groups. Such macroinitiator can be obtained by simple titration of commercially available poly(ethylene glycol) bis-(carboxymethyl)s. However, ESI-MS analysis of selected PEG-$(COOH)_2$ ($M_n = 600$) presented in Figure 1 shows bimodal distribution of singly charged positive ions. The ions appearing in the first distribution at lower mass range (m/z 500–1050) (series A) correspond to the sodium adducts of linear bis(carboxy)-terminated PEG oligomer chains. The second distribution of ions visible in the mass range m/z 1050–1600 (series C) corresponds also to the sodium adducts of PEG oligomers (the differences between the neighboring peaks are equal to 44 Da) but possessing hydroxyl and aldehyde end groups.

Due to the fact that this commercially available PEG-$(COOH)_2$ contains some amount of oligomers with hydroxyl and aldehyde end-groups which can not be converted by titration with base to carboxylate anions the commercial product can not be used as a starting material for preparation of PEG macroinitiator.

In this work we decide to use another approach for preparation of the PEG macroinitiator i.e., esterification of PEG with succinic anhydride and subsequent titration of resulting product with tetrabutylammonium hydroxide (Scheme 1). The addition reaction has been monitored using ^{13}C NMR spectroscopy and it has been carried out until the PEG end groups signals i.e., $\mathbf{C}H_2$–OH at $\delta = 61.60$ ppm and –$\mathbf{C}H_2$–CH_2–OH at $\delta = 72.45$ ppm disappeared (Figure 2).

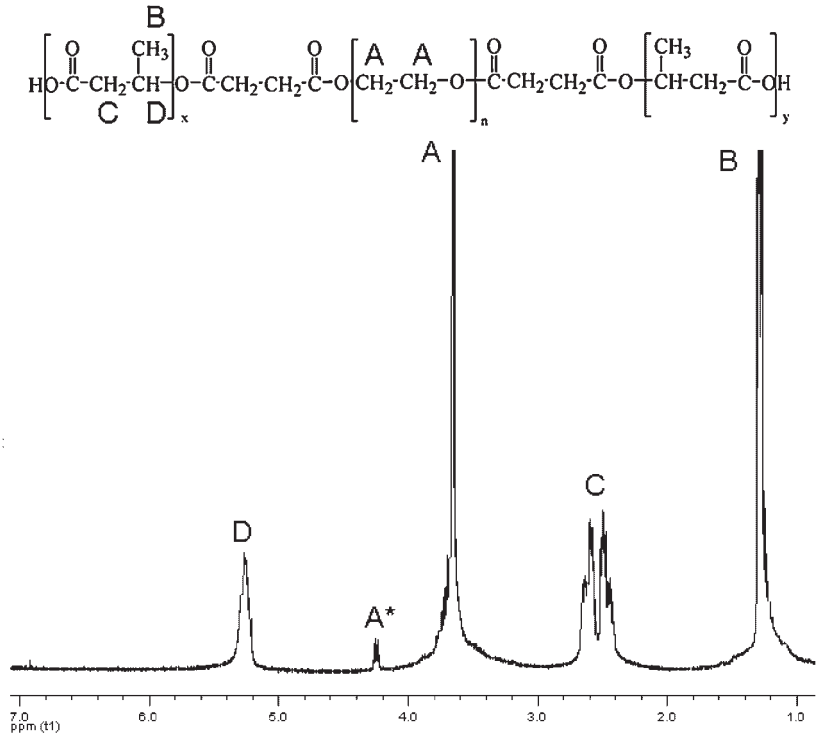


Figure 3.
^1H NMR spectrum of poly[(R,S)-3-hydroxybutyrate-*block*-ethylene glycol-*block*-(R,S)-3-hydroxybutyrate] (Entry 1, Table 1).

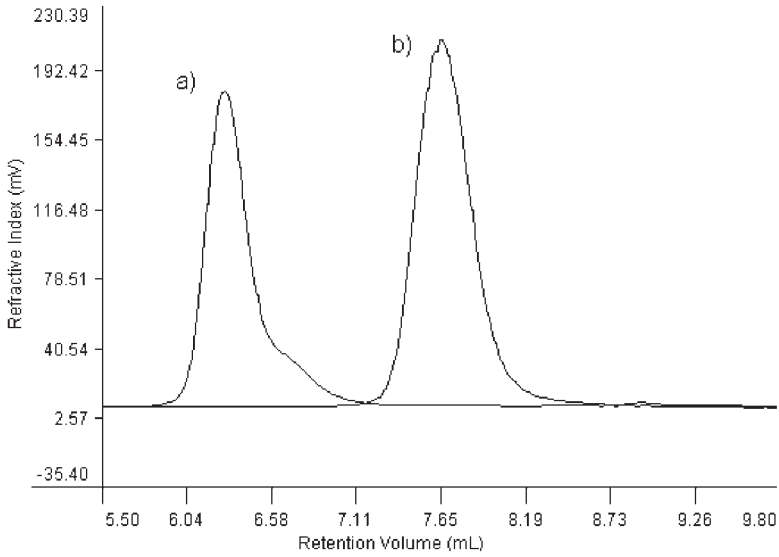


Figure 4.
Overlay of SEC traces of a) copolymer (Entry 3, Table 1) and b) esterified PEG.

Complete disappearance of the signals of PEG end groups has been proof of quantitative esterification. The resulting PEG bis(succinate) was next titrated with solution of NBu_4OH and the PEG macroinitiator with two active -$COONBu_4$ groups has been obtained. Then the block copolymer has been obtained by anionic polymerization of racemic BL initiated by PEG macroinitiator (Scheme 1). The obtained product has been analyzed using NMR and SEC techniques. The presented in Figure 3 ^{1}H NMR spectrum of sample Entry 3, Table 1 reveals signals ascribed to a-PHB block (C**H**$_3$ at $\delta = 1.2$–1.4 ppm, C**H**$_2$ at $\delta = 2.4$–2.7 ppm, C**H** at $\delta = 5.2$–5.3 ppm) and PEG block (O–C**H**$_2$–C**H**$_2$–O at $\delta = 3.6$–3.8 ppm, C**H**$_2$–OC(O) at $\delta = 4.2$–4.3 ppm which is denoted as A* in Figure 3). The signal corresponding to succinate C**H**$_2$ protons overlaps with much more intensive signal of methylene group of a-PHB block. SEC analysis indicates that the product obtained has possessed unimodal molecular weight distribution and M_n higher than applied PEG initiator (Figure 4) proving PEG/a-PHB triblock copolymer formation.

The results of anionic polymerization of racemic BL initiated with PEG macroinitiator are presented in Table 1.

Results presented in Table 1 reveal relatively good control of copolymer com-

Table 1.
Results of BL polymerization initiated with PEG macroinitiator in THF solution at room temperature.[a)]

Entry	Theoretical data		Experimental data		
	[HB]/[EO]	M_{nT}	[HB]/[EO][b)]	M_n[c)]	M_w/M_n[c)]
1.	0.51	2200	0.49	2250	1.24
2.	1.02	3200	0.97	2900	1.25
3.	2.05	5200	1.91	4800	1.17
4.	4.40	9800	4.19	8900	1.20

a) $[BL]_0 = 1$ mol/dm^{-3}, M_n of esterified PEG was equal to 1200;
b) determined using ^{1}H NMR spectroscopy;
c) from SEC measurements.

position and molecular weight achieved by applying such a macroinitiator. However, molecular weight and composition of resulting block copolymers are slightly different than theoretical ones. It is caused probably by chain transfer reaction (traces of crotonate-terminated oligo-BL have been found in diethyl ether filtrate). Such side reaction occurring in BL anionic polymerization initiated with carboxylates was reported previously.[5,13–15]

Conclusions

The method presented above allows to obtain a-PHB-*b*-PEG-*b*-a-PHB copolymers via relatively simple process. The structure of obtained copolymers is clearly proved by NMR and SEC techniques. It should be pointed out that using this method the length of hydrophilic block of resulting copolymer is controlled by molecular weight of PEG macroinitiator used while hydrophobic block length is controlled by fitting of molar ratio of (R,S) BL/PEG macroinitiator. It has been found, that esterification of PEG, although is more time consuming, is a better method for PEG macroinitiator preparation due to quantitative esterification of hydroxyl terminal groups of PEG. Moreover, the copolymers obtained by this synthetic route possess carboxylic terminal groups. The copolymers obtained thereof may constitute promising candidates for further modification either with other polymers (*e.g.* consecutive esterification) or by attaching drugs, proteins, amino acids, growth factors, etc.

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