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Controlled synthesis and characterizations of amphiphilic poly[(*R*,*S*)-3-hydroxybutyrate]-poly(ethylene glycol)-poly[(*R*,*S*)-3-hydroxybutyrate] triblock copolymers

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Abstract

Well-defined biodegradable amphiphilic triblock copolymers consisting of atactic poly[(*R*,*S*)-3-hydroxybutyrate] (PHB) and poly(ethylene glycol) (PEG) as the side hydrophobic block and middle hydrophilic block were synthesized via ring opening polymerization of (*R*,*S*)-β-butyrolactone from PEG macroinitiators and characterized using NMR, GPC, FT-IR, XRD, DSC and TG analyses. The controlled synthesis was made possible by the facile synthesis of pure PEG macroinitiators through a TEMPO-mediated oxidation. Constituting 40–70 wt% of the copolymer content, PHB blocks grown were amorphous while PEG formed crystalline phase when segment was sufficiently long. While hindering PEG crystallization, atactic PHB mixed well with amorphous PEG to give single T_g in all the copolymers. The copolymers exhibited two-step thermal degradation profile starting with PHB degradation from 210 to 300 °C, then PEG from 350 to 450 °C.

Keywords: Poly[(*R*)-3-hydroxybutyrate]; Poly(ethylene glycol); Anionic ring opening polymerization

1. Introduction

Amphiphilic block copolymer is formed by coupling hydrophilic and hydrophobic polymers into one macromolecule. The incorporation of different polymers into one macromolecular entity presents an attractive approach in modulating polymer properties [1–3]. Moreover, in the presence of selective solvents, amphiphilic block copolymers self-assemble into micelles or vesicles of different shapes [4–6], as a result of disparate interactions between soluble and insoluble blocks with the solvent. These fascinating properties have made them valuable in the bottom-up approach of obtaining and controlling nanostructure formation that has many practical applications in pollution control, gene therapy and drug delivery [7–11].

Many amphiphilic block copolymers comprising biodegradable polyesters and poly(ethylene glycol) (PEG), as hydrophobic and hydrophilic segments, respectively, have been explored to these ends and their use as a component block is particularly desirable in the context of biomaterials, as this imparts and regulates biodegradability of the whole block copolymer [12,13]. With their excellent biocompatibility, polylactic acid (PLA), polycaprolactone (PCL), polyglycolic acid (PGA), polyhydroxybutyrate (PHB) and their copolymers are amongst the more popular biodegradable polyesters investigated to date. Block copolymers of PLA–PEG, PGA–PEG and PCL–PEG of various macromolecular architectures from linear diblock, triblock, multiblock to multi-arm star shaped have been reported [14–19]. PHB–PEG block copolymers, on the other hand, are less investigated. The PHB-based block copolymers are generally synthesized from high molecular weight microbial PHB, which is found in many microorganisms as carbon source and energy storage material [20], and examples include diblock [21], triblock [22] and multiblock PHB–PEG

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copolymers [23,24] reported recently. The coupling of hydrophilic, soft PEG segment with the hard crystalline isotactic PHB afforded copolymers with better mechanical properties than their precursors [23] and when the coupling was carried out in a controlled manner, interesting self-assembly behavior was observed, as in the case of diblock PHB–PEG [25], triblock PEG–PHB–PEG [26,27] and alternating multiblock of PHB–PEG [24] copolymers.

An alternative synthetic approach to PHB-based block copolymers is via ring opening polymerization of β-butyrolactone monomer from a macroinitiator. An important feature of this strategy is the ability to regulate microstructure of the PHB chain by varying the ratio of β-butyrolactone stereoisomers in the monomer feed, leading to atactic, isotactic or even syndiotactic PHBs, in contrast to microbial PHB that exists only in isotactic *R* configuration [28,29]. Various catalytic systems have been explored for the polymerization of β-butyrolactone monomer, including organometallic catalysts, such as tin(IV) complexes [30–32], yttrium complexes [33], zinc complexes [34,35], aluminum complexes [28,36], and dibutylmagnesium [37], to *N*-heterocyclic carbene based organocatalysts [38], as well as carboxylate salts with or without crown ether activation [39–41]. To obtain PHB block copolymers with well-defined architecture, the catalytic system employed must allow growth of PHB chain on the macroinitiator in a controlled manner such that good control of molecular weight and molecular weight distribution can be achieved along with good end group integrity. For biomedical application, a non-toxic system is preferred to ensure biocompatibility of the final polymers.

Amphiphilic block copolymers consisting PEG and PHB are of great interest as they represent a biodegradable analog to Pluronics or reverse Pluronics, which are commonly used as polymeric surfactant [42]. With atactic PHB segment that is completely amorphous, we would have a closer mimic to the poly(propylene glycol)-containing (PPG) block copolymers, in which the PPG segments are usually atactic and amorphous in nature. To this end, an anionic ring opening polymerization strategy, that eliminates the use of toxic crown ether, as recently reported by Juzwa and Jedlinski on the preparation of poly(3-hydroxybutyrate) [41], is used. A synthesis of triblock PHB–PEG–PHB using anionic ring opening polymerization was reported previously [43]. However, the synthetic approach involved the use of toxic crown ether, which might have adverse effect on the biocompatibility of resultant triblock copolymer. Moreover, there was no report on physical properties of the polymers prepared. During the course of our investigation, a synthesis of PHB–PEG–PHB via tin-mediated ring opening polymerization at high temperature was reported [44,45]. Studies on drug encapsulation, drug release as well as enzymatic degradation of the polymers were carried out. However, the synthesis lacks control on the block copolymer architecture, as evidenced by the presence of substantial amount of crotonate end group on the polymer chains; hence it is difficult to conclude whether the characterization data are representative of the triblock architecture.

More recently, Kawalec et al. [46] polymerized β-butyrolactone on PEG macroinitiators via a crown ether-free anionic ring opening polymerization to obtain triblock PHB–PEG–PHB and achieved a good control on molecular weight and molecular weight distribution of the final triblock copolymer. Despite its similarity with our work, they focused on the controlled synthesis of PHB–PEG–PHB triblock copolymer that ended with carboxylic acid functionality and no physical properties on the obtained polymers were reported. Additionally, our work employed an efficient yet mild and environmentally friendly TEMPO-mediated oxidation to prepare PEG macroinitiator, in contrast to the conventional grafting of succinic anhydride. Moreover, our triblock PHB–PEG–PHB copolymers are decorated with telechelic hydroxyl groups, which can be conveniently used for further modification using well established coupling chemistry.

Herein, we report the successful synthesis of well-defined triblock PHB–PEG–PHB copolymers based on crown ether-free anionic ring opening polymerization on PEG macroinitiators, which were prepared using a convenient yet efficient TEMPO-mediated oxidation. The molecular architecture of the block copolymers was ascertained using GPC, ^{1}H NMR, ^{13}C NMR and FT-IR analyses while their thermal properties were studied using DSC and TGA, along with XRD.

2. Experimental section

2.1. Materials

Poly(ethylene glycol)s (PEGs) with M_n of ca. 1000 and 3000 were purchased from Aldrich, and purified by dissolving in dichloromethane followed by precipitation in diethyl ether and further dried under high vacuum at 40 °C for 48 h before use. Their M_n and polydispersity index (PDI) were found to be 1010 and 1.03, 2990 and 1.02, respectively. (*R*,*S*)-β-Butyrolactone (>95%) was supplied by Tokyo Kasei Inc and purified by drying over and vacuum distilled from CaH_2 twice before use. 2,2,6,6-Tetramethyl-1-piperidinyloxy (TEMPO, 99%, Aldrich), sodium hypochlorite (NaClO, available chlorine, ≥4%, Aldrich), hydrochloric acid (HCl, 1 N, Aldrich), 2-bromoethanol (95%, Aldrich), sodium bromide (NaBr, 99%, Alfa Aesar), sodium hydroxide (NaOH, 99%, Merck), sodium carbonate (Na_2CO_3, 99.9%, BDH), dimethylsulfoxide (DMSO, anhydrous, ≥99.9%, Aldrich), ethanol (99.9%, Merck), diethyl ether (99.9%, J. T. Baker), hexane (99.8%, J. T. Baker) and chloroform (99.8%, Tedia) were used as received.

2.2. Synthesis of PEG macroinitiators

The PEG macroinitiators were prepared from commercially available PEG via a TEMPO-mediated oxidation under ambient aqueous condition [47] and subsequent neutralization with Na_2CO_3. Preparation of the PEG salt of M_n ca. 3000 (PEG3K-($CO_2Na)_2$) is detailed below: hydroxyl-terminated PEG (4 g, 1.33 mmol) was first dissolved in 100 ml water along with TEMPO (41.7 mg, 0.27 mmol) and NaBr (41.7 mg, 0.41 mmol). NaClO (28 ml) was then added after pH adjustment to 12 with NaOH (1.0 M). The oxidation was quenched after 1 h by addition of ethanol (7 ml) and acidification to pH 1–2 with hydrochloric

acid solution. The aqueous solution was extracted with chloroform, and the combined extract was concentrated, filtered and precipitated into diethyl ether to afford the PEG-diacid after freeze drying. The PEG-diacid was then neutralized with slight excess of Na_2CO_3 under ambient aqueous condition to obtain the desired PEG macroinitiator. After removal of water, the crude product was dissolved in ethanol and filtered to remove excess Na_2CO_3. The process was repeated with chloroform as solvent and pure macroinitiator was obtained after solvent removal and freeze drying. Yield: 3.06 g (75%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.44–3.82 (m, $-OCH_2$-CH_2O-), 3.96 (s, $-OCH_2$-CO_2Na). ^{13}C NMR (100 MHz, $CDCl_3$): δ 68.9 ($-OCH_2$-CO_2Na), 69.6–71.0 ($-OCH_2CH_2O-$), 175.3 ($-OCH_2CO_2Na$).

2.3. Synthesis of PHB–PEG–PHB triblock copolymer

The PHB–PEG–PHB triblock copolymers were obtained from anionic ring opening polymerization of (*R*,*S*)-β-butyrolactone on the PEG macroinitiators in DMSO at room temperature under dry N_2 atmosphere, similar to the method reported by Juzwa and Jedlinski [41]. In all the polymerization reactions, the initial concentration of β-butyrolactone was kept at 0.6 mol/dm^3, while the initiator concentration varied from 1.0×10^{-2} to 2.8×10^{-2} mol/dm^3, according to the required monomer to initiator ratio for a particular M_n of the final block copolymer. As a typical example, 0.766 g of dried PEG macroinitiator (M_n 3062, 0.25 mmol) was first dissolved in 10 ml of DMSO followed by addition of 0.5 ml of β-butyrolactone (6.13 mmol) at room temperature. The reaction mixture was sampled periodically to determine monomer conversion using NMR. Upon reaching monomer conversion of >90%, the polymerization was quenched by adding excess of 2-bromoethanol. DMSO was then removed by distillation, and the residue re-dissolved in chloroform, filtered, before precipitated into *n*-hexane to afford the final copolymer. Yield: 1.022 g (70.3%). GPC (THF): $M_n = 7530$, PDI = 1.05. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.26–1.31 (m, $-OCH(CH_3)CH_2CO_2-$ of PHB block), 2.43–2.63 (m, $-OCH(CH_3)CH_2CO_2-$ of PHB block), 3.46–3.70 (m, $-OCH_2CH_2O-$ of PEG block), 3.81 (t, $-CO_2CH_2$-CH_2OH of end group), 4.10 (s, $-OCH_2CO_2-$ of PEG block), 4.21 (t, $-CO_2CH_2CH_2OH$ of end group), 5.23–5.25 (m, $-OCH(CH_3)CH_2CO_2-$ of PHB block). ^{13}C NMR (100 MHz, $CDCl_3$): δ 19.8 ($-OCH(CH_3)CH_2CO_2-$ of PHB block), 40.8–40.9 ($-OCH(CH_3)CH_2CO_2-$ of PHB block), 60.8 ($-CO_2CH_2CH_2OH$ of end group), 66.3 ($-CO_2CH_2CH_2OH$ of end group), 67.7 ($-OCH(CH_3)CH_2CO_2-$ of PHB block), 68.6 ($-OCH_2CO_2-$ of PEG block), 70.6–70.9 ($-OCH_2CH_2O-$ of PEG block), 169.1–169.4 ($-OCH(CH_3)CH_2CO_2-$ of PHB block), 169.7 ($-CO_2CH_2CH_2OH$ of end group), 170.3 ($-OCH_2CO_2-$ of PEG block).

2.4. Molecular characterizations

Gel permeation chromatography (GPC) measurements were carried out at 45 °C and at a flow rate of 0.2 ml/min, with a Shimadzu SCL-10A and LC-8A systems equipped with two Phenogel 5μ 100 and 1000 Å columns (size: 300 × 4.6 mm) connected in series and a Shimadzu RID-10A refractive index detector. Tetrahydrofuran (THF) was selected as the mobile phase and the system was calibrated with monodispersed poly(ethylene glycol) standards. ^{1}H NMR (400 MHz) and proton-decoupled ^{13}C NMR (100 MHz) spectra were obtained on a Bruker AV-400 NMR spectrometer at room temperature and chemical shifts reported in ppm with reference to solvent peaks ($CHCl_3$: δ 7.26 for ^{1}H NMR and δ 77.2 for ^{13}C NMR). Fourier transform infrared (FT-IR) spectra of the polymers coated on CaF_2 plate were recorded on a Bio-Rad 165 FT-IR spectrophotometer; 16 scans were signal-averaged with a resolution of 2 cm^{-1} at room temperature.

2.5. Thermal analysis

Differential scanning calorimetry (DSC) measurements were performed on a TA Instruments Q100 differential scanning calorimeter equipped with an auto-cool accessory and calibrated using indium. The following protocol was used for each sample: heating from room temperature to 150 °C at a heating rate of 5 °C min^{-1}, holding at 150 °C for 3 min, cooling from 150 to −80 °C at a heating rate of 5 °C min^{-1}, isothermal at −80 °C for 3 min and finally reheating from −80 to 150 °C at a heating rate of 5 °C min^{-1}. Data collected from both first cooling and second heating runs were analyzed and peak maxima were taken as transition temperatures. Thermogravimetric analyses (TGA) were done on a TA Instruments SDT 2050, by heating the samples at a rate of 20 °C min^{-1} from room temperature to 800 °C in a dynamic nitrogen atmosphere (flow rate = 120 ml/min).

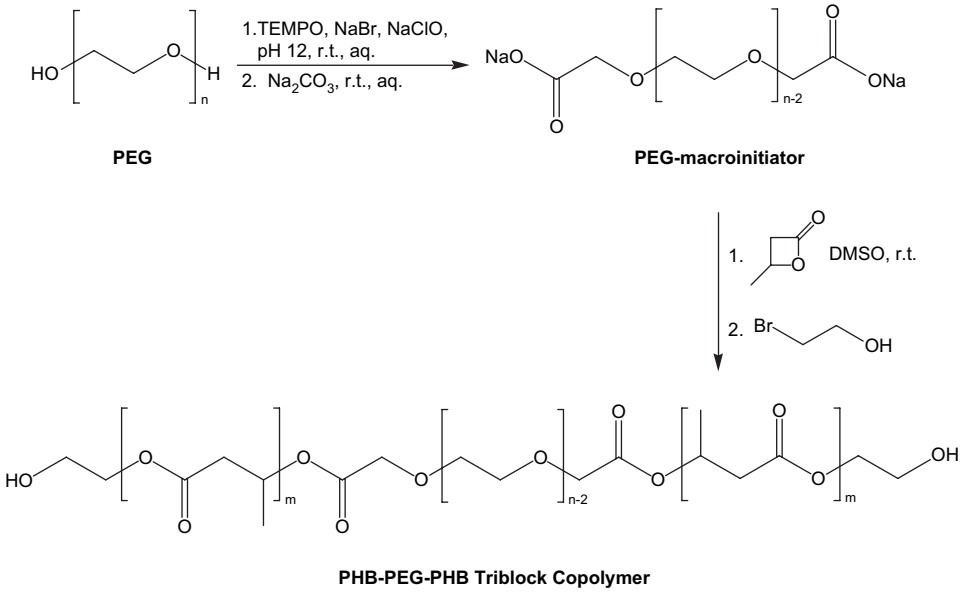
2.6. Wide-angle X-ray diffraction (XRD)

X-ray diffraction measurements were carried out by using a Bruker GADDS diffractometer with an area detector operating under Cu Kα (1.5418 Å) radiation (40 kV, 40 mA) at room temperature. Polymer samples were first deposited on glass slides and then mounted onto a sample holder with double-sided adhesive tape. The samples were scanned from 5 to 40° (2θ).

3. Results and discussion

3.1. Synthesis and molecular characterization of PHB–PEG–PHB triblock copolymers

PHB–PEG–PHB triblock copolymers with atactic PHB blocks were synthesized via anionic ring opening polymerization of (*R*,*S*)-β-butyrolactone from telechelic-carboxylated PEG macroinitiators. The synthetic route is outlined in Scheme 1. Telechelic-carboxylated PEGs were obtained from TEMPO-mediated oxidation that converted the two hydroxyl end groups of PEG to carboxylic acid functionality in aqueous ambient condition. This highly efficient process has allowed us to prepare PEG macroinitiators of high purity after treatment with Na_2CO_3, which transformed the acid moieties to sodium carboxylate that is active for polymerization of β-butyrolactone. PEGs with M_n of 1010 and 2990 have been used to prepare



Scheme 1. Synthesis of PEG macroinitiator and PHB–PEG–PHB triblock copolymers.

the required macroinitiators and their end group functionality was confirmed using ^{1}H NMR and ^{13}C NMR analyses (see Fig. 1). Polymerization of β-butyrolactone was initiated by a nucleophilic attack on the chiral carbon of β-butyrolactone by the solvent activated carboxylate anion [41] attached to the PEG macroinitiator, which simultaneously generated a new carboxylate anion through the alkyl–oxygen bond scission on the monomer. The newly formed anion provided an avenue for chain propagation until all monomers were consumed or when the capping agent, 2-bromoethanol, was added. The progress of polymerization was monitored using ^{1}H NMR, based on the intensity of methine proton of monomer β-butyrolactone at δ 4.7 ppm to that of PHB at δ 5.2 ppm. When monomer conversion went beyond 90%, the polymerization was quenched through addition of capping agent. After removal of solvent, unreacted monomer and excess capping agent by vacuum distillation, the resultant polymer was purified by precipitation into hexane and filtration to remove salt formed in the capping step. A series of PHB–PEG–PHB triblock copolymers with varying PHB and PEG block lengths have been synthesized in good yield and their molecular characteristics summarized in Table 1. The triblock copolymers are named according to the H–E–H(m–n–m) notation, where H–E–H represents the PHB–PEG–PHB block order while m and n reflect the theoretical number of repeating units present in each PHB and PEG block, respectively.

^{1}H and ^{13}C NMR have been used to elucidate the chemical structure of the obtained triblock copolymers. Fig. 2(a) shows the ^{1}H NMR spectrum of H–E–H(30–68–30), in which all signals can be ascribed to protons belonging to either PHB, PEG or end group. Signals belonging to the PHB block can be found at 1.26–1.31, 2.43–2.63 and 5.23–5.25 ppm and they can be identified as the methyl, methylene and methine protons present along the PHB chain. Methylene protons along the PEG chain contribute to the signals at around 3.46–3.70 ppm while those adjacent to the ester linkages appeared at 4.10 ppm. The end group protons can be seen at 3.81 and 4.21 ppm. PEG macroinitiators have signal at around 3.96 ppm that is attributed to neighboring methylene protons of the carboxylate end groups and it shifted completely down field to 4.10 ppm in the triblock copolymers. The shift was observed even before capping was done and provides a strong indication that the block copolymers obtained are of PHB–PEG–PHB triblock architecture, instead of a mixture containing triblock PHB–PEG–PHB and diblock PEG–PHB with some unreacted carboxylate functionality. The presence of end group methylene protons at 3.81 and 4.21 ppm points to the successful end capping of the growing PHB chain and hence confirmed that the triblock copolymers synthesized were end-functionalized with primary hydroxyl groups, which may be useful for further modification through some well established coupling methodologies such as isocyanate and CDI-mediated coupling. The peak intensities of end group protons at 4.21 ppm as well as those next to ester linkage of the PEG block at 4.10 ppm were used to estimate the average degree of polymerization (DP) of β-butyrolactone together with the peak intensity of PHB methine proton and this value was further used to calculate M_n of the final triblock copolymers as listed in Table 1. The intensity ratio between the methine proton of PHB and PEG's methylene proton has been used to estimate PEG or PHB content in the triblock copolymers.

The triblock architecture of the PHB–PEG–PHB copolymers is further substantiated by ^{13}C NMR analyses. ^{13}C NMR spectrum of H–E–H(30–68–30) along with all the peak assignments are shown in Fig. 2(b). Briefly, signals of PHB carbons can be found at 19.8 (methyl carbon), 40.8–40.9 (methylene carbon), 67.7 (methine carbon) and 169.1–169.4 ppm (carbonyl carbon). The PEG carbons, on the other hand, can be clearly seen at 68.6 (methylene carbon next to ester linkage), 70.6–70.9 (all other methylene carbons) and 170.3 ppm (carbonyl carbon of ester linkage). Carbons of end group are also present at 60.8 and 66.3 ppm. The disappearance of carboxylate

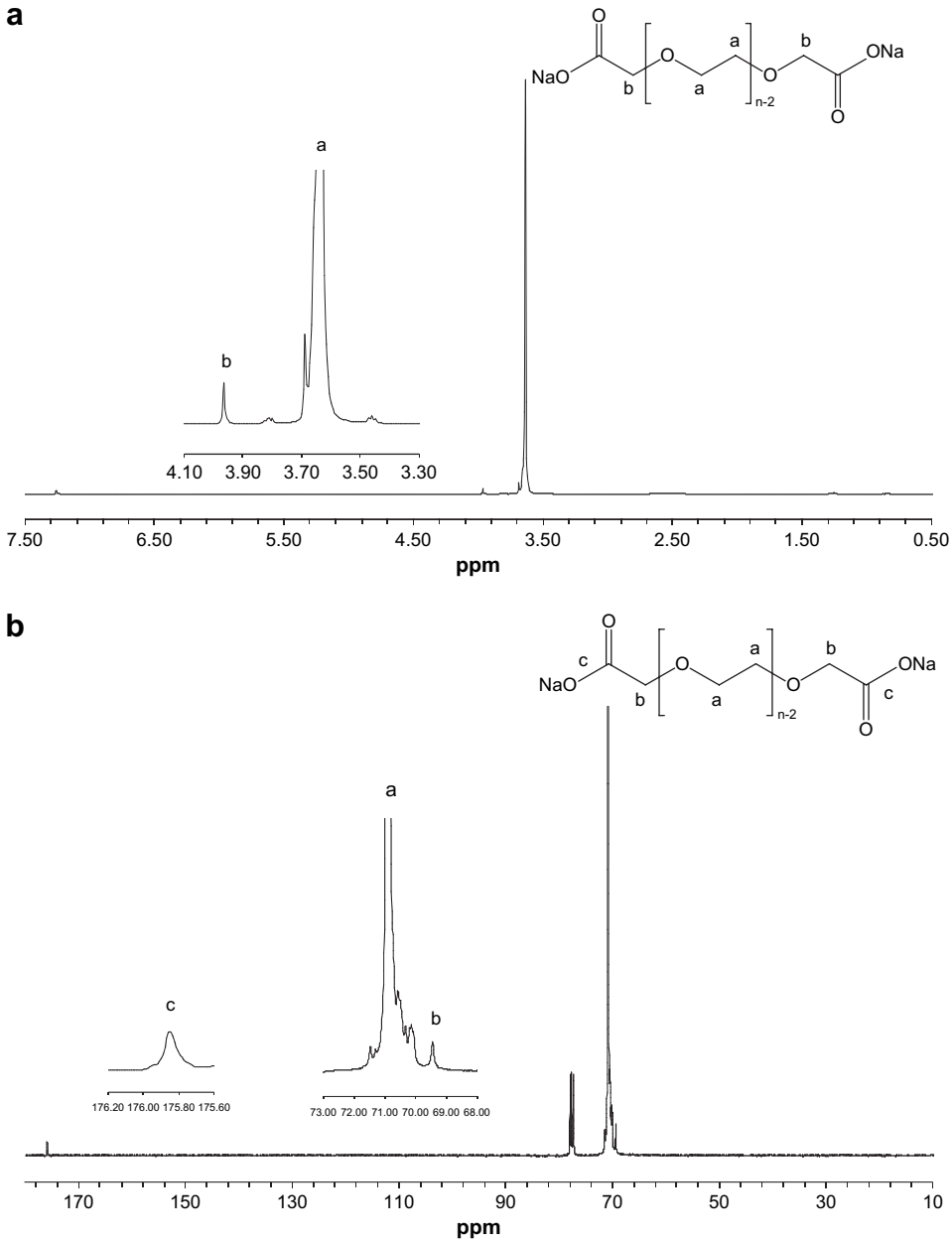


Fig. 1. (a) 400 MHz ^{1}H NMR and (b) 100 MHz ^{13}C NMR spectra of PEG3K–$(CO_2Na)_2$ in $CDCl_3$.

peak at 175.3 ppm along with the detection of a new carbonyl peak at 170.3 ppm is in line with the observation from ^{1}H NMR that all carboxylate moieties on the PEG macroinitiator have formed ester linkages through the ring opening of β-butyrolactone. Hence, implies the formation of PHB–PEG–PHB triblock architecture. An expansion at the PHB carbonyl carbon region revealed two relatively strong peaks that are due to different microstructures of the PHB chain. Syndiotactic diads of both (*R*)- and (*S*)-3-hydroxybutyrate repeating units have their carbonyl carbon signal slightly downfield, at 169.2 ppm, than those of isotactic diads at 169.1 ppm [37]. The two signals appeared in almost equal proportion and thus confirmed the atactic nature of PHB block.

For precise synthesis of triblock PHB–PEG–PHB copolymers, a controlled polymerization of β-butyrolactone is essential. Chain transfer reaction has been identified as a major detrimental factor to this as it not only terminates chain propagation prematurely but also simultaneously generates additional active sites for chain propagation. These lead to a loss of control in molecular weight of polymer as well as their end group integrity. Experimentally, these translate to lower polymer molecular weight from that expected based on monomer to initiator ratio, broadening of molecular weight distribution as well as formation of crotonate group at the chain end of polymer [48]. In our ^{1}H and ^{13}C NMR analyses, a negligible amount of crotonate end group was detected and this indicates that our synthetic approach, although unable to completely suppress chain transfer reaction, is capable of yielding PHB–PEG–PHB triblock copolymer of acceptable purity. This is demonstrated by the reasonably good agreement between the M_n values obtained by

Table 1
Molecular characteristics of PHB–PEG–PHB triblock copolymers

PHB–PEG–PHB copolymers[a]	$[M]_0/[I]_0$[b]	X[c] (%)	DP[d]	M_n			PDI[g]	PHB Content (wt%)		Yield (%)
				Theoretical[e]	NMR[f]	GPC		NMR[h]	TGA[i]	
H–E–H(12–68–12)	12	98.9	25	5200	5260	5490	1.10	41.0	44.5	79.0
H–E–H(24–68–24)	24	100.0	46	7280	7060	6880	1.08	56.5	59.2	80.6
H–E–H(30–68–30)	30	97.0	55	8160	7840	7530	1.05	61.3	63.1	70.3
H–E–H(10–23–10)	10	96.6	18	2840	2670	2030	1.15	63.4	66.9	88.2

[a] PHB–PEG–PHB triblock copolymers are represented by the notation H–E–H(m–n–m) where m and n denote theoretical number of 3-hydroxybutyrate and ethylene oxide repeating units, respectively.
[b] Initial monomer to initiator ratio in reaction mixture. Initiator concentration is twice the concentration of PEG macroinitiator.
[c] Monomer conversion of β-butyrolactone as calculated from ^{1}H NMR data.
[d] Degree of polymerization of PHB block; it was determined from the intensity ratio of PHB methine proton (δ 5.2 ppm) and methylene protons of end group and those at α and ω positions of PEG block (δ 4.0–4.2 ppm).
[e] Calculated from $M_n = M_n(\mathrm{PEG}) + 86 \times 2m \times X + 90$.
[f] Calculated from $M_n = M_n(\mathrm{PEG}) + 86 \times \mathrm{DP} + 90$.
[g] Obtained from GPC measurement.
[h] Calculated based on intensity ratio of PHB methine proton and PEG methylene proton.
[i] Calculated from TGA results.

GPC with the theoretical ones as well as with those determined by ^{1}H NMR, as listed in Table 1, and with narrow PDI values ranging from 1.05 to 1.15. GPC traces for all the triblock copolymers as well as their PEG macroinitiators are shown in Fig. 3. The unimodal traces all appeared at earlier retention time than their respective precursors, indicating proportionate growth of PHB chain from the respective PEG macroinitiators, with respect to initial monomer to initiator ratios. Although small peaks at higher molecular weight region, probably due to transesterification between growing chains, were observed in H–E–H(12–68–12) and H–E–H(24–68–24), they amounted only to 7 and 5% of total peak area and thus could still be considered essentially unimodal.

As an independent method, FT-IR spectroscopy was used to confirm findings from ^{1}H and ^{13}C NMR spectroscopies. FT-IR spectra of the PEG macroinitiators and the triblock copolymers are shown in Fig. 4. The triblock copolymers all bear a strong carbonyl C=O stretching vibration at 1738 cm^{-1} that is characteristics to amorphous PHB. Additionally, the characteristic C–O–C stretching vibration that belongs to ethylene oxide repeating units of PEG is also evidently present at 1102 cm^{-1} with increasing intensity as the PEG content in block copolymer increases. Coupled with the disappearance of strong C=O stretching vibration of carboxylate moiety of PEG macroinitiator at 1601 cm^{-1} in the copolymer spectra, we can conclude that the PHB chains have indeed grown on both sides of PEG macroinitiator, yielding PHB–PEG–PHB triblock copolymer bearing hydroxyl end group, that is represented by a broad absorption band centering at around 3500 cm^{-1}.

3.2. Crystallization behavior and thermal properties

Besides chemical information, FT-IR spectroscopy is able to offer us some insight into the crystallization behaviors of triblock copolymers. Crystalline phase of PEG is known to have absorption bands at 963 and 843 cm^{-1} [49]. In varying degree, they are found in the two PEG macroinitiators as well as triblock copolymers H–E–H(12–68–12), H–E–H(24–68–24) and H–E–H(30–68–30). The two absorption bands decrease in intensity with increasing PHB content and hence imply retardation in PEG crystallization by PHB chain. On the part of PHB block, its amorphous nature is apparent from the intense carbonyl C=O stretching vibration at 1738 cm^{-1} and a lack of absorption at 1723 cm^{-1} that is due to crystalline PHB carbonyl C=O stretching vibration. Further indications would be the presence of strong absorption at 1186 cm^{-1} that is characteristics of amorphous PHB and absence of absorptions at 1279 and 1228 cm^{-1} that are distinctive in crystalline PHB [50]. The complete lack of crystallinity on the PHB block is due to the atactic microstructure of PHB chain that prevents any chain packing.

XRD is also used to study the crystallinity of PHB–PEG–PHB triblock copolymers, and their diffraction patterns are shown in Fig. 5. It has been demonstrated that crystalline PEG showed X-ray diffraction peaks at $2\theta = 19.3$ and 23.5°, while PHB crystalline phase has diffraction peaks at $2\theta = 13.6$ and 17.0° [24]. In the case of the triblock copolymers, diffraction peaks at 19.3 and 23.5° are clearly seen in the H–E–H(12–68–12), H–E–H(24–68–24) and H–E–H(30–68–30) samples but not in H–E–H(10–23–10). The presence of diffraction peaks at these two positions implies that crystalline phase of PEG is present in the three triblock copolymers. Diffraction peaks of crystalline PHB, on the other hand, are not observed in any of the polymer samples and hence indicate that PHB block exists in amorphous state. The observations corroborate well with findings from FT-IR measurements. Comparing the peak intensity across the three copolymers that contain PEG segment of same molecular weight, one can find that PEG crystallinity decreases with increasing PHB chain length. This suggests that formation of PEG crystalline phase is hindered by PHB chain and the inhibition becomes more pronounced with longer PHB chain. The ability of PEG segment to form crystalline phase is affected not only by the chain length of PHB. As shown by the completely amorphous H–E–H(10–23–10) that has PHB blocks comparable in length to that of H–E–H(12–68–12), as well

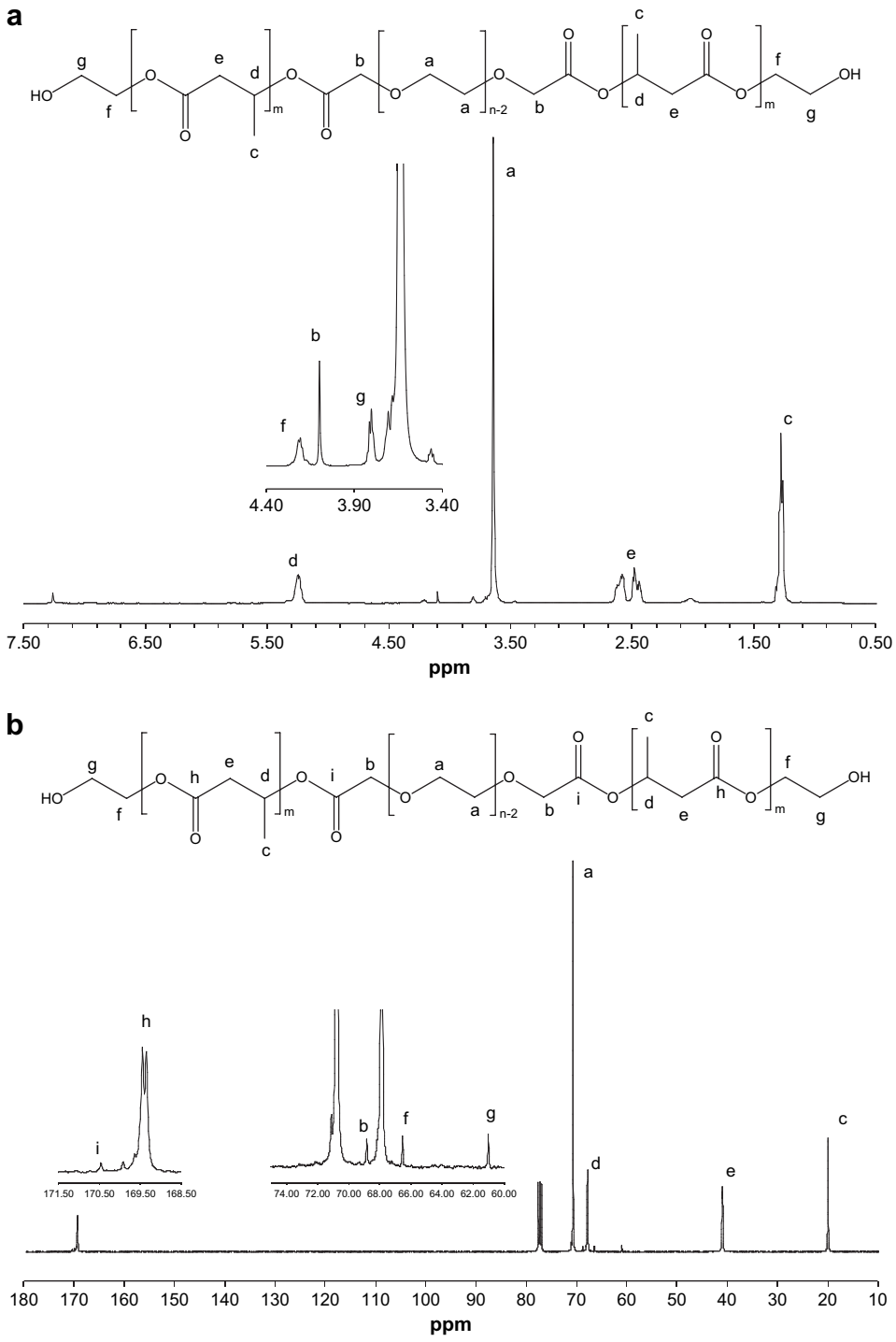


Fig. 2. (a) 400 MHz ^{1}H NMR and (b) 100 MHz ^{13}C NMR spectra of H–E–H(30–68–30) in $CDCl_3$.

as similar PEG content to that of H–E–H(30–68–30), the absence of PEG crystalline phase is also due to the lower molecular weight of PEG segment.

DSC study was carried out to further understand the interactions between PEG and PHB within the triblock copolymers. All copolymer samples were first heated to 150 °C to erase thermal history and subsequently cooled to −80 °C at a rate of 5 °C min^{-1} and heated back to 150 °C at the same rate. Analyses are based on first cooling and second heating runs. Thermal transitions of all the copolymers can be seen in Figs. 6 and 7 while their numerical values as well as corresponding enthalpies and PEG crystallinity (X_c) are presented in Table 2. Both PEG macroinitiators with M_n 1080 and 3060 were found to be crystalline with melting temperatures (T_m) at around 41 and 48 °C, respectively. In addition, the macroinitiator with lower molecular weight exhibited a glass transition temperature (T_g) at around −48 °C. Atactic PHB homopolymer is known to be amorphous with T_g around 0 °C [51]. Melting endotherms for copolymers

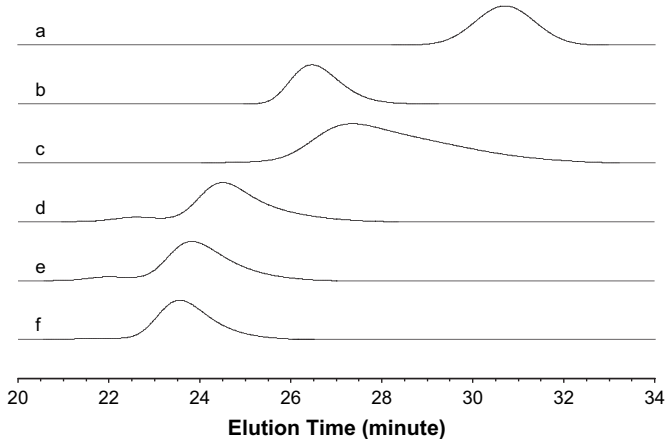


Fig. 3. GPC traces of PHB–PEG–PHB triblock copolymers and their PEG precursors: (a) PEG (M_n 1010, PDI 1.03); (b) PEG (M_n 2990, PDI 1.02); (c) H–E–H(10–23–10) (M_n 2030, PDI 1.15); (d) H–E–H(12–68–12) (M_n 5490, PDI 1.10); (e) H–E–H(24–68–24) (M_n 6880, PDI 1.08); (f) H–E–H(30–68–30) (M_n 7530, PDI 1.05).

consisting of longer PEG block were observed at 35.4, 32.2 and 27.7 °C for H–E–H(12–68–12), H–E–H(24–68–24) and H–E–H(30–68–30), respectively. The corresponding PEG melting enthalpies (ΔH_m) for the copolymers were 114.4, 97.5 and 92.2 J/g. The observed melting temperatures, corresponding enthalpies and hence PEG crystallinity were all lower than their PEG precursors and moreover these reductions are greater when PHB content increases. Hence, the observed changes are due to amorphous PHB present that hinders PEG crystallization. The effect of such hindrance is also reflected by the suppression of PEG crystallization during the first cooling run at a rate of 5 °C min^{-1}. When the copolymers were cooled from their completely amorphous state, only the PEG segment in H–E–H(12–68–12) managed to form crystalline phase, albeit at a much lower temperature than its precursor. The crystallization of PEG was kinetically hindered to a greater extent with higher PHB content and thus no PEG crystallization was observed in H–E–H(24–68–24) and H–E–H(30–68–30). However, in the second heating run, when the copolymers were heated above

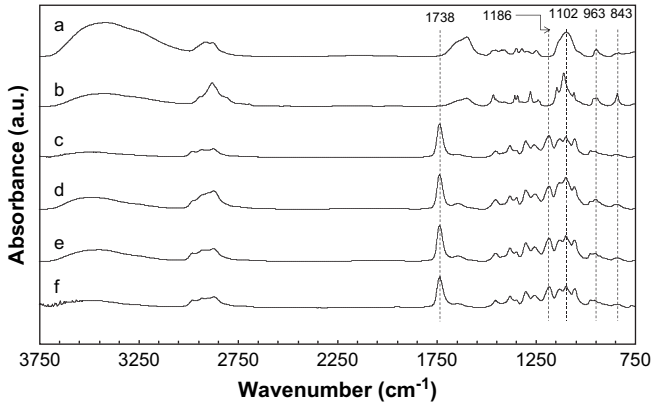


Fig. 4. FT-IR spectra of PEG macroinitiators and PHB–PEG–PHB triblock copolymers: (a) PEG1K–$(CO_2Na)_2$; (b) PEG3K–$(CO_2Na)_2$; (c) H–E–H(10–23–10); (d) H–E–H(12–68–12); (e) H–E–H(24–68–24); (f) H–E–H(30–68–30).

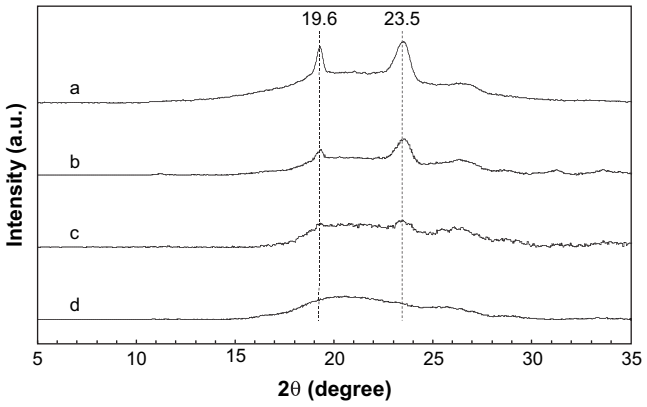


Fig. 5. XRD patterns of PHB–PEG–PHB triblock copolymers: (a) H–E–H(12–68–12); (b) H–E–H(24–68–24); (c) H–E–H(30–68–30); (d) H–E–H(10–23–10).

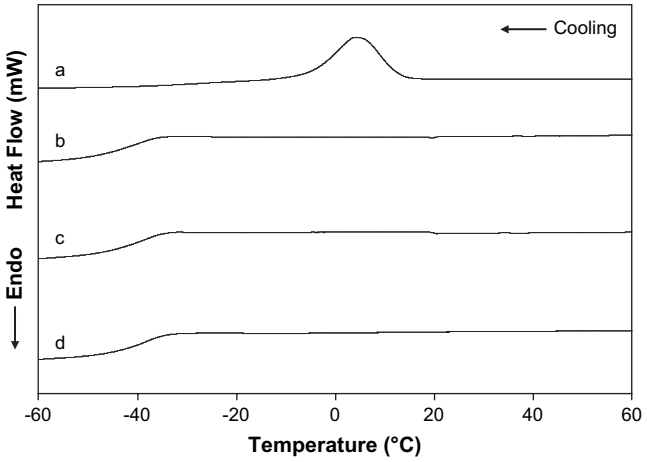


Fig. 6. DSC first cooling curves of (a) H–E–H(12–68–12); (b) H–E–H(24–68–24); (c) H–E–H(30–68–30); (d) H–E–H(10–23–10).

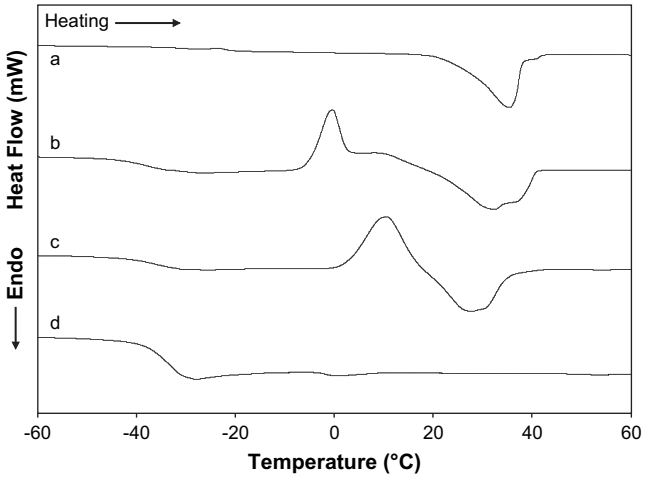


Fig. 7. DSC second heating curves of (a) H–E–H(12–68–12); (b) H–E–H(24–68–24); (c) H–E–H(30–68–30); (d) H–E–H(10–23–10).

Table 2
Glass transition temperatures, crystallization temperatures, cold crystallization temperatures, melting temperatures, corresponding enthalpies, PEG crystallinity and decomposition temperatures for PHB–PEG–PHB triblock copolymers

PHB–PEG–PHB copolymers	T_g[c] (°C)	T_c[d] (°C)	ΔH_c[d,i] (J/g)	T_{cc}[e] (°C)	ΔH_{cc}[e,i] (J/g)	T_m[f] (°C)	ΔH_m[f,i] (J/g)	X_c[g] (%)	T_d[h] (°C)	
									PHB	PEG
PEG MI[a] (M_n 1080)	−48.1	–	–	−11.4	75.68	41.1	85.83	41.9	–	363.2
PEG MI[a] (M_n 3060)	–	24.4	125.3	–	–	48.2	125.0	61.0	–	387.5
H–E–H(12–68–12)	−23.3	4.1	107.0	−26.6	1.06	35.4	114.4	55.8	222.4	389.2
H–E–H(24–68–24)	−38.0	–	–	−0.5	62.2	32.2	97.5	47.6	219.8	384.9
H–E–H(30–68–30)	−36.1	–	–	10.6	91.4	27.7	92.2	44.5	236.7	386.3
H–E–H(10–23–10)	−32.6	–	–	–	–	–	–	–	226.7	353.7
PHB[b] (M_n 1952)	−10.8	–	–	–	–	–	–	–	224.2	–

[a] PEG macroinitiator.
[b] Obtained from ring opening polymerization of β-butyrolactone under similar polymerization condition, except that sodium (*R*)-3-hydroxybutyrate was used as initiator.
[c] Glass transition temperatures determined in DSC second heating run.
[d] Crystallization temperatures of PEG and corresponding enthalpies determined in DSC first cooling run.
[e] Cold crystallization temperatures of PEG and corresponding enthalpies determined in DSC second heating run.
[f] Melting temperatures of PEG and corresponding enthalpies determined in DSC second heating run.
[g] PEG crystallinity calculated from melting enthalpies. Reference value of 205.0 J/g for completely crystallized PEG was used [22].
[h] Temperature at which 10% of segmental mass loss has occurred from TGA curves.
[i] Enthalpy changes were calculated based on the formula $\Delta H = \Delta H_i/W_i$, where ΔH_i is the area of the exothermic or endothermic peak for PEG read from DSC thermograms, and W_i is the weight fraction of PEG segment.

their T_gs, the gain in sufficient chain mobility allowed the copolymers to reorganize and thus lead to crystallization of PEG blocks. This can be seen from the large cold crystallization exotherms in both H–E–H(24–68–24) and H–E–H(30–68–30) in the second heating run. The kinetic restraint imposed by PHB chain on PEG cold crystallization can be seen from the delay in PEG cold crystallization from −26.6 to 10.6 °C when PHB content increases. H–E–H(10–23–10) was found to be completely amorphous at all temperatures and such a lack in PEG crystallinity is not only due to the amount of PHB present but also due to the lower molecular weight of PEG. This is inferred from the presence of 44.5% PEG crystallinity in H–E–H(30–68–30) although it has about the same PHB content as H–E–H(10–23–10).

All four triblock copolymers demonstrated a single transition from glassy to rubbery state in the second heating run and at temperatures between T_gs of PEG macroinitiators and PHB homopolymer. The observation points to the favorable mixing of PHB and PEG in their amorphous state. In fact, with greater PHB content, T_gs of the copolymer increase from −38.0 to −32.6 °C, as in the case of H–E–H(24–68–24), H–E–H(30–68–30) and H–E–H(10–23–10). The T_g of H–E–H(12–68–12), however, is unexpectedly high at −23.3 °C. This can be explained by the presence of lesser amount of PEG in the amorphous state as most have been crystallized in the first cooling run.

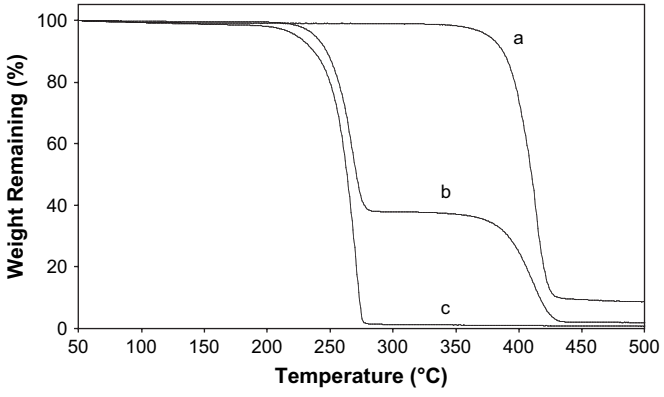


Fig. 8. Thermal degradation profiles of (a) PEG3K–$(CO_2Na)_2$; (b) H–E–H(30–68–30); (c) PHB (DP = 30).

Thermal stability of the triblock copolymers was evaluated using TGA under dynamic N_2 atmosphere. Fig. 8 shows the thermal degradation profile of H–E–H(30–68–30), that is typical of all copolymers, along with those of its PEG precursor and a PHB homopolymer with around 30 repeating units. The copolymer exhibited a two-step degradation behavior, with the first step occurring between 180 and 310 °C followed by the second step from 310 to 450 °C. Thermal degradation of the PEG precursor occurred from 330 to 450 °C while PHB homopolymer degraded from 180 to 310 °C. Comparison amongst the three weight loss profiles allows us to attribute the first degradation step to the degradation of PHB chains within the triblock copolymer and the second step being the loss of PEG block. Good resolution between the two degradation steps enables us to estimate the amount of PHB that is present in the copolymers and the values are tabulated in Table 1. The results are in good agreement with the estimation based on ^{1}H NMR data. Temperature at which 10% weight loss has occurred is taken as the degradation temperature (T_d) of each block [52]. The values are listed in Table 2 and it can be seen that thermal stability of PHB and PEG segments within the triblock copolymers is comparable to those of the corresponding homopolymers.

4. Conclusions

A series of well-defined B–A–B amphiphilic triblock copolymers consisting of atactic PHB as the hydrophobic segment (B) and PEG as the middle block (A) were synthesized through

crown ether-free anionic ring opening polymerization of (*R*,*S*)-β-butyrolactone from PEG macroinitiators. PEG macroinitiators were prepared from commercially available PEGs through a facile TEMPO-mediated oxidation as the key step to obtain pure macroinitiator that allows controlled synthesis of the triblock copolymers. Molecular characterizations based on ^{1}H NMR, ^{13}C NMR, GPC and FT-IR analyses confirmed the well-defined structure of the telechelic-hydroxylated PHB–PEG–PHB triblock copolymers. PHB content ranging from 40 to 70% was attained by varying the β-butyrolactone to PEG macroinitiator ratio or by using PEG macroinitiator of different molecular weights. In the context of biomaterials, this synthetic approach is particularly attractive as the use of toxic crown ether or metal catalyst is totally eliminated and hence avoids the contamination of these catalysts in the final copolymer.

FT-IR, XRD and DSC analyses coherently revealed the coexistence of crystalline PEG and amorphous PHB in the copolymers, except for H–E–H(10–23–10) which was found to be completely amorphous. Crystallinity of PEG decreases with increasing PHB content and thus implying restraint imposed by PHB chains on the crystallization of PEG. Single T_gs were detected on most copolymers and all lie between T_gs of PEG and PHB homopolymers. These signified the favorable mixing of PEG and PHB in the amorphous state.

Acknowledgements

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