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ORIGINAL PAPER

Novel poly(3-hydroxy butyrate) macro RAFT agent. Synthesis and characterization of thermoresponsive block copolymers

Baki Hazer[1,2] · Melike Eren[2] · Yetkin Senemoğlu[2] · Tina Modjinou[3] · Estelle Renard[3] · Valérie Langlois[3]

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Abstract

A novel reversible addition fragmentation transfer (RAFT) agent based on the poly-3-hydroxy butyrate (PHB) with three hydroxyl groups (PHB-R2) and thermoresponsive amphiphilic block copolymers derived from N-isopropyl acryl amide (NIPAM) are described. Hydroxylated PHB is obtained by the reaction between PHB and diethanol amine (DEA) to prepare hydroxylated PHB (PHB-DEA). It is then reacted with a RAFT agent, 2-(dodecylthiocarbonothioylthio)-2-methylpropionic acid (DDMAT). Hydroxyl ends of the hydroxylated PHB are capped with carboxylic acid end of the trithiocarbonate. The block copolymers obtained by the polymerization of NIPAM initiated by PHB-R2 were characterized by ^{1}H NMR and physicochemical techniques. PHB content in the obtained block copolymers is varying between 14 and 45 wt%. The thermo-responsive PHB-PNIPAM block copolymers show the lower critical solution temperature (LCST) 28 and 30 °C while LCST of the pure PNIPAM is 32 °C.

Keywords Poly (3-hydroxy butyrate) · RAFT polymerization · Polymerization kinetics · Thermoresponsive polymer · PNIPAM

Introduction

Some microorganisms accumulate poly(3-hydroxy alkanoic acid) (PHA) into their cell as energy reserve material from carbon sources [1]. Regarding pendant groups, PHA can be classified in three types: short chain length (scl) PHA, medium chain length (mcl) PHA and long chain length (lcl) PHA [2–8]. However, they need modification reactions because of their limited properties for industrial and medical applications [9–16].

PHAs are biodegradable, biocompatible hydrophobic semi crystalline polyesters. Mcl-PHAs are soft elastomeric polyesters and show low thermal resistant (ca. T_m 50 °C) while scl-PHAs are brittle solid materials and show higher thermal resistance (ca. T_m 170 °C). There are fine modification reactions to enhance hydrophilicity of the mcl-, and scl-PHAs. Among them, thiol-ene reactions [17, 18], esterification with PEG [19, 20], water soluble PHA-sulfonate [21, 22], and aminolysis reactions [23] were successfully used in order to obtain mcl-PHA with enhanced hydrophilicity.

There are some modification reactions of mcl-PHAs. In a combined reaction of thiol terminated poly (N-isopropyl acryl amide) (PNIPAM) with thiol-ene reaction of unsaturated mcl-PHA results in a thermoresponsive copolymer of PHA. In this manner, chain transfer agent terminated poly (N-isopropyl acryl amide) (PNIPAM) converted to PNIPAM with thiol end is used in the thiol-ene click reaction with a random copolymer of 3-hydroxydodecanoate (3HDD) and 3-hydroxy-10-undecylenate [24]. In a recent work, a novel elastomer, polystyrene copolymer of a mcl-PHA, have been described [25]. There are also some reports on PHB modification reactions. Chlorination reactions of PHB result in chloride derivative of PHB for further modification reactions [26, 27]. A recent published article reports optical properties of blend of the chlorinated PHB and PMMA [28]. PEG is a hydrophilic and biocompatible polymer. It is widely used in preparation of amphiphilic PHB derivatives [29]. Atom transfer radical polymerization of NIPAM and dimethyl amino ethyl

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✉ Baki Hazer
bhazer2@yahoo.com; bkhazer@beun.edu.tr

✉ Valérie Langlois
langlois@icmpe.cnrs.fr

[1] Department of Aircraft Airframe Engine Maintenance, Kapadokya University, 50420 Ürgüp, Nevşehir, Turkey

[2] Department of Chemistry, Department of Nano Technology Engineering, Bülent Ecevit University, 67100 Zonguldak, Turkey

[3] CNRS, Inst Chim & Mat Paris Est, UMR 7182, 2-8 Rue Henri Dunant, University of Paris Est, F-94320 Thiais, France

methacrylate initiated by brominated PHB-macroinitiator rendered the related responsive block copolymers which could be used for the encapsulation and release of therapeutic drugs in the biological systems [30, 31]. Dihydroxylated PHB (PHB-diol) prepolymer is prepared by transesterification from the natural PHB with 1,10-decane diol [32] and diethylene glycol [33]. Multiblock-elastomeric PHB-PEG-PHO copolymers can be obtained by the amination reaction of PHB with the amine terminated PEG [34]. Recently, hydroxylated PHB (PHB-DEA) was derived from the PHB and diethanol amine. Then, a radiopaque biodegradable polymer was obtained by the capping reaction of hydroxyl ends of PHB-DEA with iodo benzoyl chloride [35]. In this work, hydroxyl ends of the PHB-DEA were capped with the carboxylic acid end of the tri thio carbonate, 2-(dodecyl thio carbonothioylthio)-2-methylpropionic acid. RAFT polymerization of NIPAM initiated by PHB-macro RAFT agent, resulted in PHB-PNIPAM thermoresponsive amphiphilic graft copolymers. Physicochemical characterization of series of the PHB-PNIPAM thermoresponsive block copolymers were characterized by ^{1}H NMR and physicochemical methods.

Experimental

Materials

Poly (3 - hydroxy butyrate) (PHB), microbial polyester (*M*n 187,000 g/mol, *M*w/*M*n 2.5, Biomer Inc.) was supplied from BIOMER (Germany) [36]. 2-(Dodecylthiocarbonothioylthio)-2-methylpropionic acid (DDMAT) was synthesized according to the procedure in cited reference [37]. Dimethyl formamide (DMF), N,N′-dicyclohexylcarbodiimid (DCC), dimethyl amino pyridine (DMAP), stannous 2-ethyl hexanoate (Sn-oct), diethanol amine (DEA), N-isopropyl acryl amide (NIPAM), $NaBH_4$ and the other chemicals were purchased from Sigma-Aldrich. NIPAM was crystallized from boiling n-hexane before use.

Synthesis of PHB-DEA

Hydroxylated PHB was obtained by the reaction of PHB with DEA according to the procedure reported in the cited reference [35]. Briefly: A mixture of 60.3 g of vacuum dried PHB, 102 g of DEA and 2.1 g of Sn-oct in 250 mL of $CHCl_3$ was stirred at room temperature for 24 h. Then, it was refluxed for 3 h. The solvent was distilled under atmospheric condition (not in the rotary evaporator). Then the crude product was cured in 125 °C for 30 min. The product was leached with excess methanol and filtered. The crude product was dried under vacuum at 40 °C. For further purification, the obtained polymer was dissolved in 600 mL of $CHCl_3$ and filtered. The solvent was evaporated in a rotary evaporator. The obtained polymer was leached excess methanol again. Methanol was removed via filtration. The pure PHB-DEA was dried under vacuum at 40 °C for 24 h. Yield was 36 g. This was coded as PHB-DEA-13. Characteristic FTIR signals: 1567 cm^{-1} amide carbonyl; 3301 cm^{-1} primary hydroxyl groups of DEA; 1721 cm^{-1} belongs to ester carbonyl of PHB. The characteristic chemical shifts of the PHB-DEA sample in ^{1}H NMR spectrum were observed at 1.3 ppm for $–CH_3$, 2.4–2.6 ppm for $–CH_2$-COO–, 3.0 ppm for –N-CH_2–, 3.5–3.8 ppm for –CH_2-OH, 4.1 ppm for –CH-OH and 5.1–5.3 ppm for –CH-O–.

Synthesis of PHB macro-raft agent (PHB-mR)

The PHB-macro-RAFT agent (PHB-R2–131) was obtained by the reaction between PHB-DEA-22 and 2-(Dodecylthiocarbonothioylthio)-2-methylpropionic acid (DDMAT), using the procedure reported by Steglich esterification method [38]. Briefly, PHB-DEA-22 (9.8 g) was dissolved in a mixture of CH_2Cl_2 (100 mL) and DMF (5 mL). To this solution were added continuously stirring DDMAT (6.3 g, 0.017 mol), DCC (9.2 g, 0.045 mol) and DMAP (0.81 g, 0.0066 mol) under Argon. After stirred at room temperature for 24 h, the precipitated side product, dihexyl urea, was filtered. The solvent of the filtered solution was evaporated, the crude product was leached with excess methanol, and the PHB-R2 was purified via filtration. The white solid product was dried under vacuum at 40 °C for 24 h. The GPC result (in DMF) was Mn 3800 Da, Mw 4700 Da, PDI 1.225.

RAFT polymerization of NIPAM initiated by PHB-R2

RAFT polymerization of NIPAM was carried out at 80 °C in DMF. For example, the mixture of 0.507 g of PHB-R2, 19 mg of AIBN and 1.22 g of NIPAM was dissolved in 5 mL of DMF in a glass bottle. Argon was introduced through a needle into the tube for about 1 min to expel the air. The tightly capped bottle was then put into a water bath at 80 °C for 220 min. Then, the crude polymer solution diluted with 5 mL of $CHCl_3$ was precipitated in 200 mL of methanol. The graft copolymer samples were dried overnight under vacuum at 40 °C for 24 h. The dried copolymer (1.0 g) was stirred in THF (20 mL) for 2 h. The mixture was filtered from the insoluble copolymer residue. PHB-PNIPAM copolymer was isolated by evaporating the solvent. The THF soluble polymer was dried under vacuum at 40 °C for 24 h.

Characterization

^{1}H NMR and FT-IR ^{1}H NMR spectra of the obtained products were taken at 25 °C with an Agilent NMR 600 MHz NMR (Agilent, Santa Clara, CA, USA) spectrometer equipped with a 3 mm broadband probe. Acquisition parameters included a

45° hard pulse angle, a sweep width of 14 ppm, 1.7 s acquisition time, 0.1 s pulse delay and continuous WALTZ - 16 broadband 1H decoupling. Up to 2000 scans were collected per sample, corresponding to ~1 h of collection time. FT- IR spectra of the polymer samples were recorded using Perkin-Elmer FT - IR Spectrometer 100.

Size exclusion chromatography (SEC) analysis Two different SEC instruments were used in this work; eluents were THF and DMF, separately. For THF eluent, molecular weights were determined by size exclusion chromatography instrument, Viscotek GPCmax Auto sampler system, consisting of a pump, three ViscoGEL GPC columns (G2000H HR, G3000H HR and G4000H HR), and a Viscotek differential refractive index (RI) detector with a THF flow rate of 1.0 mL/min at 30 °C. A calibration curve was generated with four polystyrene (PS) green standards: 8450, 2960, 50,400, 200,000 and 696,500 Da, of low polydispersity. The polymer sample solution containing 0.05 g in 10 mL of THF was filtered and injected automatically into the instrument. Data was analyzed using Viscotek Omni SEC Omni 01 software.

Another Size Exclusion Chromatography using DMF as eluent with Flow rate 0.5 mL/min was also used. It was TOSOH EcoSEC – WYATT HELEOS-II – WYATT DynaPro Nanostar GPC System using. Column: Tosoh HHR5000-HHR3000-HHR Guard Column Set. Low polydispersity index polystyrene standards purchased from Polysciences were used to calibrate the system (483.000; 184,900; 60,450; 19,720; 8450; 3370, 580 g.mol^{-1}). The samples were dissolved at a concentration of 10 mg.mL^{-1} and filtered on a PTFE filter of 0.5 μm prior analysis.

Thermal analysis Thermal analysis of the obtained polymers was carried out under nitrogen using a TA Q2000 DSC and Q600 Simultaneous DSC - TGA (SDT) series thermal analysis systems. Differential Scanning Calorimeters (DSC) measures temperatures and heat flows associated with thermal transitions in the polymer samples obtained. The dried sample was heated from - 60 to 120 °C under nitrogen atmosphere heating from 20 to 600 °C at a rate of 10 °C/min.

The mass loss of the samples was determined by TGA under nitrogen atmosphere using a Setaram Labsys Evo 1150 apparatus by heating from 30 °C to 550 °C at 10 °C.min^{-1}.

Proton NMR spectra in $CDCl_3$ solutions of the samples were taken at a temperature of 25 °C with an Agilent NMR 600 MHz NMR (Agilent, Santa Clara, CA, USA) spectrometer equipped with a 3 mm broadband probe.

LCST determination At fixed temperature, vacuum dried copolymer film (200 mg) was soaked into water. After 3 h, the swollen polymer film gently wiped with a paper tissue and weighed. The water uptake of the film was calculated using the following equation:

$$\text{Water uptake (WT\%)} = \frac{(m_s - m_d)}{m_d} \times 100 \tag{1}$$

Where, m_s is weight of swollen polymer film and m_d is weight of dried polymer film.

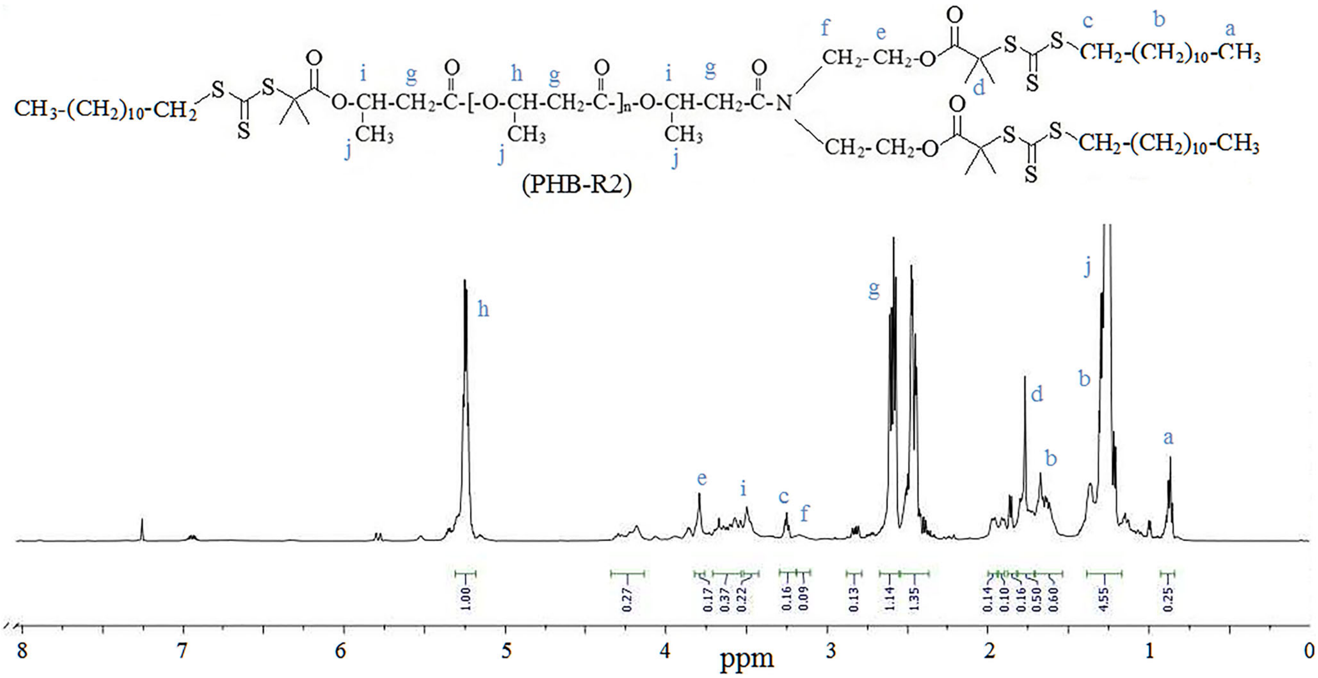
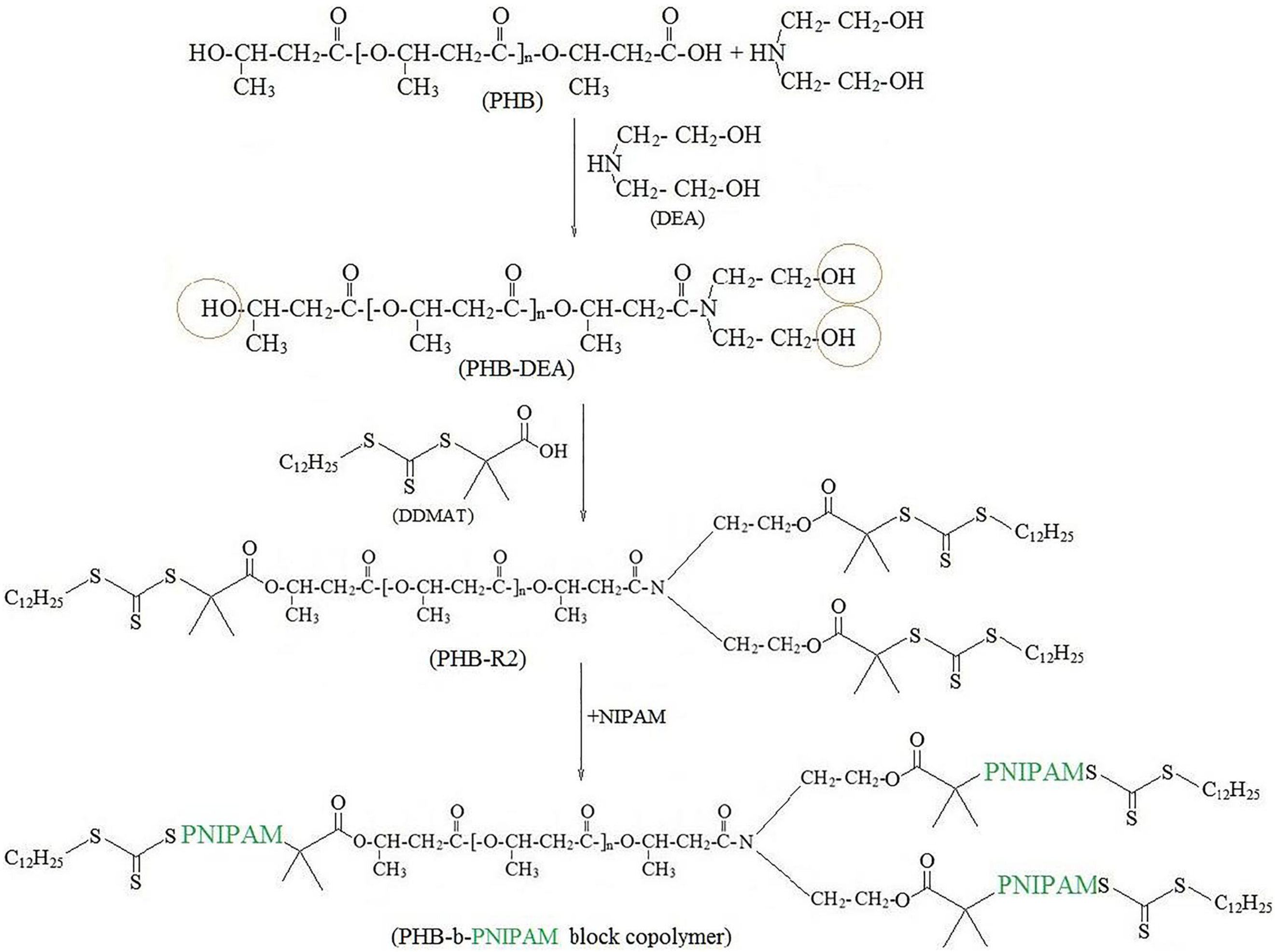


Fig. 1 ^1H NMR spectrum of the PHB-macro raft agent, PHB-R2



Scheme 1 Reaction steps for the synthesis of the PHB-R2 macro raft agent

The same swelling procedure was repeated at 4, 22, 27, 29, 32, and 34 °C. Water uptake against temperature was plotted. The strong drop in sigmoidal curve offers us LCST for this thermoresponsive copolymer.

Micelle formation and dynamic light scattering analysis Micelles were prepared according to the Cheng [39] method. 10 mg of the PHB-PNIPAM-6 were dissolved in 1 mL of DMF under stirring during 2 h. Then, 10 mL of Milli-Q ultra purified water were added dropwise to the solution under stirring. After 3 h of stirring at room temperature, the DMF was removed by dialysis against water for 3 days.

DLS measurements were performed at 633 nm on a Malvern Instrument Zetasizer Nano ZS provided with a He-

Table 1 RAFT polymerization of NIPAM using PHB-R2 macro raft agent (0.50 g) and AIBN (20 mg) in 5 mL of DMF at 80 °C for 205 min. (*calculated from their NMR spectra)

Code	PHB-R2 (wt%)	NIPAM (g)	NIPAM wt%	Copolymer Yield (g)	Mn (D)	Mw (D)	Copolymer Yield PDI	PHB content* in cop.(%)
PHB-PNIPAM-0	50	0.51	50	0.84	5400	6600	1.2	45
PHB-PNIPAM-1	29	1.22	71	1.67	9900	11,200	1.1	33
PHB-PNIPAM-2	18	2.25	82	2.61	14,700	16,800	1.1	24
PHB-PNIPAM-3	12	3.84	88	3.69	15,600	19,300	1.2	18
PHB-PNIPAM-4	9	5.20	91	4.77	16,600	20,600	1.2	14
PHB-R2					3800	4700	1.2	(in DMF)

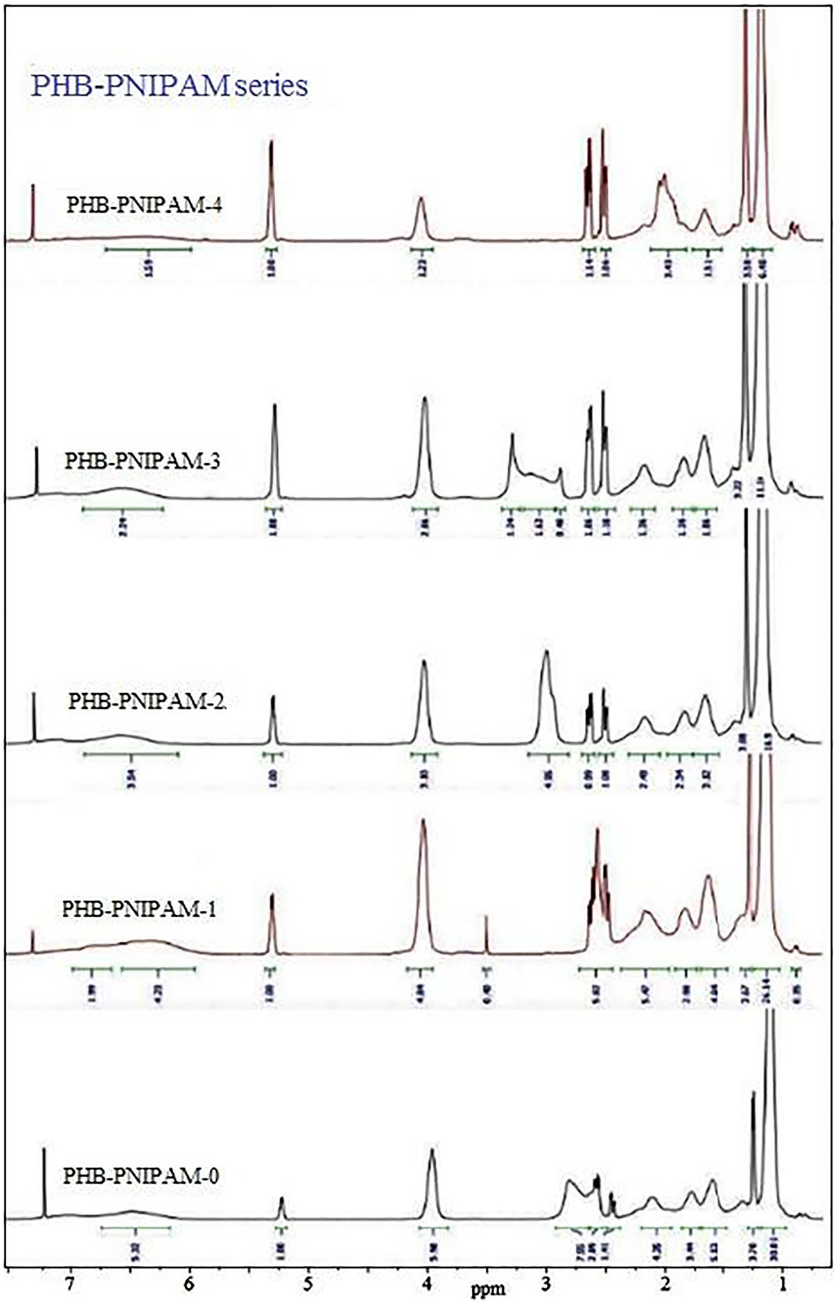


Fig. 2 ^{1}H NMR spectra of the obtained PHB-PNIPAM block copolymers

Ne laser source. Measurements were conducted at an angle of 173° at 25 °C. The cumulant method was used to analyze the autocorrelation functions to determine dimeters and distributions.

Results and discussion

The main objective of this work is to prepare a new PHB-macro RAFT agent and to study the polymerization of NIPAM. For this purpose, we started with the synthesis of hydroxylated PHB using DEA to prepare the novel macro RAFT agent. PHB was refluxed with DEA and stannous octoate as catalyst in chloroform in an oil bath at 80 °C, after solvent was distilled; PHB-DEA crude product was cured at 125 °C for 30 min. During the hydroxylation process at least 20% of the starting material remains undissolved in chloroform. The unreacted starting material was removed from the chloroform solution via filtration [32]. Then, the hydroxyl ends of the pure PHB-DEA were capped with DDMAT using Steglich reaction process. Because, trithiocarbonate (DDMAT) have been previously shown to be effective for

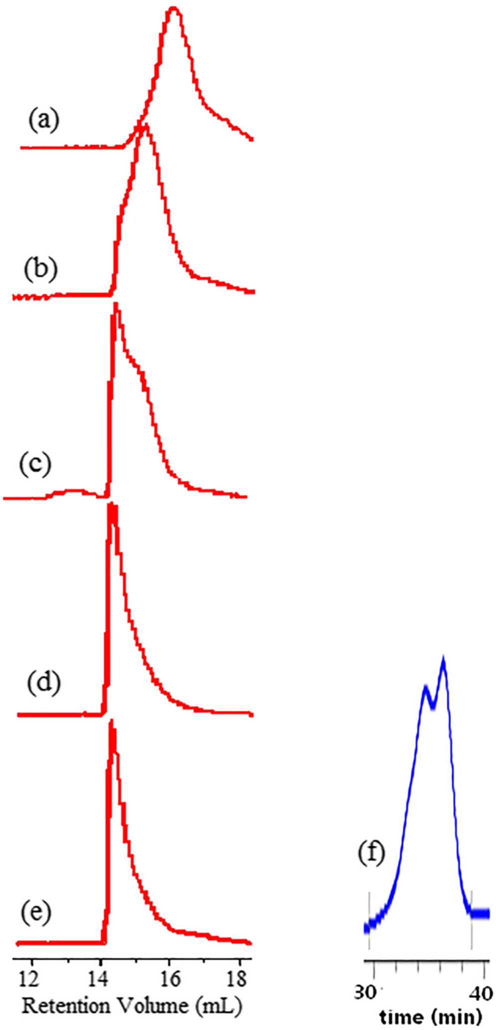


Fig. 3 SEC measurements of the PHB-PNIPAM block copolymers: **a** PHB-PNIPAM-0, **b** PHB-PNIPAM-1, **c** PHB-PNIPAM-2, **d** PHB-PNIPAM-3, **e** PHB-PNIPAM-4, **(f)** PHB-R2

the controlled polymerization of a variety of monomers [40]. The characteristic signals of PHB-macro RAFT agents (PHB-R2) were observed in its ^{1}H NMR spectrum. Figure 1 shows the all labeled signals of the macro RAFT agent. The characteristic signals of the PHB-RAFT agent in the 1H NMR spectra were observed: δ ppm: for PHB blocks: 1.25 (–CH3, j), 2.5 [–CH2-C(O)-, g], 5.25 (–CH-O-, h); for DEA units: 3.1 (–N-CH2-, f), 3.8 (-CH2-O-, e); for the trithiocarbonate units 0.85 ppm (CH3, a), 1.65 ppm [−(CH2)10-, b], 1.75 ppm [-C(CH3)2, d], 3.25 ppm (-S-CH2-, c).

Synthesis of PHB-NIPAM thermoresponsive copolymers PHB macro RAFT agent was used in RAFT polymerization of NIPAM as chain transfer agent at 80 °C. Scheme 1 shows the reaction steps of the RAFT polymerization of NIPAM by PHB-2R and AIBN at 80 °C. A series of the block copolymers were obtained by the RAFT polymerization of NIPAM using different amount of PHB-R2 at 80 °C. The ratio of the RAFT agent to AIBN was varying from 25/1 to 55/1 while polymerization time was kept at 205 min.

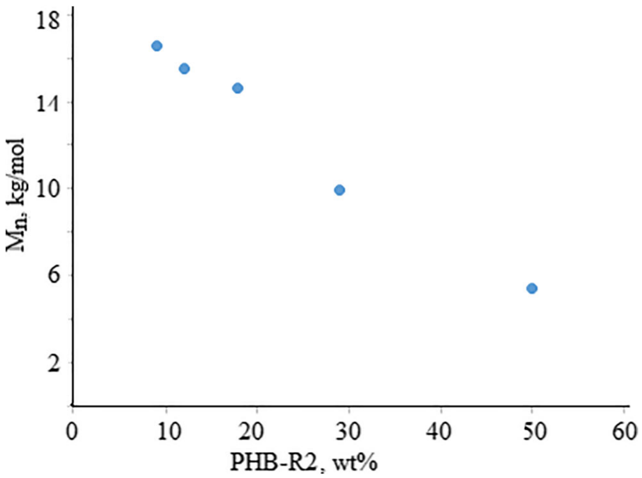


Fig. 4 RAFT polymerization of NIPAM. Variation of PHB-R2 concentration (wt%) in feeding against molar masses of the obtained copolymers

Polymerization conditions and results were listed in Table 1. PHB content in the beginning was proportional with the PHB content in copolymer determined from their NMR spectra. Small difference in PHB content may come from that NIPAM monomer used in the beginning does not convert to polymer completely. Then the PHB content is found to be slightly higher than the beginning one. In case of the highest concentration of PHB, the slightly less PHB content was observed. Presumably, some degraded PHB copolymer can be solubilized in non-solvent media.

^{1}H NMR technique was used for the structural analysis of the obtained PHB-PNIPAM block copolymers. As expected, PHB inclusion of the obtained copolymer was proportional with the PHB-R2 used in feeding. Figure 2 shows the comparative ^{1}H NMR spectra of the copolymer series. As macro RAFT agent, PHB-R2, decreases in feeding, much PNIPAM but low PHB introduces into the obtained copolymer. PHB contents in the obtained copolymers were calculated by comparing chemical shifts at 5.2 ppm (-C**H**-O-, PHB) with 4.0 ppm (>C**H**-NH-, PNIPAM) in their ^{1}H NMR spectra. The calculated PHB contents in the obtained copolymers were listed in Table 1 as well.

We used SEC instrument with eluent THF for the THF soluble PHB-PNIPAM −0-5 series. PHB-R2 was soluble in DMF as eluent, and then we used another SEC instrument with eluent DMF. For the PHB-PNIPAM series, molar masses were measured in range between from 5400 to 16,600 Da while molar mass of PHB-R2 chain transfer agent 3800 Da. SEC results and the PHB contents in the copolymers were tabulated in Table 1. The GPC chromatograms of the obtained PHB-PNIPAM block copolymer −0-4 series can be seen in Fig. 3. There are smooth correlation with PHB contents in feeding composition and molar masses. Increase in NIPAM

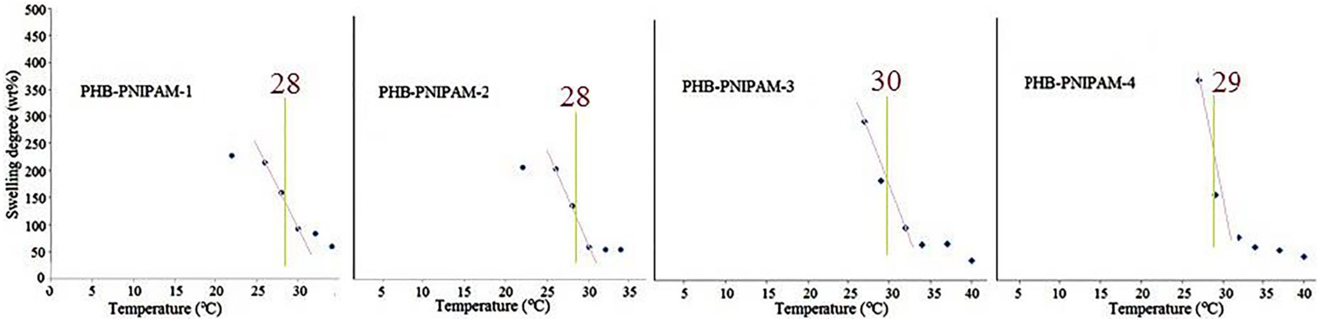


Fig. 5 Variation of the swelling degrees in water against temperature: PHB-PNIPAM-1, −2, −3, and − 4

feeding causes increase in polymer yield and increase in molar mass. The dispersity is around 1.2 for monomer conversions above 40%. Interestingly, SEC analyses revealed bimodal molar mass distributions for some samples. One could speculate that the PHB-R2 chain transfer agent can behave with varying activity in the monomer solution, which clearly requires extensive additional studies [41].

In this manner, in the highest PHB-R2 concentration leads to copolymer with the lowest Mn (5400 g/mol) while the lowest concentration of the raft agent yields the block copolymer with

Fig. 6 TGA analysis of the PHB-PNIPAM samples

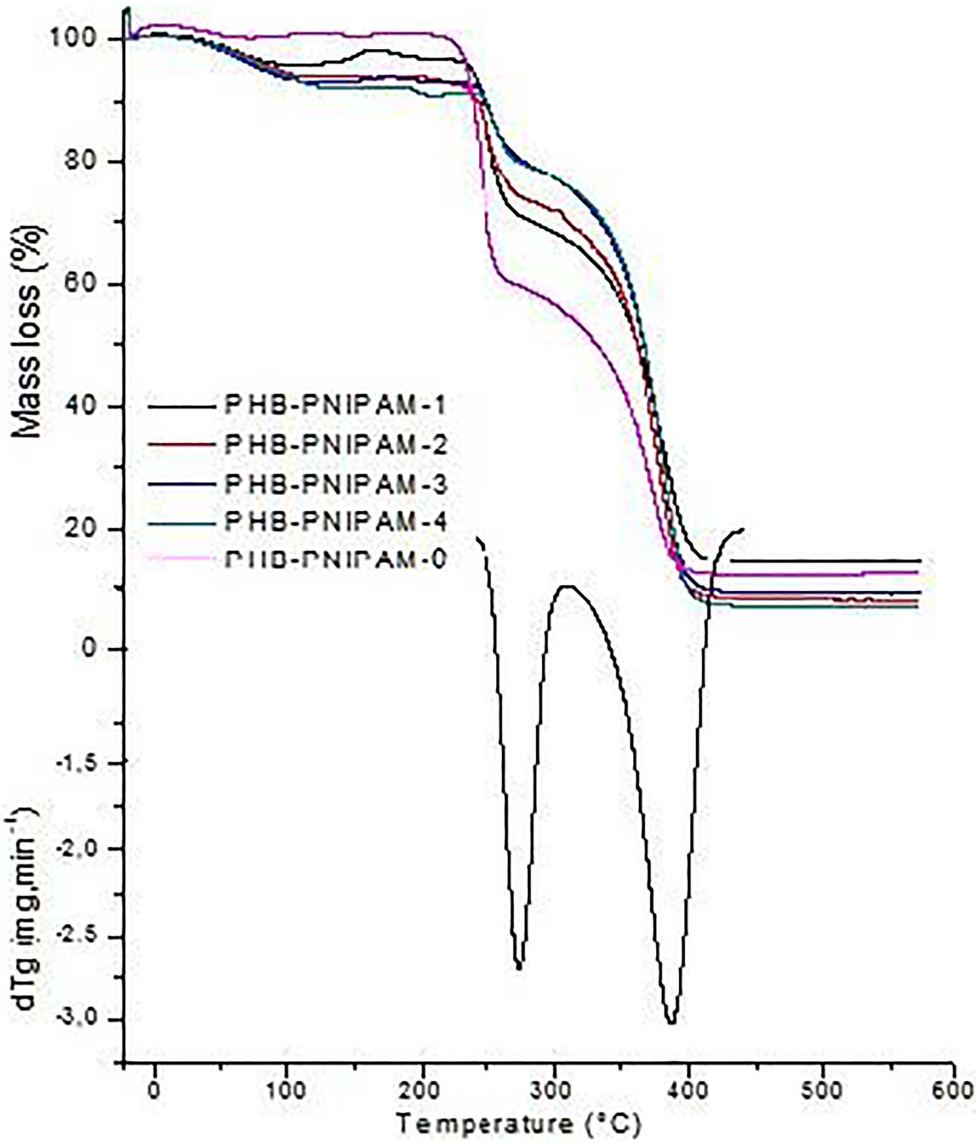


Table 2 Results of the thermogravimetric analysis of the PHB-PNIPAM samples

Sample	T_{d1} 245 °C (wt loss%)	T_{d2} 380 °C (wt loss%)
PHB-PNIPAM-0	44	45
PHB-PNIPAM-1	27	55
PHB-PNIPAM-2	20	65
PHB-PNIPAM-3	14	70
PHB-PNIPAM-4	11	73

the highest Mn (16,600 g/mol). Figure 4 shows the variation of the macro RAFT agent concentration (PHB-R2, wt%) in feeding against the molar masses of the obtained block copolymers.

Lower critical solution temperatures of PHB-PNIPAM block copolymers were determined by the swelling measurements at the temperatures between from 40 to 22 °C. The samples had the LCSTs between 28 to 30 °C. The enhanced hydrophobicity leads to lower LCST of NIPAM [42]. Variation of the swelling degrees in water against temperature can be seen in Fig. 5.

Thermogravimetric analysis The PHB-PNIPAM0–4 samples were found to be thermally stable below 200 °C, exhibiting two main weight loss steps (Fig. 6). The evaporation of the small and soluble compounds was observed at lower temperature than 240 °C. The first stage of degradation with a T_{max} of 260 °C occurs between 245 and 300 °C can be attributed to the thermal degradation of PHB main chains. The second stage of degradation with a T_{max} of 380 °C associated to the weight loss of PNIPAM blocks occurs between 300 and 420 °C. The TGA results can be seen in Table 2.

Micelle formation PHB-PNIPAM copolymers were synthesized by RAFT mechanism. The solution behaviors of some

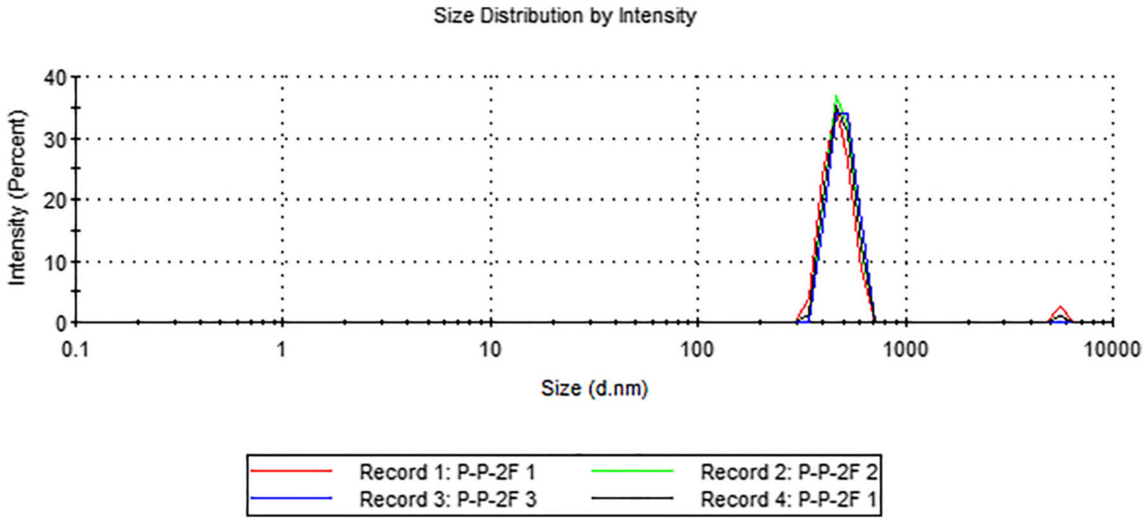


Fig. 7 Particle size distribution analyzed by dynamic light scattering (DLS) of PHB-PNIPAM-2 before filtration

Table 3 Polymerization of NIPAM (500 mg, 2.21 M) initiated by PHB-R2: 200 mg (26 mM), AIBN: 8 mg, DMF: 2.0 mL, at 70 °C)

Code	Time	Yield	Conv.	PHB		Water	Inslble yield	
	(min)	(mg)	(%)	(%)	$Ln[M_o]/[M]$	uptake(%)	in H_2O(%)	(mg)
PHB-NIPAM-740	5	293	18.6		0.206	156	80	236
PHB-NIPAM-741	10	368	33.6	52	0.41	132	46	170
PHB-NIPAM-746	15	487	57.4		0.85	173	47	231
PHB-NIPAM-742	20	533	66.6	34	1.10	167	37	197
PHB-NIPAM-743	30	568	73.6	27	1.33	239	35	198
PHB-NIPAM-744	42	617	83.4	29	1.80	202	37	227
PHB-NIPAM-747	50	618	83.6	1.81	205	51	315	
PHB-NIPAM-745	55	634	86.8	24	2.025	248	24	155

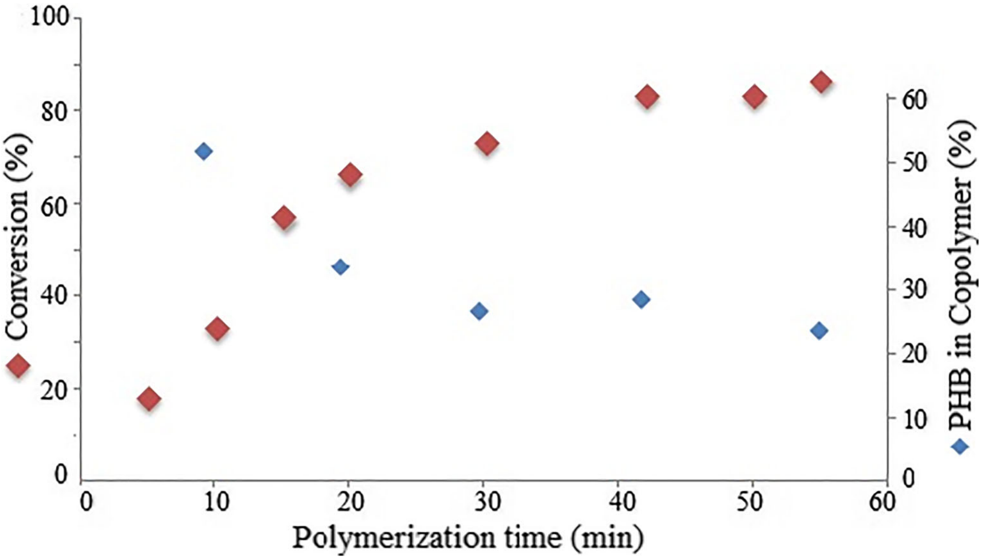


Fig. 8 The plots for the monomer conversion (NIPAM) and PHB content in copolymer as a function of time. RAFT polymerization of NIPAM initiated by PHB-macro RAFT agent with AIBN at 70 °C

samples were studied by the determination of the micelle size by DLS. For the sample PHB-PNIPAM-2, micelles were measured by DLS. Without filtration, main peak can be distinguish, with a diameter of 744 nm represent 99% of the micelles and PDI of 0.511 (Fig. 7).

Polymerization kinetics The second set of experimental runs was established to evaluate the RAFT polymerization kinetics of NIPAM with the macro chain transfer agent, PHB-R2 at 70 °C (Table 3). The fixed PHB-R2, NIPAM and AIBN concentration in DMF solution, polymerization was carried out in different times starting from 5 min to 55 min. Polymer was recovered by precipitating in hexane after given polymerization times. From the Table 3, we can conclude that water uptake of the copolymer obtained increases as PNIPAM content increases; and water solubility of the obtained copolymer increases as PNIPAM content increases.

Conversion of monomer to polymer was calculated by the equation below:

$$Coversion(\%) = [(Yield{-}200)/500] \times 100$$

Evolution of monomer conversion as a function of time with fixed chain transfer agent concentration in NIPAM polymerization at 70 °C was plotted in Fig. 8. The polymer yield increase proportionally by the time in the beginning. And then, the rate was retarded by the RAFT agent nearly the constant yield. As polymerization time increases, PHB content decreases similarly.

Ln $[M_o]/[M]$ was calculated using the $[M_o] = 500$ mg and $[M]$ =500 mg-(yield-200 mg) and plotted against

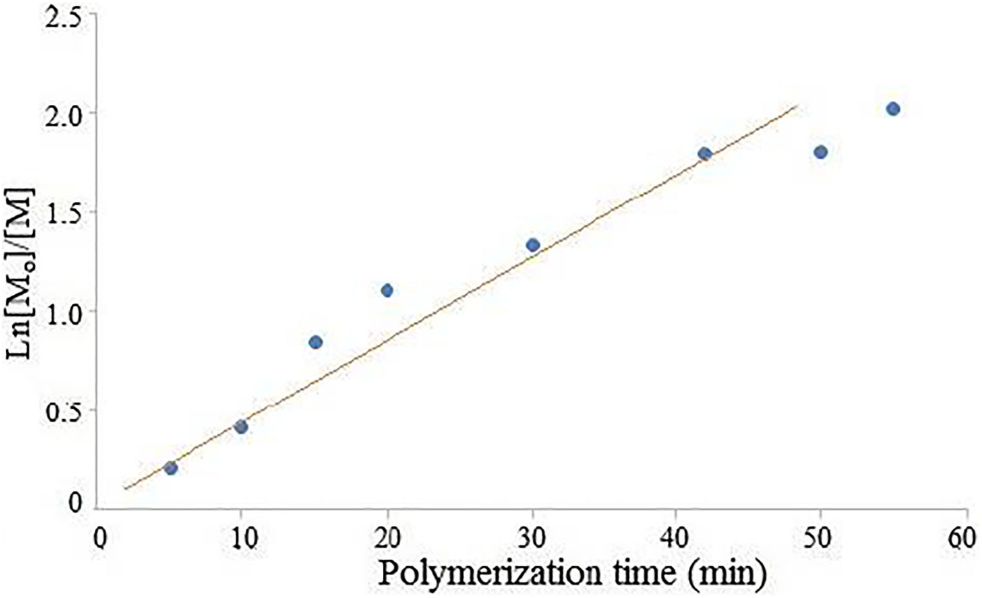


Fig. 9 The plot of the Ln $[M_o]/[M]$ of NIPAM as a function of time. ($k = 6.86 \times 10^{-4}$ L/mols at 70 °C)

polymerization time. The data presented in Fig. 9 indicate that the conditions reported above lead to controlled living NIPAM polymerization. Slope of the pseudo first order kinetic plot gives us the rate constant of the RAFT polymerization of NIPAM as k = 6.86 × 10^{-4} L/mols at 70 °C.

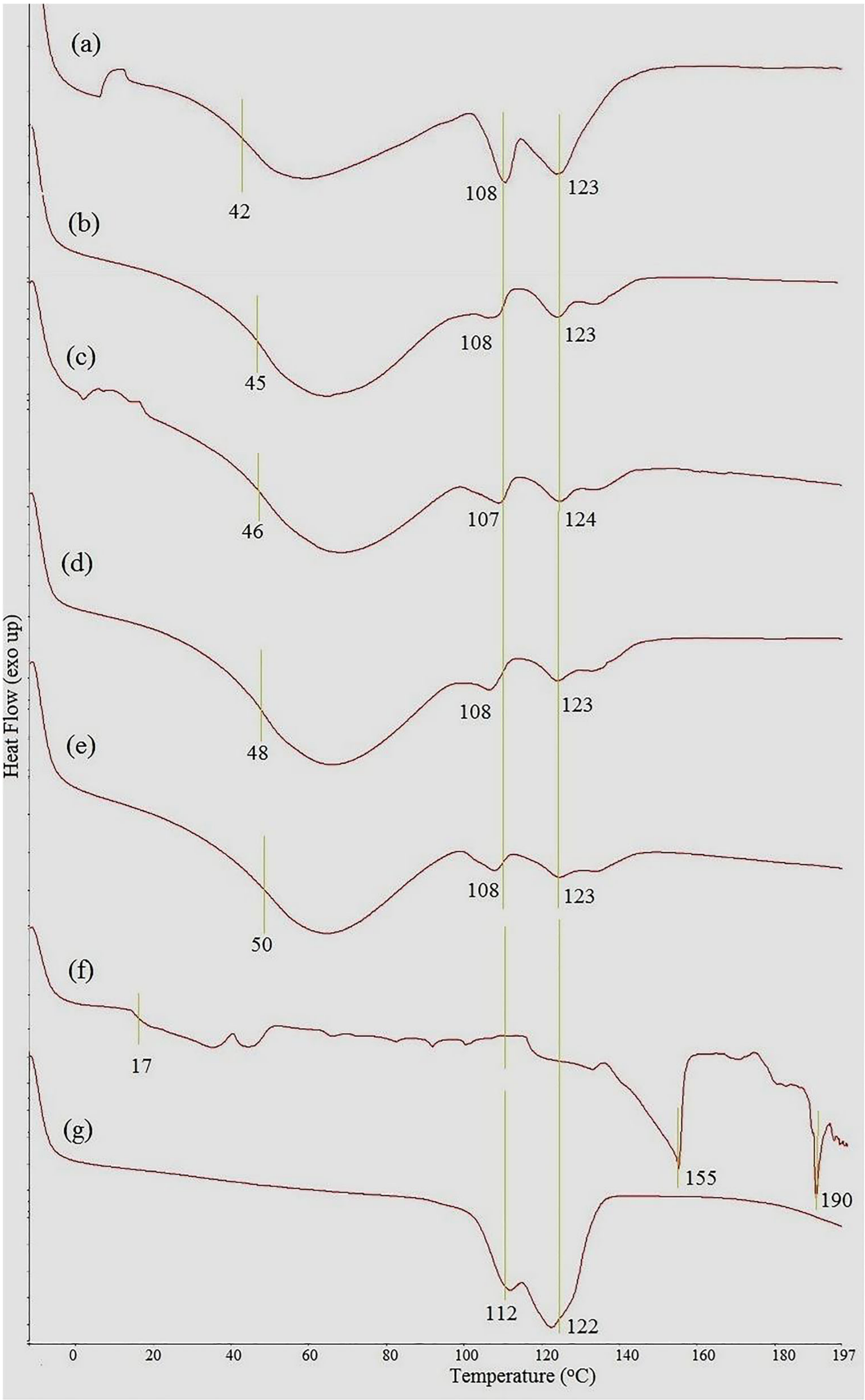


Fig. 10 DSC curves of PHB-PNIPAM block copolymers: **a** PHB-PNIPAM-741, **b** PHB-PNIPAM-742, **c** PHB-PNIPAM-743, **d** PHB-PNIPAM-744, **e** PHB-PNIPAM-745, **f** PHB-DEA-281, **g** PHB-R2–131

DSC Thermal Analysis was carried out using DSC to measure thermal behavior of the obtained copolymers of the second series in Table 3. DSC thermograms of the precursor PHB-DEA, PHB-R2 and the obtained PHB-PNIPAM copolymers. DSC traces of the polymers (Fig. 10), very wide Tg and several Tms were observed. While pure PHB had a melting transition at around 170 °C, the PHB derivatives showed lower melting transitions. Increase in PNIPAM content in copolymer slightly increases the Tg of PNIPAM in the copolymer. This result is in good agreement with the cited reference [43].

Several Tm's in DSC thermograms for poly-3-hydroxy alkanoates in the modification reactions can be seen in some cited references [28, 34, 44, 45], as well. This small melting transition may result from different combinations of the block copolymer with the development of micro-heterogeneities.

Conclusion

A member of commercially available microbial polyester, poly(3-hydroxy butyrate) derivatization opens a new gate to prepare PHB copolymers for medical applications. This novel PHB-macro raft agent makes possible to synthesis wide variety of the PHB derivatives. The carboxyl end of PHB was reacted with diethanol amine in order to obtain PHB with three hydroxyl functionalities in the chain ends. The hydroxyl ends were then reacted with the RAFT agent containing carboxylic acid end to prepare first time PHB-macro raft agent which was successfully initiated the polymerization of NIPAM. Because polymerization rate at 80 °C was very fast, the polymerization was conducted at 70 °C to study the kinetic parameters. Then, RAFT polymerization kinetics of NIPAM at 70 °C showed the typical controlled living polymerization aspects. Physicochemical characterization of the PHB-PNIPAM thermoresponsive block copolymer was investigated in detail.

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