Synthesis of Graft Copolymers Containing Biodegradable Poly(3-hydroxybutyrate) Chains

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Introduction

Graft copolymers present a wide range of interesting properties, which are designed in a flexible way through selection of the polymer backbone and the side chains. The properties depend on a variety of molecular parameters including the polymerization degrees of the main (DP_n) and side chains (DP_{sc}), graft density, main chain topology, and chemical composition. The combination of two or more comonomers with different properties gives the possibility of forming various materials, which have found numerous applications as thermoplastic elastomers, hydrogels, surface-modifying agents, stabilizers, dispersants, emulsifiers, or compatibilizers in polymer blends.[1]

Moreover, the introduction of biodegradable components into nontoxic polymer backbones allows the expansion of potential application areas to medicine and environmental protection. The most common biocompatible and biodegradable oligomers/polymers, which were used as the side chains for the preparation of graft copolymers with (meth)acrylic backbone, consist of a hydrophilic poly((meth)acrylic acid), poly((meth)acrylamide), poly(vinyl alcohol), poly(vinylpyrrolidone), poly(ethylene oxide) (PEO), and/or a hydrophobic poly(glycolic acid), poly(lactic acid), poly(lactone), and poly(hydroxyvalerate). Poly(3-hydroxybutyrate) (PHB) is a more interesting starting material in biomedical applications (drug release, bone replacement therapy, fracture treatment, and bone cements) because of its slowly hydrolyzing, biodegradability without cytotoxic effect and thermoplastic biocompatibility. It is known that copolymers of bacterial PHB and PMMA show enhanced mechanical properties with improved biocompatibility in comparison to PMMA/PHB blends.[2]

Literature reports a few examples of PHB graft copolymers which were prepared by various polymerization methods using different techniques. One of them is synthesis of P(MMA-*graft*-aPHB) containing atactic PHB side chains, where β-butyrolactone was anionically polymerized by *grafting from* modified poly(methyl methacrylate) used as the multifunctional macroinitiator.[3] The incorporation of isotactic PHB segments into backbone performed by the conventional free radical polymerization (FRP) of methyl methacrylate with methacrylic macromonomers of PHB yielded a poorly controlled PMMA-*graft*-PHB (unpredictable molecular weights and broad polydispersity indices).[4] However, the use of a controlled method, i.e., atom transfer radical polimerization (ATRP),[5−7] significantly improved these parameters.[8] Moreover, low molecular weight bacterial PHB was also grafted onto chitosan and cellulose acetate by coupling reactions.[9] The reverse structure of graft copolymers PHB-*graft*-PMMA obtained by radiation-induced graft polymerization of MMA on bacterial PHB was also reported.[10] In addition, the other monomers (isoprene, styrene, 2-hydroxyethyl methacrylate, and acrylic acid) were grafted from bacterial PHB in a similar way.[11−13]

In this paper, the synthesis of the PHB grafted copolymers modified by introduction of hydrophobic or hydrophilic segment via grafting through by ATRP is described. The reactions were initiated by ethyl 2-bromoisobutyrate (EtBriBu) using copper(I) bromide/1,1,4,7,10,10-hexamethyltriethylenetetramine (CuBr/HMTETA) as a catalyst/ligand system in organic solvent. Atactic or isotactic PHB macromonomers functionalized by methacrylate end groups were copolymerized in a one-step procedure with several methacrylates: methyl methacrylate (MMA), ethylene glycol methyl ether methacrylate (MeOEMA), and poly(ethylene glycol) methyl ether methacrylates with various lengths of PEO chains (PEOMA5 and PEOMA23; MW = 300 and 1100 g/mol, respectively), which are shown in Scheme 1. The following monomers were selected because they form a series of methacrylates with increasing number of EO units. In consequence, differential MW of comonomers had influence on the compositions of PHB graft copolymers (gradient or statistical). These are completely hydrophobic homografted with more or less distributed PHB side chains P(MMA-*graft*-PHBMA) or heterografted with hydrophobic polymethacrylic backbone and biodegradable PHB side chains, which accompany hydrophilic poly(ethylene oxide) segments generating amphiphilic behavior of the yielded material.

Experimental Section

Materials. (*R,S*)-β-Butyrolactone (Aldrich) was dried over calcium hydride and distilled under vacuum. 18-Crown-6 (18C6, Fluka, 99%) was thoroughly dried at 70 °C under vacuum. Tetrahydrofuran (POCH) was purified by a standard method[14] and dried over sodium−potassium alloy just before use. Natural poly(3-hydroxybutyrate) with high molecular weight (HMW PHB, M_n = 187 000 g/mol, M_w/M_n = 2.5, Biomer Inc.) was used for degradation to prepare well-defined oligomers as precursors of PHB macromonomers. Distilled 2-hydroxyethyl methacrylate (HEMA, Aldrich, 99%) as well as *N,N′*-dicyclohexylcarbodiimide (DCC, Aldrich, 99%) and 4-(dimethylamino)pyridine (DMAP, Aldrich, 99%) without further purification were applied for esterification of PHB oligomers. Methyl methacrylate (MMA, Aldrich, 99%) and ethylene glycol methyl ether methacrylate (MeOEMA, Aldrich, 99%) were stirred over calcium hydride for 2 h, distilled under vacuum, and stored at −15 °C under an inert gas atmosphere. Poly(ethylene glycol) methyl ether methacrylates, $H_2C{=}C(CH_3)COO{-}(CH_2CH_2O)_nCH_3$ (PEOMA5, MW_{av} = 300 g/mol, n = 5; PEOMA23, MW_{av} = 1100 g/mol, n = 23), were also obtained from Aldrich. Antioxidant inhibitors MEHQ and BHT were removed from liquid PEOMA5 by passing through an alumina column. In the case of PEOMA23, which is a solid at room temperature, before polymerization it was dissolved in THF, passed through an alumina column to remove the antioxidant inhibitor, and then the solvent was evaporated and the macromonomer was dried under vacuum to a constant mass. Copper(I) bromide (CuBr, Aldrich, 98%) was purified by stirring with glacial acetic acid (Aldrich, 99.8%), followed by filtration and washing the solid three times with ethanol and twice with diethyl ether. The solid was dried under vacuum for 6 h. Anisole (Aldrich, 99%) was distilled and stored over molecular sieves. 1,1,4,7,10,10-Hexamethyltriethylenetetramine (HMTETA, Aldrich, 97%), ethyl 2-bromoisobutyrate (EtBriBu, Aldrich, 98%), *tert*-butylammonium hydroxide (1.5 M aqueous solution, Fluka), 2,6-di-*tert*-butyl-4-methylphenol, methyl iodide (Aldrich, 99.5%), sodium methacrylate (Aldrich, 99%), and all other solvents were used without purification.

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Scheme 1. Structures of Copolymerized Methacrylates

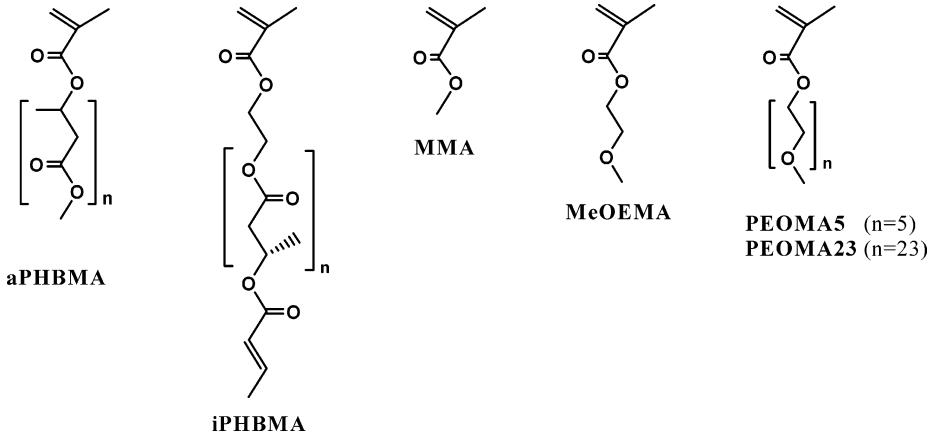

Table 1. PHB Macromonomers Used for ATR Copolymerization

PHB macromonomers	preparation	estrification yield (%)	n_{PHB}[d]	$M_{n,NMR}$[d] (g/mol)	$M_{n,GPC}$[e] (g/mol)	M_w/M_n[e]
aPHBMA (atactic)	AP[a]		25	2300	2200	1.22
iPHBMA1 (isotactic)	DCh[b]	81	16	1550	1600	1.21
iPHBMA2 (isotactic)	DT[c]	84	20	1800	1900	1.39
iPHBMA3 (isotactic)	DT[c]	70	13	1300	1500	1.28

[a] Anionic polymerization in the presence of potassium methacrylate as the initiator and 18C6 as ligand. [b] Chemical degradation of bacterial HMW PHB by *tert*-butylammonium hydroxide at 50 °C. [c] Thermal degradation of bacterial HMW PHB at 200 °C. [d] Calculated from the NMR spectrum on the macromonomer after purification. [e] Measured by GPC with polystyrene standards.

Synthesis. Preparation of PHB Macromonomers. *aPHBMA by Anionic Polymerization*. aPHBMA was prepared in similar way to the previously described procedure using another anionic initiator.[15] Briefly, sodium methacrylate (5.4 g, 0.05 mol) and 18-crown-6 (13.2 g, 0.05 mol) were dissolved in 30 mL of tetrahydrofuran and stirred until the formation of a complex. Then, (*R,S*)-β-butyrolactone (100 g) and inhibitor 2,6-di-*tert*-butyl-4-methylphenol (0.02 g, 10^{-4} mol) were added, and the polymerization was carried out at room temperature in the dark. The reaction was stopped by methyl iodide, when signals corresponding to CH protons in lactone ($\delta = 4.70$ ppm) completely disappeared in the ^{1}H NMR spectrum. A new signal at 5.24 ppm generated by CH protons in the polymer was used to calculate and confirm the assumed value of molecular weight for the resulting poly(3-hydroxybutyrate) (aPHBMA, Table 1).

iPHBMA by Degradation and Esterification. The preparation of PHB oligomers end-capped by carboxylic acid and unsaturated crotonate groups, which were consequently used as the precursors of PHB macromonomers, was done in two ways: (i) chemical and (ii) thermal degradation. First method (i) was performed via alkaline depolymerization of original PHB biopolyester according to the procedure described for polyhydroxyalkanoates in ref 16. The reaction was achieved in chloroform in the presence of 0.375 mol/L aqueous solution of *tert*-butylammonium hydroxide at 50 °C for 2 h. The fraction containing low molecular weight oligomers was protonated by hydrochloric acid. The other route (ii) of HMW bacterial PHB degradation was carried out in laboratory scale at 200 °C.[8,17] Pyrolysis was completed in 27 and 34 h, yielding products with different low molecular weights.

In the next step, the natural PHB oligomers obtained by chemical or thermal degradation were esterified with HEMA. The reaction was activated by DCC and accelerated by DMAP under optimal conditions (PHB:HEMA:DCC:DMAP = 1:3:3:0.3, $T = 25$ °C) in anhydrous methylene chloride.[8,18] It resulted in methacrylic PHB macromonomers, iPHBMA1, iPHBMA2, and iPHBMA3, which are characterized in Table 1.

General Procedure for ATR Copolymerization of aPHBMA Macromonomer (Example). aPHBMA (0.39 g, 0.177 mmol) was dissolved in anisole (3 mL), and then MMA (0.94 mL, 10.14 mmol) and HMTETA (28 μL, 0.103 mmol) were added. The homogeneous solution was purged by nitrogen. After 2 h, CuBr (15 mg, 0.103 mmol) was added and the mixture was purged again for 30 min at room temperature. Next, the Schlenk flask was placed in a thermostated oil bath at 70 °C. After 1 min, EtBr*i*Bu (15 μL, 0.103 mmol) was added to start the reaction. During polymerization, samples were periodically removed to determine the molecular weight of the polymer by GPC and conversion by NMR. The polymerization was stopped by exposing the solution to air, and mixture was then diluted with chloroform and filtered through an activated (neutral) alumina column to remove the copper catalyst.

The same procedure was also applied for the (co)polymerization with MeOEMA, PEOMA5, and PEOMA23. However, the conditions have been changed for PEOMA5 (mon/EtBriBu/CuBr/HMTETA = 100/1/2/2) and PEOMA23 (mon/EtBriBu/CuBr/HMTETA = 100/1/3/3).

In the case of copolymerization with iPHBMA, which indicated lower solubility than aPHB methacrylate, the larger amount of anisole was used that is mon/solvent = 1/10 (w/v).

Characterization. Gel permeation chromatography (GPC) measurements were conducted in chloroform at 30 °C at a flow rate of 1 mL/min using a Spectra-Physics 8800 solvent delivery system with 10^4, 10^3, and 500 Å Styragel columns in series and detection systems: a differential refractometer (Shodex) and UV detectors (Spectra-Physics). The apparent molecular weights ($M_{n,app}$) and polydispersity indices (M_w/M_n) of the graft copolymers were determined on the basis of linear polystyrene (PS) standards with low polydispersity index.

^{1}H nuclear magnetic resonance (NMR) spectroscopy was performed on a Varian 300 MHz spectrometer in chloroform-*d* at room temperature. Characterization of (macro)monomers and (co)polymers is presented in the Supporting Information.

Results and Discussion

Copolymerization of aPHBMA with Methacrylates. PHB graft copolymers P(MMA-*graft*-aPHB) containing polymethacrylic backbone with atactic PHB side chains have been prepared via the *grafting from* technique by anionic polymerization of β-butyrolactone.[3] In this case, the synthesis consisted of three steps: (i) the preparation of PMMA backbone, (ii) its transformation to multifunctional macroinitiator by partial saponification (KOH/18C6), and finally (iii) grafting of β-butyrolactone to acive centers of the backbone. Such a procedure required the

Table 2. Copolymerization of aPHBMA with Various Methacrylates (M)

						conversion[b] (%)							
				aPHBMA[a] (%)				total					
	M	M/In/CuBr/L anisole, 70 °C	reaction time (h)	mol	wt	x_{aPHBMA}	x_M	x_{av}[c]	x_{est}[d]	DP_n[e] aPHB/M	$M_{n,app}$[f] (g/mol)	M_w/M_n[f]	$M_{n,abs}$[g] (g/mol)
IA	MMA	100/1/1/1	25	0	0		83		83	0/83	11 700	1.23	8 300
IB			48	1.7	28	99.9	99	99	~100	2/97	15 600	1.41	14 100
IC			48	9.1	69	90	71	84	76	8/76	17 200	1.24	25 200
IIA	MeOEMA	100/1/1/1	68	0	0		99		99	0/99	16 300	1.51	14 300
IIB			44	1.8	22	99	98	98	99	2/96	15 600	1.60	18 200
IIC			46	6.2	50	67	67	67	70	4/63	15 500	1.30	17 900
IID			48	11.6	67	66	62	64	56	7/57	10 000	1.17	23 600
IIIA	PEOMA5	100/1/2/2	2.5	0	0		97		97	0/97	13 600	1.34	29 100
IIIB			42	4.3	25	92	91	91	89	4/87	11 300	1.13	34 900
IIIC			45	18.2	62	81	77	79	79	14/65	10 200	1.12	50 300
IIID			18	29.2	75	66	62	65	61	19/46	9 200	1.13	55 600
IVA	PEOMA23	100/1/3/3	45	0	0		96		96	0/96	8 700	1.46	105 600
IVB			48	2.6	5	97	98	98	96	2/96	4 900	1.14	110 000
IVC			45	14.4	25	76	71	72	76	10/62	3 400	1.12	90 200
IVD			48	33.3	50	45	46	45	49	15/30	3 800	1.13	66 000
VA		100/1/3/3	48	100	100	25			25	25/0	9 100	1.18	55 000
VB			91	100	100	27			27	27/0	9 600	1.21	59 400

[a] Initial molar and weight fraction of PHB macromonomer used for copolymerization. [b] Calculated from the NMR spectrum on the copolymer before purification. [c] Conversion of both comonomers as average value calculated according to the following equation: conv(aPHBMA) × mole fraction(aPHBMA) + conv(M) × mole fraction(M). [d] Estimated by comparison of integration peaks becoming to formed copolymer (3H, 0.8−1.2 ppm) with unreacted comonomers (2H, $\delta = 5.6$ and 6.1 ppm). [e] Polymerization degree of backbone containing units of PHB and M defined via conversion. [f] Measured by GPC with polystyrene standards. [g] Calculated on the basis of conversion determined by NMR.

determination of the precise number of initiating sites, which was done by reaction of saponificated PMMA with benzyl bromide. Additionally, in the polymerization step the length of PHB side chains had to be controlled, whereas the polymerization degree of backbone was well-defined. The *grafting through*, which is the other widely applied method for the preparation of graft copolymers, is proposed to be more convenient because of the one-step procedure. The technique can be used for copolymerization of macromonomer with low molecular weight comonomer, resulting in loosely distributed grafts among the backbone[8,19−26] or (co)polymerization of (two) macromonomer(s) yielding densely grafted copolymers,[27−29] also called molecular brushes.

The procedure of *grafting through* was also adopted for our studies on the synthesis of PHB graft copolymers by ATR copolymerization of atactic PHB methacrylate and several comonomers. The PHB macromonomer was prepared by anionic polymerization of β-butyrolactone initiated by potassium methacrylate in the presence of ligand 18-crown-6 and terminated by methyl iodide. It resulted in atactic PHB functionalized by methacrylate and methoxy end groups (aPHBMA, Table 1, Scheme 1). The following methacrylates, that is low molecular weight MMA, methacrylate with one unit of ethylene oxide end-capped by methyl group (MeOEMA), and two methacrylates with different lengths of PEO chains (PEOMA5 $n = 5$ and PEOMA23 $n = 23$), were selected as a comonomers (Scheme 1). The PEOMA23 has been previously used for copolymerization with several (meth)acrylic monomers[25,28−31] and is especially interesting because of its crystalline nature and ability to generate elastomeric behavior in the graft copolymers, which could also improve properties of the synthesized atactic PHB graft copolymers.

The copolymerizations were initiated by EtBriBu in the presence of CuBr/HMTETA as catalyst/ligand system in anisole at 70 °C, changing the ratio of monomer to CuBr/HMTETA to obtain optimal conditions for corresponding methacrylate pairs. The results are presented in Table 2. The reactions indicated that a small amount of aPHBMA copolymerized in the feed of 25 wt % with MMA, MeOEMA, and PEOMA5 (**IB**, **IIB**, **IIIB**) or 5 wt % in the case of PEOMA23 (**IVB**) yielded the highest conversions in the range 89−100%, which were very closed to the values obtained for homopolymerization of coresponding comonomers (**IA**, **IIA**, **IIIA**, **IVA**). However, the conversion was reduced at comparatively higher initial feed ratios with 50−75 wt % of aPHBMA added into the reaction, especially for graft copolymers **IID**, **IIID**, and **IVD** (49−61%). Furthermore, the comparison of copolymerizations performed with the same amount of aPHBMA with various comonomers showed a decrease in conversion with the length of substituent chain in the comonomer. For example, for the ratio aPHBMA/M = 25/75 wt % the graft copolymer P(MMA-*graft*-aPHBMA) **IB** was obtained with almost 100%, and then for P(MeOEMA-*graft*-aPHBMA) **IIB**, P(PEOMA5-*graft*-aPHBMA) **IIIB**, and P(PEOMA23-*graft*-aPHBMA) **IVC** 99, 91, and 76% conversions were observed, respectively. The limitation of both comonomer conversion at larger amount of aPHBMA in the copolymerization was confirmed by low conversion of atactic PHB macromonomer in the homopolymerization **VB** (27% after 91 h).

During polymerization the conversion for each comonomer pairs was determined using ^{1}H NMR, which spectra are presented in Figure 1a−d. The comparison of the peak area of the signal assigned to the repeat unit protons $-[O-CH(CH_3)-CH_2-COO]_n-$ ($\delta = 5.25$ ppm, $n = 25$) in the PHB macromonomer and graft copolymer with the vinyl protons in the methacrylate end group $CH_2{=}C(CH_3)-$ ($\delta = 5.54$ and 6.10 ppm) in the unreacted monomer allowed calculation of aPHBMA conversion. For comonomers MeOEMA, PEOMA5, and PEOMA23, the signal of methoxy protons $-OCH_3$ ($\delta = 3.4$ ppm) in monomer and copolymer was compared with monomer signals ascribed to the vinyl protons $CH_2{=}C(CH_3)-$ ($\delta = 5.47$ and 6.03 ppm). In the case of MMA, the peak generated by the methoxy protons at 3.75 ppm was shifted during polymerization to that at 3.6 ppm, which corresponds to protons in the methoxy group of incorporated MMA into the copolymer. The calculated values of corresponding monomer conversion were used for estimation of total comonomer conversion into the graft copolymers. Additionally, it was also evaluated by the integral

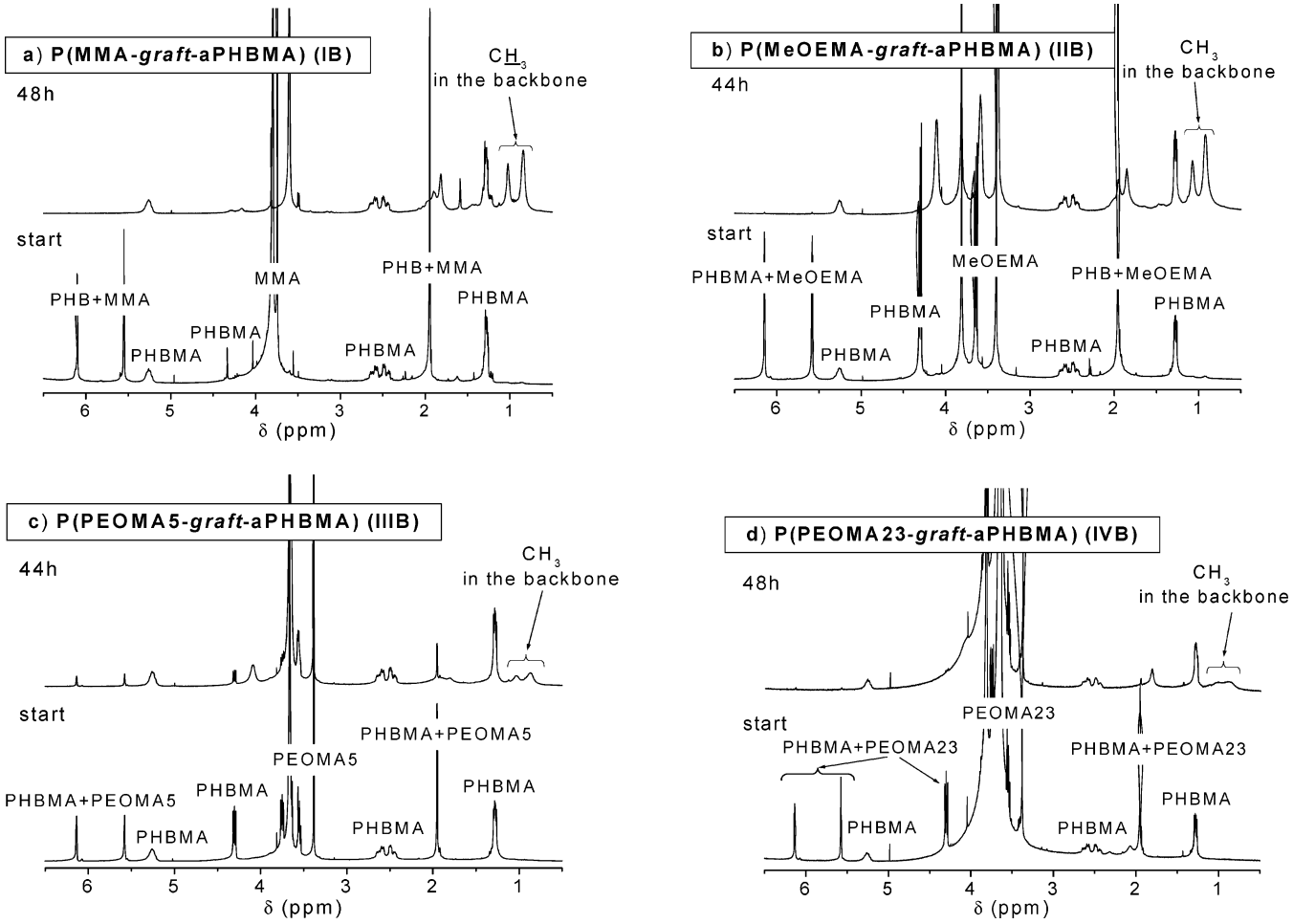

Figure 1. ^{1}H NMR spectra for the synthesis of graft copolymers containing atactic PHB segments: (a) P(MMA-*graft*-aPHBMA) (**IB**), (b) P(MeOEMA-*graft*-aPHBMA) (**IIB**), (c) P(PEOMA5-*graft*-aPHBMA) (**IIIB**), and (d) P(PEOMA23-*graft*-PHBMA) (**IVB**). Conditions are given in Table 2.

ratio of peaks (δ = 6.1 and 5.6 ppm) assigned to the vinyl protons in the methacrylate group $CH_2{=}C(CH_3)-$ in the unreacted comonomers to the methyl protons in the formed backbone $-CH_2-C(CH_3)-$ (δ = 0.8−1.1 ppm), which appeared after starting of copolymerization and subsequently were growing with the progress of reaction. Both values of total conversion were in a good agreement (up to 10% difference).

Furthermore, the conversion calculations performed on the basis of NMR allowed the determination of the absolute molecular weights ($M_{n,abs}$) of the graft copolymers, which were significantly different than apparent value ($M_{n,app}$) obtained by conventional GPC (Table 2). This difference was caused by underestimation of GPC values by linear standards, which have lower hydrodynamic volumes than loosely or densely grafted copolymers. Figure 2 shows that, with the exception of P(MMA-*graft*-aPHBMA), the apparent M_n of graft copolymers was decreasing with the increase in aPHBMA content. Moreover, the difference between absolute and apparent M_n is larger when the molecular weight of used comonomer is higher, as with the brush P(PEOMA23-*graft*-aPHBMA) compared with loosely grafted copolymer P(MMA-*graft*-aPHBMA).

Additionally, the GPC chromatograms provided a means of monitoring the improvement of the reaction, which is shown for representative synthesis of P(PEOMA5-*graft*-aPHBMA) in Figure 3. The intensity of the signals assigned to the comonomers decreased as the reaction progressed whereas the copolymer peak increased in intensity as the reaction shifted to comparatively higher MWs. All polymers displayed monomodal GPC traces with polydispersity indices 1.1−1.6.

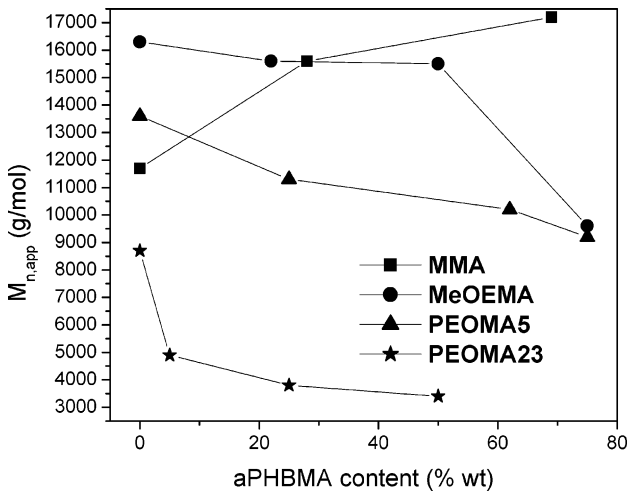

Figure 2. Dependence of apparent molecular weight ($M_{n,app}$) of copolymers containing aPHB segments on the content of aPHBMA in the graft copolymer.

Copolymerization of iPHBMA with Methacrylates. The other series of experiments was performed with isotactic PHB macromonomers. They were prepared via PHB oligomer precursors with carboxylic acid and crotonate end groups, which were obtained in two ways of degradation. One of them was based on the pyrolysis at high temperature, whereas the chemical depolymerization by *tert*-butylammonium hydroxide was applied in the second procedure. It is known that radical polymerization

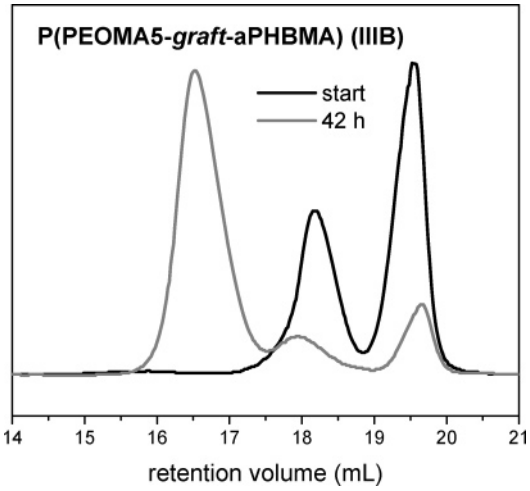

Figure 3. GPC traces monitored during synthesis of P(PEOMA5-*graft*-aPHBMA) (**IIIB**).

of such oligomers via the unsaturated crotonate end groups have some difficulties because the presence of the β-methyl substituent on the unsaturation induces a steric hindrance with the α-substituent and also a degradative chain transfer.[32] It means that further chemical modification of carboxylic acid end group had to be done to yield new terminal groups, which could be radically polymerized. The esterification at these end groups of PHB oligomers with HEMA resulted in the isotactic PHB macromonomers functionalized by methacrylate end groups: iPHBMA1 (from chemically degraded bacterial PHB) as well as iPHBMA2 and iPHBMA3 (from thermally degraded bacterial PHB), which are presented in Scheme 1 and Table 1.

The copolymerizations with MMA, MeOEMA, PEOMA5, and PEOMA23 as comonomers were performed under similar conditions to the ones used in the case of aPHB methacrylate (Table 2). However, the larger amount of solvent (5 times) was necessary to use because of lower solubility of isotactic PHB in comparison to atactic macromonomer. The same rules, which are the decrease in conversion with the increasining amount of iPHBMA (**VIC** and **VID**) and the length of substituent chain in comonomer (**VIA**, **VIB**, **VIC**, and **VIE**), were also observed. The results for graft copolymers P(MMA-*graft*-iPHBMA1) are in a good agreement with previously reported studies by Marchessault.[8] Furthermore, the copolymerization of MMA (75−80 wt %) with iPHBMA1 and iPHBMA2 macromonomers, prepared by various degradation procedures (chemical DCh and thermal DT) with similar estrification yields (above 80%), resulted in graft copolymers P(MMA-*graft*-iPHBMA) **VIA** and **VIIA** with almost complete conversion of both comonomers. It indicated no influence of the preparation route of the PHB macromonomer precursor on the occurrence of polymerization, whereas the lower estrification yield in the case of iPHBMA3 (**VIIIA**) significantly decreased conversion to 47%. Reaction of 75 wt % PEOMA5 with iPHBMA2 (**VIC**) yielded 78% comonomer conversion, which was reduced to 32% when iPHBMA2 was replaced by iPHBMA3 (**VIIIB**).

The progress of copolymerization was also monitored by GPC chromatograms and ^{1}H NMR spectra, which are presented for representative P(PEOMA5-*graft*-iPHBMA1) **VIC** in Figure 4a,b. The total conversion as well as conversion of iPHBMA and comonomer were calculated from NMR in the same way like it was described for the copolymerization with atactic PHB macromonomer.

Although the yields of iPHB graft copolymers were lower than for graft copolymers containing atactic segments of PHB,

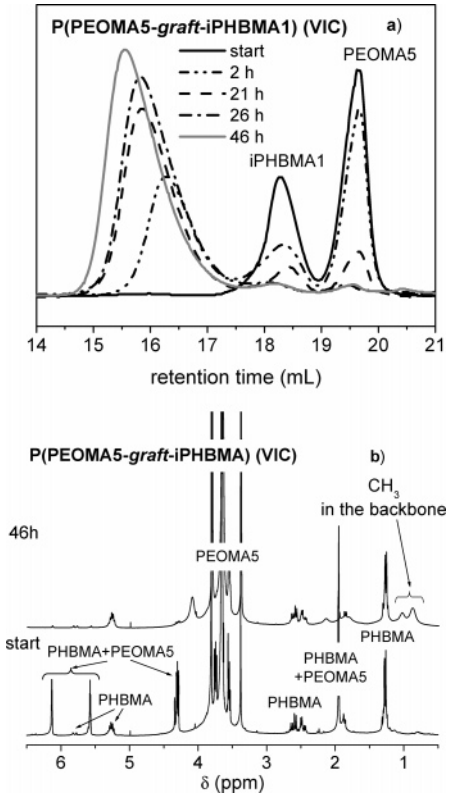

Figure 4. GPC traces (a) and ^{1}H NMR spectra (b) for the ATRP of iPHBMA and PEOMA5 (**VIC**). Conditions are given in Table 3.

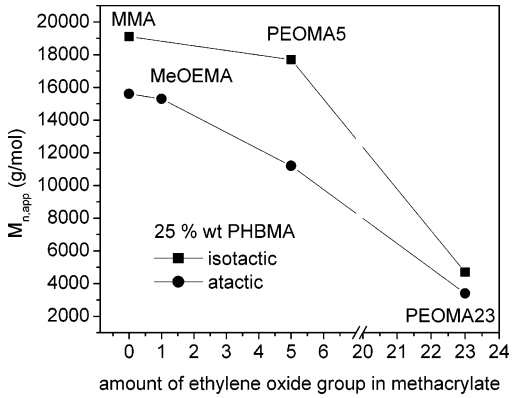

Figure 5. Apparent molecular weight ($M_{n,app}$) of copolymers containing atactic or isotactic PHB segments vs amount of ethylene oxide units in methacrylate comonomer.

the apparent M_n determined by GPC presented higher values (Figure 5), which caused lighter difference between $M_{n,asb}$ and $M_{n,app}$ (Table 3). It can be explained by various hydrodynamic volumes for both (i/a)PHB graft copolymers. Molecular weight distributions for all monomodal GPC traces were relatively narrow in the range 1.12−1.28.

Homopolymerization of iPHBMA2 was performed with much lower ratio monomer/initiator = 10/1 to obtain higher conversion, which was closed to 60%, yielding graft copolymer **IX** with low polymerization degree ($DP_n = 6$) and polydispersity index $M_w/M_n = 1.28$ (Table 3).

Relative Reactivities of PHB Macromonomers. The kinetic plots are presented for the reactions of atactic PHB methacrylate with another methacrylate M (M = MMA, MeOEMA, PEOMA5, and PEOMA23) in Figure 6a. They demonstrate faster consumption of aPHBMA than MMA or MeOEMA and slightly faster than PEOMA5, whereas aPHBMA and PEOMA23 were copolymerized at similar rates. In the ATR copolymerizations

Table 3. Copolymerization of iPHBMA with Various Methacrylates (M) (VIA-E: iPHBMA1; VIIA-B, IX: iPHBMA2; VIIIA-B: iPHBMA3)

				iPHBMA[a] (%)		conversion[b] (%)							
								total					
	M	M/In/CuBr/L anisole, 70 °C	reaction time (h)	mol	wt	x_{iPHBMA}	x_M	x_{av}[c]	x_{est}[d]	DP_n[e] iPHB/M	$M_{n,app}$[f] (g/mol)	M_w/M_n[f]	$M_{n,abs}$[g] (g/mol)
VIA	MMA	100/1/1/1	48	2.0	25	97	89	91	99.9	2/89	19 100	1.24	12 100
VIB	MeOEMA	100/1/1/1	48	2.9	25	74	62	65	68	2/63	10 800	1.25	12 300
VIC	PEOMA5	100/1/2/2	46	5.9	25	82	71	74	78	4/70	17 700	1.28	27 400
VID	PEOMA5	100/1/2/2	48	15.8	50	50	51	50	59	8/42	10 500	1.15	25 400
VIE	PEOMA23	100/1/3/3	48	18.6	25	30	27	28	27	5/23	4 700	1.13	33 300
VIIA	MMA	100/1/1/1	48	1.3	20	99	90	92	99.8	2/90	16 100	1.18	12 800
VIIB	PEOMA23	100/1/3/3	48	2.5	4.3	81	92	91	86	2/89	5 100	1.12	101 700
VIIIA	MMA	100/1/1/1	48	6.2	50	50	45	47	46	3/44	9 700	1.16	8 900
VIIIB	PEOMA5	100/1/2/2	48	6.2	25	33	32	32	32	2/30	7 100	1.23	12 000
IX		10/1/2/2	67	100	100	59			59	6/0	7 700	1.28	11 400

[a] Initial molar and weight fraction of PHB macromonomer used for copolymerization. [b] Calculated from the NMR spectrum on the copolymer before purification. [c] Conversion of both comonomers as average value calculated according to the following equation: conv(iPHBMA) × mole fraction(iPHBMA) + conv(M) × mole fraction(M). [d] Estimated by comparison of integration peaks becoming to formed copolymer (3H, $\delta = 0.8-1.2$ ppm) with unreacted comonomers (2H, 5.6 and 6.1 ppm). [e] Polymerization degree of backbone containing units of PHB and M defined via conversion. [f] Measured by GPC with polystyrene standards. [g] Calculated on the basis of conversion determined by NMR.

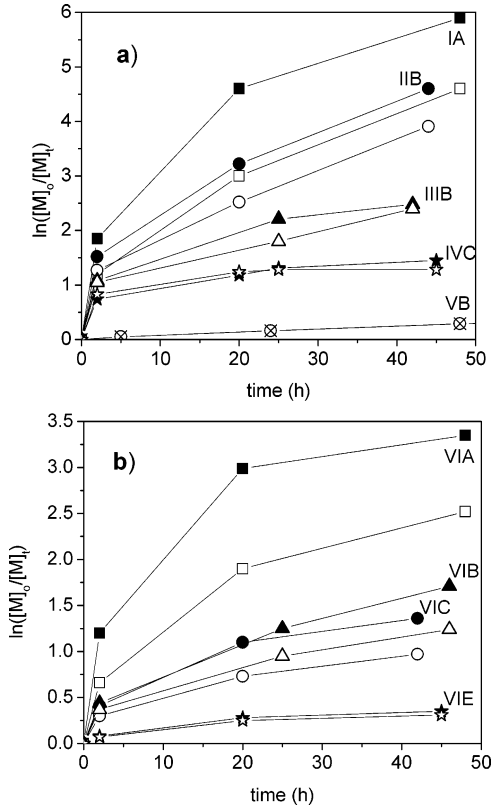

Figure 6. Kinetic plots for the ATRP of (a) atactic and (b) isotactic PHBMA (black symbols) with corresponding methacrylate: MMA **IA**, **VIA** (□), MeOEMA **IIB**, **VIB** (○), PEOMA5 **IIIB**, **VIC** (Δ), and PEOMA23 **IVC**, **VIE** (☆). Homopolymerization of aPHBMA **VB** (⊗). Conditions are given in Table 2.

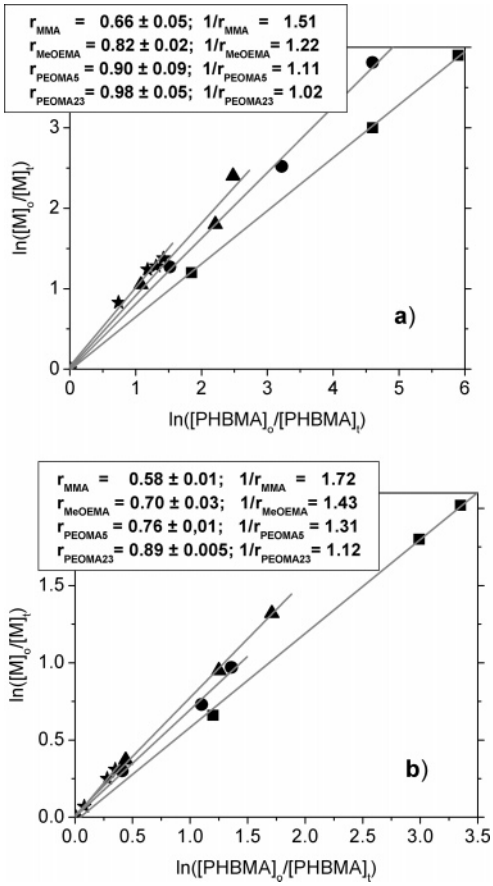

Figure 7. Jaacks plots for the ATRP of (a) atactic and (b) isotactic PHBMA and corresponding methacrylate M: MMA (■), MeOEMA (●), PEOMA5 (▲), and PEOMA23 (★). Conditions are given in Table 3.

performed with low PHB macromonomer feed compositions (1.7−4.3 mol %), the reactivity ratios were estimated using the Mayo and Lewis method[33] simplified by Jaacks.[34] As a result of plotting kinetic data of M against those of PHBMA (Figure 7a), the value r_M was obtained from the slope determined by following equation:

$$r_M = \ln([M]_0/[M]_t)/\ln([PHBMA]_0/[PHBMA]_t)$$

The relative reactivity of the PHB macromonomer was evalueted by $1/r_M$, which means that the rate constant for the reaction of the methacrylate radical at growing chain with PHBMA is $1/r_M$ times higher than that with M comonomer.

The crystalline isotactic PHBMA macromonomer containing crotonate end group was copolymerized with methacrylate comonomers under similar ATRP conditions using larger amount of reaction solvent because of its lower solubility than atactic PHBMA. The reactivity ratios of methacrylates ($r_{MMA} = 0.58 \pm 0.01$, $r_{MeOEMA} = 0.70 \pm 0.03$, $r_{PEOMA5} = 0.76 \pm 0.01$, $r_{PEOMA23} = 0.89 \pm 0.05$) were slightly lower in comparison

to the reactivity of comonomers in the reactions with atactic PHBMA ($r_{MMA} = 0.66 \pm 0.05$, $r_{MeOEMA} = 0.82 \pm 0.02$, $r_{PEOMA5} = 0.9 \pm 0.09$, $r_{PEOMA23} = 0.98 \pm 0.05$), and therefore $1/r_{MMA}$ gave relatively higher values (Figure 7a,b). This indicates that the compatibility of the PHB macromonomer does not significantly affect the copolymerization reactivity as long as the reaction mixture is homogeneous. The relative reactivity of comonomers was differed by using of various comonomers in the feed for ATR copolymerization with PHB macromonomer. It decided about graft copolymer microstructure, i.e., the distribution of corresponding grafts in the copolymer chains. Higher reactivity of PHBMA in the copolymerization with MMA or MeOEMA can conclude the formation of copolymers with spontaneous gradient, which means PHB chains were more concentrated at the beginning of the polymer chain, whereas comonomer was gradually incorporated into the backbone. The gradient composition was weaker for P(PEOMA5-*graft*-PHBMA) than for P(MMA-*graft*-PHBMA) and P(MeOEMA-*graft*-PHBMA). Finally, the kinetic for copolymerization of PEOMA23 and PHB macromonomer revealed that comonomers were converted almost simultaneously (Figure 6a,b), indicating random composition of the graft copolymer.

Conclusion

PHB graft copolymers were prepared via macromonomer method, i.e., *grafting through* copolymerization. Methacrylate end-capped atactic or isotactic PHB macromonomers were copolymerized with various methacrylates (MMA, MeOEMA, PEOMA) under ATRP conditions (CuBr/HMTETA). It yielded graft polymers with $DP_n < 100$ having narrow molecular weight distribution ($M_w/M_n = 1.12-1.6$). The conversion of comonomers was lower for copolymerization with isotactic PHBMA because of limited solubility, which needed a larger amount of solvent than in the case of atactic PHB macromonomer. Moreover, the increase in initial feed of PHBMA also caused reduction of conversion for both comonomers.

The using of various methacrylate comonomer pairs (MMA/PHBMA, MeOEMA/PHBMA, PEOMA5/PHBMA, PEOMA23/PHBMA) in the copolymerization indicated differential relative reactivity ratios of comonomers. The calculated values suggested the formation of PHB graft copolymers with various compositions, i.e., spontaneous gradient for P(MMA-*graft*-PHBMA), P(MeOEMA-*graft*-PHBMA), and P(PEOMA5-*graft*-PHBMA), whereas the random distribution of heterografts is proposed for P(PEOMA23-*graft*-PHBMA).

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Supporting Information Available: NMR characterization of (macro)monomers and (co)polymers. This material is available free of charge via the Internet at http://pubs.acs.org.

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