Atom Transfer Radical Copolymerization of Bacterial Poly(3-hydroxybutyrate) Macromonomers and Methyl Methacrylate

Sophie Nguyen and Robert H. Marchessault*

Department of Chemistry, McGill University, 3420 University Street, Montreal, QC H3A 2A7, Canada

Received June 3, 2004; Revised Manuscript Received September 30, 2004

ABSTRACT: PHB macromonomers and methyl methacrylate were copolymerized by atom transfer radical polymerization (ATRP) methods. Kinetic studies are reported and compared to ones obtained for conventional free radical polymerization (FRP). Graft copolymers produced by ATRP were of lower polydispersity (ca. 1.2) than the ones prepared by FRP (1.6−2.1), but also of lower molecular weight (ca. 20 000 for ATRP, 31 800−84 100 for FRP). With both techniques, the addition of PHB macromonomers, compared to MMA, was faster on a propagating chain with an MMA active center at low molar feed ratio of PHB macromonomers. The gradual decrease of PHB macromonomers reactivity compared to the one for MMA, with increasing proportion of macromonomers in the comonomer feed, affected the microstructures of the graft copolymers produced by ATRP.

Introduction

Poly(3-hydroxybutyrate), PHB, is a carbon reserve material in bacterial cells which accumulates when an essential nutrient is limited.[1,2] This 100% isotactic biopolyester can represent up to 90% of the cell dry weight.[2] Since depolymerase enzymes excreted by some bacteria and fungi were shown to degrade PHB to water-soluble oligomers subsequently used as nutrients by the microorganisms, PHB is a sustainable material.[1] Furthermore, its monomeric degradation product, D(−)-3-hydroxybutyric acid, is found in human blood as a normal metabolite, and PHB was shown to be biocompatible, with no cytotoxic effect.[1,2] Since 1962, when Baptist proposed the use of PHB as an absorbable suture, it has been considered as potentially useful material in the field of medicine.[3] The combination of PHB, which hydrolyzes slowly in vivo,[1,2,4−6] with PMMA could have potential applications as material for orthopedic surgery. The most obvious use is in bone cements[4−6] since PMMA is their main constituent.[7]

Blends of bacterial, high molecular weight PHB and PMMA, PHB/PMMA, were found to be partially miscible, with a solubility limit of 20 wt % PHB.[8] However, when mixed with graft copolymers of PMMA in the backbone and atactic PHB grafts, at 67 mol % hydroxybutyrate content in the copolymer, the ductility of PHB/PMMA blends increased significantly.[9] For bone cement formulations, a copolymer of bacterial PHB and PMMA is therefore expected to show improved mechanical properties compared to cements with a PMMA/PHB blend, while bringing enhanced biocompatibility. In addition, as PHB would partially and progressively erode in vitro, bone cells could grow around and into the created channels in the cement, thereby reducing loosening of the cement.[4−6]

Graft copolymers containing PHB blocks were reported using either the "grafting onto" or the "grafting from" technique. Low-molecular-weight bacterial PHB was grafted on chitosan and cellulose acetate by coupling reactions.[10] Isoprene, styrene, 2-hydroxyethyl methacrylate, and acrylic acid were grafted on bacterial PHB by radiation-induced polymerization.[11,12] To our knowledge, the only examples of graft copolymers of PMMA and PHB were also obtained by "grafting from" methods: PMMA-*graft*-atactic PHB, with the side chains randomly distributed along the methacrylate backbone, was produced by anionic grafting of racemic β-butyrolactone on poly(methyl methacrylate),[13] and PHB-*graft*-PMMA was obtained by radiation-induced graft polymerization of MMA on bacterial PHB.[14]

Bacterial PHB macromonomers can be copolymerized with methyl methacrylate to obtain graft copolymers of PMMA and isotactic PHB. This synthesis approach is called the "grafting through" or macromonomer method and is the third main technique to yield graft copolymers. Compared to the other methods, the benefit is a more controlled structure, as the macromonomer size can be chosen prior to the polymerization, and their distribution along the main graft copolymer chain is defined by the comonomer reactivity ratios.[15−17] This system has been increasingly studied,[18] especially after 1975, with macromonomers of a wide range of repeat unit and polymerizable end groups.

Among the different polymerization methods, free radical polymerization (FRP) allows a wide range of monomers, is relatively tolerant to impurities, and is extensively used in industry.[19,20] The conventional FRP in solution of methyl methacrylate with methacrylic macromonomers of PHB to yield PMMA-*graft*-PHB was reported in a previous paper.[21] This method inherently produces a poorly controlled polymer structure,[22,23] e.g., molecular weights are usually unpredictable, and polydispersity indexes are rather broad. Over the past decade or so, controlled or "living" free radical polymerization techniques have been developed,[24,25] such as nitroxide-mediated polymerization (NMP),[26] reversible addition−fragmentation chain transfer (RAFT),[27] and atom transfer radical polymerization (ATRP).[19,20,22,23] The latter was shown to polymerize methacrylate monomers in a controlled fashion.[19,22,23]

The present work reports kinetic studies on the polymerization of methacrylic PHB macromonomers

* To whom correspondence should be addressed: Fax (514) 398-8254; e-mail robert.marchessault@mcgill.ca.

10.1021/ma048899i CCC: $30.25
Published on Web 12/16/2004

with MMA, using ATRP in comparison with conventional FRP. The reactivities of PHB macromonomer and MMA in the copolymerizations will be discussed.

Experimental Section

Materials. High molecular weight bacterial PHB (referenced 16M) was obtained from Biomer Inc. (Forst-Karsten-Strasse 15, D-82152, Krailing, Germany) and used "as received". Number-average molecular weight, M_n, and polydispersity index, PDI, were measured by gel permeation chromatography (PMMA standards) and were 242 500 and 1.4, respectively. 2-Hydroxyethyl methacrylate, HEMA (Aldrich, 97%), was distilled under reduced pressure and stored at −20 °C. *N,N′*-Dicyclohexylcarbodiimide, DCC (Aldrich, 99%), was dried under vacuum for at least 2 days. Methylene chloride, CH_2Cl_2 (Fisher, HPLC grade), was dried over calcium hydride for over 2 days and quickly filtered just before use as solvent for the preparation of PHB macromonomers. MMA (Aldrich, 99%) was stirred over calcium hydride, distilled under reduced pressure, and stored at −20 °C. Copper(I) bromide, CuBr (Aldrich, 98%), was purified by stirring and washing with glacial acetic acid (Fisher) and then washed with anhydrous ethyl alcohol (Commercial Alcohols Inc.) and anhydrous diethyl ether (Fisher). The reagent was then kept under a nitrogen atmosphere. 4-(Dimethylamino)pyridine, DMAP (Aldrich, 99%), was used as received. 1,1,4,7,10,10-Hexamethyltriethylenetetramine, HMTETA (Aldrich, 97%), and ethyl 2-bromoisobutyrate (Aldrich, 98%) were stored at 3 °C and used without further purification. 2,2′-Azobis(isobutyronitrile), AIBN (BDH Chemicals Ltd.), was recrystallized from methanol and stored at 3 °C. Anisole (Aldrich, anhydrous, 97%) was washed with a 2 M sodium hydroxide aqueous solution and then with distilled water and was dried over calcium chloride. The solvent was then distilled under reduced pressure from barium oxide and stored at −20 °C. Tetrahydrofuran, THF (EMD Chemicals, Omnisolv grade), used for gas chomatography analyses was used "as is". For the recovery and purification of products, methanol, CH_3OH, and methylene chloride (both from Fisher, HPLC grade) were not further purified.

Measurements. *Gas Chromatography (GC).* The measurements were obtained with a Hewlett-Packard 5890A gas chromatograph equipped with a J&W Sci. DB-200 column (30 m, 0.53 mm i.d., 1 μm thickness) and a flame ionization detector. The injector and detector temperatures were respectively 150 and 250 °C; the oven temperature was kept at 26 °C for 5 min, raised to 160 °C at 5 °C/min, then to 220 °C at 20 °C/min, and finally kept at 220 °C for 2 min. The injection volume was 1 μL.

Gel Permeation Chromatography (GPC). The analyses were performed at room temperature with chloroform as eluent, at a flow rate of 1.0 mL/min, using two Waters Styragel columns HR3 and HR4 connected in series. A Hewlett-Packard refractive index HP 1047 RI was the detector used. If not mentioned otherwise, calibration was performed using poly(methyl methacrylate) standards (Polymer Laboratories for molecular weights, MW, 254 100, 11 100, 5200, and 1310 and Polysciences for MW 74 000 and 33 500).

Proton Nuclear Magnetic Resonance (^{1}H NMR). ^{1}H NMR experiments were carried out with a Varian Unity 500 MHz at room temperature, with deuterated chloroform $CDCl_3$ as solvent. The internal standard was tetramethylsilane.

Preparation of PHB Macromonomers. PHB oligomers with carboxylic acid and unsaturated (crotonate-type) end groups were obtained according to a pyrolysis procedure: high molecular weight bacterial PHB (130 g) was thermally degraded at 200 °C for 5 h to yield oligomers (after purification: 49 g, M_n(^{1}H NMR) = 1860, PDI(GPC) = 1.4).[28,29] The oligomers were esterified with HEMA, using DCC and DMAP,[30] in a PHB:HEMA:DCC:DMAP molar ratio of 1:3:3:0.3, with anhydrous CH_2Cl_2 as solvent to yield methacrylic PHB macromonomers, PHB* (M_n(^{1}H NMR) = 2010, PDI(GPC) = 1.2, functionality F = 100%).[21]

Preparation of PMMA-*graft*-PHB. *Atom Transfer Radical Polymerizations.* PHB* (0.560 g, 0.279 mmol), CuBr (0.040 g, 0.278 mmol), MMA (2.88 g, 28.8 mmol) (0.96 mol % PHB* in the comonomer feed ratio), and HMTETA (77.0 μL, 0.283 mmol) were placed in a round-bottom flask, and the suspension was purged with argon (Ar). Anisole (8.2 mL) was transferred under Ar to the reaction flask, which was purged back with Ar. After the initiator, ethyl 2-bromoisobutyrate (42.0 μL), was transferred to the reaction flask, a sample of the reaction medium (300 μL) was taken under Ar (time = 0). The reaction flask was then placed in a 70 °C hot bath and kept there for 45 h under magnetic stirring. Samples of the reaction medium (300 μL) were taken at regular intervals (2, 4, 6, 24, 30, and 45 h) under Ar. For each sample, 50 μL was diluted in THF (total volume 1.00 mL) and then analyzed by GC, with three injections per sample, with anisole as internal standard; the remaining 250 μL was transferred to a 10 mL beaker equipped with a magnetic stirrer, and 2.5 mL of methanol was added dropwise to the liquid, under magnetic stirring. The obtained suspension was rinsed three times with methanol and centrifuged, and the clear supernatant was removed. The obtained solid was then vacuum-dried to constant mass. After 45 h, the reaction flask was removed from the hot bath and cooled with cold tap water, and the reaction medium was transferred to an Erlenmeyer flask. Methanol (volume ratio: reaction medium: CH_3OH:1:10) was added dropwise under magnetic stirring. The obtained suspension was filtered, and the final, solid product was dried under vacuum to a constant mass. It was then purified by dissolution in methylene chloride and precipitation in methanol (volume ratio: CH_2Cl_2:CH_3OH:1:7), followed by filtration and vacuum-drying.

Copolymerizations with PHB* to MMA molar feed ratios of 3, 5, 7, and 10 mol % and homopolymerizations of MMA in anisole were carried out using this procedure, with the following molar ratios: $([MMA]_0 + [PHB^*]_0)/[initiator]_0 = 100/1$ and $[CuBr]_0/[HMTETA]_0/[initiator]_0 = 1/1/1$. The initial concentration of PHB* was kept at ca. 0.050 g/mL.

Free Radical Polymerizations (FRP) Initiated by AIBN. The procedure was identical to the ATRP one, except that CuBr and HMTETA were not used, and the initiator was AIBN ($([MMA]_0 + [PHB^*]_0)/[AIBN]_0 = 200/1$) instead of ethyl 2-bromoisobutyrate. AIBN was charged in a vial with a cap equipped with a septum, purged with Ar, dissolved with a portion of anisole, and purged again with Ar.

Results and Discussion

ATRP System Used in the Copolymerization of PHB* and MMA. In ATRP, initiation should occur rapidly and quantitatively, at least at an apparent rate constant similar to the one of propagation, but an excessively fast initiation would generate a large number of radicals which would undergo irreversible termination.[19] An initiator containing a carbon−halogen bond, activated for radical generation by electronic and steric effects, and having a chemical structure resembling the one of the polymer dormant chains is therefore recommended.[22] Hence, ethyl 2-bromoisobutyrate, with its ester function stabilizing the initiator radical produced, was used to initiate ATRP of methacrylates[22] and MMA in particular.[22,31,32] Shinoda and Matyjaszewski also used it for the ATRP of macromonomers of methacrylic poly(lactic acid) with methyl methacrylate.[33] The catalyst system, or reversible termination agent CuBr/HMTETA, which are two commercially available reagents, was shown to polymerize MMA rapidly and in a controlled manner.[34] In general, this monomer is polymerized in solution, using a solvent that keeps the PMMA formed in solution.[19] Anisole, which dissolves PHB oligomers and macromonomers at 70 °C in the concentration used for the copolymerizations, was reported as solvent for the ATRP of MMA, using CuBr/HMTETA as catalyst system,[34] and therefore was used in the polymerizations reported in this paper.

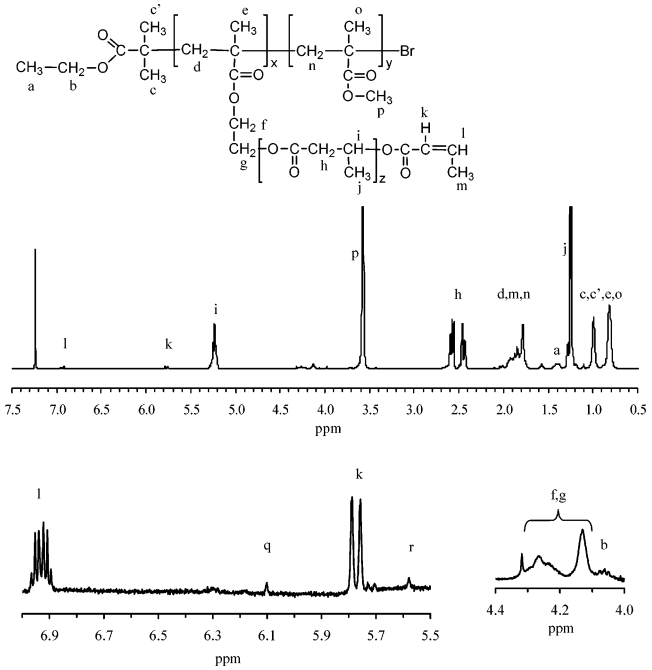


Figure 1. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) for ATRP copolymerization of MMA and PHB* (M_n = 2010, PDI = 1.16, F = 100%) at 2.8 mol % of PHB* in the comonomer feed. Polymerization conditions: ethyl 2-bromoisobutyrate as initiator, $[PHB^*]_0 = 2.49E^{-2}$ mol L^{-1} (0.0500 g mL^{-1}), $[MMA]_0/[PHB^*]_0/[CuBr]_0/[HMTETA]_0/[initiator]_0 = 98.9/2.8/1.3/1.0/1.0$.

PMMA-*graft*-PHB Copolymers. Figure 1 shows an example of the ^{1}H NMR spectrum of PMMA-*graft*-PHB copolymers, obtained by ATR copolymerization with 2.8 mol % PHB* in the comonomer feed. Peaks corresponding to methyl methacrylate and PHB macromonomer repeat units, with end groups derived from the initiator ethyl 2-bromoisobutyrate, were observed. The PHB peaks corresponded to the ones of a typical spectrum of the PHB macromonomers used,[21] except for the ones of the methacrylate end group. The absolute M_n of the PHB macromonomers after copolymerization (inserted in the graft copolymers or unpolymerized) could be calculated from the average integral ratio of the crotonate end group protons to the repeat unit protons corresponding to peaks h and i in Figure 1. The M_n value was found to be close to the one of the macromonomers before copolymerization, with an average discrepancy of 5%, for all the copolymerizations. Therefore, the crotonate end group protons did not undergo any reaction during the copolymerization reaction. The presence of unpolymerized PHB macromonomers was detected with the peaks for the protons of the methacrylate end CH_2=C(CH_3)−C(O)O−(CH_2)$_2$− (peaks q and r in Figure 1), appearing at ca. 6.1 and ca. 5.6 ppm. Their average peak integral represented only 3% of the one for the crotonate end protons (peaks k and l in Figure 1). The four methylene protons at the methacrylate end CH_2=C(CH_3)−C(O)O−(CH_2)$_2$−, appear in one very small peak at 4.3 ppm, and the two multiplet peaks at 4.15 and 4.25 ppm can be attributed to these methylene protons (peaks f and g in Figure 1) in macromonomers inserted in graft copolymers. Their differentiation in the polymerization product spectrum, along with the methacrylate peak integrals considerably reduced (peaks q and r in Figure 1), would show that most of the PHB macromonomers, more precisely 97%, copolymerized with MMA. The conversion of PHB

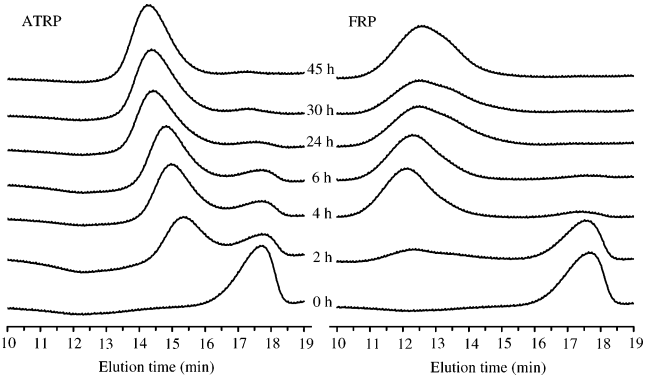


Figure 2. GPC curves for copolymerizations of MMA and PHB* (M_n = 2010, PDI = 1.16, F = 100%) by ATRP and FRP at 2.8 mol % of PHB* in the comonomer feed. ATRP conditions: ethyl 2-bromoisobutyrate as initiator, $[PHB^*]_0 = 2.49E^{-2}$ mol L^{-1} (0.0500 g mL^{-1}), $[MMA]_0/[PHB^*]_0/[CuBr]_0/[HMTETA]_0/[initiator]_0 = 98.9/2.8/1.3/1.0/1.0$. FRP conditions: AIBN as initiator, $[PHB^*]_0 = 2.51E^{-2}$ mol L^{-1} (0.0504 g mL^{-1}), $[MMA]_0/[PHB^*]_0/[AIBN]_0 = 196/5.7/1$.

macromonomers into the graft copolymers was calculated by the integral ratio of the methacrylate end protons peaks to the ones of the crotonate end, taking into account the macromonomer functionality.[21] The small peak at 4.0 ppm is an artifact produced by the instrument, while the one at 1.6 ppm corresponds to the traces of urea from the macromonomer synthesis.

The evolution of the molecular weight of the copolymer in ATRP and conventional FRP is shown on the GPC curves displayed in Figure 2 for the copolymerizations with 2.8 mol % PHB* in the comonomer feed. For both polymerization methods, an increase of molecular weight of the product accompanied by macromonomer peak disappearance indicated that PHB* was inserted in the polymer chains, producing graft copolymers. In the case of ATRP, molecular weights increased with a polydispersity kept relatively low (1.2 or less), which indicates that most of the polymer chains are growing at the same time, as expected for a controlled polymerization method. In addition, PHB* traces were more reduced than the ones for FRP after 2 h of polymerization time but disappeared more progressively than in conventional FRP afterward, which would produce a copolymer with a gradient of PHB grafts along the polymer chains. For conventional FRP, the product trace appears at high molecular weight already after 2 h of polymerization time, indicating fast propagation. Its broadening with time on the lower molecular weight side would show late formation of small polymer chains, possibly due to chain transfer and termination.

Results of the different ATR and conventional FRP copolymerizations are shown in Table 1, with homopolymerizations of MMA in the same conditions as references. The molecular weights given were obtained by GPC, with a refractometer as detector. The values are therefore relative to the polymer standards used (linear PMMA), and most probably underestimated, as the hydrodynamic volumes of graft copolymers are generally smaller than the ones of linear polymers of same molar mass.[18] With ATRP, molecular weights and PDI were rather low, respectively around 20 000 g/mol and 1.2 or less. Values up to 84 100 for M_n and 2.1 for PDI could be obtained at low PHB* content with conventional FRP using AIBN as initiator. Graft copolymer compositions were derived from ^{1}H NMR and GPC analyses. The

Table 1. Synthesis of PMMA-*graft*-PHB Copolymers by Atom Transfer Radical Copolymerization

		comonomer conversion			copolymer[e] (after purification)			
no.	feed ratio MMA:PHB*[a] mol (wt)[b]	MMA[c]	PHB*[d]	total monomer	M_n (GPC)	PDI (GPC)	MMA:PHB* mol (wt)[b]	MMA:HB mol[f]
1	100:0 (100:0)	90		90	18 300	1.1	100:0 (100:0)	100:0
2	99.0:0.96 (84:16)	90	98	91	20 300	1.2	99.0:1.0 (83:17)	82:18
3	97.2:2.8 (63:37)	84	91	87	23 200	1.2	96.3:3.7 (57:43)	55:45
4	95.4:4.6 (51:49)	78	75	77	23 100	1.2	94.9:5.1 (48:52)	46:54
5	93.5:6.5 (42:58)	57	47	51	15 000	1.2	92.0:8.0 (37:63)	36:64
6	91.0:9.0 (33:67)	52	41	45	16 400	1.2	90.6:9.4 (32:68)	32:68

[a] PHB*: PHB macromonomer, $M_n = 2010$, PDI = 1.16, $F = 100\%$. [b] MMA:PHB* means molar fraction (%) of monomer MMA: molar fraction (%) of macromonomer PHB*, the values given in parentheses being weight fractions. [c] Calculated from GC analyses on the copolymer before purification. [d] Calculated from the NMR spectrum on the copolymer before purification. [e] Polymerizations were performed in anisole at 70 °C, for 45 h, with $[PHB^*]_0$ ca. 0.050 g/mL, $([MMA]_0 + [PHB^*]_0)/[initiator]_0 = 100/1$, and $[CuBr]_0/[HMTETA]_0/[initiator]_0 = 1/1/1$. [f] MMA:HB means molar fraction (%) of repeat unit MMA: molar fraction (%) of repeat unit HB.

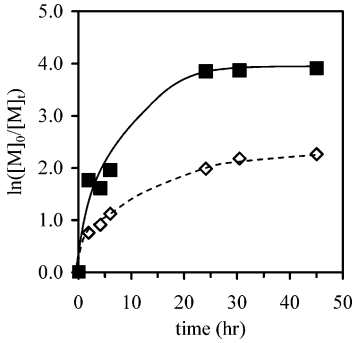


Figure 3. Kinetic plot for the copolymerization of MMA and PHB* ($M_n = 2010$, PDI = 1.16, $F = 100\%$) by ATRP. White diamonds: MMA; black squares: PHB*. Conditions: $[MMA]_0 = 3.46$ mol L^{-1}, $[MMA]_0/[PHB^*]_0/[CuBr]_0/[HMTETA]_0/[initiator]_0 = 100/0.97/0.97/0.99/1.0$. Initiator: ethyl 2-bromoisobutyrate. $[PHB^*]_0 = 2.44E^{-2}$ mol L^{-1} (0.0492 g mL^{-1}). $[M]_0$ and $[M]_t$ are respectively the initial monomer concentration and the one at time t.

determinations of PHB macromonomer molar content in the copolymers[21] and of the molar ratio of 3-hydroxybutyrate (HB) to methyl methacrylate were obtained from the ^{1}H NMR analyses of the purified copolymers. The weight ratio in the purified copolymers of PHB* was calculated from both ^{1}H NMR and GPC and is only indicative, due to the probable underestimation of the molar masses by GPC. The presence of free PMMA polymers was not evidenced by GPC, where only one peak for the product, with in some cases the macromonomer peak, was observed.

Copolymerization Kinetics. Each sample taken from the polymerization medium was analyzed for comonomer conversion and molecular weight. MMA conversion was calculated from GC analyses, whereas PHB* conversion was determined from ^{1}H NMR. The kinetic plot for the copolymerization with a 0.96 mol % of PHB* in the comonomer molar feed ratio by ATRP is shown in Figure 3. PHB* was consumed rapidly, and faster than MMA, during the early stages of polymerization, reaching ca. 80% conversion after 2 h and 98% conversion after 24 h. For comparison, MMA was converted at 53% after 2 h and 86% after 24 h.

The reactivity ratio for MMA comonomer, r_{MMA}, was derived using the method of Mayo and Lewis simplified by Jaacks.[15−18,33,35] The Mayo−Lewis equation for copolymerization, in steady-state conditions, is the following:

$$\frac{d[MMA]}{d[PHB^*]} = \frac{1 + r_{MMA}\dfrac{[MMA]}{[PHB^*]}}{1 + r_{PHB^*}\dfrac{[PHB^*]}{[MMA]}} \quad (1)$$

where

$$r_{MMA} = \frac{k_{MMA,MMA}}{k_{MMA,PHB^*}} \quad \text{and} \quad r_{PHB^*} = \frac{k_{PHB^*,PHB^*}}{k_{PHB^*,MMA}}$$

The propagation rate constant $k_{i,j}$ corresponds to the reaction of a propagating chain ending in monomer i which reacts with a monomer j, i and j being MMA or PHB*.

In the case where the ratio [MMA]/[PHB*] is very large during the copolymerization, this eq 1 reduces to

$$\frac{d[MMA]}{d[PHB^*]} \approx r_{MMA}\frac{[MMA]}{[PHB^*]} \quad (2)$$

The integration of this equation gives

$$\frac{\ln[MMA]_0}{\ln[MMA]_t} \approx r_{MMA}\frac{\ln[PHB^*]_0}{\ln[PHB^*]_t} \quad (3)$$

In the ATR copolymerization with 0.96 mol % of PHB* in the comonomer feed, the excess of MMA compared to PHB* was large enough: $[MMA]_0/[PHB^*]_0$ at 103, and since PHB* was converted faster than MMA (Figure 3), this ratio remained large throughout the copolymerization. The value of r_{MMA} was therefore obtained by the slope of the plot of $\ln[MMA]_0/\ln[MMA]_t$ as a function of $\ln[PHB^*]_0/\ln([PHB^*]_t$, until PHB* reached 98% conversion, and found to be 0.52 ± 0.14. The relative reactivity of the PHB macromonomers, defined as $1/r_{MMA}$,[15−17,33] was therefore 1.9, i.e., the rate constant of the addition of PHB* to a propagating chain with a MMA active center (k_{MMA,PHB^*}) was 1.9 times the rate constant of the homoaddition of MMA to the same propagating chain ($k_{MMA,MMA}$).

Figure 4 shows the dependence of the molecular weight (measured by GPC, PMMA standards) with the total monomer conversion for three of the ATR copoly-

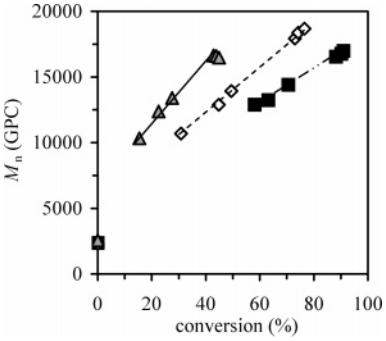


Figure 4. Molecular weight trend with total monomer conversion for the ATRP of MMA and PHB* at 0.96, 4.6, and 9.0 mol % PHB* in the comonomer feed. Black squares: 0.96 mol % PHB*; white diamonds: 4.6 mol % PHB*; gray triangles: 9.0 mol % PHB*. Conditions: $[MMA]_0 + [PHB^*]_0/[CuBr]_0/[HMTETA]_0/[initiator]_0 = 100/1/1/1$, initiator: ethyl 2-bromoisobutyrate, $[PHB^*]_0 \sim 0.050$ g mL^{-1}.

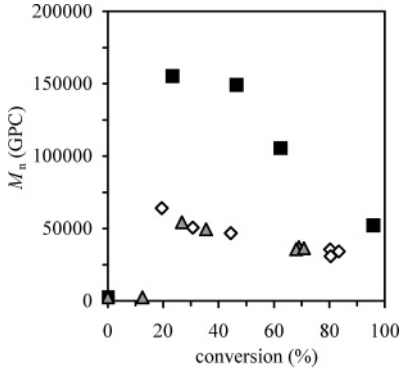


Figure 5. Molecular weight trend with total monomer conversion for the conventional free radical copolymerization of MMA and PHB* at 1.0, 4.8, and 8.6 mol % PHB* in the comonomer feed. Black squares: 1.0 mol % PHB*; white diamonds: 4.8 mol % PHB*; gray triangles: 8.6 mol % PHB*. Conditions: $[MMA]_0 + [PHB^*]_0/[AIBN]_0 = 200/1$, $[PHB^*]_0 \sim 0.050$ g mL^{-1}.

merizations. M_n of graft copolymers increased linearly, with increasing slope for higher PHB* content in the comonomer feed ratio. PDIs were low throughout the polymerizations (1.10−1.27). This would indicate that there is little chain transfer during the polymerizations. The M_n values reported for 0% monomer conversion corresponded to the macromonomers, unpolymerized at polymerization time 0. They are lower than the y-intercepts of the M_n curves, which could arise from the change in polymer architecture and nature: linear macromonomer at 0 conversion, which is a PHB homopolymer, and branched structure for graft copolymers of PMMA and PHB after. This will induce a change in hydrodynamic volumes and in M_n values since molecular weights were measured by GPC and given relative to a calibration with linear PMMA standards.

With conventional free radical copolymerization methods using AIBN as initiator, PHB* was also consumed faster than MMA, with 100% PHB* conversion and 95% MMA conversion after 24 h. In the first 6 h of polymerization, both comonomers were converted less rapidly with conventional FRP than with ATRP. The Jaacks method was used for the conventional free radical copolymerization of PHB* with MMA at 1.0 mol % of PHB* in the comonomer feed ($[MMA]_0/[PHB^*]_0$ at 99, and stayed high, since PHB* was consumed faster than MMA). An r_{MMA} value of 0.59 ± 0.09 was found, which is rather close from the one found with our ATRP experiments, and gives a relative PHB* reactivity, $1/r_{MMA}$, of 1.7.

The M_n of the graft copolymers tended to decrease with total monomer conversion after ca. 20% of conversion (Figure 5), along with PDI values increasing during the polymerizations (up to 2.36). These trends are typical for conventional free radical polymerizations, where termination and transfer lead to molecular weight distribution broadening.

The reactivity ratios r_{MMA} obtained for the copolymerizations of MMA and PHB* by ATRP and FRP were rather close to each other, with the one for FRP higher than the one for ATRP (0.52 ± 0.14 for ATRP, 0.59 ± 0.09 for FRP). For relative comparison, in copolymerizations of MMA with methacrylic poly(L-lactic acid) (PLLA), of M_n 2800, in a mixture of xylene and diphenyl ether at 90 °C, Shinoda and Matyjaszewski obtained a r_{MMA} value of 0.57 ± 0.02 with ATRP and 1.09 ± 0.05 in the same conditions with conventional free radical polymerization using benzoyl peroxide as initiator.[33] Eguiburu and co-workers determined a reactivity ratio for MMA at 1.01 ± 0.17 in the conventional free radical copolymerization of methacrylic PLLA (M_n 4500) and MMA, with AIBN as initiator, in dioxane at 60 °C.[16] These values for FRP were significantly higher than the one that we found; however, the copolymerizations conducted were performed in solvents and reaction temperatures different than the ones of the copolymerizations reported here.

Reactivity ratios of MMA in copolymerizations with small comonomers of structures resembling the one of the methacrylate end of PHB* are only given here as indications, since these data were obtained from polymerizations performed in different conditions (solvent, temperature). Eguiburu et al. reported an $r_{MMA} = 0.85 \pm 0.01$ in the conventional FRP of 2-acetoxyethyl methacrylate with MMA, performed in the same conditions as for the copolymerizations of PLLA with MMA.[16] In the ATR copolymerization of MMA with HEMA, Shinoda and Matyjaszewski reported an $r_{MMA} = 0.67 \pm 0.02$ with the same conditions as the ones for PLLA macromonomers and MMA, except the solvent was different (100% xylene instead of diphenyl ether/xylene/1/1.7/w/w), and the initial concentration of MMA was doubled.[33] Grassie and co-workers derived an r_{MMA} of 1.09 ± 0.01 for the conventional bulk free radical copolymerization of MMA with ethyl methacrylate at 60 °C.[36]

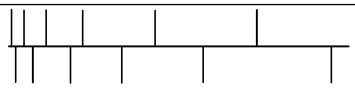
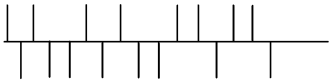
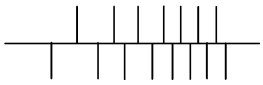
When monomer conversions of MMA and PHB* were compared with each other, for each copolymerization, it was found that for both polymerization methods the increase of PHB* fraction in the comonomer feed ratio slowed down PHB* conversions compared to MMA ones. Up to 2.8 mol % PHB* in the comonomer feed, the macromonomers were polymerized faster than MMA. Above 4.6 mol % PHB* in the comonomer feed, PHB* conversions reached a maximum value after 24 h of polymerization, while MMA was still being converted. During the first hours of polymerization, the comonomers conversions were nearly identical at 4.6−7.1 mol % PHB* in comonomer feed, whereas MMA was converted faster than PHB* at 8.6−9.0 mol % PHB* in comonomer feed. In the final graft copolymer products, as the reaction time was the same for all polymerizations, PHB* final conversion values decreased with increasing PHB* fraction in the comonomer feed (Table 1). Hence, with 2.8 mol % PHB* in the feed ratio or

Table 2. Synthesis of PMMA-*graft*-PHB Copolymers by Conventional Free Radical Copolymerization

		comonomer conversion			copolymer[e] (after purification)			
no.	feed ratio MMA:PHB*[a] mol (wt)[b]	MMA[c]	PHB*[d]	total monomer	M_n (GPC)	PDI (GPC)	MMA:PHB* mol (wt)[b]	MMA:HB mol[f]
1[g]	100:0 (100:0)	90		90	55 600	1.8	100:0 (100:0)	100:0
2[g]	99.0:1.0 (83:17)	95	100	96	84 100	2.1	99.0:1.0 (83:17)	82:18
3	97.2:2.8 (63:37)	93	98	95	63 000	1.6	97.5:2.5 (66:34)	62:38
4	95.2:4.8 (50:50)	85	82	83	44 200	1.6	93.1:6.9 (40:60)	37:63
5	92.9:7.1 (40:60)	80	73	76	31 800	1.7	90:10 (31:69)	29:71
6	91.4:8.6 (35:65)	77	67	71	40 600	2.0	87:13 (25:75)	23:77

[a] PHB*: PHB macromonomer, $M_n = 2010$, PDI = 1.16, $F = 100\%$. [b] MMA:PHB* means molar fraction (%) of monomer MMA: molar fraction (%) of macromonomer PHB*, the values given in parentheses being weight fractions. [c] Calculated from GC analyses on the copolymer before purification. [d] Calculated from the NMR spectrum on the copolymer before purification. [e] Polymerizations were performed in anisole at 70 °C, for 45 h, with $[PHB^*]_0$ ca. 0.050 g/mL, $([MMA]_0 + [PHB^*]_0)/[AIBN]_0 = 200/1$. [f] MMA:HB means molar fraction (%) of repeat unit MMA: molar fraction (%) of repeat unit HB. [g] Polymerizations 8 and 9 were respectively stopped after 25 and 24 h of reaction time due to the high viscosity of the reaction medium.

Table 3. Proposed Microstructures for PMMA-*graft*-PHB Copolymers Synthesized by Atom Transfer Radical Copolymerization

PHB* molar content in comonomer feed	Proposed graft copolymer microstructure
0.96, 2.8	
4.6, 6.5	
9.0	

more, free PHB* could be detected, in increasing amount with higher proportion of PHB* in the comonomer feed. MMA final conversions also decreased, but to a lesser extent than the ones of PHB* (Table 1).

Graft copolymer microstructures, i.e., the distribution of the grafts in the copolymer chains, could therefore be derived for the different PHB* contents in the feed for the ATR copolymerizations, as they were shown to have little chain transfer. The microstructures are shown in Table 3. For comonomer feed content of PHB* at 0.96 mol % and also, but less markedly, at 2.8 mol %, the graft copolymers would be spontaneous gradient copolymers. At the intermediate PHB* feed contents of 4.6 and 6.5 mol %, the copolymers would have polymer grafts regularly located along most of the backbones and, at one end, a PMMA segment. At 9.0 mol % PHB*, chains with a gradient of PHB* branches, less concentrated at the start, with the other end being a PMMA block.

The decrease of macromonomer reactivity with increasing PHB* content may be explained by some incompatibility between the macromonomer chain and the propagating comonomer chain. The latter is related to thermodynamic repulsive interactions[17,33,37] and influences the degree of interpenetration of the two types of polymer chains[17,37] and therefore the concentration of the macromonomers around the propagating chains.[17] The large size of the macromonomer compared to the comonomer can also affect the macromonomer reactivity, particularly when the macromonomer fraction in the comonomer feed is increased. In the conventional free radical copolymerization of methacrylic PMMA macromonomers and MMA, Radke and Müller observed a decrease in macromonomer reactivity compared to the one for MMA.[38] The macromonomer and comonomer used there were of same chemical structure, so the incompatibility effect was annulled. For a given macromonomer size, at low initial polymer concentration, it was found that the macromonomer reactivity decreased with increasing the macromonomer fraction in the comonomer feed ratio.[38] The composition of the propagating chains toward the terminal polymerizable unit, and not only the terminal unit, seems therefore to alter the copolymerization by excluded-volume effects between the propagating chains and the macromonomers. Since the graft copolymers compositions were similar to the comonomer feed ratios, even at higher PHB* contents in the feed, the incompatibility and excluded-volume effects should vary with comonomer feed composition and affect monomer conversions as well as their reactivities.

The diffusion control effect, which is also another major factor, is related to the high molecular weight of macromonomers compared to traditional monomers and reported to increase with higher concentrations of macromonomers.[33,38] In this report, since PHB* initial concentrations were kept constant, this factor would reduce the macromonomer reactivity to the same extent in all the copolymerizations.

Conclusions

Graft copolymers prepared by ATRP were less polydisperse than the ones prepared by free radical polymerization and also of lower molecular weight. At low molar feed ratio of PHB macromonomers, with both techniques, the reactivity of PHB macromonomers was higher than for MMA. From the evolution of the comonomer conversions with time, and product compositions, it was observed that the reactivity of PHB macromonomers decreased compared to the one for MMA with increasing proportion of macromonomers in the comonomer feed. This could be explained by the incompatibility between the macromonomer chain and the propagating comonomer chain having a major influence on their degree of interpenetration as well as excluded-volume effects around the terminal polymer-

izable group of the growing chains. The change in polymerization behavior of PHB* and MMA comonomers with comonomer feed composition would lead to variable microstructures of the graft copolymers produced by ATRP.

Acknowledgment. Thanks are addressed to Drs. Hung Anh Nguyen, Alain Le Borgne, and Pr. Philippe Guérin, who kindly received me at the Laboratoire de Recherche sur les Polymères (CNRS, UMR C7581), Thiais, France. Prof. Michael Georges is thanked for helpful comments on the manuscript. The Natural Sciences and Engineering Research Council and Labopharm Inc. provided financial support.

References and Notes

(1) Doi, Y. *Microbial Polyesters*; VCH Publishers: New York, 1990.
(2) Hocking, P. J.; Marchessault, R. H. In *Biopolymers from Renewable Resources*; Kaplan, D. L., Ed.; Springer: Berlin, 1998; Chapter 9, p 220.
(3) Baptist, J. N. W.R. Grace & Co.: U.S. 3036959, 1962.
(4) Bunel, C.; Le Saux, V.; Vairon, J.-P. Science et Medecine, Fr.: FR 2670114, 1992.
(5) Bunel, C.; Le Saux, V.; Vairon, J.-P. Science et Medecine, Fr.: FR 2670115, 1992.
(6) Jiang, Y.; Roby, M. United States Surgical Corp.: US 5847046, 1998.
(7) Kühn, K.-D. *Bone Cements, Up-to-Date Comparison of Physical and Chemical Properties of Commercial Materials*; Springer: Berlin, 2000.
(8) Lotti, N.; Pizzoli, M.; Ceccorulli, G.; Scandola, M. *Polymer* **1993**, *34*, 4935−4940.
(9) Ceccorulli, G.; Scandola, M.; Adamus, G. *J. Polym. Sci., Part B: Polym. Phys.* **2002**, *40*, 1390−1399.
(10) Yalpani, M.; Marchessault, R. H.; Morin, F. G.; Monasterios, C. J. *Macromolecules* **1991**, *24*, 6046−6049.
(11) Jiang, T.; Hu, P. *Polym. J.* **2001**, *33*, 647−653.
(12) Bahari, K.; Mitomo, H.; Enjôji, T.; Hasegawa, S.; Yoshii, F.; Makuuchi, K. *Angew. Makromol. Chem.* **1997**, *250*, 31−44.
(13) Kowalczuk, M.; Adamus, G.; Jedlinski, Z. *Macromolecules* **1994**, *27*, 572−575.
(14) Mitomo, H.; Enjôji, T.; Watanabe, Y.; Yoshii, F.; Makuuchi, K.; Saito, T. *J. Macromol. Sci., Pure Appl. Chem.* **1995**, *A32*, 429−442.
(15) Ito, K.; Kawaguchi, S. *Adv. Polym. Sci.* **1999**, *142*, 129−178.
(16) Eguiburu, J. L.; Fernandez, M. J.; San Roman, J. *Polymer* **1996**, *37*, 3615−3622.
(17) Meijs, G. F.; Rizzardo, E. *J. Macromol. Sci., Rev. Macromol. Chem. Phys.* **1990**, *C30*, 305−377.
(18) Rempp, P. F.; Franta, E. *Adv. Polym. Sci.* **1984**, *58*, 1−53.
(19) Matyjaszewski, K.; Xia, J. *Chem. Rev.* **2001**, *101*, 2921−2990.
(20) Davis, K. A.; Matyjaszewski, K. *Adv. Polym. Sci.* **2002**, *159*, 2−166.
(21) Nguyen, S.; Marchessault, R. H. *Macromol. Biosci.* **2004**, *4*, 262−268.
(22) Kamigaito, M.; Ando, T.; Sawamoto, M. *Chem. Rev.* **2001**, *101*, 3689−3745.
(23) Coessens, V.; Pintauer, T.; Matyjaszewski, K. *Prog. Polym. Sci.* **2001**, *26*, 337−377.
(24) Matyjaszewski, K. *Controlled/Living Radical Polymerization. Progress in ATRP, NMP, and RAFT. (Proceedings of a Symposium on Controlled Radical Polymerization held on 22−24 Aug 1999, in New Orleans)*; American Chemical Society: Washington, DC, 2000; Chapter 1.
(25) Bisht, H. S.; Chatterjee, A. K. *J. Macromol. Sci., Polym. Rev.* **2001**, *C41*, 139−173.
(26) Hawker, C. J.; Bosman, A. W.; Harth, E. *Chem. Rev.* **2001**, *101*, 3661−3688.
(27) Moad, G.; Mayadunne, R. T. A.; Rizzardo, E.; Skidmore, M.; Thang, S. H. *Macromol. Symp.* **2003**, *192*, 1−12.
(28) Nguyen, S.; Yu, G.-e.; Marchessault, R. H. *Biomacromolecules* **2002**, *3*, 219−224.
(29) Marchessault, R. H.; Nguyen, S.; Yu, G.-e. McGill University: US 6534599B2, 20003.
(30) Neises, B.; Steglich, W. *Angew. Chem.* **1978**, *90*, 556−557; *Angew. Chem., Int. Ed. Engl.* **1978**, *17*, 522−524.
(31) Wang, J.-S.; Matyjaszewski, K. *Macromolecules* **1995**, *28*, 7901−7910.
(32) Haddleton, D. M.; Jasieczek, C. B.; Hannon, M. J.; Shooter, A. J. *Macromolecules* **1997**, *30*, 2190−2193.
(33) Shinoda, H.; Matyjaszewski, K. *Macromolecules* **2001**, *34*, 6243−6248.
(34) Xia, J.; Matyjaszewski, K. *Macromolecules* **1997**, *30*, 7697−7700.
(35) Jaacks, V. *Makromol. Chem.* **1972**, *161*, 161−172.
(36) Grassie, N.; Torrance, B. J. D.; Fortune, J. D.; Gemmel, J. D. *Polymer* **1965**, *6*, 653−658.
(37) Tsukahara, Y.; Hayashi, N.; Jiang, X.-L.; Yamashita, Y. *Polym. J.* **1989**, *21*, 377−391.
(38) Radke, W.; Müller, A. H. E. *Makromol. Chem., Macromol. Symp.* **1992**, *54/55*, 583−594.

MA048899I