Monohydroxylated Poly(3-hydroxyoctanoate) Oligomers and Its Functionalized Derivatives Used as Macroinitiators in the Synthesis of Degradable Diblock Copolyesters

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The presence of a hydroxyl group at the end of poly(3-hydroxyoctanoate) oligomers, noted PHO oligomers, is required to prepare diblock copolymers with improved properties by ring-opening polymerization of cyclic monomer as ϵ-caprolactone. Several chemical methods such as basic hydrolysis, acid-catalyzed reaction with APTS, and methanolysis were used to prepare well-defined low molar masses PHO oligomers. The methanolysis reaction was allowed to proceed for 10−60 min to produce PHO oligomers with M_n values ranging from 20 000 to 800 g mol^{-1} with low polydispersity index. Detailed analysis of the MALDI-TOF mass spectra of the obtained oligomers has revealed the presence of linear structures bearing methyl ester on one side and hydroxyl end group on the other side. The same procedure was applied to poly(3-hydroxyoctanoate-*co*-3-hydroxyundecenoate), PHOU, a poly(3-hydroxyalkanoate) containing unsaturated units in its side chains. These oligomers were further used to initiate the polymerization of ϵ-caprolactone by varying the PHO (or PHOU) and PCL lengths. By copolymerization with ϵ-caprolactone, the properties of PHO or PHOU have been improved. The crystallinity of the obtained copolymers was modified by controlling the length of the two different blocks. The unsaturations in the side chains of the PHOU block were oxidized in acid carboxylic functions to obtain a novel artificial biopolyester. Moreover, degradation was followed to study the influence of carboxylic groups on the hydrolysis of the copolymers.

Introduction

Poly(3-hydroxyalkanoate)s (PHAs) can be accumulated intracellularly by a large number of bacteria as energy and carbon reserve. Using various types of substrates, a great variety of PHAs have been synthesized.[1−3] PHAs have properties ranging from those of elastomers (medium chain length PHA) to thermoplastics (short chain length PHA) according to their composition and their monomer sequence. Because of their biodegradability and biocompatibility,[4−6] PHAs have been considered as polymers with high potential for applications as carriers for long-term dosage of active biomolecules or for degradable implant materials.[7−10] They have also been used in bone plates or blood vessel replacements. To improve their potential applications, copolymers have been prepared. A possible approach to obtain copolymers is to prepare them by bioconversion.[11−14] The biosynthesis of block copolymers of PHAs has not actually been achieved. This prompted us to explore block copolymer preparation by chemical methods. Block copolymers were previously obtained by direct condensation between oligomers[15,16] or by ring-opening polymerization of lactones.[17] The chemical process involved the preparation of oligomers containing reactive end groups which were used for copolymers preparation. Various methods have been developed for preparing low molecular weight of poly(3-hydroxybutyrate) copolymers (PHB copolymers) like acid-catalyzed methanolysis,[17,18] thermal degradation,[19] and transesterification reaction, for example.[20,21] Telechelic dihydroxylated PHB, PHBHV (poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate)), and poly(3-hydroxyoctanoate) (PHO) were prepared by a transesterification procedure with ethyleneglycol. These diols were used as segments in the synthesis of copoly(ester-urethane)s.[16]

The purpose of the present study is to prepare novel diblock copolymers on the basis of mcl PHAs as PHO (poly(3-hydroxyoctanoate)) or its functionalized derivative, noted PHOU (poly(3-hydroxyoctanoate-*co*-3-hydroxyundecenoate)). This first block is considered as a soft segment. To improve thermal properties of these mcl PHAs, the second block is constituted by poly(ϵ-caprolactone) which is also biodegradable but more crystalline than PHO and PHOU. The interest for synthesizing diblock P(HOU-*b*-CL)s is their potential for chemical modification. The quantitative conversion of the unsaturated groups of PHOU into carboxylic groups[22,23] has already permitted to improve the hydrolytic degradation of these biopolyesters[24] and to graft PEG or PLA.

The preparation of diblock copolymers proceeds in two steps. In the first step, we have investigated the preparation of monohydroxylated PHO and PHOU oligomers with different methods: basic hydrolysis, acid-catalyzed hydrolysis, and methanolysis. The identification of the structure was realized to investigate the nature of functional end groups formed. In a second step, the hydroxyl end group of the oligomers was used to initiate the ring-opening polymerization of ϵ-caprolactone. In our study, copolymers were prepared through the controlled coordination−insertion ring-opening polymerization of lactones initiated by oligomers of PHAs using trialkyl metal as catalyst, Et_3Al. The synthesized diblock copolymers were characterized with regard to their molar masses, thermal properties, and molar

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compositions by SEC, differential scanning calorimetry (DSC), and ^{1}H NMR. The degradation of these new copolyesters has been studied to compare the influence of the copolymer compositions on the abilities of being hydrolyzed.

Experimental Section

Materials. Poly(3-hydroxyoctanoate), PHO, and poly(3-hydroxyoctanoate-*co*-3-hydroxyundecenoate), PHOU, were carried out using *Pseudomonas sp Gpo1* (CNRS, Cermav, Grenoble, France) as described in a previous report. PHO, with a number average molecular weight of 80 000 g mol^{-1} (Ip = 2.0), was produced from sodium octanoate.[25] PHOU was produced from a mixture of sodium octanoate and 10-undecenoic acid (80:20).[26] The percentage of unsaturated units corresponds to the proportion of alkenoic acid in the mixture of substrate. PHOU prepared contains 25% of reactive olefinic groups. Bacterial polyesters were extracted from lyophilized cells with chloroform in a Soxhlet extractor and then were purified by precipitation in methanol and were dried under vacuum.

ϵ-caprolactone (Aldrich, 99%) and toluene (SDS, 99.3%) were dried over calcium hydride at room temperature for 48 h and then were distilled under reduced pressure just before being used. Triethylaluminium, Et_3Al, (1.8 mol L^{-1} in toluene from Fluka) was used without further purification.

Preparation of Oligomers. *Basic Hydrolysis.* Fifty milligrams of PHA was solubilized in 3 mL of CH_2Cl_2 during 3 h at room temperature. Seven hundred fifty microliters of alcoholic NaOH at pH = 14 (0.6 g of NaOH dissolved in 3 mL of water and 3 mL of ethanol) was introduced into the solution. Hydrolysis was stopped at different times by addition of concentrated chlorohydric acid aqueous solution (6 N). The organic solvent elimination by evaporation induces the precipitation of the polymer in the aqueous phase. After water elimination, the polymer was solubilized in 5 mL of CH_2Cl_2. The organic layer was washed three times with 3 mL of water and then was dried on $MgSO_4$. The organic layer was concentrated under vacuum. The same procedure was used for basic hydrolysis at pH = 10 or pH = 12.

Acid-Catalyzed Reaction. *para*-Toluenesulfonic acid monohydrate (APTS) was obtained from Aldrich. Solvents were used without further purification.

In a typical experiment of hydrolysis in solution, 50 mg of PHO was dissolved in 1.075 mL of solvent (toluene or dichloroethane). Five milligrams of APTS was added, and the reactor was then plunged into a preheated oil bath at 120 °C. The reaction was stopped by cooling it in an ice bath. The solvent was removed and the product obtained was dissolved in 2 mL of CH_2Cl_2. APTS was removed by two filtrations on regenerated cellulose membrane (0.45 μm). The solution was evaporated and concentrated under vacuum to a solid product.

A similar hydrolysis was carried out in bulk with similar conditions as reported above. The method implies a melt process, thus without organic solvent. After stopping the reaction by cooling, products were solubilized in CH_2Cl_2 to remove APTS by filtration.

Methanolysis Catalyzed by H_2SO_4. Two hundred fifty milligrams of PHO was dissolved in 10 mL of dichloromethane. Ten milliliters of methanol was mixed with the solution of PHO, and 0.75 mL of H_2SO_4 was added. The solution was stirred on a magnetic stirrer at 100 °C for a determined time to obtain the desired molar masses. After cooling in an ice bath, 5 mL of distilled water was added, and after decantation, the organic layer was washed again with distilled water, was dried under $MgSO_4$, and was filtered. The solvent was evaporated and the resulting product was dried under vacuum at 40 °C.

Diblock Copolymers Synthesis. In a previously flamed and argon-purged three times round-bottom flask, 160 mg of PHO oligomers (M_n = 800 g mol^{-1}) was dried by three successive azeotropic distillations of dried toluene (3 × 2 mL). Then, 1.2 equiv of triethylaluminium compared to the content of hydroxyl functions (2.4 10^{-4} mol, 133 10^{-3} mL) was added into the flask equipped with a rubber septum, under argon atmosphere, using a syringe. The mixture was allowed to stir for 2 h at 50 °C. Emission of ethane evolved through a connected oil valve to produce aluminum alkoxide macroinitiator ($Et_2AlO-PHO$). After cooling to room temperature, ϵ-caprolactone (8.8 10^{-3} mol, 0.97 mL) was added using a syringe and was polymerized for 24 h. Deactivation of aluminum alkoxide growing sites and deprotection of hydroxyl groups were carried out by dissolution in toluene and addition of a few drops of a HCl aqueous solution (0.1 mol L^{-1}). The polymer solution was poured twice into 10 volumes of heptane (20 mL). The copolymer was recovered by filtration and was dried under reduced pressure at 40 °C until constant weight (606 mg) (yield = 73%). The resulting products of PHO−PCL diblock copolymers were obtained through adjusting the molar feed ratio of ϵ-CL monomers to PHO oligomers macroinitiator length.

Oxidation of Diblock Copolymers P(HOU-*b*-CL). One hundred milligrams of P(HOU-*b*-CL) copolymer was dissolved in 2 mL of dichloromethane to obtain a concentration of 20 g L^{-1}. One hundred thirty milligrams of crown-ether (3 equiv compared to the content of unsaturated units), 104 mg of potassium permanganate (4 equiv compared to the content of unsaturated units), and 0.34 mL of acetic acid (17% of the total volume) were added. The mixture was stirred at room temperature for 4 h 30 min. Then, 4.4 mL of CH_2Cl_2 and 4.4 mL of distilled water were added. The addition of sodium bisulphite was then realized to complete reduction of unreacted $KMnO_4$. After decantation, the aqueous layer was extracted with 4.4 mL of CH_2Cl_2. Organic layers were concentrated. For purification, the crude oxidized product was transformed into nanoparticles using the nanoprecipitation−solvent evaporation method in acetone/water mixture (40 mL of acetone into 40 mL of water). The resulting nanoparticle suspension was transferred into a regenerated cellulose ester dialysis membrane (6−8000 Da) and was dialyzed by using deionized water for 48 h. The water was changed twice a day. The product was then recovered by evaporation of the water.

Hydrolytic Degradation. *Preparation of the Films.* The films of PHO and P(HO−COOH) were prepared by the method of dissolution/evaporation of solvent. Copolymers were dissolved in chloroform (concentration 10 mg mL^{-1}). Eight hundred microliters of the solution was spread over a glass plate. The films were recovered by evaporation of the solvent.

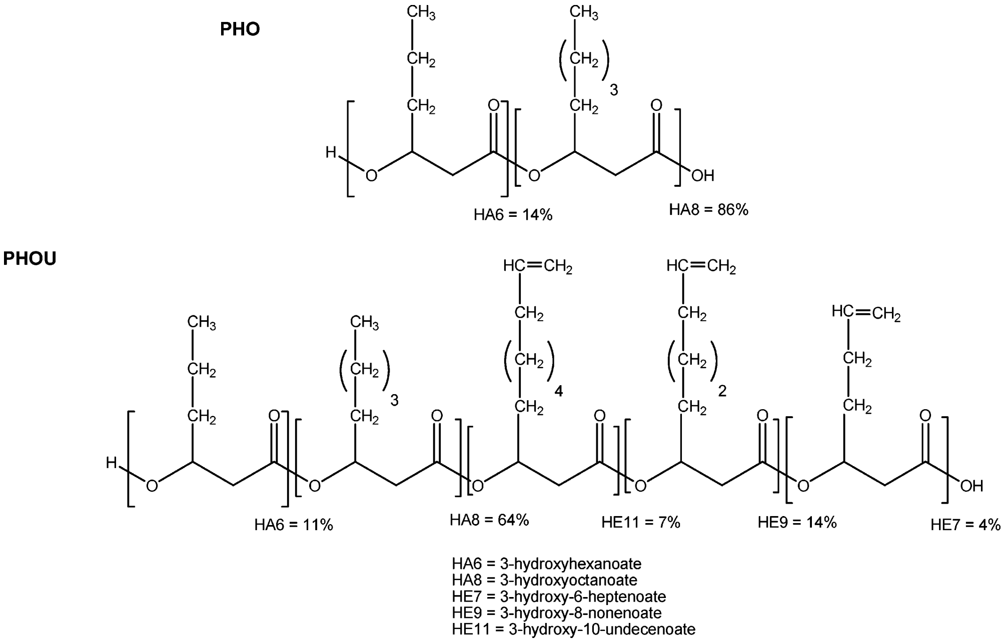
The films based on copolymers of P(HA-*b*-CL) were prepared by compression. Eighty milligrams of copolymers, placed between two sheets of Teflon, was heated at 50−80 °C for 30 s and was pressed during 2 s. The obtained films have a diameter of 3.5 cm and a thickness comprised between 40 and 80 μm.

Study of the Degradation. The films were plunged into 50 mL of a buffer solution with a pH = 7.3 at 37 °C. Each week, the buffer solution was changed to avoid the development of microorganisms. The film was periodically washed with distilled water, was dried, and was analyzed by SEC. The aqueous solution was also analyzed by SEC after being lyophilized. For the study of films of P(HO−COOH), the solution was centrifuged twice for 50 min at 10 000 tr min^{-1}. The precipitate was washed with distilled water and the precipitate and the supernatant were both analyzed by SEC.

Analytical Techniques. *Molar Mass Measurements*. Average molecular masses were determined by SEC using a Kontron 420 pump with two styragel columns connected in series which type is PL gel (mixte C, 5 μm) from polymer laboratories and a Shodex RI-71 model refractive index detector. The system was calibrated by using polystyrene standards of low polydispersity index purchased from Polysciences (M_n (in g mol^{-1}): 2656 000, 841 700, 320 000, 148 000, 59 500, 28 500, 10 850, 2930, and 580). The samples were dissolved at a concentration of 10 mg mL^{-1} in THF. Aliquots of 50 μL of the polymer solution were chromatographed with pure THF as the solvent phase at a flow rate of 1.0 mL min^{-1}.

Thermal Analysis. Thermal analysis was conducted by differential scanning calorimetry (DSC). Samples of 8−12 mg of polymers were weighted into aluminum pans and were analyzed using a DSC-2010 TA instrument. The samples were scanned from 20 °C to 100 °C with

Scheme 1. Structural Formula of PHO and PHOU



a heating rate of 10 °C min^{-1} and then cooling at −120 °C, and they were scanned again from −120 °C to 20 °C with a heating rate of 10 °C min^{-1}. The glass-transition temperature (T_g) was taken as the midpoint of the transition, in the second heating run. Melting temperature (T_m) was taken as the summit of melting peak and the melting enthalpy (ΔH_m) was calculated from the area of the endothermic peak after the first run.

Nuclear Magnetic Resonance Spectroscopy. Nuclear magnetic resonance spectra were recorded on a Bruker Avance 300 spectrometer at room temperature. For proton (^{1}H) NMR spectra, 20 mg of product was dissolved in 0.7 mL of chloroform-*d*, and 200−300 mg of product dissolved in 2−3 mL of chloroform-*d* was used for carbon (^{13}C) spectra. Chemical shifts were given in parts per millions (ppm) relative to the signal of chloroform as internal reference (^{1}H NMR, 7.26 ppm; ^{13}C NMR, 77.7 ppm).

Quantitative ^{13}C NMR measurements were carried out by using a sequence with a pulse of 30°, an acquisition time of 0.7 s, and a delay of 3 s between scans. The quantitative ^{13}C NMR spectra were recorded in the inverse gated mode.

MALDI-TOF Mass Spectrometry. MALDI-TOF mass spectrometry was performed using a Perceptive Biosystems Voyager Elite time-of-flight mass spectrometer equipped with a nitrogen laser (λ = 337 nm). Spectra were recorded in a reflector-delayed extraction mode at an acceleration voltage of 20 kV. The matrix, 2.5-dihydroxybenzoïc acid, was dissolved in THF (15 g.L^{-1}). NaI was added for control experiments (0.1 g L^{-1}). The SEC polymer fractions were evaporated to dryness and were diluted with 50 μL of THF (3 g L^{-1}). Five microliters of the polymer solution was mixed with 50 μL of the matrix solution. One-microliter portion of the final solution was deposited onto the stainless steel sample slide and was allowed to dry in air at room temperature. The MALDI-TOF mass spectra represent averages of 256 consecutive laser shots.

Results and Discussion

Preparation of Mmonohydroxylated PHO and PHOU Oligomers. Different methods were used to produce well-defined mcl PHA oligomers from natural mcl PHAs which could be used as macroinitiators to build diblock copolymers. These macroinitiators have to contain a specific hydroxylic end group to initiate the ring-opening polymerization of lactones like ϵ-caprolactone. Depending on the nature and length of the blocks, the copolymer properties of crystallinity and degradability could be modified. Monohydroxylated PHO and PHOU oligomers have been prepared from natural PHO and PHOU (Scheme 1) using different methods. Basic hydrolysis, acid-catalyzed reactions, and methanolysis were chosen to prepare PHO oligomers. The synthesis of low molar mass PHO was carried out by performing basic hydrolysis (pH = 10, 12, and 14) at room temperature or acid-catalyzed reaction on natural PHO with *para*-toluenesulfonic acid (APTS) in dichloroethane or in toluene. The kinetics of the PHO oligomer formation depends on reaction conditions. The variation of molar masses was investigated by SEC following hydrolysis for various periods (Figure 1). In basic solutions (pH = 10 and 12), ester bonds were stable because no change in the molecular mass was observed confirming the very slow hydrolytic degradation of hydrophobic PHO. However, hydrolysis was faster in very strong conditions when pH was equal to 14, and degradation occurred immediately. In this case, two populations of oligomers were formed with different molar masses (Figure 2). ^{1}H NMR analysis showed that the PHO oligomers obtained by basic

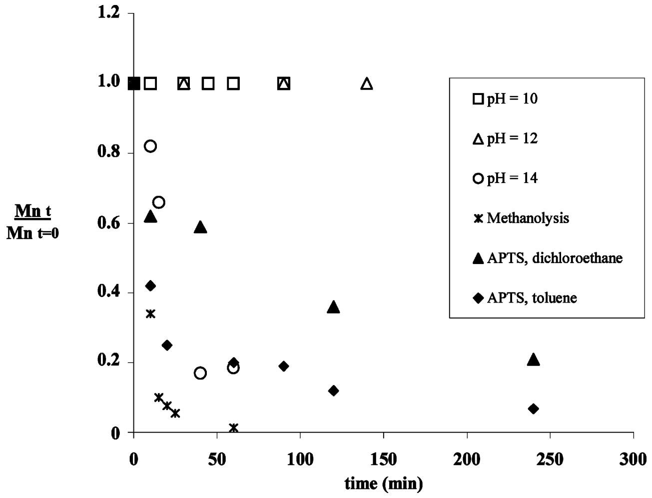


Figure 1. Evolution of $M_{n\ t}/M_{n\ t=0}$ of PHO during basic (pH = 10, 12, and 14), acid-catalyzed reaction (in dichloroethane, in toluene) and methanolysis.

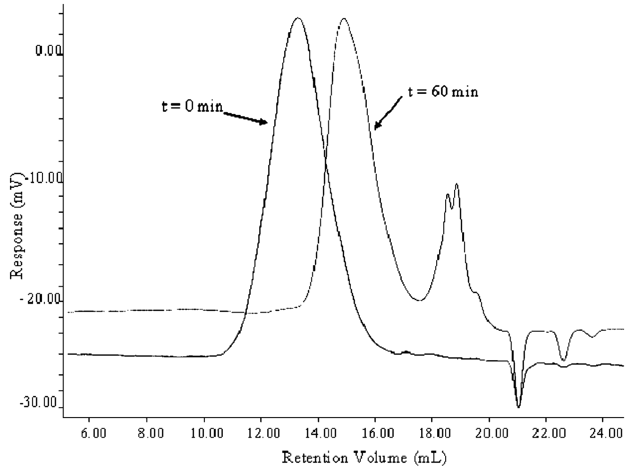
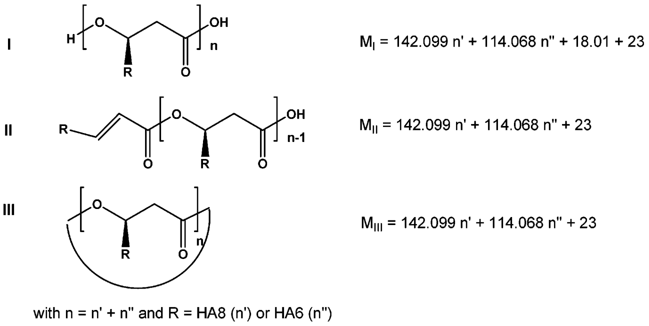


Figure 2. SEC profiles of initial PHO and PHO degraded after 60 min of basic hydrolysis at pH = 14.

Scheme 2. Proposed Structures of PHO Oligomers Prepared by Acid-Catalyzed Reaction



degradation at pH = 14 contained an unsaturated end group resulting from the cis elimination reaction (Mc Lafferty rearrangement) previously described during PHB degradation.[19]

Acid-catalyzed reaction and methanolysis were more efficient to produce PHO oligomers. In acid-catalyzed conditions (APTS, 10 wt % in toluene or in dichloroethane), a decrease of the PHO molar mass was observed depending on the solvent nature (Figure 1). The degradation occurred more rapidly in toluene (20 000 g mol^{-1} after 20 min of reaction) than in dichloroethane (60 000 g mol^{-1}) which can be explained by a better solubility of PHO in toluene. MALDI-TOF mass spectrometry was used to determine more precisely the nature of the end groups present in PHO oligomers obtained by acid-catalyzed reaction. Polydispersed PHO oligomers were first fractionated by SEC in THF to obtain oligomers with a lower molecular mass distribution. Fractionation by SEC has given 56 fractions. The MALDI-TOF mass spectrum of an SEC fraction of PHO oligomers obtained after 4.5 h of APTS catalyzed reaction in THF (fraction 42, M_n = 2000 g mol^{-1}, Ip = 1.02) showed two series of peaks centered on two different values. The first was centered at *m*/*z* 1250 and the second was centered at *m*/*z* 1750. Scheme 2 shows the structural assignments of sodiated ions (I−III) which could appear in the MALDI-TOF spectra where *n* represents the total number of repetitive units, *n*′ the number of HA8 repetitive units, and *n*″ the number of HA6 repetitive units. Figure 3 represents the expansion of the region centered at *m*/*z* = 1750 of the MALDI-TOF spectrum and the mass assignments. Only two types of structure (I and III) were detected in MALDI-TOF spectra. These results have confirmed that the formation of oligomers by acid-catalyzed hydrolysis has given linear oligomers (structure I, Scheme 2) with a hydroxyl group at one end and a carboxylic group at the other end and cyclic structure (structure III, Scheme 2) in a very low proportion.

PHO and PHOU of high molar masses (M_n = 80 000 g mol^{-1}) were also exposed to methanolysis in the presence of sulfuric acid as catalyst to form PHO and PHOU oligomers (Scheme 3). The reactions were stopped at different times and the evolution of molar masses during reaction was studied by SEC. As shown in Table 1, the molar masses of the produced oligomers were controlled by adjusting reaction times. The methanolysis reaction was allowed to proceed for 10−60 min to produce PHO oligomers with an M_n value obtained from

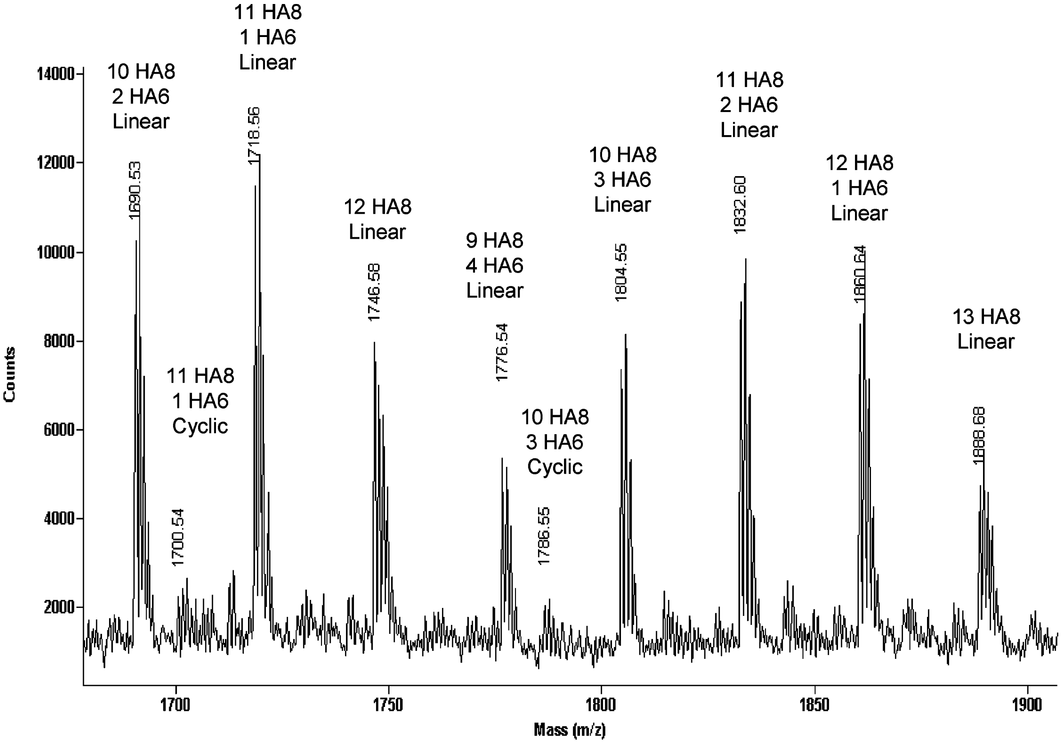


Figure 3. MALDI-TOF spectrum of a fraction of PHO oligomers (M_n = 2000 g mol^{-1}, Ip = 1.02) obtained after 4.5 h of acidic treatment in toluene.

Scheme 3. Methanolysis of mcl PHA

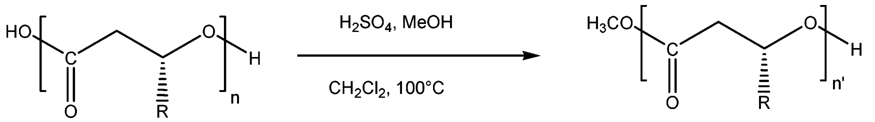


Table 1. Comparison of Molar Masses of the PHO and PHOU Oligomers at Different Times of Methanolysis Determined by ^{1}H NMR and by SEC and Their Thermal Characteristics

polymer	T (min)	M_n[a] (g mol^{-1})	M_n[b] (g mol^{-1})	Ip	T_g (°C)	T_m (°C)	ΔH_m (J g^{-1})
PHO	0		80 000	2.0	−40	53	27
	10	20 000	27 700	1.9	−40	50.5	18.5
	15	8000	8600	2.1	−40	47	18
	20	5300	6100	2.1	−44		
	25	2500	4400	2.0	−48		
	60	800	1100	1.9	−53		
PHOU	0		85 800	2.0	−39	40	6
	7	20 100	35 600	1.9	−39		
	10	17 700	23 900	1.9	−39		
	15	9600	16 500	1.9	−39		
	20	7700	9300	2.0	−39		
	25	4800	6000	2.2	−42		

[a] Determined by ^{1}H NMR. [b] Determined by SEC (THF, polystyrene standards).

20 000 to 800 g mol^{-1}. The degradation of PHO was faster by this method than by the others described before (Figure 1). The rate of oligomer formation is nearly the same for PHO and PHOU.

The chemical structure of PHO and PHOU oligomers prepared by methanolysis was confirmed by ^{1}H and ^{13}C NMR. The ^{13}C NMR spectrum (Figure 4) of oligomers of PHO obtained after 20 min of methanolysis gives information about the oligomers end groups. The signal corresponding to the CH−OH end group at 68.4 ppm, noted 3′, and the OCH_3 signal at 51 ppm, noted 9, not detected in the natural PHO, were here clearly distinguished. Different peaks were observed between 169.7 and 173 ppm. The signal of the ester group of the natural PHO backbone is located at 169.7 ppm (noted β). To determine the nature of the novel peaks at 169.9 (χ), 170.9 (ϵ), and 172.6 (α) ppm, the ^{13}C NMR of the carbonyl region of the products obtained after different treatments was analyzed and compared (Figure 5). The presence of the ester end group at 170.9 ppm (ϵ) was confirmed by the reaction of esterification by trimethylsilyldiazomethane. We have modified the carboxylic end group of PHO oligomer produced by acid hydrolysis (Figure 5c) by esterification with trimethylsilyldiazomethane (Figure 5d). The peak at 170.4 ppm (δ) attributed to free carboxylic group disappeared and a peak centered at 170.9 ppm (ϵ) because of the methyl ester end group appeared. The signal at 172.6 ppm (α) is attributed to the ester group of the repeating unit located near the hydroxyl end group. Complete assignments of the CO resonance in PHO oligomers were realized and are shown in Figure 5.

Figure 6 shows the ^{1}H NMR spectrum of the PHOU oligomers with an M_n of 9600 g mol^{-1}. The CH−OH end group, noted 3′, has a resonance at 3.9 ppm, while the CH of the

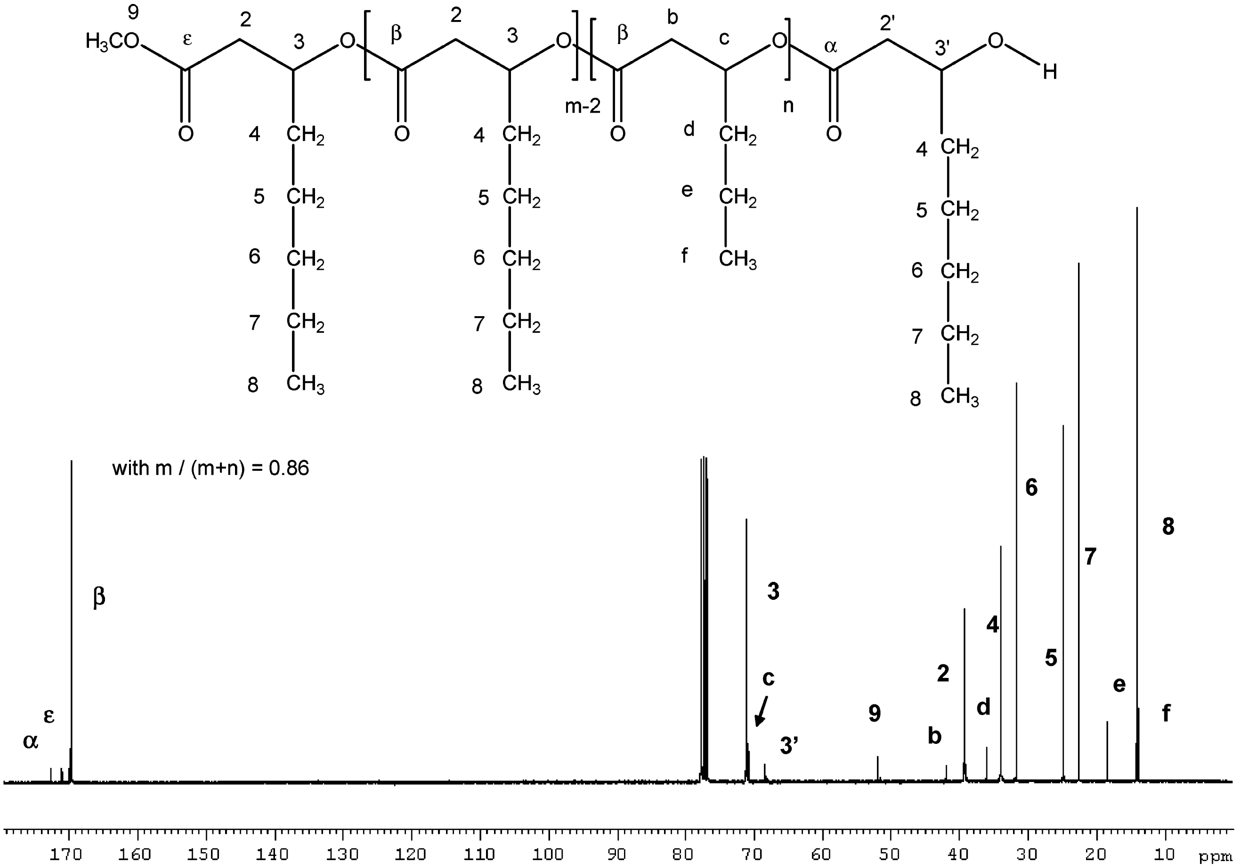


Figure 4. ^{13}C NMR ($CDCl_3$) of PHO oligomers (M_n = 5300 g mol^{-1}) obtained after 20 min of methanolysis.

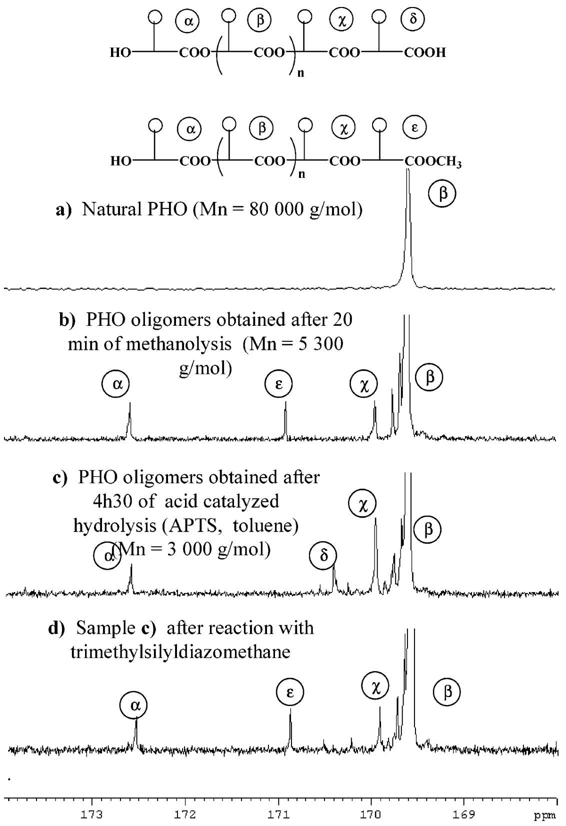


Figure 5. Comparison of the CO region of the ^{13}C NMR spectra of PHO oligomers in $CDCl_3$.

backbone, noted 3, has a resonance at 5.1 ppm. On the basis of the integrations of signals 3 and 3′, the number-average molecular weights can be calculated and compared with the M_n values determined by SEC (Table 1). The results are in good agreement. The peak at 3.6 ppm was assigned to methyl ester, noted 9, formed during methanolysis. By comparison of the integration at 3.6 and 3.9 ppm, we can conclude that the yield of methanolysis is quantitative. The presence of proton signals at 4.9 and 5.7 ppm, characteristics of the unsaturations in the side chains, clearly proved that the unsaturations were not modified during methanolysis. On the basis of the integrations, the percentage of PHOU oligomers unsaturations, determined using eq 1, is equal to 24% like in natural PHOU (25%).

$$\%C = C = \frac{I_\gamma}{I_3} \times 100 = \frac{I_\eta}{2I_3} \times 100 \qquad (1)$$

To confirm the well-defined structure of oligomers obtained by methanolysis, MALDI-TOF analyses were conducted. Figure 7 shows the MALDI-TOF mass spectrum of an SEC fraction (in THF) of PHO oligomers obtained after 20 min of methanolysis. In this spectrum, only one ion distribution centered at *m*/*z* 1800 was observed. The mass assignments and the results confirm the presence of only one structure which is attributed to linear PHO oligomers. PHO oligomers have been analyzed by DSC (Table 1). The value of T_g decreases with the length of PHO oligomers from −40 °C to −53 °C. These PHO oligomers become amorphous when their molar masses are inferior to 8600 g mol^{-1}. Natural PHOU is a semicrystalline polyester with a very low melting enthalpy (6 J g^{-1}). Consequently, the PHOU oligomers prepared by methanolysis are amorphous. The objective was to improve the thermal properties of these interesting PHOU oligomers, which can be easily functionalized by copolymerization with poly(ϵ-caprolactone).

Preparation of Diblock Copolymers P(HO-*b*-CL) and P(HOU-*b*-CL). The rapid one-step synthesis of low molecular weights PHO and PHOU containing a hydroxyl end group at

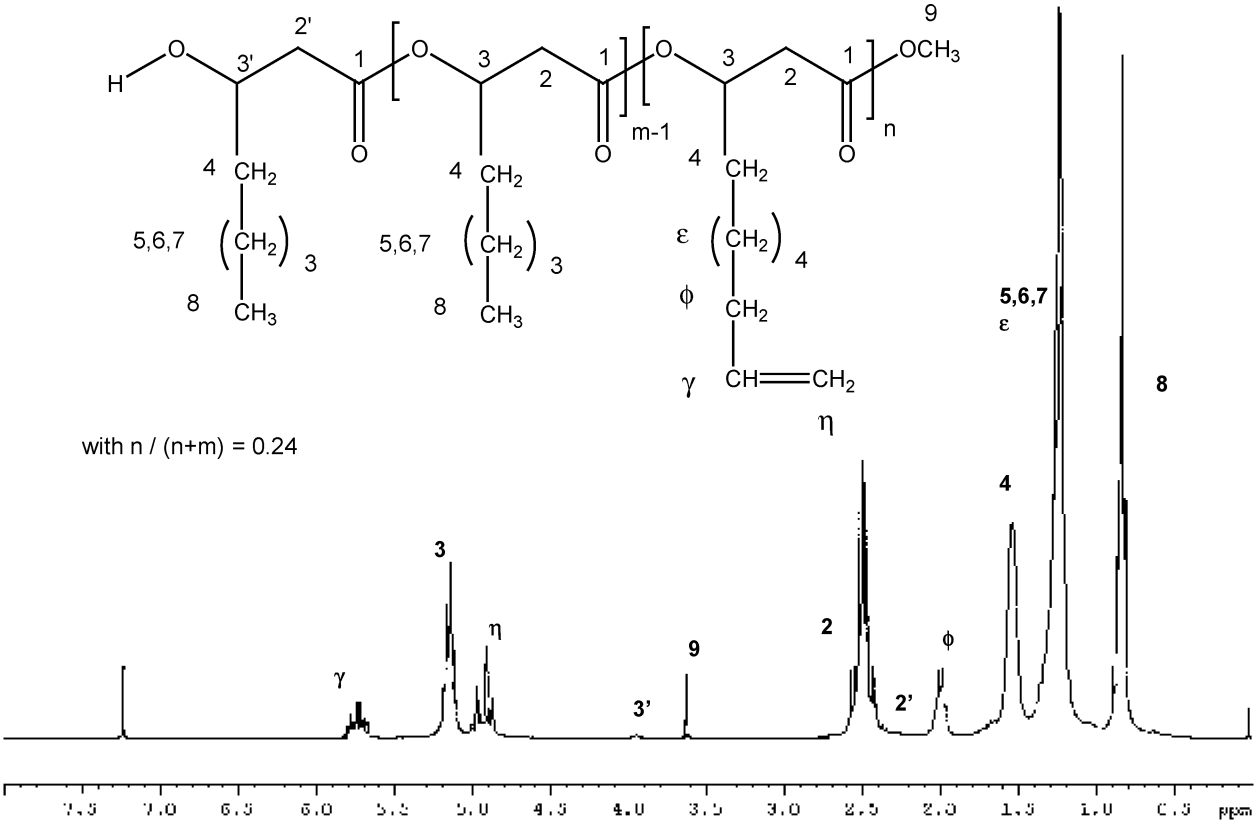


Figure 6. ^{1}H NMR ($CDCl_3$) of PHOU oligomers (M_n = 9600 g mol^{-1}) obtained after 15 min of methanolysis.

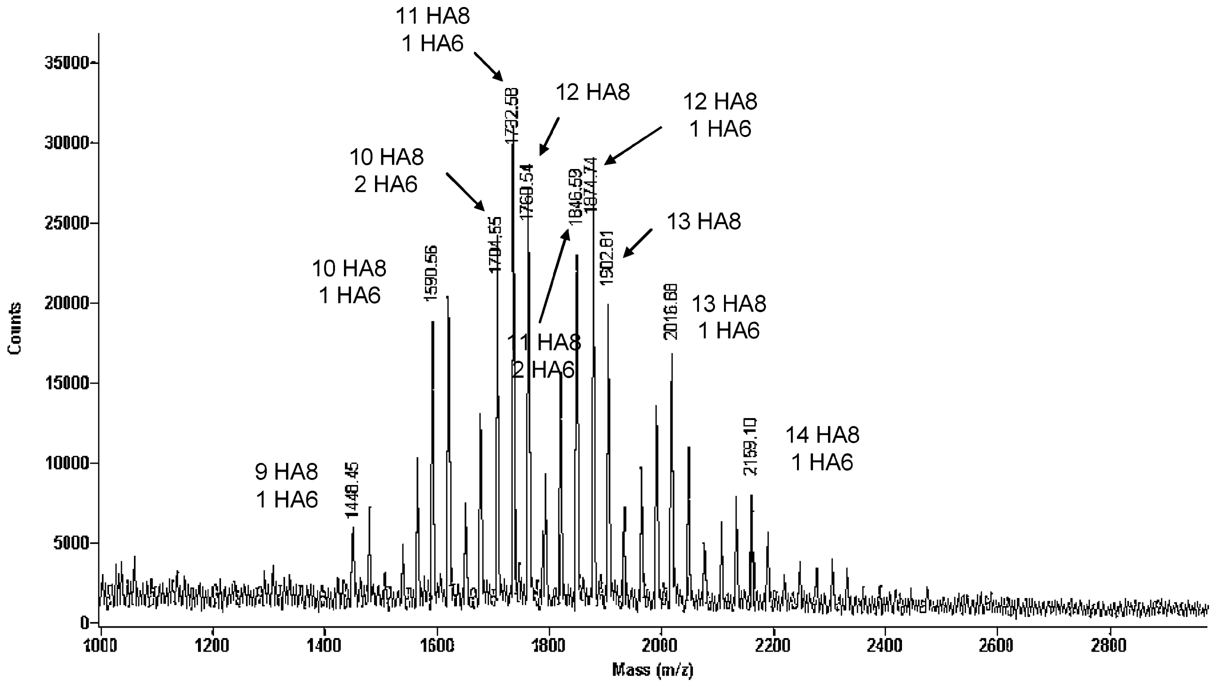


Figure 7. MALDI-TOF spectrum of a fraction of PHO oligomers (M_n = 2000 g mol^{-1}, Ip = 1.02) obtained by methanolysis.

Table 2. Characteristics of P(HO-*b*-CL) Copolymers with PHO Blocks of Different Lengths

copolymer	$M_{n\ PHO}$[a] (g mol^{-1})	$M_{n\ exp\ PCL}$[a] (g mol^{-1})	$M_{n\ cop}$[b] (g mol^{-1})	Ip	T_g (°C)	T_m (°C)	ΔH_m (J g^{-1})	theo$_{HO}$ (mol %)	exp$_{HO}$[a] (mol %)
1	800	16 100	15 000	1.8	−58	63	89	11	4
2	2500	13 500	18 200	1.6	−57	63	81	29	14
3	3800	11 700	18 400	1.5	−60	66	80	38	19
4	5300	18 400	16 700	1.5	−57	63	71	46	24
5	8000	18 100	14 400	1.6	−59	62	54	56	30
6	20 000	18 500	19 500	1.8	−63 −41	62	57	76	37

[a] Determined by ^{1}H NMR. [b] Determined by SEC (THF, polystyrene standards), theo: theoretical, exp: experimental, cop: copolymer.

Table 3. Characteristics of P(HO-*b*-CL) Copolymers with PCL Blocks of Different Lengths

copolymer	$M_{n\ PHO}$[a] (g mol^{-1})	$M_{n\ theo\ PCL}$ (g mol^{-1})	$M_{n\ exp\ PCL}$[a] (g mol^{-1})	$M_{n\ cop}$[b] (g mol^{-1})	Ip	T_g (°C)	T_m (°C)	ΔH_m (J g^{-1})	theo$_{HO}$ (mol %)	exp$_{HO}$[a] (mol %)
7	5700	5000	12 300	17 600	1.5	−58	66	74	48	28
8	5700	15 000	20 900	18 000	1.3	−60	64	88	23	10
9	5700	25 000	26 400	30 900	1.4	−61	61.5	78	15.5	8
10	12 000	15 000	25 700	13 700	1.4	−61	67	80	39	7.5
11	12 000	25 000	47 700	21 500	1.4	−61	66	82.5	28	7

[a] Determined by ^{1}H NMR. [b] Determined by SEC (THF, polystyrene standards), theo: theoretical, exp: experimental, cop: copolymer.

one side and an ester end group on the other side was undertaken with success using methanol and sulfuric acid. The hydroxyl end group of the oligomers was used to initiate the ring-opening polymerization of ϵ-caprolactone in the presence of triethylaluminium as catalyst.[27] These reactions must be done in anhydrous conditions to avoid the initiation of the polymerization by water. PHAs are known to be thermally instable polyesters,[21] and consequently, the use of trialkylaluminium (Et_3-Al) as catalyst was appropriate because the polymerization of ϵ-CL initiated by aluminum alkoxide macroinitiator (Et_2AlO-PHO) was realized at room temperature. The resulting diethylaluminium alkoxide functions initiate the polymerization of ϵ-CL. We have modified PHO or PHOU block lengths by methanolysis (800−20 000 g mol^{-1}) and then PCL block lengths (from 5000 to 50 000 g mol^{-1}) by varying the proportion of monomer/initiator (Tables 2−4). The ring-opening polymerization of ϵ-caprolactone was carried out for 24 h in toluene. The copolymers were precipitated twice in heptane to remove the unreacted PHO (or PHOU) oligomers and residual ϵ-caprolactone. The conversion of ϵ-caprolactone is quantitative.

Figure 8 illustrates the ^{1}H NMR spectrum of the copolymer P(HOU-*b*-CL) obtained from PHOU oligomers with a molar mass of 13 500 g mol^{-1}. The presence of the double bonds is confirmed in the ^{1}H NMR spectrum, with a similar amount as the one determined in PHOU oligomers. The reaction of copolymerization kept intact these unsaturations. Typical signals of both PHOU and PCL units were detected. On the basis of the integrations of signals obtained by ^{1}H NMR, the molar masses of each block were calculated. On the basis of the integrations of signals CH_2O of the PCL backbone, noted f (4.0

Table 4. Characteristics of P(HOU-*b*-CL) Copolymers

copolymer	$M_{n\ PHOU}$[a] (g mol^{-1})	$M_{n\ theo\ PCL}$ (g mol^{-1})	$M_{n\ exp\ PCL}$[a] (g mol^{-1})	$M_{n\ cop}$[b] (g mol^{-1})	Ip	yield (%)	T_g (°C)	T_m (°C)	ΔH_m (J g^{-1})	theo$_{HOU}$ (mol %)	exp$_{HOU}$[a] (mol %)
12	4800	5000	12 000	19 800	1.4	92	−61 −45	63	64	42	23
13	7200	5000	12 600	24 200	1.4	78	−60 −41	64	65	52	30
14	9600	5000	14 900	16 200	1.7	69	−59 −43	70	73,5	59	32
15	4800	25 000	31 300	29 600	1.6	95	−60	63	98.5	12.5	7.5
16	7700	25 000	47 200	24 000	1.4	84	−61	67	86.5	19	4
17	13 500	25 000	55 800	54 300	2.5	70	−58	67	63.5	29	12
18	20 500	25 000	35 300	38 500	1.4	56.5	−61	64	77	38	11

[a] Determined by ^{1}H NMR. [b] Determined by SEC (THF, polystyrene standards), theo: theoretical, exp: experimental, cop: copolymer.

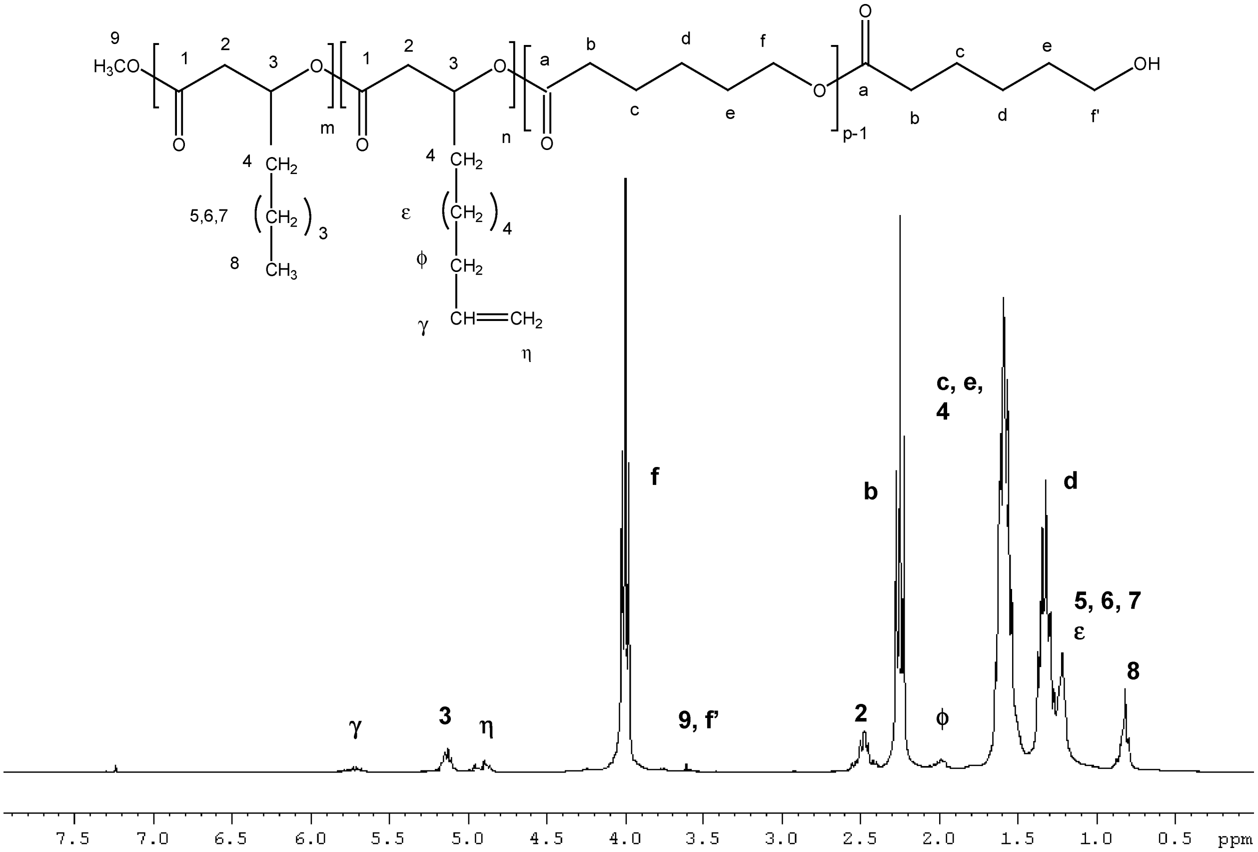


Figure 8. ^{1}H NMR spectrum of the copolymer P(HOU-*b*-CL), 17, with $M_{n,blockPCL}$ = 55 800 g mol^{-1}, $M_{n,blockPHOU}$ = 13 500 g mol^{-1}.

ppm), and the end group CH_2OH signal, noted f′ (3.6 ppm), the molar mass of the PCL block can be calculated using eq 2,

$$M_{n,PCL} = \left(\frac{I_f}{2}\right) \times \left(\frac{5}{I_{f,9}}\right) \times MW_{CL} \qquad (2)$$

where MW_{CL} represents the molecular weight of ϵ-caprolactone. In the same way, the molecular weight of the PHOU block can be determined using the integration of the signals CHO of the backbone of PHOU, at 5.1 ppm, noted 3, and the signal of the methyl ester end group of PHOU at 3.6 ppm, noted 9. The signal at 3.6 ppm is also attributed to the end group CH_2O of PCL, noted f′. The molar mass of the PHOU block can be calculated using eq 3,

$$M_{n,PHOU} = I_3 \times \frac{5}{I_{f,9}} \times MW_{HOU} \qquad (3)$$

where MW_{HOU} represents the molar mass of the repetitive unit of PHOU. The molar percentage of HOU units in the copolymers was determined using the integration of the signal CHO of the backbone of PHOU, at 5.1 ppm noted 3, and of the signal CH_2O of the backbone of PCL, at 4.0 ppm noted f, using eq 4.

$$\%\ HOU_{theoretical} = \frac{DP_{n\ PHOU\ oligomer}}{(DP_{n\ PHOU\ oligomer} + DP_{n\ PCL\ theoretical})} \times 100 \qquad (4)$$

These experimental values were compared to the theoretical values, noted theoretical in Table 4. We noticed that the experimental data were smaller than the expected data and the difference increased with the molar mass of PHOU block. We supposed that the reactivity of hydroxyl end group of PHOU oligomer decreased with the molar mass and that it became difficult to avoid the polymerization of PCL.

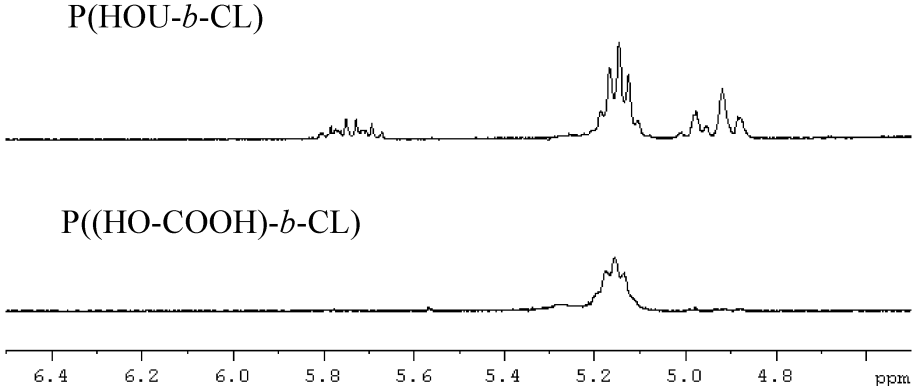


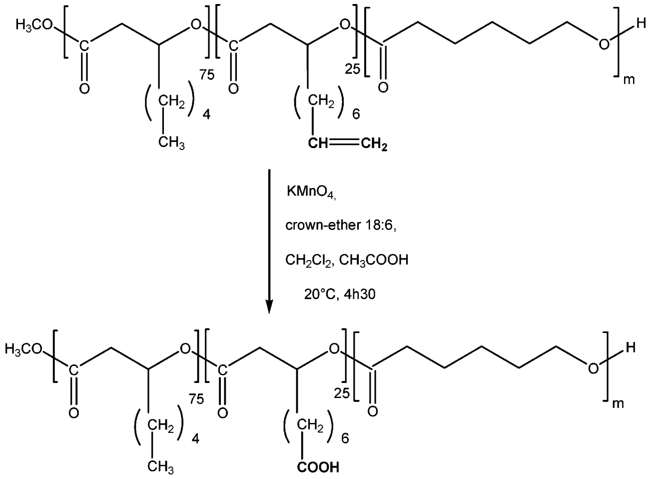
Figure 9. ^{1}H NMR spectrum of the copolymer P((HO−COOH)-*b*-CL), 27, with $M_{n,blockPCL}$ = 35 300 g mol^{-1}, $M_{n,blockP(HO-COOH)}$ = 20 500 g mol^{-1}.

Table 5. Characteristics of P((HO−COOH)-*b*-CL) Copolymers

copolymer	$M_{n\ P(HO-COOH)}$[a] (g mol^{-1})	$M_{n\ exp\ PCL}$[a] (g mol^{-1})	$M_{n\ cop}$[b] (g mol^{-1})	Ip	T_g (°C)	T_m (°C)	ΔH_m (J g^{-1})
24	4800	26 800	20 400	1.5	−58	61.5	70
25	7700	47 200	27 000	1.4	−60	60	65
26	9600	14 900	15 500	1.4	−57	60	62.5
27	20 500	35 300	23 900	1.6	−57	61.5	80.5

[a] Determined by ^{1}H NMR. [b] Determined by SEC (THF, polystyrene standards), exp: experimental, cop: copolymer.

Scheme 4. Chemical Modifications of Diblock Copolymer P(HOU-*b*-CL)



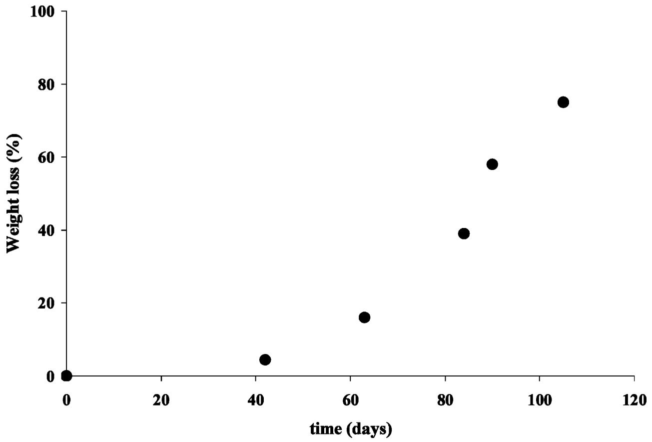


Figure 10. Weight loss of P(HO−COOH) film during the hydrolytic degradation at pH = 7.3 and at 37 °C.

The molar mass of the block of PCL determined by ^{1}H NMR (16 000 g mol^{-1}) is superior to the one expected (5000 g mol^{-1}) for copolymers 1−6. This result showed that the activation of the hydroxyl end group is different than the one expected in the used conditions. The titration of the catalyst is done for all Al^{3+} species, the one belonging to Et_3Al and the one from $Al(OH)_3$ resulting from the reaction between Et_3Al and traces of water or impurities, so the amount of active species is estimated. The molar percentage of the experimental units of PHO increases (4−37%) with the PHO block length (800−20 000 g mol^{-1}). The molecular weight distribution of diblock copolymers is monomodal and shifted to lower retention volume compared to the PHO macroinitiator confirming the synthesis of diblock copolymers. All the P(HO-*b*-CL) copolymers 1−6 are semicrystalline and present a very interesting aspect as white powder compared to the aspect of natural PHO. The thermal characteristics of the diblock copolymers were investigated using DSC. It indicates that the presence of PHO short block did not disturb the crystallinity of the PCL. The melting point (65 °C) was constant when the PHO molecular weight was below 5300 g mol^{-1}. Increasing PHO length resulted in the decrease of the crystallinity of PCL block. This is due to a hindrance of PCL crystallization by the presence of long PHO block, and the melting enthalpy decreases from 89 to 54 J g^{-1} when the molecular weight of PHO block increases from 800 to 20 000 g mol^{-1}. The PHO glass-transition temperature is difficult to detect in copolymers because of the low amount of PHO in copolymers except for the one containing a PHO block of 20 000 g mol^{-1}. The presence of two glass-transition temperatures at −63 °C and −41 °C corresponding to PCL and PHO, respectively, shows that these two polymers are not compatible.

We have demonstrated the possibility of synthesizing diblock copolymers with blocks of PHO of different lengths. In a second step, the length of the PHO block was kept constant (5700 or 12 000 g mol^{-1}), and the length of the PCL block was changed from 5000 to 47 700 g mol^{-1} (copolymers 7−11, Table 3). The reaction time was increased from 24 to 48 h to obtain total conversion. The experimental molar percentage of PHO units is inferior to the one expected, which can be explained by the presence of PCL homopolymers. The amount of PCL homopolymers is more important when the synthesis of the

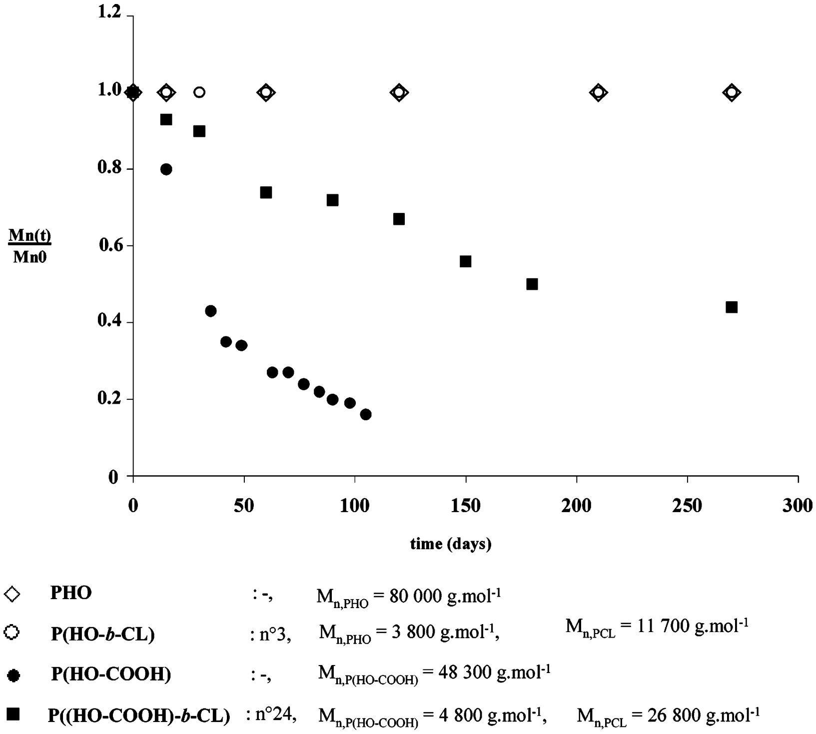


Figure 11. Study of the hydrolytic degradation of different copolymers at pH = 7.3 and at 37 °C.

copolymer requires a longer block of PCL. The molecular masses of the copolymers determined by SEC are in good agreement with the one obtained by ^{1}H NMR ($M_{\mathrm{n\,theo}} = M_{\mathrm{n\,PHO}} + M_{\mathrm{n\,PCL}}$) when copolymers have been obtained from PHO oligomers of 5700 g mol^{-1} (copolymers 7 and 9). When copolymers are obtained from oligomers of PHO of 12 000 g mol^{-1}, the obtained results are different because of the difference of the hydrodynamic volumes of the two blocks. All the copolymers synthesized have a polydispersity index in the range of 1.3 and are semicrystalline. Because of the longer length of the PCL block and the small percentage of PHO units in copolymers, the only T_g detected is attributed to the PCL block.

Similar reactions were carried out with PHOU oligomers. As with PHO oligomers, length of both blocks has been modulated. The length of the PCL block ranged from 5000 to 25 000 g mol^{-1} and the length of the PHOU block ranged from 4800 g mol^{-1} to 20 500 g mol^{-1}. The characteristics of the P(HOU-*b*-CL) diblock copolymers are presented in Table 4. All copolymers synthesized are semicrystalline. As expected, the presence of the PCL block has improved the properties of the amorphous PHOU oligomers. Moreover, the copolymerization process keeps intact the double bonds of the PHOU block. These double bonds will be further used for chemical modifications.

Synthesis of P((HO−COOH)-*b*-CL) by Oxidation of Diblock Copolymers P(HOU-*b*-CL). The presence of unsaturated units in the side chains of the PHOU block was used for chemical modifications to produce functionalizable diblock copolymers containing acid carboxylic functions (Scheme 4). Previous studies[22] have demonstrated the feasibility of chemical modifications of the unsaturations in the side chains of PHOU into acid carboxylic functions in the presence of potassium permanganate. Potassium permanganate was employed as oxidant agent and crown-ether as dissociation agent of potassium permanganate. The reaction was carried out in biphasic medium, in acidic conditions, to avoid further oxidations.[23] The concentration used is very high (20 g L^{-1}) to avoid formation of diols. The purification of the product was done by dialysis. The product, named P((HO−COOH)-*b*-CL), was recovered by lyophilization. Figure 9 represents ^{1}H NMR spectrum between 4.7 and 6.5 ppm of the copolymer P((HO−COOH)-*b*-CL), noted 27. This spectrum shows the disappearance of signals at 4.9 and 5.7 ppm, characteristics of unsaturations of the side chains of PHOU. The conversion of double bonds into acid carboxylic functions is total. Characteristics of oxidated copolymers are described in Table 5. The molecular weights of copolymers analyzed by SEC are the same before and after oxidation. Consequently, no degradation occurs during the oxidation process. Moreover, all copolymers are semicrystalline contrary to previous P(HO−COOH) which was completely amorphous.[22]

Hydrolytic Degradation. PHO films are known to be very hydrophobic and to not degrade at pH = 10 at 37 °C. Blends with PEG and PLA did not facilitate water penetration, and consequently, hydrolytic degradation rate of blends was very long whereas the oxidized PHO, noted P(HO−COOH), was actually the only mcl PHA easily hydrolyzable (few hours at pH = 10).[24] We have concluded that the presence of carboxylate groups increased the hydrolytic degradation. In this study, we compare the hydrolytic comportment of novel diblock copolyesters P(HO-*b*-CL) and P((HO−COOH)-*b*-CL) with natural PHO and P(HO−COOH) at pH 7.3 and 37 °C to study the effect of the presence of carboxylate groups. We presented first the hydrolytic degradation of the P(HO−COOH) in Figure 10. Results concerning degradation of the diblock copolymers are presented in Figure 11. The weight loss of P(HO−COOH) film is about 4% after 42 days of hydrolysis and reached 75% after 105 days. The molar masses of the supernatant were nearly the same during this period (10 000 and 7000 g/mol). As expected, the copolymer P(HO-*b*-CL) (copolymer 3, Table 2) was not degraded because the two blocks were not hydrolyzable in our conditions. On the other hand, the copolymer P((HO−COOH)-*b*-CL) (copolymer 24, Table 5) and the P(HO−COOH) were degraded because of the presence of carboxylate groups which catalyzed the hydrolytic degradation. The presence of PCL block limited the hydrolysis rate while the molecular weights decreased after 15 days more rapidly for the P(HO−COOH) than for the P((HO−COOH)-*b*-CL). When the chain scission is completely random, a linear relationship between the number of chain scissions and the time is predicted as described in eq 5,[28]

$$N_{(t)} = k_d P_{n(0)} t \quad \text{with } N_{(t)} = \frac{M_{n(0)}}{M_{n(t)}} - 1 \qquad (5)$$

where k_d is the rate constant of hydrolytic degradation, $P_{n(0)}$ is the number-average degree of polymerization at time 0, $M_{n(0)}$ is the molecular weight at time 0, and $M_{n(t)}$ is the molecular weight at time t. In our study, linear relationships were obtained for P(HO−COOH) and P((HO−COOH)-*b*-CL) (results not shown), demonstrating the random process of chain scission for polymers containing carboxylic functions in their side chains. The rate constant of hydrolytic degradation of P(HO−COOH) is calculated to be 13.8 10^{-5} day^{-1} whereas for the diblock copolymer P(HO−COOH-*b*-CL), the k_d value is smaller (=1.84.10^{-5} day^{-1}).

Conclusions

Methanolysis is a process which can be applied to different types of PHAs to prepare well-linear defined monohydroxylated oligomers with a protected carboxylic acid function as ester on the other side. The rapid one-step synthesis of PHO and PHOU linear oligomers was characterized. The hydroxylic end group of the oligomers was used to initiate the ring-opening polymerization of ϵ-caprolactone in the presence of triethylaluminium as catalyst. The results have demonstrated the possibility of the synthesis of diblock copolymers for which it is possible to modify the length of each block. The PHA length block is controlled by methanolysis step depending on the reaction time used, whereas the length of the PCL block is modified by the ratio monomer to initiator used in the reaction. All diblock copolymers synthesized are semicrystalline, so the thermal properties of PHAs have been improved by copolymerization with ϵ-caprolactone. The presence of unsaturated units in P(HOU-*b*-CL) allowed us to synthesize novel block copolymers which could be further functionalized. Presence of the pendent carboxylic groups on P(HO−COOH)-*b*-CL) leads to functional copolymers and could facilitate modifications, such as linking drug molecules, short peptides, and oligosaccharides. Moreover, they enhanced the degradability of the polymers. These diblock copolyesters could be easily used to form novel degradable and functionalizable nanoparticles because of the presence of carboxylic groups, and they present high potential as drug delivery systems.

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