Biomacromolecules **2007,** *8,* 579−584

Autoxidation of Medium Chain Length Polyhydroxyalkanoate

Manfred Schmid,* Axel Ritter, Andreas Grubelnik, and Manfred Zinn

*Empa, Swiss Federal Laboratories for Materials Testing and Research, Laboratory for Biomaterials,
Lerchenfeldstrasse 5, CH-9014 St. Gallen, Switzerland*

Received August 10, 2006; Revised Manuscript Received November 2, 2006

Polyhydroxyalkanoates (PHAs) are a class of biopolymers that are currently the subject of intensive research for various applications (packaging, consumer products, medical applications, etc.). It is known from synthetic polymers that all plastic materials show more or less pronounced autoxidation (aging induced by UV radiation, temperature, heavy metal ions, etc.). There is less knowledge as yet regarding the autoxidation behavior of biopolymers. The autoxidative behavior of medium chain length poly[(*R*)-3-hydroxyalkanoate] (mcl-PHA) was therefore investigated. mcl-PHA (co)polymers with amounts of 0, 10, 50, and 75 mol % of olefinic side chains with terminal double bonds were tempered at 60 °C in air for 3 months. After 1, 2, 4, 8, and 12 weeks, samples were removed and analyzed for changes in chemical and physical properties by sol−gel analysis (Soxhlet extraction), size exclusion chromatography (SEC), infrared analysis (IR), and gas chromatography/flame ionization detection (GC/FID). It became apparent that the content of double bonds greatly influences the autoxidation of mcl-PHA. A low amount of unsaturated moiety (0 and 10 mol %) resulted in chain scission, whereas samples with 50 and 75 mol % olefinic side chains showed cross-linking and became insoluble after a few weeks. Kinetic data of oxidation behavior were investigated by performing isothermal DSC experiments at elevated temperatures. The kinetic data combined with the experiment enabled the gelation time to be predicted and the shelf-life of mcl-PHA to be estimated. Because of the detected sensitivity of mcl-PHA regarding autoxidation, it is recommended that these biopolymers should be stored cold (at least −5 °C) and in an inert gas atmosphere or stabilized by suitable additives (antioxidants).

Introduction

Polyhydroxyalkanoate (PHA) is a class of biopolymers accumulated by many different microorganisms as a carbon/energy storage compound.[1−4] Because of its inherent biodegradability and biocompatibility, PHA is regarded as a promising bio-based polymer for use in tackling the problem of plastic waste in the future[5] and also as a suitable polymer for high-tech applications (e.g., medical applications in tissue engineering and drug delivery systems).[6−9] Regarding the length of their carbon chains, PHAs are divided into three groups: short (scl), medium (mcl), and long chain length (lcl) PHA (scl: 4−5 carbon atoms; mcl: 6−12 carbon atoms; and lcl: more than 12 carbon atoms).[10] Besides scl-PHAs (poly[(*R*)-3-hydroxybutyrate] (PHB) and its copolymers with (*R*)-3-hydroxyvalerate (3-HV) and (*R*)-3-hydroxyhexanoate (3-HHx) units), which have already been widely studied, there has recently been increased interest in mcl-PHA. The possibility of introducing olefinic side chains in mcl-PHAs in a terminal position[11] and in a well-defined amount during biosynthesis[12] is of particularly great interest. Covalent linkage of functional groups onto the double bonds generates biocompatible materials with different and finely adjustable properties. For example, the formation of hydroxyl functions,[13] the bonding of natural antifouling agents,[14] the synthesis of comb polymers,[15] and the synthesis of organic/inorganic hybrid polymers[16] have recently been accomplished and reported. The formation of PHA duromers with[17] or without[18−21] prior epoxidation of the olefin function has also already been described with lateral unsaturated mcl-PHA.

Organic polymers are known to be unstable in the presence of oxygen, heat, and UV radiation. This phenomenon is known as autoxidation or aging of plastics.[22] Figure 1 shows a general scheme (cycle) of the process of polymer autoxidation. The polymer (R−H) decays under the influence of temperature (ΔT) and/or UV-light ($h\nu$) into free radicals (R*). In the presence of oxygen, these radicals are transformed to highly active peroxy species (ROO*) that support the accelerated deterioration by generating further radicals. Every cycle of this process produces at least two new polymer radicals (R*), so that a chain reaction takes place.

Autoxidation could be simulated and investigated in laboratory aging tests in drying ovens, climate chambers, by using QUV test equipment, or even by combinations of these.[23] Polymers with double bonds in the main or side chains in particular are heavily affected by oxygen, heat, and/or UV irradiation due to the reactive olefin function.[24] The long-term stability and shelf-life of polymers is thus of great relevance and is usually tested in accelerated autoxidation tests under aggravated conditions (higher temperatures, intensified UV irradiation, or pure oxygen instead of air). Chain scission and/or unwanted and uncontrolled cross-linking may result from autoxidation reactions. In the case of cross-linking, one consequence will be that fractions of the aged samples become insoluble and tend to form gels. Chemical gelation occurs when covalent bonds connect across the whole volume of a curing material for the first time and a macroscopic network is formed. Thus, gelation is related to network formation and occurs in free radical chain growth cross-linking systems.[25] The first time polymers show gel particles is known as the gel point. At this point, the maximum shelf-life of a polymer is achieved as it is no longer soluble and reasonably processable beyond it. Thus, the knowledge of the gel point of a polymeric system is of great relevance as properties change dramatically and spiral downward.[26]

---

* Corresponding author. E-mail: manfred.schmid@empa.ch.

10.1021/bm060785m CCC: $37.00

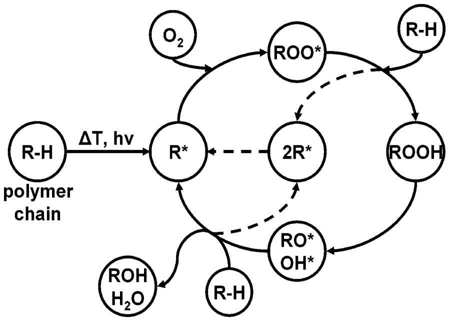

Figure 1. Schematic autoxidation process of polymers.

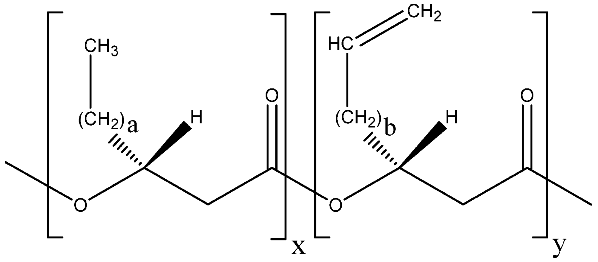

Figure 2. Structure of PHOU copolymers (y = amount of unsaturated part).

We report the accelerated autoxidation behavior of partially unsaturated mcl-PHA stored at 60 °C in air for 3 months. Periodically taken samples enable the oxidation (aging) process to be conducted in detail and the material conversion in relation to physical and chemical properties to be documented. Furthermore, DSC investigations regarding the kinetics of cross-linking will be described with one selected PHA type. By combining the results from laboratory investigations with the kinetic data calculations of critical gel formation time and shelf-life, estimations of different mcl-PHAs in different conditions are possible, and some significant examples will be presented.

Materials and Methods

Biosynthesis. Poly[(*R*)-3-hydroxyalkanoate-co-(*R*)-3-hydoxyalkenoate] samples used in this study were produced by fermentation of *Pseudomonas putida* GPol (ATTC 29347). The composition of the polymers was adjusted by feeding the cells with different mixtures of octanoic and undecenoic acid under dual (C,N) limited growth conditions in a chemostat.[27] As well as pure octanoic acid, mixtures with an amount of 10, 50, and 75 mol % undecenoic acid were fed. It is well-known that the composition of feed correlates linearly with the amount of olefin in the polymer.[20] Biosynthesis by continuous culture fermentation at 30 °C, purification of the product by reprecipitation from a methylene chloride/methanol system, and analysis of the copolymer composition by NMR and GC/MS are described elsewhere.[12] The common structure of the resulting isotactic copolymer is shown in Figure 2.

As well as the main monomer units (a, $b = 4$), the PHOU polymer can also exhibit small amounts of other side chain length units ($a = 2$, $b = 6$ and 2) due to beta-oxidation of monomers during biosynthesis (shortening the monomers in steps of C_2H_4 units due to cell metabolism). The polymers used for the tests are designated by the mol fraction of unsaturated moieties (y in Figure 2). For example, poly[(*R*)-3-hydroxyoctanoate-co-(*R*)-3-hydoxyundecenoate] with about 10% unsaturated side chains ($y \approx 0.1$ in Figure 2) is designated as PHOU(10). The materials subjected to the test were PHOU(0), PHOU(10), PHOU(50), and PHOU(75). The exact compositions of the materials used, regarding the amount of different side chain lengths, determined by GC and NMR analysis,[12] is summarized in Table 1. Additionally, the medium molar mass of the repeating units (M_g) and the concentration of unsaturated units (c_u) necessary for kinetic calculations thereinafter are given in Table 1 along with the thermal properties. The thermal properties indicate that the material, due to the low crystallinity (small ΔH_m values) and low glass transition temperature (far below room temperature), is sticky and not easy to handle in ambient conditions.

Autoxidation and Analysis. Several specimens of each polymer (PHOU(0), PHOU(10), PHOU(50), and PHOU(75)) were prepared by solvent casting from chloroform in Teflon dishes. The thickness of the resulting films was 500 ± 50 μm. All specimens were placed in a hot air cabinet at 60 °C in a normal atmosphere (air). After 1, 2, 4, 8, and 12 weeks, two samples of each polymer were removed from the heating chamber and stored in a freezer (−20 °C), until all samples were available. They were subsequently analyzed by repeat determinations with the following: sol−gel analysis (Soxhlet extraction), size exclusion chromatography (SEC), infrared analysis (IR), gas chromatography/flame ionization detection (GC/FID), and differential scanning calorimetry (DSC).

Sol−Gel Analysis. The sol−gel analysis[28] was conducted to determine if cross-linking had occurred and to what extent. Around 1 g of every sample was placed in a filter cartridge and extracted in a Soxhlet apparatus for 24 h with chloroform as the liquid phase. The resulting solution with the soluble part of the material was evaporated, thoroughly dried, and analyzed by determining weight (gravimetric analysis).

Size Exclusion Chromatography (SEC). All molecular weight data were obtained by size exclusion chromatography using a refractive index detector (SEC apparatus: Viscotek, Houston, TX). Around 30 mg of every sample was dissolved in 10 mL of tetrahydrofurane (THF) overnight. Aliquots of 100 μL of the polymer solution were injected and separated on three linear coupled SEC columns (PSS, Mainz, Germany), 10^3, 10^5, and 10^6 Å (300 mm × 8 mm) at 35 °C and a flow rate of 1 mL/min. The system was calibrated by using 10 polystyrene standards with known molecular weights (M_w) (2×10^3 g/mol to 2.13×10^6 g/mol) and low polydispersity ($M_w/M_n \leq 1.09$).

Infrared Analysis (IR). IR analysis was performed on an IR spectrometer (BIO-RAD FTS 175) using a technique with a single reflection mode (Golden Gate Diamond ATR, Graseby Specac Limited, Kent, UK). Samples could be used as they were, without any sample preparation.

Gas Chromatography/Flame Ionization Detection (GC/FID). Monomers obtained from PHOU by propanolysis were analyzed quantitatively in dichloromethane solution on a Hewlett-Packard 5890/II gas chromatograph. The separation was made by a polar fused silica capillary column (Supelcowax 10, 30 m, i.d. 0.25 mm, film thickness 0.5 μm, polyethylene glycol coating). The method of propanolysis was developed by Riis et al.[29] and was optimized at the Empa laboratory for mcl-PHA and complete quantification. About 10 mg of PHOU samples and about 10 mg of 3-hydroxyisovaleric acid (internal standard) were weighed exactly into a 35 mL ace pressure tube. One milliliter of dichloromethane and 1 mL of a mixture of *n*-propanol/hydrochloric acid (37%) (80:20 v/v) were added with a pipette to the ace pressure tube. The tube was capped and heated for 3 h at 100 °C. No side reaction occurred when the reaction temperature was kept near 100 °C. After cooling, 2 mL of water was added, and the solution was shaken vigorously on a laboratory mixer. The solution was transferred to a centrifuge tube. The lower organic layer was drawn through a single-use needle into a 2 mL single-use syringe filled with anhydrous sodium sulfate (up to about 0.3 mL volume) and shaken vigorously by hand. The solution was filtered through a Nylon filter into a 1 mL crimp vial for GC analysis.

Differential Scanning Calorimetry (DSC). Thermal analysis was conducted using a DSC apparatus (Type: DSC822e) from Mettler-Toledo (Schwerzenbach, Switzerland). Isothermal measurements performed between 110 and 180 °C (steps of 10 °C) in an oxygen atmosphere were made for the determination of Arrhenius plots, to calculate kinetic data of the oxidation process of PHOU(50).

Table 1. Molecular Composition and Thermal Properties of PHAs Used for This Study

PHA composition (mol %)	PHOU(0)	PHOU(10)	PHOU(50)	PHOU(75)
∑ 3-hydroxyalkanoates (3-HO)	*100*	*89.8*	*46.7*	*23.3*
3-hydroxyoctanoate	*86.2*	*76.1*	*39.6*	*19.3*
3-hydroxyhexanoate	13.8	13.7	7.1	4.0
∑ 3-hydroxyalkenoates (3-HU)	*0*	*10.2*	*53.3*	*76.7*
3-hydroxy-10-undecenoate		*1.8*	*11.0*	*13.7*
3-hydroxy-8-nonenoate		*8.4*	*36.8*	*52.6*
3-hydroxy-6-heptenoate			5.5	10.4
medium molar mass of polymer repeating unit M_g (g/mol)	138.1	139.9	148.0	151.0
concentration of unsaturated units c_u (mmol/g of polymer)		0.73	3.60	5.08
Thermal properties				
melting point T_m (°C)	58.1	50.8	39.9	
melting enthalpy ΔH_m (J/g)	14.5	10.2	0.2	
glass transition temperature T_g (°C)	−33.1	−35.9	−44.6	−49.3

Table 2. Summary of Sol Fraction and SEC Data of Different PHOU Polymers at Beginning (Starting Material) and After Different Aging Times[a]

	PHO U(0)			PHO U(10)			PHO U(50)			PHO U(75)		
aging time (weeks)	sol^b (%)	M_w^{c} (kD)	PD^d	sol^b (%)	M_w^{c} (kD)	PD^d	sol^b (%)	M_w^{c} (kD)	PD^d	sol^b (%)	M_w^{c} (kD)	PD^d
	cs^e	211.5	1.99	cs^e	164.0	1.98	cs^e	206.5	2.13	cs^e	185.5	2.02
1	cs^e	218.7	2.02	cs^e	157.4	1.82	cs^e	211.9	2.33	95.0	210.3	2.54
2	cs^e	207.4	1.95	cs^e	153.8	1.99	95.0	234.6	2.52	85.8	236.8	2.79
4	cs^e	186.9	2.01	cs^e	149.4	1.96	96.0	401.6	4.79	20.4	nm^g	nm^g
8	cs^e	181.1	2.17	cs^e	131.5	2.03	31.0	144.4^f	2.90^f	11.4	nm^g	nm^g
12	cs^e	167.2	2.12	98.3	119.3	2.04	14.7	nm^g	nm^g	6.5	nm^g	nm^g

[a] All data are mean values from repeat determinations. [b] sol = soluble fraction in Soxhlet extraction. [c] M_w = weight average molecular weight (1 Da = 1 g/mol). [d] PD = polydispersity (PD = M_w/M_n). [e] cs = completely soluble. [f] M_w and PD values of the soluble part. [g] nm = not measurable.

Results and Discussion

Autoxidation. Table 2 summarizes the results of the sol−gel analyses and the SEC data of the samples after oven storage in comparison with the corresponding starting materials. The soluble fraction of a specimen (sol in Table 2) indicated whether a material was cross-linked and if so to what extent. All PHOU(0) and PHOU(10) samples were completely soluble, even after 12 weeks of autoxidation. At the same time, the weight average molecular weight (M_w) showed a clear decrease and the polydispersity (PD) a slight increase for both sample types. The M_w of PHOU(0) dropped from 211.5 to 167.5 kDa, while PD rose from around 2 to 2.12. Almost identical behavior was found for PHOU(10) (M_w decreased from 164.0 to 119.3 kDa; PD increased from 1.98 to 2.04). The entire solubility of both samples over the whole storage period with a synchronous drop in M_w and a slight increase in PD means that autoxidation of PHOU(0) and PHOU(10) samples was determined mainly by chain scission with little or no cross-linking.

Opposite behavior was found for the samples with a greater amount of unsaturated side chains (PHOU(50) and PHOU(75)). Solubility declined rapidly, whereas molecular weight rose sharply. In the case of PHOU(50), after 4 weeks of storage, the specimen was almost completely soluble, but the average molecular weight (M_w) and PD were doubled. After 8 weeks of autoxidation, only around one-third of the PHOU(50) sample was still soluble. This point of insolubility was already achieved between 2 and 4 weeks of storage for PHOU(75). Here, the increase in M_w was not as great as in the former case, but restricted solubility was reached much earlier. The diminished solubility combined with the significant rise in molecular weight revealed that PHOU(50) and PHOU(75) had a distinct tendency toward cross-linking. Figure 3 shows the development of M_w data for all samples, as a relative change (%) in M_w with respect to M_w of the starting material. The completely different behavior of PHOU(0) and PHOU(10) as compared to PHOU(50) and PHOU(75) is evident.

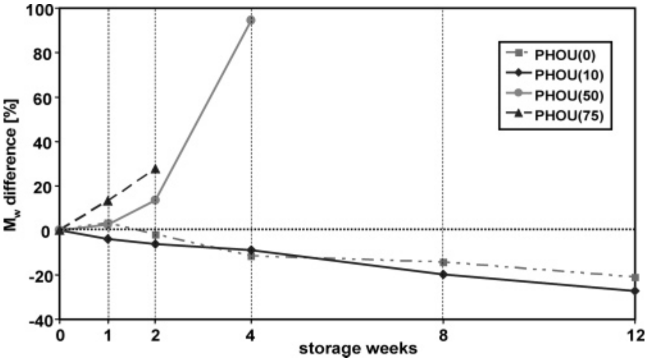

Figure 3. Relative change in molecular weight (M_w difference) of the different PHOU copolymers with respect to aging time (storage weeks).

As an example of the evolution of molecular weights, Figure 4 shows the 4 and 8 week samples of PHOU(50) indicated by the respective SEC curves. The 4 week sample showed a significant additional shoulder in the high molecular region (low retention volume at around 13 mL). This signal indicated the beginning of cross-linking, which was linked to the described increase in M_w. In contrast, the 8 week sample was shifted toward low molecular weights due to the insolubility of the higher cross-linked fraction and the chain scission of the still soluble part.

Infrared (IR) spectroscopy offered a smart and easy tool with which to detect the existence of double bonds in organic materials. In the case of terminal double bonds in polymer side chains, two distinct signals for the $=CH_2$ stretch (around 3100 cm^{-1}) and the C=C stretch vibration (around 1640 cm^{-1}) could

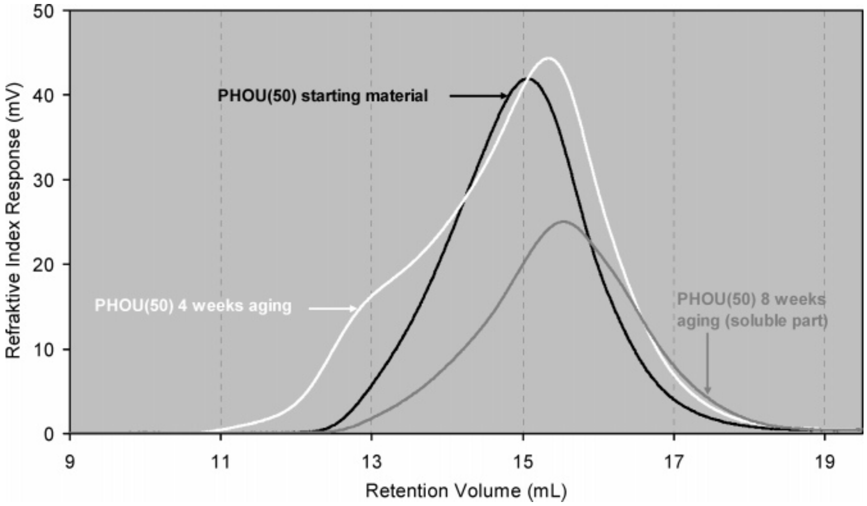

Figure 4. SEC curves of PHOU(50) after different storage times.

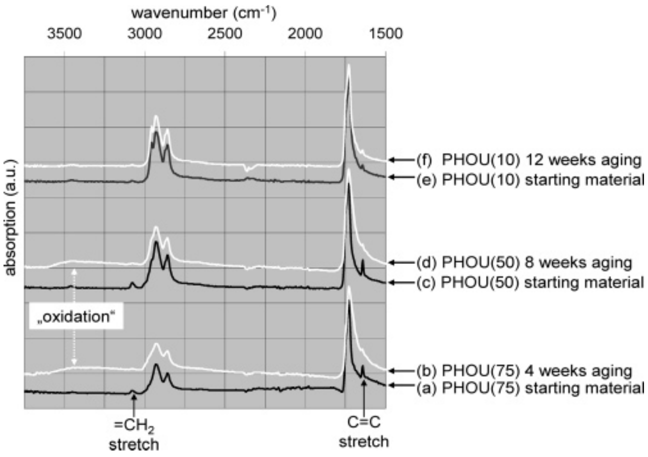

Figure 5. Infrared spectra of different PHOU copolymers.

be observed. The evolution of double bonds during PHOU storage was controlled qualitatively over the whole storage period with these characteristic IR signals. It was found that the IR signals of the double bonds almost vanished at the same time when the samples became partially insoluble. Figure 5 summarizes the IR spectra of PHOU(75), PHOU(50), and PHOU(10) at the beginning of the autoxidation process (spectra a, c, and e in Figure 5) and after different storage times: PHOU(75) after 4 weeks autoxidation (spectrum b); PHOU(50) after 8 weeks autoxidation (spectrum d); and PHOU(10) after 12 weeks autoxidation (spectrum f).

Comparing IR spectrum a with b, respectively, and c with d, it can be seen that the typical double bond IR signals (3100 and 1640 cm^{-1}) are no longer present after 4 weeks (PHOU(75)) and 8 weeks (PHOU(50)) of autoxidation. At the same time, for both aged samples (spectrum b and d), an additional broad signal was detected between 3600 and 3100 cm^{-1}, which is typically found for oxidized polymers (C−OH groups were formed during oxidation; see Figure 1). PHOU(10) did not show similar behavior even after 12 weeks of storage. The whole IR spectra for the starting material e and the 12 week aged material f are nearly congruent. The concentration of double bonds in PHOU(10) was evidently too low to generate the same effects as were found in PHOU(50) and PHOU(75).

In addition, the monomer amounts of PHOU(10) and PHOU(50) were determined quantitatively by GC/FID after propanolysis. It was found that in the case of PHOU(10) after 12 weeks of storage, the total amount of all propylester monomers was almost 100% with respect to the theoretically expected value, whereas in the case of PHOU(50), only around 87% of propylester monomers could be detected. This again indicated, in agreement with the previously described results, cross-linking of PHOU(50) during storage. The missing 13% belongs to cross-linked aggregates not detectable by GC.

Kinetic Measurements. To make some theoretical estimations of gelation time and long-term stability of different PHOU polymers and to compare them with the experimental findings, it is necessary to determine the activation energy (E_A) of the reaction and the rate constants (k_T) of the cross-linking reaction at different temperatures. For this reason, PHOU(50) was the subject of accelerated oxidation by isothermal DSC measurements between 110 and 180 °C in steps of 10 °C (Figure 6 shows the isothermal DSC curves obtained). It can easily be seen from Figure 6 that the rate of reaction increases with temperature, as expected.

From these measurements, it is possible to calculate the rate constants (k_T) by integration of the kinetic equation: $d\alpha/dt = k_T(1 - \alpha)^n$ ($d\alpha/dt$ = reaction rate; α = conversion at a certain time; and n = order of reaction). As autoxidation is a radical process, the order of reaction (n) can be assumed as one (first order). Thus, the equation is simplified to $d\alpha/dt = k_T(1 - \alpha)$, and the following logarithmic values of the rate constants ($\ln(k_T)$) were found for the different temperatures: $\ln(k_{180}) = -6.7$, $\ln(k_{170}) = -7.1$, $\ln(k_{160}) = -7.6$, $\ln(k_{150}) = -8.2$, $\ln(k_{140}) = -8.7$, $\ln(k_{130}) = -9.2$, $\ln(k_{120}) = -9.6$, and $\ln(k_{110}) = -10.5$.

Assuming that autoxidation follows the theory of Arrhenius ($k_T = k_0 \exp(-E_A/(RT))$, E_A and k_{60} can be calculated by plotting $\ln(k_T)$ versus $1/T$ (K^{-1}) (see Figure 7). This leads to the following values: $E_A = 78$ kJ/mol and $\ln(k_{60}) = -14.2$ (60 °C was the temperature where the accelerated oven autoxidation was performed).

These findings are in good agreement with values for autoxidation processes of nonstabilized polyolefin given in the literature (E_A = 60−70 kJ/mol; temperature range 40−100 °C).[30,31] The activation energy is more or less the same for saturated and unsaturated polyolefin because the first step of oxidation is the free radical abstraction of a proton from the polymer chain (Figure 1). However, in the case of unsaturated polymers, the proton abstraction happens preferentially in the α-position to the double bonds. Thus, the olefin function is activated in a second step and mainly involved in the reactions that take place. In comparison with this, the activation energy

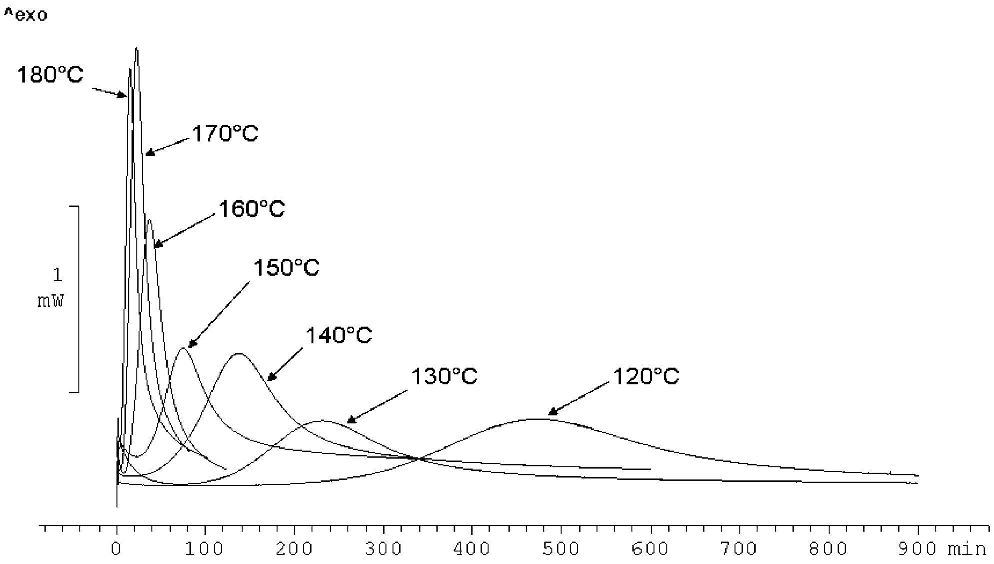

Figure 6. DSC thermograms of isothermal kinetic measurements of PHOU(50).

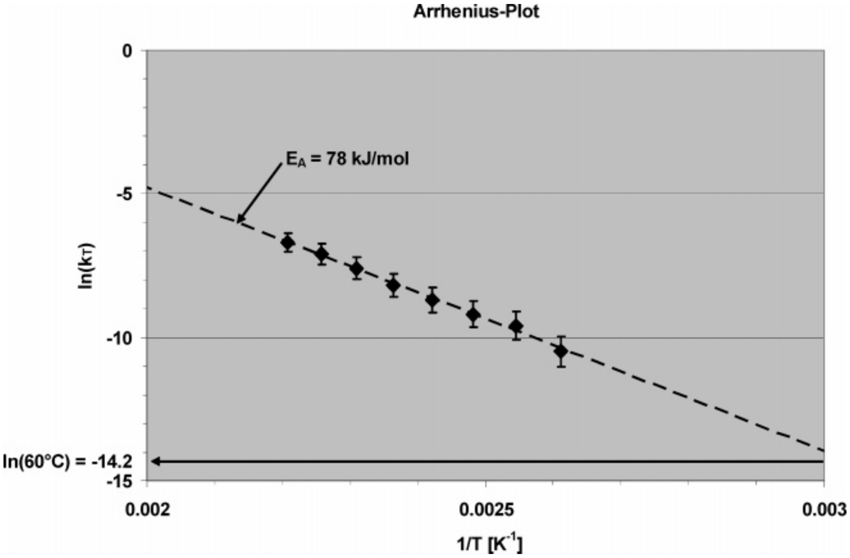

Figure 7. Arrhenius plot for determination of activation energy (E_A) and rate constant (k_{60}) of PHOU(50).

for the thermal degradation of the ester function of PHAs is $E_A = 212$ kJ/mol.[32] The nearly three-fold E_A value for ester cleavage explains the dominance of thermally induced cross-linking at the olefin moiety as compared to chain scission in PHOU copolymers.

Shelf-Life Calculations. With the kinetic data obtained from DSC measurements and the result from SEC and sol−gel determinations that PHOU(50) was very close to the gel point after 4 weeks of storage at 60 °C (incipient insolubility with a subsequent dramatic increase in molecular weight), it is possible to calculate the critical consumption of double bonds (c_{crit}) for gelation. The initial concentration (c_0) of double bonds in PHOU(50) is $c_0 = 3.60$ mmol/g of polymer (Table 1). The concentration of the double bonds at a certain time (c_t) can then be calculated for a first-order reaction using the following formula:

$$c_t = c_0 \exp(-k_T t)$$

This means for PHOU(50) after 4 weeks (2 419 200 s) storage at 60 °C ($k_{60} = 6.81 \times 10^{-7}$ s^{-1})

$$c_t = 3.60 \text{ mmol/g of polymer} \exp(-6.81 \times 10^{-7} \text{ s}^{-1} \times 2\,419\,200 \text{ s}) = 0.7 \text{ mmol/g of polymer}$$

According to this calculation, the concentration of remaining nonreacted double bonds in PHOU(50) at the gel point (c_{gel}) is 0.7 mmol/g of polymer. The critical consumption of 2.9 mmol/g of polymer double bonds (c_{crit}) is thus connected with the beginning of gel formation in PHOU copolymers ($c_{crit} = c_0 - c_{gel}$). Conversely, it can be calculated from this result that a PHOU copolymer with around 40% double bonds (PHOU(40)), which has an approximate initial concentration (c_0) of double bonds of 2.9 mmol/g of polymer, is the limiting copolymer where gel formation is just possible. For copolymers with a lower concentration of double bonds, chain scission will therefore be the predominant autoxidation effect. This theoretical estimation was in good agreement with the experimental result (Figure 3).

Furthermore, knowledge of the marginal consumption of double bonds for gelation allows the time for gelation (t_{gel}) for other PHOU polymers to be calculated. For example, PHOU-(75) has an initial concentration of double bonds of $c_0 = 5.08$ mmol/g of polymer (Table 1). The critical concentration leading to gelation (c_{gel}) in this case is thus 2.18 mmol/g of polymer

($c_{\mathrm{gel}} = c_0 - c_{\mathrm{crit}} = 5.08 - 2.9 = 2.18$ mmol/g of polymer). By rearranging the previous formula, t_{gel} can be calculated as

$$t_{\mathrm{gel}} = -[\ln(c_{\mathrm{gel}}/c_0)]/k_{60} = -[\ln(2.18/5.08)]/6.81 \times 10^{-7}\ \mathrm{s}^{-1} = 1\,242\,270\ \mathrm{s}\ \text{(around 14 days)}$$

The critical time for gelation of PHOU(75) is thus approximately 14 days at 60 °C. This is also in good agreement with the experimental finding that PHOU(75) was almost entirely (nearly 85%) soluble after 2 weeks and rather insoluble after 4 weeks of storage (see Table 2).

As it can be assumed that the activation energy (E_A) for the radical process is constant for temperatures far above the glass transition temperature (T_g) of PHOU (T_g is between −50 and −30 °C for PHOU; Table 1), the estimation of shelf-life for PHOU materials at room temperature is also possible. It was calculated in this way that PHOU(75) will reach gelation after around 13 months at room temperature and that the critical gel time for PHOU(100) is only around 8 months in ambient conditions.

Conclusion

All the analytical results showed a clear dependency between the autoxidation behavior of the different polymers and their amount of olefin side chains. The content of double bonds fundamentally influences the autoxidative behavior of PHOU. Above a certain olefin concentration, cross-linking is predominant, and the PHOU polymers become insoluble at a certain time. Below this amount of double bonds, chain scission prevails.

The kinetic measurements in connection with the analytical results allow the gel time and shelf-life of PHOU (co)polymers to be calculated. According to these investigations, mcl-PHA with a higher concentration of olefin side chains (>50 mol%) in particular already tend to form gels at room temperature in finite time frames. Gelation will be achieved, for example, after around 1 year for PHOU(75) in ambient conditions. Because of this distinct sensitivity of PHA copolymers in relation to autoxidation, it is recommended that these polymers should be stored in an inert gas atmosphere (e.g., nitrogen) and at low temperatures (<−5 °C) to keep them fresh for further processing, particularly the ones with substantial unsaturated fractions. Another way of tackling the oxidation problem would be the stabilization of materials by suitable additives (e.g., antioxidants), but these additives are often not suitable due to the biocompatible character of PHA.

Acknowledgment. We thank Angela Hinz and Franziska Ammann for performing the sample storage and the routine analyses. Additionally, we thank Ernst Pletscher and Thomas Ramsauer for their efforts in fermentation and purification of the polymers. Special thanks to Veronika Meyer for revision of the manuscript.

References and Notes

(1) Steinbuechel, A.; Valentin H. E. *FEMS Microbiol. Lett.* **1995**, *128*, 219.
(2) Sudesh, K.; Abe H.; Dio Y. *Prog. Polym. Sci.* **2000**, *25*, 1503.
(3) Reddy C. S. K.; Ghai R.; Rashmi Kalia V. C. *Bioresour. Technol.* **2003**, *87*, 137.
(4) Salehizadeh, H.; Van Loosdrecht, M. C. M. *Biotechnol. Adv.* **2004**, *22*, 261.
(5) Haenggi, U. J. *FEMS Microbiol. Rev.* **1995**, *16*, 213.
(6) Zinn, M.; Witholt, B.; Egli, T. *Adv. Drug Delivery Rev.* **2001**, *53*, 5.
(7) Williams, S. F.; Martin, D. P. In *Biopolymers, Vol. 4: Applications of PHAs in Medicine and Pharmacy*; Steinbuechel, A., Ed.; VCH Verlagsgesellschaft: Weinheim, 2003; pp 91.
(8) Köse, G. T.; Kenar, H.; Hasirci, N.; Hasirci, V. *Biomaterials* **2003**, *24*, 1949.
(9) Sendil, D.; Gursel, I.; Wise, D. L.; Hasirci, V. *J. Controlled Release* **1999**, *59*, 207.
(10) O'Leary, N. D.; O'Connor, K. E.; Ward, P.; Goff, M.; Dobson, A. D. W. *Appl. Environ. Microbiol.* **2005**, *71* (8), 4380.
(11) Kim, Y. B.; Lenz, R. W.; Fuller, R. C. *J. Polym. Sci., Part A: Polym. Chem.* **1995**, *33* (8), 1367.
(12) Hartmann, R.; Hany, R.; Geiger, T.; Egli, T.; Witholt, B.; Zinn, M. *Macromolecules* **2004**, *37* (18), 6780.
(13) Renard, E.; Poux, A.; Timbart, L.; Langlois, V.; Guerin, P. *Biomacromolecules* **2005**, *6*, 891.
(14) Hany, R.; Böhlen, C.; Geiger, T.; Schmid, M.; Zinn, M. *Biomacromolecules* **2004**, *5*, 1452.
(15) Hany, R.; Böhlen, C.; Geiger, T.; Hartmann, R.; Kawada, J.; Schmid, M.; Zinn, M.; Marchessault, R. H. *Macromolecules* **2004**, *37*, 385.
(16) Hany, R.; Hartmann, R.; Böhlen, C.; Brandenberger, S.; Kawada, J.; Löwe, C.; Zinn, M.; Witholt, B.; Marchessault, R. H. *Polymer* **2005**, *46*, 5025.
(17) Ashby, R. D.; Foglia, T. A.; Solaiman, D. K. Y.; Lui, C. K.; Nunez, A.; Eggink, G. *Int. J. Biol. Macromol.* **2000**, *27* (5), 355.
(18) Chung, C. W.; Kim, H. W.; Kim, Y. B.; Rhee, Y. H. *Int. J. Biol. Macromol.* **2003**, *32*, 17.
(19) Dufresne, A.; Reche, L.; Marchessault, R. H.; Lacroix, M. *Int. J. Biol. Macromol.* **2001**, *29*, 73.
(20) Gagnon, K. D.; Lenz, R. W.; Farris, R. W.; Fuller, R. C. *Polymer* **1994**, *35* (20), 4368.
(21) de Koning, G. J. M.; van Bilsen, H. M. M.; Lemstra, P. J.; Hazenberg, W.; Witholt, B.; Preusting, H.; van der Galien, Z.; Schirmer, A.; Jendrossek, D. *Polymer* **1994**, *35* (10), 2090.
(22) Zweifel, H. *Stabilization of Polymeric Materials*; Springer-Verlag: Berlin, 1998; p 2.
(23) Ibid, p 139.
(24) Riga, A.; Collins, R.; Mlachak, G. *Thermochim. Acta* **1998**, *324*, 135.
(25) Van Assche, G.; Verdonck, E.; Van Mele, B. *Polymer* **2001**, *42* (7), 2959.
(26) Halley, P. J.; Mackay, M. E. *Polym. Eng. Sci.* **1996**, *36*, 593.
(27) Zinn, M.; Witholt, B.; Egli, T. *J. Biotechnol.* **2004**, *113* (1−3), 263.
(28) Rabek, J. F. *Experimental Methods in Polymer Chemistry*; Wiley: New York, 1980; p 8.
(29) Riis, V.; Mai, W. *J. Chromatogr.* **1988**, *445*, 285.
(30) Burnett, J. D. *Chem. Abstr.* **1960**, *54*, 4125.
(31) Tago, K.; Tsuchiya, M.; Gondo, Y.; Ishimaru, K.; Kojima, T. *J. Appl. Polym. Sci.* **2000**, *77*, 1538.
(32) Kunioka, M.; Doi, Y. *Macromolecules* **1990**, *23*, 1933.

BM060785M