Chlorination of Poly(3-hydroxy alkanoates) Containing Unsaturated Side Chains

Ali Hakan Arkin,[†] Baki Hazer,*[,†,‡] and Mehlika Borcakli[‡]

Department of Chemistry, Zonguldak Karaelmas University, 67100, Zonguldak, Turkey; and TÜBİTAK, Marmara Research Center, Food, Science and Technology Institute, Gebze, 41470, Kocaeli, Turkey

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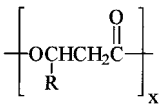
ABSTRACT: Unsaturated biopolyesters, (PHA−DB), obtained from pure anchovy (hamci), hazelnut, soybean oily acids and the mixtures of octanoic acid (in weight ratios of 50/50 and 70/30) were chlorinated up to 54 wt % Cl content. The molecular weights (MW) of the chlorinated biopolyesters were between 1.3 $\times 10^4$ and 3.0 $\times 10^4$ and decreased with the increase in chlorine content in PHA−DB. Melting transitions of the chlorinated biopolyesters were between 62 and 125 °C depending on the chlorine content when compared with those of the original PHAs, 44−55 °C.

Introduction

During past few decades there has been much interest in the wide variety of biopolyesters especially poly(β-hydroxyalkanoates) (PHAs) since the isolation of the original homopolymer poly[(R)-(−)-(3-hydroxybutyrate)] (PHB) by Lemoigne in 1925.[1] PHAs are stored within the cytoplasm of many prokaryotic organisms under conditions of limiting one of the nutrients such as nitrogen, oxygen, or other essential nutrients in the presence of an excess carbon source.[2] As published elsewhere, various types of PHAs with diverse physical properties have been produced using alkanols, alkanoic acids, edible oily acids, and bromo derivatives of alkanoic acids.[3−10] The general sturucture of the repeating unit of these polyesters shown below, in which R = $-(CH_2)_n-CH_3$ for most of naturally occurring PHAs depending on the substrates and the type of the bacteria.



Pseudomonas oleovarans produces medium chain length PHAs of random copolymers of R−3-hydroxy repeating units containing 6−12 carbon atoms. The physical properties of PHAs vary from crystalline−brittle to soft−sticky as the length of the side chain on β-carbon increases. Most biopolyesters, because of their low melting transitions, have narrow processabilities, and to overcome some of the nonapplicable mechanical and industrial natures of these PHAs, chemical modifications are needed. On the other hand, to insert the biopolyester into commodity polymers, such as polystyrene and poly(methyl methacrylate), is important to gain biodegradability for such polymers. So far, several attempts have been published for this purpose.[10−15] Hazer[10−11] has reported grafting reactions of poly(3-hydroxynonanoate) (PHN) with polystyrene and poly(methyl methacrylate) by peroxidic initiators onto unsaturated PHAs. Poly(ethylene glycol) was thermally grafted onto the PHAs containing double bonds by a polyazoester.[16] Synthesis of PHA−carbonhydrate conjugates was performed by Marchessault et al.,[17] and extensive studies on this subject were published.[18] PHN-*g*-PMMA graft copolymers were obtained by γ-irradiation of the mixture of PHN and methyl methacrylate (MMA) directly.[12] Lenz and co-workers[13−15] obtained PHAs with double bonds from *P. Oleovarans* co-feeding with 10-undecenoic acid and octanoic or nonanoic acid; subsequently, they performed the modification of the biopolyesters via irradiation and epoxidation. The rate of epoxidation[19] and cross-linking properties[20] of the unsaturated PHAs were studied.

The aim of the study is to modify PHAs containing double bonds from edible oily acids[10] via chlorination in order to prepare useful intermediates for blends of polymers and for further chemical reactions such as substitution reactions or grafting.

Experimental Section

Materials. Na^0, CH_3OH, $CHCl_3$, CCl_4, and $KMnO_4$ were purchased from Aldrich and used as received.

PHA Biosynthesis. Stock cultures of *P. oleovarans* (ATCC 29347) were used in all growth and polymer production experiments. *P. oleovarans* was grown, and the resulting polymer was extracted by using methods in the literature.[7,8,16,22] Table 1 includes the results and conditions of the PHA productions.

Substrates. Nonanoic acid and octanoic acid were supplied from Aldrich and used as received. Hamci oil was extracted from hamci (anchovy) fish from the Black Sea. Hazelnut and soybean oils were extracted from the related agricultural products grown in Turkey. The acids obtained from the hydrolysis of hazelnut and hamci oil included both saturated and unsaturated acids in different weight percentages.[10] Unsaturated acids, including both oleic and linoleic acids, comprised approximately 90 wt % of the acids from the plant oils (hazelnut and soybean[21]) with lesser amounts in the hamci oil. In the latter, saturated acids, including both palmitic and stearic acids, were approximately 20 wt % of the samples used. In hamci oily acid, unlike the others, was included unsaturated moieties containing di-, tri-, tetra-, penta-, and hexaenes up to 30%. Soybean oil is also very useful to produce unsaturated PHA. In this oil, likely sesame oil, was included unsaturated moieties: diene (50−57 wt %) and triene (6−10 wt %).[21]

Chlorination of the PHA. To the $KMnO_4$ crystals (890 mg, 5.632 mmol) placed in two-necked round-bottomed flask

* Corresponding author. E-mail: hazer@karaelmas.edu.tr.
† Zonguldak Karaelmas University.
‡ TÜBİTAK, Marmara Research Center, Food, Science and Technology Institute.

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Table 1. PHA−DB Productions from Edible Oily Acids

run no.	substrate	polymer yield (g/10 L)	unsaturation (mol %)	$M_w \times 10^{-4}$	$M_n \times 10^{-4}$	MWD[c]
80	soybean oily acid	4.5	40[a]	11.5	6.2	1.9
59	70/30 (octanoic acid/hamci oily acid)	1.2	3.5[a]	9.4	4.8	2.0
64	50/50 (octanoic acid/hamci oily acid)	2.2	5.5[a]	12.7	4.9	2.6
235	hazelnut oily acid	2.3	55[b]	8.7	4.2	2.1
60	hamci oily acid	3.0	10[b]	9.2	5.3	1.7

[a] Calculated from ^{1}H NMR spectra. [b] Calculated from ^{1}H NMR spectra (ref 10). [c] Molecular weight distribution.

Table 2. Results and Conditions of the Chlorination Reactions of the PHA−DBs

FEED			PHA−Cl		copolymer fractionation				
run no.	PHA−DB (g)	amount of Cl_2 reacted (g)	wt (g)	physical property	sample no.	γ	wt (g)	wt (%)	Cl in polymer (wt %)
PHA−DB from Hamci Oily Acid[a,b] (PHA Hamci)									
2	0.510	10	0.850	crystalline	21	0.5−0.8	0.612	72	22
					22	0.9−1.7	0.075	9	
					24	2.3−2.8	0.158	19	19
PHA−DB from 50/50 (Octanoic Acid/Hamci Oily Acid)[c,d] (PHA 50/50)									
5	0.500	0.5	0.332	elastic, soft	52	0.9−1.7	0.136	41	2
					53	1.8−2.2	0.034	10	3
					54	2.3−2.8	0.138	41	3
7	0.500	1.3	0.418	sticky	72	0.9−1.7	0.276	66	14
					74	2.3−2.8	0.075	18	7
PHA−DB from 70/30 (Octanoic Acid/Hamci Oily Acid)[e,f] (PHA 70/30)									
3	0.535	5	0.622	elastic, soft	31	0.5−0.8	0.440	71	18
					32	>1.0	0.182	29	
4	0.500	10	0.728	elastic, soft	41	0.5−0.8	0.590	81	21
					42	1.0−2.8	0.138	19	
10	1.412	37	3.203	crystalline	101	0.3−0.6	2.787	87	35
					102	>0.9−1.5	0.416	13	
PHA−DB from Soybean Oily Acid[g,h] (PHA−Soybean)									
11	0.905	12	2.180	crystalline	111	0.3−0.6	1.984	91	54
					112	>0.9−1.5	0.196	9	
PHA−DB from Hazelnut Oily Acid[g,h] (PHA−Hazelnut)									
12	0.745	5.5	1.670	crystalline	121	0.3−0.6	1.470	88	21
					122	>0.9−1.5	0.200	12	

[a] Pure Hamci biopolyester has waxy property. [b] By fractional precipitation. Solvent: $CHCl_3$. Nonsolvent: methanol. γ: volume ratio of nonsolvent to solvent, 1.0−1.7 for pure hamci biopolyester. [c] Pure 50/50 biopolyester has elastic property. [d] By fractional precipitation. Solvent: $CHCl_3$. Nonsolvent: methanol. γ: volume ratio of nonsolvent to solvent, 1.45−1.95 for 50/50 biopolyester. [e] Pure 70/30 biopolyester has elastic property. [f] By fractional precipitation. Solvent: $CHCl_3$. Nonsolvent: Methanol. γ: Volume ratio of nonsolvent to solvent, 1.15−1.70 for 70/30 biopolyester. [g] Pure soybean biopolyester has sticky, soft property. [h] By fractional precipitation. Solvent: $CHCl_3$. Nonsolvent: methanol. γ: volume ratio of nonsolvent to solvent, 1.0−1.68 for soybean biopolyester. [i] Pure hazelnut biopolyester has slippery, elastic property. [j] By fractional precipitation. Solvent: $CHCl_3$. nonsolvent: methanol. γ: volume ratio of nonsolvent to solvent, 0.8−1.5 for hazelnut biopolyester.

was added excess HCl dropwise to produce 1 g of Cl_2. The produced gas was passed through the solution of PHA ($M_n \approx$ 100 000) in CCl_4 (15 mL) at room temperature under sunlight with a rate that bubbled per second. The estimated amount of chlorine is shown in Table 2 for each run. Chlorinated PHA solution was left in the refrigerator overnight. The solvent was evaporated and the crude polymer was dried under vacuum.

Fractional Precipitation of the Chlorinated Samples. A vacuum-dried chlorinated biopolyester sample was dissolved in 5 mL of $CHCl_3$. Into the stirring solution, was added MeOH dropwise until completion of the first precipitation. After decantation, the upper solvent was followed by addition of MeOH for the second fraction. The same procedure was attempted until precipitation ended. γ values were calculated as the ratio of the total volume of MeOH used for each fraction to the volume of $CHCl_3$ in which the chlorinated biopolyester was dissolved. The polymer fractionated was dried under vacuum.

Determination of Chlorine Content. Chlorinated polymer (50 mg) was fused with a small piece of Na^0 to transform −Cl to NaCl. The reaction content was acidified with diluted HNO_3. Afterward, the analytical content of NaCl was determined by the Volhard method.[22]

Polymer Characterization

^{1}H and ^{13}C NMR Analysis. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ with a TMS internal standard using a Varian XL 200 NMR. The polymer concentration for ^{1}H NMR spectroscopy was 10 mg/mL and was 100 mg/mL for ^{13}C NMR. Chemical shifts are given in ppm downfield from TMS.

IR Analysis. IR spectra were obtained from Perkin-Elmer 177 IR spectrometers.

GPC Analysis. Molecular weights were determined by gel permeation chromatography (GPC) with a Waters model 6000A solvent delivery system with a model 401 refractive index detector and a Mode 730 data module and with two Ultrastyragel linear columns in series. THF was used as the eluent at a flow rate of 1.0 mL min^{-1}. Sample concentration of 2−3 mL and injection volumes of 150 μL were used. A calibration curve was generated with six polystyrene standards (MW's: 3×10^6, 2.33×10^5, 2.2×10^5, 2150, 580, and 92). The correlation coefficient was 0.994.

Methanolysis and GC−MS Analysis. The methanolysis reaction was carried out in chloroform/methanol/sulfuric acid (1 mL/0.85 mL/0.15 mL) at 100 °C for 140 min following a procedure identical with that described previously.[23] The methyl esters obtained were assayed by gas chromatography and mass spectroscopy (GC−MS analysis) using Hewlett-Packard HP 5890 gas chromatograph with He carrier gas. After injection the apolar column was maintained at 60 °C for 4 min and then heated at 10 °C/min to 270 °C. A temperature program was used which efficiently separated the different methyl 3-hydroxy alka(e)noates. Each peak in the chromatogram was analyzed with a mass spectrometer. The data were

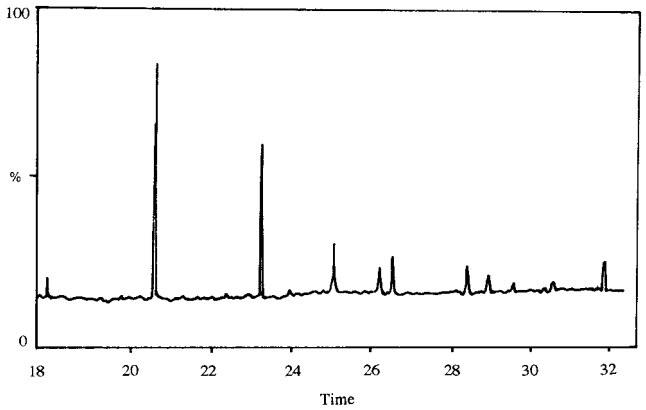


Figure 1. GC−MS spectrum of PHA−soybean (no. 80 in Table 1).

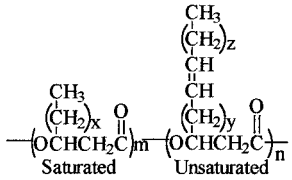
processed with a Hewlett-Packard laboratory data system (including more than 250 000 GC−MS spectra of chemicals).

Thermal Analysis. DSC analysis of the polymers obtained was performed on a DuPont 2910 instrument to determine the glass transition temperatures (T_g), and the melting transitions (T_m). Samples were heated from −100 to +200 °C in a nitrogen atmosphere at a rate of 10 °C/min, quenched, and heated a second time using the same range and heating rate. The T_g reported was the onset temperature in the thermogram.

Results and Discussion

Several different biopolyesters with double bonds (PHA−DB) were obtained by feeding *P. oleovarans* with edible oily acids such as hamci (anchovy), hazelnut, soybean, and mixtures of octanoic and hamci oily acids (50/50, 70/30 in weight respectively). Fermentation yields of PHA−DB from edible oily acids are summarized in Table 1.

Biopolyesters obtained were soft and sticky materials so they are difficult to handle. Biopolyesters obtained containing double bonds in range between 3.5 and 20 mol %. In this manner, unsaturation of biopolyester in repeating units was 10% for PHA from Hamci,[10] 55 % for PHA from hazelnut oily acids.[10] Unsaturation was found to be 40% for PHA from soybean oily acids from the GC−MS spectrum given in Figure 1. Saturated units were C-6, C-8, and C-10 units. However, unsaturated repeating units were expected to be C-10 and C-12 repeating units. Structures of corresponding PHAs, obtained from different edible oily acids, are shown below.



Chlorination was carried out by chlorine addition to the double bonds and substitution reactions with the saturated hydrocarbons. In this study, different types of chlorinated PHAs were synthesized. Table 2 shows the variable physical properties obtained for PHA samples and chlorination results for the unsaturated PHAs. Due to an increase in the chlorination degree when the amount of chlorine in the PHA sample was allowed to rise, the morphology of the polymer changed from soft, sticky to crystalline, brittle. For instance, 70/30 (octanoic/hamci acid) polyester is an elastic soft polymer. Its elasticity changed to crystalline when PHA contained 21 wt % Cl, (run 4). The same situation were also observed for pure hamci samples (run 2). In contrast, for run no. 7 for 50/50 samples, for small changes in the chlorine amount, the physical property of the polymer changed to sticky. However, a lesser chlorine content in run 5, PHA−DB 50/50, did not change the original elasticity of the polyester very much. The same trend is observed in 70/30 samples. A small amount of chlorine first made the elastic polyester sticky and then elastic and crystalline, respectively. Properties of soybean and hazelnut biopolyesters have been presented in runs 11 and 12, respectively. To obtain PHA with higher crystallinity, a higher chlorine amount was needed.

Table 3. Molecular Weight Measurements of the Chlorinated PHAs

sample no.[a]	$M_w \times 10^4$	$M_n \times 10^4$	MWD[b]
31	3.0	2.0	1.5
101	2.5	1.6	1.6
111	1.3	1.0	1.3
121	2.5	1.5	1.7

[a] Samples were eluted with THF. [b] Molecular weight distribution.

Fractional precipitation of the chlorinated PHAs was performed by means of precipitation with methanol as a nonsolvent from their chloroform solutions. γ values were calculated from the ratio of the volume of the nonsolvent methanol to that of the chloroform solution. γ values of PHA−DB vary from 0.8 to 2.0. γ values of the chlorinated PHA samples were lower than that of original PHA, 0.3−0.8. This difference in the γ values was also a typical property of the chlorinated PHAs. For instance, samples 21, 31, and 41 have the same range of γ values (e.g., 0.3−0.8) as samples 101, 111, and 121, as can be seen in Table 2. Naturally, the second and the third fractions belong to the oligomers formed by the hydrolysis during chlorination. Hence, they have grater γ values than those of the first fractions despite nearly the same amount of chlorine content (samples 21 and 24, in Table 2).

GPC was used to determine the molecular weights of fractions of highly chlorinated products, namely samples 31, 101, 111, and 121. Molecular weight distributions of samples are tabulated in Table 3. When we compared the molecular weights of the original PHA−DB, those of chlorinated samples were half or one-third of the original ones. We can conclude that a noticeable amount of hydrolysis is unavoidable during chlorination reactions. Molecular weights of the chlorinated samples also depend on the chlorine content. Decrease in chlorine content is in the order 111 (54%), 101 (35%), 121 (21%), and 31 (18%). Molecular weights of the samples change similarly. 111 (MW: 1.3×10^4), 101 (MW: 2.5×10^4), 121 (MW: 2.5×10^4), and 31 (MW: 3.0×10^4).

GC−MS analysis of the chlorinated samples failed because the chlorinated PHA repeating units need a temperature higher than 250 °C at which the polar column used is damaged.

^{1}H NMR spectra of the chlorinated samples show characteristic peaks (δ ppm): 0.84, −C*H*$_3$; 2.7, −C*H*$_2$−COO−; 3.5−4.5, −C*H*Cl−; 5.2−5.4, −C*H*−O−. A typical ^{1}H NMR spectrum of the chlorinated sample 31 can be seen in Figure 2. The broad peak at 3.5−4.5 ppm indicates that the −C*H*Cl− groups occurred by the addition reaction of Cl_2 with double bonds or from the substitution reaction with the saturated hydrocarbons. The decrease in the methyl peaks at 0.9−1.2 ppm can

Table 4. Chemical Shift Data (in ppm) from the ^{13}C NMR Spectra of the PHA−Soybean and Chlorinated PHA Samples: 41 and 101

samples	$-CH_3$	$-CH_2$ (side chains)	$O-CHCH_2-C=O$	$-CH_2CO_2$	$-CH=CH-$	$-C=O$	$-CH_2Cl$ $-CHCl$ $-CCl$
PHA−soybean	14, 15.8	23.6, 26, 22.5, 24.7, 25, 25.7, 27.2, 29.3, 31.5, 31.7, 32.8, 33.8	70.9	38.4, 39.1	122.5, 123.3, 127.9, 131.1, 132.0, 135.4	169.4	
HK 41	25.5	31.9, 33.8			128.2, 129.0	169.0	39.1, 41.5, 48.1, 46.4
HK 101	20.8					168.5, 166.1	47, 39, 36, 69.2, 60.9, 67.8, 69.4, 70.0, 72.3
	23.0						

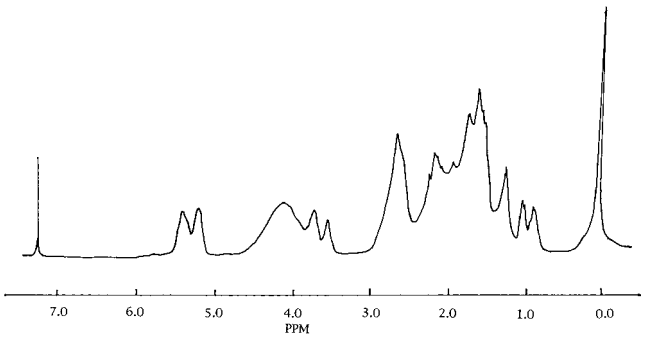


Figure 2. ^1H NMR spectrum of the chlorinated PHA (no. 31 in Table 2).

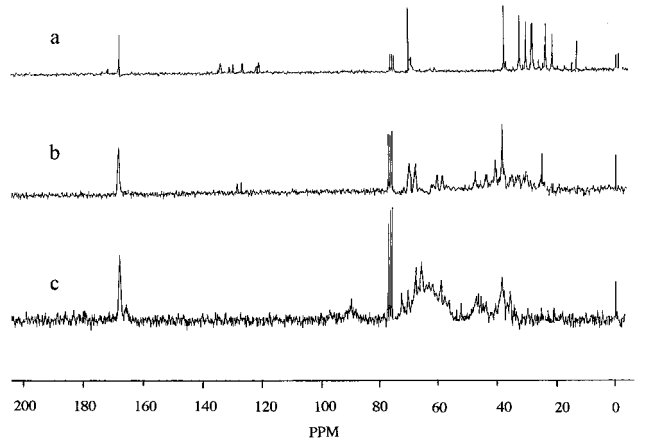


Figure 3. FT-IR spectra of the PHN (a), PHA−soybean (no. 80 in Table 1) (b) and chlorinatedPHA (no. 31 in Table 2) (c).

be attributed to substitution of Cl_2 with methyl groups. Thus, methyl peaks decrease.

^{13}C NMR spectra of the chlorinated samples were compared with that of PHA from soybean oily acid in Figure 3 and the signals were listed in Table 4. Methyl peaks of the original sample were 14 and 15.8 ppm while those of chlorinated PHA shifted to 20−25.5 ppm. For the higher chlorinated sample 101, saturated side CH_2− groups were all chlorinated. So, CH_2 signals at 23.6−33.8 ppm completely disappeared. Similarly, olefinic signals at 122.5−135.4 ppm disappeared. The new C−Cl, CH−Cl, CH_2Cl bands appeared at 39−72 ppm. Less chlorinated PHA sample 41 contains still double bonds at around 130 ppm.

Characteristic FT-IR spectra of the chlorinated samples show typical C−Cl stretching at 780 cm^{-1} strong and sharp.[24] Signals of double bonds[10,25] in PHA−soybean at 800, 910, 1640, and 3010 cm^{-1} disappeared in chlorinated sample 111. Carbonyl stretching appears between 1735 and 1750 cm^{-1}. Figure 4 shows the FT-IR spectra of poly(3-hydroxynonanoate) (PHN), PHA−soybean and, 111,

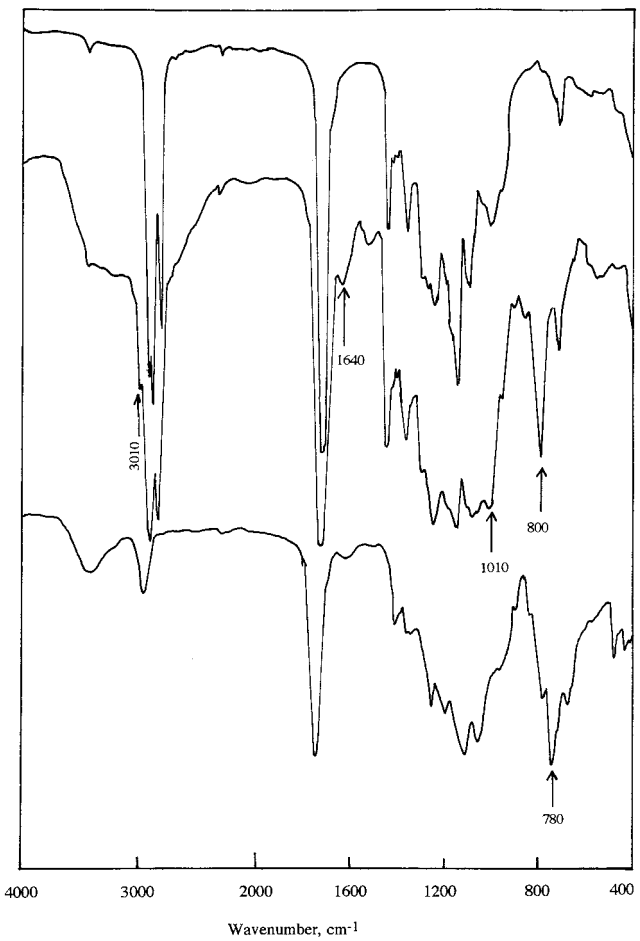


Figure 4. ^{13}C NMR spectra of (a) the PHA−soybean (no. 80 in Table 1) and chlorinated samples: (b) no. 41 and (c) no. 101 in Table 2.

Table 5. Thermal Properties of the First Fractions of the Chlorinated PHA Samples

sample no. in Table 2	γ [a]	Cl % in polymer	T_g (°C)	T_m (°C)
31	0.5−0.8	18	2	109
101	0.3−0.6	35	34	104
111	0.3−0.6	54	50	
121	0.3−0.6	21	58	115, 134

[a] For the symbol, see the Experimental Section.

Thermal analysis of the chlorinated samples was performed using DSC. Higher glass transition (T_g) and melting transition (T_m) for the chlorinated PHAs were observed when compared with the original PHAs (ca. $T_g = -50$ °C). DSC results are listed in Table 5. T_g's of the chlorinated samples were between 2 and 58 °C depending on the chlorine content of the samples. Sample 31 showed a very sharp T_m at 109 °C while the

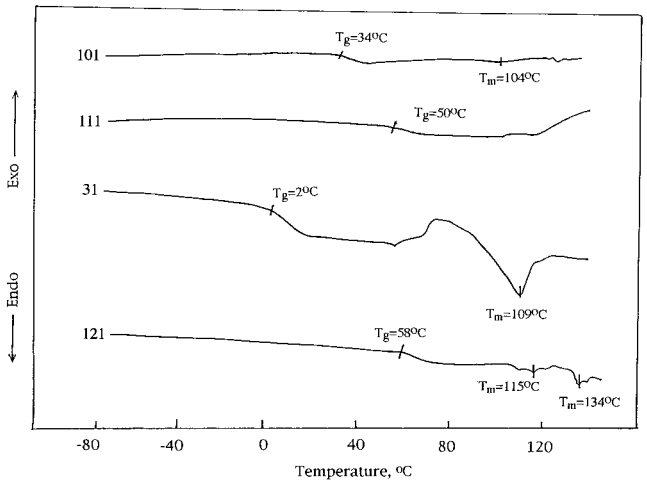


Figure 5. DSC thermograms of the chlorinated biopolyesters (samples 101, 111, 31, and 121 in Table 1).

others had small or no melting peaks. Figure 5 illustrates first scans of the DSC traces of the samples.

Conclusion

Chlorination of the sticky, soft PHAs with double bond have given hard, brittle, and crystalline physical properties depending on the chlorine content. Chlorination was performed by the addition to double bonds and substitution reactions with saturated hydrocarbon groups. Hydrolysis is also unavoidable during chlorination. As a conclusion, chlorinated PHAs can be very useful intermediates for polymer blends and further modification reactions.

References and Notes

(1) Lemoigne, M. *Ann. Inst. Pasteur* **1925**, *39*, 144.
(2) Doi, Y. *Microbial Polyesters*; WCH: New York, 1990.
(3) Anderson, A. J.; Dawes, E. A. *Microbiol. Rev.* **1990**, *54*, 450.
(4) Brandl, H.; Gross, R. A.; Lenz, R. W.; Fuller, R. C. *Adv. Biochem. Eng./Biotech.* **1990**, *41*, 77.
(5) Hocking, P. J.; Marchessault, R. H. In *Biodegradable Polymer*; Griffing, G. J. L., Ed.; Blake Academic & Professional: London, 1994.
(6) Steinbüchel, A. Valentin, H. E. *FEMS Microbiol. Lett.* **1995**, *128*, 219.
(7) Hazer, B.; Lenz, R. W.; Fuller, R. C. *Polymer* **1996**, *37*, 5951 and references therein.
(8) Curley, J. M.; Hazer, B.; Lenz, R. W.; Fuller, R. C. *Macromolecules* **1996**, *29*, 1762 and references therein.
(9) Kim, Y. B.; Lenz, R. W.; Fuller, R. C. *Macromolecules* **1992**, *25*, 1852.
(10) Hazer, B.; Torul, O.; Borcakli, M.; Lenz, R. W.; Fuller, R. C.; Goodwin, S. D. *J. Environ. Polym. Degrad.* **1998**, *6*, 109.
(11) Hazer, B. *Macromol. Chem. Phys.* **1996**, *197*, 431. Hazer, B. *Polym. Bull.* **1994**, *33*, 431.
(12) Eroglu, M. S.; Çaykara, T.; Hazer, B. *Polym. Bull.* **1998**, *41*, 53.
(13) Babu, G. N.; Hammar, W. J.; Rutherford, D. R.; Lenz, R. W.; Richards, R.; Goodwin, S. D. *1996 International Symposium on Bacterial Polyhydroxyalkanoates*; Eggink, G., Steinbuchel, A., Poiner, Y., Witholt, B., Eds.; NRC Research Press: Ottawa, 1997; pp 48−55.
(14) Gagnon, K. D.; Lenz, R. W.; Faris, R. J.; Fuller, R. C. *Polymer* **1995**, *35*, 4358.
(15) Bear, M.; Durand, M. L.; Langlois, V.; Lenz, R. W.; Goodwin, S. D.; Guerin, P. *React. Funct. Polym.* **1997**, *34*, 65.
(16) Hazer, B.; Lenz, R. W.; Çakmakli, B.; Borcakli, M.; Koçer, H. *Macromol. Chem. Phys.* **1999**, *200*, 1903.
(17) Yalpani, M.; Marchessault, R. H.; Morin, F. G.; Monasterials, C. J. *Macromolecules* **1991**, *24*, 6046.
(18) Yu, Ga-er, Morin, F. G.; Nobes, G. A. R.; Marchessault, R. H. *Macromolecules* **1999**, *32*, 518.
(19) Park, W. H.; Lenz, R. W.; Goodwin, S. D. *J. Polym. Sci., Part A: Polym. Chem.* **1998**, *36*, 2381.
(20) Park, W. H.; Lenz, R. W.; Goodwin, S. D. *J. Polym. Sci., Part A: Polym. Chem.* **1998**, *36*, 2389.
(21) *Codex Alimentarius*; FAO-WHO Food Standards Program, FAO: Rome, 2. Auflage, Band 8−1992[2]. Codex Standard für Sojaspeiseöl[3].
(22) Christian, G. D. *Analytical Chemistry*, 5th ed.; John Wiley & Sons: Toronto, Canada, 1994; p 279.
(23) Gross, R. A.; De Mello, C.; Lenz, R. W.; Brandl, H.; Fuller, R. C. *Macromolecules* **1989**, *22*, 1106.
(24) Nisoli, E.; Braglia, R.; Castiglioni, C.; Meroni, E.; Pegoraro, M.; Severini, F. *Macromolecules* **1999**, *32*, 7263.
(25) Lageveen, R. G.; Huisman, G. W.; Preusting, H.; Ketelaar, P.; Eggink, G.; Witholt, B. *Appl. Environ. Microbiol.* **1989**, *55*, 1949.

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