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Factorial Design in Optimization of PHAs Processing

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Summary: Poly(hydroxyalkanoate)s (PHA)s, as all melt-processed polymers, may undergo thermal degradation at temperatures close to the melting point. The primary purpose of the present study is to screen out process variables on the basis of a factorial design for two variables at two levels. The investigated parameters for PHA polymer samples processed in a torque rheometer were structure of PHA, processing temperature, and rotor speed. For polymers processed by compression molding, only the first two variables were considered and purification. The main effects and possible interactions were verified by the changes of molecular weight and thermal properties, as detected by DSC and TGA. It was observed that molecular weight decreased up to about 50 % while the thermal properties did not appreciably change.

Keywords: melt processing; poly(3-hydroxybutyrate); poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate); size exclusion chromatography; thermal properties

Introduction

The most common bacterial polyesters, poly(3-hydroxybutyrate) (PHB) and poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) when melt-processed undergo thermal degradation with decreasing of the molecular weight. The dominant reaction pathway is a random chain scission reaction (*cis*-elimination) with low probability of production of volatile species.[1] Lehrle *et al.*[2] proposed that other mechanisms, such as scission at the chain end could be kinetically favored at early stage of thermal degradation. Gogolewski *et al.*[3] processed PHB and PHBV (5.5 to 22% hydroxyvalerate [HV]) by injection molding. It was observed that molecular weight can decrease from 4 to 53 % over temperature range of 135-160 °C and residence time of 41 s. Less degradation was promoted with increasing content of HV in PHBV copolymer. The lower process temperature adopted for PHBV copolymers explained this result. Melik *et al.*[4] reported the melt behavior of PHBV (12% of HV) from a torque rheometer, characterizing the effect of temperature and shear on the degradation kinetics. Although the activation energy for chain scission was independent of shear rate, it was observed that the degradation rate increased with increasing shear. It was inferred that this behavior could be caused by viscous heat dissipation. The influence of temperature and

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screw speed on the molecular weight and mechanical properties of PHBV (6-7 % of HV) was investigated by Renstad *et al.*[5] Temperature ranged from 150 to 180 °C and screw speed from 5 to 40 rpm. A decrease of the molecular weight up to ca. 50 % was observed for the higher temperature and lower screw speed.

The melt processing of PHAs have been studied in experiments where each variable was investigated in turn, while all others were held constant. The present report is part of a project of melt-processing optimization. The goal of this study is to find the best settings for melt processing of PHB and PHBV and to detect which are the most important in affecting thire physical-chemical characteristics. A full two-levels factorial design[6] was chosen to screen out process variables using a torque rheometer and compression mold as melt-processing techniques.

Experimental

Materials. Poly(3-hydroxybutyrate) (PHB) (Mw = 344124 ± 4.0%, MWD = 2.8; MFI = 14.7 ± 1.2% g/10 min at 190 °C/2.16 kg) and poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV), containing 13 % of hydroxyvalerate units (Mw = 196500 ± 3.6%, MWD = 3.6; MFI = 99.7 ± 4.8% g/10 min at 190 °C/2.16 kg], were kindly supplied by Copersucar (Brazil).

Sample processing. Polymers were processed in a torque rheometer W 50 EHT (with roller blade) connected to a Plastograph Can-Bus Brabender and in a laboratory mold press. Table 1 presents the selected settings for a 2^2 full factorial design for compression molding and torque rheometry at residence times of 2 and 7 minutes, respectively. Sample identification code corresponding to experiment trials are shown in the Table 2.

Table 1. Settings for the two-levels factorial design.

Factor	Level	
(independent variable)	low (–)	high (+)
X1: Temperature	160 to 170 °C[a)]	180 °C
X2: Rotor Speed or purification[b)]	30 rpm or no	60 rpm or yes

[a)] 160 °C to PHBV and 170 °C to PHB in torque rheometry and 165 °C for both polymers in compression molding. [b)] Rotor speed in the case of torque rheometry experiment and purification for compression molding experiment.

Table 2. Sample identification codes.

B	Poly(3-hydroxybutyrate) (PHB) as received
BFn	Compression molded PH**B** as received **F**ilm at different temperatures (n = 165 °C; and 180 °C)
Bp	PH**B** **p**urified (chloroform solution and precipitation in ether)
BpFn	Compression molded PH**B** **p**urified **F**ilm at different temperatures (n = 165 °C; and 180 °C)
BBn-m	PH**B** processed on **B**rabender mixer at different temperature (n = 17 for 170 °C and 18 for 180 °C) and speed (m = 3 for 30 rpm and 6 for 60 rpm)
V	Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) as received
VFn	Compression molded PHB**V** as received **F**ilm at different temperatures (n = 165 °C; and 180 °C)
Vp	PHB**V** **p**urified (chloroform solution and precipitation in ether)
VpFn	Compression molded PHB**V** **p**urified **F**ilm at different temperatures (n = 165 °C; and 180 °C)
VBn-m	PHB**V** processed on **B**rabender mixer at different temperature (n = 16 for 160 °C and 18 for 180 °C) and speed (m = 3 for 30 rpm and 6 for 60 rpm)

Characterization. Molecular weights were obtained by Jasco PU-1580 HPLC liquid chromatograph connected to Jasco 830-RI detector and equipped with two PLgel 5 μ mixed-C columns. Chloroform was used as eluent at 1.0 ml·min^{-1} flow rate. Monodisperse polystyrene standards were used for calibration.

Thermal analyses were performed by a Mettler TA 4000 System instrument consisting of DSC-30 differential scanning calorimeter, TGA-50 furnace with a M3 microbalance, and TA72 GraphWare software. DSC samples of 10–15 mg were weighed in 40 μl aluminum pan and an empty pan was used as reference. Measurements were carried out under 80 ml·min^{-1} nitrogen flow rate according to the following protocol: first and second heating from -30 to 210 °C at 10 °C·min^{-1}; first cooling (quenching after the first heating) from 210 to -30 °C at 100 °C·min^{-1}. TGA evaluations were performed on ca. 20 mg samples at 10 °C·min^{-1} from 25 to 700 °C, under 200 ml·min^{-1} nitrogen flow rate.

Results and Discussion

Polymeric molecular weight and its distribution are very important for the end product performance. Besides, their changes can delineate the effects of process conditions on polymer degradation. Weight average molecular weight (Mw) of PHB and PHBV after purification by solution-precipitation, changed from 344 ± 14 to 364 ± 29 kD and from 196 ± 8 to 189 ± 23 kD at 95 % confidence interval, respectively. The Student's *t*-test of the two samples demonstrated that both mean pairs are equal. Consequently, purification does not change significantly molecular weight of not processed polymers. Table 3 illustrates the molecular weight characteristics of PHAs samples processed by both Brabender and compression mold. The results are arranged in a Yates order[6] for convenience of statistical graphical analysis.

Table 3. Experiment design (EXD), weight average molecular weight (Mw), and molecular weight distribution (MWD) for both types of melt-processing.

EXD			Process					
	Factor		Brabender			Compression molding		
Trial[a)]	X1	X2	Sample	Mw (kD)	MWD	Sample	Mw (kD)	MWD
(1)	–	–	BB17-3	275	2.1	BF165	184	3.9
a	+	–	BB18-3	219	2.4	BF180	264	3.1
b	–	+	BB17-6	199	2.8	BpF165	277	3.4
ab	+	+	BB18-6	183	2.3	BpF180	178	2.5
(1)	–	–	VB16-3	129	2.7	VF165	161	3.7
a	+	–	VB18-3	105	2.5	VF180	107	3.0
b	–	+	VB16-6	117	2.9	VpF165	99	2.9
ab	+	+	VB18-6	116	2.6	VpF180	51	2.6

[a)] (1) The low level of both factors; a, b, the primary effects; ab, first order interactions.

Data from Table 3 were analyzed graphically (Figs. 1 and 2) and by analysis of variance (ANOVA). EXD scatter plots clearly indicate that temperature is important for processed PHBV by both Brabender and compression molding (Figs. 1b and 1d, respectively). This means, at 180 °C all Mw's are low and vice-versa at 160 °C. However, temperature was less important for processed PHB (Figs. 1a and 1c). For Brabender processed PHB, Mw values at 180 °C are low and slightly superior for that processed at 170 °C (Fig. 1a). Besides, temperature does not appear to be important on compression molding, Mw values being only slightly higher at 165 °C than at 180 °C.

The Mw of PHB processed at 30 rpm decreased less than at 60 rpm. However, for processed PHBV, rotor speed does not seems to be important. Purification seems to affect only the processed PHBV. Polymers not purified presented higher Mw values.

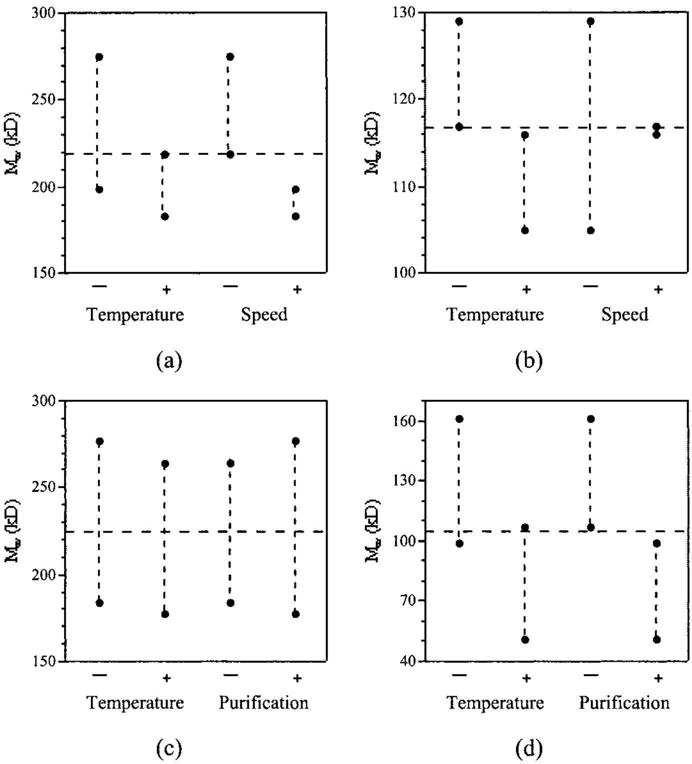
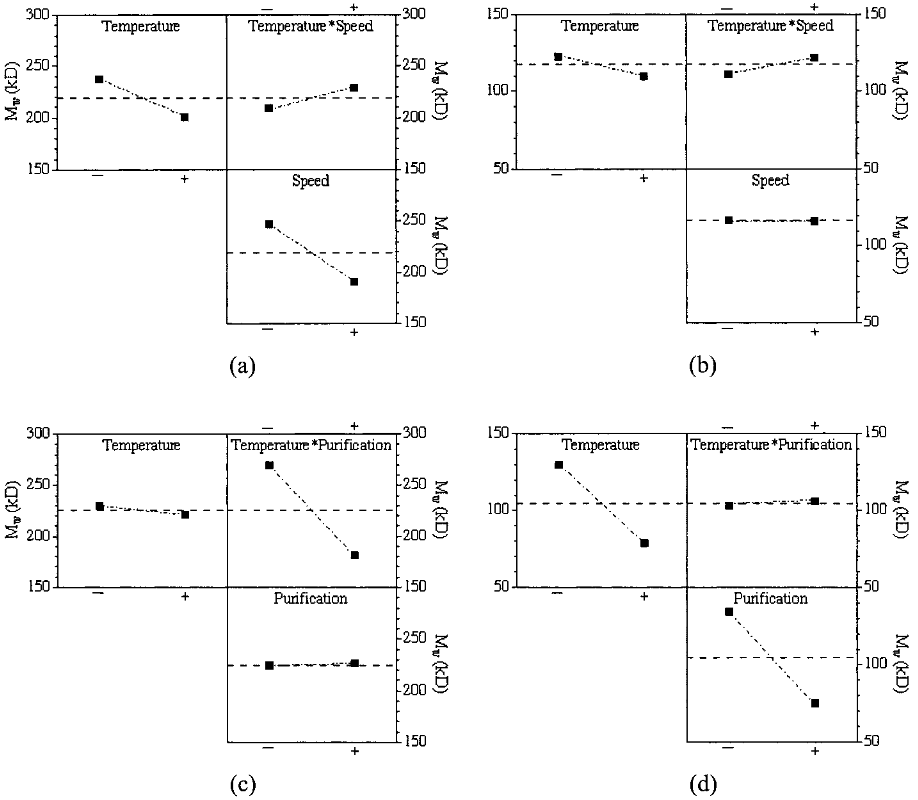


Figure 1. EXD Scatter Plots for Brabender processed PHB (a) and PHBV (b) and compression molded PHB (c) and PHBV (d) samples.

The EXD Mean Interaction Plots (Fig. 2) confirms the detected important factors from EXD Scatter Plot and two-way ANOVA substantiating the reported indications. The best settings from this preliminary experiment for both Brabender processed PHB and PHBV are low factors levels [(170 °C; 30 rpm) and (160 °C; 30 rpm), respectively]. In addition to these main effects, it was observed that temperature-speed interaction (Fig. 2a) is important for PHB and relatively unimportant for PHBV (interaction mean difference was 20 for PHB and 11 for PHBV). Compression molded polymers showed different behaviors. For PHB, no significant difference was observed between factor levels but temperature-purification interaction is important. However, PHBV showed that there is a significant difference between factor levels and that the best conditions are temperature 165 °C and not purified polymer; no factor interactions were detected.



(a) (b)

(c) (d)

Figure 2. EXD Mean Interaction Plots for Brabender processed PHB (a) and PHBV (b) and compression molded PHB (c) and PHBV (d) samples.

Renstad *et al.*[5] processed PHBV (7 % HV) by extrusion in the 146-177 °C temperature range and at screw speed from 5 to 40 rpm. They found that Mw values decreased with increasing temperature in agreement with results of the present study. However, both studies are divergent as far as the speed (screw or rotor) effect on Mw is concerned. In our study on torque rheometer processed polymers, process residence time was fixed at 7 minutes where was verified that both torque and temperature were at equilibrium. On the other hand, extrusion residence time changes with the screw speed, therefore the residence time was not controlled in Renstad *et al.* work[5].

Table 4 presents thermogravimetric data of both as received and processed PHAs. Although the Mw of processed PHAs decreased up to ca. 50 % for PHB and up to ca. 70 % for PHBV, no significant change of the thermal stability was detected. Performing Student's *t*-test at 95

% confidence, only T_2 and T_d mean values of the pair as received-purified PHB (B-Bp) revealed a significant difference. PHB as received was more stable than that purified. Kopinke *et al.*[7] observed that different fermentation conditions produce crude PHB's of different thermal stability. Accordingly, it can be supposed that residual materials from the culture medium (for instance proteins and lipids) present in as received PHB may have an important role in thermal degradation. On the other hand, the mean thermal stabilities of the pair as received-purified PHBV (V-Vp) are not significantly different from a statistical point of view.

Table 4. Thermogravimetric data of PHB and PHBV samples.[a)]

Sample	T_2 (°C)	T_d (°C)	T_p (°C)
B	276.2	280.8	302.7
Bp	260.7	269.6	294.2
BB17-3	272.4	275.9	297.0
BB17-6	274.0	277.6	299.8
BB18-3	270.6	275.4	297.0
BB18-6	277.0	279.1	302.7
V	255.6	269.6	295.6
Vp	260.3	269.1	292.7
VB16-3	259.5	271.9	297.0
VB18-3	267.7	278.1	302.7

T_2 = temperature at which 2% weight loss occurs; T_d = onset decomposition temperature at the tangent crossover drawn on both side of the decomposition trace; T_p = peak temperature of the first derivative trace.

Considering T_2 and T_d, PHBV copolymer was less stable than PHB. This result is in contrast with the increased thermal degradation temperature of PHAs reported by Li *et al.*[8] on increasing of hydroxyvalerate content. On the other hand, T_p values of both PHAs are not significantly different and they are in agreement with literature data.[7] These results point out that not only the presence of impurities but also their kind and very likely their amount are important in determining the thermal stability of PHAs.

Typical DSC traces from the second heating after quenching of both PHB and PHBV samples are shown in Figures 3 and 4, respectively. The corresponding thermal parameters are presented in Tables 5 and 6, respectively.

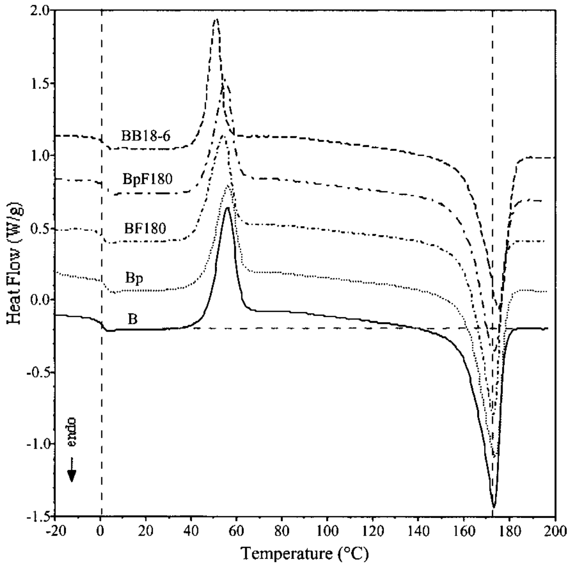


Figure 3. Typical second heating DSC traces for PHB samples.

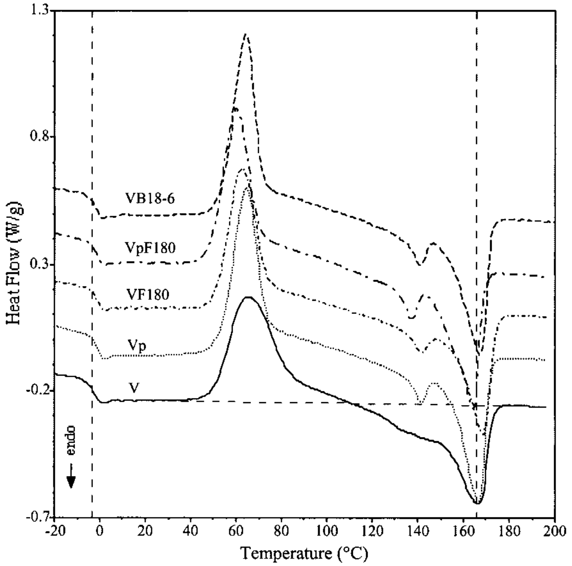


Figure 4. Typical second heating DSC traces for PHBV samples.

Apparently PHA polymers are 100 % amorphous after quenching, as suggested by the equivalent areas of cold crystallization and melting peaks. In general, glass transition temperatures have a tendency to slightly increase after the processing. T_g values of PHB and PHBV samples ranged from 0.4 °C to 2.6 °C and from -3.3 °C to -1.1 °C, respectively.

The melting enthalpy of 146 J/g was used to determine the degree of crystallinity[9] of both PHA's. In the case of PHBV, the area of both peaks was considered. Peak temperatures of the first narrow cold crystallization of PHB samples were around 53 °C and those of PHBV samples were about 10 °C higher. However, the second broad cold crystallization peak occurred at about 73 °C for both PHA's. In general, melting temperatures and crystallization degree of both PHAs did not change after processing. Average melting temperatures of PHB samples were 172 °C and 174 °C for compression molding and torque rheometer process, respectively. Processed samples crystallinity remains basically equivalent to that of original polymer.

Table 5. Thermodynamic parameters of processed PHB.[a)]

Sample	T_g (°C)	T_{cc1} (°C)	H_{cc1} ($J \cdot g^{-1}$)	T_{cc2} (°C)	H_{cc2} ($J \cdot g^{-1}$)	T_m (°C)	H_m ($J \cdot g^{-1}$)	X_c (%)
B	0.9	56.5	53.3	nd	33.3	173.1	82.5	56.5
BF165	0.9	52.5	47.1	62.9	28.8	171.1	73.7	50.5
BF180	0.4	52.5	49.5	70.0	31.1	172.1	77.4	53.0
Bp	1.9	56.3	53.0	nd	36.5	173.4	82.5	56.5
BpF165	0.9	52.4	53.2	64.9	32.2	169.2	82.8	56.7
BpF180	2.6	55.8	51.1	nd	29.3	173.1	84.3	57.7
BB17-3	1.1	52.0	44.7	80.3	34.6	174.0	81.4	55.7
BB17-6	1.4	51.9	50.7	72.5	34.5	174.6	81.7	55.9
BB18-3	0.8	52.5	50.6	63.9	33.2	174.6	83.0	56.9
BB18-6	1.2	51.6	45.1	78.3	31.5	174.3	82.1	56.2

[a)] T_g, T_{cc}, and T_m are glass transition, cold crystallization, and melting temperatures, respectively; H_{cc} and H_m are cold-crystallization and melting enthalpies; X_c is the degree of crystallinity; nd = not detected.

The melting transitions of processed PHBV appeared at ca. 141 °C and 168 °C. However, purified samples (VpF165 and VpF180), corresponding to lower Mw samples showed a slight decrease of about 3 °C.

Table 6. Thermodynamic parameters of processed PHBV.[a)]

Sample	T_g (°C)	T_{cc1} (°C)	H_{cc1} ($J{\cdot}g^{-1}$)	T_{cc2} (°C)	H_{cc2} ($J{\cdot}g^{-1}$)	T_{m1} (°C)	H_{m1} ($J{\cdot}g^{-1}$)	T_{m2} (°C)	H_{m2} ($J{\cdot}g^{-1}$)	X_c (%)
V	-3.3	65.2	62.5	—	—	nd	nd	166.3[b)]	56.7	38.8
VF 165	-2.2	62.8	60.0	—	—	140.6	14.8	168.2	41.1	38.3
VF 180	-2.2	63.3	50.5	79.8	7.4	141.6	14.7	168.4	40.4	37.7
Vp	-1.1	64.3	55.7	nd	10.0	141.7	15.3	166.5	43.5	40.3
VpF165	-2.0	58.4	64.2	—	—	137.9	14.2	164.8	45.6	41.0
VpF180	-2.2	60.2	52.5	73.9	10.8	137.7	15.6	164.4	45.6	41.9
VB16-3	-1.2	62.4	51.9	78.3	8.7	141.6	15.7	168.3	49.1	44.4
VB16-6	-2.2	62.7	53.6	75.9	9.3	141.2	15.9	168.0	43.2	40.5
VB18-3	-2.2	64.4	52.6	78.8	8.9	141.1	15.8	167.2	42.9	40.2
VB18-6	-1.7	64.3	52.6	nd	8.5	141.1	16.6	167.8	42.8	40.7

[a)] T_g, T_{cc}, and T_m are glass transition, cold crystallization, and melting temperatures, respectively; H_{cc} and H_m are cold-crystallization and melting enthalpies; X_c is the degree of crystallinity; nd = not detected. [b)] Two overlapping peaks.

Conclusions

Poly(3-hydroxybutyrate) and poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) were melt-processed and molecular weight characteristics and thermal parameters were analyzed from a statistical point of view. The best settings for torque rheometer processed PHB and PHBV were found to be 30 rpm at 170 and 160 °C, respectively. Besides, in the investigated variable range, an interaction between temperature and rotor speed seems to exist for PHB. In other words, the effect of temperature on Mw depends on the rotor speed and vice-versa. For compression molding processed polymers, only the Mw of PHBV was affected by both the temperature and the polymer purification.

The polymer thermal stability appeared to be independent of processing conditions but it was very likely affected by the presence of impurities. The same behavior was observed for thermodynamic parameters, with a possible influence by the molecular weight polydispersity.

Acknowledgements

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