

Thermal Degradation of Poly(3-hydroxybutyrate) and Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) in Drying Treatment

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ABSTRACT: This study examines the isothermal treatment of poly(3-hydroxybutyrate) (PHB) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) powders and films. The PHB and PHBV crystallinities were determined using X-ray diffractometry, and shown to increase with temperature (130–150°C) and then decreased from 55% to 45% at 180°C. The crystal morphology of crystal planes (101) and (111) became sharp at a high temperature. The weight average molecular weight (M_w) of PHB decreased from 1,028,000 to 41,800 g/mol when heated at 180°C for 30 min. The molecular weight of PHB decreased more rapidly than that of PHBV with time. No peak signal was observed in gel permeation chromatography after heating at 150°C because the solubility of PHB changed with crystallinity. The thermal behaviors of PHB and PHBV were analyzed by differential scanning calorimetry and thermogravimetric analysis. The roughness, contact angle, and surface morphology of PHB and PHBV films were also measured to determine the surface properties. © 2013 Wiley Periodicals, Inc. *J. Appl. Polym. Sci.* 130: 3659–3667, 2013

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INTRODUCTION

Polyhydroxyalkanoates (PHAs) are semicrystalline polymer and have material properties that are similar to those of petrochemical polymers.^{1–3} Therefore, it may be a suitable alternative to traditional plastic for various applications. Extensive studies have been conducted on PHAs. They have been evaluated for various medical applications, such as controlled drug release, wound dressing, surgical implants, biomaterials for tissue engineering, and other medical purposes.^{4–6}

A number of separation processes have been proposed to separate PHAs from biomass, including solvent extraction, digestion, mechanical disruption, and supercritical fluid.^{7–16} After separation, the PHA product must be dried with heat and this can lead to PHAs degradation.⁴ An understanding of the thermal stability and thermal degradation behavior of PHAs is essential for processing, industrial application, and thermal recycling. The thermal degradation of PHAs has been examined in prior studies.^{17–20} In most cases, the thermal decomposition of polymers has been examined using non-isothermal techniques, such as thermogravimetric analysis (TGA) or differential scanning calorimetry (DSC), which tend to reflect phenomena at higher temperatures.^{4,21,22} However, the drying process is conducted using isothermal treatment at low temperature, which may also degrade the polymer. Hence, the mechanical properties of the

PHAs were influenced when examining the isothermal degradation of PHAs.²⁰ This study examined the effect of isothermal heating on the molecular weights and crystallinities of poly(3-hydroxybutyrate) (PHB) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV).

EXPERIMENTAL

Materials

PHB ($M_w = 1 \times 10^6$ g/mol, 98%, 35–60 mesh) was produced using Recombinant *Escherichia coli* XLI blue in our laboratory.¹⁵ PHBV (5 wt % hydroxyvalerate) was purchased from Aldrich (USA), and CHCl_3 (99%) was purchased from J. Mallinckrodt (USA).

Isothermal Treatment of PHAs

Polymer thin films were prepared using a solvent-casting method. The polymer powder (0.26 g) was first dissolved in CHCl_3 (13 mL) under vigorous stirring at 60°C for 3 days. The polymer solution (16 g) was poured into a Φ 10 cm glass Petri dish and dried to obtain thin films (~ 30 μm thicknesses), and further dried for 3 days.

The PHA powder and thin films were placed in an oven at 60°C, 100°C, 130°C, and 150°C, or temperatures higher than the melting point of PHB (180°C) and PHBV (170°C) for selected times of 10 min to 6 h. After heating, the molecular weight and crystallinity of the samples were measured,

Table I. Effect of Isothermal Treatment on Molecular Weight of PHB and PHBV Powders

Temperature (°C)	Heating time (min)	PHB powder		PHBV powder	
		$M_w (\times 10^3)$ g/mol	PDI	$M_w (\times 10^3)$ g/mol	PDI
Original		1028 ± 48.1	1.85 ± 0.1	620 ± 15.9	3.18 ± 0.5
60	60	1006 ± 13.3	1.85 ± 0	591 ± 21.7	4.38 ± 1.4
	360	973 ± 46.8	1.78 ± 0.1	571 ± 39.3	4.65 ± 0.3
100	60	987 ± 36.2	1.64 ± 0.2	564 ± 39.0	3.78 ± 0.1
	360	976 ± 37.6	1.70 ± 0.2	492 ± 56.3	4.24 ± 0.8
130	10	974 ± 26.4	2.02 ± 0.1	488 ± 78.4	4.22 ± 0.9
	30	605 ± 79.6	2.40 ± 0.2	395 ± 77.3	4.69 ± 0.6
150	10	402 ± 70.7	4.68 ± 0.7	176 ± 73.8	5.43 ± 0.1
	30	98 ± 38.9	4.04 ± 0	40.0 ± 22.6	4.15 ± 2.2
	60	N.D.	N.D.	14.0 ± 0.5	4.17 ± 1.7
180 for PHB (170 for PHBV)	10	N.D.	N.D.	20.4 ± 6.4	4.11 ± 2.0
	20	N.D.	N.D.	7.5 ± 3.2	3.81 ± 1.0
	30	41.8 ± 14.5	3.61 ± 0.8	5.4 ± 3.3	3.39 ± 0.4
	40	20.4 ± 13.6	3.07 ± 1.3	N.D.	N.D.
	50	18.6 ± 9.2	3.31 ± 1.2	N.D.	N.D.
	60	11.5 ± 4.3	2.39 ± 0.5	2.9 ± 2.1	2.76 ± 0.8

N.D.: not detected; M_n : number average molecular weight; M_w : weight average molecular weight; PDI: polydispersity index = M_w/M_n .

respectively, using a gel permeation chromatography (GPC) and X-ray diffractometry (XRD).

Determination of Molecular Weight Using GPC

The molecular weights of polymers were determined using a GPC (Shimadzu, Model LC 20A, Japan) equipped with a refractive index detector (Shimadzu, RID-10A, Japan). Two columns in series were used for analysis (PLgel 50 Å–10⁶ Å and PLgel 5 μm MIXED-C, Polymer Laboratories, England) on elution with CHCl₃ (1 mL/min) at 50°C. The polymer was dissolved in hot CHCl₃ (60°C) for 6 h to a concentration of 2%, and filtered (0.45 μm poly(vinylidene fluoride)). Universal calibration of the columns was accomplished using five polystyrene standards with a narrow range of molecular weight (Polymer Laboratories, England, M_w = 1,471,974 g/mol, 990,604 g/mol, 777,499 g/mol, 492,493 g/mol, 288,204 g/mol). Data analysis was performed using Peak-ABC software (Great Tide Instrument, Taiwan).

Determination of Crystallinity Using an X-ray Diffractometer

The crystallinity and crystal structure of PHA polymers were determined using an XRD (Shimadzu Labx, XRD-6000, Japan). Crystallinity was calculated by separating the intensities because the amorphous and crystalline phases on the diffraction phase were resolved using the computer-aided curve technique. After separation, the total area of the diffracted pattern was divided into crystalline (A_c) and amorphous components (A_a). The percentage of crystallinity X_c was calculated from the ratio of the crystalline area and total area, as shown in eq. (1):

$$X_c (100\%) = \left(\frac{A_c}{A_c + A_a} \right) \times 100 \quad (1)$$

where A_c , A_a , and X_c denote the area of crystalline phase, area of amorphous phase, and percentage of crystallinity, respectively.²³

Morphology Observed Using Scanning Electron Microscope

A scanning electron microscope (SEM) (JEOL, JSM-5600, 1 eV, Japan) was used to determine the surface structure of the polymer films. The samples were mounted onto brass stubs using double-sided adhesive tape and vacuum-coated twice with a thin layer of gold using the auto fine coater for 40 s prior to examination. The pore diameter was calculated using SEM software to draw the diameter of the pore. Each film was measured at least five times on various surface locations to determine common phenomena.

Atomic Force Microscope Observations

An atomic force microscope (AFM) (Shimadzu, SJ9500, Japan) was used to determine the surface roughness of the polymer films.²⁴ An AFM is a high-resolution scanning probe microscope with resolution of fractions of a nanometer, which is more than 1000 times higher than the resolution of partial diffraction. Each film (30 μm × 30 μm) was measured at least five times on various surface locations to obtain comprehensive results.

Contact Angle Measurement

Hydrophilicity and hydrophobicity of the polymer films were measured using a static water droplet contact angle meter (First Ten Angstroms, FTA125, USA) with analytical software. Each film was measured at least five times on various surface locations for evaluation.

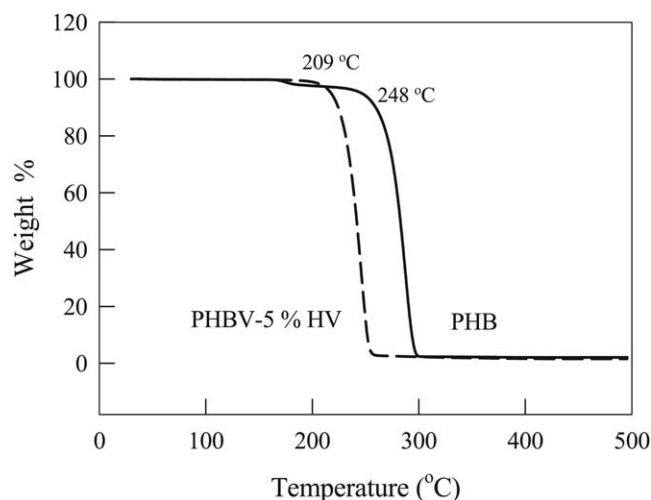


Figure 1. TGA thermograms of PHB and PHBV.

Puncture Test of PHB and PHBV

Puncture tests were performed using a tensile (and compressive) strength tester (PT1066, PROTEST, Taiwan) with a probe of 5.1×10^{-3} m in diameter and a speed of 1×10^{-3} m/s. The film sample was fixed in a mold of 5.0×10^{-2} m in diameter. Three samples of each type of film were prepared for testing. The data was correctly reproduced within 5% of the values reported in this work.

Thermal Analysis

TGA was performed on a Q50 instrument (TA instrument, USA) calibrated with indium. The temperature program from 30°C to 400°C at a heating rate of 10 °C/min was employed under nitrogen flow (40 mL/min). The weight-loss behavior of each specimen was measured in a dynamic scanning mode.

DSC (TA instrument, Q10, USA) was used to measure the endotherms and exotherms of different specimens. The polymer sample was first heated from -40°C to 200°C (10°C/min) (first heating scan), then the temperature was held for 5 min before cooling to -40°C (10°C/min) under a nitrogen flow rate of 40 mL/min. Temperature was also held for 5 min before a second heating in the same condition as the first step. Three specimens prepared from hermetic aluminum (Al) pans were tested for each DSC measurement. The obtained thermograms were calibrated with the baseline obtained from empty Al pan and indium standard.

Statistical Analysis

Statistical comparisons were performed using student's *t*-test with SigmaPlot software. *P* values < 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

The isothermal treatments of PHB and PHBV powders (or films) were conducted at specific temperatures (below T_m) and time

Effect of Isothermal Treatment on Molecular Weight of PHB and PHBV

The effect of temperature and heating on the weight-average molecular weights (M_w) and polydispersity index (PDI) of PHB

and PHBV powders are given in Table I. The M_w of PHB decreased slightly upon heating at 60°C and 100°C for 360 min. The M_w decreased by 40% and 90% at 130°C and 150°C for 30 min, respectively. Moreover, the M_w decreased rapidly, by up to 95%, at 180°C for 30 min. The depolymerization trend of PHBV was similar to that of PHB. The M_w of PHBV slightly decreased at 60°C; however, it gradually decreased by approximately 20% (from 620,000 to 500,000 g/mol) at 100°C for 360 min. The decomposition degree increased with heating time. The M_w of PHBV decreased rapidly by $\sim 97\%$ (from 620,000 to 20,000 g/mol) at 170°C, which is higher than T_m of PHBV. The results in Table I indicate that the M_w of PHB or PHBV were not influenced when the temperature was below 60°C. However, at higher temperatures and longer heating times, the M_w of PHB and PHBV decreased. A statistical analysis showed that the M_w of PHB and PHBV on temperature and heating time were significantly different from each other ($P < 0.05$).

Lo et al. indicated that various molecular weights of PHB affect the thermal press application.²⁵ A lower M_w (such as 220,000 g/mol) cannot make a film using the isothermal press process. When the M_w reaches 500,000 g/mol, a film can be prepared by processing. In addition to the M_w of PHAs, the thermal history/profile must be considered when applying the thermal process for manufacturing.

No peak signal of PHB was observed in GPC after heating for 30 min at 150°C, as shown in Table I. However, the molecular

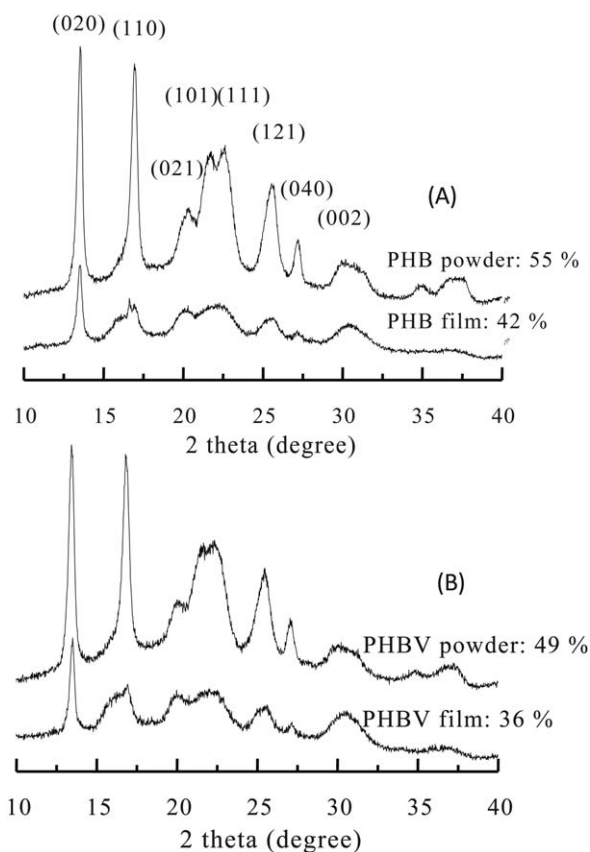


Figure 2. XRD diffractograms of PHB and PHBV (a) powder and (b) films without thermal treatment.

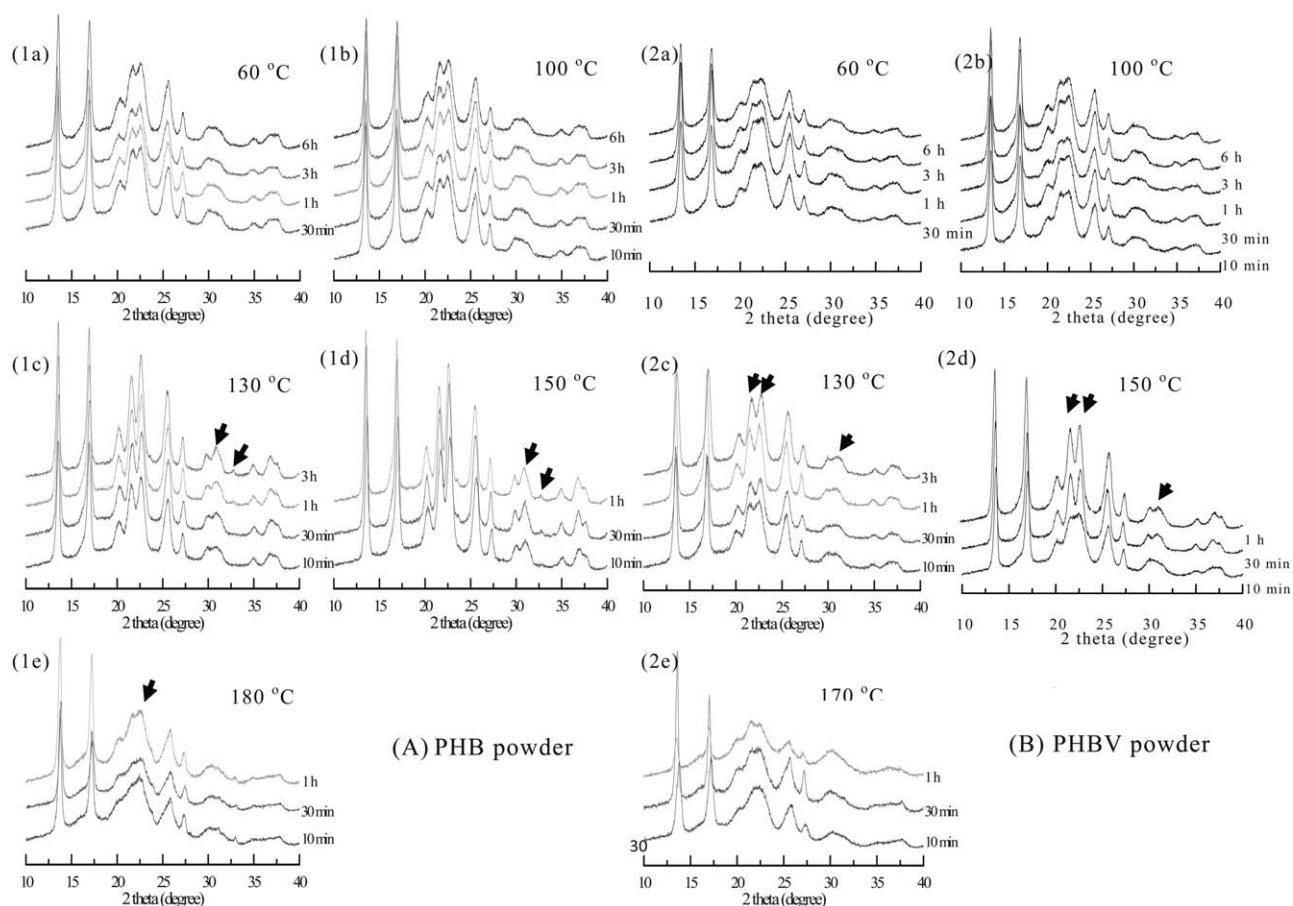


Figure 3. XRD diffractograms of (A) PHB and (B) PHBV powder on different temperatures and times.

weight was observed in GPC after heating for 30 min at 180°C. This was attributed to the change of solubility of PHB at 150°C because of an increase in crystallinity. It was difficult to dissolve the PHB powders in chloroform after isothermal treatment.

In most cases, the thermal decompositions of PHAs polymers have been investigated using non-isothermal techniques, such as using TGA in nitrogen. The PHB and PHBV backbones cleaved in a one-stage process, in which the decomposing temperatures ranged from 190 to 300°C²⁵ and 190 to 250°C, and the onset temperatures were 240°C and 210°C, respectively. Figure 1 shows TGA thermograms of PHB and PHBV. This finding corresponds to that of Lo et al.²⁵ Aoyagi et al. (2002) indicated that less volatile products were produced by isothermal degradation of PHB at 170°C¹⁷; that is, weight loss is unobvious <220°C. However, the M_w may be reduced at a low temperature, such as 100°C. A random chain scission reaction has been proposed for PHB.²⁶ When the chain scission is completely random, a linear relationship between number-average degrees of polymerization (P) and time (t) is derived by

$$\frac{1}{P_{n,t}} = k_d t + \frac{1}{P_{n,0}} \quad (2)$$

where $P_{n,0}$ and $P_{n,t}$ are the number-average degrees of polymerization at time 0 and t , respectively, and k_d is the degradation rate constant. Plot $1/P_n$ vs. t to obtain the slope, k_d . The results in Table I show that a linear relationship was obtained. The rate

constants of isothermal degradation calculated by eq. (2) were $6 \times 10^{-10} \text{ s}^{-1}$ at 100°C, $1 \times 10^{-7} \text{ s}^{-1}$ at 130°C, and $1 \times 10^{-6} \text{ s}^{-1}$ at 150°C for PHB, and $2 \times 10^{-8} \text{ s}^{-1}$ at 100°C, $4 \times 10^{-7} \text{ s}^{-1}$ at 130°C, and $7 \times 10^{-6} \text{ s}^{-1}$ at 150°C for PHBV. This finding is consistent with the result obtained by Aoyagi et al. (2002) of $k_d = 1.84 \times 10^{-6} \text{ s}^{-1}$ at 170°C.¹⁷ The deactivation energies calculated using Arrhenius' law were 177 kJ/mol and 136 kJ/mol for PHB and PHBV, respectively.

Effect of Isothermal Treatment on Crystallization of PHB and PHBV

The effects of isothermal treatment on crystallization of PHB and PHBV were analyzed using XRD. Figure 2 shows the crystal plane and crystallinities of PHB and PHBV powders and films without thermal treatment. Figures 3 and 4 show the crystal plane and crystallinities of PHB and PHBV powders and films, respectively, with isothermal treatment. The crystallinities of PHB and PHBV powders and films were correctly reproduced within 2% of the values reported in this work. A statistical analysis showed that the crystallinities of PHB and PHBV on temperature were significantly different from each other ($P < 0.05$). The crystallinity of PHB was higher than that of PHBV, as shown in Table II. The crystallinity of PHB or PHBV powder was higher than that of the PHB or PHBV film.

The peaks in Figure 3(1a,1b) for PHB with isothermal heating at 60°C and 100°C are similar to those in Figure 2(A).

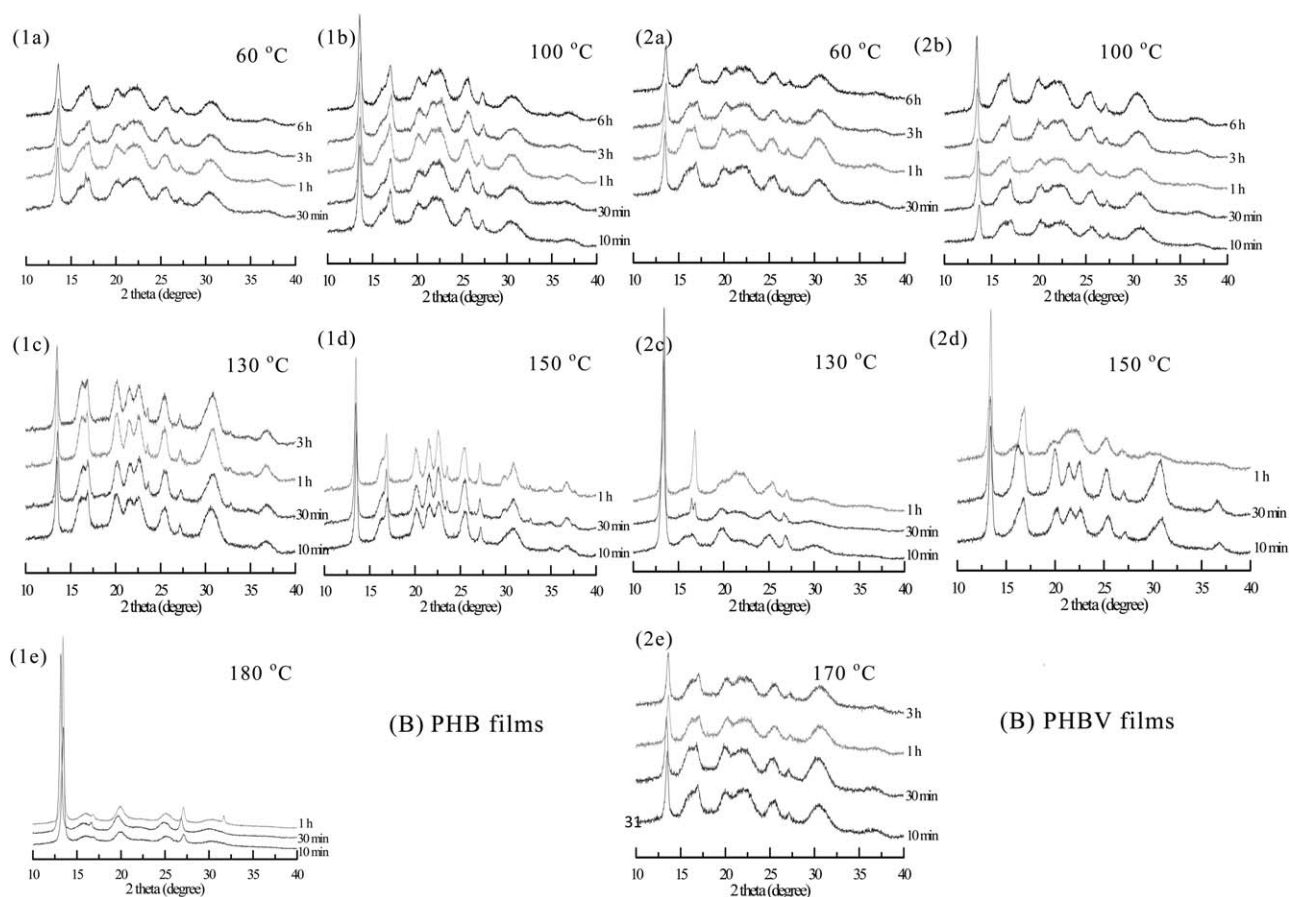


Figure 4. XRD diffractograms of (A) PHB and (B) PHBV films on different temperatures and times.

Table II. Effect of Isothermal Treatment on Crystallization of PHB and PHBV as Determined by XRD

Temperature (°C)	Heating time (min)	PHB		PHBV	
		Powder (%)	Film (%)	Powder (%)	Film (%)
Original		55.4	42.3	49.6	36.4
60	30	57.6	45.1	49.4	36.6
	60	58.3	43.9	52.8	34.5
	180	58.9	48.5	53.9	35.9
	360	57.9	43.5	50.7	36.6
100	10	55.5	36.8	52.3	40.3
	30	57.3	42.1	55.3	36.9
	60	57.3	40.6	49.0	40.8
	180	59.8	39.1	56.2	35.4
130	360	57.3	43.2	58.1	38.6
	10	57.6	38.9	56.7	37.1
	30	59.1	39.7	52.8	35.5
	60	59.5	40.7	55.9	38.2
150	180	62.4	36.7	57.3	37.0
	10	57.6	39.6	52.5	46.1
	30	61.1	46.2	57.3	48.4
	60	66.3	43.9	61.1	43.7
180 for PHB (170 for PHBV)	10	45.4		47.6	
	30	44.9		50.9	
	60	45.9		40.3	

The data was correctly reproduced within 2% of the values reported in this work.

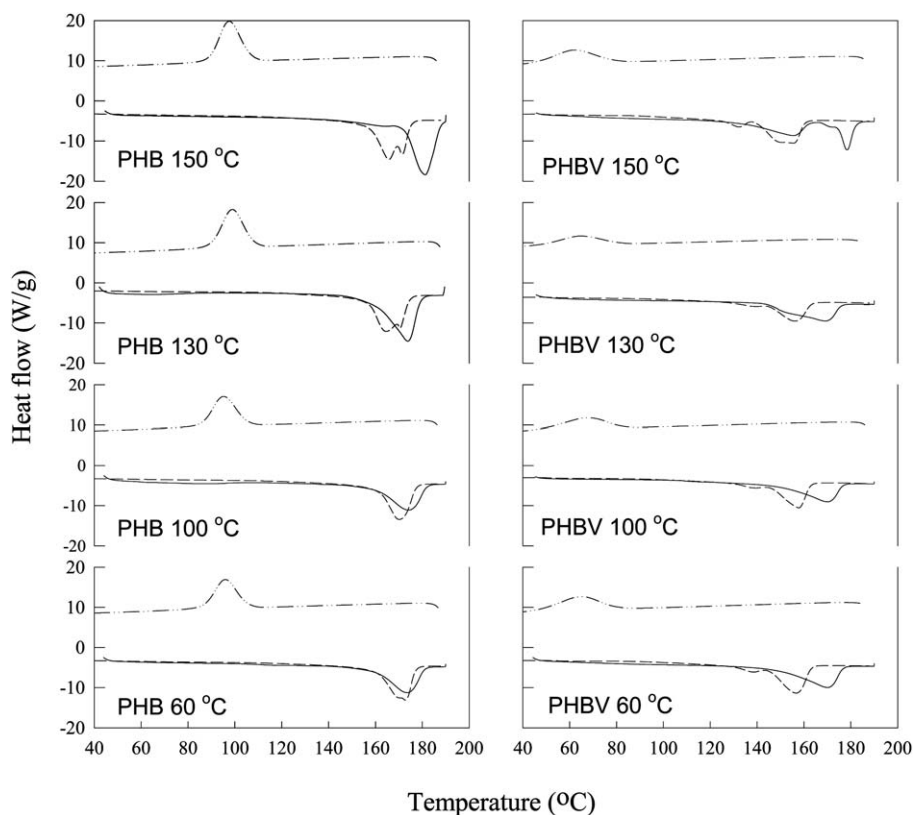


Figure 5. DSC thermograms of PHB and PHBV at different heating temperature for 1 h (scanning rate: 10 °C/min): solid-first heating; dash dot-cooling; dash-second heating.

However, the (101) and (111) peaks are sharper in Figure 3(1d) for PHB, and the (002) crystal plane ($2\theta = 30^\circ$) separated into two peaks at 130 °C after 30 min and 150 °C after 10 min. However, the crystal morphology of the (101) and (111) crystal planes changed at 180 °C after 10 min for PHB [Figure 3(1e)], and the crystallinity decreased.

The crystallinity trend of PHBV was similar to that of PHB. The peaks became obvious with increasing temperature, as shown in Figure 3(2c,2d). The (002) crystal plane ($2\theta = 30^\circ$) also had higher crystallinity and separated at 130 °C and 150 °C. The peak of the (101) and (111) crystal planes broadened at 170 °C after 10 min for PHBV, as shown in Figure 3(2e).

The crystallinities of PHB and PHBV were calculated using eq. (1), and are listed in Table II. The crystallinities of PHB and PHBV increased in conjunction with the temperature from 130 to 150 °C. The crystallinity decreased rapidly at 180 °C for PHB and 170 °C for PHBV. This occurred because PHB and PHBV melted at these temperatures. In addition, the M_w s of PHB and PHBV decreased considerably, as shown in Table I.

The crystallinities of PHAs were higher at 150 °C than those at other temperatures. It was difficult to dissolve PHAs of higher crystallinity in the CHCl_3 solution. Gogolewski et al. (1993) proposed a two-stage process for the non-isothermal degradation of polymers.⁴ First, the chain scission occurs in the

amorphous regions of a polymer. The ongoing chain scission results in a decline in the degree of entanglement, which enhances the mobility of non-degraded chain segments in these regions and promotes their recrystallization. Second, the crystallinity may be reduced using hydrolysis to degrade the chains in the crystalline regions; however, these stages cannot be strictly distinguished. The results of this study are consistent with those obtained by Gogolewski et al.⁴

Figure 4 shows XRD diffractograms of PHB and PHBV films at various treatment temperatures and times. After film processing, the crystallinity of PHB and PHBV decreased. The high crystallinity appears during the temperature range from 130 °C to 150 °C.

Figure 5 shows DSC thermogram for PHB and PHBV at various temperatures for heating 1 h. The melting temperatures (T_m) of PHB and PHBV during the first heating are 174 °C and 172 °C, respectively. Two T_m 's (170 °C and 156 °C) are present during the second heating process. The crystallization Temperature (T_c) of PHB and PHBV are around 99 °C and 67 °C, respectively. Because of less crystallization time for the PHA sample, the crystallized sample with less perfect crystals will melt at lower temperature during the second heating than the T_m of the first heating process. The peak of T_m for first heating shift to right with increasing heating temperature, and otherwise, that for second heating shifts to left.

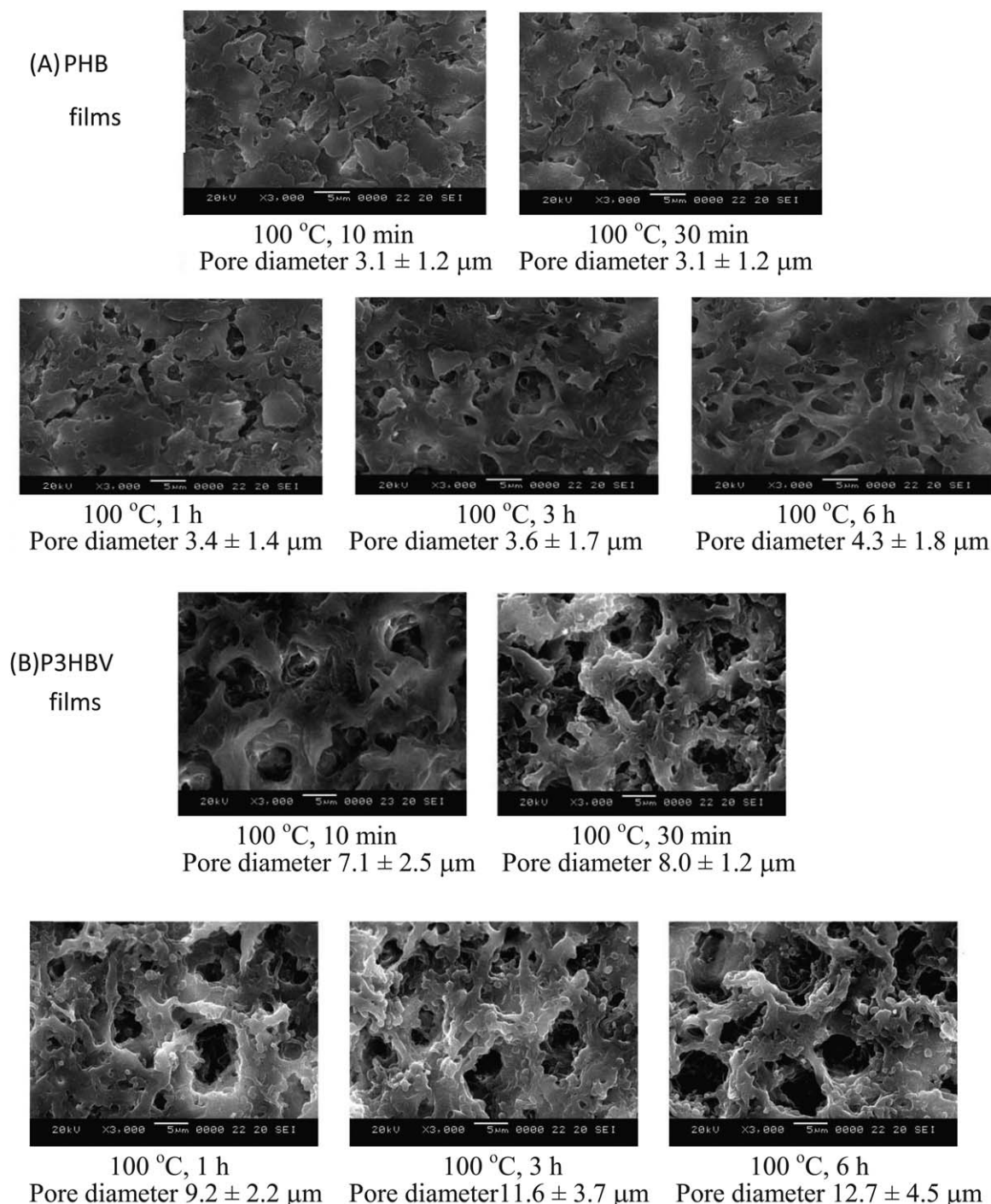


Figure 6. SEM micrographs and surface pore size of (A) PHB and (B) PHBV films heated at different times at 100°C.

Effect of Isothermal Treatment on the Surface Morphology of PHB and PHBV Films

PHB and PHBV of high M_w are suitable for various medical and environmental applications, due to biodegradability, biocompatibility, and non-cytotoxicity in films and scaffolds. Therefore, the surface properties of PHB and PHBV films after thermal treatment were examined. The puncture force of the

PHB film was approximately 108 kPa at a thickness of 16 μm , which is slightly higher than that reported by Parra et al.²⁷ The attachment of the cells on film is dependent on the surface pore size. The pore diameters on the surface of PHB and PHBV casting films were measured using SEM (Figure 6). PHB films had a smaller pore diameter than PHBV. The pore diameter increased in conjunction with isothermal heating time at 100°C.

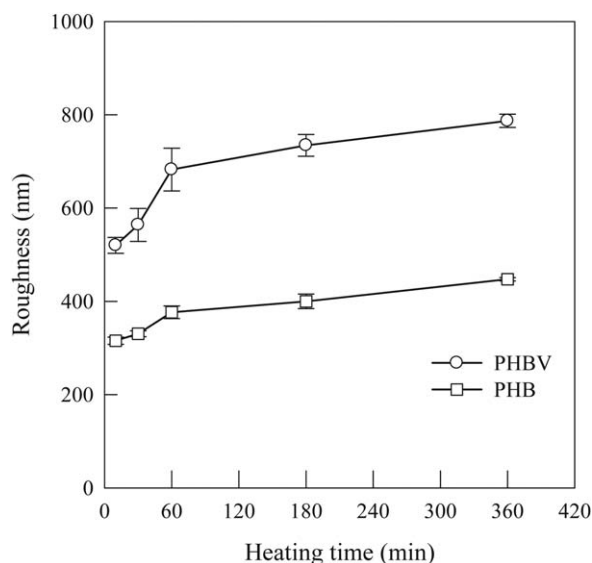


Figure 7. Effect of isothermal treatment on roughness of PHB and PHBV films heated at 100°C as detected using AFM.

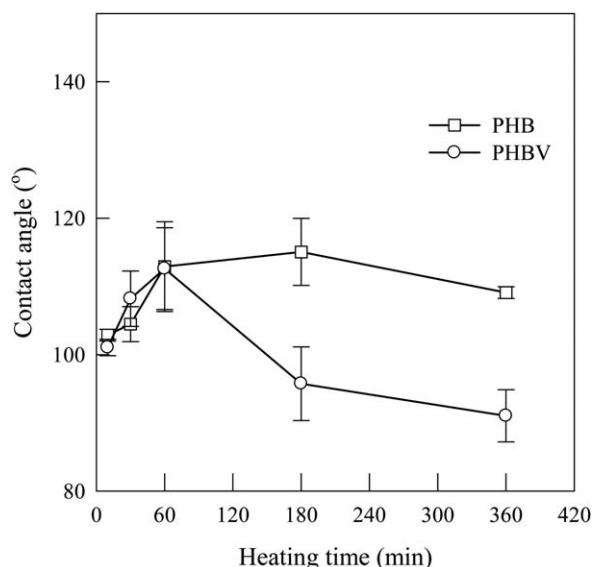


Figure 8. Effect of isothermal treatment on contact angle of PHB and PHBV films heated at 100°C.

The attachment of the cells on the film is also dependent on the surface roughness; and was measured using AFM. Figure 7 shows the roughness of PHBV that was higher than that of PHB. The surface roughness increased with heating time at 100°C. The degradation trend of both PHB and PHBV decreased after heating at 100°C for 1 h.

Hydrophilicity on the film surfaces for cell attachment plays a crucial role in cell growth on film and therefore the contact angle was measured. Figure 8 shows the contact angles for PHB and PHBV films and increased with a heating time (1 h) at 100°C. The hydrophilicity of PHB and PHBV decreased before heating 1 h because their crystallinity increased, and increased after heating 1 h because the surface roughness is increased, and they were degraded (Figure 6)

CONCLUSION

This study examined the chemical, physical thermal properties of PHB and PHBV on heat treatment. The results indicated that the properties of PHB and PHBV were not changed at temperatures below 60°C. High heating temperatures significantly increase PHB and PHBV degradation. Result of this study strongly suggests that PHAs must be dried and processed at heating temperatures below 60°C to minimize polymer degradation properties.

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