Macromol. Symp. **2005**, *224*, 11-19

Mechanical Properties of Uniaxially Cold-Drawn Films of Poly[(*R*)-3-hydroxybutyrate] and Its Copolymers

Tadahisa Iwata,[*1] *Yoshiharu Doi*[1,2]

[1] Polymer Chemistry Laboratory, RIKEN Institute, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan
Fax: (+81) 48 462 4667; E-mail: tiwata@riken.jp
[2] Department of Innovative and Engineered Materials, Tokyo Institute of Technology, 4259 Nagatsuta, Midori-ku, Yokohama 226-8502, Japan

Summary: Uniaxially oriented films of poly[(*R*)-3-hydroxybutyrate] (P(3HB)) and two kind of copolymers, poly[(*R*)-3-hydroxybutyrate-*co*-8%-[*R*]-3-hydroxyvalerate] (P(3HB-*co*-8%-3HV)), and poly[(*R*)-3-hydroxybutyrate-*co*-[*R*]-5%-3-hydroxyhexanoate] (P(3HB-*co*-5%-3HH)), were prepared by cold-drawing from amorphous preforms at temperatures near to the respective glass transition temperatures. Melt-quenched films in a rubber state could be stretched reproducibly to a draw ratio of 500%~1800%, and subsequent annealing under tension led to improvement of the tensile strength and Young's modulus. Two-step drawing resulted in further improvement of the mechanical properties. The mechanical properties remained unchanged after storing for 6 months at room temperature, suggesting that high orientation and crystallinity suppress the secondary crystallization.

Keywords: cold-drawn films; crystal orientation; mechanical properties; poly[(*R*)-3-hydroxybutyrate] and its copolymers; X-ray diffraction

Introduction

Poly([*R*]-3-hydroxybutyrate) (P(3HB)) is accumulated by a wide variety of microorganisms as intracellular carbon and energy storage material.[1] P(3HB) and its copolymers have been extensively studied as a biodegradable and biocompatible thermoplastic.[2-4] However, the mechanical properties are rather low and deteriorate due to a process of secondary crystallization, thereby limiting the range of application.[5,6] Some groups have attempted to improve the mechanical properties of P(3HB) films. Holmes tried to obtain uniaxial and biaxial oriented films of P(3HB) to improve the mechanical properties.[4] However, the P(3HB) films were difficult to draw uniaxially and biaxially. De Koning et al. reported that the stiffness and brittleness of P(3HB) film was avoided by an annealing procedure at elevated temperatures.[7] An alternative method of improving the ductility of brittle P(3HB) films is a cold-drawing procedure, which was reported by Barham and Keller.[8] However, the authors did not report on the

DOI: 10.1002/masy.200550602

reproducibility of the cold-rolling procedure. In the case of fibers, three research groups have succeeded in obtaining melt-spun fibers with a tensile strength of 190-330 MPa from bacterial P(3HB).[9-11] However, the strength of films and fibers was not enough for industrial and medical application.

Recently, we succeeded in obtaining films with good mechanical properties by the hot-drawing of ultra-high-molecular-weight P(3HB) (UHMW-P(3HB)) produced by a recombinant *Escherichia coli*.[12-15] The hot-drawing process is, however, limited by molecular weight, whereas new techniques of cold-drawing and two-step drawing allow the application against wild-type P(3HB) with relatively low molecular weight.[16] These methods yielded useful films with mechanical properties similar to common plastics. The cold-drawing and two-step-drawing methods are very attractive for obtaining a strong UHMW-P(3HB) fiber with a tensile strength of 1.32 GPa, an elongation to break of 35 %, and Young's modulus of 18 GPa.[17]

A second strategy to improve the mechanical properties of P(3HB) is copolymerization with different secondary monomer units. Poly([*R*]-3-hydroxybutyrate-*co*-[*R*]-3-hydroxyvalerate) (P(3HB-*co*-3HV)) is a successful copolyester, which has been produced commercially under the trade name of Biopol. P(3HB-*co*-3HV), however, is a relatively unusual copolymer because 3HB and 3HV units are isodimorphous, i.e. due to similarity in shape and size, the 3HV units are incorporated into the P(3HB) crystal-lattice.[18] To produce copolymers with improved properties, copolymerization of 3HB with longer chain hydroxyalkanoic acids, which cannot fit into a crystalline lattice with each other and which therefore avoid isodimorphism, is necessary. An example for this type of copolymer showing the exclusion of the longer chain monomer is copolymers of 3HB and 3-hydroxyhexanoate (3HH).[19] Poly([*R*]-3-hydroxybutyrate-*co*-[*R*]-3-hydroxyhexanoate) (P(3HB-*co*-3HH)) shows high elongation to break, but low tensile strength. As we have shown, cold-drawing is a good technique to improve mechanical properties.[16,17] Therefore, at first we performed cold-drawing against P(3HB-*co*-3HH) films to combine the benefits of cold-drawing and copolymerization.[20] More recently, we succeeded in obtaining uniaxially cold-drawn P(3HB-*co*-3HV) films with high tensile strength.

In this paper, we describe the processing and structures of P(3HB), P(3HB-*co*-3HV), and P(3HB-*co*-3HH) films with high tensile strength by cold-drawing from amorphous preforms.

Experimental

Materials. The P(3HB) (weight-average molecular weight (M_w) = 0.41 $\times 10^6$ and polydispersity (DPI) = 2.6) sample was supplied by Monsanto. The glass transition temperature (T_g) and melting temperature (T_m) of P(3HB) is 4 ˚C and 177 ˚C, respectively. The poly[(*R*)-3-hydroxybutyrate-*co*-8%-(*R*)-3-hydroxyvalerate) (P(3HB-*co*-8%-3HV)) with $M_w = 1.0 \times 10^6$ and DPI = 2.8 was supplied by Monsanto. The T_g and T_m of P(3HB-*co*-8%-3HV) is -4.3 ˚C and 143 ˚C, respectively. The poly[(*R*)-3-hydroxybutyrate-*co*-5%-(*R*)-3-hydroxyhexanoate) (P(3HB-*co*-5%-3HH)) with $M_w = 0.82 \times 10^6$ and DPI = 2.2 was supplied by Procter & Gamble Company. The T_g and T_m of P(3HB-*co*-5%-3HH) is 4 ˚C and 144 ˚C, respectively. All samples were purified before investigation by dissolving in chloroform, filtering, and reprecipitating with *n*-hexane. All solvents were obtained from Kanto Chemical Co. and used without prior purification.

Cold-drawing, two-step-drawing and annealing. The films were prepared by a conventional solvent-casting technique from a chloroform solution using glass petri dishes as a casting surface. The amorphous preforms of P(3HB) films were prepared by the melting of solvent-cast films in a hot press at 200 ˚C for 30 sec and subsequently quenching in iced water. In the cases of P(3HB-*co*-8%-3HV) and P(3HB-*co*-5%-3HH), the temperatures of the hot press were set at 180 ˚C and 160 ˚C, respectively. These amorphous preforms were oriented by cold-drawing 200% - 1800% of their initial length in iced water and annealed in an autoclave at 50 ˚C - 160 ˚C with weak tension to increase the crystallinity. In the case of two-step drawing, before the annealing procedure, further drawing at room temperature was applied against cold-drawn films. All samples were used after aging for at least 3 days at room temperature.

X-ray diffraction. X-ray fiber diagrams of films were recorded in an evacuated flat-pale camera on Fuji Imaging Plate BAS-SR 127, using Ni-filtered Cu-Kα radiation with a Rigaku RINT UltraX 18 type X-ray generator operated at 40 kV and 110 mA. Crystal orientation was evaluated from the width of the azimuthal direction of the (020) reflection.

Stress-strain test. Stress-strain tests of films were performed at room temperature with a strain rate of 20 mm/min on an Imada tensile machine model SV-50. A specimen length of 30 mm and width of 3 mm were used.

Molecular weight measurement. Molecular weight distribution data of P(3HB) samples were obtained by gel-permeation chromatography (GPC) at 40 ˚C, using a Shimadzu 10A

GPC system and a 6A refractive-index detector with joint columns of Shodex K-802 and K806M. Chloroform was used as an eluent at a flow rate of 0.8 mL/min, and a sample concentration of 0.5 mg/mL was applied. Eight polystyrene standard samples with low polydispersity ($M_p = 1.3 \times 10^3 - 6.5 \times 10^6$) were used to construct a calibration curve.

Results and Discussion

Mechanical properties of cold-drawn and annealed films. Independent of molecular weight and sample, the cold-drawing of all melt-quenched films (amorphous preform) of P(3HB), P(3HB-*co*-8%-3HV) and P(3HB-*co*-5%-3HH) succeeded easily and reproducibly at a temperature below, but near to, the glass transition temperature of 4 °C in iced water. The amorphous preform of film was transparent, while during cold-drawing the oriented films showed slightly translucent, indicating that the crystallization together with chain orientation occurs near to the glass transition temperature. The films were easily drawn at very low stress by more than 500%, but elastic recovery occurred on release from the stretching machine. Accordingly, the annealing procedure is required for fixing the extended polymer chains. The maximum values of tensile strength were obtained at 75 °C for P(3HB) films, at 75 °C for P(3HB-*co*-8%-3HV) films, and at 120 °C for P(3HB-*co*-5%-3HH) cold-drawn films.

Figure 1 shows the tensile strength of cold-drawn and annealed films of P(3HB), P(3HB-*co*-8%-3HV), and P(3HB-*co*-5%-3HH), as functions of the draw ratio. The tensile strength

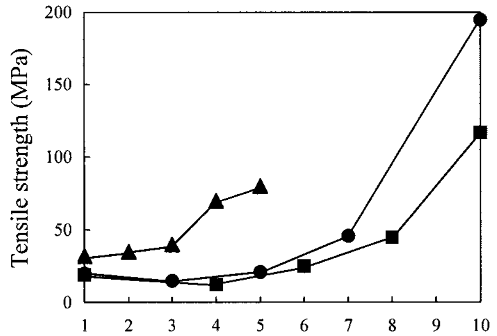

Figure 1. Tensile strength of cold-drawn and annealed films, as functions of draw ratio: (●) P(3HB) cold-drawn films annealed at 75 °C for 2 h, (■) P(3HB-*co*-8%-3HV) cold-drawn films annealed at 75 °C for 2 h, and (▲) P(3HB-*co*-5%-3HH) cold-drawn films annealed at 120 °C for 2 h.

of P(3HB) and P(3HB-*co*-8%-3HV) films was drastically increased up to 195 Mpa and 117 Mpa, respectively, when the film was stretched at the draw ratio of 10 and annealed at 75 °C for 2 h. In the case of P(3HB-*co*-5%-3HH) films, melt-quenched films in a rubber state could be stretched reproducibly to a draw ratio of 5. The 5 times cold-drawn films has the tensile strength of 80 Mpa with high elongation to break of 258%. Mechanical properties of cold-drawn films are summarized in Table 1, together with crystal orientation and crystallinity.

The crystal structure, crystal orientation and crystallinity of cold-drawn and annealed films were determined by X-ray fiber diagrams as shown in Figure 2. All reflections of X-ray fiber diagrams were indexed with orthorhombic unit cell parameters of P(3HB) homopolymer (α-form: a = 0.576 nm, b = 1.320 nm and c(fiber axis) = 0.596 nm, $P2_12_12_1$ space group) as reported by Yokouchi et al.[21] and by Okamura and Marchessault,[22] indicating that all cold-drawn and annealed films have the same crystal structure.

The crystal orientation evaluated from the width in the azimuthal direction of the (020) reflection and the crystallinity of cold-drawn and annealed films are summarized in Table 1, together with mechanical properties. The crystal orientation in 10 times cold-drawn P(3HB) and P(3HB-*co*-8%-3HV) films is 0.97 and 0.95, respectively, and the crystallinity is 80% and 77%, respectively. It is difficult to allow the comparison between these values and that of 5 times cold-drawn P(3HB-*co*-5%-3HH) films. However, the crystallinity (47%) of 5 times cold-drawn P(3HB-*co*-5%-3HH) is much lower than those of P(3HB) and P(3HB-*co*-8%-3HV), despite the crystal orientation (0.87) being slightly lower. These results indicate that the molecular chains and lamellar crystals of P(3HB-*co*-5%-3HH) are oriented by cold-drawing; however, the lamellar crystals are very thin. Thus, the amorphous regions between lamellar crystals exist much in these cold-drawn films, and these unrestricted molecular chains in amorphous regions generate high elongation to break (258%).

Mechanical properties of two-step-drawn and annealed films. Two-step drawing was achieved against cold-drawn films to the further improvement of mechanical properties. Thus, 5 or 10 times cold-drawing was applied at iced water temperature against an amorphous preform; then the cold-drawn film was kept at room temperature for several minutes to increase the crystallinity. This slight crystalline film was further stretched by 1.4 ~ 2.5 times at room temperature, and then annealed. The tensile strength of P(3HB) increased 50% by this two-step drawing procedure to reach 287 MPa with an elongation to

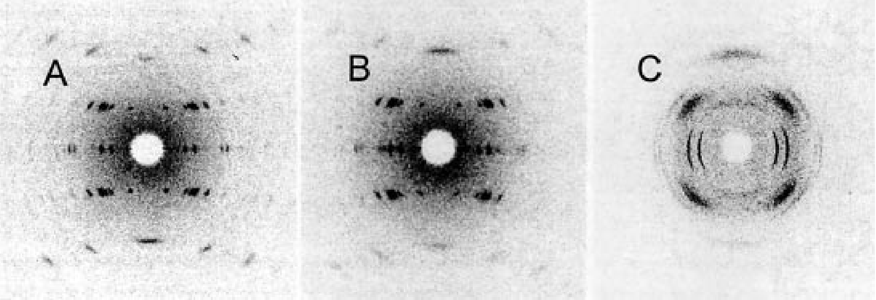

Figure 2. X-ray fiber diagrams of cold-drawn and annealed films: (A) 10 times cold-drawn P(3HB) film annealed at 75 °C for 2 h, (B) 10 times cold-drawn P(3HB-*co*-8%-3HV) film annealed at 75 °C for 2 h, and (C) 5 times cold-drawn P(3HB-*co*-5%-3HH) film annealed at 120 °C.

Table 1. Mechanical properties, crystal orientation, and crystallinity of P(3HB), P(3HB-*co*-8%-3HV), and P(3HB-*co*-5%-3HH) cold-drawn and annealed films.

Sample	Draw ratio (λ)	Tensile strength (Mpa)	Elongation to break (%)	Young's modulus (Gpa)	Crystal orientation	Crystallinity (%)
P(3HB)[a]	1	15	5	0.5	-	60
	10	195	100	2.0	0.97	80
	15[d]	287	53	1.8	0.97	>85
P(3HB-*co*-8%-3HV)[b]	1	19	35	0.1	-	53
	10	117	109	0.5	0.95	77
	14[e]	185	63	1.4	0.94	82
P(3HB-*co*-5%-3HH)[c]	1	32	267	0.5	-	42
	5	80	258	0.9	0.87	47
	10[f]	140	116	1.5	0.92	65

[a] P(3HB): Poly[®-3-hydroxybutyrate], all cold-drawn films were annealed at 75 °C for 2 h.

[b] P(3HB-*co*-8%-3HV)): Po®(*R*)-3-hydroxybutyrate-®8%-(*R*)-3-hydroxyvalerate], all cold-drawn films were annealed at 75 °C for 2 h.

[c] P(3HB-*co*-5%-3HH)): Poly[(*R*)-3-hydroxybutyra®*co*-5%-(*R*)-3-hydroxyhexanoate], all cold-drawn films were annealed at 120 °C for 2 h.

[d] Total draw ratio after two-step-drawing; first drawing is 10 times and second drawing is 1.5 times.

[e] Total draw ratio after two-step-drawing; first drawing is 10 times and second drawing is 1.4 times.

[f] Total draw ratio after two-step-drawing; first drawing is 4 times and second drawing is 2.5 times.

break of 53%. The tensile strength of P(3HB-*co*-8%-3HV) and P(3HB-*co*-5%-3HH) increased 117 to 185 MPa and 80 to 140 MPa, respectively. Mechanical properties of cold-drawn films are summarized in Table 1, together with crystal orientation and crystallinity. Figure 3 shows the X-ray fiber diagrams of two-step drawn films. One sees the new reflection on the equatorial line of Figure 3(A) and 3(B), derived from the planar zigzag conformation (β-form) of the P(3HB) chain, together with α-form reflections. This β-form reflection was observed in a strong P(3HB) fiber produced by combining methods of cold-drawing and two-step-drawing.[17] The β-form seems to be introduced from the orientation of free chains in amorphous regions between α-form lamellar crystals; that is, the development of a β-form is not really a transformation from the α-form. This conclusion supports the proposal model of Clark modified by Orts et al.[23] that the noncrystalline domains are transformed to the extended chain conformation during cold-drawing. In our case, by two-step drawing at room temperature, molecular chains between lamellar crystals were strongly extended and as a result planar zigzag conformation was generated. The high elongation to break of cold-drawn film seems to be due to the alignment of polymer chains parallel to the drawing direction in amorphous regions between lamellar crystals and the "molecular spring" phenomenon of polymer chains with extended conformation.

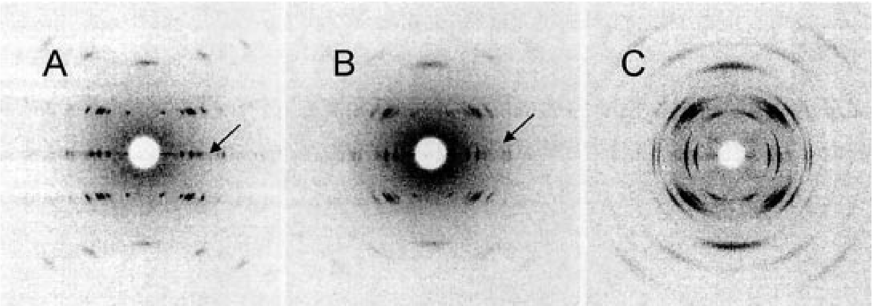

Figure 3. (A) X-ray fiber diagram of P(3HB) two-step-drawn and annealed film. Two-step-drawing of 1.5 times at room temperature was applied to 10 times cold-drawn film, which was then annealed at 75 ˚C for 2 h. Arrow indicates the equatorial reflection derived from β-form. (B) X-ray fiber diagram of P(3HB-*co*-8%-3HV) two-step-drawn and annealed film. Two-step-drawing of 1.4 times at room temperature was applied to 10 times cold-drawn film, which was then annealed at 75 ˚C for 2 h. Arrow indicates the equatorial reflection derived from β-form. (C) X-ray fiber diagram of P(3HB-*co*-5%-3HH) two-step-drawn and annealed film. Two-step-drawing of 2.5 times at room temperature was applied to 4 times cold-drawn film, which was then annealed at 120 ˚C for 2 h.

Table 2. Mechanical properties of cold-drawn and annealed P(3HB) films stored for 7 days and 4 months.

Aging time (days)	Tensile strength (MPa)	Elongation to break (%)	Young's modulus (GPa)
7	195	100	2.0
120	198	95	2.2

In the case of P(3HB-*co*-5%-3HH) two-step-drawn film, one can not see a *β*-form reflection, as shown in Figure 3(C). This result indicates that tie molecular chains in amorphous region could not be strongly extended, because of low crystallinity and of 3HH units excluded from the P(3HB) lamellar crystals. However, the high elongation to break of two-step-drawn film (ca. 100%) seems to be due to the alignment of polymer chains parallel to the drawing direction in amorphous regions between lamellar crystals.

Effect of storage time on mechanical properties. It is well known that the mechanical properties of P(3HB) and its copolymer films markedly deteriorate due to stiffness and brittleness by a process of secondary crystallization. The cold-drawn and annealed films of P(3HB), P(3HB-*co*-8%-3HV), and P(3HB-*co*-5%-3HH) were stored for 4 months at room temperature to study the time dependent change of the mechanical properties, and the stress-strain test was performed. The tensile strength and elongation to break of cold-drawn and annealed P(3HB) films remained unchanged for 4 months as shown in Table 2. The tensile strength of P(3HB-*co*-8%-3HV) and P(3HB-*co*-5%-3HH) also remained unchanged for 4 months (data not shown). It is of importance to note that the mechanical properties of the cold-drawn and annealed film did not deteriorate over the 4 month period. It is concluded that a highly oriented and crystallized P(3HB) and its coplymer films keep superior mechanical properties for long periods.

Conclusion

Uniaxially oriented films of P(3HB), P(3HB-*co*-8%-3HV), and P(3HB-*co*-5%-3HH) were produced by cold-drawing. Melt-quenched film (amorphous preform) was easily and reproducibly stretched at a temperature below, but near to, the glass transition temperature of 4 °C, independent of molecular weight and second monomer units. The cold-drawn and annealed film had acceptable mechanical properties with high tensile strength and elongation to break. The mechanical properties were even more improved by two-step orientation at room temperature. Mechanical properties of two-step-drawn films increased not only with the orientation of the crystal domains formed by the 2_1 helix conformation (α-form) but also with generating the planar zigzag conformation (β-form).

In addition, the mechanical properties did not deteriorate over a 4 month time period. It has been suggested that the embrittlement of P(3HB) and its copolymer films due to secondary crystallization is avoided by its high crystallinity and high degree of orientation. It is possible to produce ductile P(3HB) and its copolymer sheets with high mechanical properties reproducibly by cold-drawing and annealing processes, independent of the molecular weight.

Acknowledgements

This work has been supported by a Grant-in Aid for Young Scientists (A) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan (No. 15685009), by a SORST (Solution Oriented Research for Science and Technology) grant from Japan Science and Technology Corporation (JST), and by the grant for Ecomolecular Science Research, RIKEN Institute.

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