Effect of Orotic Acid as a Nucleating Agent on the Crystallization of Bacterial Poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) Copolymers

Nicolas Jacquel,[1,2] Koichirou Tajima,[2] Nobuo Nakamura,[3] Toshio Miyagawa,[4] Pengju Pan,[2] Yoshio Inoue[2]

[1]*Chemistry and Process Engineering Department, Ecole Supérieure de Chimie, Physique et Electronique de Lyon, France*
[2]*Department of Biomolecular Engineering, Tokyo Institute of Technology, Midori-ku, Yokohama 226-8501, Japan*
[3]*Frontier Materials Development Laboratories, Kaneka Corp., 5-1-1 Torikai-Nishi, Settsu, Osaka 566-0072, Japan*
[4]*Foam Plastics Technology Laboratories, Kaneka Corp., 5-1-1 Torikai-Nishi, Settsu, Osaka 566-0072, Japan*

ABSTRACT: The kinetics of crystallization induced by orotic acid (OA) and boron nitride (BN) as nucleating agents were investigated for bacterial poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate)s (P(HB-*co*-HH)s) containing from 0 to 18% HH monomer units. The nucleation efficiency of these two chemicals was investigated by differential scanning calorimetry (DSC) and polarized optical microscopy (POM). It was found that both orotic acid and boron nitride are able to nucleate the crystallization of PHB. In the case of P(HB-*co*-HH) copolymers, orotic acid showed an outstanding nucleating effect. The comparison of half-crystallization times shows that for P(HB-*co*-10% HH), the crystallization initiated by orotic acid is more than three time faster than the one induced by boron nitride ($t_{1/2BN}/t_{1/2OA}(60°C) = 3.7$ and $t_{1/2BN}/t_{1/2OA}(90°C) = 4.5$). According to the fact that orotic acid is a biodegradable, biocompatible and a nontoxic chemical, this nucleating agent is a promising solution for PHAs used in medical applications such as implants. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 114: 1287–1294, 2009

Key words: boron nitride; crystallization; nucleation; orotic acid; poly(3-hydroxybutyrate-co-3-hydroxyhexanoate)

INTRODUCTION

Due to their convenient physical properties, polymer materials are useful for a large range of applications. Because most of these plastics are used during short periods, biodegradable polymers produced from renewable resources have been envisaged. For that purpose, different kinds of polyesters, such as bacterial poly(3-hydroxyalkanoates) (PHAs), have been intensively studied these last decades. Unfortunately, the most common of these polymers, poly(3-hydroxybutyrate) (PHB), has two major drawbacks: its brittleness and its thermal degradation around its melting temperature.[1] This brittleness has been attributed by several research groups to the morphology of PHB crystals (production of large spherulites) and to the secondary crystallization of the polymer.[2–4]

To solve this problem and to improve the polymer physical properties, PHA copolymers were investigated. One of the first copolymer studied, poly(hydroxybutyrate-*co*-hydroxyvalerate) (P[HB-*co*-HV]),

did not show much improvement. In fact, as HB and HV (3-hydroxyvalerate) monomers are quite similar, a high crystallinity is observed on the whole co-monomer composition range. This behavior has been attributed to the co-crystallization (or isodimorphism phenomenon) of HB and HV monomers.[5–10] Significant improvements were obtained by using 3HH (3-hydroxyhexanoate) monomers (see chemical structure in Fig. 1): Doi et al.[6] reported a decrease of the crystallinity from 60% to 18% and an increase of the elongation at break from 5% to 850%. Unfortunately, the crystallization of P(HB-*co*-HH)s containing high HH contents (>10%) is very slow, which make them difficult to use in industry. Moreover, it has been shown that the induction of the enzymatic degradation of such polyesters requires the presence of a crystalline phase or a rigid phase. Therefore its biodegradability will be affected by crystallinity, spherulite morphology, and glass transition.[11–14]

To improve the morphology of PHAs crystals and to accelerate the crystallization, nucleation mechanisms have been studied. Barham et al. from the University of Bristol first investigated the nucleation of PHB[15] and P(HB-*co*-HV)[16] with saccharine[15,17,18] and nitrogen compounds.[19] Later, more conventional chemicals such as boron nitride or talc[20–22] were

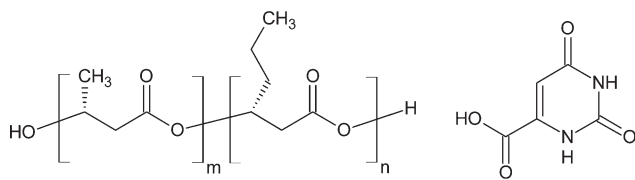*Correspondence to:* Y. Inoue (inoue.y.af@m.titech.ac.jp).

Figure 1 Chemical structure of P(HB-co-HH) and orotic acid.

shown to have higher nucleation efficiency. The addition of such nucleating agents increase both crystallization temperature and crystallization rate, but some of these components are undesirable in finished articles that may come in contact with humans, animals, or the environment; that is why the nucleating agent must by carefully chosen. More recently, Inoue et al. from the Tokyo Institute of Technology have studied green and nontoxic substitution chemicals for the nucleation of PHAs. These chemicals include cyclodextrins[23,24] or ligin.[25] To date, no nucleants have shown better nucleating abilities than boron nitride for PHB and its copolyesters.

In this study, different P(HB-co-HH) samples with different monomeric unit contents were nucleated with orotic acid (Fig. 1), a green chemical manufactured in the body by intestinal flora. As far as we know, the nucleation effect of orotic acid is first reported in the present work. For comparison purposes, studies on isothermal and nonisothermal crystallization of P(HB-co-HH) copolymers containing boron nitride and orotic acid were realized.

EXPERIMENTAL SECTION

Materials and sample preparation

The P(HB) homopolymer sample and a series of bacterial P(HB-co-HH) copolymers with HH unit content of 5, 10, and 18 mol % were supplied by Sigma-Aldrich Chemie GmbH (Steinheim, Germany) and Kaneka Corporation, (Osaka, Japan) (Table I).

All polymer samples were purified by dissolution in hot chloroform and precipitated in cold ethanol before use.[26] Orotic acid monohydrate and boron nitride were, respectively, purchased from the Tokyo Chemical Industry (Japan) and Nacalai Tesque Inc. (Kyoto, Japan).

Films were prepared via a solvent casting method: The polymer was first dissolved in chloroform at 60°C under vigorous stirring during 3 h and was casted on Teflon Petri dishes. The solvent was then evaporated during 1 day at room temperature. For the nucleated samples, nucleating agents were dispersed by ultrasonication in chloroform and the purified polymer was added to the solution (the amount of nucleating agent used for all polymers

samples was 1 wt %). To create a homogenous material, the shift of nucleating agents during the film casting was reduced by using high polymer concentrations (100 g/L). Moreover, chloroform was selected by a solvent screening method[27] to have a good solvent for P(HB-co-HH) copolymers that could be easily removed by evaporation.

Differential scanning calorimetry

Crystallization kinetics were investigated with Pyris Diamond differential scanning calorimetry (DSC) (Perkin-Elmer, Yokohama, Japan) under a nitrogen atmosphere. For the nonisothermal crystallization, the 6- to 8-mg samples were melted at 190°C for 2 min to erase thermal history and then cooled to 50°C at the cooling rate of 10°C/min. The temperature was then held during 1 minute and the sample was reheated to 190°C at 10°C/min. For isothermal crystallization, samples were first heated to 190°C for 2 min and the temperature was rapidly decreased (100°C/min) to the predetermined crystallization temperature. The temperature was then held until completion of the crystallization ($t > 4t_{1/2}$, where $t_{1/2}$ is the half-crystallization time).

Polarized Optical Microscopy

Polarized optical microscopy (POM) observation of thin polymer films was performed with an Olympus BX90 polarizing microscope (Olympus Company, Tokyo, Japan) equipped with a digital camera. The thin films were realized by melting the polymer between the microscope coverslips at 190°C during 2 min and by quenching the sample to the predetermined temperature for isothermal crystallization. The temperature program was performed with a Mettler FP82HT hot stage.

RESULTS AND DISCUSSION

Nonisothermal crystallization

Figure 2 shows the DSC thermograms of nonisothermal melt crystallization of P(HB-co-HH) copolymers and their blends with nucleating agents. All the melt

TABLE I
Molecular Weight and Polydispersity of PHB and P(HB-co-HH)s Used in This Study

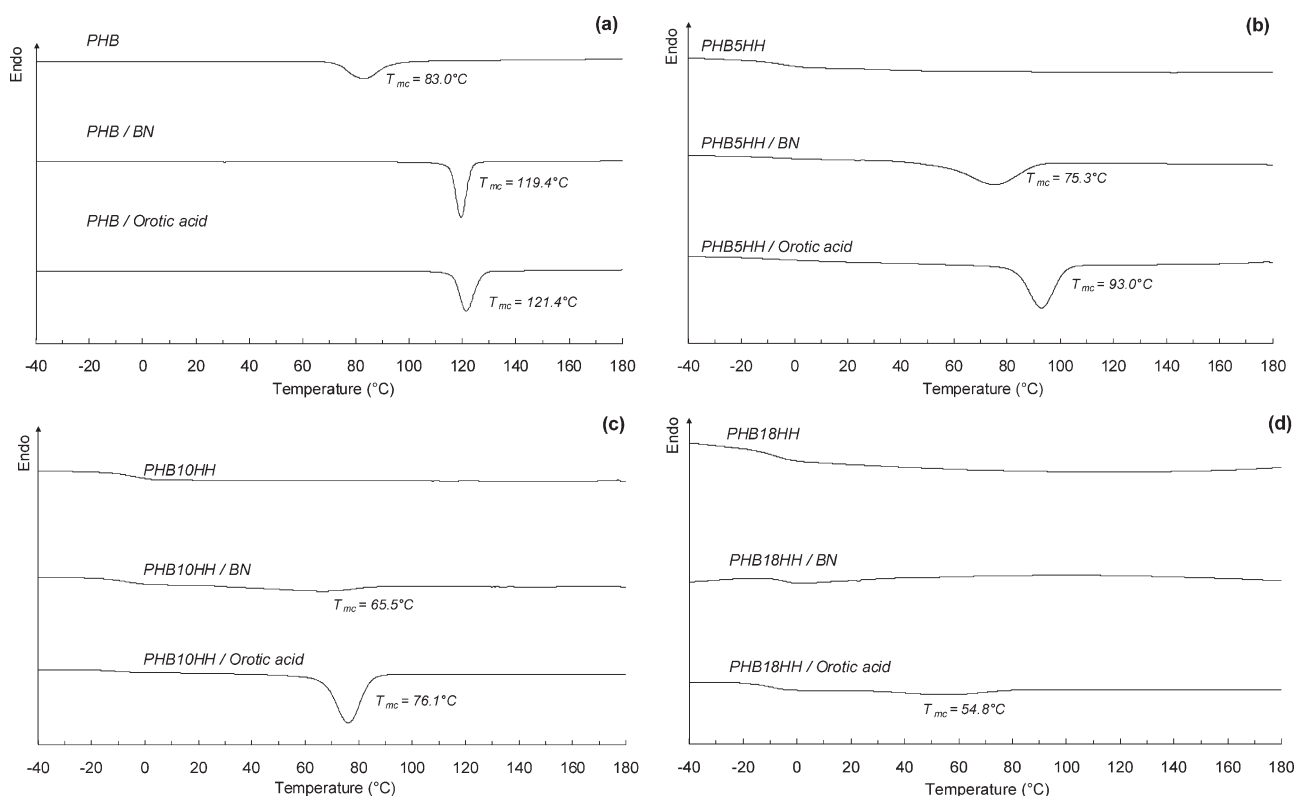| Sample code	Company	HH unit content (mol %)	M_w (× 10^{4}Da)	M_w/M_n
PHB	Aldrich	0.0	41.9	2.85
PHB5HH	Kaneka Corp.	5.0	35.5	2.02
PHB10HH	Kaneka Corp.	10.0	20.5	2.29
PHB18HH	Kaneka Corp.	18.0	28.6	2.40

Figure 2 DSC curves recorded during nonisothermal melt crystallization at 10°C/min of: (a) PHB, (b) PHB5HH, (c) PHB10HH, and (d) PHB18HH and their blends with boron nitride (BN, 1 wt %) and orotic acid (1 wt %).

crystallization temperatures (T_{mc}) and the enthalpies of crystallization (ΔH_{mc}) are summarized in Table II. By comparing the results obtained for neat polymers, it is obvious that only PHB is able to crystallize to a significant degree during the temperature scan. It has been shown by Doi et al.[6] that the degree of crystallinity of neat PHB-*co*-HH decreases rapidly with the comonomer content. In this case, the presence of only 5% HH monomer makes the melt crystallization not visible. On that subject, Chen et al.[11] reported that the crystallization of PHB-*co*-HH copolymers is too slow to be observed by nonisothermal crystallization at 10°C/min; some lower cooling rates such as 2.5°C/min should be used to that end.

These thermograms also show a general enhancement of the crystallization by adding nucleating

agents to the polymer. In Figure 2(a), the crystallization peaks of nucleated PHBs shifted to higher temperatures, which indicated a faster crystallization of the polymer. In addition, the enthalpy of crystallization of both PHB containing nucleating agents has been increased. Considering these two aspects, the effects of boron nitride and orotic acid on PHB are very similar. In the case of the copolymer samples, orotic acid demonstrates a good ability to increase both crystallization temperature and the degree of crystallinity, whereas boron nitride shows a much lower efficiency with copolymers containing more than 5% HH.

Figure 3 shows the heating scans of P(HB-*co*-HH) copolymer and their blends with nucleating agents obtained after melt crystallization. Table III shows

TABLE II
Nonisothermal Melt Crystallization Temperature and Enthalpy of P(HB-co-HH) at 10°C/min and Their Blends with 1 wt % of Nucleating Agent

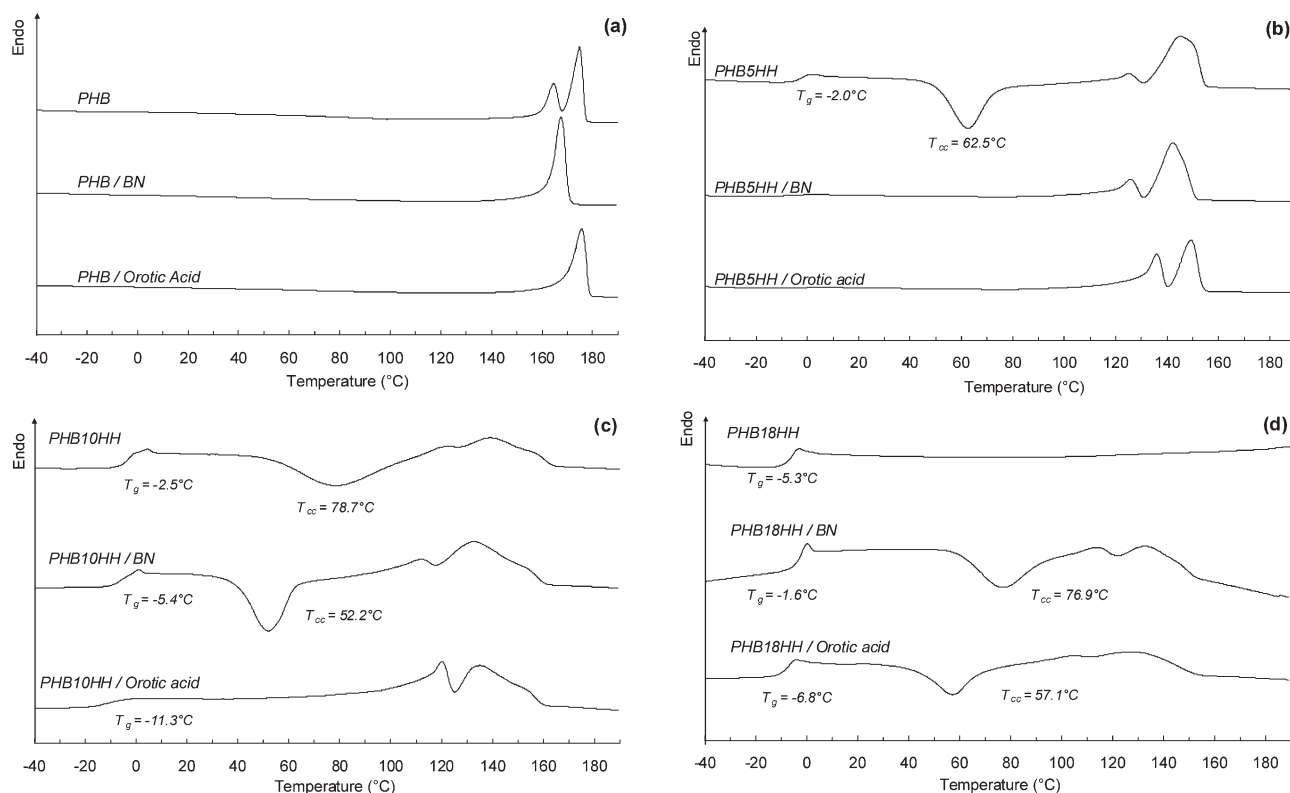|	PHB		P(HB-*co*-5%HH)		P(HB-*co*-10%HH)		P(HB-*co*-18%HH)	
Nucleating agent	T_{mc} (°C)	ΔH_{mc} (J/g)	T_{mc} (°C)	ΔH_{mc} (J/g)	T_{mc} (°C)	ΔH_{mc} (J/g)	T_{mc} (°C)	ΔH_{mc} (J/g)
None	83.0	65.5	None*	None	None	None	None	None
Boron nitride	119.4	81.0	75.3	40.7	65.5	8.9	None	None
Orotic acid	121.4	76.6	93.0	37.5	76.1	38.2	54.8	9.9

Figure 3 DSC heating scans after the nonisothermal melt crystallization (10°C/min) of: (a) PHB, (b) PHB5HH, (c) PHB10HH, and (d) PHB18HH and their blends with boron nitride (BN, 1 wt %) and orotic acid (1 wt %).

the cold crystallization temperatures (T_{cc}) and the enthalpies of crystallization (ΔH_{cc}) obtained in these heating scans. In some of these plots [Fig. 3(b–d)] cold crystallization peaks could be observed at temperatures higher than glass transition. This behavior is explained by the fact that the polymer did not have enough time to complete its whole crystallization during the cooling scan. On the opposite, for the samples shown in the Figure 3(a), the melt crystallization has been fully completed; that is why no glass transition is observed on the heating scan. By comparing the scans obtained with or without nucleating agents [Fig. 3(b,c)], it becomes obvious that the nucleating agents enhance the crystallization rate. Moreover, the fact that the P(HB-*co*-10% HH) blend sample containing orotic acid [Fig. 3(c)] did not

show any cold crystallization proves the higher nucleating efficiency of orotic acid in comparison with boron nitride. In the case of P(HB-*co*-18% HH) [see Fig. 3(d)], the presence of a glass transition combined with the absence of a crystallization peak suggests that the cold crystallization was too slow to be observed on a heating scan at 10°C/min. As in the previous study, the fact that cold crystallization temperatures (Table III) of samples containing nucleating agents have been shifted to lower values confirms that the crystallization rate has been increased.

The double melting peak observed in most of these cases has been identified in previous studies as the melting of primary crystals formed in the crystallization process (lower peak) and to the crystals

TABLE III
Cold Crystallization Temperature (T_{cc}) and Enthalpy (ΔH_{cc}) of PHB and P(HB-*co*-HH) at 10°C/min and Their Blends with Boron Nitride and Orotic Acid (Data Obtained from the DSC Curves Shown in Fig. 4)

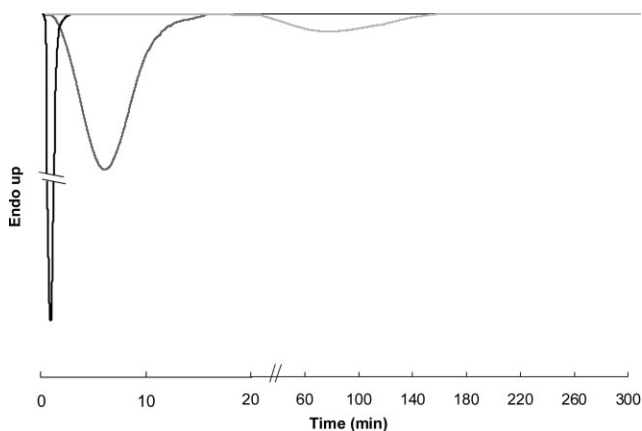| Nucleating Agent	PHB		P(HB-*co*-5%HH)		P(HB-*co*-10%HH)		P(HB-*co*-18%HH)	
	T_{cc} (°C)	ΔH_{cc} (J/g)	T_{cc} (°C)	ΔH_{cc} (J/g)	T_{cc} (°C)	ΔH_{cc} (J/g)	T_{cc} (°C)	ΔH_{cc} (J/g)
None	None*	None	62.5	44.3	78.7	23.0	None	None
Boron nitride	None	None	None	None	52.2	43.7	76.9	13.0
Orotic acid	None	None	None	None	None	None	57.1	5.8

Figure 4 Typical isothermal crystallization curves obtained for neat PHB, PHB5HH, and PHB18HH at 70°C.

that recrystallized during the heating.[22] According to the fact that the nucleated PHB shows only one melting peak, it could be deduced that the perfectness of polymer crystals have been enhanced. On the contrary, in the nucleated P(HB-*co*-HH) copolymers, the presence of two melting peaks could be explained by the fact that HH monomers (the minor monomer unit) caused imperfect crystals.

Isothermal crystallization

Figure 4 shows the typical isothermal crystallization behavior of neat P(HB-*co*-HH) copolymers. Jugging from that figure, it is obvious that the higher the HH monomeric content, the slower the crystallization.

The kinetic analysis of isothermal crystallization of the polymer sample has been accomplished by using the Avrami equation (1).[28–30] In this relationship, X_{rel} refers to the relative degree of crystallinity, k to the rate constant and n to the Avrami index.

$$1 - X_{rel} = \exp(-k \cdot t^n) \qquad (1)$$

X_{rel} could be evaluated from DSC thermograms with the following formula:

$$X_{rel} = \int_0^t \frac{dH(t)}{dt}dt \Big/ \int_0^\infty \frac{dH(t)}{dt}dt \qquad (2)$$

If eq. (1) is transformed into the double-logarithmic form (3), the equation of a straight line is obtained, and the parameters k and n could be determined from the intercept and the slope, respectively.

$$\log[-\ln(1 - X_{rel})] = \log k + n \log t \qquad (3)$$

The half-crystallization time ($t_{1/2}$), (time when half of the crystallization has been completed $X_{rel} = 0.5$), could also be stated from the parameters k and n as follows:

$$t_{1/2} = \left(\frac{\ln 2}{k}\right)^{1/n} \qquad (4)$$

For the different polymer/nucleating agent systems, the Avrami kinetics were evaluated for several temperatures around the melt crystallization peaks of the nonisothermal study (Table II and Fig. 2). The Avrami double-logarithmic plots are shown in Figures 5 and 6 and the Avrami parameters calculated from these plots are shown in Table IV. For all the samples, the different isothermal curves are almost straight and parallel. The average Avrami index obtained for the PHB and PHB10HH sample are,
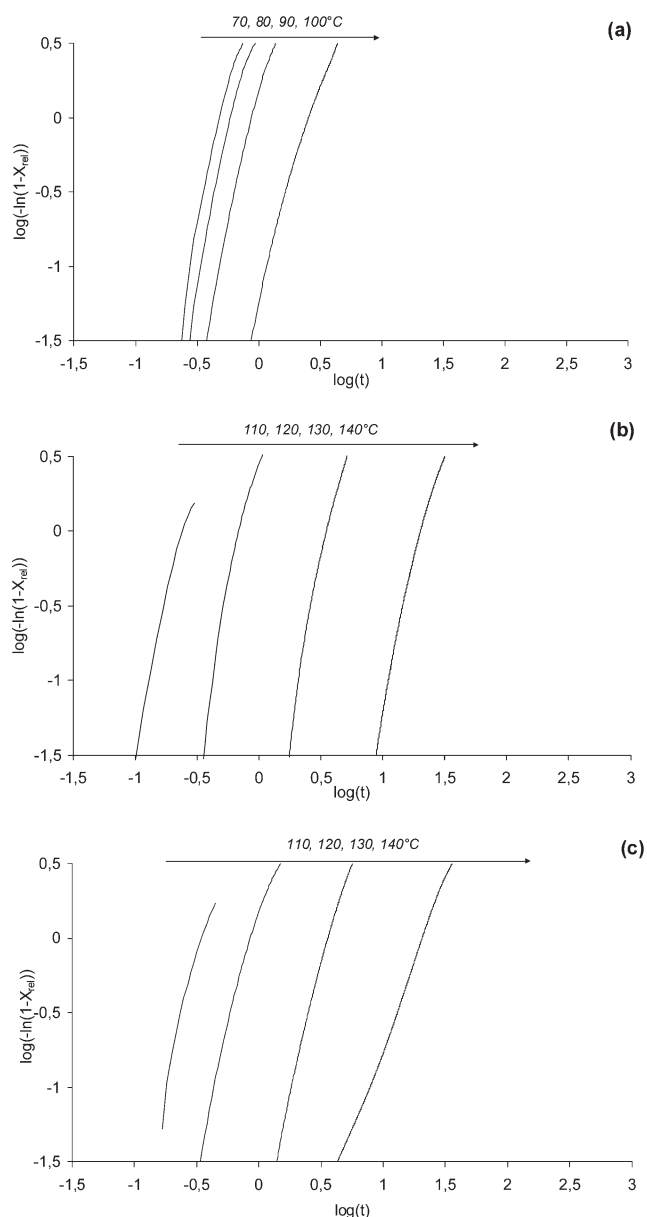

Figure 5 Isothermal Avrami plots of PHB and its blends with nucleating agents (1 wt %): (a) neat PHB, (b) PHB/BN, and (c) PHB/orotic acid.
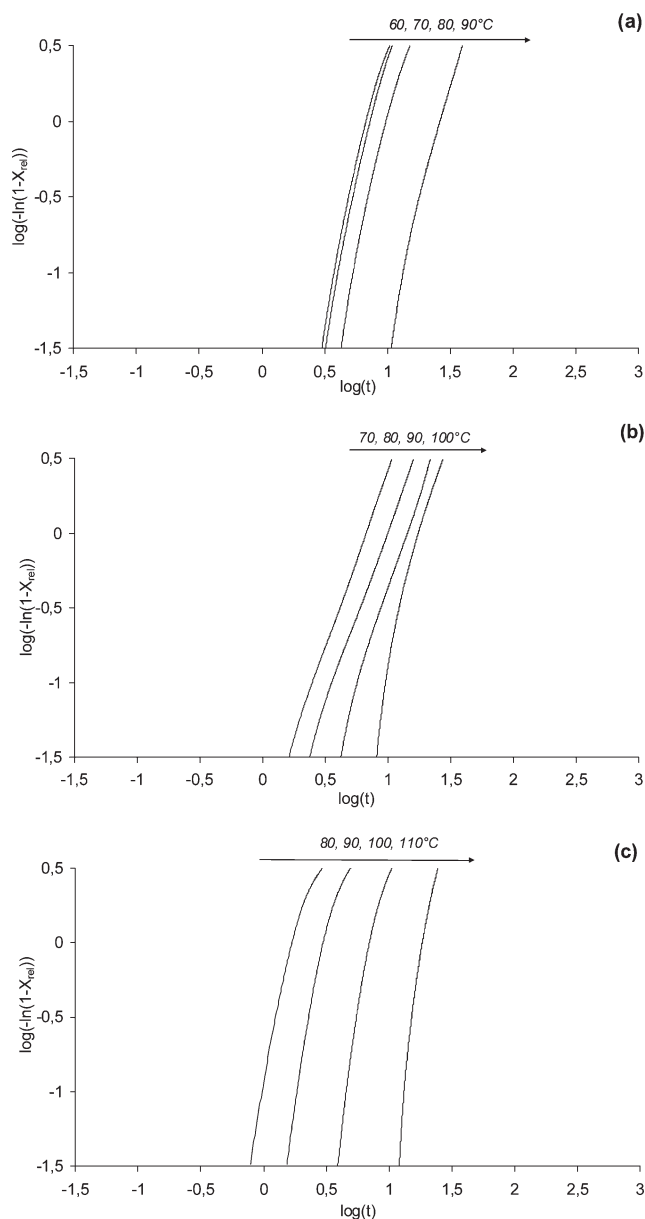
Figure 6 Isothermal Avrami plots of PHB10HH and its blends with nucleating agents (1 wt %): (a) neat PHB10HH), (b) PHB10HH/BN, and (c) PHB10HH/orotic acid.

respectively, 3.4 and 3.5, suggesting that polymer spherulites are germinated via combined germination processes. The half-crystallization times obtained from these isothermal crystallizations are plotted against the crystallization temperature in Figure 7.

The data from Table IV show that for a given temperature, the Avrami rate constant k is increased by the addition of the nucleant, indicating that the crystallization rate has been increased. As it has been shown for the nonisothermal study, the efficiency of boron nitride and orotic acid are compara-

ble for PHB. For PHB containing HH monomeric unit, the orotic acid leads to a much faster crystallization (example: for P(HB-co-18% HH) at 50°C, k_{OA} $\sim 4000\ k_{BN}$).

The same observation could also be made with the half-crystallization time, which is much shorter for the samples nucleated with orotic acid (example: for P(HB-co-18%HH) at 50°C, the half-crystallization time obtained with orotic acid is almost four times shorter than the one obtained with boron nitride). Moreover, Figure 7 shows that the temperature corresponding to the minimum of the half-crystallization time decreases when the HH content increases. This trend is less important for polymers nucleated with orotic acid, which indicate their faster crystallization.

Polarized Optical Microscopy

The melt crystallizations of PHB copolymers and their blends with nucleating agents were further investigated using a polarized optical microscope. In these experiments, the PHB, PHB5HH, PHB10HH, and PHB18HH samples were isothermally crystallized at 90, 80, 70, and 50°C, respectively. Figure 8

TABLE IV
Values of Avrami Parameters, n, k, and $t_{1/2}$ for Isothermal Crystallization of P(HB-co-HH) Copolymers and Their Blends with Nucleating Agents

Sample	Temperature (°C)	N	K (min^{-n})	$t_{1/2}$ (min)
PHB	70	3.75	1.3E + 01	0.43
	80	3.65	5.7E + 00	0.53
	90	3.50	1.4E + 00	0.78
	100	2.66	7.5E − 02	2.18
PHB/BN	110	3.61	1.6E + 02	0.22
	120	3.99	3.6E + 00	0.62
	130	3.95	6.2E − 03	3.15
	140	3.44	2.9E − 05	17.93
PHB/orotic acid	110	3.22	2.9E + 01	0.30
	120	2.94	1.3E + 00	0.75
	130	3.17	1.6E − 02	3.18
	140	2.30	9.2E − 04	17.78
PHB10HH	60	3.55	1.0E − 03	6.05
	70	3.62	6.9E − 04	6.53
	80	3.44	3.5E − 04	10.50
	90	3.23	2.5E − 05	23.12
PHB10HH/BN	70	2,38	1.1E − 02	5.77
	80	2.31	5.0E − 03	8.50
	90	2.55	1.2E − 03	12.20
	100	3.33	6.4E − 05	19.40
PHB10HH/orotic acid	80	3,31	1.4E − 01	1.52
	90	3.73	1.3E − 02	2.70
	100	4.37	1.6E − 04	6.38
	110	5.82	3.6E − 08	17.10

Values of $t_{1/2}$ were obtained from the plot $X_{rel} = f(t)$ at $X_{rel} = 0.5$).
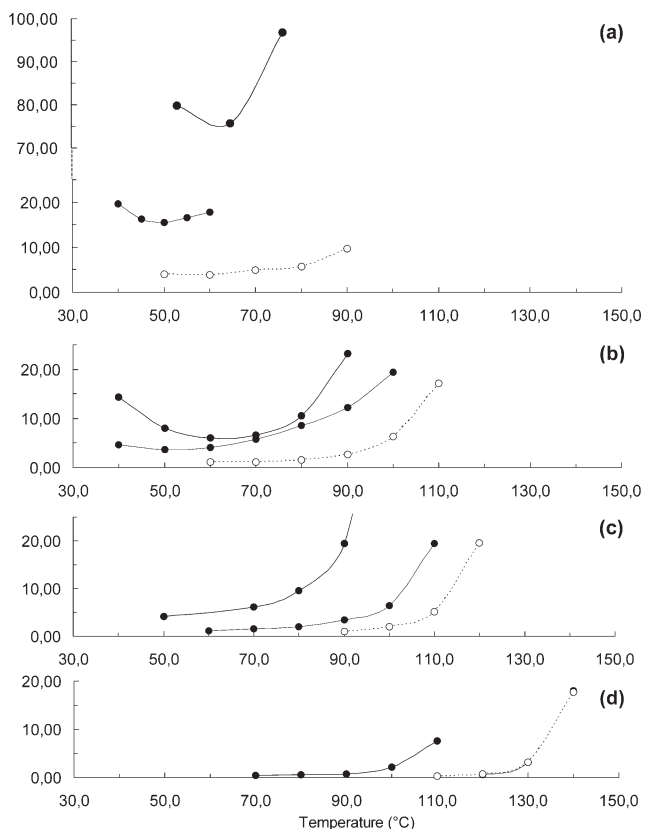
Figure 7 Variation of the half-crystallization time with the temperature for P(HB-*co*-HH) copolymers [(a) PHB18HH, (b) PHB10HH, (c) PHB5HH, and (d) PHB] and their blends with nucleating agent (● neat polymer, ◐ boron nitride, and ○ orotic acid).

shows these POM micrographs. By comparing neat samples with the nucleated ones, it is obvious that the nucleation density has been increased by the addition of the nucleant. As a consequence, the size of spherulite is decreased. Moreover, the diversity of crystal size seems to become narrower by the addition of the nucleating agent. The sharpness of the DSC peaks obtained for the nucleated samples under nonisothermal crystallization confirms this statement. As suggested by the DSC measurements, whereas the efficiency of boron nitride is deeply decreasing with the 3HH monomer content, the orotic acid seems to have a constant nucleating effect.

It can be seen that the ring spherulitic pattern tends to disappear when the % HH content increases. This behavior could be explained by the fact that HH monomers are excluded from HB crystals and then create some irregularities in the regularly twisted PHB crystals.

The size of the nucleating agent and its dispersion in the polymer affect nucleation. In fact, small nucleant particles tend to create more nuclei. To get equivalent size, both nucleating agents used in this study were milled in a ball mill (Retsch MM 200) at 20 Hz during 10 min. The average size of boron nitride and orotic acid, estimated by optical microscopy, were between 7 and 15 μm. Moreover, in Figure 8, nucleating agents seem to be well dispersed in polymer; only a few agglomerates were observed.
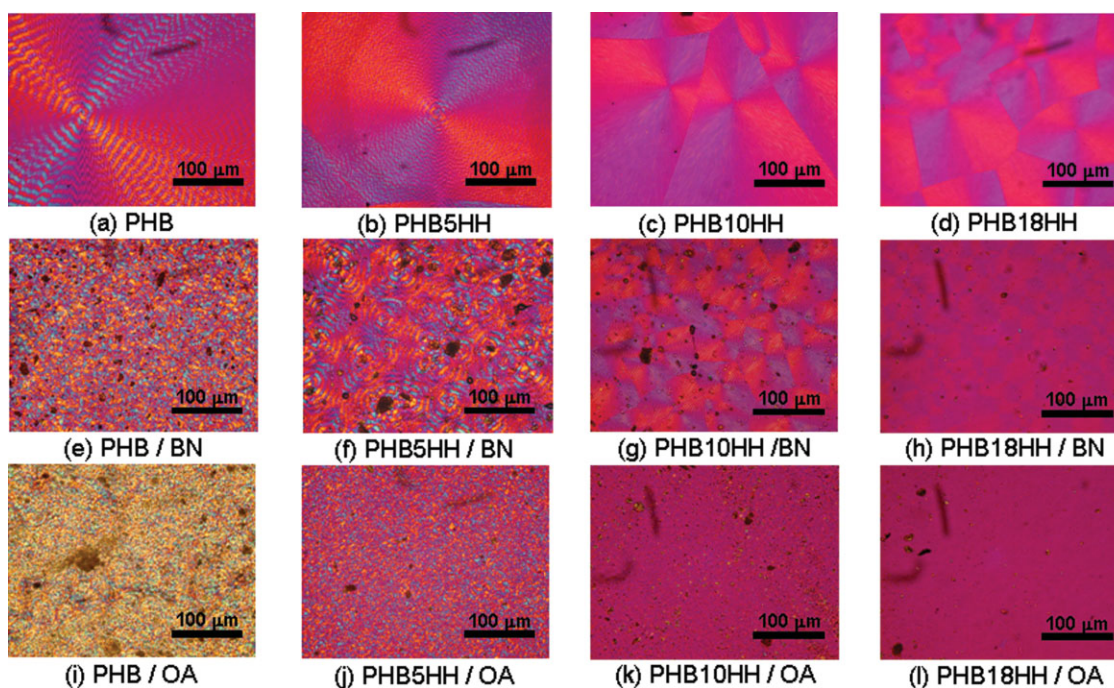

Figure 8 Polarized optical micrographs of P(HB-*co*-HH) copolymers and their blends with nucleating agents after isothermal melt crystallization. [Color figure can be viewed in the online issue, available at www.interscience.wiley.com.]

CONCLUSIONS

Orotic acid has an outstanding nucleating effect on P(HB-*co*-HH) copolymers. The results obtained from isothermal and nonisothermal crystallization show that the slow crystallization of P(HB-*co*-HH) polymers has been accelerated. In comparison, boron nitride was only able to nucleate P(HB-*co*-HH) copolymers with low HH contents. The POM micrographs also show an improved crystalline morphology of the polymer: Smaller spherulites and higher nuclei densities were obtained.

According to the fact that orotic acid is a biodegradable and a nontoxic chemical, its use for the nucleation of a biodegradable polymer is a real improvement in comparison with commonly used nucleants that are undesirable in articles that may come in contact with humans, animals, or the environment. Moreover, this biocompatible nucleating agent has a promising future for PHAs used in medical applications such as implants.

Further studies to investigate the nucleation mechanism of orotic acid are currently under investigation.

References

1. Grassie, N.; Murray, E. J.; Holmes, P. A. Polym Degrad Stab 1984, 6, 95.
2. Steinbüchel, A.; Füchtenbusch, B. Trends Biol Technol 1998, 16, 419.
3. Anderson, A. J.; Dawes, E. A. Microbiol Rev 1990, 54, 450.
4. Barham, P. J.; Keller, A. J Polym Sci Polym Phys Ed 1986, 24, 69.
5. Bluhm, T. L.; Hamer, G. K.; Marchessault, R. H.; Eyfe, C. A.; Veregin, R. P. Macromolecules 1986, 19, 2871.
6. Doi, Y.; Kitamura, S.; Abe, H. Macromolecules 1995, 28, 4822.
7. Kamiya, N.; Sakurai, M.; Inoue, Y.; Chujo, R.; Doi, Y. Macromolecules 1991, 24, 2178.
8. Kamiya, N.; Sakurai, M.; Inoue, Y.; Chujo, R. Macromolecules 1991, 24, 3888.
9. Yamada, S.; Wang, Y.; Asakawa, N.; Yoshie, N.; Inoue, Y. Macromolecules 2001, 34, 4659.
10. Wang, Y.; Yamada, S.; Asakawa, N.; Yamane, T.; Yoshie, N.; Inoue, Y. Biomacromolecules 2001, 2, 1315.
11. Chen, C.; Cheung, M. K.; Yu, P. H. F. Polym Int 2005, 54, 1055.
12. Scandola, M.; Focarete, M. L.; Frisoni, G. Macromolecules 1998, 31, 3846.
13. He, Y.; Shuai, X.; Kasuya, K.; Doi, Y.; Inoue, Y. Biomacromolecules 2001, 2, 1045.
14. Qian, J.; Zhu, L.; Zhang, J.; Whitehouse, R. S. J Polym Sci Part B: Polym Phys 2007, 45, 1564.
15. Barham, P. J. J Mater Sci 1984, 19, 3826.
16. Organ, S. J.; Barham, P. J. J Mater Sci 1991, 26, 1368.
17. Barham, P. J.; Keller, A.; Otun, E. L.; Holmes, P. A. J Mater Sci 1984, 19, 2781.
18. Black, S. N.; Dobbs, B.; Dempsey, P. S.; Davey, R. J. J Mater Sci Lett 1990, 9, 51.
19. Organ, S. J.; Barham, P. J. J Mater Sci 1992, 27, 3239.
20. Withey, R. E.; Hay, J. N. Polymer 1999, 40, 5147.
21. Liu, W. J.; Yang, H. L.; Wang, Z.; Dong, L. S.; Liu, J. J. J Appl Polym Sci 2002, 86, 2145.
22. Kai, W.; He, Y.; Inoue, Y. Polym Int 2005, 54, 780.
23. He, Y.; Inoue, Y. Biomacromolecules 2003, 4, 1865.
24. He, Y.; Inoue, Y. J Polym Sci Part B: Polym Phys 2004, 42, 3461.
25. Kai, W.; He, Y.; Asakawa, N.; Inoue, Y. J Appl Polym Sci 2004, 94, 2466.
26. Jacquel, N.; Lo, C. W.; Wu, H. S.; Wei, Y. H.; Wang, S. S. Biochem Eng J 2008, 39, 15.
27. Jacquel, N.; Lo, C. W.; Wu, H. S.; Wei, Y. H.; Wang, S. S. AIChE J 2007, 53, 2704.
28. Avrami, M. J Chem Phys 1939, 7, 1103.
29. Avrami, M. J Chem Phys 1940, 8, 212.
30. Avrami, M. J Chem Phys 1941, 9, 177.