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Isothermal crystallization kinetics and morphology of biodegradable poly(3-hydroxybutyrate-co-4-hydroxybutyrate)

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Abstract Isothermal crystallization kinetics and morphology of poly(3-hydroxybutyrate-co-4-hydroxybutyrate) [P(3HB-co-4HB)] with different 4-hydroxybutyrate (4HB) molar fraction were investigated by differential scanning calorimetry (DSC), wide angle X-ray diffraction (WAXD), and polarized optical microscopy (POM). The results show that the crystallization mechanism and crystal structure of P(3HB-co-4HB) copolymers are the similar as those of poly(3-hydroxybutyrate) (PHB). While the equilibrium melting point and crystallization rate decrease with the increase of 4HB molar fraction. Banded spherulites are observed in neat PHB and P(3HB-co-4HB) copolymers, and morphology is influenced apparently by the crystallization temperature and 4HB unit.

Introduction

Concerns over the persistence of plastics in the environment, shortage of landfill space, emissions during incineration, and negative impact on wildlife through ingestion and entrapment have increased research and development efforts on biodegradable polymers [1]. Among various biodegradable plastics available, only poly(3-hydroxyalkanoates), PHA, possesses thermoplasticity and mechanical properties similar to those of synthetic polymers [2]. PHA is biologically synthesized polyesters produced by a number of microorganisms and is completely biodegradable under aerobic and anaerobic conditions [3].

So far there are more than 100 known PHA [4], which have different structures and, consequently, significantly differ in their physical–mechanical properties and kinetics of biodegradation. They are high crystallinity and thermoplastic materials as well as elastic and rubber-like polymers with low melting points [5]. Poly(3-hydroxybutyrate) (PHB) is one of the first well-studied bacterial polyesters in the PHA family [6] and can be processed by traditional processing such as extrusion and injection moulding [7]. The thermal properties [8–10] and crystal structure [11, 12] of PHB have been investigated by several research groups.

Natural origin PHB has perfect stereoregularity, high purity, and high degree of crystallinity [13] as well as low nucleation density and crystallizes slowly to form large spherulites [9, 14], so that it has been considered for the study of isothermal crystallization kinetics and morphology [15]. However, the high crystallinity and large spherulites make it rigid and brittle, not necessarily well suited for certain applications as a commodity plastic [16]. It is also thermally unstable during the conventional melt processing due to the high melting temperature [17]. Another disadvantage that lessens its attractiveness for industrial mass production is secondary crystallization process that occurs in samples stored at room temperature, which leads to an increasing embrittlement of the material [18].

To reduce the excess crystallinity and modify the overall physical properties of PHB [19], copolymerization of 3HB units with other 3-hydroxyalkanoate (3HA) units is an effective way. The copolymerization of 3HB with 3HV (3-hydroxybutyrate) (PHBV), 3HP (3-hydroxypropionate) (PHBP), 4HB (4-hydroxybutyrate) [P(3HB-co-4HB)], and 3HHx (3-hydroxyhexanoate) (PHBHHx), respectively, has been successfully carried out by microbial fermentation [20]. These PHB-based copolymers show a wide range of physical properties depending on the chemical structure of

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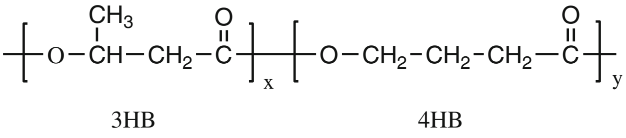
the comonomer units as well as the comonomer composition [21].

Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) is the first commercialized bacterial copolyester under the trade name of Biopol [22]. However, PHBV shows an unusual phenomenon of isodimorphism, due to the similarity in the shape and size between the 3HB and 3HV units, they can incorporate into the crystal lattice each other, and cause high crystallinity over the entire range of comonomer composition [23, 24]. Thus, the properties of PHBV are not significantly improved in comparison to those of PHB homopolymer.

While P(3HB-co-4HB), whose chemical structure is shown below,



$$\left[O-\overset{CH_3}{CH}-CH_2-\overset{O}{\overset{\|}{C}} \right]_x \left[O-CH_2-CH_2-CH_2-\overset{O}{\overset{\|}{C}} \right]_y$$

exhibits substantially reduced crystallinity and melting temperature when compared to the PHB homopolymer [25]. Both mechanical properties and melt temperature could be adjusted by changing 4HB molar fraction. As the 4HB monomer has one more backbone carbon atom than those of 3HB, 3HV, 3HHx, and 3HP, it was found that P(3HB-co-4HB) copolymer shows unique crystallization behaviors [20].

However, similar to most PHA materials, P(3HB-co-4HB) faces the drawbacks of slow crystallization and secondary crystallization phenomena. This would cause the problems for processing instability and low efficiency [26]. Therefore, much more attention should be paid directly to the crystallization kinetics study since it affects not only the crystalline structure and morphology but also the processing and final physical properties of P(3HB-co-4HB) [27]. In this article, isothermal crystallization kinetics, crystal structure, and spherulites morphology of neat PHB and P(3HB-co-4HB) copolymers were investigated by DSC, WAXD, and POM. It is expected that the results will be helpful for a better understanding of the relationship between structure and properties of biodegradable P(3HB-co-4HB) copolymers.

Experimental

Sample purification

Three samples, neat PHB ($M_w = 4.03 \times 10^5$, $M_w/M_n = 1.15$), P(3HB-co-5%4HB) ($M_w = 3.94 \times 10^5$, $M_w/M_n = 1.21$), and P(3HB-co-10%4HB) ($M_w = 4.06 \times 10^5$, $M_w/M_n = 1.15$) copolymers which contain 0, 5, and 10 mol% 4HB, respectively, were supplied by Green Biological Material Co. Ltd. from Tianjin, China.

Each sample (1 g) was dissolved in 50 mL of chloroform and filtered by vacuum filtration at room temperature to remove any insoluble fraction or impurities to obtain a clear solution. Pure P(3HB-co-4HB) was finally obtained by precipitating the solution into absolute ethanol, filtering, and drying in vacuum at 60 °C for 24 h to remove residual solvent and moisture [25].

Differential scanning calorimetry

The DSC experiment of isothermal crystallization and subsequent melting behaviors were performed with a NETZSCH 200 F3 DSC, which was calibrated with indium before use. The samples were first heated to 200 °C, remained for 5 min to eliminate the thermal and mechanical history, and then cooled at a rate of −80°C/min to predetermined crystallization temperature (T_c) and were maintained at the T_c until the crystallization was completed. The samples were subsequent heated to 200 °C at a rate of 20°C/min. During DSC scanning, the temporal development of crystallinity and melting temperature (T_m) at various T_c was recorded.

Wide angle X-ray diffraction

Wide angle X-ray diffraction measurements were performed for the powder samples of neat PHB and P(3HB-co-4HB) copolymers at room temperature with a Rigaku D/max 2500 PC X-ray diffractometer using CuKa radiation ($\lambda = 0.154$ nm), which was operated at 40 kV and 100 mA. WAXD data were collected from $2\theta = 0$–$60°$ with a step interval of 0.02°.

Polarized optical microscopy

The spherulitic morphology was monitored with a XPR-500D polarized optical microscopy. A thin film of each sample obtained by solution casting was inserted between two microscope cover slides and heated to molten state, then quickly transferred to a hot stage and equilibrated at the desired isothermal crystallization temperature (T_c) to isothermally crystallize, and monitored the spherulite growth. The radial growth rate of the spherulites was calculated from the slope of the plots of spherulite radius versus crystallization time.

Results and discussion

Isothermal crystallization kinetics

The well-known Avrami equation was used to analyze the overall isothermal crystallization kinetics of neat PHB, P(3HB-co-5%4HB), and P(3HB-co-10%4HB). It assumes that the relative crystallinity $X(t)$ develops as a function of crystallization time t as follows:

$$X(t) = 1 - \exp(-kt^n) \qquad (1)$$

where $X(t)$ is the relative crystallinity at time t, k is the overall crystallization rate constant depending on nucleation and growth rate, and n is the Avrami exponent which depends on the nucleation and growth mechanism of the crystals [28].

Figure 1 shows the development of $X(t)$ as a function of crystallization time t for three samples at various T_c. It can be seen that the half time of crystallization ($t_{1/2}$) of the copolyesters increases with the increase of 4HB molar fraction at the same T_c, indicating that the incorporation of 4HB unit reduces the crystallization rate of PHB in the copolyesters. As shown in Fig. 1, the $t_{1/2}$ values of neat PHB, P(3HB-co-5%4HB), and P(3HB-co-10%4HB) at 65 °C are 1.3, 3.3, and 6.1 min, respectively.

The Avrami equation could be further deduced as:

$$\lg[-\ln(1 - X(t))] = \lg k + n \lg t \qquad (2)$$

Thus, the Avrami parameters (n and k) at each isothermal crystallization temperature T_c can be determined from the linear plots of $\lg[-\ln(1-X(t))]$ against $\lg t$.

Figure 2 displays the Avrami plot of three samples. It can be seen that all curves maintain the linearity even at longer crystallization times, which indicates that the crystallization kinetics of neat PHB and its copolymers follows the Avrami equation. The Avrami parameters n and k can be obtained from the slope and intercept, respectively, in the plots of $\lg[-\ln(1-X(t))]$ versus $\lg t$ as shown in Fig. 2. The values of n and k are summarized in Table 1. All n values are ranging from 1.8 to 2.2, which imply two-dimensional spherulite growth initiated by heterogeneous nucleation mechanism. Furthermore, the n values change slightly with the increase of T_c and 4HB molar fraction, indicating that the introduction of 4HB dose not change the crystallization mechanism of PHB within the crystallization temperature range of 55–80 °C.

For all samples, the k values in Table 1 vary with the increasing of T_c, suggesting that melt crystallization of copolyesters exhibits the temperature dependency. With the increase of 4HB molar fraction, the temperature dependency of copolyesters depresses due to the decrease of crystallinity. The higher 4HB molar fraction of copolyesters finally leads to a relative lower k at the same T_c.

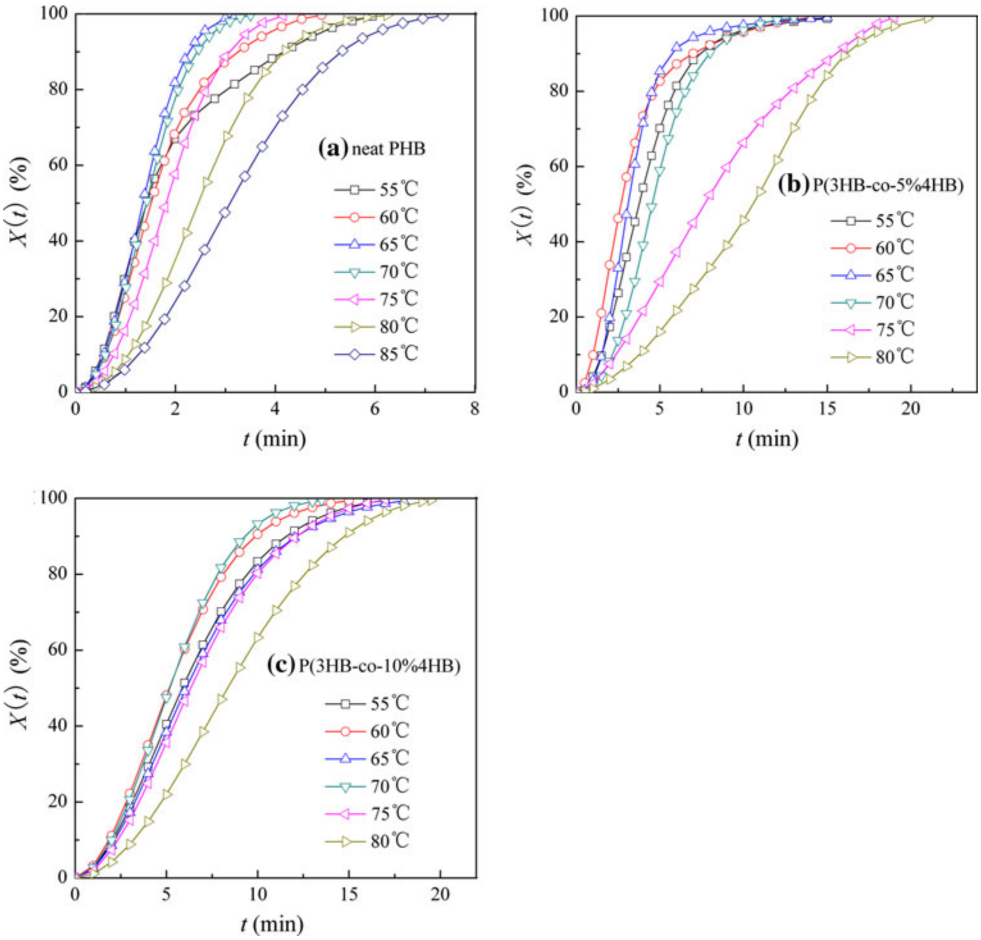


Fig. 1 Temporal development of $X(t)$ of neat PHB and P(3HB-co-4HB) copolymers at various T_c: **a** neat PHB, **b** P(3HB-co-5%4HB), and **c** P(3HB-co-10%4HB)

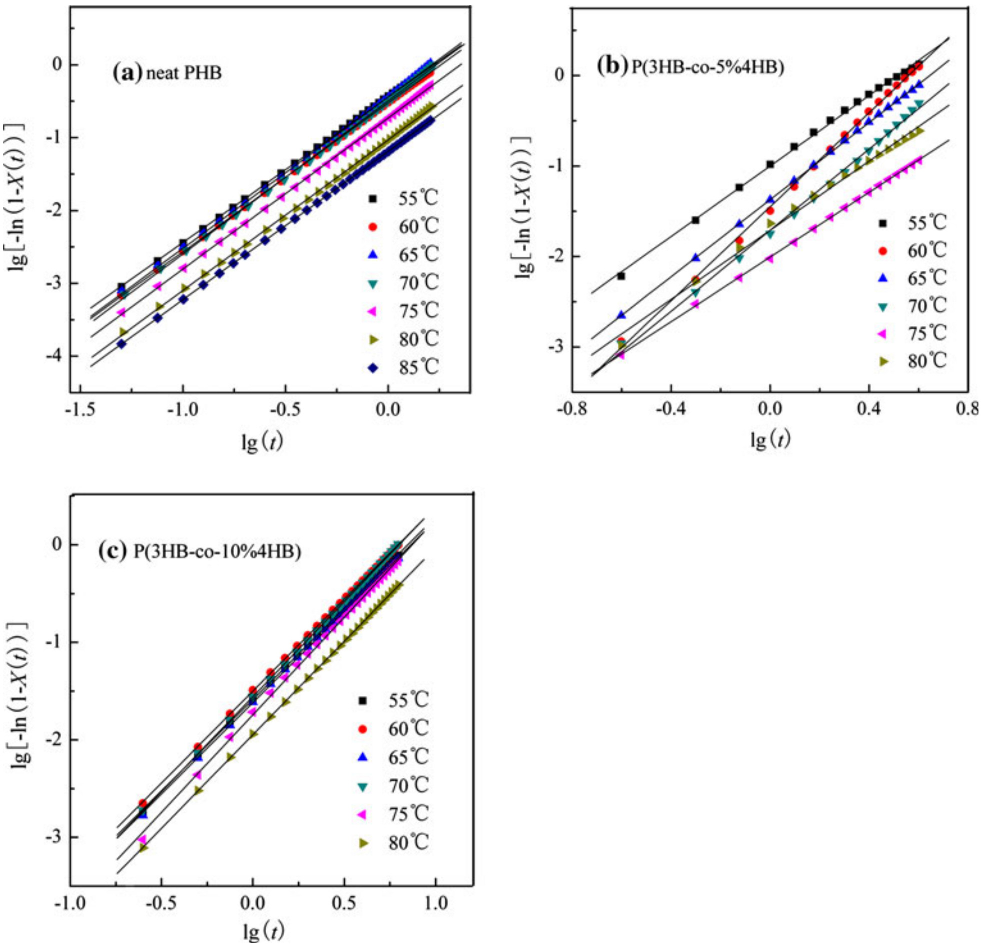


Fig. 2 Avrami plot of neat PHB and P(3HB-co-4HB) copolymers at various T_c: **a** neat PHB, **b** P(3HB-co-5%4HB), and **c** P(3HB-co-10%4HB)

Furthermore, the crystallization half time ($t_{1/2}$) was obtained from following equation and data included in Table 1.

$$t_{1/2} = (\ln 2/k)^{1/n} \tag{3}$$

The overall crystallization rate (g) calculated from the reciprocal of crystallization half time, listed in Table 1.

$$g = 1/t_{1/2} \tag{4}$$

Figure 3 displays the variation of g on crystallization temperatures for the samples. It is clearly observed that the trend of g is similar to that of k. There is a maximum value ($g^{\max}$) for each curve of g versus T_c, and the corresponding temperature of $g^{\max}$ varies with 4HB molar fraction. In addition, the g value decreases with the increase of 4HB molar fraction at the same $T_{c,}$ indicating the reduction of crystallization rate.

The reduction of crystallization rate of P(3HB-co-4HB) is mainly caused by several following factors. First, the existence of 4HB unit can destroy the crystal regularity of PHB [29], resulting in descending of crystallization ability of P(3HB-co-4HB). Second, the equilibrium melting temperature of copolyesters is lower than that of neat PHB, which may reduce the thermodynamic driving force required for the growth of PHB spherulites [27]. Third, 4HB unit may play a role of a diluent to PHB in the copolyesters [30], resulting in the dilution of PHB chains at the spherulites growth front.

In addition, PHB has a particular C–H···O=C hydrogen bonding between the C=O group and the CH_3 group [31], which can stabilize the chain folding in the lamella structure of PHB, and the high crystallinity partly comes from the C–H···O=C hydrogen bonding [32]. While the existence of 4HB unit makes the C–H···O=C hydrogen bondings break much more easily in P(3HB-co-4HB) than in PHB because of the bulkiness of large amounts of amorphous parts [33]. Therefore, the interaction of intermolecular hydrogen bonding is weakened by the introduction of 4HB unit, which is unfavorable to crystallization.

Equilibrium melting point

Equilibrium melting point T_m^o, the melting temperature of infinitely thick lamellae, is one of the important parameters for the analysis of crystallization kinetics [34]. It can be obtained from the relationship between the observed

Table 1 Crystallization parameters for neat PHB and P(3HB-co-4HB) copolymers at various T_c

Sample	T_c (°C)	n	k	$t_{1/2}$ (min)	g (min^{-1})
PHB	55	1.898	0.32307	1.4951	0.6689
	60	1.909	0.26984	1.6393	0.6100
	65	2.118	0.39482	1.3044	0.7666
	70	2.177	0.34135	1.3845	0.7223
	75	2.195	0.20300	1.7497	0.5715
	80	2.192	0.10060	2.4119	0.4146
	85	2.143	0.06651	2.9857	0.3349
P(3HB-co-5%4HB)	55	2.0900	0.04263	3.7974	0.2633
	60	1.8147	0.10088	2.8923	0.3457
	65	2.1906	0.03581	3.2860	0.3043
	70	2.2248	0.02080	4.8359	0.2068
	75	1.8100	0.01881	7.3355	0.1363
	80	2.0424	0.00755	9.1399	0.1094
P(3HB-co-10%4HB)	55	1.873	0.02534	5.8525	0.1709
	60	1.885	0.03146	5.1598	0.1938
	65	1.851	0.02404	6.1472	0.1627
	70	2.023	0.02634	5.0340	0.1986
	75	1.979	0.01801	6.3283	0.1580
	80	2.041	0.01006	7.9597	0.1256

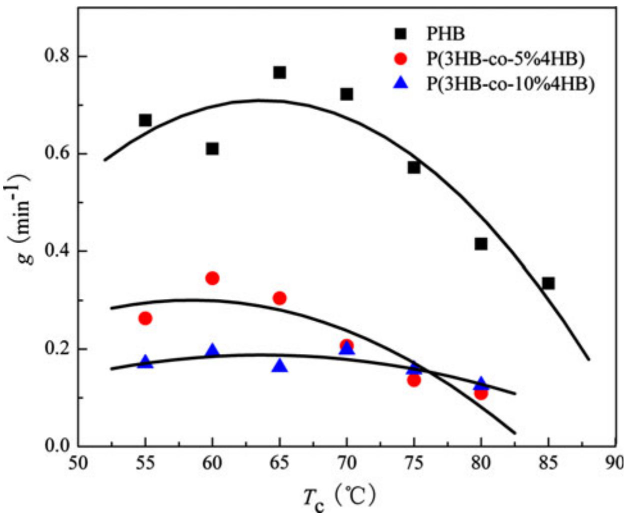


Fig. 3 Variation of g as a function of crystallization temperature for neat PHB and P(3HB-co-4HB) copolymers

melting temperature T_m and the isothermal crystallization temperature T_c through Hoffman–Weeks equation [35] as given by:

$$T_m = T_m^o\left(1 - \frac{1}{\gamma}\right) + \frac{T_c}{\gamma} \tag{5}$$

where γ is the ratio of the initial to final lamellar thickness. T_m^o is obtained by the extrapolation to the plot of T_m versus T_c, where $T_m = T_c$.

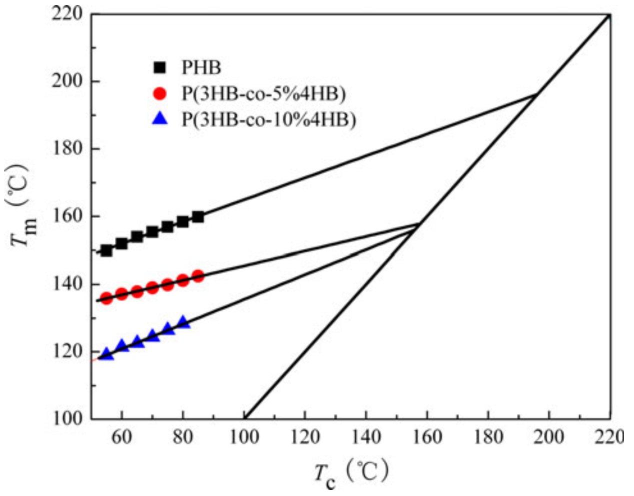


Fig. 4 Plot of the Hoffman–Weeks equation for neat PHB and P(3HB-co-4HB) copolymers

As is shown in Fig. 4, the measured T_m linearly increases with increasing T_c for neat PHB, P(3HB-co-5%4HB), and P(3HB-co-10%4HB).

It is clearly seen that T_m^o decreases with the increase of 4HB molar fraction. In general, the T_m^o depression implies a reduction in the thickness of lamellar crystals as well as an increase in the level of crystal defects [34]. Therefore, it can be concluded that the incorporation of 4HB unit into the PHB chain reduces the lamellar crystal thickness and/or increases the crystal defects.

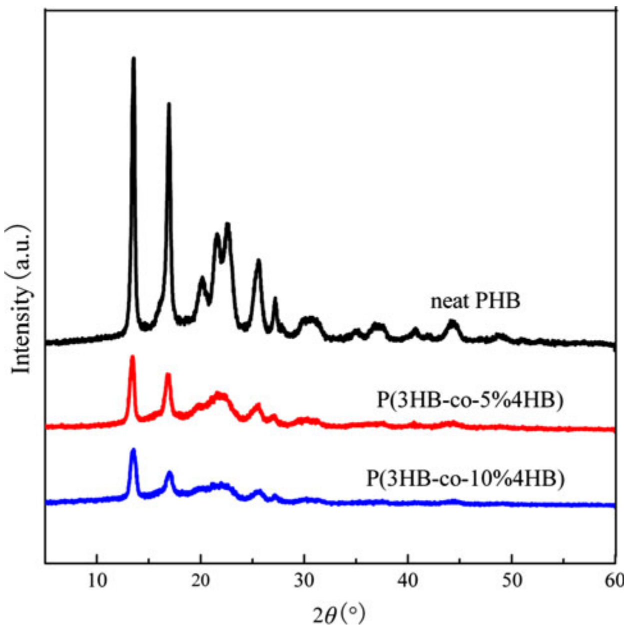


Fig. 5 WAXD patterns of neat PHB and P(3HB-co-4HB) copolymers

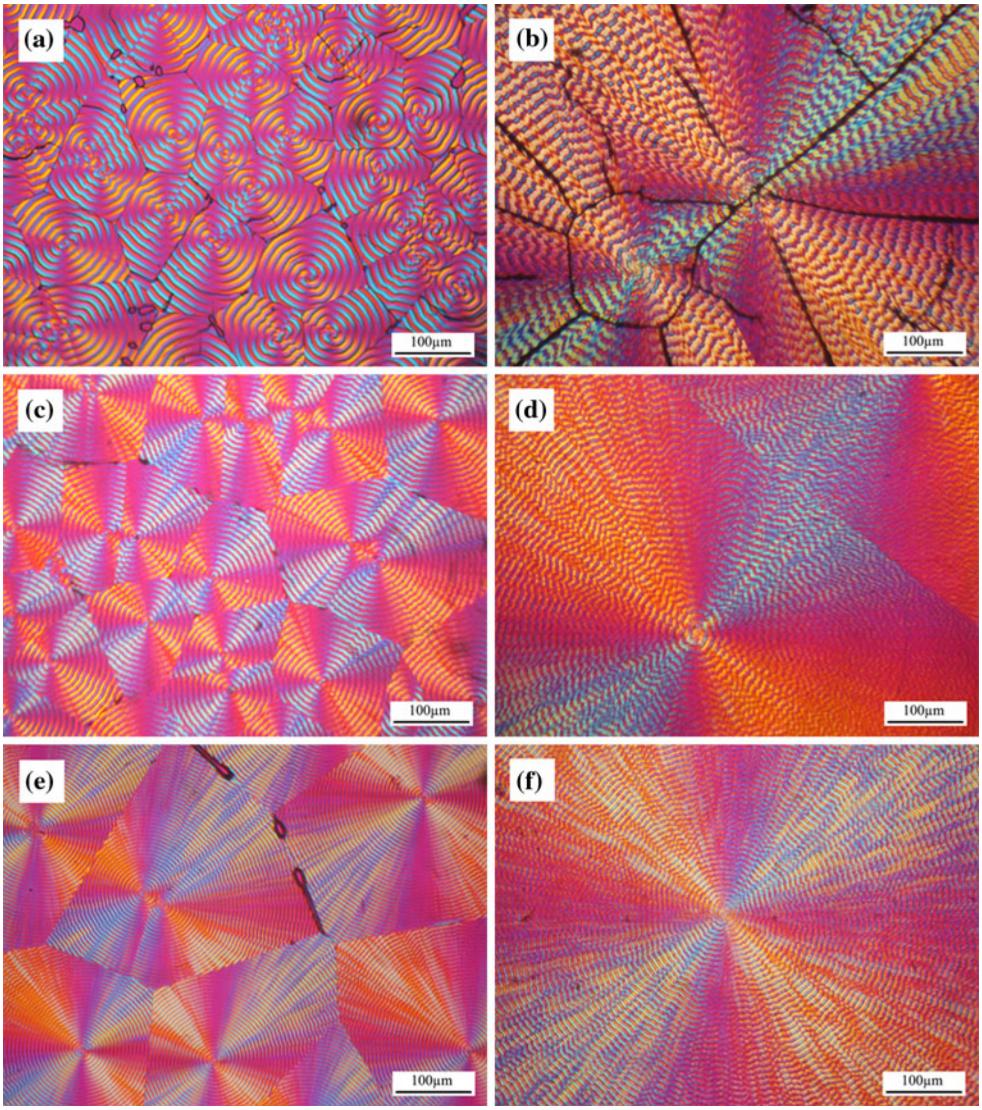


Fig. 6 Optical micrographs of the spherulitic morphology of neat PHB and P(3HB-co-4HB) copolymers crystallized at various temperatures: **a** neat PHB at 60 °C, **b** neat PHB at 80 °C, **c** P(3HB-co-5%4HB) at 60 °C, **d** P(3HB-co-5%4HB) at 80 °C, **e** P(3HB-co-10%4HB) at 60 °C, and **f** P(3HB-co-10%4HB) at 80 °C

Crystal structure

Figure 5 indicates the WAXD patterns for neat PHB and P(3HB-co-4HB) copolymers. The crystal unit cell of PHB is an orthorhombic system with lattice parameters of $a = 0.576$, $b = 1.320$, and $c = 0.596$ nm [36], and two prominent diffraction peaks of (020) and (110) planes are observed at 2θ of 13.3° and 16.6°, respectively [37]. In addition, several strong sharp peaks in the scattering angle range of $2\theta = 18–30°$ are also present in the diffraction pattern of neat PHB.

For P(3HB-co-4HB) copolymers, their WAXD patterns show similar diffraction peaks at the same 2θ, suggesting that the copolymers have the same crystal structure as that of PHB. However, with the increase of 4HB molar fraction, the intensity of diffraction peaks depresses sharply and the diffraction peaks at larger 2θ become broaden, meaning that crystallinity of copolyesters decreases due to the existence of 4HB unit [38]. This indicates that 4HB unit exists as defects in the PHB crystalline regions and is excluded from the PHB crystal lattice.

Spherulitic morphology

Figure 6 shows the spherulitic morphology of neat PHB and P(3HB-co-4HB) copolymers crystallized at 60 and 80 °C, respectively. Both neat PHB and P(3HB-co-4HB) copolymers show banded spherulites with concentric extinction bands. It can be found that the size of spherulites increases with the increase of 4HB molar fraction and T_c, because of the decrease of nucleation density and the improvement of macromolecular chains mobility. On one hand, the diluent effect of 4HB on the crystallization of PHB and more flexible of molecular chain segments at higher T_c make PHB molecular chains much difficult to stack in an ordered manner, resulting in the decrease of nucleation density [30]. On the other hand, the introduction of 4HB unit and higher T_c also can improve the macromolecular chains mobility, which is beneficial to crystallization.

The spherulites grown isothermally at 80 °C have larger band spacing than those grown at 60 °C, and the band spacing becomes small with the increase of 4HB molar fraction at the same T_c. It is generally believed that the formation of the banded spherulite is attributed to the crystalline lamellar twisting along the spherulitic radius during crystal growth [39]. 4HB unit can act as diluents excluded from the PHB crystal lattice so as to cause the imbalance surface stress at crystalline lamellar fold surfaces and enhance the tendency of the lamellar twisting. Whereas increased crystallization temperature results in decreased magnitude of lamellar twisting [40].

In addition, two types of cracks are found in pure PHB spherulites: radial cracks and circumferential cracks, as illustrated in Fig. 6b, which formed during cooling due to differences in radial and circumferential thermal expansion coefficients [41]. These spherulites with cracks of PHB are responsible for the poor mechanical properties. However, no cracks appear in P(3HB-co-4HB) banded spherulites, that is, the incorporation of 4HB unit can inhibit the emergence of crack.

Conclusions

Isothermal crystallization kinetics and morphology of neat PHB and P(3HB-co-4HB) copolymers were investigated by DSC, WAXD, and POM.

The existence of 4HB unit does not change the crystallization mechanism and morphology of PHB in the investigated crystallization temperature range. While the crystallization rate and equilibrium melting point (T_m^o) decrease with the increase of 4HB molar fraction. WAXD result shows that the crystal structure of PHB is not modified by 4HB unit.

Banded spherulites are observed in neat PHB and P(3HB-co-4HB) copolymers. The size of spherulites increases, and the band spacing decreases with the increase of 4HB molar fraction. The spherulites grown isothermally at 80 °C have larger band spacing size than those grown at 60 °C. The incorporation of 4HB unit inhibits the emergence of crack.

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