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Crystallization behaviors and morphology of biodegradable poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate)

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Abstract Crystallization behaviors and spherulitic 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 and polarized optical microscopy. Crystallization behaviors of P(3HB-*co*-4HB) are significantly affected by 4HB molar fraction. The melting temperature (T_m), glass transition temperature (T_g), and crystallinity (X_c) decrease with the increase of 4HB molar fraction. Banded spherulites are observed in poly (3-hydroxybutyrate) (PHB) and P(3HB-*co*-4HB) copolymers. The band spacing decreases with the increase of 4HB molar fraction. The morphology and growth rate of the spherulites strongly depend on 4HB molar fraction and the crystallization temperatures. The introduction of 4HB unit can inhibit the emergence of cracks in PHB spherulites.

Keywords Poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) · Crystallization behaviors · Spherulitic morphology · Band spacing · Spherulitic growth rate

Introduction

Biodegradable polymers have received much more attention in the last two decades due to their potential applications in the fields related to environmental protection and the maintenance of physical health [1]. Among various biodegradable plastics available, there is a growing interest in the group of poly(3-hydroxyalkanoates) (PHA) [2]. The physical properties of thermoplastic PHA can range from hard rigid solids to elastomers [3]. PHA is manufactured via bacterial fermentation and degraded completely by aerobic microorganisms to water and carbon dioxide [4, 5]. Its chemical structure is: $-[O–CH(R)–CH_2(C=O)]_n-$, with $R=CH_3$ for poly(3-hydroxybutyrate) (PHB) and $R=C_2H_5$ for PHV [6].

Poly(3-hydroxybutyrate), the most common representative of PHA [7], has attracted enormous attention in agriculture, pharmaceutical, and medical industries due to its biocompatibility and biodegradability [8]. It can be produced intracellularly in many bacteria as a carbon and energy storage compounds when growth is restricted by an essential nutrient and if a carbon source is available in excess [9]. It is an ideal model for studying the crystallization morphology due to its perfect stereoregularity, high purity and crystallinity [10]. Having a similar melting temperature, strength, and modulus to isotactic polypropylene [11], it could be processed using conventional extrusion and molding process [12].

However, the inherent brittleness and inferior thermal stability (in addition to cost) have blocked the popular usage of PHB [13]. The brittleness of PHB is generally resulted from its high crystallinity, large spherulites [14], and post-crystallization behaviors [15], which result in irregular pores that formed on the surface and limit the flexibility of amorphous chains. Furthermore, the processing temperature should be in a narrow processing temperature range because thermal degradation proceeds rapidly above the melting temperature, so the acceptable residence time in processing equipment is only a few minutes [16]. PHB degradation occurs through random chain scission resulting in terminations of crotonic acid and vinyl groups [17].

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To overcome these shortcomings of PHB, several approaches have been investigated as follows [18].

(i) An interesting solution to the problem relies in the biosynthesis of copolymers containing hydroxyalkanoate units other than 3-hydroxybutyrate.
(ii) Chemical modification aims to introduce other structural units into the PHB backbone. The process can be carried out by transesterification reactions of PHB with other polyesters in solution or melt.
(iii) The third approach involves physical modification, i.e., blending of PHB with other polymers using compatibilizers.

The most effective method to reduce brittleness of PHB is copolymerization that is incorporating other copolymerized components to PHB macromolecular chains. Previous research shows that [19] crystallinity and melting temperature can be decreased by including other hydroxyalkanoate units in PHB, such as 3-hydroxyvalerate (3HV) unit to form poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) via fermentation [20]. It has been reported that the 3HB and 3HV components in PHBV can form cocrystallization due to the similar in lattice indexes of respective unit cell [21], which leads to the impact toughness of PHBV with 3HV molar fraction ranging from 0 to 95 mol% is still not satisfactory [22].

While P(3HB-*co*-4HB), a copolyester of 3HB and 4HB, showed decrease melting temperature, crystallinity, and brittleness with the increase of 4HB molar fraction [23]. It has received considerable attention and become a new research focus of biodegradable materials.

It is well known that crystallinity plays an important role in physical properties and biodegradability of PHB, of which the crystalline structure and morphology are influenced greatly by the thermal history. Therefore, much more attention should be directed to the crystallization behaviors since it affects not only the crystalline structure and morphology but also the final physical properties and biodegradability of PHB [24]. In this study, the crystallization behaviors and spherulitic morphology of P(3HB-*co*-4HB) copolymers are characterized by differential scanning calorimetry (DSC) and polarized optical microscopy (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

Six samples, Neat PHB, P(3HB-*co*-5%4HB), P(3HB-*co*-10%4HB), P(3HB-*co*-15%4HB), P(3HB-*co*-20%4HB), and P(3HB-*co*-30%4HB) copolymers which contain 0, 5, 10, 15, 20, and 30 mol% 4HB, respectively, were supplied by Green Biological Material Co. Ltd. from Tianjin, China.

Each sample (1g) 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

Differential scanning calorimetry measurement was performed with a NETZSCH 200 F3 DSC, which was calibrated with indium prior to use. Each sample was first heated at a heating rate of 10 °C/min to 200 °C and kept 2 min. Then the sample was cooled with liquid nitrogen at a cooling rate of 10 °C/min to −50 °C and kept 2 min. Subsequently, the sample was undergone second heating from −50 to 200 °C at the same heating rate. During DSC scanning, melting temperature (T_m), melting enthalpy (ΔH_m), glass transition temperature (T_g), and crystallization temperature (T_c) were measured.

The crystallinity was calculated by

$$X_c\,(\mathrm{PHB}) = \Delta H^*_{\mathrm{PHB}}/\Delta H^{\mathrm{o}}_{\mathrm{PHB}} \tag{1}$$

$$X_c\,(\mathrm{copolymers}) = \Delta H^*_{\mathrm{copolymers}}/\Delta H^{\mathrm{o}}_{\mathrm{PHB}} \tag{2}$$

$\Delta H^{\mathrm{o}}_{\mathrm{PHB}}$ is the enthalpy of melting per gram of 100% crystalline 146 J/g [9] and $\Delta H^*{}_{\mathrm{PHB}}$ and $\Delta H^*{}_{\mathrm{copolymers}}$ are the measured enthalpy of melting for PHB and copolymers, respectively.

Polarized optical microscopy

The spherulitic morphology was monitored with a XPR-500D POM. 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

Nonisothermal crystallization and melting behaviors

Figure 1a shows the melting temperature (T_{m1}) and enthalpy (ΔH_{m1}) of neat PHB and P(3HB-*co*-4HB)

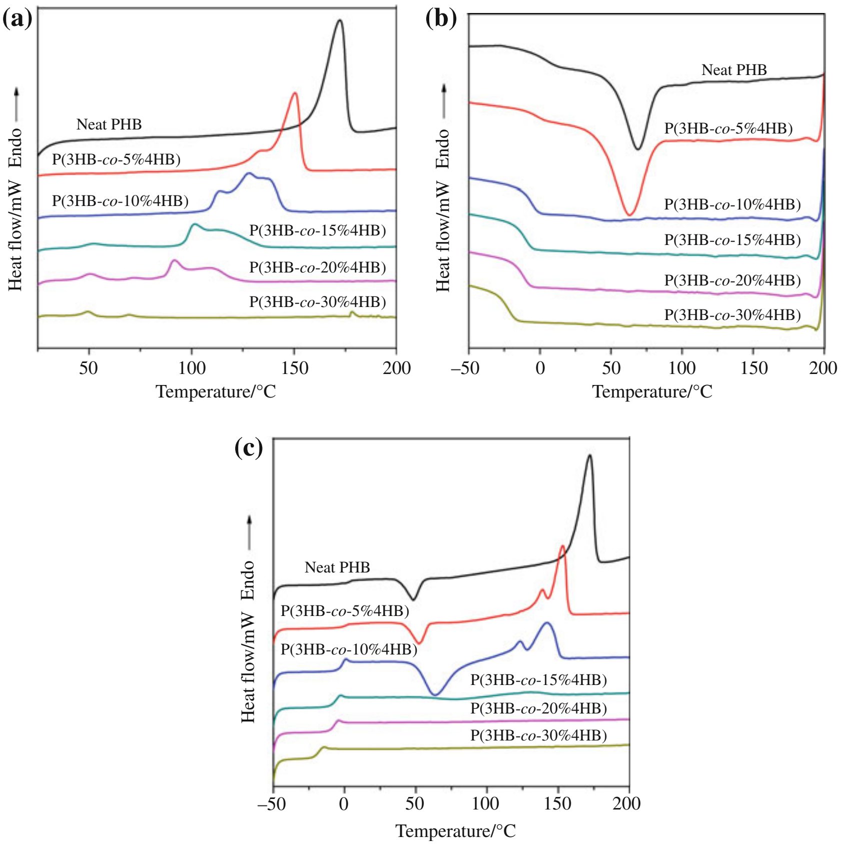

Fig. 1 **a** First heating curve of neat PHB and P(3HB-*co*-4HB) copolymers. **b** Cooling curve of neat PHB and P(3HB-*co*-4HB) copolymers. **c** Second heating curve of neat PHB and P(3HB-*co*-4HB) copolymers

copolymers in the first DSC heating scans. A single sharp melting peak appears in neat PHB, while multiple melting peaks are observed in P(3HB-*co*-4HB) copolymers. As 4HB molar fraction increases, all melting peaks shift to lower temperature. In particular, the intensity of the melting peak in the higher temperature decreases, while that of the melting peak in the lower temperature increases.

There are six mechanisms to relate to the multiple melting behaviors [26, 27]: (1) melting, recrystallization and remelting (mrr) during heating, (2) presence of more than one crystal modifications (polymorphism), (3) different crystalline morphology (lamellar thickness, distribution, perfection, or stability), (4) physical aging and relaxation of the rigid amorphous fraction, (5) different molecular weight species, (6) orientation effects, and so on.

From WAXD observation [28], there is no crystal modification change for PHB with different 4HB molar fraction. Therefore, the multiple melting behavior of P(3HB-*co*-4HB) in Fig. 1a does not originate from the different crystal modification. In first heating scans, the multiple melting behavior of P(3HB-*co*-4HB) can be explained reasonably by the micro-phase separation behavior. With the introduction of 4HB unit, the incompatible two-phase containing 3HB-rich micro area and 4HB-rich micro area, respectively are formed [29], the melting peak in the higher temperature corresponded to the melting of crystals generated in 3HB-rich micro area and the melting peak in the lower temperature attributed to the melting of crystals formed in 4HB-rich micro area. While in second heating scans, the multiple melting peaks may be caused by melting, recrystallization and remelting (mrr) process as well [30].

In addition, the melting enthalpy of the copolyesters is much smaller than that of neat PHB, indicating that the crystallinity (X_c) decreases due to the introduction of 4HB unit, which exists as defects in the PHB crystalline regions and is excluded from the PHB crystal lattice.

The cooling scans and the second heating scans are shown in Fig. 1b, c, displaying the glass transition temperature (T_g), melt crystallization temperature (T_c), cold crystallization temperature (T_{cc}), and second melting temperature (T_{m2}), respectively. These relative values of T_{m1}, X_c, T_g, T_c, T_{cc}, T_{m2}, and ΔH_{m1} are listed in Table 1. It can be seen that T_g decreases with increasing of 4HB molar fraction, meaning that the flexibility of molecular chain segments is improved by 4HB unit. This is attributed to the presence of 4HB unit offering a larger free volume of molecular movement [20].

T_c and T_{cc} reflect the nucleation rate and crystal growth rate, respectively [20], the higher T_c, the rapider nucleation

Table 1 Values of T_{m1}, T_g, T_c, T_{cc}, T_{m2}, ΔH_{m1}, and X_c of PHB and P(3HB-*co*-4HB) copolymers

Samples	First heating process			Cooling process		Second heating process		
	T_{m1}/°C	ΔH_{m1}/J/g	X_c/%	T_g/°C	T_c/°C	T_g/°C	T_{cc}/°C	T_{m2}/°C
Neat PHB	172.3	98.16	67.23	2.6	69	3.8	48	173.6
P(3HB-*co*-5%4HB)	150.4	76.98	52.73	−3.5	63.1	0.4	52.0	153.1
P(3HB-*co*-10%4HB)	128.4	71.53	48.99	−6.4	50.7	−2.5	63.7	142.1
P(3HB-*co*-15%4HB)	101.6	41.03	28.10	−11.2	–	−7.1	78.2	130.5
P(3HB-*co*-20%4HB)	91.8	38.43	26.32	−12.1	–	−8.1	–	–
P(3HB-*co*-30%4HB)	69.7	3.04	2.08	−22.5	–	−18.8	–	–
P(4HB) [31]	54.0	–	–	−50.0	–	–	–	–

P(4HB) poly (4-hydroxybutyrate)

rate and the better crystallization ability. While the lower T_{cc}, the rapider crystal growth rate and the better crystallization ability. It can be observed in Table 1 that T_c decreases whereas T_{cc} increases with the increase of 4HB molar fraction. This indicates that crystallization ability of PHB is strongly reduced by the presence of 4HB unit.

It should be noted that at the selected cooling and heating rate, T_c and T_{cc} of P(3HB-*co*-20%4HB) and P(3HB-*co*-30%4HB) cannot be detected, suggesting when 4HB fraction is up to 20 mol%, the crystallization ability of copolyesters markedly decreases.

Spherulitic morphology

Figure 2 shows the polarized optical micrographs of neat PHB and P(3HB-*co*-4HB) copolymers after isothermal crystallization at 60 °C for 2 h. It is observed that the typical spherulite morphology with ‘‘Maltese Cross’’

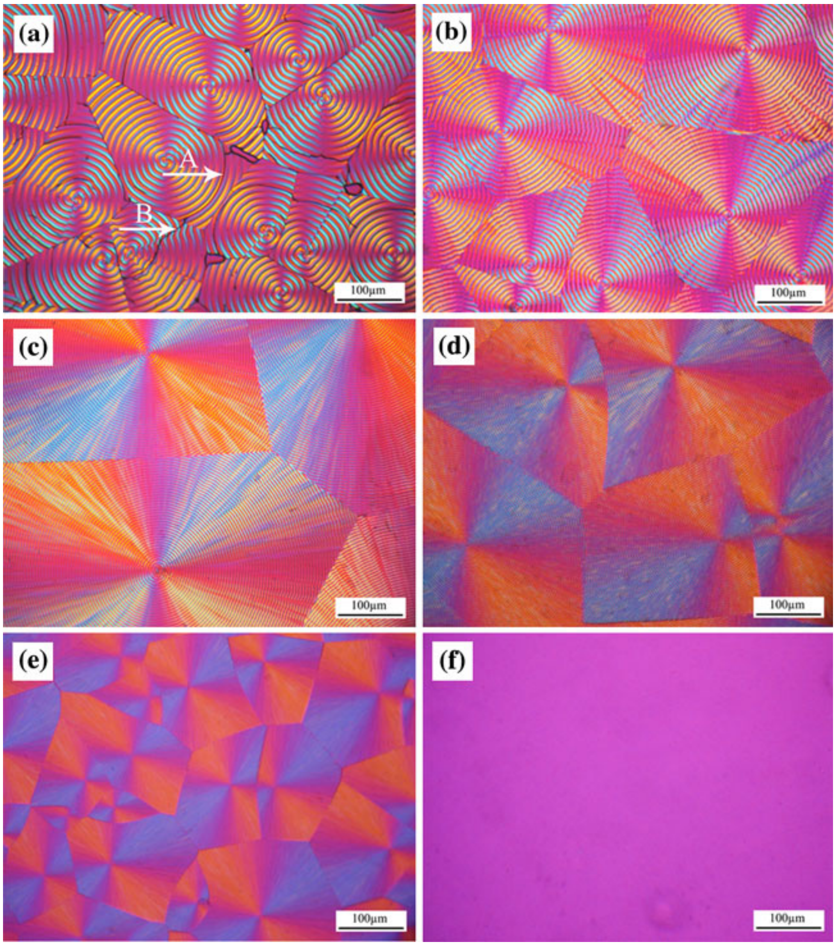

Fig. 2 Optical micrographs of neat PHB and P(3HB-*co*-4HB) copolymers crystallized at T_c = 60 °C. **a** Neat PHB, **b** P(3HB-*co*-5%4HB), **c** P(3HB-*co*-10%4HB), **d** P(3HB-*co*-15%4HB), **e** P(3HB-*co*-20%4HB), **f** P(3HB-*co*-30%4HB). *Arrow A* and *B* indicate radial and circumferential cracks, respectively

extinction pattern can be found in neat PHB, P(3HB-*co*-5%4HB), P(3HB-*co*-10%4HB), P(3HB-*co*-15%4HB) and P(3HB-*co*-20%4HB) (Fig. 2a–e). Also, those spherulites exhibit concentric extinction bands in POM, which has been found in PHB [25] and PHBV [32] spherulites as well. In addition, with 4HB fraction lower than 10 mol%, the size of spherulite increases with the increase of 4HB molar fraction. While the spherulite size decreases with 4HB fraction higher than 15 mol%. By contrast, no spherulite is observed in P(3HB-*co*-30%4HB) (Fig. 2f).

The results indicate that the influence of 4HB unit on P(3HB-*co*-4HB) crystallization behaviors is more complicated. The crystallization of P(3HB-*co*-4HB) copolymers is determined by the competition between two factors. On the one hand, the crystal regularity of neat PHB is undermined by the existence of 4HB unit, which resulting in decreasing of crystallization ability. On the other hand, the introduction of 4HB unit could improve the macromolecular chains mobility, which is beneficial to crystallization. Therefore, with 4HB fraction lower than 10 mol%, it has less effect on the regularity reduction of copolyesters and stronger macromolecular chains mobility. Thus, the growth rate of the spherulites increases with increasing of 4HB molar fraction, and the large and perfect spherulites is formed. Whereas with 4HB fraction higher than 15 mol%, the regularity of the copolyesters is greatly destroyed leading to the decrease of crystallization ability, and molecular chain segments are too flexible to form the stable crystal lattice. PHB molecular chains are diluted by 4HB unit and are more difficult to stack in an ordered manner than those of neat PHB [33]. Thus, the degree of perfection of the spherulites is disrupted and the spherulite size decreases. When 4HB fraction is up to 30 mol%, the spherulitic morphology could not be clearly discerned.

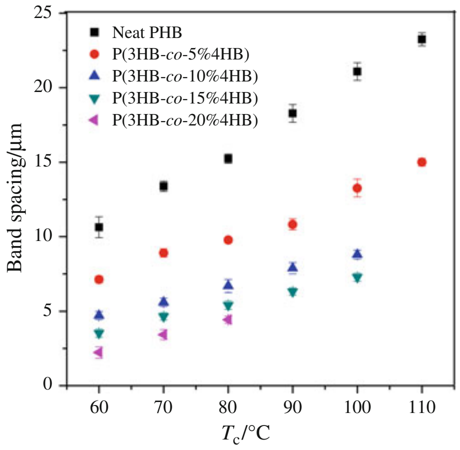

Fig. 3 Variation of band spacing as a function of T_c in neat PHB and P(3HB-*co*-4HB) spherulites

Furthermore, according to the average measurement on the periodicity of the extinction bands for at least 50 spherulites, the band spacing of the banded spherulite decreases with the increase of 4HB molar fraction (Fig. 3). Banded spherulite (i.e., the presence of concentric extinction bands in POM) is a common morphological feature in crystalline polymers [34, 35]. 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. Various mechanisms of the lamellar twisting have been proposed [36–40]. The lamellar twisting is attributed to the formation of screw dislocations and/or continuous twisting for the growth of lamellae. Imbalanced stress at lamellar fold surfaces is usually regarded as the cause of the lamellar twisting.

The band spacing corresponds to the half-pitch length of twist lamellae. Also, the inversed value of the helical pitch length of twisting ribbons is defined as helical twisting power (HTP) [35]. In other words, the HTP increases with the increase of 4HB molar fraction, and P(3HB-*co*-4HB) copolymers possess higher HTP for the formation of twist lamellae. 4HB unit is excluded from the PHB crystal lattice that can act as diluents to cause the imbalance surface stress at crystalline lamellar fold surfaces. Thus, the tendency of the lamellar twisting is considerably enhanced.

In addition, two types of cracks are found in neat PHB spherulites: radial cracks and circumferential cracks, as illustrated in Fig. 2a. These cracks are responsible for the embrittlement of PHB [41], which formed during cooling because of large internal stress generated by high-volume shrinkage [42]. However, no cracks appear in P(3HB-*co*-4HB) banded spherulites. On one hand, the lower crystallinity of P(3HB-*co*-4HB) copolymers leads to the increase of amorphous region and chain segments mobility, which results in the internal stress produced during cooling dissipated completely in the amorphous region. On the other hand, the lamellae contraction is restricted by 4HB unit. Therefore, the incorporation of 4HB unit inhibits the emergence of cracks.

Figure 4 shows a series of polarized optical micrographs for P(3HB-*co*-5%4HB) spherulites isothermally crystallized at various T_c for 2 h. As observed, all images show the banded spherulites and morphology of P(3HB-*co*-5%4HB) spherulites is strongly dependent upon T_c.

In certain crystallization temperature ranging from 60 to 90 °C, the size of spherulites increases with the increase of T_c due to the decrease in nucleation density. The size of spherulites decreases when the temperature is up to 100 °C. Also, with the increase of T_c, the band spacing increases (Fig. 3), resulting from the decreased in magnitude of lamellar twisting [34].

Moreover, concentric rings are observed in the banded spherulites of P(3HB-*co*-5%4HB) (Fig. 4d, e). The rings

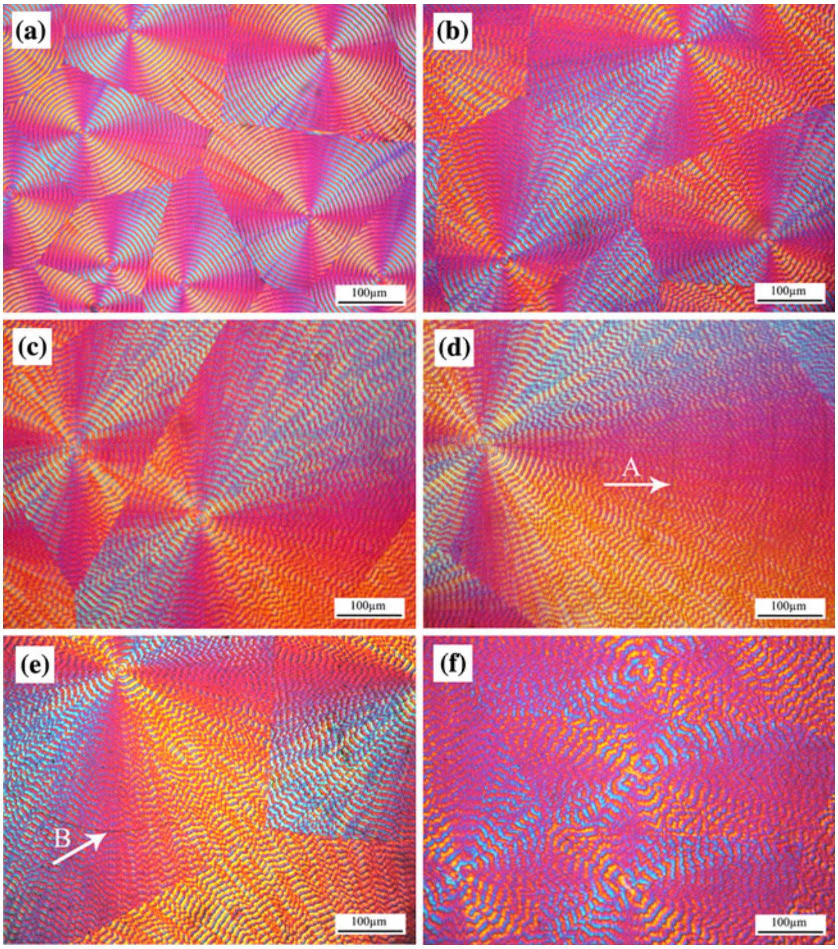

Fig. 4 Optical micrographs of P(3HB-*co*-5%4HB) at different crystallization temperatures: **a** 60 °C, **b** 70 °C, **c** 80 °C, **d** 90 °C, **e** 100 °C, **f** 110 °C. *Arrow A* and *B* indicate concentric rings

used to be considered as cracks [43]. Xu [44] confirmed that these concentric rings with varying spacing were growth terraces rather than cracks. The height of the terraces reached up to several hundred nanometers. The formation mechanisms of concentric rings will be investigated in our future study.

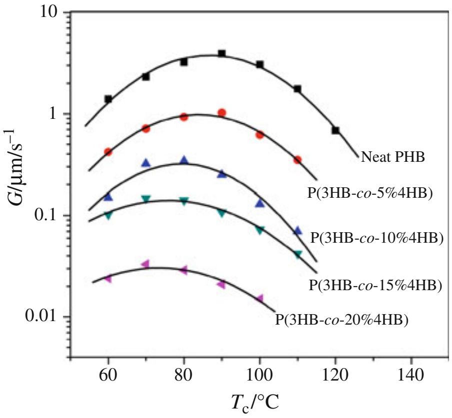

Fig. 5 Variation of spherulite growth rate (G) as a function of T_c of neat PHB and P(3HB-*co*-4HB) copolymers

Spherulitic growth rate

The radial growth rate of the spherulite (G) was determined by monitoring the spherulite radius R as a function of time during isothermal crystallization in the hot stage of a polarizing microscope. For each sample, R was found to increase linearly in time up to the point of impingement, indicating a constant growth rate throughout the crystallization process. Figure 5 shows the radius growth rate (G) of spherulites as a function of T_c. The curve of G is considerably reduced with the increase of 4HB molar fraction, indicating that 4HB unit retarded the crystallization of 3HB unit. This may be attributed to the steric effect of 4HB unit and the depression of equilibrium melting point (T_m^o) [20]. A maximum value, G_{max} is observed in each curve and the G_{max} exhibits a gradual shift toward lower T_c with the increase of 4HB molar fraction, which may partially be attributed to the depression in T_m^o.

Therefore, the crystallization rate of P(3HB-*co*-4HB) copolymers can be adjusted by varying the crystallization temperature or copolymer composition.

Conclusions

Crystallization behaviors and spherulitic morphology of neat PHB and P(3HB-*co*-4HB) copolymers were investigated using DSC and POM, respectively.

The incorporation of 4HB reduces the regularity of PHB chain structure to depress the melt temperature and crystallinity. The higher 4HB molar fraction, the more pronounced is the reduction of T_m and crystallinity. The depression of T_m is beneficial, because the material can be processed at a temperature much lower than the thermal decomposition temperature of PHB. The reduction of crystallinity of P(3HB-*co*-4HB) imparts the desired ductility to the material compared to the excessively crystalline and brittle PHB. The incorporation of 4HB unit also increases the local segmental mobility of P(3HB-*co*-4HB) copolymers, indicated by the depression of their glass transition temperatures.

Micro-phase separation behavior and mrr process are predominant in multiple melting peaks of P(3HB-*co*-4HB) during heating process.

Banded spherulites are observed in PHB and P(3HB-*co*-4HB) copolymers. The band spacing decreases with the increase of 4HB molar fraction and the decrease of crystallization temperatures. Morphology and growth rate of the spherulites strongly depend on 4HB molar fraction and the crystallization temperatures. With the increase of 4HB molar fraction, the spherulites growth rate decreases and the temperature at the maximum growth rate shifts to lower temperature. The introduction of 4HB unit can inhibit the emergence of cracks in PHB spherulites.

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