A Synchrotron in situ X-ray Study on the Multiple Melting Behaviors of Isomorphous Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (P(HB-co-HV)) with Middle HV Content

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Abstract The multiple endothermic peaks without observable recrystallization phenomenon of isomorphous poly(3-hydroxybutyrate-co-3-hydroxyvalerate) [P(HB-co-HV)] with the middle HV content of 19.4 mol% and 28.7 mol% were confirmed by differential scanning calorimetry (DSC), and the evolutions of crystal structure and lamellar morphology in the heating and melting process were tracked by in situ synchrotron wide angle X-ray diffraction (WAXD) and small angle X-ray scattering (SAXS) techniques. The emergence of asymmetric features of both the diffraction peaks and scattering curves indicates the coexistence of different lamellar crystals with varied unit cell parameters. Based on the in situ WAXD and SAXS measurements, we calculated the evolutions of the unit cell parameters a and b as well as the long period and lamellar thickness upon heating. The comparative analysis of WAXD and SAXS data confirms that the multiple endothermic peaks of P(HB-co-19.4%HV) and P(HB-co-28.7%HV) result from the melting of different lamellae rather than the melting/recrystallization. The thinner, unstable uniform lamellae with HV counts total inclusion melt first and the thicker, stable sandwich lamellae with HV counts partial inclusion melt last. In addition, the large second melting peak in P(HB-co-19.4%HV), differing from that of samples with HV content of 28.7 mol% and 36.2 mol%, is due to the unique state of HV content leading to a transition of sandwich lamellae to uniform lamellae. The present study establishes the relationship between the different lamellae structure and multiple melting behaviors of isomorphous copolymer.

Keywords P(HB-co-HV); Isomorphous; Middle HV content; Synchrotron radiation

INTRODUCTION

With the rapid decrease of nonrenewable petroleum and the aggravating environmental pollution of plastics waste, the development and application of traditional petroleum-based polymer materials are being restricted. Aliphatic polyesters have received increasing attention due to their excellent biodegradability and eco-friendly feature. It is known that material properties and degradation behavior of crystalline polyesters are highly related to crystal structure and morphology, which can be controlled by crystallization conditions. Recently, much attention has been paid to random copolyesters, in which the introduced comonomers can further regulate the crystallization behavior and thus improve the material performance. The copolyesters consisting of two crystalline comonomers can crystallize in two different ways: (a) the crystals are only formed by the major comonomers with the minor component total exclusion; (b) the minor monomers can be included in the crystal lattice of major comonomers, resulting in occurrence of isomorphism. Only a few copolyesters, such as poly(3-hydroxybutyrate-co-3-hydroxyvalerate) [P(HB-co-HV)], poly(ε-lactic acid-co-glycolic acid), poly(butylene succinate-co-cis-butene succinate), poly(butylene succinate-co-e-caprolactone), and poly(1,6-hexanediol-co-1,12-dodecanediol), can crystallize with the second manner. It is now well recognized that the formation of isomorphism requires the different comonomers reflecting the similar chemical structure, chain conformation, and crystal lattice. The existence of isomorphism allows the copolyesters to crystallize in the large range of comonomer content, and has a profound influence on the thermal behavior, crystal structure, and lamellar morphology of resultant polymer materials.

P(HB-co-HV) is one of the excellent biocompatible and biodegradable copolyesters, which has been widely studied in fields of biological scaffold and food packaging materials. Owing to the similar chemical structure, chain conformation, and unit cell parameters between the two monomers of HB and HV, the isomorphism of P(HB-co-
HV) has been confirmed by wide angle X-ray diffraction (WAXD), nuclear magnetic resonance (NMR), and infrared spectroscopy (IR). The crystal structure transforms from PHB-type to PHV-type at HV content higher than 40 mol%. Meanwhile, the crystallization behavior of P(HB-co-HV) with PHB-type crystal is also deeply affected by HV content. For example, the single crystal morphology of P(HB-co-HV) with HV content lower than 10 mol% is similar to that of PHB, but different from that of P(HB-co-HV) with HV content higher than 10 mol%. In addition, the ratio of the HV content in crystalline phase to the overall HV content increases with HV content, e.g., the sample with 27 mol% HV content can include 2/3 HV units in the crystalline phase. To explain the discontinuity of change of melting point, crystallization rate, and long period with increasing HV content, Yoshie et al. have proposed two lamellar models to describe the structure of crystalline phase with HV counts inclusion. The sandwich lamellae with the core of pure HB units and HV counts inclusion in the edge form at the HV content lower than 10 mol%, and the uniform lamellae with the HV counts uniform inclusion generate at the HV content range from 10 mol% to 40 mol%.

Emergence of multiple endothermic peaks is a generic phenomenon in P(HB-co-HV), which has also been reported to occur in other isomorphous copolymers. It is usually believed that this behavior is attributed to the melting-recrystallization-induced lamellar thickening. However, with the exception of the evidence that the cold crystallization peak is observed among melting peaks, more observations appear to relate to it to the isomorphism structure of P(HB-co-HV) crystalline lamellar crystals. On the one hand, the HV content in crystalline phase increases with decreasing isothermal temperature, and the crystallization rate depresses with increasing HV content. On the other hand, the lamellar morphology alters with different HV content range, and it has been confirmed that the rapid increase of long period originates from the melting of the thin and unstable lamellae rather than the gradual thickening of lamellar thickness. It has also shown in other isomorphous copolymers that the competition of comonomer units affects the isomorphism behavior of lamellae.

Recently, we have investigated the multiple endothermic peaks in P(HB-co-HV) by synchrotron X-ray diffraction and scattering techniques. It has confirmed that the different lamellar structures with HV counts total exclusion and HV counts partial inclusion result in the double melting peaks of P(HB-co-HV) with HV content lower than 10 mol%, and the different lamellar crystals with HV counts total inclusion and HV counts partial inclusion lead to the dual endothermic peaks of P(HB-co-HV) with HV content higher than 30 mol%. The thermal behavior of P(HB-co-HV) with middle HV content of 10 mol% to 30 mol% is different from that of P(HB-co-HV) with HV content lower than 10 mol% or higher than 30 mol%. So the underlying mechanism leading to multiple endothermic peaks of P(HB-co-HV) with middle HV content deserves further investigation. Herein, we chose P(HB-co-19.4%HV) and P(HB-co-28.7%HV) samples to further study the structural evolution of isomorphous P(HB-co-HV) crystals with middle HV content. By a comparative analysis of the effect of HV content upon the crystal lattice size and lamellar morphology, the structural evolution of P(HB-co-HV) lamellar crystals resulting in the multiple endothermic peaks was proposed.

**EXPERIMENTAL**

**Materials**

Two P(HB-co-HV) samples with HV content of 19.4 mol% and 28.7 mol%, measured by Bruker Advance III NMR spectrometer at 400 MHz, were provided by Ningbo Tianan Biologic Co., Ltd. in China. Both samples were polymerized by bacterial fermentation, and the HV counts distributed randomly in sequence. Gel permeation chromatography (GPC) calibrated with a standard sample of polystyrene was employed to characterize the weight-average molecular weight ($M_w$) and polydispersity index (PDI). We found that $M_w$ and PDI of P(HB-co-19.4%HV) were 164.5 kg/mol and 3.17, respectively, while those of P(HB-co-28.7%HV) were 52.6 kg/mol and 3.64, respectively, as given in Fig. S1 (in the electronic supplementary information, ESI).

**DSC Measurement**

Thermal behavior of P(HB-co-HV) was analyzed by the Pekin Elmer DSC8000 under nitrogen atmosphere. The instrument was calibrated by indium, and the baseline was collected by an empty alum pan performing the same procedure as samples. To obtain the melting temperature ($T_m$) and fusion enthalpy ($ΔH$), the two samples were firstly heated to 190 °C and held for 3 min to remove the thermal history, and then cooled from 190 °C to 30 °C at a constant rate of 10 °C/min and subsequently kept isothermal for 24 h. Finally, the second heating under a rate of 5 °C/min from 30 °C to 190 °C was performed to reveal the melting behavior of P(HB-co-HV).

**X-ray Measurement**

In situ synchrotron WAXD and SAXS measurements were applied to track the evolution of structure and thickness of P(HB-co-HV) lamellar crystals upon heating by using a Linkam 650 high temperature stage. The temperature procedure is the same as that of the second DSC scanning. The X-ray measurements were implemented at the BL16B1 beamline in Shanghai Synchrotron Radiation Facility (SSRF) with the X-ray wavelength of 0.1239 nm, and a MAR165 X-ray detector with 2048 × 2048 pixels and size of 79 μm × 79 μm was employed to collect two dimensional (2D) WAXD and SAXS patterns. The sample-to-detector distance was 250 mm for WAXD and 5130 mm for SAXS testing, respectively, and the acquisition time of each image of both WAXD and SAXS was 20 s. All WAXD and SAXS data were captured by the background scattering and air scattering.

**WAXD and SAXS Data Analysis**

The analyses of 2D WAXD and SAXS data were carried out by the Fit2d software package. The 1D WAXD curves were verified and corrected after transforming by 2D WAXD data. Based on WAXD measurement, the evolution of crystal lattice can be detected. The orthorhombic unit cell parameters $a$ and $b$ of P(HB-co-19.4%HV) and P(HB-co-28.7%HV) were calculated according to the two main diffraction peaks of (020) and (110) planes by Eq. (1):

$$
\frac{1}{d_{hkl}^2} = \frac{h^2}{a^2} + \frac{k^2}{b^2} + \frac{l^2}{c^2}
$$
where \( h, k, \) and \( l \) are the Miller indices and \( d_{\text{hkl}} \) is the interplanar spacing, defined by Bragg’s law:

\[
d_{\text{hkl}} = \frac{\lambda}{2\sin \theta}
\]

(2)

where \( \lambda \) is the X-ray wavelength and \( \theta \) is the diffraction angle.

SAXS is an established method for probing the evolution of long period and lamellar thickness. In crystalline polymer systems, the scattering ring of SAXS patterns reflects the long period \( (L = d_c + d_a) \) formed by the crystalline layer with thickness of \( d_c \) and amorphous layer with thickness of \( d_a \). In unoriented polymer systems, the electron density periodically fluctuates along the normal of the crystalline layer, corresponding to the periodic alternation of crystalline and amorphous regions. So one-dimensional correlation function \( K(z) \) provides an approach to describe the variation of electron density as a function of distance, which can be used to evaluate the average values of \( L, d_c \), and \( d_a \), the linear crystallinity, and the scattering invariant.\(^{[52,53]} \)

\[
K(z) = \int_0^\infty i(q) q^2 \cos(qz) dq \int_0^\infty i(q) q^2 dq
\]

(3)

where \( z \) is the distance along the normal direction beyond original crystalline layer, and \( q \), defined as \( 4\pi \sin \theta / \lambda \), is the scattering vector.

RESULTS AND DISCUSSION

Fig. 1 presents the second DSC heating curves to display the melting behavior of the two P(HB-co-HV) samples. Multiple endothermic peaks are observable in both P(HB-co-19.4%HV) and P(HB-co-28.7%HV), especially in P(HB-co-19.4%HV), which can be divided into three melting peaks. The corresponding melting temperature and enthalpy of fusion of the multiple melting peaks is discernible in Fig. 1, and the finite thickening of amorphous regions. So one-dimensional correlation function \( K(z) \) provides an approach to describe the variation of electron density as a function of distance, which can be used to evaluate the average values of \( L, d_c \), and \( d_a \), the linear crystallinity, and the scattering invariant.\(^{[52,53]} \)

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Gaussian function (Fig. S3, in ESI).\[56,57\] Then the unit cell parameters $a_1$ and $b_1$ are calculated by the low 2$\theta$ peaks of (110) and (020) crystallographic planes, respectively, and the unit cell parameters $a_2$ and $b_2$ are calculated by the high 2$\theta$ peaks of (110) and (020) diffractions, respectively, as presented in Figs. 4(c) and 4(d). Although the melting behavior of P(HB-co-19.4%HV) is similar to that of P(HB-co-28.7%HV), the (110) and (020) diffraction peaks of P(HB-co-19.4%HV) are difficult to divide into two peaks, and the large error was found in the results of $a_1$ and $b_1$ when we tried to calculate unit cell parameters after the peak separated. In Figs. 4(a) and 4(b), at the early heating procedure, both $a$ and $b$ increase almost linearly, which is caused by thermal expansion of crystals. The slope of $a$ and $b$ decreases with the temperature approaching to the melting range around $T_{m1}$, indicating that some crystals with large crystal lattice melt first. Due to the existence of isomorphism, the first melted crystals must contain the HV counits, which has been confirmed previously by that the HV counits inclusion expands both $a$ and $b$.\[22,23\] The decrease of the slope of $b$ happens at 80.4 °C, which is earlier than the first melting range around $T_{m1}$ and can be ascribed to the slight evolution of crystal corresponding to the early occurrence of the first melting process. The earlier change in $b$ is because the direction of side ethyl of HV units in PHB type lattice is
closer to b axis. From 139.5 °C to 161.4 °C, b increases linearly again, meaning that the crystal structure related to the second and third melting peaks is similar. When temperature is higher than 161.4 °C, b decreases with the increasing temperature, indicating that most crystals have molten and the remaining ones with higher stability contain less HV counts inclusion. As can be seen in Figs. 4(c) and 4(d), a1 and b1 first increase linearly, then begin to decrease at 65.0 °C, and finally disappear at 111.0 °C, which can be attributed to the early melting of some crystals with large crystal lattice. The change of a2 and b2 above 111.0 °C is related to the crystals melted at the range around Tm2 and Tm3, which increases linearly below 111.0 °C. The slope of b2 decreases at melting range between Tm1 and Tm2, meaning that the unstable crystals with large HV counts inclusion melt first, while the slope of a2 decreases in the melting range around Tm1 with the slope calculated to be k4 = 6.17 × 10−5, as shown in Fig. 4(c). It is due to the complex distribution of HV counts inclusion in crystals. The two types of lamellar crystals are the ideal model to describe the evolution of crystals with different HV counts inclusion; when one kind of crystals melts, the other must be affected since the separation of the peak during the first melting range around Tm1 is quite difficult in Fig. 3(b). According to the above analyses, it is clear that not only P(HB-co-19.4%HV) but also P(HB-co-28.7%HV) has two types of lamellar crystals, and the one with larger size of unit cell melt first while the other with the smaller crystal lattice melt subsequently.

Comparing the results of samples with other HV contents, it is apparent that the evolution of unit cell parameters of P(HB-co-28.7%HV) is similar to that of P(HB-co-36.2%HV). The values of a1, a2, b1, and b2 of P(HB-co-28.7%HV) are 0.597, 0.582, 1.347, and 1.328 nm, and those of P(HB-co-36.2%HV) are 0.603, 0.581, 1.351, and 1.329 nm at 41.0 °C, respectively. It is obvious that the first melted crystals of P(HB-co-28.7%HV) are the lamellae with HV counts total inclusion. The similar Tm1 of P(HB-co-19.4%HV) and P(HB-co-28.7%HV) and the decreased size of crystal lattice of P(HB-co-19.4%HV) around Tm1 indicate that the first kind of melting crystals of P(HB-co-19.4%HV) is also the crystals with HV counts total inclusion. Furthermore, the values of a and b of P(HB-co-9.4%HV), P(HB-co-9.4%HV), P(HB-co-19.4%HV), P(HB-co-28.7%HV), P(HB-co-36.2%HV) are 0.584, 0.584, 0.592, 0.589, 0.588 nm and 1.326, 1.327, 1.340, 1.332, 1.333 nm at 150.5 °C, respectively. At this temperature, the samples with HV content lower than 10 mol% contain a large number of crystals with HV counts partial inclusion and a small number of crystals with HV counts total exclusion. So, the last melted crystals of P(HB-co-19.4%HV) and P(HB-co-28.7%HV) are the crystals with HV counts partial inclusion, for which the values of a and b are higher than those of samples with low HV contents. The length of HB sequence reduces with the in-

Fig. 4 The evolution of unit cell parameters (a) a and (b) b of P(HB-co-19.4%HV) and (c) a and (d) b of P(HB-co-28.7%HV) as a function of temperature from 30.0 °C to 170.2 °C. k is the slope of unit cell parameters.

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increasing HV content, meaning the absence of crystals with HV counits total exclusion in samples with HV content higher than 10 mol%. It is interesting that the values of $a$ and $b$ of P(HB-co-19.4%HV) are the largest among all the samples after $T_{m1}$, which can be attributed to the melting of crystals with more HV counits inclusion earlier than other samples. The values of $a$ and $b$ of remaining crystals of P(HB-co-28.7%HV) and P(HB-co-36.2%HV) are similar at 150.5 °C. This indicates that the HV content from 19.4 mol% to 28.7 mol% is the transition state from the crystals with HV partial inclusion to the crystals with HV counits total inclusion, for the melting behavior and change of unit cell parameters of P(HB-co-4.9%HV) and P(HB-co-9.4%HV) are quite similar, but those of P(HB-co-19.4%HV), P(HB-co-28.7%HV), and P(HB-co-36.2%HV) show the tendency of generating more crystals with HV counits inclusion with the increase of HV content.

In situ SAXS measurements can also provide the evidence to support the evolution of different lamellar crystals with HV counits total or partial inclusion. The 1D SAXS patterns are obtained from 2D SAXS patterns by the same method as that of 1D WAXD patterns, and the applied Lorentz correction ($Iq^2$ versus $q$) SAXS patterns are shown in Fig. 5. Before melting, the scattering peaks are asymmetrical and board double peaks. With the increasing temperature, the high $q$ scattering peak weakens and the low $q$ scattering peak shifts to the lower $q$. At $T_{m1}$, the high $q$ scattering peak disappears, hence the shape of scattering peak changes to the symmetrical single peak. It is therefore evident that the two different kinds of periodic structures melt at varied melting range, i.e., the small periodic structure with high $q$ scattering peak melts first and the large periodic structure with low $q$ scattering peak melts last. A comparison of the scattering behavior of the two samples demonstrates that the shift of scattering peaks of P(HB-co-19.4%HV) is larger than that of P(HB-co-28.7%HV), indicating that the evolution of lamellar morphology of P(HB-co-19.4%HV) upon heating is more complex than that of P(HB-co-28.7%HV), being well in line with the WAXD results.

To reveal the evolution of different periodic structures in the heating process, the scattering peaks are divided into two correlative peaks by Gaussian function. One dimensional correlation function is used to calculate the long period and lamellar thickness by each independent peak. The long period-1 ($L_1$) and thickness of lamellae-1 ($d_{c1}$) are calculated from the peak at high $q$, and the long period-2 ($L_2$) and thickness of lamellae-2 ($d_{c2}$) are calculated from the peak at low $q$. It can be seen from Fig. 6 that the evolution of long period and lamellar thickness of the two samples is similar before $T_{m1}$, and the increase of long period is larger than that of lamellar thickness during melting, implying that the amorphous layer increases substantially. As shown in Figs. 6(a) and 6(b), $d_{c1}$ of P(HB-co-19.4%HV) is almost unchanged before melting, and then increases with temperature close to $T_{m1}$, and disappears at $T_{m2}$, indicating that the thinner and less stable lamellae with HV counits total inclusion melt first. $d_{c2}$ still increases during the melting range around $T_{m2}$ and $T_{m3}$, which corresponds to the melting process of the remaining thicker lamellae with HV counits partial inclusion on the basis of the WAXD results. As can be seen in Figs. 6(c) and 6(d), the evolution of long period and lamellar thickness of P(HB-co-28.7%HV) is similar to those of P(HB-co-19.4%HV), indicating that the lamellar structures are similar in P(HB-co-HV) with this range of HV content. It is clear that the uniform lamellae with HV counits total inclusion melt at the first melting peak and the sandwich lamellae with HV counits partial inclusion melt at the second and the third melting peaks. It seems that the sandwich lamellae containing much HV counits melt earlier at $T_{m2}$, corresponding to the decrease of slope of $b$ and increase of $d_{c2}$ of both P(HB-co-HV) samples closing to $T_{m2}$.

By comparing samples with different HV contents,[49,50] several points need to be further discussed. The values of $d_{c2}$ of P(HB-co-4.9%HV), P(HB-co-9.4%HV), P(HB-co-19.4%HV), P(HB-co-28.7%HV), and P(HB-co-36.2%HV) are 7.92, 5.41, 5.10, 5.18, and 5.87 nm at 41.0 °C and 8.88, 7.37, 10.02, 6.86, and 8.71 nm at 150.5 °C, respectively. When the HV content is lower than 10 mol%, $d_{c2}$ decreases with the increase of HV content, which is due to the decrease of HB sequence with increasing HV counits. When the HV content is higher than 10 mol%, the most crystals are formed at the isothermal temperature of 30.0 °C, which are difficult to crystallize at the cooling rate of 10 °C/min. This point is further supported by the obvious cold crystallization peaks appearing during heating procedure.[46] It is clear that the amount of HV counits in crystals increases with the decrease of isothermal crystallization temperature,[24] therefore the sandwich lamellae formed

![Fig. 5](https://doi.org/10.1007/s10118-020-2427-5) 1D SAXS patterns of (a) P(HB-co-19.4%HV) and (b) P(HB-co-28.7%HV) heating from 30.0 °C to 170.2 °C.
at 30.0 °C may include more HV counits. So, $d_{c2}$ increases with HV content, corresponding to $a_2$ and $b_2$, increasing with HV at 41.0 °C in WAXD analysis. In other words, the crystals include more HV counits with the increase of HV content. The lamellar thickness and unit cell parameters of P(HB-co-19.4%HV) are higher than the samples with other HV contents at 150.5 °C, and the increase of $d_{c2}$ of P(HB-co-28.7%HV) happens around $T_{m1}$, while the increase of $d_{c2}$ of P(HB-co-19.4%HV) takes place around $T_{m2}$, which is the main melting peak as the most crystals melted. It is clear that the evolution of unit cell parameters and lamellar thickness of the HV content range from 19.4 mol% to 28.7 mol% is different from that of the HV content range from 28.7 mol% to 36.2 mol%, which has been mentioned at the analysis of WAXD and SAXS data. This point may correspond to the transition state of sandwich lamellae to uniform lamellae. Cuesta et al. and Barker et al. have offered an equation to estimate the amount of HV counits in crystals by unit cell volume,[22,24] i.e., $x_{hv} = (v_v - v_b) / (v_V - v_b)$, wherein $x_{hv}$ is the fraction of HV counits included in crystals, $v$ is the unit cell volumes of sample (parameter $c$ cited from the PHB single crystal, $c = 0.596$ nm),[19] $v_b$ and $v_V$ are the unit cell volume of PHB and PHV, which can be provided by the single crystals of PHB and PHV as $a = 0.576$ nm, $b = 1.320$ nm, $c = 0.596$ nm,[19] and $a = 0.932$ nm, $b = 1.002$ nm, $c = 0.556$ nm,[25] respectively. $x_{hv}$ of P(HB-co-19.4%HV) is calculated to be 0.18 at 30.0 °C, closing to the HV content. $x_{hv}$ of P(HB-co-28.7%HV) is calculated to be 0.38 and 0.10, corresponding to the crystals with HV counits total inclusion and the crystals with HV unit partial inclusion, respectively. It further explains the above phenomena including $\Delta H_v$ of P(HB-co-19.4%HV) larger than that of P(HB-co-28.7%HV), the difficulty of separated peaks of P(HB-co-19.4%HV), and the larger unit cell parameter $b$ of P(HB-co-19.4 mol%) than other HV content at 150.5 °C as the amount of HV counits in sandwich lamellae is close to that in the uniform lamellae. It also seems that the HB sequences with fewer HV counits crystallize first into sandwich lamellae, while the HB sequences with more HV counits are first accumulative in amorphous regions and then crystallize slowly into uniform lamellae with HV total inclusion, which is related to the samples with HV content higher than 19.4 mol%. So, the larger value of $x_{hv}$ of P(HB-co-28.7%HV) than the overall HV content in P(HB-co-28.7%HV) is because that the crystals with HV total inclusion are composed of HB sequences with more HV counits accumulative in amorphous regions, while P(HB-co-19.4%HV) contains the suitable length of HB sequence with HV counits to form the sandwich lamellae with more HV counits inclusion.

Based on above discussion and analysis, it can be concluded that the multiple endothermic peaks of P(HB-co-19.4%HV) and P(HB-co-28.7%HV) are ascribed to the formation of two kinds of lamellae, as illustrated in Fig. 7. The uni-

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Fig. 6 The (a, c) long period and (b, d) lamellar thickness from separated peaks calculated by the 1D correlation function as the function of temperature for (a, b) P(HB-co-19.4%) and (c, d) P(HB-co-28.7%HV).
form lamellae with HV counits total inclusion (Fig. 7I) melt first and the sandwich lamellae with HV counits partial inclusion (Fig. 7II) melt last. The evolution of different lamellae can mainly be divided into four different stages: (a) before melting, the sandwich lamellae and uniform lamellae with HV counits total inclusion coexist in P(HB-co-HV), and the slight increase of both lamellar layer and amorphous layer are caused by thermal expansion; once the temperature is close to the first melting range around $T_{m1}$, uniform lamellae with HV counits total inclusion begin to melt; (b) in the temperature range between $T_{m1}$ and $T_{m2}$, the uniform lamellae with HV counits total inclusion melt completely, and the thinner sandwich lamellae also begin to melt, but the thicker sandwich lamellae remain stable; (c) before approaching $T_{m3}$, most of sandwich lamellae are also melted, but the residual sandwich lamellae showing the largest lamellae thickness and least HV counits inclusion can exist, which is supported by the gradual decrease of the unit cell parameters; (d) all the residual sandwich lamellae are melted and thus all HB sequences with HV counits are disorderly distributed in amorphous region when the temperature is higher than $T_{m3}$.

Furthermore, there are some differences between the two samples during the heating procedure. At low temperatures, $d_1$ and $d_2$ are similar in both samples. At temperature closing to the first melting range around $T_{m1}$, the value of $d_2$ of P(HB-co-19.4%HV) increases earlier than that of P(HB-co-28.7%HV), which may be related to that some uniform lamellae transform into sandwich lamellae via melting/recrystallization process. It is worth noting that though melting/recrystallization exists during melting range, the two kinds of lamellar crystals are the main reason for the occurrence of multiple endothermic peaks. At the second melting range around $T_{m2}$, the increase of $d_2$ of P(HB-co-19.4%HV) is larger than that of P(HB-co-28.7%HV), while $d_2$ of P(HB-co-28.7%HV) obviously increases at the range around $T_{m3}$. It is clear that sandwich lamellae of P(HB-co-19.4%HV) contain more HV units than those of P(HB-co-28.7%HV), which is related to the main melting peaks of $T_{m2}$ of P(HB-co-19.4%HV) and that of $T_{m3}$ of P(HB-co-28.7%HV). Compared to the evolution of P(HB-co-36.2%HV), P(HB-co-19.4%HV) and P(HB-co-28.7%HV) contain much thinner sandwich lamellae melted at around $T_{m2}$, especially for thinner sandwich lamellae with large unit cell parameter of P(HB-co-19.4%HV), indicating that P(HB-co-HV) with middle HV content contains the transition structure from sandwich lamellae to uniform lamellae.

CONCLUSIONS

In this work, the multiple endothermic peaks of P(HB-co-19.4%HV) and P(HB-co-28.7%HV) are investigated by DSC, in situ WAXD and SAXS measurements. Firstly, the diffraction peaks of (020) and (110) crystallographic planes are asymmetrical and the width is more slanted to low 20 with the increase of HV content, indicating the possible existence of different lamellar crystals. Subsequently, the change of unit cell parameters $a$ and $b$ further reveals that the crystals with large crystal lattice melt first and the crystals with small crystal lattice melt last. Compared with other samples with varied HV contents, it is apparent that the first melted crystals are the ones with HV units total inclusion and the last melted crystals are those with HV partial inclusion. Meanwhile, as the temperature is raised to $T_{m1}$, the asymmetrical scattering peak changes into symmetrical peak with the high $q$ peak disappearing, further confirming the existence of different periodic structures and the first melting of the periodic structure with the small long period. By calculating long period and lamellar thickness of the two separated peaks via one-dimensional correlation function, it again provides the evidence of two kinds of lamellae and that the thinner lamellae melt at first and the thicker lamellae melt last. Finally, by combining the data analyses of WAXD and SAXS, it is evident that the thinner, unstable uniform lamellae with HV units total inclusion melt at $T_{m1}$ and the thicker, stable sandwich lamellae with HV units partial inclusion melt at $T_{m2}$ and $T_{m3}$ respectively. Moreover, the sandwich lamellae with the largest thickness and least HV units melt at last, corresponding to $T_{m3}$. This is the main origin of multiple endothermic peaks of P(HB-co-19.4%HV) and P(HB-co-28.7%HV). It is also interesting that P(HB-co-19.4%HV) contains larger crystal lattice and sandwich lamellae thickness than other samples with different HV contents, which may be related to the much HV counits inclusion in sandwich lamellae, closing to the amount of HV counits in uniform lamellae. The present study establishes the relationship between lamellae structure and melting behavior in isomorphous copolymer. Furthermore, we are investigating the band spherulite morphology based on the isomorphism, as the different isomorphous structures formed at different temperatures.

Fig. 7 Schematic diagram of the evolution of different lamellae of P(HB-co-HV) during heating procedure: (I) uniform lamellae formed by the crystal with HV counits total inclusion, (II) and (II') sandwich lamellae formed by the crystal with HV counits partial inclusion. The black dots are the HV counits.
Electronic Supplementary Information

Electronic supplementary information (ESI) is available free of charge in the online version of this article at https://doi.org/10.1007/s10118-020-2427-5.

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