

# Twisting of Lamellar Crystals in Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) Ring-Banded Spherulites

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**Introduction.** Ring-banded spherulites, as interesting and representative morphological feature of polymer crystalline aggregates, have been attracting considerable attention for 50 years. It is generally believed that the formation of ring-banded spherulites is attributed to the periodical lamellae twisting along the radial growth direction of the spherulites.<sup>1–9</sup> The rhythmic crystal growth of ring-banded spherulites has been observed experimentally in some polymers,<sup>10–14</sup> but such rhythmic crystal growth is generally encountered in thin films of semicrystalline polymers when the diffusion of molecular chains and the growth of spherulites are competitive because of the mass and spatial confinement.<sup>15</sup> Ho et al.<sup>16</sup> and Wang et al.<sup>17</sup> confirmed the periodical continual twisting of lamellar crystals in poly(trimethylene terephthalate) ring-banded spherulites by electron diffraction (ED). Gazzano et al.<sup>18,19</sup> and Tanaka et al.<sup>20</sup> confirmed the regular lamellae twisting in poly(3-hydroxybutyrate) (PHB) ring-banded spherulites by microfocus X-ray diffraction. Xu et al.<sup>21</sup> found the lamellae twisting during crystals growth of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBHHx) ring-banded spherulites by real-time atomic force microscopy (AFM) observation in thin film. The origin of the lamellar twisting was recently extensively studied by Lotz and Cheng in a comprehensive review.<sup>22</sup>

Although ring-banded spherulites have been widely investigated over the years, analyzing the origin of lamellar twist in ring-banded spherulites remains a challenge because the mere observation of the building lamellae in such complex three-dimensional entities is a difficult task.<sup>22</sup> Major contributions to the observation of the internal morphology of spherulites had to await the development by Olley et al.<sup>23–26</sup> of permanganic acid and potassium hydroxide etching techniques. Organ et al.<sup>27</sup> also contributed to the etching techniques by suspending PHB spherulites in methylamine aqueous solution. The etching techniques mentioned above are useful, but more mild ones are needed to provide more clear observation for the lamellar shape, orientation, and organization within ring-banded spherulites.

It is known that polymers with configurational chirality frequently form ring-banded spherulites, which is sometimes described as the “chirality effect”. An obvious question then comes to mind: does the configurational chirality of

polymers determine the twist sense of lamellar crystals in ring-banded spherulites? A generally accepted correlation between them suggests that configurational chirality plays a major role in inducing twisting of lamellar crystals. This, however, turns out not to hold true when considering a series of polymers that have the same chiral center but different chain constitutions. Li et al.<sup>28</sup> found that only a methylene difference in the main chain chiral liquid crystalline polyesters, PET(R\*-9) and PET(R\*-10), leads to the opposite twist sense of the lamellar crystals although the two polymers have the same configurational chirality. Saracovan et al.<sup>29</sup> found that PHB and poly(3-hydroxyvalerate) (PHV), which have identical configurational chirality, but different side group lengths, were left-handed and right-handed twist in lamellar crystal, respectively. Therefore, what is the twist sense of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) lamellae in ring-banded spherulites is much significant to study the link between configurational chirality and twist sense of lamellar crystals.

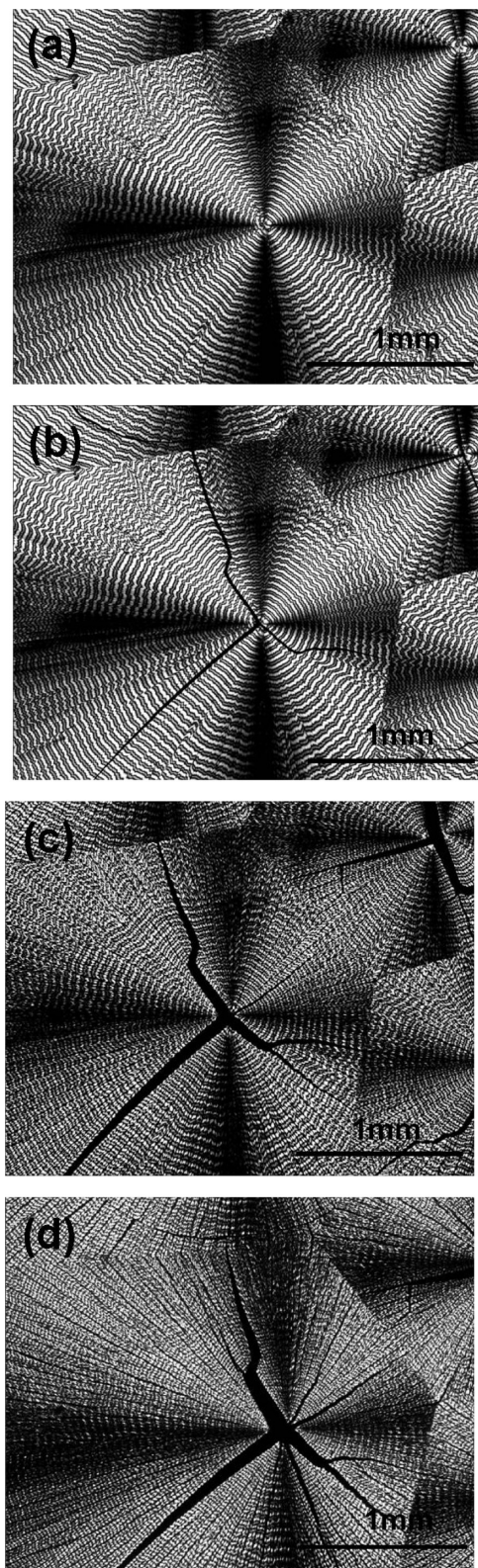
In this Communication, using methylamine vapor etching technique, the distinct periodical cooperative lamellae twisting in PHBV ring-banded spherulites was observed, and the twist sense of PHBV ring-banded spherulites containing different amount of HV was studied.

**Experimental Part.** PHBV samples used in experiments were kindly provided by Ningbo Tianan Biologic Material Co., Ltd. PHB was purchased from Aldrich Chemical Co. PHBV samples were purified before further investigation. The samples were first dissolved in chloroform and then precipitated with methyl alcohol. The precipitates were collected by centrifugation and dried in vacuum at room temperature for 24 h. The configurational chiralities of all samples (including PHB and PHBV) were measured to be right using a polarimeter. The number-average molecular weight ( $M_n$ ) and polydispersity index ( $M_w/M_n$ ) of PHBV with 12 mol % HV were measured to be 182 000 g/mol and 1.52, respectively. The glass transition temperature ( $T_g$ ) and melting point ( $T_m$ ) of PHBV with 12 mol % HV were about 2 and 167 °C, respectively. The PHBV films were prepared by casting three drops of PHBV chloroform solution (20 mg mL<sup>-1</sup>) onto a clean glass slide. The isothermal crystallization of the films was performed by heating the sample at 190 °C for 3 min and then cooling it to 90 °C at a cooling rate of 100 °C min<sup>-1</sup>. Before observation, the crystal films were treated by a solvent vapor etching using 30% (w/w) methylamine aqueous solution in sealed container at room temperature.

The films were observed using an Olympus BX51 polarized optical microscope (POM) equipped with a CCD camera. The scanning electronic microscopy (SEM) experiments were performed on a Hitachi TM1000 SEM at an accelerating voltage of 15 kV.

**Results and Discussion.** The global morphology of the PHBV ring-banded spherulites was first investigated by polarized optical microscopy (POM). Both extinction bands and Maltese cross are clearly observed under crossed-polarized light (Figure 1a), which are similar to the classical ring-banded spherulites in other different polymeric materials. The size of the perfect ring-banded spherulites is greater than 1 mm. The band spacing is quite uniform, and it is calculated to be about 72 μm.

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**Figure 1.** Polarized optical micrographs of PHBV ring-banded spherulites formed at 90 °C etched by methylamine vapor for different times: (a) 0, (b) 18, (c) 30, and (d) 45 h.

In order to identify the orientation of lamellar crystals in the ring-banded spherulites, the films were etched by methylamine vapor. Figure 1 shows the POM images of PHBV ring-banded spherulites etched for 0, 18, 30, and 45 h. Because spherulitic eyes at the central part of spherulites are thinner and more easily eroded by methylamine than

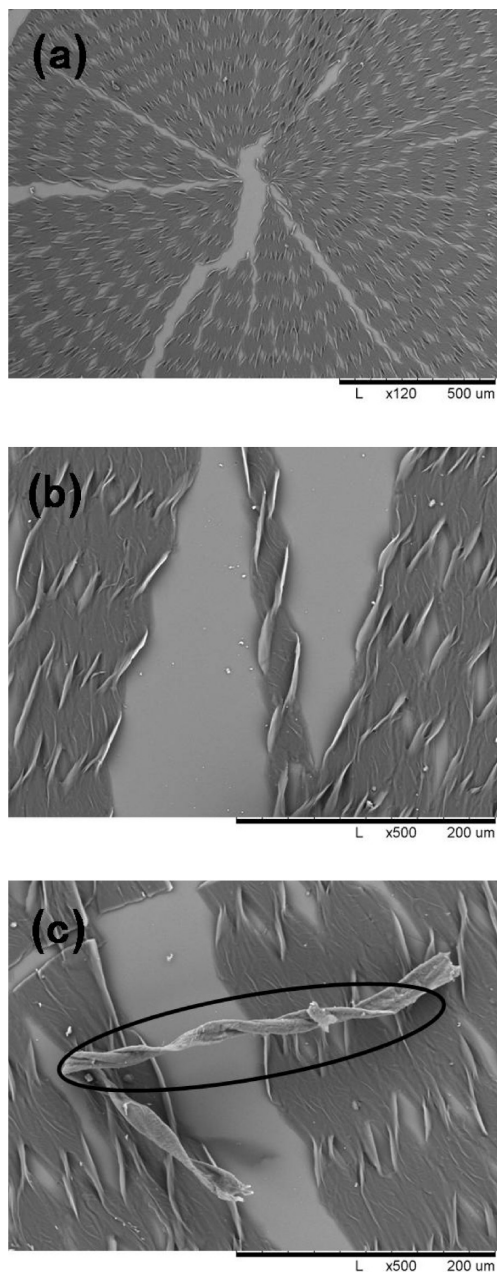
lamellar crystals in other regions, the etching starts from the central part of PHBV ring-banded spherulite. Three narrow slits first occur at the central part of the spherulites and the width of slits gradually increases, and more narrow slits appear as the etching proceeds (Figure 1). The birefringent rings are still clear at the beginning (Figure 1b) because methylamine washed out most of the flat-on lamellae of the spherulites and more edge-on lamellae remain. This is understandable because the amorphous parts of the spherulite are easier to be eroded than the crystalline lamellae. For flat-on lamellae in the spherulite, the amorphous surfaces of lamellar crystals are exposed to methylamine and can relatively easily be eroded, while for edge-on crystals, methylamine must penetrate between the crystals in order to reach the amorphous surfaces. Iwata et al.<sup>30</sup> observed similar phenomena in the studies of enzyme erosion of PHB crystals. With the development of etching, the edge-on crystals are gradually exposed to methylamine so that the etching rates of crystals in different regions are almost similar. As a result, the birefringent rings of the PHBV spherulites turn fuzzy as the etching proceeds (Figure 1c,d).

A more distinct morphology of lamellar crystals can be seen by SEM experiment. Figure 2 displays SEM images of PHBV ring-banded spherulites formed at 90 °C etched by methylamine vapor for 45 h. As shown in Figure 2b, edge-on lamellar crystals (the bright strip) protrude from the film surface, after growing to a certain length edge-on lamellae twist and lamellae with tilt orientations can be seen, and the lamellae twist to the flat-on lamellar crystals with further growth. More obvious periodical cooperative twisting of lamellar crystals can be seen in lamellar crystals (indicated by the ellipse in Figure 2c), detached from substrate, which may be originated from the serious erosion of lamellar crystals, around the slit, by methylamine. The etching effect was manifested by gel permeation chromatography (GPC). The number-average molecular weight of the PHBV after methylamine etching for 45 h is calculated to be 3200 g/mol, which is much smaller than the molecular weight of non-etched sample ( $M_n = 182\,000$  g/mol). Because the methylamine vapor etching is much mild, the periodical cooperative twisting of lamellar crystals in ring-banded spherulites can be clearly observed.

As suggested by Keith and Padden, nonadjacent re-entry of chains and loose folds generally introduce overcrowding and inefficient packing in lamellar crystals. The different conditions at the opposite fold surfaces may lead to the difference in the degree of congestion, and hence the difference in the magnitude of compressive stresses, which can result in a bending moment responsible for twisting of lamellar crystals.<sup>6</sup> Recently, Lotz and Cheng suggested that lamellar twist in chiral polymers was a consequence of unbalanced stresses resulted from the different chain conformations on the opposite surfaces of the lamellae.<sup>22</sup> Configurational chirality probably prefer particular fold conformations; if these preferred fold conformations are segregated on one side of the lamella, a small difference in fold encumbrance due to differences in fold conformations on the lamellar surface causes the lamellar crystals periodically twisted to release stresses.

It should be noted at this point that the etching-induced splitting is along radial direction of the ring-banded spherulites. Related results worthy to be mentioned are that PHB or PHBV spherulites can form circular fractures along the tangential direction of spherulites when they were cooled rapidly from high crystallization temperature. Martinez-Salazar et al.<sup>31</sup> confirmed that circumferential features in PHB or PHBV spherulites derived from the large difference

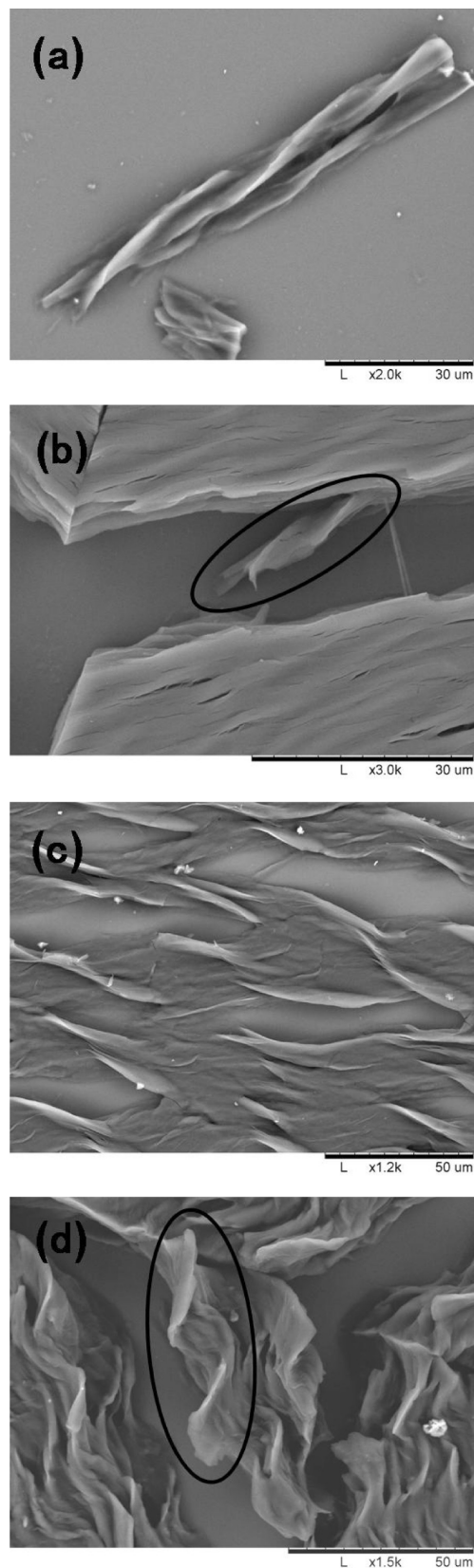




**Figure 2.** Scanning electronic micrographs of PHBV ring-banded spherulites formed at 90 °C etched by methylamine vapor for 45 h.

of thermal expansion coefficients between the substrate and the radial direction of spherulites. When the ring-banded spherulites were cooled slowly to room temperature, to release the internal stress, the etching-induced splitting was still along radial direction. It can be concluded, therefore, that the slits in the etched film are not caused by difference of thermal expansion coefficients. When PHB ring-banded spherulites with circular fractures were etched by methylamine, the splitting in the radial direction can also occur although the circular fractures provide enough regions for initial attack zone by methylamine. Therefore, the splitting may reflect the removal of tangential stress which origin from the periodical cooperative twisting of lamellar crystals in the formation of ring-banded spherulites. The problem is probably significant to study the formation of ring-banded spherulites, and further work is in progress.

It can be clearly found that the twist sense is right-handed in PHBV ring-banded spherulites from the lamellae strip



**Figure 3.** Scanning electronic micrographs of PHBV, containing different amount of HV, ring-banded spherulites formed at 90 °C etched by methylamine vapor: (a) 0, (b) 8, (c) 12, and (d) 40 mol %.

detached from substrate (Figure 2c). It should be noted that PHBV samples, used in our experiment, were measured to be have the same configurational chirality with PHB (with (R)

chiral center in the main chain). Therefore, a series of PHBV samples containing different amount of HV were studied to investigate the link between configurational chirality and twist sense of lamellar crystals. Figure 3 shows the SEM images of PHBV lamellar crystals in ring-banded spherulites etched by methylamine vapor. Although the etching effect of ring-banded spherulites is not very fine, the twist senses can be clearly identified from the SEM results (indicated by the ellipse in Figure 3). The twist senses of PHBV lamellae with 0 mol % (PHB) and 8 mol % HV are left-handed, but the ones of PHBV with 12 and 40 mol % HV are right-handed which are the same as that of PHV. The PHBV samples used in our experiments, only increasing HV amount from 8 to 12 mol %, reverse the twist sense. Analogous results worthy to be mentioned are that the ring-banded spherulites of PHBHHx, containing 17 mol % HHX, give completely opposite twist sense compared to PHB. From the results mentioned above, it can be concluded that the configurational chirality is not the sole decisive factor in fixing the lamellar twist sense, which accords with the opinion of Lotz and Cheng.<sup>22</sup> What on earth determines the twist sense of the lamellar crystals of the nonracemic chiral polymers? The question is still open. It is necessary to point out that for chiral polymers configurational chirality exists at the angstrom scale, but the "final" chirality apparent in the lamellar crystals exists at the micrometer scale; therefore, the chirality information may or may not be transferred.

**Conclusion.** By using mild methylamine vapor etching method, the periodical cooperative twisting of lamellar crystals in PHBV ring-banded spherulites was clearly observed. The twist sense of PHBV, with 12 mol % HV, ring-banded spherulites was found to be right-handed, which is completely opposite to PHB and PHBV containing 8 mol % HV, but the same as PHBV containing 40 mol % HV and PHV.

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