

Infrared Spectroscopy and X-Ray Diffraction Studies on the Structure and Thermal Behavior of Biodegradable Polyhydroxyalkanoates

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Summary: The structure and thermal behavior of new types of bacterial copolyester, poly(3-hydroxybutyrate-co-3-hydroxyhexanoate); P(HB-co-HHx) (HHx=2.5, 3.4, and 12 mol %) have been explored by means of wide-angle x-ray diffraction (WAXD), differential scanning calorimetry (DSC), and infrared (IR) spectroscopy. The WAXD pattern of P(HB-co-HHx) (HHx=12 mol %) copolymer measured at room temperature has revealed that it has an orthorhombic system ($\alpha = \beta = \gamma = 90^\circ$) with $a=5.76 \text{ \AA}$, $b=13.20 \text{ \AA}$, $c=5.96 \text{ \AA}$ (fiber repeat), which is identical to that of poly(3-hydroxybutyrate) (PHB). It has been found from the temperature-dependent variations of the WAXD pattern that only the a lattice parameter shows the thermal expansion, while the b lattice parameter changes little with temperature in the crystalline

P(HB-*co*-HHx) (HHx=12 mol %). This observation suggests that there are inter and intramolecular interactions between C=O groups and alkyl groups along the *a* axis and that interactions are broken little by little with temperature. IR spectra were measured for the four kinds of polymers over a temperature range from 30°C to high temperatures (200°C; PHB, 180°C; P(HB-*co*-HHx) (HHx=2.5 mol %), 180 °C ; P(HB-*co*-HHx) (HHx=3.4 mol %), 150 °C ; P(HB-*co*-HHx) (HHx=12 mol %)). Temperature-dependent IR spectral variations were analyzed for the CH, C=O, and C-O-C stretching band regions, and bands characteristic of crystalline and amorphous parts were identified in each region. It has been found from the IR study that the strength of interaction between the C=O group and the CH₃ (or CH₂) group is similar among the four polymers and that the population of C=O groups that are not involved in the interaction becomes higher with the increase in HHx. Both WAXD and IR studies have revealed that the crystallinity of P(HB-*co*-HHx) (HHx=12 mol %) decreases gradually starting from relatively low temperature (about 60°C) while that of PHB remains high up to 170 °C.

Keywords: biodegradable polymer; differential scanning calorimetry; infrared spectroscopy; poly(3-hydroxybutyrate); thermal behavior; x-ray diffraction

Introduction

Recently, poly(3-hydroxybutyrate) (PHB) and related bacterial poly(hydroxyalkanoates)s (PHA)s (Figure 1) have attracted much attention as new environmentally friendly materials. PHAs are completely biodegradable under both aerobic and anaerobic conditions and can be produced from renewable resources.^[1-5] Among all natural polymers, only PHA polymers show thermoplasticity and mechanical properties similar to those of synthetic polymers.^[6-9]

PHB is one of the simplest and largely produced bacterial polyesters in the PHA family. It possess similar glass transition temperature (*T_g*), melting point, and comparable ultimate tensile strength to those of isotactic polypropylene (iPP).^[8, 10-13] However, because of high crystallinity,^[14, 15] PHB is rigid and stiff, not necessarily well suited for

certain applications as a commodity plastics. To overcome the shortcomings of PHB, there have been a number of trials in designing PHB-based copolymers aimed at reducing crystallinity and better physical properties.^[16] The copolymerization of PHB with 3-hydroxyvalerate (3HV) was one of the early attempts to reduce the excess crystallinity and melting temperature (T_m).^[17] However, due to the crystalline isodimorphism of 3HB and 3HV units, the incorporation of 3HV did not improve the physical properties of PHB to a large extent.^[18]

Recently, several kinds of new PHA copolymers with a small amount of medium length side groups have been introduced by Procter and Gamble Company (Cincinnati, USA)^[19] and Kaneka Corporation (Settsu, Osaka, Japan). These PHA copolymers show significantly reduced crystallinity, increased flexibility, and excellent compatibility with other biodegradable polymers and synthetic polymers.

Figure 1 shows structures of PHB and P(HB-*co*-HHx). The physical properties of P(HB-*co*-HHx) can be controlled by changing the comonomer composition and the percentage of HHx. It is also possible to generate polymer blends of P(HB-*co*-HHx). To design new types of PHA copolymers with more desirable physical properties, it is very important to further explore structure, molecular interaction, and thermal behavior of P(HB-*co*-HHx). Comparison of these properties between PHB and P(HB-*co*-HHx) is also useful.

The purpose of the present study is to investigate temperature-dependent variations in the structure and the inter- and intramolecular interaction between a C=O group and a CH₃ (or CH₂) group for PHB and P(HB-*co*-HHx) (HHx=2.5, 3.4, and 12 mol %) by using infrared (IR) spectroscopy, wide-angle x-ray diffraction (WAXD), and differential scanning calorimetry (DSC). WAXD is very useful not only in the investigation of a crystal structure but also as a tool to explore thermally-induced changes in the unit cell dimensions as a result of thermal expansion. IR spectroscopy is powerful to monitor temperature-dependent changes in the inter- and intramolecular interactions and thermally induced conformation changes. So far, the WAXD measurements of PHA copolymers were carried out only at room temperature to investigate their crystallinity and crystal

structure.^[20, 21] However, thermal behavior of PHA copolymers has never been investigated by WAXD. IR studies of thermally induced structural changes of PHA copolymers were reported. However, the IR spectra were measured only for several temperatures, and the extent of spectral analysis was not sufficient.^[22, 23]

We have recently undertaken combined use of WAXD and IR to studies a series of PHA copolymers.^[24-26] The combined method is useful because WAXD and IR spectroscopy are quite complementary to each other.

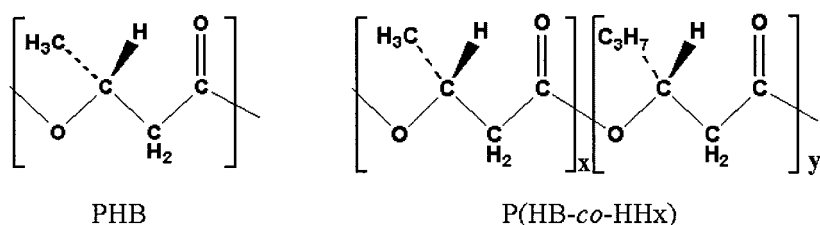


Figure 1. Structures of poly(3-hydroxybutyrate) (PHB) and poly(3-hydroxybutyrate-co-3-hydroxyhexanoate), P(HB-co-HHx) (HHx = 2.5, 3.4, and 12 mol %).

Experimental Section

Samples. Bacterially synthesized PHB and P(HB-co-HHx) (HHx=12 mol %) were obtained from the Procter and Gamble Company, USA. P(HB-co-HHx) (HHx=2.5 and 3.4 mol %) were provided by Kaneka Corporation, Japan. They were dissolved in hot chloroform, re-precipitated in methanol as fine powder, and vacuum-dried at 60°C. The purified samples thus obtained were used for all the experiments. Some of the purified powder samples was re-dissolved in chloroform and then cast as a film by evaporating the solvent.

Wide Angle X-ray Diffraction (WAXD). The WAXD data were measured for the precipitated powder samples of PHB and P(HB-co-HHx) (HHx= 12 mol %) over a temperature range from 25 to 110 °C for PHB and 30.1 to 105.8 °C for P(HB-co-HHx) (HHx= 12 mol %) in the scattering angle range of $2\theta = 5-13^\circ$ by using a two-circle Rigaku x-ray diffractometer equipped with a scintillation detector. Mo-K α radiation

(wavelength, 0.71069 Å) was used as an incident x-ray source (40kV, 240 mA). The contribution from the amorphous part to the x-ray scattering was estimated by computer fitting using series of linear functions.

Differential Scanning Calorimetry (DSC). DSC measurements of the P(HB-*co*-HHx) (copolymers were performed with a Perkin Elmer Pyris 6 DSC over a temperature range from 25 to 200°C for PHB, 180°C for P(HB-*co*-HHx) (HHx=2.5 mol %), 180°C for P(HB-*co*-HHx) (HHx=3.4 mol %), and 150°C for P(HB-*co*-HHx) (HHx=12 mol %) at heating and cooling rates of 10 °Cmin⁻¹.

IR measurement. The transmission IR spectra were measured at a 2 cm⁻¹ resolution using a Nicolet Nexus 480 Fourier transform IR spectrometer with a liquid-nitrogen-cooled mercury-cadmium-telluride detector. A total of 512 scans were co-added for each spectrum. The temperature of the IR cell was controlled by a thermoelectric device (MODEL SU, CHINO) with an accuracy of ± 0.1 °C. After changing the temperature, the cell was maintained for 15 min to make the sample equilibrate at that temperature.

Data processing such as the calculation of second derivative spectra was performed by a home-made program composed by Yukiteru Kastumoto (Hiroshima University) written in C++ language (Visual C++ 6.0, Microsoft).

Results and Discussion

Differential Scanning Calorimetry (DSC).

Figure 2 shows DSC curves at the second heating for PHB and P(HB-*co*-HHx) (HHx=2.5, 3.4, and 12 mol %). It is noted that PHB shows a single peak at 169.51 °C while the rest yield double peaks. There are two possibilities for the origin of two peaks in the second heating cycle of DSC. One possibility is the observation of melt and re-crystallization of the sample. The other possibility is that there are two types of lamella in the crystal.

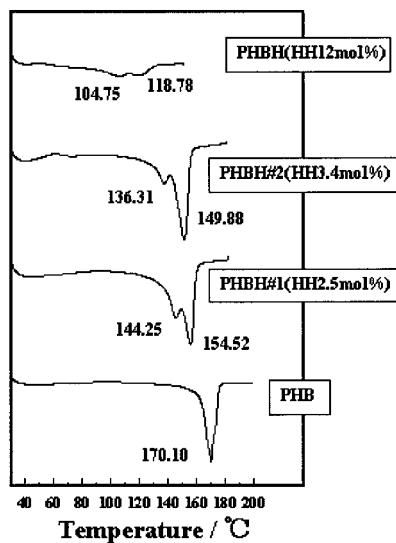


Figure 2. DSC curves at the second heating for PHB and P(HB-co-HHx) (HHx=2.5, 3.4, and 12 mol %).

Wide Angle X-Ray Diffraction (WAXD)

Figures 3 (A) and (B) compare a temperature dependence of the WAXD of P(HB-co-HHx) (HHx=12 mol %) precipitated sample over a temperature range from 25 to 110 °C with that of PHB homopolymer over a temperature range from 30.1 to 105.8 °C.^[24] Comparison of the results of the x-ray diffraction between P(HB-co-HHx) (HHx=12 mol %) and PHB reveals that the crystallinity of P(HB-co-HHx) (HHx=12 mol %) is considerably lower than that of PHB. It was found from the analysis of the WAXD measurement that P(HB-co-HHx) powder sample assumes an orthorhombic system $P2_12_12_1$ (D_2^4), ($\alpha = \beta = \gamma = 90^\circ$) with $a=5.76 \text{ \AA}$, $b=13.20 \text{ \AA}$, $c=5.96 \text{ \AA}$ (fiber repeat), which is the same as the PHB crystal system.

Figure 4 (A) shows temperature-dependent variations in the lattice parameters, a and b , of P(HB-co-HHx) (HHx=12 mol %), while Fig. 4 (B) presents those of PHB.^[24] It can be seen from Fig. 4 (A) that the a lattice parameter changes gradually with temperature, while the b lattice parameter changes little. It seems that the increase in

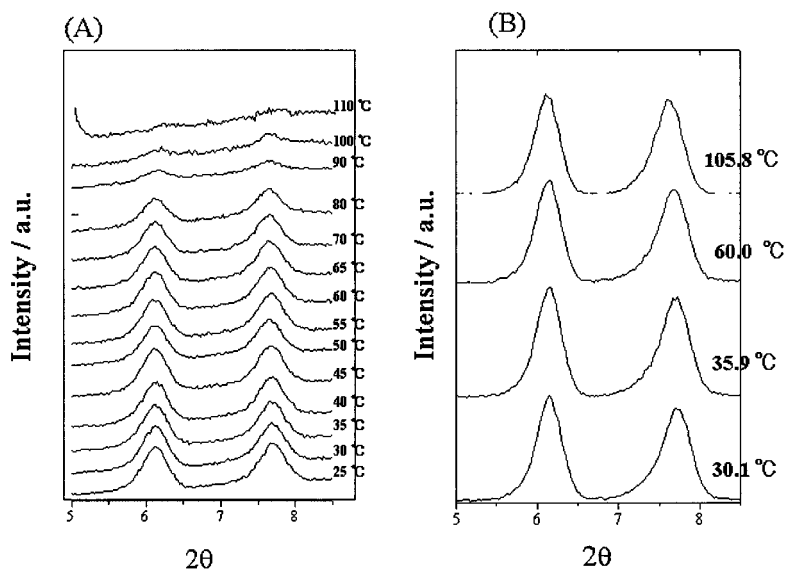


Figure 3. Temperature dependences of the x-ray diffraction of (A) P(HB-*co*-HHx) (HHx=12 mol %) and (B) PHB.

the a lattice parameter results from the decrease in the inter- and intramolecular interactions between the C=O group and the alkyl group along the a axis of crystalline P(HB-*co*-HHx) (HHx=12 mol %). Similar result was observed for PHB as shown in Figure 4 (B). Both lattice parameters, a and b , show thermal expansion in PHB; however, a change in the b lattice parameter is much smaller than that in the a lattice parameter.

Marked differences were observed for the temperature-dependent changes in the (110) and (020) peak areas between PHB and P(HB-*co*-HHx) (HHx=12 mol %).^[24] Both peak areas of PHB change little from 30.1 °C till 105.8 °C while those of P(HB-*co*-HHx) (HHx=12 mol %) decrease significantly even from just above room temperature and decrease largely from about 60 °C. Therefore, one can conclude that the crystallinity of PHB is high at least up to 105.8 °C, but that of P(HB-*co*-HHx) (HHx=12 mol %) decreases from much lower temperature.

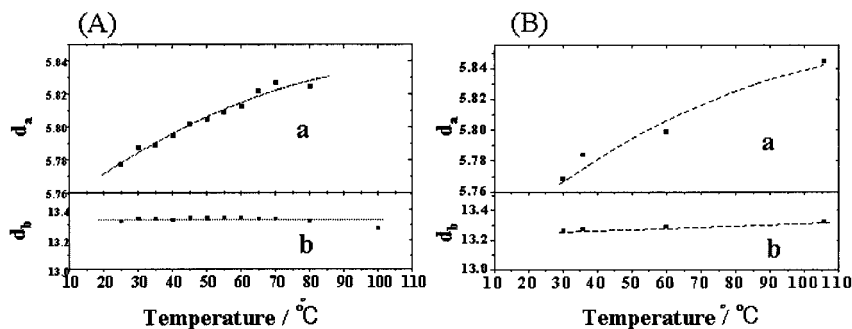


Figure 4. Changes in the lattice parameters, a and b , of (A) P(HB-co-HHx) (HHx=12 mol%) and (B) PHB versus temperature.

Infrared (IR) Spectroscopy

Figures 5 (A) and (B) show IR spectra in the C=O stretching band region of P(HB-co-HHx) (HHx=2.5, 3.4, and 12 mol %) and PHB measured at room temperature and high temperature, respectively.^[26]

Figures 6 (A) and (B) present second derivatives of the spectra shown in Figs. 5 (A) and (B), respectively.^[26] Figs. 7 (A) and (B) display temperature-dependent spectral variations in the C=O stretching band region of PHB and P(HB-co-HHx) (HHx=12 mol%), respectively.^[26] It can be seen from Figs. 5, 6, and 7 that there are three major bands in the C=O stretching band region. One is located at 1723 cm⁻¹ (Fig. 5 (B) and 6(B)). This band decreases with temperature and eventually disappears at high temperature (Fig. 5 (B) and 6(B)), being very much characteristic of the crystalline form. The other band is found near 1750 cm⁻¹ (Fig. 5 and 6). This band is assigned to the stretching mode of free C=O groups of both the crystalline and amorphous parts. Yet another band is observed around 1735 cm⁻¹ (Fig. 5 (B) and 6(B)). This band is intense in the spectra measured at high temperatures but weak in those obtained at room temperature, being characteristic of the amorphous forms. The crystalline band at 1723 cm⁻¹ shows a downward shift by about 25 cm⁻¹ compared with the free C=O

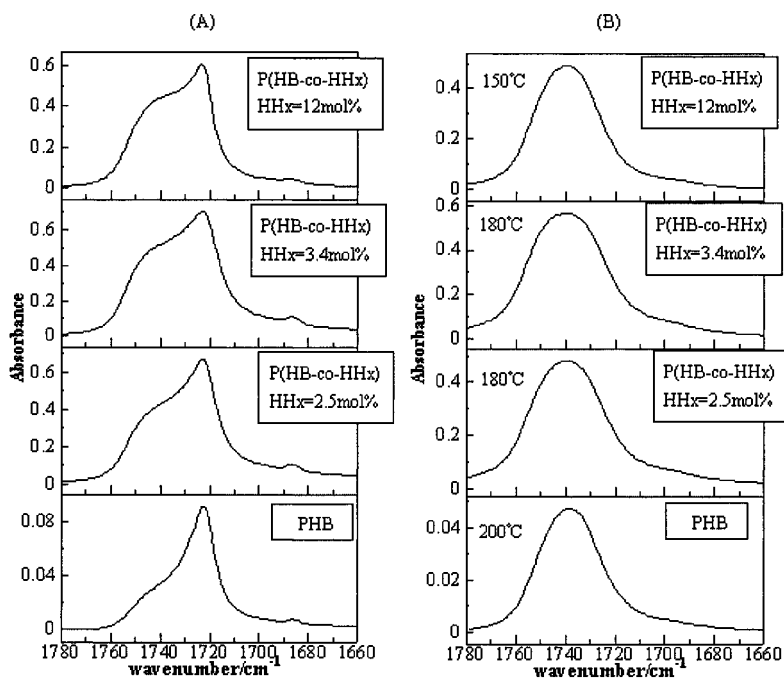


Figure 5. IR spectra in the C=O stretching band region of P(HB-*co*-HHx) (HHx=2.5, 3.4, and 12 mol %) and PHB at (A) room temperature and (B) high temperatures.

stretching band. This shift may be caused by the interaction between the C=O group and the CH₃ or CH₂ group. Another possibility of the shift is vibrational couplings of C=O stretching modes in the crystalline part. The band near 1735 cm⁻¹ may be assigned to the stretching mode of the C=O group that is involved in a weaker interaction with a CH₂ group.

Of note in comparison of the four spectra in the C=O stretching band region at room temperature is that the relative intensity of the band due to the free C=O group increases with the increase in the HHx content. This result is in good agreement with the fact that the order of crystallinity is PHB, P(HB-*co*-HHx) (HHx=2.5 mol %), P(HB-*co*-HHx) (HHx=3.4 mol %), and P(HB-*co*-HHx) (HHx=12 mol %). At high temperatures, the four spectra are more or less similar. It can be seen from Figs. 7 (A) and (B) that the

intensity of the crystalline band at 1723 cm^{-1} of PHB decrease a little from 20 to 180°C and decreases markedly upon going from 180 to 190°C and that the intensity of the corresponding band of P(HB-co-HHx) (HHx=12 mol %) changes gradually from even fairly low temperature (60°C). These results are in good agreement with the results for the temperature-dependent variations of the (110) and (020) peak areas.^[26]

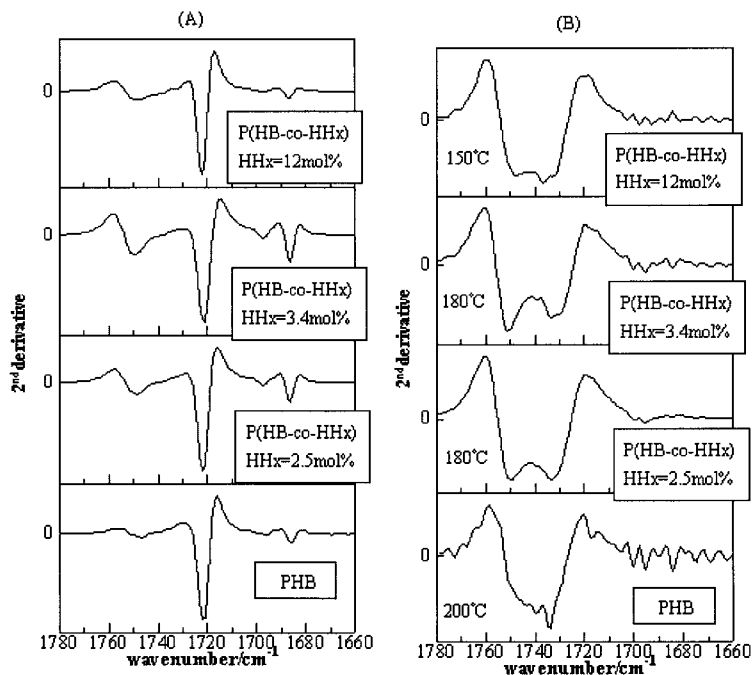


Figure 6. (A) and (B). Second derivatives of the spectra are shown in Figs. 5 (A) and (B), respectively.

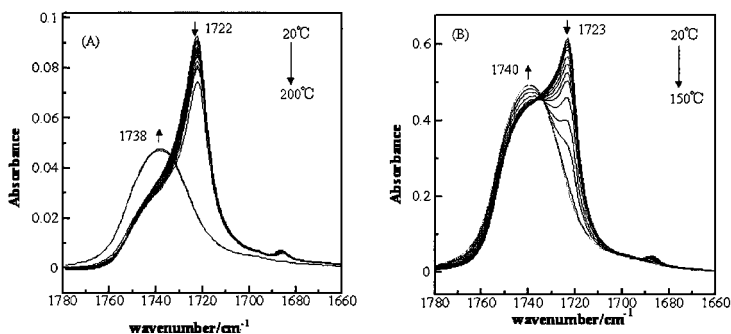


Figure 7. Temperature-dependent IR spectral variations in the C=O stretching band region of (A) PHB and (B) P(HB-*co*-HHx) (HHx=12mol %), respectively.

Figure 8 (A) depicts IR spectra in the CH stretching band region of PHB (HHx=12 mol %) and P(HB-*co*-HHx) (HHx=2.5, 3.4, and 12 mol %) measured at room temperature, and Fig. 8 (B) shows their second derivatives.^[26] Figs. 9 (A) and (B) display the corresponding spectra and their second derivatives, respectively, obtained at high temperatures.^[26] The spectra at the high temperatures are much simpler than those at room temperature. Bands at 2984, 2937, 2908, and 2876 cm^{-1} (Fig. 9(B)) are assigned to asymmetric CH_3 , antisymmetric CH_2 , symmetric CH_3 , and symmetric CH_2 stretching modes of the amorphous parts, respectively. It is noted that the intensity of a band at 2962 cm^{-1} increases with the increase in the HHx content. Accordingly, we assign this band to the asymmetric stretching mode of the CH_3 group in the branched alkyl groups.

It is noted in the CH stretching band region at room temperature that the asymmetric CH_3 stretching band of the crystalline form is split into the four bands at 3008, 2996, 2974, and 2967 cm^{-1} (Fig. 8 (B)). These splittings are ascribed to the crystal field splittings of bands associated with inter- or intramolecular interaction between the CH_3 groups along the polymer chain. This sort of band splittings are often observed for polymers with a helical structure.

Bands due to the amorphous parts are seen at 2984, 2937, and 2876 cm^{-1} in the spectra at room temperature (Fig. 8). The 2984 cm^{-1} band is clearly observed even in the original spectrum of P(HB-*co*-HHx) (HHx=12 mol %) (Fig. 8 (A)).

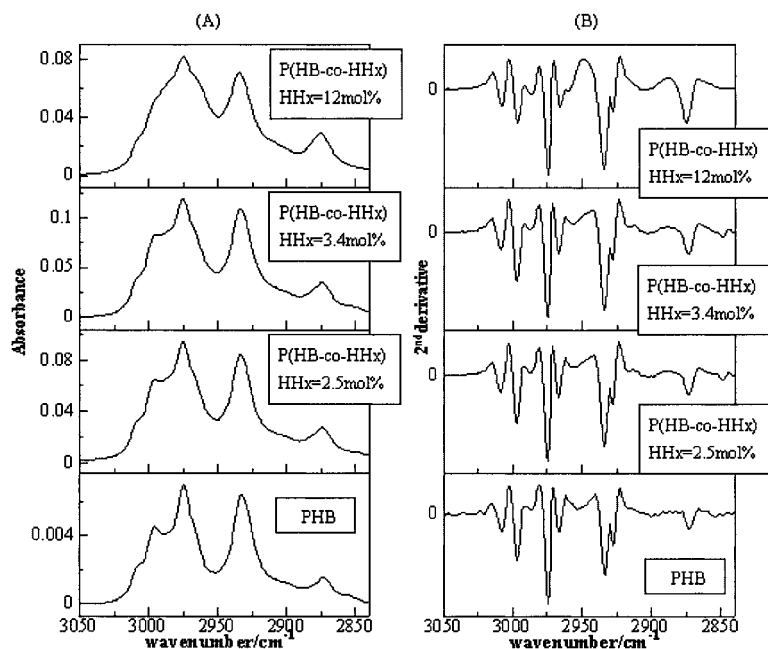


Figure 8. (A) IR spectra in the CH stretching band region of PHB and P(HB-*co*-HHx) (HHx=2.5, 3.4, and 12 mol %) measured at room temperature. (B) Second derivatives of the spectra shown in (A).

Figures 10 (A) and (B) depict the C-O-C stretching band region of IR spectra of PHB and P(HB-*co*-HHx) (HHx=12 mol %), respectively.^[26] Bands at 1289, 1279, 1263, and 1228 cm^{-1} are readily assigned to the crystalline forms while those at 1302 and 1257 cm^{-1} are due to the amorphous forms. It seems that the former four bands are due to C-O-C stretching modes of the helical structures whereas the latter two bands are ascribed to those of random structures. Marked differences in the spectral changes in

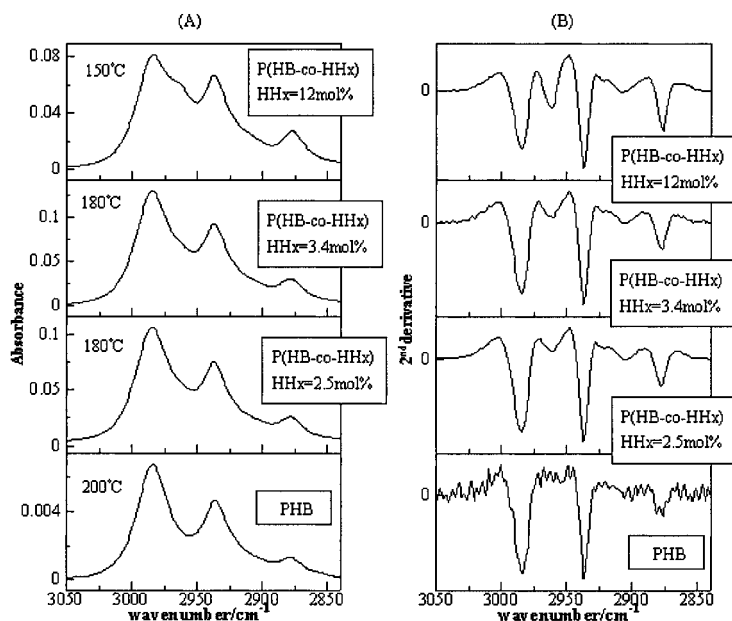


Figure 9. (A) IR spectra in the CH stretching band region of PHB and P(HB-co-HHx) (HHx=2.5, 3.4, and 12 mol %) measured at high temperatures. (B) Second derivatives of the spectra shown in (A).

the C-O-C stretching region between PHB and P(HB-co-HHx) (HHx=12 mol %) are that the intensities of the crystalline bands change gradually in the spectra of P(HB-co-HHx) (HHx=12 mol %) while they change a little from 20 °C to 180 °C and vary markedly from 180 to 190 °C in the spectra of PHB.^[26] These results suggest that the helical structure is destroyed gradually in P(HB-co-HHx) (HHx=12 mol %) while it is kept until nearly 180 °C and rather suddenly disappears in PHB.

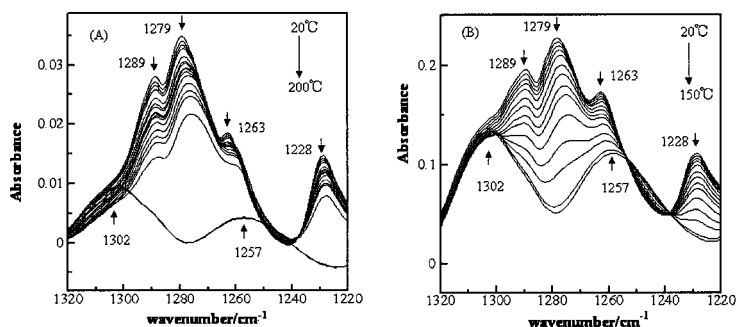


Figure 10. Temperature-dependent spectral variations in the C-O-C stretching band region of (A) PHB and (B) P(HB-co-HHx) (HHx=12 mol %).

Conclusion

Based on the above results from WAXD and IR spectroscopy studies, we propose structural modes for PHB and P(HB-co-HHx) in crystalline and amorphous states. Fig. 11 (A) depicts proposed helical structures for PHB and P(HB-co-HHx) and Fig. 11 (B) shows the structure of amorphous form of both PHB and P(HB-co-HHx).

In the crystalline state, PHB has a rigid helical structure where most of the C=O groups are interacted with the CH₃ group; there are very few free C=O groups in the crystalline state of PHB. P(HB-co-HHx) also has a regulated helical structure, but the incorporation of HHx groups induces some amorphous parts where some free C=O groups exist.

At a high temperature, PHB and P(HB-co-HHx) should have very similar amorphous states, where a number of C=O groups are free from interaction but still some of C=O groups are involved in weaker interaction with the CH₂ or CH₃ groups.

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