

Available online at www.sciencedirect.com



Vibrational Spectroscopy 36 (2004) 241-249

VIBRATIONAL SPECTROSCOPY

www.elsevier.com/locate/vibspec

### Thermally induced phase transition of poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) investigated by two-dimensional infrared correlation spectroscopy

Adchara Padermshoke<sup>a,b</sup>, Harumi Sato<sup>a</sup>, Yukiteru Katsumoto<sup>c</sup>, Sanong Ekgasit<sup>b</sup>, Isao Noda<sup>d</sup>, Yukihiro Ozaki<sup>a,\*</sup>

<sup>a</sup>Department of Chemistry, School of Science and Technology, Kwansei-Gakuin University, Sanda, Hyogo 669-1337, Japan <sup>b</sup>Department of Chemistry, Faculty of Science, Chulalongkorn University, Patumwan, Bangkok 10330, Thailand <sup>c</sup>Department of Chemistry, Graduate School of Science, Hiroshima University, Higashi-Hiroshima, Hiroshima 739-8526, Japan <sup>d</sup>The Procter and Gamble Company, 8611 Beckett Road, West Chester, OH 45069, USA

Received 3 October 2003; received in revised form 13 November 2003; accepted 13 November 2003 Available online 6 February 2004

### Abstract

The thermally induced crystalline/amorphous phase transition process of a newly developed biodegradable polymer, poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) (P(HB-*co*-HHx)) (HHx = 12 mol%), was investigated by using generalized two-dimensional infrared (2D IR) correlation spectroscopy. Three spectral regions, the C–H stretching (3100–2800 cm<sup>-1</sup>), C=O stretching (1780–1680 cm<sup>-1</sup>), and C– O–C stretching (1330–1200 cm<sup>-1</sup>) band regions were analyzed in order to explore the phase transition behavior of the copolymer. The asynchronous 2D spectrum generated from the temperature-induced variations in IR spectra in the C=O stretching band region clearly revealed the coexistence of two crystalline bands at 1731 and 1723 cm<sup>-1</sup>. The dominant band at 1723 cm<sup>-1</sup> may arise from the highly ordered crystalline component of the copolymer, and the weaker band at 1731 cm<sup>-1</sup> is possibly due to the minor crystalline component with a less ordered structure. The major crystalline band at 1723 cm<sup>-1</sup> shares asynchronous cross peaks with the amorphous band at 1740 cm<sup>-1</sup>. This observation suggests that the melting of the crystalline structure does not simultaneously result in the formation of the completely amorphous structure. The phase transition process of P(HB-*co*-HHx) (12 mol% HHx) takes place through an intermediate state. It is noted that the vibrational frequencies of the C=O, C–O–C, and C–H stretching bands due to the crystalline components of P(HB-*co*-HHx) (12 mol% HHx) are almost identical to those of PHB. These observations indicate that the helical structure of P(HB-*co*-HHx) (12 mol% HHx) is very similar to that of PHB. The inclusion of the HHx comonomer locally disrupts the highly ordered and helical structure of PHB from place to place, thereby reducing the crystallinity of the copolymer. It was also found that the helical structure of P(HB-*co*-HHx) (12 mol% HHx) is deformed gradually from much lower temperature than that of PHB.

© 2004 Elsevier B.V. All rights reserved.

Keywords: Polyhydroxyalkanoates (PHAs); Phase transition; 2D IR spectroscopy

#### 1. Introduction

Bacterially synthesized poly(3-hydroxyalkanoate)s (PHAs) are a class of naturally occurring biodegradable polyesters accumulated as energy-storing inclusion body granules in the cells of certain microorganisms [1–4]. Since PHAs can be biologically synthesized from renewable resources and degraded by bacteria in the soil, they have received a great attention as a new family of environmental friendly polymeric materials [2,5,6]. Despite their completely natural origin, PHAs bear a fairly close resemblance to

some petroleum-based synthetic polymers in chemical structures and physical characteristics and possess an attractive combined set of end use properties. One of the simplest and largely produced PHA polymers is poly(3-hydroxybutyrate) (PHB) [3,4]. Its ultimate tensile strength is comparable to that of isotactic polypropylene (PP) [4]. Since PHB is biocompatible, its utilization for medical purposes is also being contemplated. However, PHB is stiff and brittle due to the high degree of crystallinity, and it is also thermally unstable during processing [7]. Consequently, various efforts have been made to copolymerize PHB with other comonomers to improve its mechanical properties [7]. Recently, the Procter and Gamble Company (Cincinnati, USA) has introduced a new family of commercial PHA

<sup>&</sup>lt;sup>\*</sup> Corresponding author. Tel.: +81-79-565-8349; fax: +81-79-565-9077. *E-mail address:* ozaki@ksc.kwansei.ac.jp (Y. Ozaki).



Fig. 1. Molecular structure of P(HB-co-HHx).

copolymers under the trade name Nodax<sup>TM</sup> [6]. Nodax copolymer is comprised of 3-hydroxybutyrate and other longer side-chain 3-hydroxyalkanoates [6]. Poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) (P(HB-*co*-HHx)) with the melting temperature ( $T_m$ ) of about 110–160 °C and approximately 35–45% crystallinity are typical available commercial grades of Nodax copolymers. The molecular structure of P(HB-*co*-HHx) is shown in Fig. 1 [8,9].

The copolymer being investigated in the present study is P(HB-*co*-HHx) with 12 mol% HHx comonomer. The introduction of less crystallizable units, i.e., 3HHx, into the molecular chain of PHB dramatically improves the physical and mechanical properties of the resulting copolymer [6]. As a consequence, P(HB-*co*-HHx) possesses many attractive features, which are not only the notable physical and mechanical properties, but also the additional beneficial characteristics of polyesters. P(HB-*co*-HHx) has excellent surface properties (e.g., printability, dyeability, dispersibility, adhesion, etc.), and it is compatible with various additives and other polymers [6]. Examples of the potential applications of P(HB-*co*-HHx) include flushable products, films and flexible packaging, coated papers, synthetic papers, and bioresorbable medical devices [6].

Since the crystallinity is one of the most important key parameters for material applications of polymers and their further chemical or mechanical processing, the crystalline/ amorphous phase transition behavior of PHAs has gained keen interest [5,10-13]. We have been performing a series of studies on the structure and thermal behavior of PHB and P(HB-co-HHx) by using temperature-dependent wide-angle x-ray diffraction (WAXD) [14,15], differential scanning calorimetry (DSC) [14,15], and infrared (IR) spectroscopy [15,16]. The WAXD study [14,15] suggested that there are inter- and intramolecular interactions between the C=O and CH<sub>3</sub> groups in PHB and P(HB-co-HHx), and that the interactions decrease along the a axis of the crystal lattice of PHB and P(HB-co-HHx) with temperature. Two-dimensional (2D) IR correlation analysis [16] indicated that the phase transition of PHB involves one major and one minor crystalline components. Asynchronous 2D correlation maps generated from the temperature-dependent IR spectra of PHB revealed clearly the existence of an intermediate state during the phase transition of PHB. Since the copolymerization of the HHx comonomer with PHB results in dramatically improved physical and mechanical properties of the resulting material, it is of great interest to reveal how the HHx units affect the crystallinity of P(HB-co-HHx) copolymer. In this paper, the thermal-melting behavior of P(HB-co-HHx) (12 mol% HHx) was explored by using 2D IR correlation spectroscopy in comparison to that of PHB homopolymer, which is the basic unit of this class of biopolyesters.

IR spectroscopy is very suitable for the studies of crystalline/amorphous phase transitions of polymers because it allows one to gain insight into the phase transition behavior at the molecular functional group level [17]. The combination of IR spectroscopy with 2D correlation analysis proposed by Noda [18-20] enables a more detailed analysis on the spectral variations of polymers under various types of external perturbations [10-12,16,18-22]. 2D IR correlation spectroscopy possesses several distinctive advantages for analyzing severely overlapped bands and determining the sequential order of the intensity changes of bands caused by an external perturbation. Accordingly, the studies on the crystalline/amorphous phase transition by using 2D IR correlation spectroscopy have been carried out for some PHA polymers [10–12,16]. Wu et al. [10] and Tian et al. [12] showed, based on the 2D asynchronous spectra generated from the temperature-dependent IR spectra in the C=O stretching vibration region, the existence of an intermediate state during the phase transition of P(HB-co-HHx) (10 mol% HHx) and that of P(HB-co-HV), respectively. Recently, we investigated the changes in the crystalline structure of PHB during thermal melting process by using 2D IR correlation spectroscopy [16]. The 2D correlation maps generated from the temperature-dependent IR spectra in the C=O, C–O–C, and C–H stretching vibration regions showed a clear spectroscopic evidence for the coexistence of one major and one minor crystalline components and the presence of an intermediate state during the melting of the polymer.

The purpose of the present study is to investigate the crystalline/amorphous phase transition behavior of P(HBco-HHx) (12 mol% HHx) ( $T_m \cong 110 \,^{\circ}C$ ) by using 2D IR correlation spectroscopy. This study allows us to gain a microscopic view of the transition behavior of the copolymer at the functional group level. The present investigation is complementary to our previous studies on PHB and P(HBco-HHx) (12 mol% HHx) by DSC and WAXD [14,15]. The comparison between the phase transition behavior of P(HBco-HHx) and that of PHB homopolymer is also discussed here in order to reveal effects of the copolymerized HHx units on the structure of P(HB-co-HHx).

### 2. Experimental

### 2.1. Materials

Bacterially synthesized P(HB-*co*-HHx) (12 mol% HHx) (the Procter and Gamble Company, USA) was purified by dissolving in hot chloroform (CHCl<sub>3</sub>), re-precipitating in methanol (CH<sub>3</sub>OH), and vacuum-drying at 60 °C for 24 h. The purified P(HB-*co*-HHx) (12 mol% HHx) sample thus obtained was used in all IR experiments. Chloroform and

methanol were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and used as received. A film of P(HB-*co*-HHx) (12 mol% HHx) for IR measurements was prepared by casting its chloroform solution on a CaF<sub>2</sub> window. The film was kept at 60 °C in a vacuum-dried oven for 12 h and cooled down to room temperature before the measurements.

### 2.2. IR spectroscopic measurements

IR spectra of the film sample were measured at a 2 cm<sup>-1</sup> spectral resolution by using a Nicolet Magna-IR<sup>TM</sup> 550 spectrometer equipped with a mercury cadmium telluride (MCT) detector. To ensure a high signal-to-noise ratio, 512 scans were co-added. The temperature of the sample cell that holds the CaF<sub>2</sub> window with the P(HB-*co*-HHx) (12 mol% HHx) film was controlled by using a temperature controller unit (CHINO, model SU) with an accuracy of  $\pm 2$  °C. The temperature-perturbed dynamic IR spectra were collected throughout a temperature range of 30–140 °C with an increment of 10 °C.

### 2.3. 2D correlation analysis

The calculations of 2D correlation spectra were performed by using the 2D-Pocha software composed by Daisuke Adachi (Kwansei–Gakuin University, Japan). All IR spectra were baseline-corrected prior to the 2D correlation calculation.

### 3. Results and discussion

3.1. Changes in the C=O stretching band region during the melting process

Fig. 2 shows temperature-dependent IR spectra of P(HBco-HHx) (12 mol% HHx) in the 1780–1680 cm<sup>-1</sup> region



where bands due to the C=O stretching mode are expected to appear. A sharp band at  $1723 \text{ cm}^{-1}$  gradually decreases and eventually disappears with increasing temperature, while a broad band at  $1740 \text{ cm}^{-1}$  increases in intensity. The band shape in the C=O stretching vibration region changes greatly from a sharp peak with a broad shoulder to a symmetrical broad band in the course of temperature increase. The sharp peak near 1723 cm<sup>-1</sup> is assigned to a crystalline band associated with the ordered C=O groups in PHA polymers, while the broad band located around  $1740 \text{ cm}^{-1}$  is ascribable to an amorphous C=O band [10-12,16]. The C=O group is very sensitive to its environment [23]. Therefore, bands in the C=O stretching vibration region are particularly useful for monitoring the crystallization and melting processes of polymers in this class [10-12,16].

The splitting of the C=O stretching bands into the amorphous and crystalline bands observed at 1740 and 1723 cm<sup>-1</sup>, respectively, in the IR spectra of P(HB-*co*-HHx) (12 mol% HHx) is also observed in those of PHB [16].

To gain more detailed information about the spectral variations induced by the thermal-melting process, 2D correlation spectroscopy was employed. Fig. 3(A) and (B) shows the synchronous and asynchronous 2D correlation spectra in the spectral region of  $1790-1670 \text{ cm}^{-1}$  generated from the temperature-dependent IR spectra measured over the temperature range of 30-140 °C, respectively. Two autopeaks located at 1745 and 1723  $\text{cm}^{-1}$  reflect the spectral variations due to the amorphous and crystalline components of the polymer, respectively. A pair of negative cross peaks developed at (1745, 1723) cm<sup>-1</sup> in the synchronous spectrum indicates the opposite directions of the intensity variations in these two correlated bands. In the asynchronous spectrum (Fig. 3(B)), two pairs of cross peaks appear at (1745, 1722) and (1728, 1722) cm<sup>-1</sup>. Note that in the original one-dimensional IR spectra, only two absorption bands ascribed to the crystalline (i.e., the band at  $1723 \text{ cm}^{-1}$ ) and amorphous (i.e., the band at  $1740 \text{ cm}^{-1}$ ) parts of the copolymer are apparent, while another band is now clearly sorted out at 1728 cm<sup>-1</sup> in the 2D asynchronous spectrum. We also found the additional band in the C=O stretching vibration region for PHB at around 1731 cm<sup>-1</sup> by the use of 2D correlation analysis [16]. The existence of this band is confirmed also by the second derivative of the spectrum of P(HB-co-HHx) measured at 30 °C shown in Fig. 4. The second derivative spectrum yields clear indication for the presence of a band around  $1731 \text{ cm}^{-1}$ , which is consistent with the cross peak developed in the 2D asynchronous spectrum.

The positive sign of the synchronous spectral intensity in the region containing the (1731, 1723) cm<sup>-1</sup> coordinates indicates the same direction of the intensity variations in these two bands. The band at 1723 cm<sup>-1</sup> is much more intense than that at 1731 cm<sup>-1</sup>. Based on its frequency and band intensity, the band at 1723 cm<sup>-1</sup> may be assigned to the C=O stretching mode of the major crystalline component of





Fig. 3. Synchronous (A) and asynchronous (B) correlation spectra of P(HB-*co*-HHx) (12 mol% HHx) in the C=O stretching vibration region generated from the temperature-dependent IR spectra measured over a temperature range of 30-140 °C.

P(HB-*co*-HHx). The weaker band at  $1731 \text{ cm}^{-1}$  may arise from the C=O stretching mode of the less ordered crystalline component of the copolymer [16]. These two highly overlapped crystalline bands are distinguished only in the 2D asynchronous spectrum because an asynchronous spectrum is very powerful in differentiating bands arising from different origins, while a synchronous spectrum generally shows overall similarities of spectral variations.

Of note in the 2D asynchronous spectrum shown in Fig. 3(B) is that there are asynchronous cross peaks between the crystalline and amorphous bands. This observation clearly indicates that the melting of the crystalline component does not proceed simultaneously with the formation of the completely amorphous component. It is very likely that the melting of P(HB-*co*-HHx) takes place through an intermediate state. The existence of an intermediate state during



Fig. 4. A second derivative spectrum calculated from the IR spectrum of P(HB-co-HHx) (12 mol% HHx) in the C=O stretching vibration region at 30 °C.

the melting process was also reported for some other polymers including PHB [10,12,16]. Thus, the present study indicates that P(HB-*co*-HHx) (12 mol% HHx) undergoes the melting process through an intermediate state as other PHA polymers.

# 3.2. Changes in the C-O-C stretching band region during the melting process

Fig. 5 shows temperature-dependent IR spectra of P(HBco-HHx) (12 mol% HHx) in the 1320–1160 cm<sup>-1</sup> region. Bands in this region are attributed to the stretching vibration modes of the C–O–C groups [10–12,16]. It can be seen from Fig. 5 that these bands change largely during the melting process of the copolymer. Bands at 1289, 1278, 1264, and 1228 cm<sup>-1</sup> decrease with increasing temperature, while three broad bands at 1303, 1259, and 1183 cm<sup>-1</sup> are dominant above the  $T_{\rm m}$  of the copolymer. Therefore, the four bands at 1289, 1278, 1264, and 1228 cm<sup>-1</sup> are ascribed to



Fig. 5. IR spectra of P(HB-*co*-HHx) (12 mol% HHx) in the C–O–C stretching vibration region (1320–1160 cm<sup>-1</sup>) measured over a temperature range of 30–140 °C.



Fig. 6. Synchronous (A) and asynchronous (B) correlation spectra of P(HB-*co*-HHx) (12 mol% HHx) in the C–O–C stretching vibration region generated from the temperature-dependent IR spectra measured over a temperature range of 30–140 °C.

the crystalline component, and the three features at 1303, 1259, and 1183  $\text{cm}^{-1}$  are attributed to the amorphous part.

The synchronous and asynchronous 2D correlation spectra in the region of  $1330-1150 \text{ cm}^{-1}$  generated from the temperature-dependent IR spectra of P(HB-*co*-HHx) are shown in Fig. 6(A) and (B), respectively. There are at least three autopeaks at 1280, 1230, and 1180 cm<sup>-1</sup>. The autopeak at 1280 cm<sup>-1</sup> extends to the vicinity of 1289 and 1264 cm<sup>-1</sup>, and that at 1180 cm<sup>-1</sup> is very broad. Thus, both autopeaks may consist of more than one peak. Negative and positive 2D synchronous cross peaks at (1280, 1180), (1180, 1230), and (1280, 1230) cm<sup>-1</sup> reveal that the intensities of the bands located near 1280 and 1230 cm<sup>-1</sup> (the crystalline bands) vary in the same direction, which is opposite to the intensity variations in the bands near 1180 cm<sup>-1</sup> (the amorphous bands), in the course of melting. It is noted in Fig. 6(B)



Fig. 7. Second derivative spectra calculated from the IR spectra of P(HB*co*-HHx) (12 mol% HHx) in the C–O–C stretching vibration region over a temperature range of 30–140 °C.

that there are several asynchronous cross peaks between amorphous bands (e.g., bands near 1183 cm<sup>-1</sup>) and crystalline bands (e.g., bands near ~1289–1278 and 1230 cm<sup>-1</sup>). This observation again indicates the existence of an intermediate state during the phase transition of the copolymer. It is very likely that the appearance of the asynchronous cross peaks at (1289, 1272) and (1280, 1272) cm<sup>-1</sup> is caused by the band shift, and that at (1188, 1170) cm<sup>-1</sup> is due to the band broadening and the baseline shift [24,25]. As can be seen from the original IR spectra (Fig. 5) and the second derivatives (Fig. 7) that there exist a large shift of the band at 1278 cm<sup>-1</sup> and band broadening as well as a baseline shift of the band at 1183 cm<sup>-1</sup> in the course of temperature increase.

# *3.3. Changes in the C–H stretching band region during the melting process*

The structural difference between P(HB-co-HHx) and PHB lies in the length of the alkyl side chain attached to the polymer backbone. Accordingly, the temperature-dependent spectral variations in the CH<sub>3</sub> and CH<sub>2</sub> stretching band regions are important to reveal differences or similarities in the thermally induced microenvironmental changes of the side chains of these two polymers. Fig. 8 shows temperaturedependent IR spectra of P(HB-co-HHx) (12 mol% HHx) in the region of  $3050-2850 \text{ cm}^{-1}$ . Bands around  $2980 \text{ cm}^{-1}$ are attributed to the CH<sub>3</sub> asymmetric stretching modes, while those due to the CH<sub>2</sub> antisymmetric stretching vibrations are identified around  $2930 \text{ cm}^{-1}$ . With the temperature increase, the CH<sub>3</sub> stretching band at 2975 cm<sup>-1</sup> decreases, while that at 2984  $\text{cm}^{-1}$  increases in intensity. Similarly, the CH<sub>2</sub> stretching band at 2934 cm<sup>-1</sup> decreases and that at 2937 cm<sup>-1</sup> increases in intensity on passing from the crystalline to the amorphous phase. Bands in this region are highly overlapped. The second derivatives of the spectra of P(HB-co-HHx) at 30 and 140 °C were calculated and are shown in Fig. 9. It can be seen from the second derivative spectrum of P(HB-co-HHx) at 30 °C that there are at least



Fig. 8. IR spectra of P(HB-co-HHx) (12 mol% HHx) in the C–H stretching vibration region (3050–2850  $\rm cm^{-1})$  measured over a temperature range of 30–140  $^\circ\rm C.$ 

four major bands at 3008, 2997, 2975, and 2966 cm<sup>-1</sup> in the CH<sub>3</sub> asymmetric stretching band region. For the CH<sub>2</sub> antisymmetric stretching band region, two bands at 2934 and 2927 cm<sup>-1</sup> are shown up. These bands may be attributed to the crystalline bands arising from the crystal field splitting, which occurs in some semicrystalline polymers [16,17]. The crystal field splitting may be caused not only by the intramolecular interaction between analogous functional groups attached along a polymer chain, but also by the intermolecular interaction between those groups located closely in the crystalline lattice. The crystalline band splittings of certain vibration modes have been reported for a number of polymers especially those having a helical chain structure [16,17].



Fig. 9. Second derivative spectra calculated from the IR spectra of P(HBco-HHx) (12 mol% HHx) in the C–H stretching vibration region at 30 and 140  $^{\circ}$ C.



Fig. 10. Synchronous (A) and asynchronous (B) correlation spectra of P(HB-*co*-HHx) (12 mol% HHx) in the C–H stretching vibration region generated from the temperature-dependent IR spectra measured over a temperature range of 30-140 °C.

Fig. 10(A) and (B) shows the synchronous and asynchronous 2D correlation spectra in the  $3020-2900 \text{ cm}^{-1}$  region generated from the temperature-dependent IR spectra. Four autopeaks are observed at 2985, 2975, 2944, and 2930  $\text{cm}^{-1}$ in the synchronous spectrum. The band at 2985  $\text{cm}^{-1}$  shares three negative synchronous cross peaks with the bands at 3008, 2975, and 2930  $\text{cm}^{-1}$ . This indicates that the intensities of the bands at 3008, 2975, and those around  $2930 \text{ cm}^{-1}$  vary in the opposite direction to that of the band at 2985  $\text{cm}^{-1}$ , with the change in temperature. In the corresponding asynchronous spectrum, the amorphous band at 2985 cm<sup>-1</sup> shares asynchronous cross peaks with the crystalline bands at 3007 and 2975  $\text{cm}^{-1}$ . This observation also suggests that the completely amorphous component is not formed simultaneously with the melting of the crystalline component.

Interestingly, a band at 2962 cm<sup>-1</sup> observed in the C-H stretching region for P(HB-co-HHx) (12 mol% HHx) (Fig. 9) is absent in that for PHB. Therefore, it is very likely that this band is attributed to the CH<sub>3</sub> asymmetric stretching mode of the side-chain group (propyl, -CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>) incorporated in P(HB-co-HHx) (12 mol% HHx). It should be noted that no peak emerges at  $2962 \text{ cm}^{-1}$  in the 2D synchronous spectrum shown in Fig. 10(A). This indicates that the temperature-induced intensity change in this band is not prominent. In other words, the crystalline/amorphous phase transition of P(HB-co-HHx) does not strongly affect the behavior of the propyl side-chain groups. This is consistent with the fact that the propyl group is attached to the HHx comonomer, which functions as a less crystallizable unit in P(HB-co-HHx).

### 3.4. Spectral changes during the cooling down process

Temperature-perturbed IR spectral variations in the C=O stretching band region of P(HB-co-HHx) observed during the cooling down process were also investigated. IR spectra in the 1780–1680  $\text{cm}^{-1}$  region collected as a function of decreasing temperature are shown in Fig. 11. Fig. 12(A) and (B) shows synchronous and asynchronous 2D correlation spectra generated from the temperature-dependent IR spectra in the C=O stretching band region for the cooling down process, respectively. The pattern of the 2D asynchronous contour plot is almost the same as that for the melting process (Fig. 3(B)) except the signs of the cross peaks. It can be seen from Fig. 11 that the amorphous C=O band at  $1740 \text{ cm}^{-1}$  decreases with the temperature decrease, and that the crystalline band at  $1723 \text{ cm}^{-1}$  appears at around 110 °C and develops down to room temperature. Two pairs of asynchronous cross peaks are developed at the same coordinates as those in the asynchronous spectrum generated for the melting process. This observation also indicates the



Fig. 11. IR spectra of P(HB-*co*-HHx) (12 mol% HHx) in the C=O stretching vibration region measured over a temperature range of 140-30 °C (cooling down process).



Fig. 12. Synchronous (A) and asynchronous (B) correlation spectra of P(HB-co-HHx) (12 mol% HHx) in the C=O stretching vibration region generated from the temperature-dependent IR spectra measured over a temperature range of 30-140 °C (cooling down process).

coexistence of two crystalline C=O bands, and suggests that, in the crystallization process, the formation of the crystalline component does not proceed simultaneously with the decrease in the amorphous component.

# 3.5. Crystalline/amorphous phase transition behavior of *P*(*HB*-co-*HHx*)

The temperature-dependent IR spectra of P(HB-*co*-HHx) (12 mol% HHx) depict clearly that the crystalline C=O band observed at 1723 cm<sup>-1</sup> decreases during the heating up process and grows up during the cooling down process. Recent WAXD study showed that the crystalline part of P(HB-*co*-HHx) almost disappears above ca. 110 °C [14,15]. A good agreement is obtained between the WAXD study and

the temperature-dependent IR spectral variations observed in the melting process.

IR absorption bands are very sensitive to changes in microenvironments of molecules and the degree to which a vibrational mode is coupled to adjacent vibrations. The splitting of the C=O stretching band observed below the  $T_{\rm m}$ of the copolymer is possibly caused by some specific molecular interactions [14–16]. The analogous groups along the polymer chains are located very closely in the crystalline state of P(HB-co-HHx) because of the highly ordered and possible helical structure. As the temperature increases, the highly ordered structure collapses and, consequently, the inter- and intramolecular interactions between the repeating units along the polymer chains in a crystalline state are diminished. Therefore, the spectral changes of certain absorption bands concerning the crystalline/amorphous phase transition are clearly observed in the temperaturedependent IR spectra of P(HB-co-HHx) (12 mol% HHx).

# 3.6. Comparison between the IR spectra of P(HB-co-HHx) and those of PHB

In our previous study on PHB [16], we analyzed the temperature-dependent IR spectra of PHB in detail. In the present study, we compare the one-dimensional IR spectra and also 2D correlation spectra of P(HB-co-HHx) (12 mol% HHx) with those of PHB. In the C=O stretching band region for PHB, three bands at 1740, 1731, and 1723  $\text{cm}^{-1}$  were observed. Note that the frequencies of the bands observed in the C=O stretching region for P(HB-co-HHx) are identical to those for PHB. This suggests that C=O groups of P(HBco-HHx) and those of PHB experience very similar submolecular environments. However, the relative intensity ratio of the crystalline band at 1723 cm<sup>-1</sup> to the amorphous band at  $1740 \text{ cm}^{-1}$  for P(HB-co-HHx) (12 mol% HHx) is much lower than that for PHB. The IR spectra reveal clearly that the percentage of crystallinity for P(HB-co-HHx) is lower than that for PHB.

In the CH<sub>3</sub> asymmetric stretching band region, the bands at 3008 and 2997 cm<sup>-1</sup> for P(HB-*co*-HHx) were observed as shoulders of the band at 2975 cm<sup>-1</sup>, while those for PHB are very sharp and clear. The frequencies of the bands and the crystal field splitting pattern observed in the C–H stretching region for P(HB-*co*-HHx) are also very similar to those for PHB. These spectral features again imply a higher crystallinity of PHB and similar microenvirontments in which the C–H groups of these two polymers are located. The shapes of the bands in the C–O–C stretching region below the  $T_m$  for P(HB-*co*-HHx) are also different from those for PHB, e.g., the relative intensity of the bands in the region of 1295– 1260 cm<sup>-1</sup> are different between the two polymer samples.

The temperature-induced IR spectral changes in the C=O, C-O-C, and C-H stretching regions for P(HB-*co*-HHx) (12 mol% HHx) and those for PHB are similar. However, the temperatures at which the significant intensity changes in the three spectral regions occur are different between the two polymer systems. Marked intensity changes in the three spectral regions for P(HB-*co*-HHx) were observed at much lower temperature than those for PHB. It is also noted that the intensity changes in the IR spectra of P(HB-*co*-HHx) (Figs. 2, 5 and 8) occur gradually with increasing temperature, indicating a gradual deformation of its highly ordered and helical structure. On the other hand, the intensities of the IR spectra of PHB change abruptly in the vicinity of the  $T_m$  of PHB [16], suggesting that the highly ordered and helical structure of PHB collapses rapidly at the temperature right around the  $T_m$ .

The observations described above agree very well with the WAXD and DSC studies [14,15], which indicated that P(HB-co-HHx) (12 mol% HHx) assumes an orthorhombic crystal system, which is identical to that of PHB, and that the percentage of crystallinity of these two polymers are remarkably different. The crystallinity of PHB is higher than that of P(HB-co-HHx). We observed several remarkable differences between the temperature-dependent IR spectra of P(HB-co-HHx) and those of PHB, however, the phase transition behaviors revealed by 2D correlation spectroscopy for these two polymer systems are fundamentally similar. The present study indicates clearly that the HHx comonomer does not alter the crystalline structure of PHB. Its major role is to break down the crystalline structure from place to place, and hence increasing the amorphous entity of the copolymer.

### 4. Conclusion

The thermal melting behavior of P(HB-co-HHx)(12 mol% HHx) was investigated by using generalized 2D IR correlation spectroscopy. The C=O, C-H, and C-O-C stretching vibration regions depict marked temperature-dependent IR spectral variations. The crystalline bands decrease while the amorphous bands increase during the melting process. This is because the highly ordered and helical structure of P(HB-co-HHx) is diminished at high temperature.

The significant intensity changes in the C=O, C–O–C, and C–H stretching regions start taking place at much lower temperature in the spectra of P(HB-co-HHx) (12 mol% HHx) than in the spectra of PHB. This observation indicates that the crystallinity decreases gradually even at low temperature for P(HB-co-HHx).

The asynchronous 2D correlation spectrum generated from the temperature-induced dynamic IR spectra in the C=O stretching vibration region clearly resolves two highly overlapped crystalline bands located at 1731 and 1723 cm<sup>-1</sup>. The intense band at 1723 cm<sup>-1</sup> may be attributed to the major crystalline component of the copolymer, and the weaker band at 1731 cm<sup>-1</sup> is possibly assignable to the minor crystalline component with a less ordered structure. The appearance of the 2D asynchronous cross peaks between the crystalline and amorphous bands suggests the existence of an intermediate state during the phase transition process.

The CH<sub>3</sub> asymmetric stretching bands and CH<sub>2</sub> antisymmetric stretching bands exhibit a crystal field splitting, which is often observed for polymers with a helical structure. In comparison to PHB, this study suggests that the HHx comonomer does not alter the crystalline structure of PHB. The close similarity in the frequencies of the C=O, C–O–C, and C–H stretching bands of the crystalline components between PHB and P(HB-*co*-HHx) (12 mol% HHx) reveals that the helical structure of P(HB-*co*-HHx) is very close to that of PHB. The HHx unit locally disrupts the highly ordered and helical structure of the copolymer from place to place, increasing the amorphous entity, which gives the better physical and mechanical properties to the copolymer.

### Acknowledgements

A.P. gratefully thanks the Thailand Research Fund (TRF) for the financial support via the Royal Golden Jubilee (RGJ) PhD program (Grant contract number: PHD/0066/2544).

### References

- S.F. Williams, D.P. Martin, in: A. Steinbüchel, Y. Doi (Eds.), Biopolymers, Wiley-VCH, Wienhiem, 2002.
- [2] L.M. Lara, W.H. Gjalt, Microbiol. Mol. Biol. Rev. 63 (1991) 21.
- [3] A.J. Anderson, E.A. Dawes, Microbiol. Rev. 54 (1990) 450.
- [4] D. Jendrossek, in: A. Steinbüchel, Y. Doi (Eds.), Biopolymers, Wiley-VCH, Wienhiem, 2001.

- [5] S.Y. Lee, Biotechnol. Bioeng. 49 (1996) 1.
- [6] Web site; http://www.nodax.com.
- [7] M.M. Satkowski, D.H. Melik, J.-P. Autran, P.R. Green, I. Noda, L.A. Schechtman, in: A. Steinbüchel, Y. Doi (Eds.), Biopolymers, Wiley-VCH, Wienhiem, 2001.
- [8] M. Yokouchi, Y. Chatani, H. Tadokoro, K. Teranishi, H. Tani, Polymer 14 (1973) 267.
- [9] J. Cornibert, R.H. Marchessault, Macromolecules 8 (1975) 296.
- [10] Q. Wu, G. Tian, S. Sun, I. Noda, G.-Q. Chen, J. Appl. Polym. Sci. 82 (2001) 934.
- [11] G. Tian, Q. Wu, S. Sun, I. Noda, G.-Q. Chen, J. Polym. Sci. B 40 (2002) 649.
- [12] G. Tian, Q. Wu, S. Sun, I. Noda, G.-Q. Chen, Appl. Spectrosc. 55 (2001) 888.
- [13] K.P. Caballero, S.F. Karel, R.A. Register, Int. J. Bio. Macromol. 17 (1995) 86.
- [14] H. Sato, M. Nakamura, A. Padermshoke, H. Yamaguchi, H. Terauchi, S. Ekgasit, I. Noda, Y. Ozaki, Macromolecules, submitted.
- [15] H. Sato, A. Padermshoke, M. Nakamura, R. Murakami, F. Hirose, K. Senda, H. Terauchi, S. Ekgasit, I. Noda, Y. Ozaki, Macromol. Symp., in press.
- [16] A. Padermshoke, Y. Katsumoto, H. Sato, S. Ekgasit, I. Noda, Y. Ozaki, Appl. Spectrosc., submitted.
- [17] S.K. Mallapragada, B. Narasimhan, in: R.A. Meyers (Ed.), Encyclopedia of Analytical Chemistry, Wiley, Chichester, 2000.
- [18] I. Noda, Bull. Am. Phys. Soc. 31 (1986) 520.
- [19] I. Noda, Appl. Spectrosc. 44 (1990) 550.
- [20] I. Noda, Appl. Spectrosc. 47 (1993) 1329.
- [21] I. Noda, A.E. Dowrey, C. Marcott, G.M. Story, Y. Ozaki, Appl. Spectrosc. 54 (2000) 236A.
- [22] I. Noda, G.M. Story, C. Marcott, Vib. Spectrosc. 19 (1999) 461.
- [23] E. Galbiati, M.D. Zoppo, G. Tieghi, G. Zerbi, Polymer 34 (1993) 1806.
- [24] A. Gericke, S.I. Gadaleta, J.W. Brauner, R. Mendelshon, Biospectroscopy 2 (1996) 341.
- [25] A. Nabet, M. Auger, M. Pézolet, Appl. Spectrosc. 54 (2000) 948.