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In situ FTIR study on melting and crystallization of polyhydroxyalkanoates

Jun Xu[a], Bao-Hua Guo[a], Rui Yang[a], Qiong Wu[b], Guo-Qiang Chen[b], Zeng-Min Zhang[a,*]

[a]*Institute of Polymer Science and Engineering, Department of Chemical Engineering, School of Materials Science and Engineering, Tsinghua University, Beijing 100084, People's Republic of China*

[b]*Department of Biological Sciences and Biotechnology, Tsinghua University, Beijing 100084, People's Republic of China*

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Abstract

Melting and crystallization of two types of polyhydroxyalkanoates (PHAs), poly(3-hydroxybutyrate) homopolymer (PHB) and poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) random copolymer (PHBHHx) were studied via in situ Fourier transform infrared spectroscopy (FTIR). The absorbance variances of the crystalline and the amorphous bands revealed that melting of PHB occurred at a narrow temperature range, while melting of PHBHHx copolymer occurred at a wide temperature range, and it was shown that the latter had a much wider distribution of lamellar thickness. These results were consistent with those from DSC. The alkyl groups reached close packing prior to crystallization in PHB and followed the crystallization in PHBHHx due to the longer branch propyl group. The band at 1230 cm^{-1} was first assigned to the conformational band of the helical segments, and the absorbance ratio of the band at 1230 cm^{-1} to the reference band at 1453 cm^{-1} was used to quantitatively determine the crystallinity of PHAs after adjustment with DSC. Since further crystallization usually occurs in PHAs during heating and DSC could not reveal the original crystallinity in these samples, we suggest FTIR as an effective and non-destructive tool to evaluate crystallinity of PHAs.

Keywords: Polyhydroxyalkanoates; Fourier transform infrared spectroscopy; Crystallinity

1. Introduction

Polyhydroxyalkanoates (PHAs) are a family of microbially synthesized polymers, which have drawn much attention due to their good biodegradability and biocompatibility [1]. Ever since the first one of the family poly(3-hydroxybutyrate) (PHB) was found in 1926 [2], much work has been contributed to synthesis and physical properties of PHAs. Till now, PHAs containing over 90 types of monomers have been synthesized in different microorganisms [3]. They range from rigid plastics to tough elastomers, which predominantly depend on the molecular structure [4]. PHB homopolymer has a crystallinity up to 75–85% [5]. Consequently, it is a brittle material, which considerably limits its applications [6]. When a little amount of 3-hydroxyhexanoate is randomly incorporated into PHB, the copolymer PHBHHx is obtained. In PHBHHx, the 3-hydroxyhexanoate monomers act as impurities and are excluded from the PHB crystal lattice. Thus, PHBHHx turns into a tough plastic [7].

Different methods have been utilized to characterize the structure and physical properties of PHAs. Polarized light microscopy revealed the spherulitic texture of PHAs. It was shown that the spherulites could grow up to several millimeters [8]. Atomic force microscopy demonstrated that PHB lamellae consisted of microfibrils of 30–50 nm width [9]. Wide angle X-ray scattering revealed that the crystal structure of PHB consisted of orthorhombic unit cells $P2_12_12_1\text{-}D_2^4$, each of which contained two left-handed helical molecules in anti-parallel orientation [10].

Fourier transform infrared spectroscopy (FTIR) is sensitive to local molecular environment; consequently it has been widely applied to reveal the conformational changes of macromolecules during melting and crystallization. FTIR was utilized to characterize the structural changes in polyamide-6 fibers [11] and poly(ethylene terephthalate) films [12] during annealing and drawing. In situ FTIR has been applied to study the melting process of isotactic poly(propylene) [13] and the crystallization

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* Corresponding author. Tel.: +8610-62784550; fax: +8610-62770304.
E-mail address: zhangzm@mail.tsinghua.edu.cn (Z.M. Zhang).

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process of isotactic polystyrene [14], isotactic poly(propylene) [15], poly(phenylene sulfide) [16], polyethylene [17], poly(ethylene-2,6-naphthalate) [18]. In addition, FTIR has been applied to the qualitative studies of PHAs [19–24]. However, little study has been reported on the structural changes of the molecular level during melting and crystallization process of PHAs.

In this paper, in situ FTIR study was carried out to elucidate conformational changes during melting and crystallization of PHB and PHBHHx. Furthermore, it was applied to evaluate the crystallinity quantitatively and the results were compared with those from differential scanning calorimetry (DSC).

2. Material and experimental section

2.1. Material

PHB with M_n 522,000 and M_w 775,000 was purchased from the Institute of Microbiology, Academia Sinica. Poly(3-hydroxybutyrate-*co*-10 mol% 3-hydroxyhexanoate) (PHBHHx) was synthesized from glucose and lauric acid in cells of *Aeromonas hydrophila* 4AK4 supplied by Procter and Gamble Co., with M_n 317,000 and M_w 2,083,000.

2.2. FTIR spectroscopy

PHB and PHBHHx films were cast from 1 wt% solutions in chloroform on the KRS-5 (cocrystal of TlBr and TlI) window, respectively. After complete evaporation of chloroform, the samples sandwiched between two salt windows were studied via in situ FTIR. The experiments were carried out on a Nicolet 560 FTIR Spectrometer with a manually controlled heater. The PHB sample was heated and the FTIR spectra were collected at 50, 75, 100, 125, 150, 160, 170, 180, 190 and 200 °C by signal averaging 32 scans at a resolution of 4 cm^{-1} in the wave number range of 4000–400 cm^{-1}. After complete melting at 200 °C, the sample was cooled down and the FTIR spectra were recorded every 20 °C until it reached 120 °C. Then the sample was kept at 120 °C for isothermal crystallization and the spectra were collected every 5 min. Forty minutes later, when the FTIR spectrum did not change any longer at the temperature, the sample was then naturally cooled down to room temperature. The PHBHHx sample was heated and the spectra were collected at 50, 75, 100, 110,120, 130, 140, 150 and 160 °C. After the sample was completely melted at 160 °C, it was naturally cooled down to room temperature and the spectra were collected every 20 °C. Data were processed with a standard OMNIC software supplied by Nicolet Company.

2.3. Differential scanning calorimetry (DSC)

DSC analysis was performed to study the melting behavior of PHAs on a Dupont 2100 differential scanning calorimeter. The equipment was calibrated with indium standard and nitrogen atmosphere was used throughout. PHB samples were heated from room temperature to 200 °C and PHBHHx samples to 160 °C, at a heating rate of 10 °C/min. The melting temperature (T_m) and the apparent enthalpy of fusion (ΔH_f) were determined from the endothermal peak on the DSC curves. For comparison of crystallinity determined from FTIR with that from DSC, the PHA films were solution cast on poly(tetrafluoroethylene) (PTFE) films for ease to remove. The film removed from the PTFE substrate immediately after complete evaporation of chloroform was referred as new film and the film further stored at room temperature for 12 h was referred as old film. Different parts of each film were detected by FTIR and DSC simultaneously to assure the same crystallinity.

3. Results and discussion

3.1. Melting and crystallization of PHB

Immediately after complete evaporation of solvent, the

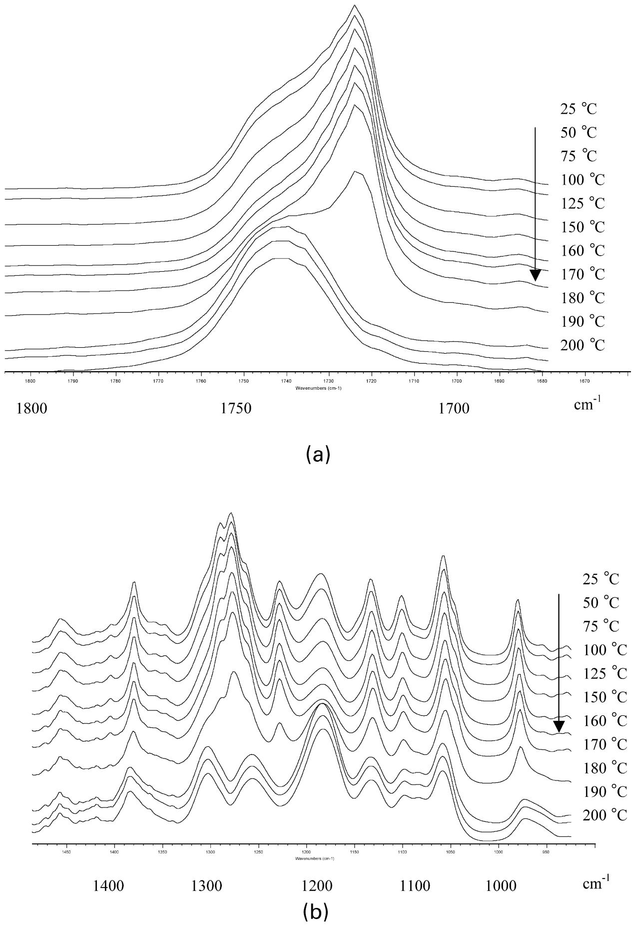


Fig. 1. In situ FTIR spectra of PHB during heating process: (a) Variance of the C=O stretching band; (b) Variance of bands of other characteristic groups.

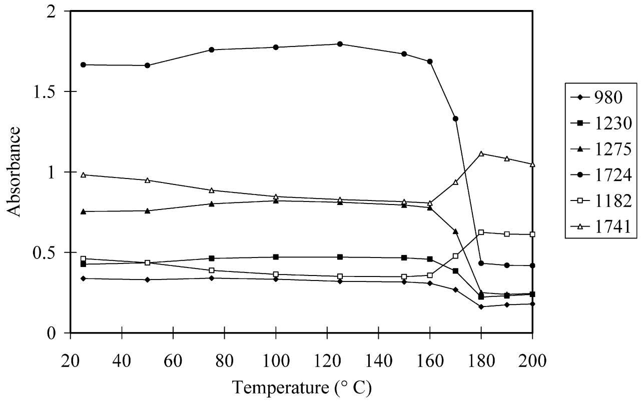


Fig. 2. Variance of the absorbance of the crystalline and the amorphous bands of PHB during heating. The curves with closed data points belong to the crystalline bands and that with open data points to the amorphous bands.

PHB film was heated, and the FTIR spectra were collected in situ during the heating process. Fig. 1 shows the variances of the bands of interest. The bands at 980, 1230, 1278 and 1724 cm^{-1} are shown to arise from the crystalline phase and those at 1186 and 1741 cm^{-1} from the amorphous phase. The bands at 1741 and 1724 cm^{-1} are assigned to the stretching vibration of the amorphous and the crystalline carbonyl group, respectively. In the crystalline phase, the segments predominantly formed 2_1 helices [10]. The oxygen atoms of the carbonyl groups are located closer to the hydrogen atoms at the other segments, thus the decreased dipole moment resulted in a lower wave number of the band [24]. During melting, the carbonyl groups were randomly surrounded by other atoms, leading to band shift from 1724 to 1741 cm^{-1}. The band at about 1380 cm^{-1} is assigned to symmetric wagging of CH_3 groups [21], which shifted from 1380 to 1384 cm^{-1} during melting. The band at 1230 cm^{-1} is proposed as the conformational band of the helical chains, since no amorphous bands of the same group could be found. The bands at 1186 and 1133 cm^{-1} are characteristic of the asymmetric and the symmetric stretching vibration of the C–O–C group, respectively [13].

The absorbance of the crystalline and the amorphous infrared bands studied in this work are plotted against temperature in Fig. 2. A bit increase in the intensities of the crystalline bands and decrease in the intensities of the amorphous bands could be observed when the new PHB film was heated to above 75 °C. It is attributed to further crystallization due to improved mobility of the molecules, since the new PHB film was heated immediately after complete evaporation of the solvent and was not yet completely crystallized. When heated to 160 °C, the absorbance of the crystalline and the amorphous bands showed abrupt changes until the sample was completed melted at 180 °C. These changes agreed well with the DSC result, as shown in Fig. 3. From the spectrum, during heating of the newly cast PHB film, it is seen that there existed a sharp endotherm and the lamellae were mostly melted between 160 and 180 °C with a maximum melting rate at 175.5 °C.

After staying at 200 °C for 2 min, the PHB sample was naturally cooled down to 120 °C, and then continued isothermal crystallization at this temperature. The temperature was chosen over the melt crystallization temperature of 97.7 °C. The spectra during the process are shown in Fig. 4.

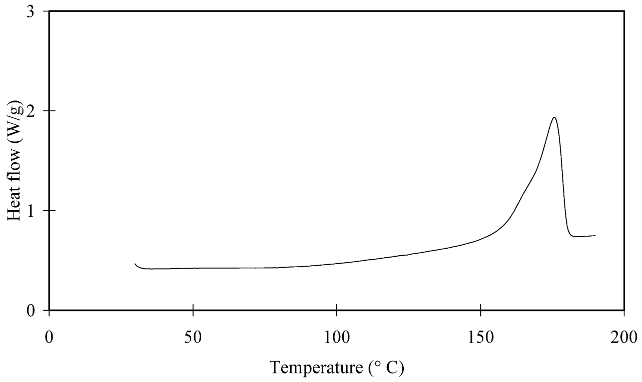
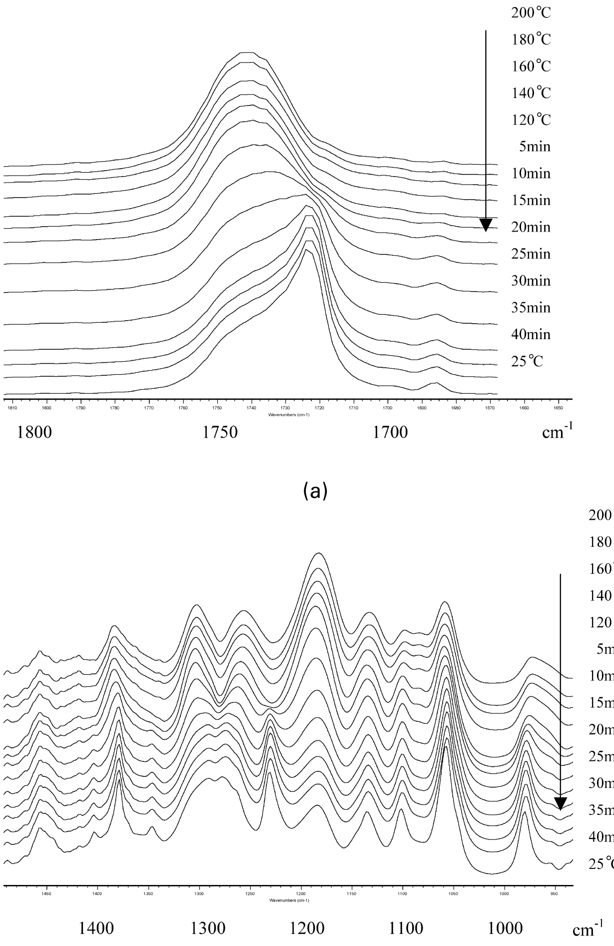


Fig. 3. The DSC curve of PHB during heating.



(a)

(b)

Fig. 4. In situ FTIR spectra of PHB during cooling process: (a) Variance of the C=O stretching band; (b) Variance of bands of other characteristic groups. The labels with unit 'min' are the time isothermally kept at 120 °C.

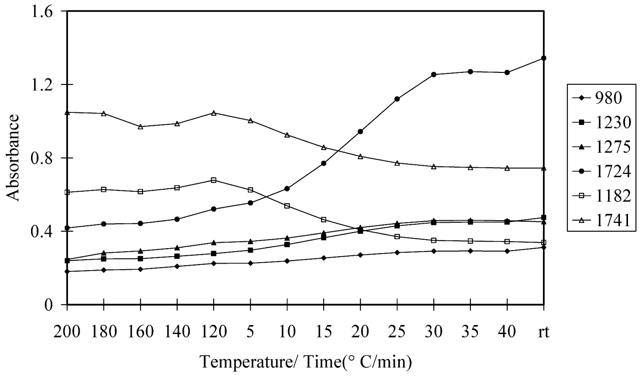


Fig. 5. Variance of the absorbance of the crystalline and the amorphous bands of PHB during cooling. The labels 5–40 with each 5 increment near the horizontal coordinate mean the time (min) kept at 120 °C. Others are temperatures, with ‘rt’ means room temperature.

The absorbance variances of the crystalline and the amorphous bands with cooling are plotted in Fig. 5. It is shown that crystallization begun when PHB melt was cooled to 120 °C. Fig. 6 demonstrates that the absorbance of all these bands, whether crystalline or amorphous, increased while cooling. The band at 1380 cm^{-1} showed an intensity increase of about 30% when cooled from 200 to 120 °C. At the first sight, the increase may be attributed to volume shrinkage with decrease of temperature. To see whether there are any other factors that play role in increasing the intensity, the cooling process of the amorphous poly(D,L-lactic acid), the monomer with only a CH_2 group different from that of PHB, was studied via in situ FTIR. It was observed that only a little increase of about 10% in the absorbance of the bands occurred when poly(D,L-lactic acid) was cooled from 200 to 120 °C (data not shown here). Consequently, it is tentatively proposed that there existed other effect, which will be described in the following text.

During crystallization, the band of CH_3 wagging shifted from 1384 to 1380 cm^{-1}, with the half-width turned narrower. The former probably arises from the amorphous phase and the latter from the crystalline phase. This phenomenon has not been noted in the literature [11,12].

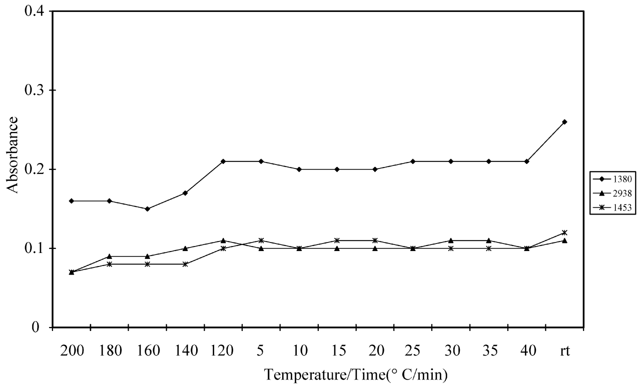


Fig. 6. The absorbance variance of the alkyl groups of PHB during crystallization.

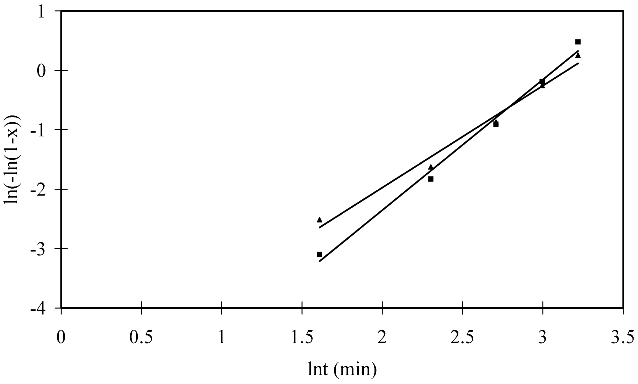


Fig. 7. Curves of the crystallization dynamics of PHB.

According to Fig. 5, the 120 °C point was taken as the start of crystallization. The dynamics of isothermal crystallization could be described with the Avrami equation

$$\ln(-\ln(1-x)) = n \ln t + \ln k \quad (1)$$

where x is the crystallinity, which could be obtained from the following equations:

$$x = A_{1230}(t)/A_{1230}(t_\infty) \quad (2)$$

or

$$x = A_{1724}(t)/A_{1724}(t_\infty) \quad (3)$$

Here $A_{1230}(t)$ and $A_{1724}(t)$ are the peak intensity of the band at 1230 and 1724 cm^{-1} at time t, respectively. $\ln(-\ln(1-x))$ is plotted against $\ln t$ in Fig. 7. The Avrami exponent was 2.08 for the band at 1724 cm^{-1} and 1.72 for the band at 1230 cm^{-1}, indicating that the crystallization started from heterogeneous nucleation.

3.2. Melting and crystallization of PHBHHx

After visible evaporation of chloroform, the cast PHBHHx film was further stored at room temperature to allow complete crystallization. After storage for 12 h, the sample was heated and the FTIR spectra were obtained in situ. The absorbance of all the crystalline bands showed steady decrease and that of the amorphous band at 1186 cm^{-1} showed steady increase, as shown in Fig. 8. It

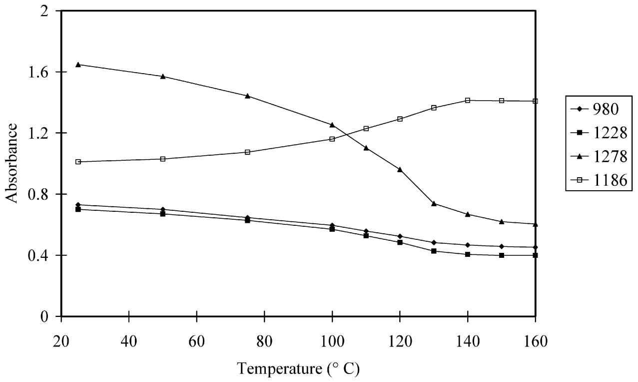


Fig. 8. Variance of the absorbance of the crystalline and the amorphous bands of PHBHHx during heating.

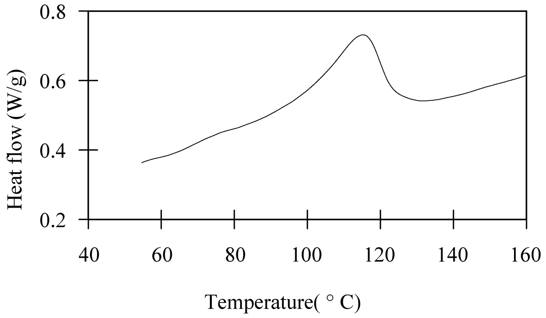


Fig. 9. DSC spectrum during melting of PHBHHx.

is demonstrated that PHBHHx melted in the temperature range between 50 and 130 °C, which is consistent with the DSC result shown in Fig. 9. Compared to PHB, the wider melting range revealed a wider distribution of the lamellar thickness in solution-cast PHBHHx film. It is seen that melting reached the maximum rate at about 120 °C, similar to the peak temperature (115.3 °C) of the endotherm on the DSC curve.

To simulate the processing conditions, crystallization of PHBHHx was carried out by naturally cooling to room temperature. The intensity variances of different bands are shown in Fig. 10, which clearly reveals that crystallization started at about 100 °C. However, the change of the bands belonging to the alkyl groups predominantly occurred at 60 °C, as shown in Fig. 11. This is distinctly different from the case of PHB, in which the transition occurred at the temperature higher than 120 °C, prior to the crystallization process. Here we tentatively propose that the difference resulted from the branched alkyl groups. In molecular level, crystallization proceeds via helical formation of the segments of the PHA macromolecules, which requires cooperative close packing of the atoms at the segments. In PHB homopolymer, the branching methyl groups have low volume and high spatial symmetry; consequently, they could easily pack together at a higher temperature. After that, the closely packed chains turned into helical conformers steadily. In the case of PHBHHx, when cooled down, the helical conformers came into being prior to the closed packing of the alkyl groups, since the latter is more difficult due to the larger volume and lower spatial symmetry of the branching propyl groups.

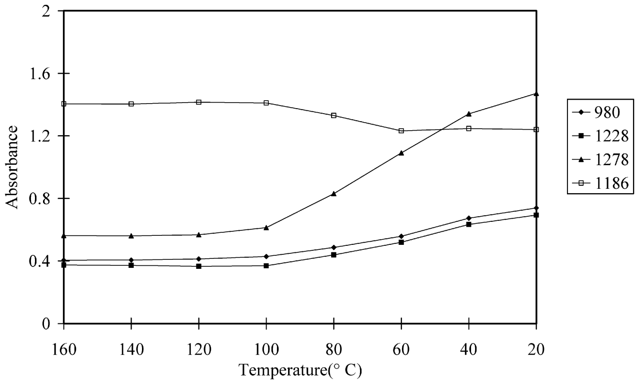


Fig. 10. Variance of the peak absorbance of the crystalline and the amorphous bands of PHBHHx during crystallization.

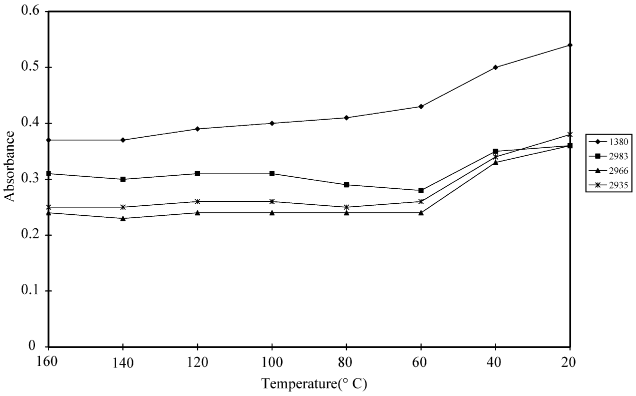


Fig. 11. Variance of the bands corresponding to the alkyl groups in PHBHHx during crystallization.

3.3. Determination of crystallinity via FTIR

It is well known that DSC is a direct method to obtain the crystallinity of a semicrystalline polymer from the area of the endothermal peak on the DSC curve. However, during the heating process, further crystallization may occur, which frequently occurs in the case of PHAs due to the relatively slow crystallization rate. Thus the crystallinity calculated from the endotherm may not be the original one. Consequently, FTIR provides a direct evaluation of the crystallinity. As described in literature, the crystallinity index defined as the absorbance ratio of the reference band at 1380 cm^{-1} to the amorphous band at 1186 cm^{-1}, was used to evaluate the crystallinity quantitatively. However, the crystallinity index is not the absolute degree of crystallinity [11,12]. In this paper, another index, defined as the absorbance ratio of the crystalline band at 1230 cm^{-1} to the reference band at 1453 cm^{-1}, is used to evaluate the crystallinity quantitatively as well. Since the absorbance of the band at 1380 cm^{-1} changed with the sample crystallinity, here the unchanged band at 1453 cm^{-1} was chosen as the reference band. The two kinds of crystallinity indices of different PHA samples and the absolute degree of crystallinity determined from DSC are shown in Table 1.

It is seen that PHBHHx has a much lower crystallinity than PHB. Consequently, PHBHHx has improved ductility. It was reported that PHBHHx with 10 mol% content of hydroxyhexanoate comonomer had elongation at break up to 400%, much larger than that of PHB, 5% [7].

It is out of question that the newly cast PHBHHx film (new film) should have a lower crystallinity than the film kept at room temperature for 12 h (old film), which is just the result from the intensity ratio of the band at 1230 cm^{-1} to that at 1453 cm^{-1}. However, the intensity ratio of the band at 1380 cm^{-1} to that at 1186 cm^{-1} and the crystallinity determined from the DSC curves showed a higher crystallinity of the new film, which apparently contradicted to the

Table 1
Crystallinity indices from FTIR and the absolute crystallinity from DSC

Sample	Crystallinity index		Absolute crystallinity from DSC
	A_{1380}/A_{1186}	A_{1230}/A_{1453}	
PHB film, immediately after solvent evaporation	0.91	2.17	0.37
PHB film, 12 h after solvent evaporation	0.95	2.57	0.47
PHBHHx film, immediately after solvent evaporation	0.82	0.24	0.30
PHBHHx film, 12 h after solvent evaporation	0.71	1.17	0.25

common sense. Here we argued that since the band at 1380 cm^{-1} is a crystalline band (shown in the above text), the intensity ratio of the band at 1380 cm^{-1} to that at 1186 cm^{-1} was not sensitive to the change of crystallinity. The abnormal higher crystallinity of the new film, determined from DSC, was attributed to further crystallization during heating in the DSC study. Due to improved mobility at higher temperature, the newly cast film will form more perfect lamellae than the film stored at room temperature, which led to a higher crystallinity. Consequently, we suggest that FTIR may be applied as a tool to determine the crystallinity of PHAs, especially those without complete crystallization.

To obtain an absolute value of crystallinity via FTIR, the standard curve is shown by plotting crystallinity from DSC curve against the intensity ratio of the crystalline band at 1230 cm^{-1} to the reference band at 1453 cm^{-1} in Fig. 12. To assure that the crystallinity from DSC was the original one, the crystallinity from the completely crystallized sample or the adjusted crystallinity was preferred. The standard curve is present as the following:

$$\text{Crystallinity} = 0.1826 \times A_{1230\ \mathrm{cm}^{-1}}/A_{1453\ \mathrm{cm}^{-1}} \tag{4}$$

It could be used to determine crystallinity of PHAs from the FTIR spectra.

Furthermore, Table 1 demonstrates that PHBHHx has a much lower crystallization rate than PHB homopolymer. The new film of PHB had a crystallinity of 0.4, a bit lower than the old film with a crystallinity of 0.47. However, the new film of PHBHHx has a crystallinity of 0.044, much lower than the old film with a crystallinity of 0.25. Furthermore, as a simple and non-destructive method, FTIR could be applied to study ageing and secondary crystallization of PHAs as well.

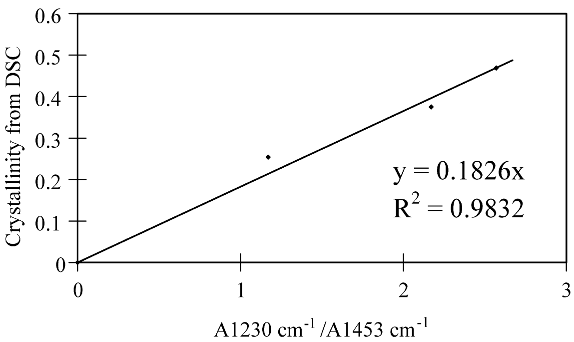


Fig. 12. Relation between the crystallization index calculated from FTIR and the absolute crystallinity determined from DSC.

4. Conclusions

Melting and crystallization of PHB and PHBHHx was studied via in situ FTIR combined with DSC. It is demonstrated that during melting, intensities of the crystalline bands at 980, 1230, 1275 and 1724 cm^{-1} decreased and those of the amorphous bands at 1186 and 1740 cm^{-1} increased, and vice versa occurred during crystallization. The spectra revealed that further crystallization occurred in the newly cast PHB film during heating and melting occurred sharply at around 170 °C, revealing a relatively perfect crystallization. Melting of PHBHHx occurred at a wider temperature range from 50 to 130 °C, demonstrating a wider distribution of lamellar thickness. The band at 1230 cm^{-1} was assigned to the conformational band of the helical chains. Avrami exponent of the crystallization dynamics of PHB was about 2, indicating heterogeneous nucleation. In PHB sample, cooperative close packing of the molecules formed prior to crystallization. On the contrary, in PHBHHx, packing of the molecules followed the crystallization due to the longer branch propyl groups.

FTIR method was utilized to evaluate crystallinity of PHAs quantitatively. It was found that the absorbance ratio of the crystalline band at 1230 cm^{-1} to the reference band at 1453 cm^{-1} could effectively and non-destructively reveal crystallinity of PHAs, especially those without complete crystallization. The standard curve between the crystallinity and the absorbance ratio of the helical band and the reference band was determined. Consequently, in situ FTIR could be further used to study ageing and secondary crystallization behavior of PHAs.

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