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# Raman microspectroscopy study of structure, dispersibility, and crystallinity of poly(hydroxybutyrate)/poly(L-lactic acid) blends

Tsuyoshi Furukawa [a,b], Harumi Sato [a], Rumi Murakami [a], Jianming Zhang [a],
Isao Noda [c], Shukichi Ochiai [b], Yukihiro Ozaki [a,*]

[a] *Department of Chemistry, School of Science and Technology, Research Center for Environment Friendly Polymers, Kwansei Gakuin University, 2-1, Gakuen, Sanda, Hyogo 669-1337, Japan*
[b] *ST Japan Inc., Minaminakaburi, Hirakata, Osaka 573-0094, Japan*
[c] *The Procter & Gamble Company, 8611 Beckett Road, West Chester, OH 45069, USA*

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## Abstract

The structure, dispersibility, and crystallinity of poly(3-hydroxybutyrate) (PHB) and poly(L-lactic acid) (PLLA) blends are investigated by using Raman microspectroscopy. Four kinds of PHB/PLLA blends with a PLLA content of 20, 40, 60, and 80 wt% were prepared from chloroform solutions. Differences in the Raman microspectroscopic spectra between the spherulitic and nonspherulitic parts in the blends mainly lie in the C=O stretching band and C–O–C and C–C skeletal stretching bands of PHB and PLLA. In addition to such bands, the Raman spectra of spherulitic structure in the blends show a band due to the $CH_3$ asymmetric stretching mode at an unusually high frequency ($3009\ cm^{-1}$), suggesting the existence of a C–H···O=C hydrogen bond of PHB in the spherulite. The existence of C–H···O=C hydrogen bond is one of the unambiguous evidence for the crystallization of PHB component in the blends. Therefore, it is possible to distinguish Raman bands due to each component in the spectra of blends. Raman spectra of the spherulitic structure in the blends are similar to a Raman spectrum of pure crystalline PHB, while those of the nonspherulitic parts in the blends have each component peak of PHB and PLLA. The present study reveals that the PHB component is crystallized in the blends irrespective of the blend ratio, and that both components are mixed in the nonspherulite parts. The crystalline structure of PHB and the nonspherulitic parts of PLLA in the blends are characterized, respectively, by the unique band of C–H···O=C hydrogen bond at $3009\ cm^{-1}$ and CCO deformation bands near $400\ cm^{-1}$.

*Keywords:* Poly(hydroxybutyrate); Poly(L-lactic acid); Raman microspectroscopy

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## 1. Introduction

Poly(3-hydroxybutylate) (PHB) is one of the biodegradable thermoplastics produced from various bacteria [1–7]. PHB has perfectly isotactic structure with only the R configuration. Such stereoregularity of PHB leads to excessively high crystallinity, so that it often ends up being very rigid and stiff. Due to such properties, it is difficult to use PHB as a replaceable material for commodity plastics. To reduce the excess crystallinity and improve overall physical properties of PHB, it is copolymerized with other kinds of hydroxyalkanoate [8–11], or blended with other polymers including biodegradable polymers [12–15]. The crystallinity and melting point of PHB copolymers and PHB blends are usually lower than those of PHB.

Poly(L-lactic acid) (PLLA) is also one of the environment friendly polymers [6,16–18]. PLLA is synthesized from renewable resources, such as starch, and it is biocompatible and degradable material. PLLA has certain superior physical and mechanical properties to other degradable polymers. Thus, its properties and synthetic methods have been investigated extensively. Because of its superior biocompatibility, PLLA has received keen interest as a promising material in biomedical applications. However, due to its relatively high cost of production in comparison with other commercial polymers, PLLA has not been employed extensively as a replaceable material. In order to reduce its cost and improve its properties, PLLA is also blended with other polymers [19–23].

Blending of polymers is an effective alternative way to acquire a new material with desired properties. Binary blends

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

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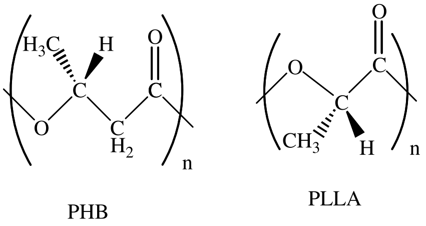

Fig. 1. Chemical structures of PHB and PLLA.

of PHB and PLLA (Fig. 1) have also been investigated by several research groups. Based on the results of polarized light microscopy, Blümm and Owen showed that PHB/PLLA blends are immiscible for high-molecular weight PHB, while they are miscible for low-molecular weight PHB [24]. Zhang et al. suggested from studies by using differential scanning calorimetry (DSC), polarized light microscopy and scanning electron microscopy that the evidence for the immiscibility of PHB/PLLA blends can be obtained from observations of constant melting point and phase crystallinity of PHB and of spherulite structure of PHB component [25].

The purpose of the present study is to investigate the structure, dispersibility, morphology, and crystallinity of PHB and PLLA in PHB/PLLA blends by using Raman microspectroscopy. In this study, four kinds of PHB/PLLA blend, i.e. 80/20, 60/40. 40/60, and 20/80 blends, are investigated.

Raman spectroscopy enables one to explore structure and interactions of molecules at the functional group level. Therefore, Raman spectroscopy has been used to elucidate the crystalline/amorphous structure, molecular interactions, and conformations of polymers [26–32]. Raman spectra of polymers show many bands due to skeletal deformation modes in the low frequency region. These bands are useful in investigating the crystalline and lamella structures of polymers. There are several Raman studies on PLLA characterization [29–32]. Qin and Kean investigated the crystallinity of PLLA by using Raman spectroscopy [27]. The degree of crystallinity was estimated by using the simple intensity ratios of two C=O stretching bands at 1766 and 1770 $cm^{-1}$ due to the crystalline and amorphous parts (an univariate analysis), as well as by using the entire spectral data in the CH stretching region and the finger print region (a multivariate analysis). Yang et al. investigated the rotational isomeric states, i.e. trans or gauche conformation, of PLLA by comparing experimental Raman spectra of PLLA with simulated ones derived from normal coordinate analysis [28,31]. Their results suggested that PLLA has the tgt conformation. Smith et al. analyzed the orientation of PLLA in drawn films with different draw ratios [32]. They found that the frequency and intensity of a band at 400 $cm^{-1}$ due to the CCO deformation mode change with the draw ratio.

In contrast to PLLA, PHB has not been studied well by Raman spectroscopy [33,34]. Moreover, to our best knowledge, no Raman study has been carried out for PHB/PLLA blends. Raman spectroscopy can be combined with microspectroscopy technique [35,36]. Raman microspectroscopy is known to be superior to IR microspectroscopy with respect to spatial resolution; the former has the spatial resolution of about 1 μm while the latter has that of 10 μm. Therefore, Raman microspectroscopy is especially suitable for structural characterization of small spots in polymer blends.

We investigated the structure, crystallinity, and thermal behavior of PHB and its copolymers by using IR spectroscopy, wide-angle X-ray diffraction, DSC, and quantum chemical calculation [37]. One of the most important findings in our studies is that crystalline structures of PHB and its copolymers, poly(hydroxybutyrate-*co*-hydroxyhexanoate) (P(HB-*co*-HHx)) have C–H···O=C hydrogen bonds between $CH_3$ groups of one helical structure and C=O groups of another helical structure. This unique C–H···O=O hydrogen bond was investigated by IR and Raman spectroscopy and quantum chemical calculations [38–41]. We also investigated interactions of poly(L-lactide) (PLLA) and poly(L-lactide)/poly(D-lactide) (PLLA/PDLA) stereocomplex by means of IR spectroscopy [42]. It was revealed that PLLA, PLLA/PDLA stereocomplex, and PHB have quite different C–H···O=C interaction.

We recently studied the structure, dispersibility, and crystallinity of PHB/PLLA blends by IR microspectroscopy, DSC, and polarized light microscopy [43]. That study was mainly concerned with bands in the C=O stretching vibration and COC stretching vibration regions of IR spectra, because the C=O and COC stretching bands are intense in IR spectra and their frequencies significantly change between the crystalline and amorphous phases. In contrast, the present Raman study discuss bands not only in these regions but also in the CH stretching band region to explore a CH band due to the C–H···O=C hydrogen bond of PHB component in PHB/PLLA blends. Moreover, Raman bands in the low frequency region (800–200 $cm^{-1}$) are analyzed partly because some low frequency Raman bands are sensitive to polymer backbone and partly because PLLA shows a clear crystalline band near 400 $cm^{-1}$.

## 2. Experiment

### 2.1. Preparation of blends

PHB and PLLA were obtained, respectively, from the Procter & Gamble Company and Shimadzu Corporation (LACTY5000). Atactic PHB was obtained from the same company to investigate the spectra of amorphous PHB. To prepare the blends of PHB and PLLA, PHB and PLLA were dissolved together in hot chloroform, and then the solution was cast on an aluminum dish as a film. To evaporate the solvent completely, the films were kept at 60 °C in a vacuum-dried oven for 12 h and cooled down to room temperature. For the measurements of polarized light microscopy and Raman microspectroscopy, the blend films were pre-treated by using a Mettler-Toledo FP82HT hot stage. The films were heated to 190 °C, kept at this temperature for 3 min, cooled to 60 °C, and then kept at this temperature for 12 h. This process was performed for the growth of spherulites. Finally, they were cooled down to room temperature. Blending ratios of PHB/PLLA were 20/80, 40/60, 60/40, and 80/20 by weight.

### 2.2. Polarized light microscopy

Polarized light microscopy images were observed with an Olympus BX51 polarizing optical microscope that was attached with a Raman microspectrometer (Kaiser Optical Systems Inc. HoloSpec f/1.8i). The sample images were observed under cross-polars with a sensitive color plate (530 nm).

### 2.3. Raman microspectroscopy

Raman spectra were measured with a HoloSpec f/1.8i spectrograph (Kaiser Optical Systems Inc.) equipped with a liquid nitrogen-cooled CCD detector (Princeton Instruments). The 785 nm line of a NIR diode laser (Invictus) was used as an excitation source for the Raman spectra. The exposure time and accumulation for measuring the Raman spectra were 10 s and 4 times, respectively. Laser power at a sample surface was ca. 15 mW.

## 3. Results and discussion

### 3.1. Polarized light microscopy

Polarized light microscopy images of PHB, PLLA, and their blends were already shown in our previous paper [43]. The polarized light microscopy image of pure PHB shows a large spherulite structure. However, due to its high crystallinity, it is difficult to observe nonspherulitic parts of PHB. On the other hand, the polarized light microscopy image of pure PLLA shows a number of small spherulitic structures along with nonspherulitic parts. Therefore, one can easily distinguish each structure in PLLA.

In the case of PHB/PLLA blends, spherulitic structures can be observed clearly in blends except for the 20/80 blend. Judging from the sizes of spherulite structures, they are ascribed to the PHB component. In contrast, the spherulite of PLLA is not observed clearly, so that the PLLA component exists in nonspherulitic parts or in very small crystalline parts that cannot be observed by polarized light microscopy.

### 3.2. Micro-Raman spectra of PHB

Fig. 2a–d shows micro-Raman spectra in the 3100–2800, 1800–1600, 1500–800, and 800–200 $\mathrm{cm}^{-1}$ regions of crystalline and amorphous PHB. The band assignments for Raman spectra of PHB are shown in Table 1. The band assignments of PHB were based partly on the assignment of PLLA, because the chemical structure of PHB is similar to that of PLLA. They were also based on a comparison of the Raman spectra of PHB with the corresponding IR spectra [34,37,43].

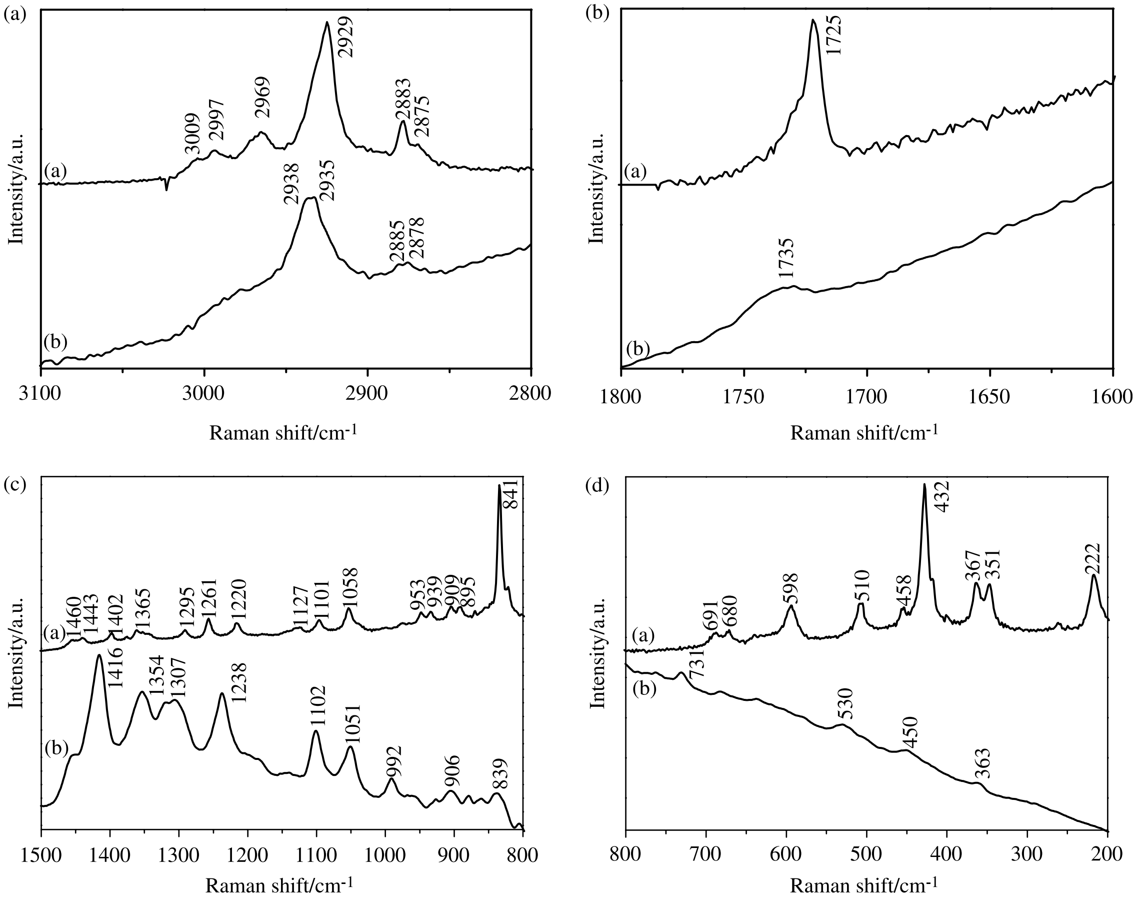

Fig. 2. Raman spectra of PHB, (a) crystalline PHB; (b) amorphous PHB.

Table 1
Raman shift (cm$^{-1}$) and assignments of the Raman bands of PHB and PLLA

| | PHB | PLLA |
|---|---|---|
| 3009 | CH$_3$ asymmetric stretching (CH···O=C) | |
| 2999 | CH$_3$ asymmetric stretching | CH$_3$ asymmetric stretching |
| 2970 | CH$_3$ asymmetric stretching | |
| 2945 | | CH$_3$ symmetric stretching |
| 2929 | CH$_3$ antisymmetric stretching | |
| 2883 | CH stretching | CH stretching |
| 1774 | | C=O stretching (C) |
| 1772 | | C=O stretching (A) |
| 1768 | | C=O stretching (C) |
| 1751 | | C=O stretching (C) |
| 1748 | | C=O stretching |
| 1732 | C=O stretching (A) | |
| 1725 | C=O stretching (C) | |
| 1460 | CH$_3$ asymmetric deformation | |
| 1454 | | CH$_3$ asymmetric deformation |
| 1443 | CH$_2$ deformation | |
| 1402 | CH$_3$ symmetric deformation | |
| 1388 | | CH$_3$ symmetric deformation |
| 1365 | CH deformation and CH$_3$ symmetric deformation | |
| 1300 | | CH deformation (A) |
| 1295 | CH deformation | CH deformation (C) |
| 1261 | C–O–C stretching and CH deformation | |
| 1220 | COC asymmetric stretching | |
| 1182 | | COC asymmetric stretching |
| 1127 | CH$_3$ asymmetric rocking | CH$_3$ asymmetric rocking |
| 1101 | COC symmetric stretching | |
| 1095 | | COC symmetric stretching |
| 1058 | C–O stretching | |
| 1044 | | C–CH$_3$ stretching |
| 953 | CC stretching and CH$_3$ rocking | |
| 939 | ? | |
| 923 | | CC stretching and CH$_3$ rocking |
| 909 | ? | |
| 895 | ? | |
| 873 | | C–COO stretching |
| 841 | C–COO stretching | |
| 737 | | C=O deformation (in-plane) |
| 708 | | C=O deformation (out-of-plane) |
| 691 | C=O deformation (in-plane) | |
| 680 | C=O deformation (out-of-plane) | |
| 598 | C–CH$_3$ deformation and CCO deformation | |
| 510 | C–CH$_3$ deformation and CCO deformation | |
| 458 | ? | |
| 432 | CCO deformation | |
| 410 | | CCO deformation (C) |
| 396 | | CCO deformation (C) |
| 367 | COC deformation +C–CH$_3$ deformation | |
| 351 | COC deformation +C–CH$_3$ deformation | |
| 301 | | COC deformation and C–CH$_3$ deformation |
| 222 | CH$_3$ torsion | |

A, amorphous; C, crystalline.

Bands at 2997, 2969, 2929, and 2883 cm$^{-1}$ in the micro-Raman spectra of the crystalline PHB may be assigned to CH$_3$ asymmetric stretching mode, CH$_3$ asymmetric stretching mode, CH$_2$ antisymmetric stretching mode, and CH stretching mode (Fig. 2a, curve (a)). Of note is the appearance of a band at 3009 cm$^{-1}$, also assignable to the CH$_3$ asymmetric stretching mode. Although it is unusual for a band due to a CH$_3$ asymmetric stretching mode to appear above at 3000 cm$^{-1}$, several research groups have reported that the appearance of the CH$_3$ band at an exceptionally high wavenumber indicates the existence of a C–H···O=C hydrogen bond [37–41]. We proposed the existence of a C–H···O=C hydrogen bond in PHB based on the observation of an infrared band at 3009 cm$^{-1}$ due to the CH$_3$ asymmetric stretching mode together with quantum chemical calculation and the measurements of wide-angle X-ray diffraction [37]. The crystal structure of PHB consists of left-handed helical molecules in the crystal lattice with antiparallel orientation [44]. This hydrogen bond is caused by the proximity of the distance between the H atom of the CH$_3$ group in one helical structure and the O atom of the C=O group in another helical structure. On the other hand, bands of amorphous PHB are observed at 2938 and 2885 cm$^{-1}$ assigned to CH$_2$ antisymmetric stretching mode, and CH stretching mode, respectively (Fig. 2a, curve (b)).

There is a clear difference in the C=O stretching band region between spectra of the crystalline PHB and amorphous PHB (Fig. 2b). The crystalline PHB spectra show a band at 1725 cm$^{-1}$ with a shoulder near 1735 cm$^{-1}$. However, the intensity of the shoulder is significantly stronger in the spectrum of the amorphous PHB. The bands at 1725 cm$^{-1}$ and the shoulder were assigned to the C=O stretching bands of the crystalline and amorphous state of PHB. Assignment are based on the temperature-dependent variation in the Raman spectra of PHB, showing that only the band at 1735 cm$^{-1}$ remains in the melt state [34,37,42,43]. Furthermore, the intensity of the band at 1735 cm$^{-1}$ of PHB copolymers with lower crystallinity is significantly stronger than that of PHB [34]. The bands at 1735 and 1725 cm$^{-1}$ have the corresponding IR bands.

Bands at 1295, 1261, and 1220 cm$^{-1}$ of PHB are assigned, respectively, to CH deformation mode, C–O–C stretching and CH deformation coupling mode, and COC asymmetric stretching mode (Fig. 2c) [29,30,34,37,43]. The peak intensities of these bands of the crystalline PHB are stronger than those of the amorphous PHB. Of note is that the relative intensity of the 1261 cm$^{-1}$ band is stronger in the spectrum of the crystalline PHB. A band at 953 cm$^{-1}$ in the PHB spectra is due to a coupling mode of C–C stretching and CH$_3$ rocking [29,30,34,37,43]. Its relative intensity is stronger in the spectrum of the crystalline PHB than in that of the amorphous PHB. We recently found from the temperature dependent variations of a Raman spectrum of PHB that the bands at 1261, 1058, and 841 cm$^{-1}$ are sensitive to the crystallinity. Therefore, the appearance of bands at 1416, 1306, and 1238 cm$^{-1}$ suggests the containment of the amorphous PHB.

Bands at 510 and 351 cm$^{-1}$ in the PHB spectra are assigned to a coupling mode of C–CH$_3$ deformation and C–C–O deformation and that of C–$CH_3$ deformation and C–O–C deformation [29,30,34,37,43]. In the low frequency region, it was found from the temperature-dependent study that the bands at 598 and 432 $cm^{-1}$ are crystalline bands.

### 3.3. Micro-Raman spectra of PLLA

Kister et al. investigated the Raman spectra of PLLA in some detail [29,30]. Their studies revealed the differences between the crystalline and amorphous spectra of PLLA. The band assignments of the Raman spectra of PLLA were made referring to Refs. [29,30], and they are summarized in Table 1.

In the case of PLLA, the evidence for a C–H⋯O=C hydrogen bond is not observed above 3000 $cm^{-1}$. PLLA have three kinds of crystal forms (i.e. $\alpha$, $\beta$, and $\gamma$ forms). The most common polymorph, $\alpha$ form, has a $10_3$ helical chain conformation and is obtained by the crystallization from the melt state, the same as this experimental condition. The distance of the H atom and the O atom would be not so short in the $10_3$ helical structure of $\alpha$ crystal of PLLA, that the C–H⋯O=C hydrogen bond is not generated.

It is well known that the crystalline PLLA yields split C=O stretching band near 1774, 1768, and 1751 $cm^{-1}$ and that the amorphous PLLA yields bands at 1772 $cm^{-1}$ [29,30]. It is noted in that the 1772 $cm^{-1}$ band is significantly stronger in the spectrum of the nonspherulitic part. Thus, it is very likely that the nonspherulitic part in the polarized light microscopy contains more amorphous states.

The Raman spectra in the 1500–800 $cm^{-1}$ region of the spherulitic and nonspherulitic part of PLLA show a few clear spectral differences. The spectrum of spherulitic structure in PLLA has a peak at 923 $cm^{-1}$. However, the corresponding peak of nonspherulitic part is missing or very weak. This peak is assigned to the C–C stretching and $CH_3$ rocking coupling mode, and the existence of this peak means that the spherulite of PLLA assumes the $10_3$ helix conformation [29].

The intensity of the 708 $cm^{-1}$ band assigned to the C=O out-of-plane deformation is stronger in the spectrum of spherulites than in that of the nonspherulitic part. Bands in the 410–395 $cm^{-1}$ region are attributed to the C–C–O deformation modes. These bands of spherulitic structure are observed as a doublet peak. However, the corresponding bands of the nonspherulitic part are observed as a broad band. Bands in the 320–290 $cm^{-1}$ region are due to COC deformation and C–$CH_3$ deformation coupling mode. The bands in this region are stronger and sharp in the spherulite spectrum than in the nonspherulite spectrum.

For both pure PHB and PLLA, the band intensities of spherulitic structure are, in general, stronger than those of the nonspherulitic part. The spherulite has high regularity of main chain conformation, so that such tendencies in the intensities are reasonable. The Raman spectra of PHB and PLLA have the strongest band due to the C–COO stretching mode at 840 and 873 $cm^{-1}$. These are very good marker bands to distinguish each component in the blends.

### 3.4. Micro-Raman spectra of PHB/PLLA blends

Fig. 3 shows ordinary or (macro-) Raman spectra of 80/20, 60/40, 40/60, and 20/80 blends. No significant shifts of bands assigned to each PHB or PLLA component are observed in the Raman spectra of the four kinds of blends and no new band emerges upon the blend formation.

### 3.5. CH stretching band region of Raman spectra

The CH stretching band region of the Raman spectrum of the 80/20 blend (Fig. 3a, curve (a)) shows bands at 3010, 2999, 2970, 2936, and 2930, 2878, and 2874 $cm^{-1}$. The spectrum of the 80/20 blend is very similar to the corresponding spectrum of pure PHB, and the $CH_3$ asymmetric stretching band of PHB due to the CH⋯O=C hydrogen bond is also observed at 3010 $cm^{-1}$. The existence of this band indicates that PHB is crystallized in the 80/20 blend. Bands assigned to PLLA are not observed clearly in this region for the 80/20 blend.

In the spectrum of the 60/40 blend (Fig. 3a, curve (b)), bands are observed at 3011, 2999, 2970, 2948, 2930, 2884, and 2876 $cm^{-1}$. Again the existence of the 3011 $cm^{-1}$ band indicates that the PHB component of the 60/40 blend is crystallized. The Raman spectrum of 60/40 blend yields bands at 2948 and 2884 $cm^{-1}$ assignable to PLLA.

The 40/60 blend (Fig. 3a, curve (c)) also has a band at 3011 $cm^{-1}$, so that the crystallization of PHB component is indicated. The intensities of bands at 2943 and 2882 $cm^{-1}$ assigned to PLLA are stronger for the 40/60 blend than for the 60/40 blend. The spectrum of the 20/80 blend (Fig. 3a, curve (d)) is very similar to that of pure PLLA. However, a band assigned to the $CH_2$ symmetric stretching of PHB is observed clearly at 2931 $cm^{-1}$.

### 3.6. C=O stretching band region of Raman spectra

The spectrum of the 80/20 blend in the C=O stretching region (Fig. 3b, curve (a)) is close to that of pure PHB. A band at 1725 $cm^{-1}$ is assigned to the C=O stretching mode of PHB. This band is characteristic of the crystalline structure [34]. Bands of PLLA are not observed clearly in this region. The spectrum of the 60/40 blend (Fig. 3b, curve (b)) yields one intense band at 1725 $cm^{-1}$ and two weak features at 1768 and 1752 $cm^{-1}$. The former is assigned to the C=O stretching mode of PHB, and the latter two band are due to those of PLLA. In the Raman spectrum of the 40/60 blend (Fig. 3b, curve (c)), bands are observed at 1774, 1769, 1762, 1752, 1745, and 1725 $cm^{-1}$. The first five bands are assigned to C=O stretching mode of PLLA and the last one is assigned to that of PHB [27,29,30]. The bands at 1774, 1762, and 1752 $cm^{-1}$ are assigned to crystalline bands, the bands at 1769 and 1745 $cm^{-1}$ are assigned to amorphous bands. The 20/80 blend (Fig. 3b, curve (d)) gives rise to bands at 1775, 1768, and 1752 $cm^{-1}$. Since these bands are assigned to crystalline PLLA bands, the observation of these bands indicates that PLLA is crystallized in the 20/80 blends.

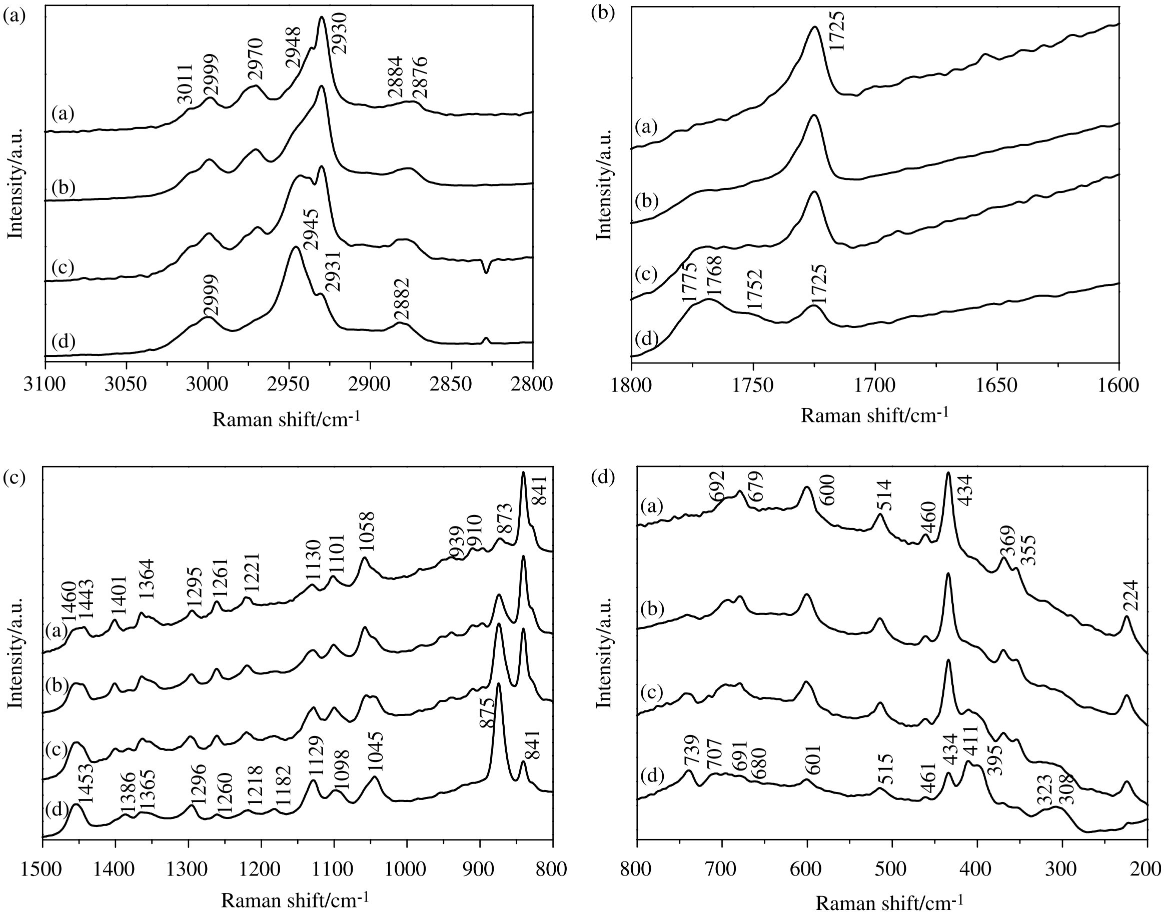

Fig. 3. Raman spectra of PHB/PLLA blends, (a) PHB/PLLA blend (80/20); (b) PHB/PLLA blend (60/40); (c) PHB/PLLA blend (40/60); (d) PHB/PLLA blend (20/80).

### 3.7. COC and CC stretching band region of Raman spectra

In the 1500–800 $\text{cm}^{-1}$ region, PHB and PLLA have a strong band at 841 and 873 $\text{cm}^{-1}$, respectively. These bands are assigned to the C–COO stretching mode. These two intense bands allow one to judge the existence of each component in the blends. The spectrum of the 80/20 blend (Fig. 3c, curve (a)) is very similar to that of pure PHB (Fig. 2c) in this region. However, a band assigned to the C–COO stretching of PLLA is observed at 873 $\text{cm}^{-1}$. The existence of this band indicates that 80/20 blend includes PLLA component. The spectra of 60/40 and 40/60 blends (Fig. 3c, curves (b) and (c)) show both PHB and PLLA bands clearly. Bands ascribed to PHB are identified at 1261, 1058, and 841 $\text{cm}^{-1}$ while those due to PLLA are observed at 1182, 1044, and 874 $\text{cm}^{-1}$. In the spectrum of the 20/80 blend (Fig. 3c, curve (d)), bands of PLLA are mainly observed. The intense band of PHB still remains at 841 $\text{cm}^{-1}$. A band at 918 $\text{cm}^{-1}$, assigned to the CC stretching and $CH_3$ rocking coupling mode of PLLA, is not observed clearly in the nonspherulite spectrum of the 20/80 blend. Since another marker band for crystalline structure of PLLA at 1295 $\text{cm}^{-1}$ is overlapped with PHB bands, it is difficult to discuss the crystallinity of PLLA in this region.

### 3.8. Low frequency region of Raman spectra

In this region, the Raman spectra of PHB and PLLA are very different. The spectrum of the 80/20 blend is so close to that of pure PHB that it is difficult to confirm PLLA bands in the spectrum of the 80/20 blend. Not only PHB bands but also PLLA bands are observed clearly in the spectra of the 60/40 and 40/60 blends. In the spectrum of the 20/80 blend, many bands assigned from PLLA are observed. Some bands due to PHB are also observed at 696, 680, 601, and 434 $\text{cm}^{-1}$. The bands of PLLA at 411–395 $\text{cm}^{-1}$ are observed as a split peak that is crystalline peak. This means that PLLA exists in the crystalline state in this blend.

The spectra of spherulite and nonspherulite parts of PLLA are obviously different from each other. However, those of PHB are not very much different. The reason why the difference of spectra of spherulite and nonspherulite parts of PHB is small may be the high crystallinity of PHB. Due to the high crystallinity of PHB, it is difficult to measure a Raman spectrum only from complete amorphous parts.

Figs. 4 and 5 show micro-Raman spectra of the four kinds of blends. The macro-Raman spectra enable one to confirm the existence of each component in the blends. On the other hand, micro-spectra allow one to investigate the structure of small

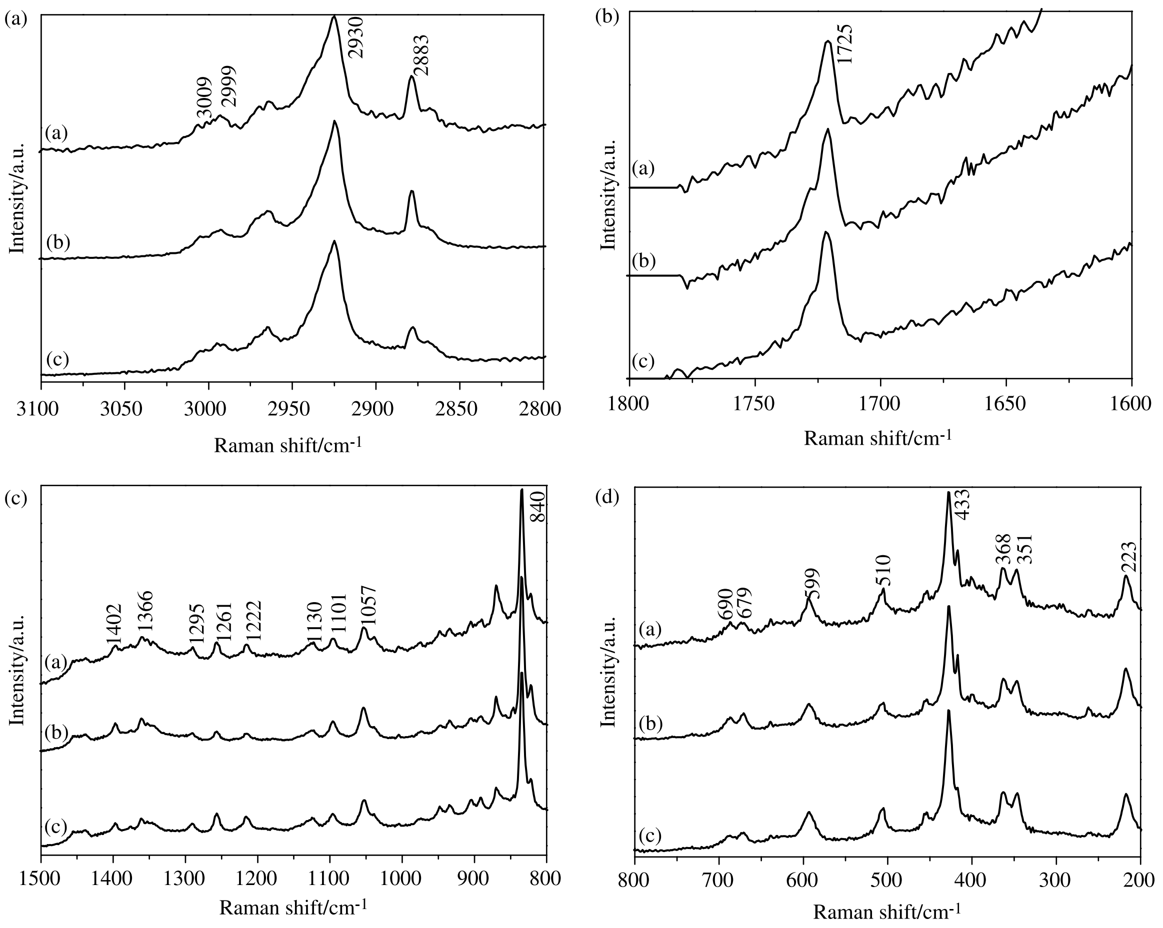

Fig. 4. Microscopic Raman spectra of PHB/PLLA blends in spherulitic parts, (a) PHB/PLLA blend (80/20); (b) PHB/PLLA blend (60/40); (c) PHB/PLLA blend (40/60); (d) PHB/PLLA blend (20/80).

spots in them. Therefore, to confirm the crystallinities of each component separately in the blends, micro-Raman spectra were measured. The Raman spectra of spherulite structures in the 80/20, 60/40, and 40/60 blends are very similar to that of spherulite structure of pure PHB. The $CH_2$ symmetric stretching band, C=O stretching band, C–O–C stretching band, C–COO stretching band, and CCO deformation band are observed as characteristic bands of PHB at 2929, 1725, 1101, 841, and 432 $cm^{-1}$. The CH stretching band due to C–H···O=C hydrogen bond is also observed at 3009 $cm^{-1}$ in these spectra. These are good evidences that the spherulite structures observed in the polarized light microscopy images of the blends are assigned to the spherulite in pure PHB.

On the other hand, in the Raman spectra of nonspherulitic parts in the four kinds of blends peaks assigned to PHB and PLLA are observed with different band intensities depending on the blending ratio. The Raman spectra of nonspherulitic parts of the four kinds of blends in the CH stretching band region show the $CH_3$ symmetric stretching band of PLLA at 2945 $cm^{-1}$ and the $CH_2$ symmetric stretching band of PHB at 2930 $cm^{-1}$. Furthermore, one can see the $CH_3$ asymmetric stretching band of C–H···O=C hydrogen bond at 3009 $cm^{-1}$ in the blends. This indicates that PHB with high crystallinity is crystallized as the very small spherulite form, or another crystalline form that is lower degree of crystalline form than spherulite structure. In the C=O stretching band region of the Raman spectra of the 80/20 and 60/40 blends, it is difficult to observe the C=O stretching band assigned to PLLA. For the 40/60 and 20/80 blends, the C=O stretching bands assigned to the crystalline and amorphous parts of PLLA are observed clearly. The C=O stretching band at 1725 $cm^{-1}$ assigned to PHB is also observed for the four kinds of blends. This band is assigned to the crystalline band of PHB. PHB may be crystallized as the very small spherulite structure or another crystalline form that is lower degree crystalline form. From the COC stretching and CC stretching band region, it is difficult to confirm the state of PLLA, because the marker bands for the spherulite structure of PLLA at 1296 and 923 $cm^{-1}$ are overlapped with PHB bands. The confirmation of the PHB state is also difficult, because the differences between bands of crystalline and amorphous state are mainly intensities of marker bands for the crystalline state at 1295, 1261, 1220, and 953 $cm^{-1}$. However, it is possible to identify PHB and PLLA bands in the Raman spectra in the C–COO stretching bands for all blends. The intensity ratios of PHB and PLLA bands depend on the blending ratio. The intensity of a band at 708 $cm^{-1}$,

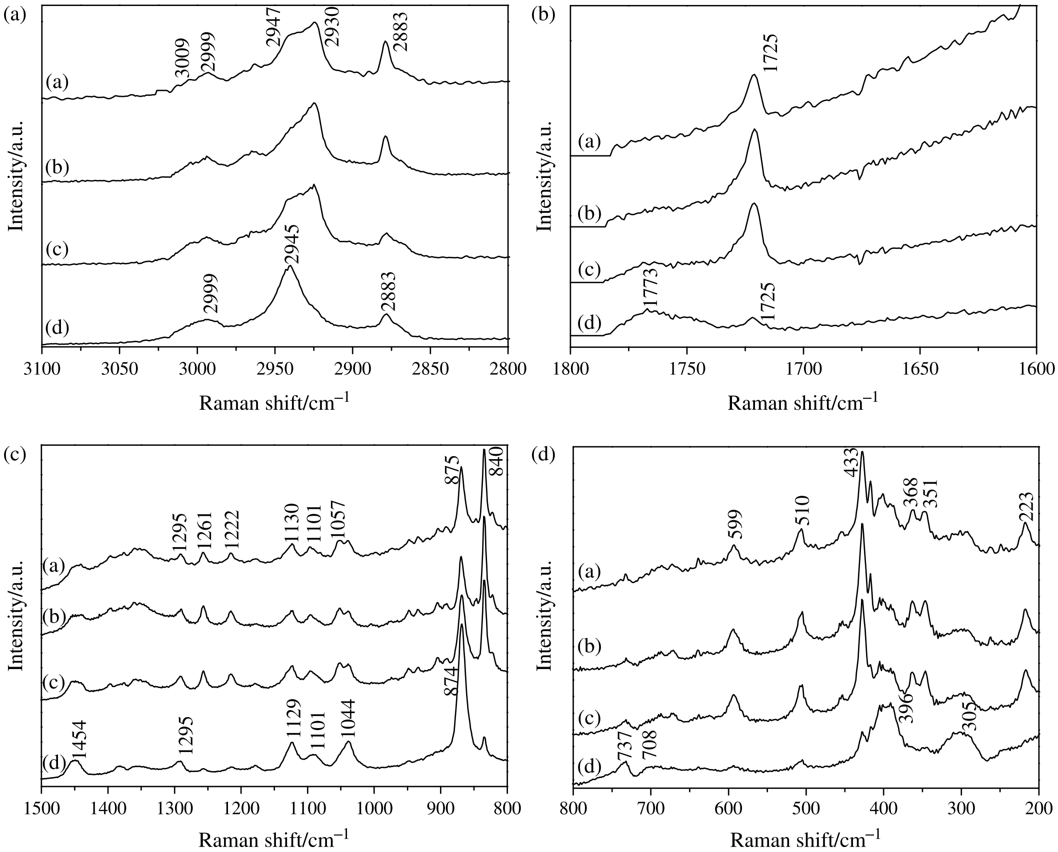

Fig. 5. Microscopic Raman spectra of PHB/PLLA blends in nonspherulitic parts, (a) PHB/PLLA blend (80/20); (b) PHB/PLLA blend (60/40); (c) PHB/PLLA blend (40/60); (d) PHB/PLLA blend (20/80).

assigned to the C=O deformation, is not so strong for the four kinds of blends. A broad band at 400 cm$^{-1}$ assigned to the CCO stretching mode is observed for the four kinds of blends. Since, these are marker bands for the crystalline state of PLLA, these bands are not observed in any blends. Therefore, it may be concluded that PLLA is in the amorphous state in these blends.

The intensities of peaks assigned to PHB and PLLA are different for each measurement spot and each blend. The intensities of PLLA bands in an edge of a cast film sample are stronger than those in a center of the sample. Therefore, PHB with high crystallinity is very likely to be crystallized dominantly in the center of sample. However, the Raman spectra of nonspherulite parts of all blends also show the band arising from the C–H···O=C hydrogen bond and C=O stretching band of crystalline PHB. Thus, PHB may be crystallized in a crystalline structure different from the spherulite structure. For the 20/80 blend, it was difficult to collect the Raman spectrum of PHB component only. The Raman spectra of the 20/80 blend yield both PHB and PLLA peaks for any measurement spots. Depending on a measurement spot, the spectrum that is similar to the pure PLLA spectrum is obtained. As can be seen in Figs. 4 and 5, all the blends have an inhomogeneous structure.

## 4. Conclusion

This study has investigated the structure, dispersibility, and crystallinity of PHB/PLLA blends by using Raman microspectroscopy. The following conclusions can be reached from the present study.

1. Raman spectra of spherulites in pure PHB show a weak band at 3009 cm$^{-1}$ due to a C–H···O=C hydrogen bond. Those of PHB/PLLA bends, except for the 20/80 blend, also demonstrate the corresponding band. This band is observed even in the spectra of nonspherulitic parts of 80/20, 60/40, and 40/60 blends, indicating that small amount of crystalline PHB may be presented in the nonspherulite parts. These results suggest that PHB with high crystallinity is crystallized as the spherulite form in the crystalline state. Meanwhile, the lower crystalline form or small structure not readily observable by polarized light microscopy is found in the nonspherulitic state in the blends.
2. The nonspherulitic parts of PLLA show a single broad band at 400 cm$^{-1}$ due to the CCO deformation mode and its spherulite structure yields split bands at 410 and 395 cm$^{-1}$. All four bands show a single broad band, suggesting that PLLA component exists in the amorphous state in the blends.

3. Raman bands at 841 and 873 $\mathrm{cm}^{-1}$ due to the C–COO stretching modes of PHB and PLLA in spherulites and nonspherulitic parts of the blends show different peak intensities. Raman spectra of the spherulitic part of blends, except for that of the 20/80 blend, are similar to the corresponding spectrum of pure PHB. Raman spectra of the nonspherulitic part of blends have both PHB and PLLA peaks. Their intensities are different from each other depending on measurement spots and blending ratios.

It has been confirmed by polarized light microscopy and Raman microspectroscopy that blends of PHB/PLLA are immiscible. The spherulite structure of PHB was observed by both polarized light microscopy and Raman microspectroscopy. However, the spherulite structure of PLLA was not observed in any blends. Raman microscopic spectra reveal that PLLA is in the amorphous state in the blends. These results have indicated that PHB with high crystallinity, and the rapid crystalline growth rate dominates in the center parts of the PHB/PLLA blends.

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