Original Communication

Molecular morphology formed by a phase separation process for poly(3-hydroxybutyrate-co-3-hydroxyvalerate)/ o-dichlorobenzene with different valerate contents

Yuto Hoki and Takahiko Nakaoki*

Department of Materials Chemistry, Ryukoku University, Seta, Otsu 520-2194, Japan

ABSTRACT

The molecular morphology and the nature of the bound solvent in poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) with 5 and 12% valerate content in o-dichlorobenzene were investigated by thermal analysis and polarized optical microscopy. In addition, porous particles were prepared through a phase separation process. When the PHBV/o-dichlorobenzene solution was quenched in ice-water, a white turbidity was obtained, suggesting the occurrence of a phase separation due to density fluctuations due to the formation of spherulites. The average spherulite diameter for the PHBV5 system was larger than that for the PHBV12 system because of the smaller amount of structural defects present in PHBV5. The melting behavior of o-dichlorobenzene trapped in PHBV/o-dichlorobenzene resulted in two endothermic DSC peaks. One has almost the same temperature as the melting temperature of pure o-dichlorobenzene, corresponding to solvent in a free state. The other peak was observed at a lower temperature than the melting temperature of pure solvent, corresponding to bound solvent. These two types of solvent are assumed to correspond to that in inter- and intra-spherulite regions, respectively. The solvent in the intra-spherulite regions for PHBV12/o-dichlorobenzene was larger than that for the PHBV5 system. This is due to the higher HV content which makes the

noncrystalline region larger. The melting temperature of the lamellar crystals was almost the same for the PHBV5 and PHBV12 systems, but increased with concentration. The lamellar thickening at a high concentration makes the noncrystalline region smaller, resulting in a decreased coagulation size of the solvent. After excluding the solvent, porous particles were obtained. The pore size as well as the particle size was governed by the concentration of PHBV and the HV content.

INTRODUCTION

Poly (hydroxyalkanoates) (PHAs) have received much attention because they are biodegradable renewable materials. Among them, poly (3-hydroxybutyrate) (PHB), having four carbons in the repeating unit is the most common [1-8]. However PHB is hard and fragile because of its high degree of crystallinity. Copolymerization with a material such as 3-hydroxyvalerate (HV) lowers the crystallinity. Bloembergen *et al.* reported the relationship between the crystallinity and the HV content of poly (3-hydroxybutyrate-co-3-hydroxyvarelate) (PHBV) [9-16]. For a copolymer with low HV content, the crystals are constructed from 3-hydroxybutyrate (HB)–sequence, while HV sequence only exists in the noncrystalline regions. It is worth noting that copolymers with a medium content of 3HV are hard to crystallize and provides the lowest crystallinity.

*Corresponding author
nakaoki@rins.ryukoku.ac.jp

The gel state is one of the most important molecular morphologies for a polymer chain. It has been established that gels arise from a cross-linked three dimensional network. For a crystalline polymer, a crystallite is formed at a cross-linking point. Many researchers, including our research group, have reported the molecular structure at a cross-linking point. However, as there are few reports concerning on bound solvent in a gel, we have recently focused on this issue for an isotactic polypropylene (iPP) /o-dichlorobenzene gel and then we reported that there were two melting peaks for the solvent o-dichlorobenzene [17, 18]. One is a sharp peak indicating melting at almost the same temperature as pure o-dichlorobenzene, and the other is a very broad peak observed at lower temperature than the melting point of the pure solvent. It was suggested that these different types of solvent molecules were located in inter- and intra-spherulite regions of iPP, respectively.

In the case of a poly (L-lactide) (PLLA) dissolved in o-dichlorobenzene, a turbid solution was obtained after quenching in ice-water [19]. Here, the system did not solidify like a gel, but contained white precipitation consisting of spherulites of the PLLA. For a crystalline polymer, gelation can be regarded as a kind of crystallization. The difference between gelation and crystallization from solution is whether the crystallites are interconnected or not. Therefore, the melting behavior of solvent trapped in the PLLA/o-dichlorobenzene system was similar to the case of iPP gel.

In this investigation, we will report the coagulation of solvent and the molecular morphology of PHBV chains formed from a PHBV/o-dichlorobenzene solution. Then we attempted to make porous PHBV particles from the PHBV/o-dichlorobenzene solution through a phase separation process.

EXPERIMENTAL

Samples

PHBV with HV contents of 5% and 12% were purchased from Sigma Aldrich Chemicals. These samples are abbreviated here as PHBV5 and PHBV12, respectively. PHBV particles were prepared as follows. PHBV and *o*-dichlorobenzene were placed in an ampoule, degassed in vacuo and

sealed. The solution was dissolved at 160°C, and then quenched at 0°C. The solution was then kept at 0°C for 24 h. The sample was then immersed in methanol, and dried in vacuo for several days in order to extract the solvent.

Measurements

Thermal analysis was performed using a Rigaku 8230D differential scanning calorimeter (DSC). A sealed aluminum pan was used and the sample was scanned at a heating rate of 5°C/min under a nitrogen flow. Measurements were carried out between -100°C and 150°C. Polarized microscopic measurements were carried out at room temperature using an Olympus BX51TF. Scanning electron microscopy (SEM) was performed using a JSM-5200 (JEOL) operating at an acceleration voltage of 20 kV. Prior to observation, the samples were coated with a thin layer of gold by vacuum deposition.

RESULTS AND DISCUSSION

### Molecular morphology of the PHBV/o-dichlorobenzene system quenched at 0°C

When a PHBV/o-dichlorobenzene solution was quenched in ice-water, the system became turbid. This is the same result as seen in the PLLA/o-dichlorobenzene system [19], in contrast to the translucent gel formed by iPP/o-dichlorobenzene [17, 18]. This shows that a phase separation is induced by density fluctuations in the PHBV system, and the crystallization would be promoted in the concentrated regions. Figure 1 shows polarized optical microscopic photographs of PHBV5 and PHBV12 / o-dichlorobenzene systems. Maltese cross shapes were clearly observed for both systems, indicating the formation of spherulites. We have reported that the iPP/o-dichlorobenzene gel was translucent, but consisted of spherulites. Therefore, in both cases of PHBV and iPP dissolved in o-dichlorobenzene spherulites formed, but the crystal-forming process between gelation and precipitation in solution is completely different. In general, a gel is characterized by a three dimensional network structure connected by crystals at cross-linking points. In the case of iPP gel, the formation of a spherulite would act as a cross-linking point. As a result, the whole system is solidified by noncrystalline chains connecting spherulites. In contrast, when there is no connection between
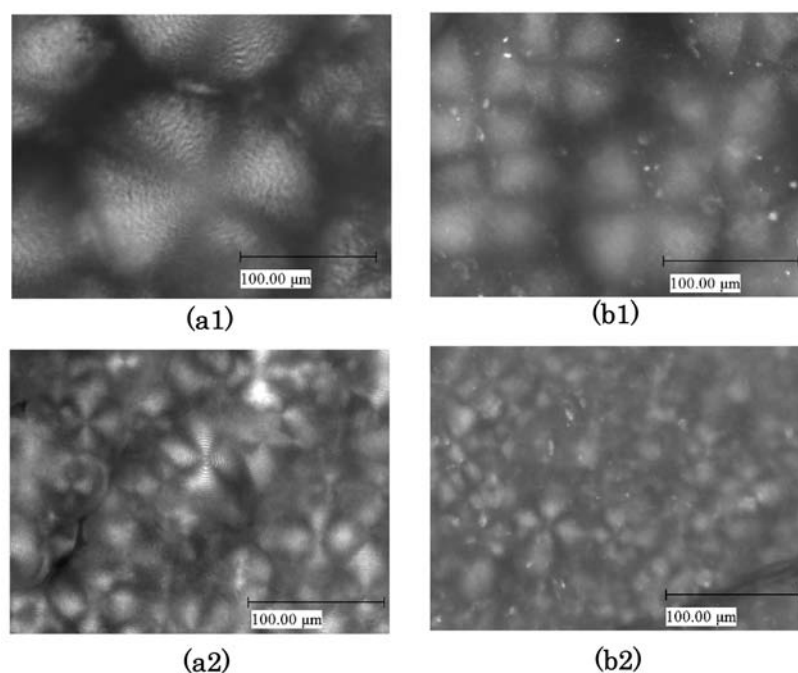
Figure 1. Polarized optical microscopic photographs of PHBV5 and PHBV12/o-dicholorobenzene systems quenched at 0°C. (a1): 10 wt% and (a2): 20 wt% for PHBV5 and (b1): 10 wt% and (b2): 20 wt% for PHBV12.

crystals, such as in the PHBV system, the crystal is precipitated.

As seen in Figure 1, the size of the spherulites for PHBV5/o-dichlorobenzene was larger than that for the PHBV12 system. For low HV content system like PHBV5 and PHBV12, the crystals consist of HB sequences. Therefore crystallization would be more efficient for PHBV5 because of the existence of long HB sequences. The small spherulite found for PHBV5 system can be explained by the number of nucleation sites. The lower number of defects due to HV units in PHBV5 promotes nucleation. Then, spherulites can only grow to a small size before they contact other spherulites. In Figure 2, the spherulite diameter is shown as a function of concentration. The spherulite size became smaller for high PHBV concentrations. The average diameters were 120 and 90 μm for PHBV5 and PHBV12 for a concentration of 10 wt%, respectively, and became less than half of these values for a 50 wt% solution. These values are comparable with that for PLLA/o-dichlorobenzene, which had spherulites quenched at 0°C with a diameter of 106 μm [19].
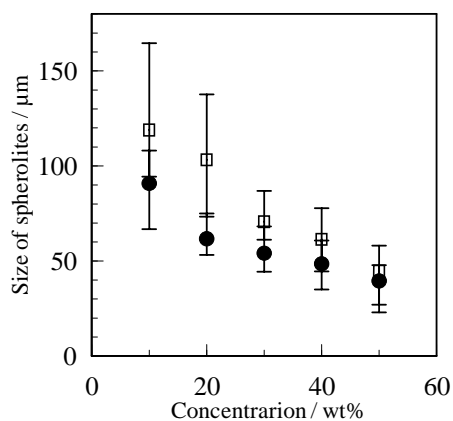
Figure 2. The diameter of spherulites for PHBV5 and PHBV12/o-dichlorobenzene systems. More than twenty spherulites were averaged to estimate the diameter. □: PHBV5 and ●: PHBV12.

Coagulation size of solvent in PHBV/o-dichlorobenzene

Figures 3(a) and (b) show the DSC plots of the melting of o-dichlorobenzene in PHBV5 and PHBV12/o-dichlorobenzene as a function of
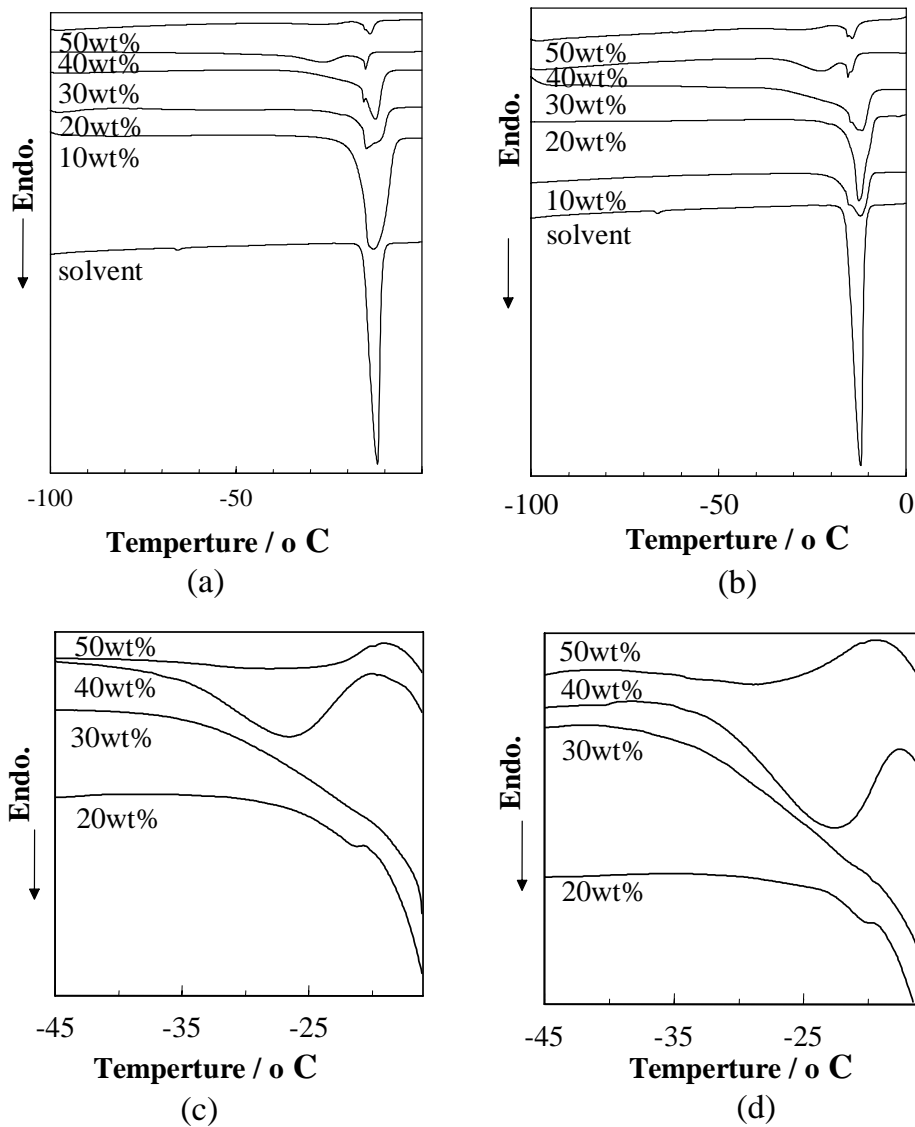
Figure 3. DSC charts of the melting behavior of o-dichlorobenzene in PHBV5 (a) and PHBV12 (b) /o-dichlorobenzene. (c) and (d) are expanded scale versions of (a) and (b).

concentration, respectively. A single melting peak was observed at -17°C for pure o-dichlorobenzene, whereas an additional endothermic peak was observed at lower temperatures, although the peak was small. An expanded view shown in Figures 3 (c) and (d) shows that the melting temperature of the low temperature peak shifted to lower temperatures with increasing concentration. The melting temperature is plotted as a function of concentration in Figure 4.

As shown in our previous report, this temperature shift is due to the coagulation size of the solvent [17, 18].

In the case of an iPP/o-dichlorobenzene gel, spherulites are constructed and there are two noncrystalline regions, that is, inter- and intra-spherulites, where the solvent exists. The inter-spherulite noncrystalline region would occupy a comparatively large volume. So there are sufficient solvent molecules involved in coagulation to produce the same melting temperature as the pure solvent. On the other hand, the solvent molecules in the intra-spherulite regions are located in the noncrystalline regions between lamellar crystals. This region is as small as a few tens of nm in size,
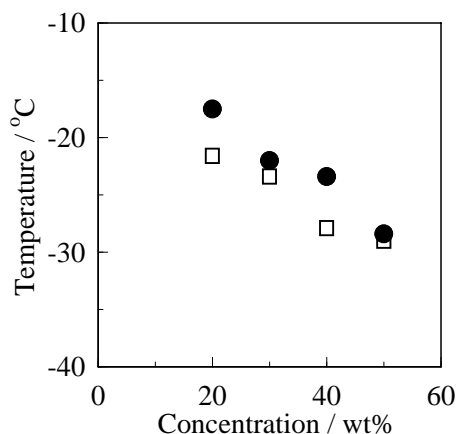
Figure 4. Melting temperature of the low temperature melting peak as a function of concentration. □: PHBV5 and ●: PHBV12.
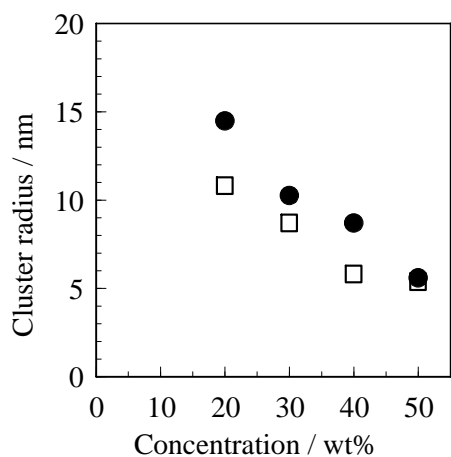
Figure 5. Dependence of coagulation radius of solvent with a low temperature melting peak on concentration. □: PHBV5 and ●: PHBV12.

so the melting temperature would shift to a low temperature. The relationship between the melting temperature depression of o-dichlorobenzene (ΔT) and the coagulation radius of o-dichlorobenzene (r) was reported in our previous paper as: [20]

$$r = \frac{0.356\Delta T + 82.9}{\Delta T} \qquad (1)$$

This equation was applied to estimate the coagulation radius of o-dichlorobenzene in the PHBV/o-dichlorobenzene systems. Figure 5 shows the concentration dependence of the coagulation

radius of solvent bound in the system. For example, the coagulation radius for PHBV12 decreased from 14 nm (20 wt%) to 5 nm (50 wt%). Since the solvent with a low melting temperature locates in the non-crystalline region between lamellae, the non-crystalline region would be smaller for high concentration system. Hence, we expect a close relation of the coagulation radius with the lamellar thickness. This relation is discussed in the next section. As for the dependence on HV content, the coagulation radius of the solvent was larger for the PHBV12/o-dichlorobenzene. The crystallization strongly depends on the HV content. The crystallization for PHBV5 is less promoted because the HV unit only locates in the non-crystalline region. Therefore the non-crystalline region for PHBV12 would be larger. This provides a larger space for solvent coagulated in the non-crystalline region between lamellae.

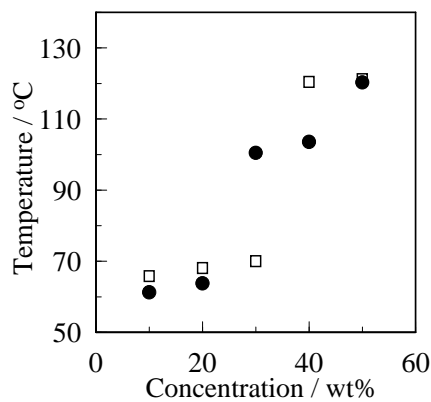**Melting behavior of lamellar crystals formed in PHBV/o-dichlorobenzene**

As shown in the previous section, spherulites consisting of lamellae are formed in the PHBV/o-dichlorobenzene system. The melting temperature of the system corresponds to the melting of lamellar crystals and has a close relationship to the lamellar thickness. Figure 6 shows the DSC chart for melting in PHBV5 and PHBV12/o-dichlorobenzene systems as a function of concentration. There are two endothermic peaks due to re-crystallization based on lamellar thickening. The low melting-point peak is regarded as due to the melting of lamellae originally formed in the system. This melting temperature is plotted as a function of concentration in Figure 7. The melting temperature increased with increasing concentration. It is worth noting that the melting temperature is almost the same for both PHBV5 and PHBV12. According to the Gibbs-Thomson equation [23], the melting temperature T_m is a function of lamellar thickness (l).

$$T_m = T_m{}^o (1 - \frac{2\sigma_s}{\Delta H_f \cdot l}) \qquad (2)$$
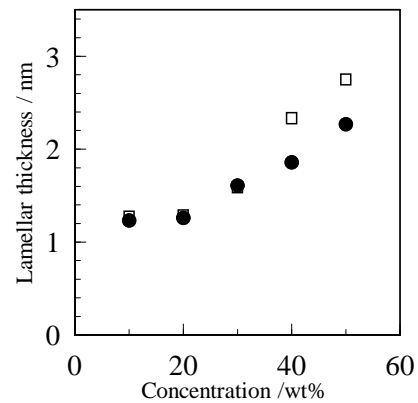
Here $T_m{}^o$, σ_s, and ΔH_f denote the equilibrium melting temperature, the surface energy, and the enthalpy of fusion. Values of these parameters
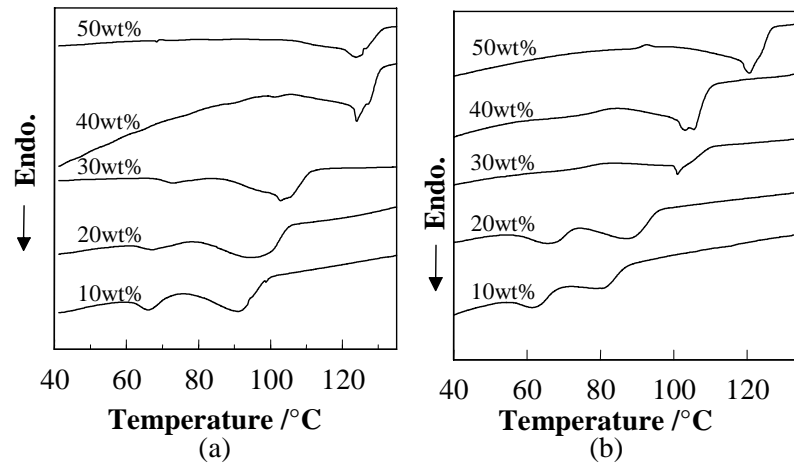
Figure 6. DSC charts of the melting behavior of PHBV/o-dichlorobenzene. (a): PHBV5 and (b): PHBV12.

Figure 7. Melting temperature of lamellar crystals formed in spherulites as a function of concentration. □: PHBV5 and ●: PHBV12.


Figure 8. Lamellar thickness formed in spherulites as a function of concentration. □: PHBV5 and ●: PHBV12.

were taken from [21, 22]. Figure 8 shows the lamellar thickness dependence on concentration. The lamellar thickness was as small as a few nm, and became larger with increasing concentration. The increase in lamellar thickness might reduce the volume of the non-crystalline region, which would make the coagulation size of the solvent smaller for a high concentration. Schematic models of bound solvent and lamellae are shown in Figure 9. The coagulation of solvent occurs in the noncrystalline region between lamellae. Since the lamellar thickness is smaller for a low concentration system, a large noncrystalline region provides a large space for the solvent.

Porous particles formed through a phase separation process

Similar to the PLLA/o-dichlorobenzene system, the spherulites in the PHBV/o-dichlorobenzene system are isolated from each other. So a particle consisting of spherulites can be obtained by dispersion in methanol. After drying in vacuo, SEM photographs were taken at room temparature. Figure 10 shows the SEM photographs of such particles derived from PHBV5 and PHBV12 depending on concentration. Spherical particles were obtained, but the particles obtained from a high concentration system were not completely spherical. This is due to the contactness with adjacent spherulites. Each particle has a porous
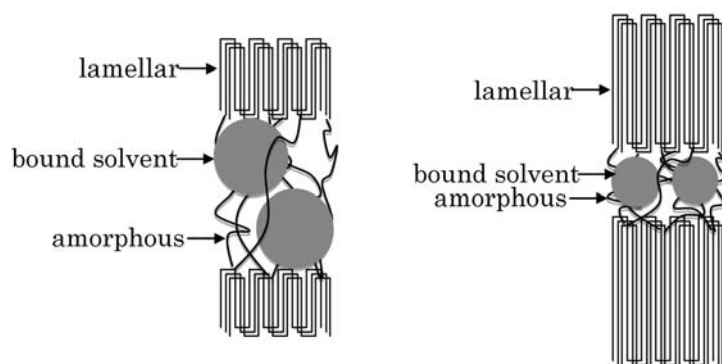
Figure 9. Schematic models of bound solvent and lamellae in the particles formed at low (a) and high (b) concentration. The lamellar thickness is smaller and the coagulation of solvent is larger for a high concentration system.
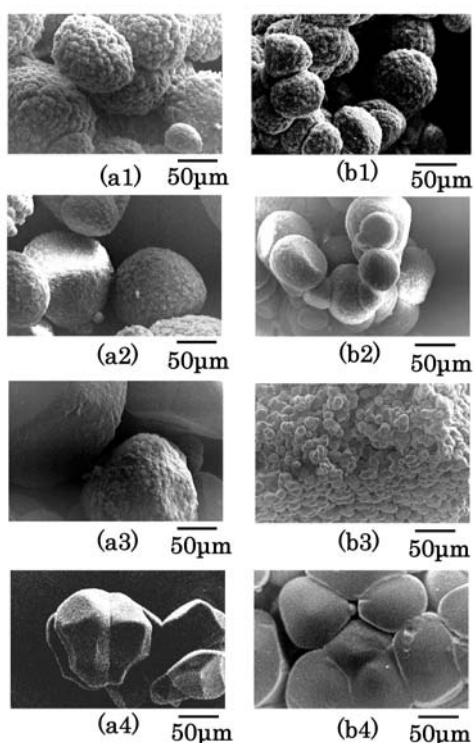
Figure 10. SEM photographs of porous particles formed after excluding o-dichlorobenzene from PHBV5 and PHBV12 / o-dichlorobenzene systems. (a1) 10wt%, (a2) 20wt%, (a3) 30 wt%, and (a4) 40 wt% for PHBV5 and (b1) 10wt%, (b2) 20wt%, (b3) 30 wt%, and (b4) 40 wt% for PHBV12.

structure, which is formed by exclusion of the solvent. Therefore, particles with a regular pore size were successfully prepared by a phase separation process.

CONCLUSIONS

The molecular structures formed from PHBV/o-dichlorobenzene was investigated by thermal analysis and polarized optical microscopy. In addition, porous particles were prepared through a phase separation process. After quenching the PHBV/o-dichlorobenzene solution in ice-water, a white turbidity ensued. This was caused by the formation of spherulites, as confirmed by polarized optical microscopy. The melting of o-dichlorobenzene in PHBV/o-dichlorobenzene showed two DSC peaks. The high melting-point peak was almost the same as pure o-dichlorobenzene. The low melting temperature peak was very broad and its temperature dropped with increasing concentration. It is reasonable to assume that these two types of solvent are-located in noncrystalline regions either between or inside spherulites. The coagulation radius of solvent in the spherulites was calculated by thermodynamic theory. For example, the coagulation radius in PHBV12/o-dichlorobenzene decreased from 14 nm at 20 wt% to 5 nm at 50wt%. In addition, the coagulation size for the PHBV12 system was larger than that for the PHBV5 system. The large noncrystalline region due to the higher presence of HV units in PHBV12 allows a larger space for the solvent. As for melting in this system, the melting temperature corresponds to the melting of lamellar crystals. The melting temperature of the crystals increased with concentration, corresponding to a larger lamellar thickness. This would reduce the non-crystalline regions between lamellar, leading to

less coagulation of solvent between lamellae. Since each spherulite is isolated from others, particles can be obtained by extracting the solvent. SEM photographs showed that these particles had a porous structure. We can conclude that the particle size and the pore size can be regulated by the concentration and HV content.

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