

# The Physicochemical Properties of Polyhydroxyalkanoates with Different Chemical Structures<sup>1</sup>

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**Abstract**—A set of polyhydroxyalkanoates are synthesized, and a comparative study of their physicochemical properties is performed. The molecular masses and polydispersities of polyhydroxyalkanoates are found to be independent of their chemical structures. It is shown that the temperature characteristics and degrees of crystallinity of polyhydroxyalkanoates are affected by the chemical compositions of the monomers and their quantitative contents in the polymers. The incorporation of 4-hydroxybutyrate, 3-hydroxyvalerate, and 3-hydroxyhexanoate units into the chain of poly(3-hydroxybutyrate) decreases its melting point and thermal degradation temperature relative to these parameters of a homogeneous poly(3-hydroxybutyrate) sample ( $175 \pm 5^\circ\text{C}$  and  $275 \pm 5^\circ\text{C}$ , respectively). The higher the content of the second monomer units in the poly(3-hydroxybutyrate) chain, the greater the changes. The degrees of crystallinity of polyhydroxyalkanoate copolymers are generally lower than that of poly(3-hydroxybutyrate) ( $75 \pm 5\%$ ). The effect on the ratio of the amorphous and crystalline phases of the copolymer samples becomes more pronounced in the series 3-hydroxyvalerate–3-hydroxyhexanoate–4-hydroxybutyrate. The prepared samples exhibit different properties ranging from rigid thermoplastic materials to engineering elastomers.

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Polyhydroxyalkanoates (PHAs), which are synthesized in natural and genetically modified organisms and are polymers of hydroxy derivatives of alkanolic acids, exhibit some physicochemical properties similar to those of synthetic polymers (PP and PE). The linear structure of PHA molecules is responsible for their thermoplasticity. In addition, these polymers exhibit optical activity, antioxidant characteristics, the piezoelectric effect, and the most important properties of biodegradability and biocompatibility. The set of properties of PHAs makes them promising for applications in various fields [1]. It is possible, in principle, to prepare a gamut of materials based on the family of polymers of this class with different physicochemical properties ranging from rigid thermoplastic materials to engineering elastomers [2, 3]. Depending on the structure of the C chain, PHAs are divided into three groups: short-chain-length PHAs composed of monomers with a C chain length of  $C_3$  to  $C_5$ , medium-chain-length PHAs ( $C_6$ – $C_{14}$ ), and long-chain-length

PHAs ( $C_{17}$  and above) [4, 5]. The most thoroughly studied representative of the PHA family is poly(3-hydroxybutyrate) (P(3HB)), which is a homopolymer of 3-hydroxybutyric acid; it is a highly crystalline thermoplastic material (the degree of crystallinity is higher than 70%). A disadvantage of P(3HB) is that it does not undergo ordered crystallization; it is very difficult to process into products that exhibit low impact resistance, rigidity, and aging [6]. PHA copolymers with different chemical compositions are more promising; however, their production is an extremely intricate problem that cannot be solved without fundamental knowledge of the laws of the structural and functional organization of the cell cycle of PHAs and the behavior of the dependence of the physicochemical properties of polymers on chemical composition.

Despite the urgency of research of PHAs, only a few short- and medium-chain-length polymers from the representative range of the identified polymers of this class (over 150) have been intensively studied. The

available results on the effect of chemical composition on the basic properties of polymers are far from providing exhaustive information. The fact is that, despite numerous reports on PHAs in general, there are a few profound studies of the properties of PHA copolymers involving modern physicochemical methods, and their results are fairly inconsistent. An analysis of studies on the physicochemical properties of PHAs with different chemical compositions showed that many important data are lacking. Moreover, the data on the effects of the compositions of PHAs on the molecular masses, degrees of crystallinity, and temperature characteristics of the polymers reported by different authors greatly differ, even in the description of samples with similar chemical compositions [3, 7]. This circumstance can be attributed to a variety of causes. Thus, the molecular-mass value is affected by not only the type of microorganism (producer) but also the culture conditions and the polymer-extraction method. The scatter in the published data on the molecular mass of the P(3HB) homopolymer is up to two orders of magnitude. The minimum number-average molecular mass of  $M_n = 58 \times 10^3$  was reported for *Comamonas acidovorans* [8], and the maximum value of  $M_n = 1580 \times 10^3$  was found for a recombinant strain of *Ralstonia eutropha* PHB-4 [9]. The scatter in the data on the  $M_n$  value for the 3HB copolymer with 4-hydroxybutyrate (4HB) with an equal ratio of monomer units likewise considerably varies: For example, for samples containing ~23–24 mol % 4HB,  $M_n$  values of 104 to 590 kDa are given in [10] and [11], respectively. A similar scatter is observed for the  $M_n$  value of the 3HB copolymers with 3-hydroxyvalerate (3HV) and 3-hydroxyhexanoate (3HH).

Important properties of PHAs that determine the conditions for preparing products via melt processing are melting point  $T_{\text{melt}}$  and thermal-degradation temperature  $T_{\text{degr}}$ . The P(3HB) homopolymer had a  $T_{\text{melt}}$  value from 159 [9] to 197°C [12]. There is no shared vision of this parameter for P(3HB-co-3HV) copolymers. For example, for a sample containing 6 mol % 3HV,  $T_{\text{melt}}$  was determined to be 186°C in [12]; for samples with this same composition, Zhao and Chen reported a  $T_{\text{melt}}$  value of 170°C [13] and Zhang et al. gave a value of 56°C [14]. The data on  $T_{\text{melt}}$  values of other types of PHAs are likewise multivalued. According to the literature data, the  $T_{\text{melt}}$  values of samples of P(3HB-co-4HB) copolymers containing 2 to 7 mol % 4HB units can be 114 to 172°C. A decrease in  $T_{\text{melt}}$  to 40–54°C with an increase in the 4HB content in the copolymer to 75–100 mol % was recorded in [11, 15]. However, it was shown by Doi that a copolymer containing 84 mol % 4HB has a  $T_{\text{melt}}$  value of no less than 130°C [16]. The published data on the temperature characteristics of P(3HB-co-3HV) copolymers are likewise controversial. For example, samples contain-

ing 5–6 mol % 3HV had melting points of 186 [12], 156 [13], and 170°C [14], and for samples with 20 mol % 3HV,  $T_{\text{melt}}$  was 114°C according to Avella et al. [17] and 145°C according to Tsuge [18].

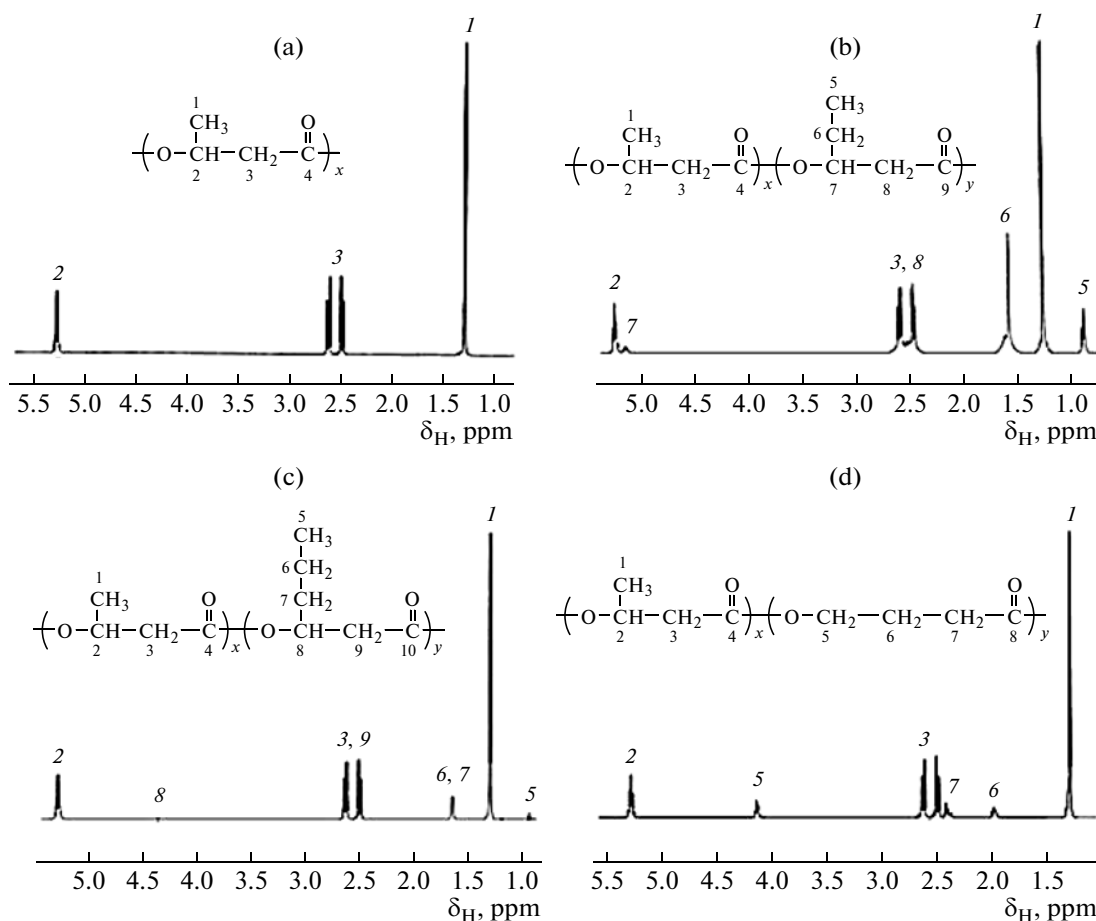
A parameter of no less importance to determination of the properties of polymers is the degree of crystallinity,  $C_x$ . However, the literature data on the degrees of crystallinity of PHA samples with different chemical compositions are scanty and fairly controversial. According to various publications, the range of variation of this parameter for P(3HB) is 59–86% [8, 19, 20]. According to Noda et al., the presence of 20–22 mol % 3HV monomers in the copolymer has hardly any effect on the degree of crystallinity [21]; however, Dai et al. showed that, for copolymers containing 29 and 32 mol % 3HV, it sharply decreases (to 5 and 9%, respectively) [22]. The data on the degrees of crystallinity of 3HB copolymers with 3HH are even scarcer and are not devoid of discrepancies. It was shown in [21] that, for a copolymer containing 12–18 mol % 3HH units, the degree of crystallinity is 38–40%. Fukui et al. recorded a similar value of  $C_x$  for a copolymer with a much lower content of 3HH (1.5 mol %) [23].

The discrepancies of the results on properties of PHAs obtained by different authors at different times are apparently due to the fact that the studied PHA samples were synthesized by different producers in different environments; samples could have different amounts of residual impurities (e.g., lipids) and differ in the methods of processing of polymers into products. Moreover, the authors used different research techniques. Thus, it is impossible to correctly determine the degrees of crystallinity and molecular masses of PHAs without X-ray diffraction analysis and high-performance liquid chromatography; however, the authors of some studies relied on the results obtained via DTA and viscometry. The ambiguous and fragmentary data on the effect of the chemical compositions of PHAs on their basic properties make it necessary to study these parameters. The Institute of Biophysics, Siberian Branch, Russian Academy of Sciences, has placed special emphasis on this aspect of the biotechnology of PHAs: Effective PHA-producing strains have been collected, and biosynthesis techniques for polymers with different chemical compositions have been developed and implemented [24–26].

The aim of this study is to examine and analyze the relationship between the chemical compositions and physicochemical properties of PHAs.

## EXPERIMENTAL

Strains of the hydrogen-oxidizing bacteria *Ralstonia eutropha* B5786 and B8562 and *Cupriavidus eutrophus* B10646 that were grown under various previously developed conditions of carbon nutrition were used as PHA producers. These strains made it possible to pre-



**Fig. 1.**  $^1\text{H}$  NMR spectra of (a) P(3HB), (b) P(3HB-co-3HV) with 17 mol % 3HV, (c) P(3HB-co-3HH) with 18 mol % 3HH, and (d) P(3HB-co-4HB) with 10 mol % 4HB.

pare PHA samples with different chemical compositions [24–30].

Four types of polymers were studied: the P(3HB) homopolymer, P(3HB-co-4HB) copolymers containing 6–24 mol % 4HB, P(3HB-co-3HV) copolymers containing 3–63 mol % 3HV, and P(3HB-co-3HH) copolymers containing 3–18 mol % 3HH.

The compositions of PHAs were determined through chromatography of fatty acid methyl esters with an Agilent Technologies 7890A gas-chromatography–mass-spectrometry system (United States). The  $^1\text{H}$  NMR spectra of PHA solutions in  $\text{CDCl}_3$  were derived with a 600-MHz Bruker Avance III NMR spectrometer (Germany).

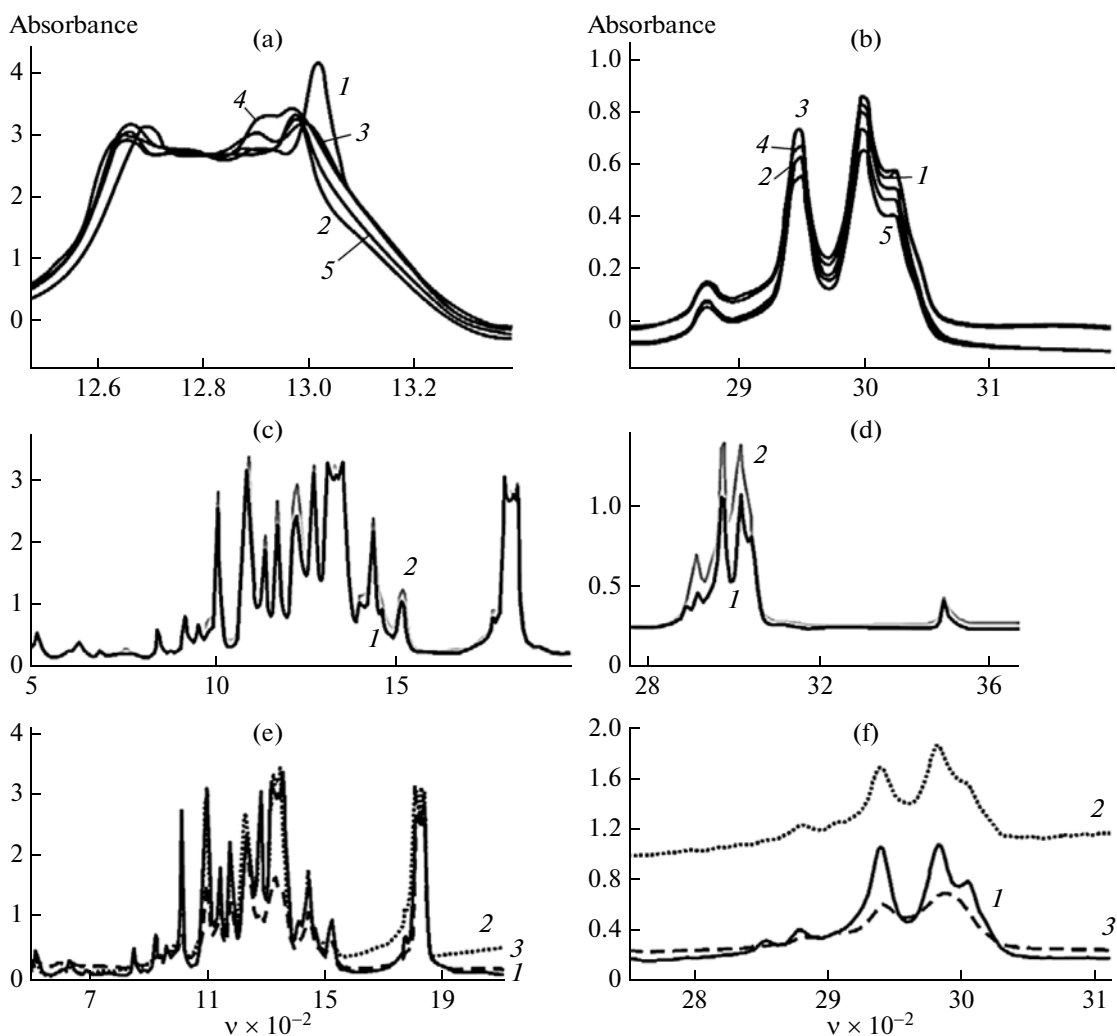
The molecular masses and the molecular-mass distributions of the polymers were determined via gel permeation chromatography (an Agilent Technologies 1260 Infinity chromatograph (United States)) relative to polystyrene standards (Fluka, Switzerland, Germany). Weight-average molecular masses  $M_w$ , number-average molecular masses  $M_n$ , and polydispersity values  $M_w/M_n$  were found.

To study the physicochemical properties of PHAs, samples in the form of powders and films and pressed samples prepared with a Carver Auto Pellet 3887 automatic pellet press were used.

The IR spectra of PHAs were recorded in the range 500–4500  $\text{cm}^{-1}$  with a Lumex InfraLUM FT-02 FTIR spectrometer (Russia).

A complex thermal analysis of PHAs was performed with a Netzsch STA 449 Jupiter synchronous thermal analyzer (Germany), which combines the simultaneous measurement of mass variation (thermogravimetry) and heat flows (DSC) and is coupled with a QMS 403 Aeolus quadrupole mass spectrometer to analyze gases released during the heating of samples.

The X-ray diffraction analysis and the determination of the degrees of crystallinity were performed on a Bruker D8 ADVANCE X-ray spectrometer (Germany) with a diffracted-beam graphite monochromator. Spectra were recorded in the step-scan mode with a step of  $0.04^\circ$  and a dwell time of 2 s to measure the



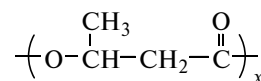
**Fig. 2.** Superimposed IR spectra of the PHA samples (a, c, e) in the range 500–2000  $\text{cm}^{-1}$  and (b, d, f) in the region of the stretching vibrations of the  $\text{CH}_3$  and  $\text{CH}_2$  groups (2840–3100  $\text{cm}^{-1}$ ): (a, b) (1) P(3HB) and (2–5) P(3HB-co-3HV) containing (2) 11, (3) 17, (4) 24, and (5) 27 mol % 3HV; (c, d) (1) P(3HB) and (2) P(3HB-co-3HH) containing 18 mol % 3HH; (e, f) (1) P(3HB) and (2, 3) P(3HB-co-4HB) containing (2) 10 and (3) 24 mol % 4HB.

intensity at a point. (The spectrometer operates at 40 kV and 40  $\mu\text{A}$ .) The degrees of crystallinity were calculated with the program Bruker AXS TOPAS 4.2 (Germany, 2008).

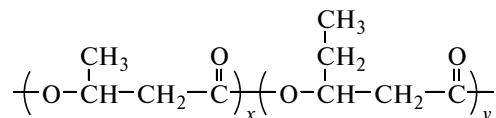
Membranes were prepared via pouring of a 1.5% PHA solution in dichloromethane heated to 35°C onto oil-free surfaces of Petri dishes preheated to the same temperature. The surface microstructures of the membranes were analyzed via scanning electron microscopy (Phillips SEM 525 M). The surface properties were calculated on the basis of measurements of water contact angle  $\theta$  (deg) through the known de Gennes equations [31]. Free surface energy  $\gamma_s$  (erg/cm<sup>2</sup>); solid/liquid-interface free energy  $\gamma_{SL}$  (erg/cm<sup>2</sup>); and the work of solid–liquid adhesion,  $W_{SL}$  (erg/cm<sup>2</sup>), were determined.

## RESULTS AND DISCUSSION

We studied a set of PHA samples purified to homogeneity that have different chemical structures and belong to four types; their <sup>1</sup>H NMR spectra are shown in Fig. 1. The first type of polymer is represented by the P(3HB) homopolymer, an isotactic polyester with regular identically oriented (head-to-tail) consecutive units of 3-hydroxybutyric acid.

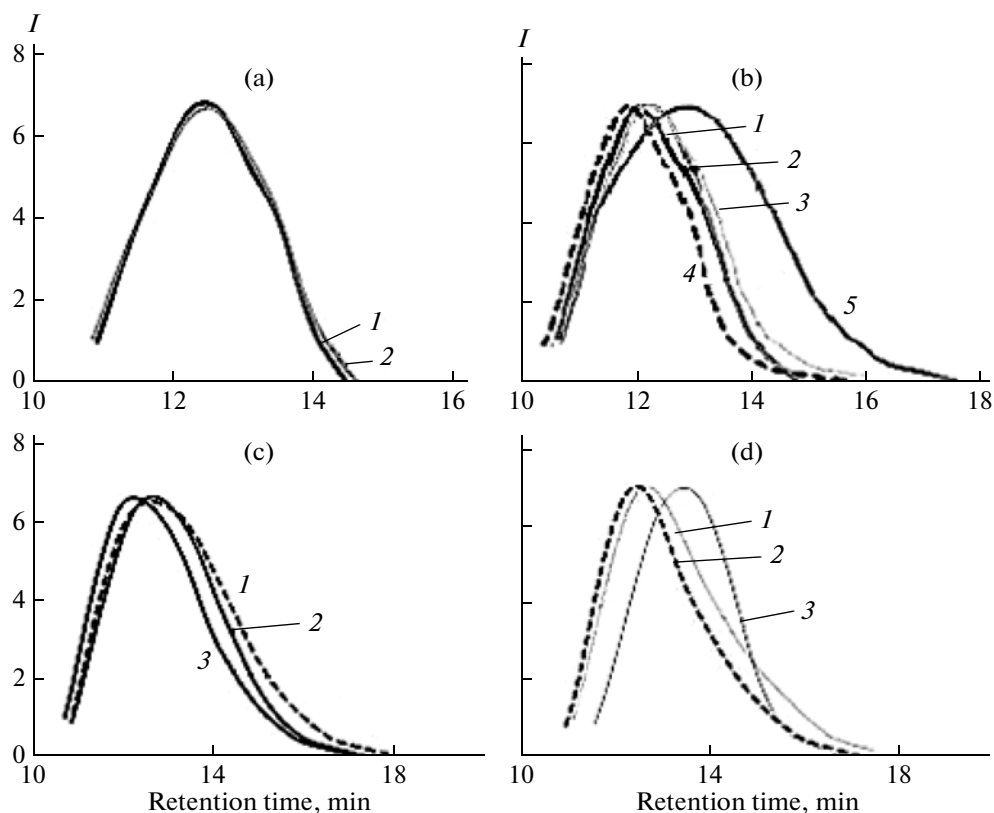


The second type comprises P(3HB-co-3HV) copolymers.



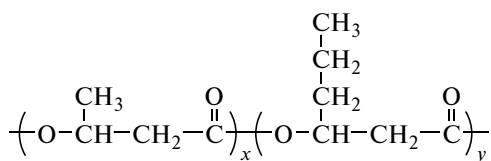
**Table 1.** IR spectra of different PHA samples. The assignment of the absorption bands is based on correlation

Absorption-band number	Wave number $\nu$ , $\text{cm}^{-1}$					Assignment
	P(3HB)	P(3HB-co-3HV) with 11 mol % 3HV	P(3HB-co-3HH) with 18 mol % 3HH	P(3HB-co-4HB)		
				10 mol % 4HB	24 mol % 4HB	
1	517	517	517	517	517	Skeletal $\nu$ and $\delta$ , X–Y (X – C, O, N, Y – C, N)
2	590	590	590	590	590	The peak is not identified
3	625	625	625	625	625	$\delta$ , C–H
4	679	679	679	679	679	Vibrations of C, N, and S atoms and/or a large number of H atoms
5	756	756	756	756	756	$\delta_{\text{nonplanar}}$ , C–H
6	826	826	826	826	826	$\delta$ , C–H
7	895	899	895	895	895	$\delta_{\text{nonplanar}}$ , terminal –CH <sub>3</sub> ; vibrations of any –OH
8	930	930	930	930	930	$\delta_{\text{nonplanar}}$ , OH dimer
9	980	980	980	980	980	$\delta_{\text{nonplanar}}$ , C–H
10	1057	1057	1057	1057	1057	$\nu$ , –C–O– (secondary alcoholic OH group)
11	1100	1103	1100	1100	1100	CH <sub>2</sub> –O–CH <sub>2</sub> vibrations of six bands result from the interaction between C=O and C–C
12	1130	1134	1130	1133	1133	Rocking vibrations of the CH <sub>3</sub> group
13	1181	1184	1181	1181	1181	Skeletal $\nu$ , –C=O
14	1227	1223	1227	1227	1227	$\nu$ , –C–O–
15	1262	1230	1262	1267	1267	$\nu_{\text{as}}$ –C–O–C–
		1269				$\nu$ , –C–O–
		1277				$\nu_{\text{as}}$ –C–O–C–;
		1281				Wagging and torsional-bending vibrations of the –CH <sub>2</sub> group
16	1302	1302	1300	1302p	1302p	$\delta$ , –C–H; $\delta_{\text{s}}$ , –CH <sub>3</sub>
		1358	1358	1351p	1351p	
		1377	1381	1377	1380	
17	1400	1404	1400	1400p	1400p	$\delta_{\text{s}}$ , –CH <sub>3</sub> ; $\delta_{\text{planar}}$ , –CH methylene group adjacent to C=O
		1423	1423p	1423p	1423p	
18	1454	1454	1454	1454	1454	$\delta_{\text{as}}$ , –CH <sub>3</sub> ; $\delta$ , –CH, –CH <sub>2</sub> , –CH <sub>3</sub>
19	1690	1690	1690	1690	1690	$\nu$ , –C=O
20	1721	1717	1717	1717	1717	Absorption band of the terminal carbonyl group
		1724	1721	1726	1726	
		1736	1736	1740	1740	
		1751	1751	1747	1747	
		1774	1774	1774	1774	
21	2874	2874	2874	2874	2874p	Bonded –OH groups
		2900p	2900p	2900p	2900p	
		2932	2932	2932	2936	
		2936	2936	2936	2936	
22	2974	2974	2974	2974	2974	$\nu_{\text{as}}$ , –CH <sub>2</sub> ; $\nu$ , –CH;
		2994	2994	2994	2994	
		2994	2994	2994	2994	
23	3438	3438	3438	3438	3438	$\nu_{\text{as}}$ , –CH <sub>3</sub>
		2994p	2994p	2994p	2996p	
23	3438	3438	3438	3438	3438	$\nu$ , –C=O; –OH

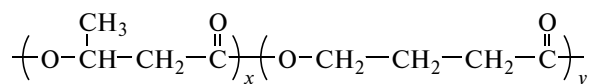


**Fig. 3.** Chromatograms of PHA samples with different chemical compositions and different molecular masses. (a) The P(3HB) homopolymers with  $M_w = (1)$   $1300 \times 10^3$  and  $(2)$   $1200 \times 10^3$ . (b) The P(3HB-co-3HV) copolymers with different 3HV contents:  $(1)$  7 mol % ( $M_w = 1700 \times 10^3$ ),  $(2)$  11 mol % ( $M_w = 1500 \times 10^3$ ),  $(3)$  20 mol % ( $M_w = 1500 \times 10^3$ ),  $(4)$  37 mol % ( $M_w = 2000 \times 10^3$ ), and  $(5)$  64 mol % ( $M_w = 1100 \times 10^3$ ). (c) The P(3HB-co-3HH) copolymers with different 3HH contents:  $(1)$  7 mol % ( $M_w = 950$  kDa),  $(2)$  11 mol % ( $M_w = 1000$  kDa), and  $(3)$  18 mol % ( $M_w = 1300$  kDa). (d) The P(3HB-co-4HB) copolymers with different 4HB contents:  $(1)$  10 mol % ( $M_w = 810 \times 10^3$ ),  $(2)$  16 mol % ( $M_w = 970 \times 10^3$ ), and  $(3)$  24 mol % ( $M_w = 550 \times 10^3$ ).

The third type comprises P(3HB-co-3HH) copolymers.



The fourth type comprises P(3HB-co-4HB) copolymers.



To reveal possible structural differences for the studied PHA types, we used IR spectroscopy (Fig. 2), which is employed to analyze the structures of macromolecules (polypeptide chains, proteins, and linear polyesters) [32]. Thus, the shapes and intensities of the bands in the low-frequency region provided information on the ratio of amorphous and crystalline phases in PE, PP, and PTFE because the spectral bands corresponding to regular portions of macromolecules that form crystalline regions become narrower and more

intense with an increase in  $C_x$ . These studies for PHAs are extremely scanty. In [33], FTIR spectroscopy was used to examine the structural features of P(3HB); the authors suggested that the hydrophobicity of P(3HB) may be attributed to the formation of cyclic H-bonded  $\pi$ -conjugated structures involving the carbonyl and methyl groups of the polymer and one water molecule.

The IR spectra of the studied PHA types were recorded in the range  $500\text{--}4500\text{ cm}^{-1}$ . Figures 2a and 2b show superimposed IR spectra of P(3HB) and P(3HB-co-3HV) copolymers containing different amounts of 3HV; Figs. 2c and 2d, superimposed IR spectra of P(3HB) and P(3HB-co-3HH) copolymers containing 18 mol % 3HH; and Figs. 2e and 2f, superimposed IR spectra of P(3HB) and P(3HB-co-4HB) copolymers containing 10 and 24 mol % 4HB.

The derived IR absorption spectra were identical down to the last detail and contained all the absorption bands corresponding to the vibrations of the main structural units of the polymers except for the bands corresponding to the vibrations of the terminal C—OH and COOH groups. The absorption bands of the

**Table 2.** Molecular-masses of the PHA samples

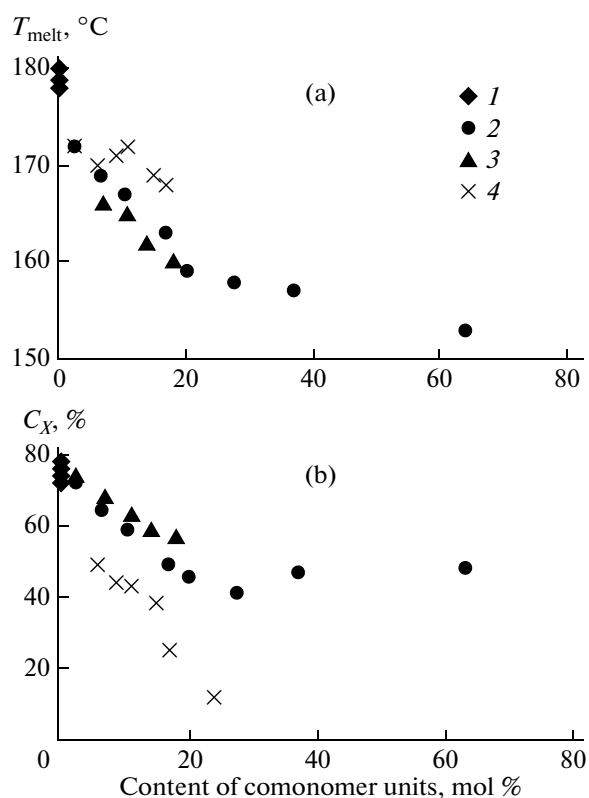
Monomer	Monomer content, mol %	$M_n \times 10^{-3}$	$M_w \times 10^{-3}$	Polydispersity $M_w/M_n$
P(3HB) homopolymer				
3HB	100	760 ± 15	1300 ± 29	1.71 ± 0.03
	100	710 ± 14	1200 ± 47	1.69 ± 0.03
P(3HB-co-3HV) copolymer				
3HV	7	890 ± 26	1700 ± 106	1.91 ± 0.04
	11	840 ± 9	1500 ± 22	1.79 ± 0.01
	20	660 ± 19	1500 ± 42	2.27 ± 0.03
	37	1000 ± 39	2000 ± 64	2.00 ± 0.01
	63	320 ± 3	1100 ± 7	3.44 ± 0.03
P(3HB-co-3HH) copolymer				
3HH	7	270 ± 3	950 ± 23	3.52 ± 0.06
	11	340 ± 9	1000 ± 16	2.94 ± 0.03
	18	420 ± 4	1300 ± 11	3.10 ± 0.04
P(3HB-co-4HB) copolymer				
4HB	10	260 ± 4	810 ± 11	3.12 ± 0.08
	16	370 ± 5	970 ± 18	2.62 ± 0.04
	24	290 ± 5	550 ± 10	1.90 ± 0.01

asymmetric stretching vibrations of the CH<sub>3</sub> and CH<sub>2</sub> groups (2994, 2974, 2936 cm<sup>-1</sup>), the symmetric stretching vibrations of the CH and CH<sub>2</sub> groups (2874, 2878 cm<sup>-1</sup>), and the stretching vibrations of the C=O groups (1700–1760 cm<sup>-1</sup>) were clearly recorded. Some differences between the P(3HB) sample and the P(3HB-co-3HV) copolymers (Fig. 2a) were observed during the comparison of the fine structures of the absorption bands on a larger scale in the range 1235–1335 cm<sup>-1</sup>. In the region of the right shoulder of the absorption band for the P(3HB) sample, a narrow local peak with a maximum at 1300 cm<sup>-1</sup> was registered; for the samples of copolymers with 3HV, the intensity of the analogous peak is more than 2 times lower and its maximum is shifted to 1296–1297 cm<sup>-1</sup>. The most characteristic difference between the IR spectra of the P(3HB-co-3HH) copolymer and the P(3HB) sample (Figs. 2c, 2d) is a slight increase in the intensity of the absorption bands in the shortwave spectral range (2800–3000 cm<sup>-1</sup>). This feature may be attributed to an increase in the fraction of stretching vibrations of the –C–H and –CH<sub>2</sub> groups in the copolymers containing 3HH. Figures 2e and 2f show the IR spectra of the P(3HB-co-4HB) copolymers containing 10 and 24 mol % 4HB in comparison to the spectrum of P(3HB). It is noteworthy that, at a 4HB content of 24 mol % (see also Figs. 2a, 2b), the intensity of the absorption band in the range 1235–1335 cm<sup>-1</sup>, which corresponds to the asymmetric stretching vibrations of the C–O–C group, significantly decreases. The more than twofold decrease in the intensity of this

band can be attributed to a decrease in the mass fraction of the C–O–C group due to an increase in the fraction of the CH<sub>2</sub> group.

PHAs are moderately hydrophobic compounds and can absorb as much as 1% water [34]. Water molecules are hydrogen bonded to a hydrogen atom of the methyl group or to an oxygen atom of the carboxyl group. With allowance for this fact, the possible versions of assignment of all the absorption bands of the IR spectra of PHAs are listed in Table 1. Narrow bands at 3436 cm<sup>-1</sup> are characteristic of a hydrogen-bonded OH group. The band at 1688 cm<sup>-1</sup> corresponds to the vibrations of the OH group. Absorption bands 22 are characteristic of a bonded OH group, probably that of the dimeric COOH group. A group of weak bands characteristic of carboxylic acid dimers is located in the range 3000–2500 cm<sup>-1</sup>. Bands 20 and 21 may be attributed to the formation of associates that is due to the interaction with sorbed water. Absorption bands 1, 4, 14, and 15 suggest that the samples contain impurities of cellular macromolecules (proteins, fatty acids) and alcohol-group-containing compounds, which result from the hydrolysis of the polymers. In regards to studying PHAs with different chemical structures, we should admit that the derived IR spectra are insufficiently informative and state that it is necessary to conduct special IR spectroscopic studies for this purpose.

It is known that the molecular masses of PHAs are extremely variable; these values depends on many factors, including the source of carbon nutrition for bac-



**Fig. 4.** Dependences of the (a) melting points and (b) degrees of crystallinity of (1) the P(3HB) homopolymer and (2) the P(3HB-co-3HV), (3) P(3HB-co-3HH), and (4) P(3HB-co-4HB) copolymers on the content of the 3HV, 3HH, or 4HB monomers, respectively.

teria, the duration of cultivation, and the polymer-extraction method.

Figure 3 shows chromatograms of the molecular-mass distributions of samples with different compositions. A clear relationship between the chemical compositions of PHAs and the  $M_w$  and  $M_n$  values has not been found. The scatter in the data for the samples of P(3HB) and three other types of PHAs was  $550 \times 10^3$  to  $2000 \times 10^3$  for  $M_w$  and  $260 \times 10^3$  to  $1000 \times 10^3$  for  $M_n$  with the polydispersity coefficients varying in the range 1.69–3.52 (Table 2); these results are consistent with the above-presented literature data.

The temperature characteristics of PHAs and their abilities to crystallize in the native state are the most important parameters because they determine the thermomechanical properties and, therefore, the possibilities of polymer-melt processing [35, 36].

The temperatures of successive phase transitions were determined via DSC. The melting temperature range of P(3HB) was 160–185°C; in fact,  $T_{melt} = 176$ –182°C. The thermal-decomposition range of P(3HB) was 275–280°C. After reheating, the melting point of the sample decreased, while its crystallization temperature remained unchanged. The heating of the polymer to the melting point and the holding of the sample at this

temperature for 15 min had no effect on the position and shape of the melting peak. No significant difference in the thermal behavior of P(3HB) in air and in an inert atmosphere (nitrogen) was found. This outcome is apparently due to the fact that the thermal degradation of the polymer occurs through the depolymerization mechanism, which is known to be more intense than oxidation. The presence of a pronounced gap between the melting point (160°C) and the initial decomposition temperature (260°C) is an important process characteristic of the polymer because it allows the production of products based on it (films, fibers, hollow forms, etc.) via conventional polymer-processing methods (solution spinning, extrusion, injection molding).

The two parameters  $T_{melt}$  and  $T_{degr}$  of the PHA copolymers had lower values. For the P(3HB-co-3HV) copolymers, the melting points are lower than the melting peak of the homogeneous P(3HB) sample and become even lower with an increase in the 3HV content (Fig. 4a). These changes are particularly significant in the sample with the dominant fraction of 3HV units (63 mol %). In addition, the incorporation of 3HV had an effect on the crystallization temperatures; the other two studied types of PHA copolymers exhibited similar behavior (Fig. 4a). In addition, the melting points and thermal-degradation temperatures of the copolymers containing 3HH and 4HB monomers were lower than those of the homogeneous P(3HB) sample. Note that the low crystallization temperatures of the homogeneous P(3HB) sample are a serious problem for its melt processing. One of the possible ways to increase the crystallization temperatures of these polymers is the targeted copolymer synthesis aimed at incorporation of units of 3HV and other monomers into the 3HB polymer chains. It is important that a decrease in the melting points and thermal degradation temperatures of PHA copolymers does not reduce the characteristic gap between these parameters. Thus, the studied types of PHA copolymers preserve their important property of thermoplasticity.

X-ray diffraction analysis revealed that the crystalline phase dominates the amorphous phase in P(3HB). The degrees of crystallinity of different P(3HB) samples slightly depended on the preparation conditions. X-ray diffraction analysis of a set of P(3HB) samples synthesized by bacteria *R. eutropha* B5786 in different biosynthesis modes on different carbon sources suggests that the degrees of crystallinity are fairly constant for this material. The spread in the values obtained for a set of a few dozen samples lies in the range 66–78%. A typical X-ray diffraction pattern of P(3HB) is shown in Fig. 5.

The degrees of crystallinity of the P(3HB-co-3HV) copolymers are lower than that of the homogeneous P(3HB) sample (Fig. 4b). The incorporation of 3HV monomers into the 3HB chain significantly decreased the degrees of crystallinity of the P(3HB-co-3HV) copolymers. The P(3HB-co-3HV) copolymers are



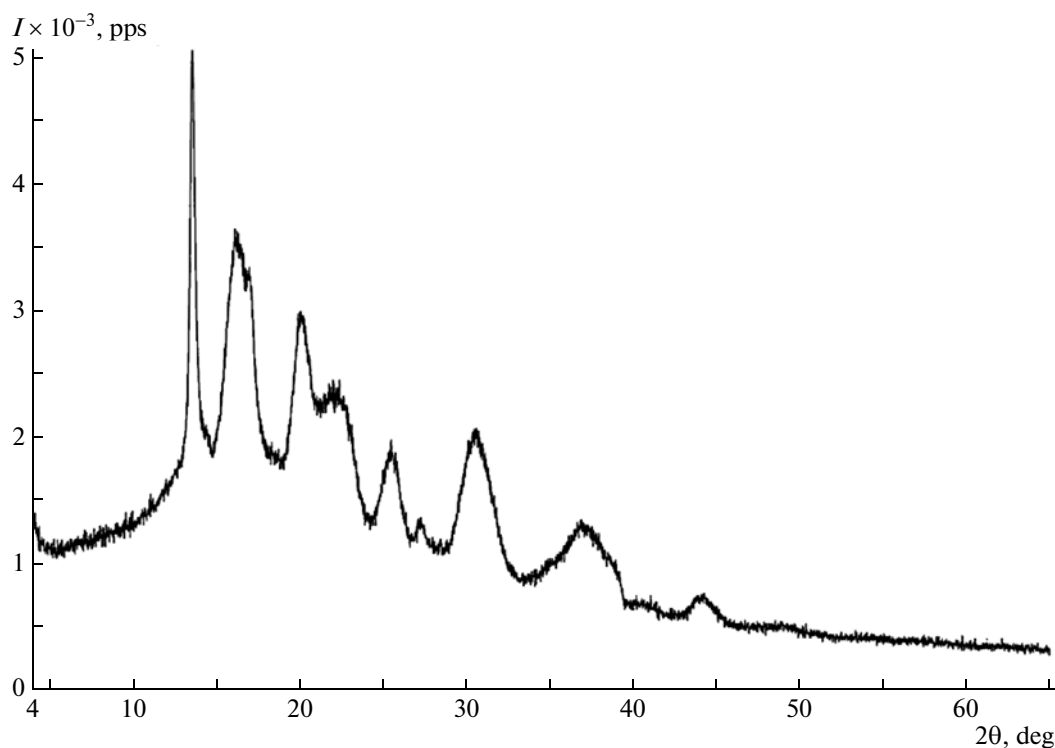


Fig. 5. X-ray diffraction pattern of P(3HB).

known to be isodimorphic owing to the cocrystallization of monomer units. It was shown that the presence of 3HV in the 3HB polymer chain has a considerable effect on the kinetics of crystallization of the material, including the process rate and the size of the resulting spherulites [37]. It is evident from Fig. 4b that the dependence of  $C_x$  on the fraction of 3HV units is linear only for the 3HV content in the range of a few units to 25–25 mol %. The samples with higher amounts of 3HV (above 35–40 mol %) had degrees of crystallinity of ~50%, a value that is close to the  $C_x$  values of the copolymers with lower contents of 3HV. To all appearances, this circumstance may be explained on the basis of the existing idea that a change in the ratio of fractions of 3HB and 3HV monomer units leads to a change in the crystal lattice. If a copolymer contains less than 35–40 mol % 3HV, its units can crystallize in the lattice of 3HB; if the amount of 3HV exceeds 40 mol %, the 3HB units can crystallize in the lattice of 3HV [38]. In the samples with dominant contents of 3HV, the monomer units cocrystallized into a single lattice; this phenomenon apparently did not affect the degree of crystallinity of P(3HB-co-3HV), despite the fact that the fraction of 3HV units increased in it.

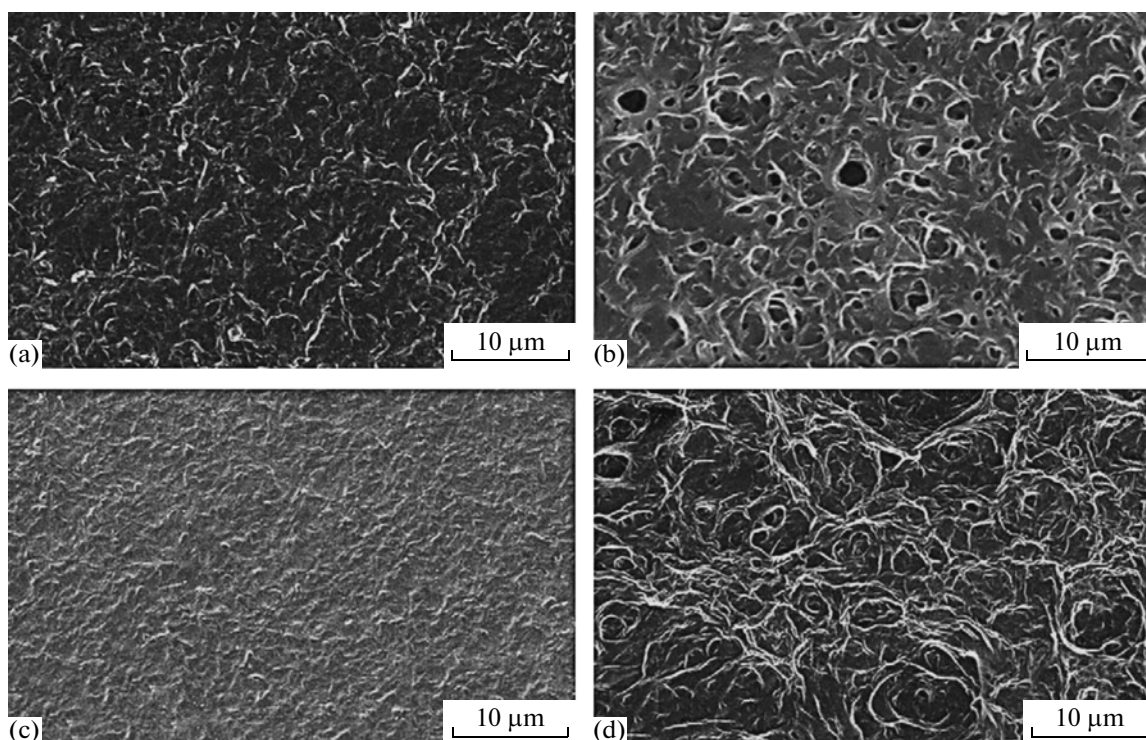
A similar change in the  $C_x$  values was observed during the study of P(3HB-co-3HH) copolymers (Fig. 4b). The incorporation of 3HH units affected the ratio of the amorphous and crystalline phases in a P(3HB-co-3HH) copolymer in a manner similar to

that for the incorporation of 3HV units: It equalized the ratio. As the amount of 3HH increased in the range 2.5–18.0 mol %, the degree of crystallinity of the copolymer monotonically decreased from 74 to 57%.

The next type of PHA includes P(3HB-co-4HB) copolymers; unlike P(3HB), they are rubbery polymers, in which the breaking elongation is up to 1000%; that is, it is two orders of magnitude higher than that of P(3HB) [39, 40]. The authors of [39, 40] showed that a single type of crystal lattice (of the P(3HB) type) is observed for these copolymers (with a fraction of 4HB units of 0–29 mol %). In contrast, the P(4HB) lattice type was recorded for these copolymers only at a high content of 4HB (78–100 mol %).

The greatest effect of composition on the degree of crystallinity was found for the P(3HB-co-4HB) copolymers (Fig. 4b). The incorporation of 4HB into the 3HB polymer chain had a dramatic effect (much more significant than that for the incorporation of 3HV or 3HH) on the ratio of the crystalline and amorphous phases in the copolymer: It significantly decreased the degree of crystallinity. PHA samples with lowered degrees of crystallinity (44 to 12%) have been prepared for the first time.

Electronic images of the surface microstructures of membranes prepared from PHA samples with different chemical compositions and different fundamental physicochemical properties revealed some differences in their structures (Fig. 6). The surfaces of the mem-



**Fig. 6.** Electronic images of the surfaces of the PHA membranes: (a) P(3HB), (b) P(3HB-co-4HB) with 15 mol % 4HB, (c) P(3HB-co-3HV) with 11 mol % 3HV, and (d) P(3HB-co-3HH) with 7 mol % 3HH.

branes from P(3HB) had minimal reliefs and were dense and almost nonporous. Numerous pores with a size of about 1  $\mu\text{m}$  are observed on the surfaces of the membranes prepared from the P(3HB-co-4HB) copolymer. The surfaces of the membranes from 3HB and 3HV copolymers are more smooth and uniform. The membranes prepared from the copolymer of 3HB and 3HH have more relief surfaces with many pores of different diameters in the range 0.5–5.0  $\mu\text{m}$ .

An important parameter characterizing the hydrophilic–hydrophobic balance of a surface [41, 42] is the water contact angle. Its measurement makes it possible to calculate some important characteristics of the surface, such as the work of adhesion, surface tension, and interface free energy. The highest value of the contact angle was found for the membranes prepared from

P(3HB) ( $70.0^\circ \pm 0.4^\circ$ ) (Table 3). The contact angle of the copolymer membranes was significantly lower; the membranes prepared from P(3HB-co-3HV) and P(3HB-co-3HH) hardly differed in this parameter ( $60^\circ$ – $62.5^\circ$ ). The contact angle of the surfaces of the membranes from the P(3HB-co-4HB) copolymer was reliably lower:  $57.4^\circ \pm 0.6^\circ$ . This is an intermediate region between hydrophobic and hydrophilic surfaces. The lowest values of surface tension and work of adhesion (31–32 and 95–97  $\text{erg}/\text{cm}^2$ , respectively) are characteristic of the membranes prepared from P(3HB), which exhibit the lowest values of hydrophilicity (Table 3); for the copolymer membranes, these parameters have higher values: 38.9–43.1 and 106.4–112.0  $\text{erg}/\text{cm}^2$ , respectively.

**Table 3.** Surface properties of the membranes prepared from PHAs of different types

Polymer	Polymer composition, mol %	Water contact angle, deg	Surface tension, $\text{erg}/\text{cm}^2$	Interface free energy, $\text{erg}/\text{cm}^2$	Work of adhesion, $\text{erg}/\text{cm}^2$
P(3HB)	P(3HB)(100)	$70.0 \pm 0.4$	32.8	7.9	97.7
P(3HB-co-3HV)	3HB : 3HV = 73 : 27	$62.5 \pm 2.0$	38.9	5.3	106.4
P(3HB-co-3HH)	3HB : 3HH = 93 : 7	$60.9 \pm 1.6$	42.4	4.1	111.1
P(3HB-co-4HB)	3HB : 4HB = 90 : 10	$57.4 \pm 0.6$	43.1	3.9	112.0

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