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MOLECULAR BIOPHYSICS

Hydrolytic Degradation of Poly(3-Hydroxybutyrate) and Its Copolymer with 3-Hydroxyvalerate of Different Molecular Weights in vitro

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Abstract—The hydrolytic degradation of polymer films of poly(3-hydroxybutyrate) of different molecular weights and its copolymers with 3-hydroxyvalerate (9 mol % 3-hydroxyvalerate in the poly(3-hydroxybutyrate) chain) of different molecular weights was studied in model conditions in vitro. The changes in the physicochemical properties of the polymers were investigated using different analytical techniques: viscometry, differential scanning calorimetry, gravimetrical method, and water contact angle measurement for polymers. The data showed that in a period of 6 months the weight of polymer films decreased insignificantly. The molecular weight of the samples was reduced significantly; the largest decline (up to 80% of the initial molecular weight of the polymer) was observed in the high-molecular-weight poly(3-hydroxybutyrate). The surface of all investigated polymers became more hydrophilic. In this work, we focus on a mathematical model that can be used for the analysis of the kinetics of hydrolytic degradation of poly(3-hydroxyaklannoate)s by noncatalytic and autocatalytic hydrolysis mechanisms. It was also shown that the degree of crystallinity of some polymers changes differently during degradation in vitro. Thus, the studied polymers can be used to develop biodegradable medical devices such that they can perform their functions for a long period of time.

Keywords: degradation, hydrolysis, poly(3-hydroxybutyrate), poly(3-hydroxybutyrate-co-3-hydroxyvalerate)

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INTRODUCTION

Poly(3-hydroxybutyrate) (PHB), which is the main polymer of the homologous series of the family of polyhydroxyalkanoates (PHA), is the best-known microbiological polyester and is a promising alternative to synthetic biodegradable thermoplastics [1–4] and other biocompatible polymers [4–11]. Unlike natural polymers (chitosan, alginate, dextran, collagen, etc.) and chemically synthesized polymers, PHB and its copolymers are obtained biotechnologically, which makes it possible to achieve a high degree of purity, as well as to set and control the physicochemical properties of biopolymers within narrow limits in the course of their biosynthesis [2]. Since PHB is characterized by biodegradability and high biocompatibility it is widely used in regenerative medicine and tissue engineering [12–21] as well as for drug formulations [22–25]. The properties of PHB allow creating composites of this biopolymer with synthetic polymers, inorganic materials, and drugs [26–29]. In addition, PHB is an environmentally friendly material that can be used in manufacturing packaging materials and in agriculture [30].

It is generally accepted that both in living systems and in the environment PHB undergoes biodegradation via enzymatic and nonenzymatic processes that proceed simultaneously in vivo. In comparison with other biodegradable polymers (e.g., polylactide and polyglycolide [31]), PHB is considered to be moderately resistant to degradation in vitro and biodegradation in animal tissues. The rate of degradation depends on the polymer characteristics such as chemical composition, crystallinity, morphology, and molecular weight [32, 33]. The analysis of the published data showed a wide variation and sometimes a considerable discrepancy in the degree of PHA hydrolytic degradation in vitro [1]. This difference is caused by various factors: different geometries of PHB samples, the

Abbreviations: PHB, poly(3-hydroxybutyrate), PHA, polyoxyalkanoates, PHBV, poly(3-hydroxybutyrate-co-3-hydroxyvalerate).

degree of purity, molecular weight, as well as differences in the origin of the polymers. As an example, there are discrepancies in the data on the weight loss of samples during their hydrolytic degradation. As an example, in the course of incubation of PHB films at 37°C and pH 7.4, the weight loss of PHB films was 7.5% for 50 days [34], did not decrease for 150 days [35], and did not change for 730 days [36] and 364 days [37]. As will be shown below, similarly to the authors of [34], we observed a considerable decrease in the weight of samples under virtually the same conditions. In addition, the weight of polymers did not change after a temperature increase to 70°C [37]. Published data on the change of the molecular weight of PHB during its hydrolytic degradation are also discrepant: a similar decrease in the molecular weight (to 68% [35] and 64% [36]) was observed for very different periods of time: for less than 6 months (150 days) and for 2 years (730 days) of incubation of samples in a buffer solution. The authors of several studies observed changes in the mechanical properties of PHB products (threads and plates) during hydrolysis [38, 39]; however, these data are also discrepant. The Young's modulus in PHB sutures did not change after incubation at 70°C for 180 days at pH 7.2; however, the tension and relative elongation at breakage decreased by 36 and 33%, respectively. A different behavior was observed at physiological temperature (37°C). For the first 3 months (90 days), the tension and relative elongation at breakage increased by 17 and 16%, respectively. In the next 90 days of the experiment, the values of these indices gradually decreased to the baseline level [38]. Measuring the mechanical properties of PHB plates showed that their Young's modulus and tension at breakage decreased by 68 and 77%, respectively [39]. A group of authors showed a dramatic decrease in the Young's modulus, tension at breakage, and hardness of PHA plates that occurred within only 1 day, by 32, 13, and 40%, respectively. In the next 28 days, the Young's modulus and hardness did not change, and the tension at breakage returned to the initial values [40].

To develop medical devices based on PHB and its copolymers, it is necessary to know how the physicochemical properties of these polymers change during degradation. To understand how the polymers change in the human body in the course of degradation, it is necessary to investigate the kinetics of changes of the main physicochemical properties during their degradation in vitro under conditions that simulate the internal environment of the body. Thus, the purpose of this study was to obtain, compare, and analyze the kinetic curves of the long-term degradation of PHB and its copolymers with a selected molecular weight and monomer composition, which were obtained by controlled microbial synthesis.

MATERIALS AND METHODS

Biosynthesis of polyhydroxyalkanoates. Poly(3-hydroxybutyrate) and its copolymers with 3-hydroxyvalerate (PHBV) with a selected molecular weight and monomer composition were obtained by controlled biosynthesis using the highly efficient PHB-producing strain *Azotobacter chroococcum* 7B. For this purpose, sodium acetate (molecular-weight regulator) and valeric acid sodium salt (precursor of 3-hydroxyvalerate monomers in the synthesized PHBV copolymer) were added to the culture medium. The producer strain was cultured for 72 h [35–37, 42, 45]. The procedure of isolation and purification of the polymer from the biomass-producing strain included chloroform extraction, filtration, precipitation with isopropyl alcohol, purification by several dissolution–precipitation cycles, and drying [42, 43].

Nuclear magnetic resonance. ^{1}H-NMR spectra of 1% (wt/vol) polymer solutions in deuterated chloroform were recorded with a 300 MHz MSL-300 spectrometer (Bruker, Germany) at the following experimental parameters: temperature 313 K, relaxation delay 2.5 s, and spectral window width 4000 Hz. Chemical shifts (in ppm) were set by the residual proton signal of $CDCl_3$ (7.24 ppm for tetramethylsilane). The percentage of 3-hydroxyvalerate monomers in the PHBV copolymer was calculated from the ratio of the integrated intensities of the signal from the methyl group of hydroxyvalerate (0.89 ppm) and the sum of the signals from the methyl group of hydroxyvalerate (0.89 ppm) and the methyl group of hydroxybutyrate (1.27 ppm).

Manufacturing of polyhydroxyalkanoate films. To study degradation in vitro, a series of films 50 ± 10 μm thick were prepared of PHB and its copolymers. The films were prepared by pouring a chloroform solution on the bottom of degreased Petri dishes. The obtained films were then cut into smaller films (3 × 1 cm).

Hydrolytic degradation in vitro. To study the degradation of PHA films, they were incubated in 15 mL of PBS (pH 7.4) [46] in a thermostat at 37°C for 183 days. This time period was selected because 6 months is the average time during which an implant is replaced with healthy tissue in the body [47]. The pH was monitored with an Orion 420+ pH meter (Thermo Electron Corporation, United States). To assess the changes in the polymer film weight, the films were removed from the solution after 1 week and 1, 3, and 6 months of incubation, dried, and weighed. The average weight of the films was 15–25 mg. Changes in the weight of films in the course of degradation were determined gravimetrically with AL-64 electronic scale balance (Max = 60 g, $d = 0.1$ mg; ACCULAB, United States). To prevent bacterial contribution to the degradation of polymers, sodium azide (2 g/L) was added to the buffer solution, and the buffer solution was replaced twice per week [33, 48].

Study of the properties of polymers. The molecular weight of PHB and its copolymers was determined viscometrically. The viscosity of polymers was measured in a chloroform solution at 30°C with a RheoTec viscometer (RheoTec Messtechnic GmbH, Germany). The molecular weight was calculated using the Mark–Kuhn–Houwink equation [49]:

$$[\eta] = 7.7 \cdot 10^{-5} M^{0.82}.$$

To determine [η], the experimentally obtained numerical values were represented in the coordinate system by plotting the concentration of the polymer solution (C) on the abscissa axis and the specific viscosity value (η_{sp}/C) on the ordinate axis. The intrinsic viscosity [η] was obtained by extrapolating the obtained line to the ordinate axis. The accuracy of determination of [η] was ~1%. The accuracy of determination of the molecular weight calculated by the Mark–Kuhn–Houwink equation is 2–5% [50].

Molecular weight is one of the most important and the most sensitive parameters for simulating degradation of biodegradable polymers [51]. A mathematical description for the noncatalytic and autocatalytic degradation of aliphatic polyesters mechanisms was proposed in [52]. Assuming that the degree of degradation is low, the authors of [52] proposed the following kinetic dependence based on the mean molecular weight of polymers:

$$1/\mathrm{MW} = 1/\mathrm{MW}_0 + kt, \quad (1)$$

where MW and MW_0 are the mean molecular weights of the polymer component at time t and at the initial time, respectively, and k is the rate constant.

An equation that took autocatalysis into account, which is the consequence of the appearance of the terminal groups of carboxylic acids, was also proposed. This process can be described by the following equation:

$$\ln\mathrm{MW} = -kt + \ln\mathrm{MW}_0. \quad (2)$$

The thermal properties of the samples of films of PHB and its copolymers were measured by differential scanning calorimetry with a DSC 204 F1 Phoenix differential scanning calorimeter (Netzsch, Germany). A polymer film (approximately 1–3 mg) was placed in an aluminum crucible. The samples were heated from 25 to 220°C at a heating rate of 10 K/min in an argon atmosphere. The crystallinity of the PHB structure (Xc) was calculated as follows:

$$X\mathrm{c} = (\Delta H_m/\Delta H_{0\_m}(\mathrm{PHB})) \cdot 100\%,$$

where ΔH_m is the changes in enthalpy caused by melting of the test specimen and ΔH_{0_m}(PHB) is the theoretical value of the thermodynamic melting enthalpy for 100% crystalline PHB samples (146.6 J/g) [11]. All calculations were performed for the second heating cycle.

The hydrophilicity of the polymer surface was assessed by measuring the wetting contact angle formed between water drops and the "smooth" surface of samples with a Contact Angle Meter 110 VAC (Cole-Parmer, United States). For this purpose, a drop of distilled water (10 μL) was applied on the surface of films and the wetting contact angle was measured. Measurements were performed ten times [53, 54].

RESULTS AND DISCUSSION

Biosynthesis of polymers. Table 1 shows the results for biosynthesis of PHB and PHBV by an *A. chroococcum* 7B culture grown on a medium containing sucrose as a main carbon source, valeric acid as an additional carbon source for the synthesis of the PHBV copolymer, and sodium acetate as an additive to regulate the molecular weight of the synthesized polymer.

We obtained PHB of different molecular weights and PHBV with nearly the same molar content of 3-hydroxyvalerate (approximately 9 mol %) and different molecular weights.

Weight loss of polymer films. In mammals, PHA is degraded as a result of combined hydrolytic and enzymatic degradation. This leads to a change in the weight of samples and their physical and chemical properties [1–3, 21–23]. The analysis of the degradation curves (Fig. 1) revealed a weight loss of all samples in the first week. As an example, the weight of the low-molecular-weight PHB 82 decreased by ~8% (to 93.6 ± 1.1%). The weight of films prepared of polymers PHB 408 and PHB 1700 decreased to 98.3 ± 0.6% and 97.5 ± 0.3% of the initial weight, respectively. The weight of copolymers also decreased: to 91.6 ± 0.2% in PHBV 9% 815 films, 92.7 ± 0.3% in PHBV 9% 1385 films, and 96.8 ± 0.6% in PHBV 9.6% 210 films. This weight loss can probably be explained by the release of water-soluble PHA oligomers from the polymer matrix [21, 22, 26].

Later, the weight of the PHA films placed in buffer solution did not change significantly even after 180 days of incubation, which is indicative of a slow hydrolytic degradation. The greatest weight loss for 6 months was observed in PHB 82 samples (to 92.1 ± 1%), PHBV 9% 815 kDa (to 92.3 ± 1.1%), and PHBV 9% 1385 (to 93.3 ± 0.7%) (Fig. 1). These results are consistent with the data on the degradation of PHB and its copolymers obtained by other authors [56], who also did not observe significant changes in the weight of PHB and PHBV. However, in our case, the weight change in the first week of degradation was more significant.

Changes in molecular weight. Conversely, the molecular weight of all PHA samples gradually decreased with the degradation time (Fig. 2). The most significant decrease was observed in the molecular weight of PHB 1700 (to 23% of the initial molecular weight), with the highest molecular weight loss (43%)

Table 1. Parameters of biosynthesis and main characteristics of PHB and PHBV synthesized by the producing strain *A. chroococcum* 7B in the culture medium containing sucrose as a main source of carbon and supplemented with valeric acid and sodium acetate

Substrate	Time of addition of valeric acid or sodium acetate to the culture medium, h	Biomass yield, g/L medium	PHA content in biomass, % of dry cell weight	Molecular weight of PHA, $\times 10^6$ Da	Content of 3-hydroxyvalerat/ 3-hydroxybutyrate in copolymer, mol %	Designation of obtained polymer
Sucrose, 50 mM	–	5.8 ± 0.6	83.4 ± 3.1	1.70	0	PHB 1700
Sucrose + 35 mM sodium acetate	0	4.3 ± 0.5*	71.7 ± 3.2*	0.48	0	PHB 408
Sucrose + 100 mM sodium acetate	0	1.9 ± 0.6*	58.8 ± 3.6*	0.08	0	PHB 82
Sucrose + 10 mM valeric acid	12	4.2 ± 0.5*	73.8 ± 3.7*	1.39	9.0	PHBV 9% 1385
Sucrose + 20 mM valeric acid	0	3.2 ± 0.4*	67.7 ± 3.0*	0.82	9.0	PHBV 9% 815
Sucrose + 20 mM valeric acid + 60 mM sodium acetate	12/0	2.6 ± 0.3*	49.5 ± 3.2*	0.21	9.6	PHBV 9.6% 210

* $p < 0.05$ compared to the Sucrose group, $n = 8$.

being observed in the first week. This was probably due to the large amount of the amorphous component in the polymer, which is degraded 20 times faster than the crystal component [57].

The significant decrease in the molecular weight of PHA in the course of biodegradation indicated that the polymer degradation proceeded primarily in the bulk of the polymer matrix. It should also be noted that the high-molecular-weight polymers lost molecular weight more rapidly than the low-molecular-weight polymers. If the polymers are grouped on the basis of their molecular weight, it can be seen that PHB 1700 and PHBV 9% 1385 lost molecular weight more rapidly than the group of PHBV 9% 815 and PHB 408, which, in turn, lost weight more rapidly than PHB 82 and PHBV 9.6% 210. This may be due to the fact that the formation of crystalline structures by the long polymer chains is hampered; therefore, such chains are more accessible to water molecules, which is consistent with the data on PHA degradation obtained by other authors [37, 56, 58, 59].

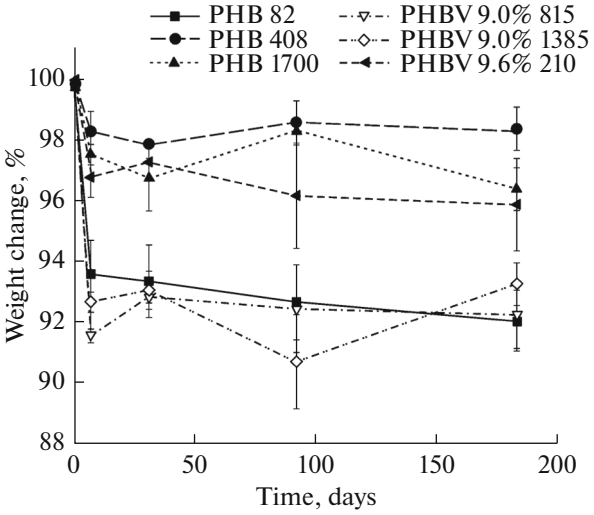

Fig. 1. Changes in the polymer weight in the course of degradation in PBS at 37°C.

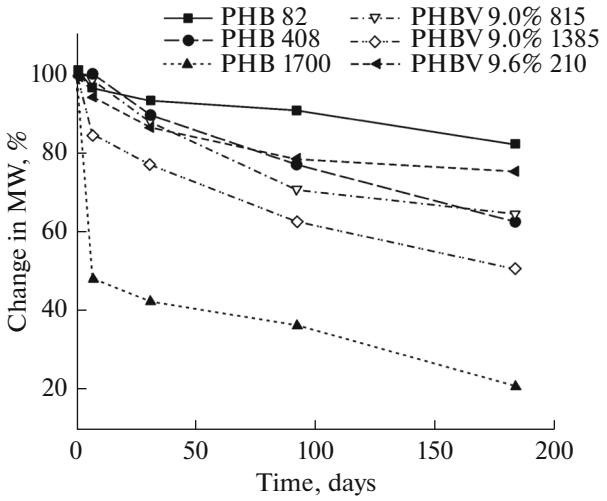

Fig. 2. Changes in the molecular weight of polymers in the course of hydrolytic degradation.

To analyze curves that describe the decrease in the molecular weight of PHB and its copolymers in the course of degradation, we used the model of degradation of partly crystalline polymers (Eqs. (1) and (2)) described in [52, 60] and constructed the plots shown in Figs. 3a, 3b.

In accordance with the models, the correlation coefficients for each curve were found (Table 2).

The analysis of the correlation coefficients shown in Table 2 showed that the highest correlation coefficients are characteristic of the noncatalytic model. However, homopolymers PHB 82 and PHB 408, as well as copolymers with the molecular weights of 815 and 1385, also showed a strong correlation for the autocatalytic model (0.93, 0.98, and 0.9, respectively). Therefore, both the noncatalytic and autocatalytic models of the decrease in molecular weight are appropriate for these polymers. A similar behavior of partly crystalline polymers is described in [52]. For the remaining two polymers, that is, PHB 1700 and PHBV 9.6% 210, the noncatalytic degradation model is more appropriate.

These results are consistent with the data on the degradation of PHB and its copolymers obtained by other authors, who also showed significant changes in the molecular weight in the course of degradation [37, 56, 58]. However, this is the first study to apply the models of the noncatalytic and autocatalytic mechanisms of degradation of partly crystalline polymers to this class of polymers. As a result, it was found that polymers PHB 82 and 408 kDa and PHBV 9% 815 and 1385 kDa obey both the autocatalytic and noncatalytic degradation models, whereas polymers PHB and PHBV 1700 9.6% 210 are fitted by the noncatalytic degradation model.

Degree of crystallinity of the polymers. The degree of crystallinity of the studied PHAs was calculated using the melting heat data for a completely crystalline PHB (146.6 J/g) [61]. Importantly, the degree of crystallinity of the copolymer was lower than that of homopolymers. This phenomenon may be due to the incorporation of 3-hydroxyvalerate monomers into the polyhydroxybutyrate chain. The incorporation of these monomers with a longer side chain CH_2–CH_3 are energetically unfavorable for the PHB chain, which leads to a decrease in the degree of crystallinity

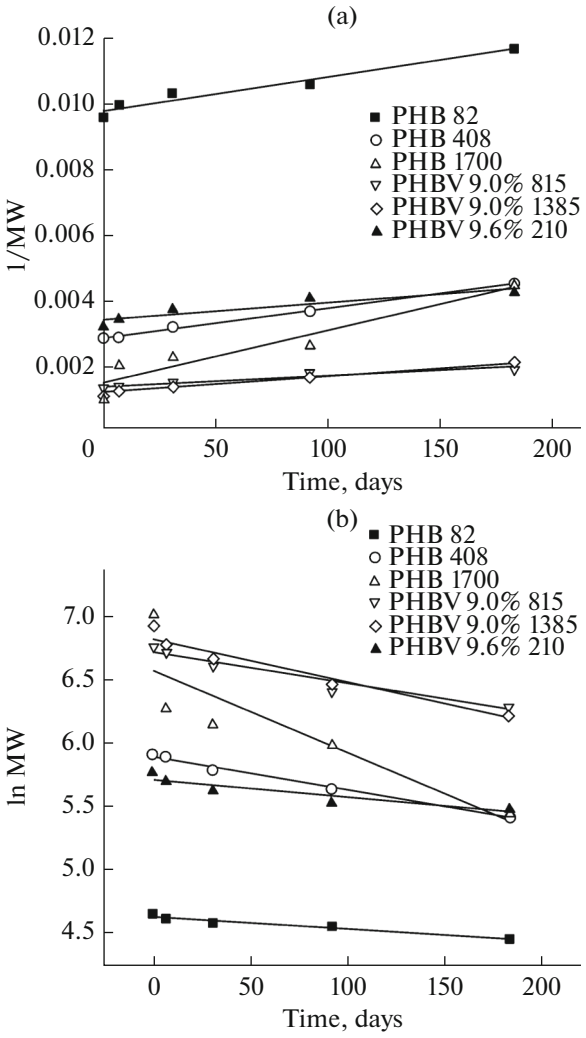

Fig. 3. (a) Noncatalytic degradation model, (b) autocatalytic degradation model.

[62]. According to our results, the degree of crystallinity of the studied polymers (except PHB 408) increased in the first week (Fig. 5). As an example, the degree of crystallinity increased from 65.9 to 67.4% for PHB 82 and from 62.8 to 66.5% for PHB 1700. For copolymers PHBV 9% 815, PHBV 9% 1385, and PHBV 9.6% 210, the degree of crystallinity increased

Table 2. Correlation coefficients for the noncatalytic and autocatalytic degradation models

Sample	R^2 (noncatalytic model)	R^2 (autocatalytic model)
PHB 82	0.94	0.93
PHB 408	0.99	0.98
PHB 1700	0.88	0.68
PHBV 9.6% 210	0.80	0.77
PHBV 9% 815	0.92	0.90
PHBV 9% 1385	0.96	0.90

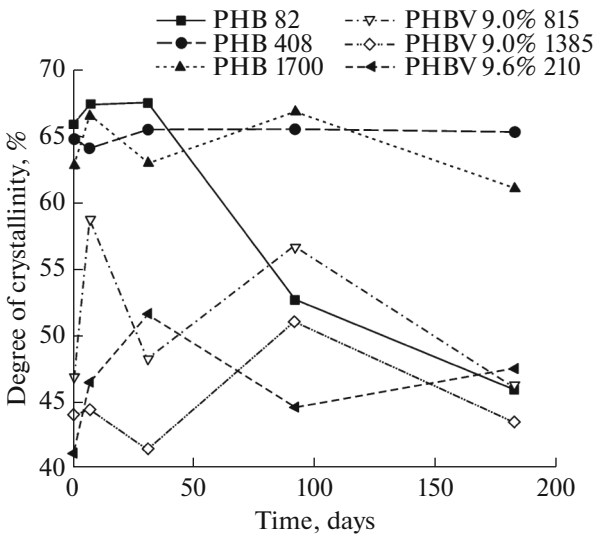

Fig. 4. Changes in the degree of crystallinity of PHA in the course of biodegradation in PBS.

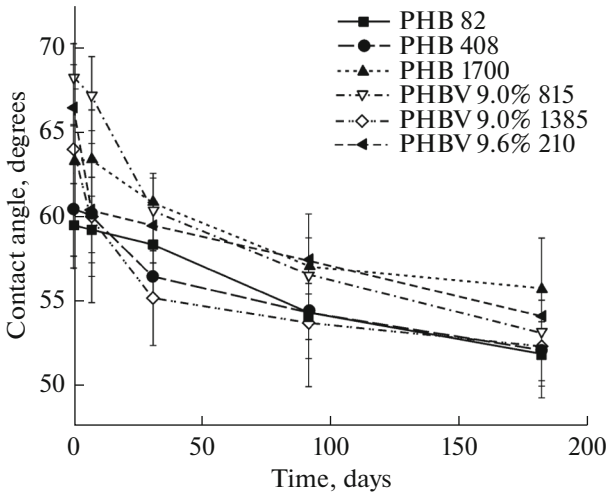

Fig. 5. Changes in the degree of hydrophobicity of the surface of PHA films.

to 58.7, 44.3, and 46.4%, respectively. Presumably, the initial increase in the degree of crystallinity is associated with the recrystallization of amorphous regions in the polymer by chain breaks in the initial period. Incubation of films for 1 month led to a decrease in the degree of crystallinity of polymers PHB 1700, PHBV 9% 815, PHBV 9% 1385, and PHBV 9.6% 210. The decrease in the degree of crystallinity is probably due to chain breaks that occur in the crystalline regions of the polymers at the later stages. A different pattern of changes in the degree of crystallinity was observed in PHB 408. After an initial slight decrease (from 64.7 to 64.1%) and subsequent slight increase (from 64.1 to 65.5%) it reached a relative plateau and then did not change for 180 days (Fig. 4). The degree of crystallinity of the low-molecular-weight PHB 82 also showed a different dependence: after incubation in phosphate buffer for 1 month, a decrease in the degree of crystallinity was observed up to the end of the experiment. Moreover, films of this polymer began to fall apart, indicating the disruption of the polymer structural integrity as a result of degradation.

In all other cases, a wavelike change in the degree of crystallinity was observed. This phenomenon can be explained by the chain break processes and subsequent recrystallization. Water molecules hydrolyzed ester bonds in the polymer chain to form highly mobile regions. Subsequently, these regions underwent recrystallization. Breaks in the crystalline parts of the chain may also occur [59].

These results were consistent with the data on the degradation of PHB and its copolymers obtained by other authors, who also observed an initial increase and subsequent decrease in the degree of crystallinity of polymers [56]. However, the curves illustrating the changes in the degree of crystallinity, which had a wavelike pattern, were first demonstrated in this study.

Changes in hydrophobicity of polymer films. The balance between hydrophobicity and hydrophilicity of the surface is one of the main parameters indicating surface biocompatibility. Biocompatibility is one of the most important characteristics of polymers that may be used in medicine because the degree of surface hydrophilicity is an important parameter for cell growth [63].

In the course of biodegradation, the contact angle between the water drop and the polymer film surface also decreased, which indicated that the degree of hydrophobicity of the studied polymers decreased; thus, their hydrophilicity increased (Fig. 5). The increase in the hydrophilicity of polymer films was probably due to the fact that the degradation of PHA films led to the cleavage of polymers on the surface with the release of polar terminal groups.

It should be noted that our data did not agree with the results on degradation of PHB and its copolymers obtained by other authors, who showed that the contact angle of PHB and PHBV films changed only slightly in the course of degradation [64]. This fact can be explained by the different conditions of film degradation.

CONCLUSIONS

In this study, changes in the characteristics of PHB and PHBV 9% of different molecular weights were investigated in great detail in the course of long-term hydrolytic degradation under model conditions in vitro. Polymer degradation was studied in PBS at 37°C for 183 days. A slight decrease in the weight of the studied polymers was found. However, changes in the molecular weight were much more significant: the

molecular weight of the high-molecular-weight PHB 1700 kDa decreased by 80%. Models of noncatalytic and autocatalytic degradation mechanism were used. As a result, it was shown that some of the polymers obeyed both autocatalytic and noncatalytic degradation mechanisms (PHB 82 and 408 kDa, PHBV 9% 815 and 1385 kDa), and only the noncatalytic mechanism was characteristic of the PHB 1700 and PHBV 9.6% 210 polymers. Changes in the degree of crystallinity of the polymers were characterized by periodicity: an initial increase was replaced by a subsequent decrease. However, the degree of crystallinity of PHB 408 kDa remained almost unchanged, whereas the degree of crystallinity of PHB 82 after the increase in the first week of incubation to 67.4% decreased to 45.9% within 6 months. A wavelike pattern of curves that illustrate the changes in the degree of crystallinity was also demonstrated for the first time. During biodegradation, films formed of the studied polymers became more hydrophilic. On the basis of the obtained physicochemical data on the degradation of PHB and its copolymers, it can be postulated that these polymers can be used to develop biodegradable medical devices that can perform their functions for a long period of time.

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REFERENCES

1. M. I. Artsis, A. P. Bonartsev, A. L. Iordanskii, et al., Mol. Cryst. Liq. Cryst. **555**, 232 (2012).
2. A. P. Bonartsev, G. A. Bonartseva, K. V. Shaitan, and M. P. Kirpichnikov, Biochem. (Mosc.) Suppl. Ser. B: Biomed. Chem. **5**, 10 (2011).
3. G.-Q. Chen and Q. Wu, Biomaterials **26**, 6565 (2005).
4. N. Pramanik, R. Das, T. Rath, and P. P. Kundu, Appl. Biochem. Biotechnol. **174**, 1613 (2014).
5. T. E. L. Douglas, G. Krawczyk, E. Pamula, et al., J. Tissue Eng. Regen. Med. **10**, 938 (2016).
6. V. Kaspárková, P. Humpolíček, Z. Capáková, et al., Colloids Surf. B: Biointerfaces **157**, 309 (2017).
7. M. M. Moisenovich, N. V. Malyuchenko, A. Y. Arkhipova, et al., Dokl. Biochem. Biophys. **463**, 232 (2015).
8. M. L. Ramiro-Gutiérrez, J. Will, A. R. Boccaccini, and A. Díaz-Cuenca, J. Biomed. Mater. Res. A **102**, 2982 (2014).
9. M. G. Raucci, M. A. Alvarez-Perez, C. Demitri, et al., J. Appl. Biomater. Funct. Mater. **10**, 302 (2012).
10. M. Stevanović, V. Pavlović, J. Petković, et al., Express Polym. Lett. **5**, 996 (2011).
11. Y. Zhang, J. Xu, Y. C. Ruan, et al., Nat. Med. **22**, 1160 (2016).
12. A. P. Bonartsev, I. I. Zharkova, S. G. Yakovlev, et al., J. Biomater. Tissue Eng. **6**, 42 (2016).
13. A. P. Bonartsev, G. A. Bonartseva, T. K. Makhina, et al., Appl. Biochem. Microbiol. **42**, 625 (2006).
14. A. A. Olkhov, O. V. Staroverova, A. P. Bonartsev, et al., Polym. Sci. Ser. D **8**, 100 (2015).
15. N. V. Andreeva, A. P. Bonartsev, I. I. Zharkova, et al., Bull. Exp. Biol. Med. **159**, 567 (2015).
16. S. K. Misra, T. I. Ansari, S. P. Valappil, et al., Biomaterials **31**, 2806 (2010).
17. T. Gredes, T. Gedrange, C. Hinüber, et al., Ann. Anat.–Anat. Anz. **199**, 36 (2015).
18. M. A. C. da Silva, R. N. Oliveira, R. H. Mendonça, et al., J. Biomed. Mater. Res. B: Appl. Biomater. **104**, 106 (2016).
19. A. P. Reyes, A. M. Torres, M. del P. Carreón Castro, et al., Sci. Rep. **6**, 31140 (2016).
20. E. I. Shishatskaya, I. V. Kamendov, S. I. Starosvetsky, et al., Artif. Cells Nanomed. Biotechnol. **42**, 344 (2014).
21. S. Ribeiro-Samy, N. A. Silva, V. M. Correlo, et al., Macromol. Biosci. **13**, 1576 (2013).
22. E. V. Filatova, S. G. Yakovlev, A. P. Bonartsev, et al., Appl. Biochem. Microbiol. **48**, 598 (2012).
23. V. A. Livshits, A. P. Bonartsev, A. L. Iordanskii, et al., Polym. Sci. Ser. B **51**, 256 (2009).
24. A. P. Bonartsev, S. G. Yakovlev, E. V. Filatova, et al., Biochem. (Mosc.) Suppl. Ser. B: Biomed. Chem. **6**, 42 (2012).
25. A. P. Bonartsev, A. L. Zernov, S. G. Yakovlev, et al., Anticancer Agents Med. Chem. **17**, 434 (2016).
26. F. M. Miroiu, N. Stefan, A. I. Visan, et al., Appl. Surf. Sci. **355**, 1123 (2015).
27. E. Biazar and S. Heidari Keshel, ASAIO J. **61**, 357 (2015).
28. A. C. Levine, G. W. Heberlig, and C. T. Nomura, Int. J. Biol. Macromol. **83**, 358 (2016).
29. A. P. Reyes, A. M. Torres, P. Carreón, et al., Nat. Publ. Gr. **6**, 1 (2016).
30. R. W. Lenz and R. H. Marchessault, Biomacromolecules **6**, 1 (2005).
31. R. N. Shirazi, F. Aldabbagh, W. Ronan, et al., J. Mater. Sci. Mater. Med. **27**, 154 (2016).
32. X. Song, F. Liu, and S. Yu, Catal. Today **276**, 145 (2016).
33. A. P. Bonartsev, A. P. Boskhomodgiev, A. L. Iordanskii, et al., Mol. Cryst. Liq. Cryst. **556**, 288 (2012).
34. I. I. Muhamad, L. K. Joon, and M. A. M. Noor, Malaysian Polym. J. **1**, 39 (2006).
35. N. Koyama and Y. Doi, Can. J. Microbiol. **41** (Suppl. 1), 316 (1995).
36. C. Kunze, H. E. Bernd, R. Androsch, et al., Biomaterials **27**, 192 (2006).
37. T. Freier, C. Kunze, C. Nischan, et al., Biomaterials **23**, 2649 (2002).

38. N. D. Miller and D. F. Williams, Biomaterials **8**, 129 (1987).
39. C. Doyle, E. T. Tanner, and W. Bonfield, Biomaterials **12**, 841 (1991).
40. S. Coskun, F. Korkusuz, and V. Hasirci, J. Biomater. Sci. Polym. Ed. **16**, 1485 (2005).
41. A. Bonartsev, S. Yakovlev, A. Boskhomdzhiev, et al., PLoS One **8**, e57200 (2013).
42. V. L. Myshkina, D. A. Nikolaeva, T. K. Makhina, et al., Appl. Biochem. Microbiol. **44**, 482 (2008).
43. V. L. Myshkina, E. A. Ivanov, D. A. Nikolaeva, et al., Appl. Biochem. Microbiol. **46**, 289 (2010).
44. A. P. Bonartsev, S. G. Yakovlev, I. I. Zharkova, et al., BMC Biochem. **14**, 12 (2013).
45. A. P. Bonartsev, I. I. Zharkova, S. G. Yakovlev, et al., Prep. Biochem. Biotechnol. **47**, 173 (2017).
46. G. A. Evans, Cell **61**, 17 (1990).
47. D. W. Hutmacher, Biomaterials **21**, 2529 (2000).
48. A. P. Boskhomdzhiev, A. P. Bonartsev, T. K. Makhina, et al., Biochem. (Mosc.) Suppl. Ser. B: Biomed. Chem. **4**, 177 (2010).
49. R. H. Marchessault, K. Okamura, and C. J. Su, Macromolecules **3**, 735 (1970).
50. S. R. Rafikov, V. P. Budtov, and Yu. B. Monakov, *Introduction to Physical Chemistry of Polymer Solutions* (Nauka, Moscow, 1978) [in Russian].
51. A. Göpferich, Biomaterials **17**, 103 (1996).
52. C. G. Pitt and Z. Gu, J. Control. Release **4**, 283 (1987).
53. A. P. Bonartsev, S. G. Yakovlev, I. I. Zharkova, et al., BMC Biochem. **14**, 12 (2013).
54. A. Bonartsev, S. Yakovlev, A. Boskhomdzhiev, et al., PLoS One **8**, e57200 (2013).
55. G. G. Choi, H. W. Kim, and Y. H. Rhee, J. Microbiol. **42**, 346 (2004).
56. J. Han, L. P. Wu, X. B. Liu, et al., Biomaterials **139**, 172 (2017).
57. K. Sudesh, H. Abe, and Y. Doi, Prog. Polym. Sci. **25**, 1503 (2000).
58. P. Kanmani, K. Kumaresan, J. Aravind, et al., Int. J. Environ. Sci. Technol. **13**, 1541 (2016).
59. *Modeling Degradation of Bioresorbable Polymeric Medical Devices*, Ed. by J. Pan (Elsevier, 2015).
60. N. A. Weir, F. J. Buchanan, J. F. Orr, and G. R. Dickson, Proc. Inst. Mech. Eng. Part H: J. Eng. Med. **218**, 307 (2004).
61. P. J. Barham, A. Keller, E. L. Otun, and P. A. Holmes, J. Mater. Sci. **19**, 2781 (1984).
62. W. J. Orts, R. H. Marchessault, and T. L. Bluhm, Macromolecules **24**, 6435 (1991).
63. M. Domínguez-Díaz, A. Meneses-Acosta, A. Romo-Uribe, et al., Eur. Polym. J. **63**, 101 (2015).
64. T. G. Volova, A. N. Boyandin, A. D. Vasiliev, et al., Polym. Degrad. Stab. **95**, 2350 (2010).

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