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Characterization of Polymeric Microparticles Based on Resorbable Polyesters of Oxyalkanoic Acids as a Platform for Deposition and Delivery of Drugs[1]

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Abstract—The effect of the preparation technique (chemical composition of a polymer, type and method of emulsion mixing, and molecular mass of a drug) on the yield, structure, and size of microparticles obtained from resorbable polyesters of microbiological origin, polyhydroxyalkanoates, is studied. It is found that the concentration of the polymer solution and the method of emulsion mixing are the most significant factors affecting the diameter of microparticles based on polyhydroxyalkanoates; the surface structure of particles depends to a higher extent on the chemical composition of the polymer. The family of microparticles from 100–200 nm to 50–70 μm in diameter is synthesized. It is shown that the rate of drug release from microparticles in vitro into the medium is higher in the case of 3-hydroxybutyrate copolymers with 3-hydroxyvalerate than in the case of the homopolymer of 3-hydroxybutyrate. This parameter increases with the content of 3-hydroxyvalerate units in the copolymer and the porosity and mass fraction of the drug in particles with a decrease in their sizes. For in vitro systems containing a phosphate buffer, variation in the preparation parameters makes it possible to obtain microparticles with various characteristics suitable for deposition of drugs. For microparticles obtained from polyhydroxyalkanoates and having different diameters, the mathematical description of the kinetics of drug release from the polymer matrix is provided.

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INTRODUCTION

The design of prolonged drug-delivery control systems (DDSs) is a topical and intensively developing direction in biotechnology and experimental pharmacology. At present, nearly 25% of the world sales volume of drugs fall to the share of drugs provided with transport–delivery systems [1]. This circumstance is due to the fact that such systems can prolong the action of drugs, increase the targeted capacity and bioavailability of drugs, and reduce the possible side effects of toxic drugs [2]. Prolonged-action drugs in the form of micro- and nanoparticles hold the greatest promise in this respect. They may be introduced into the blood flow, hypodermically, or intramuscularly and may be adapted for oral administration or inhalation [2, 3].

The key point in the design of prolonged drug systems is the search for a suitable material used as a carrier. Such materials should be absolutely harmless to humans and should possess a complex of special physicomechanical and biomedical properties. Of particular interest are biocompatible polymeric materials capable of bioresorption in vivo without formation of toxic products during destruction of the polymer matrix.

Polyhydroxyalkanoates (PHAs)—polymers of microbiological origin—deserve special attention among the many polymeric materials. These are biocompatible, bioresorbable, and thermoplastic linear polyesters that, in contrast to well-studied polylactides and polyglycolides, are characterized by a slow kinetics of biresorption; moreover, their destruction in biological media is not accompanied by a sharp change in the active response of a medium [4, 5].

The development of prolonged-drug-delivery control systems is especially urgent for the treatment of prolonged diseases. Stabilization of the desired concentration of a drug in the blood and/or tissues of a patient is frequently required in order to enhance the effect of drugs. The examples of such drugs are nonsteroid anti-inflammatory agents, antibiotics, and cytostatic preparations [6–10].

At present, prolonged-action drugs in the form of microparticles loaded with analgesics [11] and anti-

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inflammatory agents have been prepared on the basis of PHAs, and the kinetics of drug release has been studied [12–15]. It has been shown that PHAs can be used to design prolonged-action antibiotics shaped as microparticles, films, and bulky assemblies [16–19]. There are single examples of incorporation of protein compounds into composite PHA microparticles with poly(ethylene glycol) and polylactides [20].

Investigations performed at the Institute of Biophysics, Siberian Branch, Russian Academy of Sciences, revealed the high biocompatibility of high-purity PHA samples at cellular and tissue levels, including contact with blood, as well as their applicability for the design of endoprostheses of various kinds, as matrices of functioning cells, and for deposition of drugs [21, 22]. It has been shown that drugs can be deposited in PHA-based polymer carriers in the form of tablets, films, and microparticles and that the latter can be introduced intramuscularly, hypodermically, or intravenously [23–25]. The efficiency of drug action of microparticles loaded with a cytostatic drug was demonstrated on laboratory animals inoculated with the Ehrlich ascites carcinoma [26].

The goal of this study is to perform the comparative examination of polymer microparticles obtained from PHAs with the use of various methods and to ascertain factors determining their basic characteristics.

EXPERIMENTAL

Microparticles were prepared with the use of two kinds of PHA samples: the homopolymer of 3-hydroxybutyrate (PHB) and the copolymer of 3-hydroxybutyrate and 3-hydroxyvalerate (PHB–PHV) containing different amounts of hydroxyvalerate units (10.5, 20.0, and 37.0 mol %) (Table 1). The composition of PHAs was determined after preliminary methanolysis of samples through chromatography of methyl esters of fatty acids on a GCD Plus gas chromatograph–mass spectrometer (Hewlett Packard, Unites States). Parameters M_w and M_n and the molecular-mass distribution of PHAs were estimated with the use of a Waters Breeze System gel-permeation chromatograph (Waters, Unites States) relative to polystyrene standards (Sigma, Unites States).

Microparticles were obtained via solvent evaporation from two- (water–oil) and three-component (water–oil–water) emulsions. The two-component emulsion contained a 4% solution of the polymer and a 0.5% solution of PVA. The three-component emulsions were prepared through addition of an aqueous solution of gelatin to the polymer solution.

Polymer emulsions were mixed with a Heipolph RZR1 overhead-drive three-blade stirrer (Germany) at various stirring rates (300–1000 rpm), an IKA Ultra-Turrax T25 high-velocity homogenizer (Germany) (20000 rpm), and a Sonicator S3000 ultrasonic generator (United States). The ultrasound power was varied from 12 to 20 V, and the treatment duration was in the range 60–300 s. Emulsions were stabilized with various types of surfactants: PVA, polyoxyethylene (20) sorbitan monooleate (Tween® 80), and sodium dodecyl sulfate (SDS).

Emulsions were allowed to stay for a day under continuous mechanical mixing until full evaporation of the solvent was attained. The as-formed microparticles were collected through centrifugation (10000 rpm, 5 min), washed six times with distilled water, and dried on a LS-500 lyophilizer (Russia).

The yield of microparticles, a, was calculated as a percentage with respect to the weight of the polymer used for their preparation:

$$a = \frac{m_M}{m_P} \times 100\%,$$

where m_M is the weight of microparticles (mg) and m_P is the weight of the used polymer (mg).

The morphology of particles was analyzed on a scanning electron microscope (FEI Company Quanta 20, United States). The diameters and size-distribution of microparticles less than 3 μm in diameter were studied with the use of electron microscopy; particles 3 μm or greater in diameter were studied on a Casy® Automated Particle Counter (Scharle System GmBH, Germany).

In order to elaborate the technique of drug deposition in polymer microparticles, Hoffmann's violet dye (triethylrosalinine hydrochloride, $M = 338$) and antibiotics with different molecular masses and degrees of solubility in aqueous solutions—rubomycin hydrochloride ($M = 564$, a solubility in water of 0.04 mg/ml), rifampicin ($M = 823$, a solubility in water of 1.4 mg/ml), vancomycin ($M = 1440$, a solubility in water of 100 mg/ml), and tienam ($M = 320$, a solubility in water of 10 mg/ml)—were used (Table 1). The choice of the drugs is associated with their demand in clinical practice for treatment of prolonged diseases, their stability in solution, and their feasibility of drug mixing with nonpolar solvents with any change in the properties of the drugs.

In the case of the two-component emulsion, the drugs were preliminarily dissolved in dichloromethane and, then, the weighed potion of the polymer was added to the resulting solution. To obtain the three-component emulsion, the polymer was dissolved in dichloromethane, and the aqueous solution of the antibiotic was added to the resulting solution. The system was stirred until an emulsion was obtained, and the solution of PVA was slowly added.

Microparticles were loaded with rubomycin with the use of a three-component emulsion composed of a PHB solution in dichloromethane and poly(ethylene glycol) with $M_w = 4 \times 10^4$ (PEG-40) taken at a ratio of PHB : PEG = 80 : 20 (w/w). To the as-prepared solution, 1 ml of an aqueous solution of rubomycin (1% with respect to the weight of the polymer carrier) was added, and the resulting solution was homogenized for 1 min with the aid of ultrasound with a power of 12 W.

Table 1. Materials and drugs used in this study

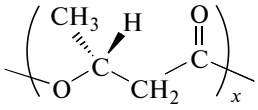
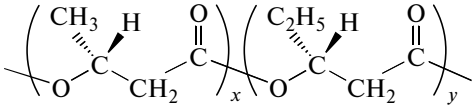
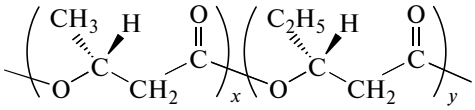
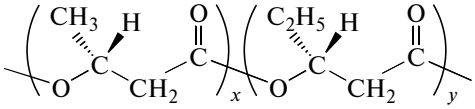
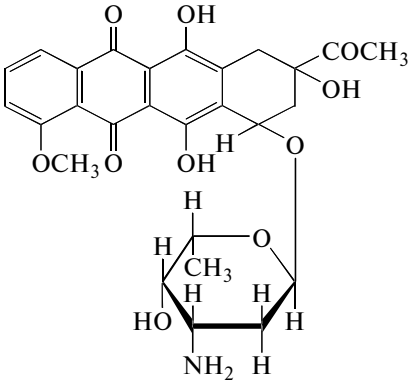
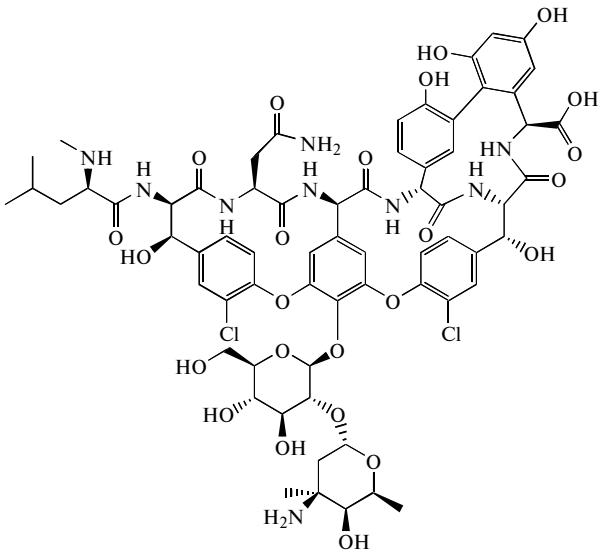
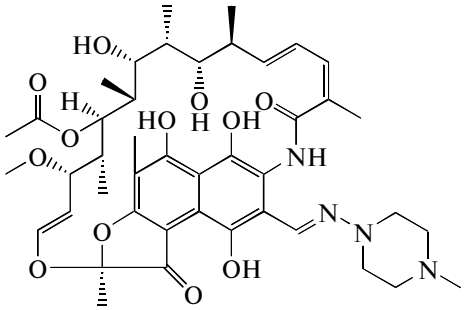
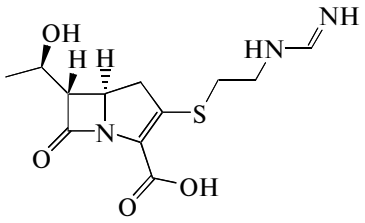
Substance	Formula	MM	Manufacturer
PHB		780000***, 487000****	Institute of Biophysics, Siberian Branch, Russian Academy of Sciences (RF Patent No. 2051967)
PHB–PHV (10.5)*		840000***, 193000****	Institute of Biophysics, Siberian Branch, Russian Academy of Sciences (RF Patent No. 2051968)
PHB–PHV (20.0)*		663000***, 292000****	
PHB–PHV (37.0)*		1026000***, 530000****	
Rubomycin (daunorubimin, rubomycin hydrochloride) (0.04)**		564	FAO Ferain, Russia
Vancomycin (>100)**		1440	Lek Farma, Russia

Table 1. (Contd.)

Substance	Formula	MM	Manufacturer
Rifampicin (1.4)**		823	FAO Ferain, Russia
Tienam (imipinem) (10)**		658	"Merck Sharp and Dohme", Netherlands

* Content of HV units in the copolymer (mol %).
** Solubility in water (mg/ml).
*** M_w of PHA.
**** M_n of PHA.

To the as-prepared emulsion, a 0.5% solution of PVA was slowly added; the polymer system was stirred at 30°C with the Heipolph RZR1 three-blade stirrer (Germany) until full evaporation of the solvent was achieved.

The degree of drug incorporation into the polymer matrix was determined through spectrophometric investigation of its initial and residual concentrations in the emulsion (Uvicon 943 spectrophotometer, Italy).

The efficiency of encapsulation, e, of the drug in the polymer matrix was calculated through the formula

$$e = \frac{m_{enc}}{m_{ini}} \times 100\%,$$

where m_{enc} is the weight of the encapsulated drug in the polymer matrix (mg) and m_{ini} is the initial weight of the drug (mg).

PHA-based microparticles loaded with drugs were sterilized with UV radiation for 20 min and placed in sterile capped centrifuge tubes containing 5 ml of a balanced phosphate buffer; the tubes were exposed at 37°C in a thermostat. In order to determine the amount of the drug released to a medium, microparticles were preliminarily precipitated from the samples via centrifugation (10 000 rpm, 5 min) and the as-prepared samples were analyzed on the spectrophotometer (Uvicon 943, Italy).

The release of drug, r_d, into the phosphate buffer was determined through the formula

$$r_d = \frac{R}{enc} \times 100\%,$$

where enc is the value of encapsulation of the drug in the polymer matrix (mg/mg) and R is the release of the drug (mg/mg).

The theoretical analysis of the experimental data on the release of drugs into a medium and on the quantitative determination of coefficients of diffusion in the polymer phase was performed through the graphical solution of equations in $(m_t/m_\infty) - t^{0.5}$ coordinates and $\ln(1 - m_t/m_\infty)$–time t semilogarithmic coordinates.

Statistical treatment of the results was performed with the programs Microsoft Excel and StatPlus. The arithmetic mean, rms deviation, and error of the arithmetic mean were calculated. The reliability of variance of mean values was verified through the Mann–Whitney U test (a significance level of 0.05).

RESULTS AND DISCUSSION

Properties of Microparticles Obtained from PHAs

The most important parameters determining the properties of microparticles and the kinetics of drug release from them are their sizes and their degrees of surface development. As opposed to particles formed from the two-component emulsion, which were loose

Table 2. Preparation conditions and characteristics of microparticles (a solution concentration of 4%, a PVA concentration of 0.5%)

PHA	Dispersion technique		Average diameter, μm	Yield of microparticles, %
	ultrasound, W (dispersion duration, min)	mechanical stirring, rpm		
PHB*	–	300	7.5 ± 0.6	74.3 ± 2.4
PHB**	–	300	12.2 ± 0.9	72.7 ± 1.5
PHB	–	300	16.0 ± 0.86	68.3 ± 1.8
PHB	–	500	9.61 ± 0.9	73.5 ± 3.8
PHB	–	1000	5.57 ± 0.8	78.6 ± 6.2
PHB	–	1000	4.25 ± 0.27	67.7 ± 3.3
ПГБ***	–	1000	13.3 ± 0.7	58.0 ± 3.4
PHB	–	1000	7.09 ± 0.4	75.4 ± 4.5
PHB*	–	20000	0.39 ± 0.08	84.6 ± 6.5
PHB	12 (1 min)	–	2.5 ± 0.14	57.0 ± 4.5
PHB	16 (1 min)	–	1.74 ± 0.13	62.0 ± 5.1
PHB	20 (1 min)	–	1.2 ± 0.08	61.0 ± 5.4
PHB*	20 (5 min)	–	0.36 ± 0.07	64.0 ± 4.8
PHB–PHV (10.5)	–	1000	4.39 ± 0.2	78.0 ± 2.2
PHB–PHV (20.0)	–	1000	4.45 ± 0.3	76.4 ± 3.2
PHB–PHV (37.0)	–	1000	4.1 ± 0.2	74.4 ± 4.7
PHB–PEG-40	–	1000	4.73 ± 0.6	69.8 ± 4.6
PHB–PHV–PEG-40 (10.5)	–	1000	4.1 ± 0.6	73.7 ± 4.5
PHB–PHV–PEG-40 (20.0)	–	1000	3.54 ± 0.5	75.2 ± 4.7
PHB–PHV–PEG-40 (37.0)	–	1000	3.6 ± 0.6	64.0 ± 5.1

Note: In the first column, the content of PHB units (mol %) is shown in parentheses.
* 1% PHB solution.
** 2% PHB solution.
*** Surfactant: 0.5% Tween®80.

and had small deformations of the surface in the form of cavities, microparticles obtained from the three-component emulsion were regular spheres. The diameters of microparticles in both variants were in the range 0.5–70 μm (Table 2). The average diameter of microparticles obtained from the two-component emulsion was 14.31 ± 1.4 μm, which is close to the diameter of particles derived from the three-component emulsion, 12.53 ± 1.1 μm.

A comparative analysis of the characteristics of the microparticles showed that the concentration of a polymer solution and the method of emulsion mixing are the most significant factors responsible for the diameters of microparticles. Thus, because of a gain in viscosity, an increase in the concentration of the polymer in solution resulted in the enlargement of microparticles. At a solution density of 10 g/l, the average diameter of microparticles was 7.5 ± 0.6 μm; at 40 g/l, the average diameter of microparticles increased to 16.0 ± 0.9 μm. In addition, the solution density affected the microparticle yield: With a decrease in the polymer concentration to 10–20 g/l, the microparticle yield reliably increased to 73% (Table 2).

The rate of mixing the polymer emulsion exerted the most pronounced effect on the sizes of microparticles. As the rate of emulsion mixing increased from 300 to 1000 rpm, the average diameter of microparticles decreased by a factor of nearly 3 to a value of 5.57 ± 0.8 μm (Figs. 1a, 1b). Variation in the conditions of mixing of the polymer emulsion made it pos-

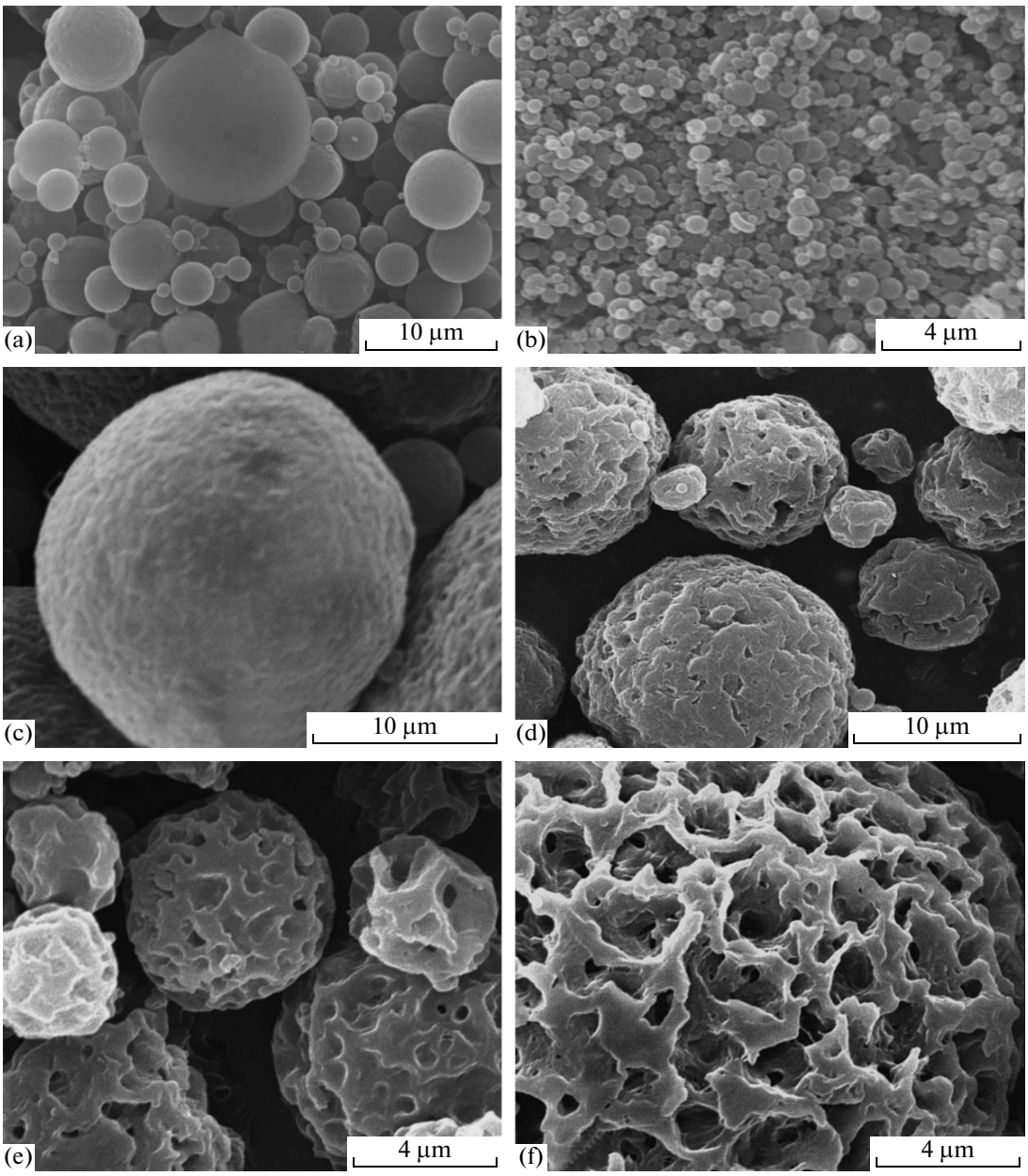


Fig. 1. SEM images of PHA microparticles: (a, b) PHB at rates of emulsion stirring of (a) 300 and (b) 20000 rpm; (c) PHB and (d) PHB–PHV (37 mol % HB units) at a rate of emulsion stirring of 1000 rpm; (e, f) microparticles obtained via mechanical stirring of the two-component emulsion containing 20% PEG-40 and either (e) PHB–PHV (20 mol % HV units) or (f) PHB–PHV (37 mol % HV units).

sible to obtain microparticles less than 1 μm in diameter. At a high rate of mixing or a high power of UV treatment, the particles were more homogeneous in size and their average diameter was on the order of 0.36–0.39 μm. It should be emphasized that microparticles obtained via sonification were characterized by insignificant deformations of the surface and their total yield was a factor of 1.5 lower than the yield of particles from emulsion under mechanical stirring (Table 2). In this case, as opposed to the mechanical stirring, during which the shape of microparticles forms and stabilizes gradually [27], the worse quality of microparticles might be related to heating of the emulsion and quicker evaporation of the solvent.

It was found that, during dissolution of PHAs and formation of emulsions, the molecular mass declined. Regardless of the technique of emulsion mixing, the molecular mass of the polymer for the formed microparticles was 30–35% lower than the corresponding value for initial polymer samples. Gel-permeation-chromatography measurements showed that the molecular mass of the polymer matrix of particles

obtained under mechanical stirring decreased to 54.6×10^4 (compared to 78.0×10^4 for the initial polymer). Sonification of the polymer emulsion exerted the same effect on the molecular mass of the polymer.

With consideration for the known data on the advantageous effect of polymeric stabilizers on the properties of microparticles [28], various surfactants were investigated and it was shown that they substantially affect the quality and yield of microparticles (Table 2). When PVA and SDS were added to the polymer emulsion, microparticles were regular spheres with an average diameter of 5.5–7.0 μm. In the case of Tween® 80, large conglomerates formed and the total yield of particles was lower (~60%). Note that an increase in the concentration of PVA from 5 to 10 g/l improved the stability of the water–oil emulsion and entailed a decrease in the average diameter of particles to 4 μm (Table 2).

In addition, the yield and diameter of microparticles were influenced by the chemical composition of the polymer. A comparative study of particles derived from a highly crystalline polymer of 3-hydroxybutyric acid and of a number of its copolymers containing different amounts of 3-hydroxyvaleric acid units was performed. Microparticles based on the PHB–PHV copolymer were regular spheres with rough and porous surfaces; the relief of the surface made itself evident during an increase in the content of 3-hydroxyvalerate in the copolymer (Figs. 1c, 1d). Although the content of 3-hydroxyvalerate units in the copolymer affected the surface structure of microparticles, it has no effect on their diameters. The average diameter of microparticles obtained from the PHB–PHV copolymer, regardless of its comonomer ratio, was 4.10–4.45 μm. These values corresponds to the sizes of microparticles obtained from the homogeneous PHB (5.57 ± 0.8 μm) (Table 2).

Complication of the composition of the polymer system via addition of the second chemical compound (20% of PEG-40 was added based on the weight of the polymer carrier) affected the surface structure of microparticles but had practically no effect on their sizes (Table 2). Thus, when PEG-40 was added to the solution of a PHB–PHV copolymer (the contents of 3-hydroxyvalerate units were 10.5, 20.0, and 37.0 mol %), the surfaces of microparticles acquired an uneven porous structure with pore diameters from 1 to 3 μm (Figs. 1e, 1f). The surfaces of microparticles obtained from PHB and a small amount of PEG-40 were less rough and contained small cavities and single pores. An increase in the temperature of emulsion facilitated an increase in porosity. When the PHB emulsion containing PEG-40 was heated to 30°C, porous particles with pore diameters of 3.5–4.0 μm were obtained.

During incorporation of drugs with different chemical structures and properties into the polymer matrix of microparticles, no changes in the surface structure of particles were observed. Thus, microparticles loaded with different drugs had folded surfaces with small cavities and pores.

The efficiency of encapsulation of drugs, e, in the matrix of microparticles depended on their preparation technique and on the weight fraction of the drug in the polymer solution–emulsion (Table 3). In the case of Hoffmann's violet dye, with an increase in its amount in the polymer matrix of microparticles from 1 to 10% with respect to the weight of the polymer matrix, the average diameter of microparticles increased from 5.8 ± 0.8 to 8.3 ± 1.1 μm, while e decreased from 78.0 ± 4.4 to 56.0 ± 3.5%.

Experiments with rubomycin hydrochloride showed that the value of e depends on the technique of preparation of microparticles. In the case of the three-component emulsion, the value of e for this drug was almost 1.5 times lower than that for microparticles prepared with the use of the two-component emulsion (Table 3). Heating of the three-component emulsion during deposition of rubomycin resulted in a decline in e to 53.0 ± 3.2%. As was found, the value of e was influenced by the chemical composition of the drug.

During encapsulation of high-molecular-mass antibiotics (for vancomycin, $M = 1440$ and, for rifampicin, $M = 823$), the values of e were 70 and 65.5%, respectively. On average, these values were a factor of 1.3 higher than the corresponding parameters for low-molecular-mass antibiotics (for rubomycin, $M = 564$ and, for tienam, $M = 320$) (Table 3).

The yield of microparticles loaded with various drugs was, on the whole, lower (~65%) than the yield from the emulsion of the unloaded particles (85%) (Tables 2, 3). In this case, a decline in the yield of microparticles is probably associated with a decrease in the surfactant properties of the aqueous solution of PVA after addition of drugs to the polymer system and possibly with a reduction in the stability of the water–oil emulsion and an increase in the probability of merging of minute microparticles into coarser conglomerates [28].

Dynamics of Drug Release from Polymer Microparticles

To estimate the dynamics of drug release from the polymer matrix, particles obtained from PHB and PHB–PHV copolymers having various diameters and loaded with various drugs to different extents were investigated.

As is known from the literature data, the release of drugs from prolonged polymer systems may occur via diffusion, during which drugs move to the edge of a polymer article and then pass to the external medium [29]. As regards the used PHAs, it is noteworthy that PHB and the PHB–PHV copolymer under in vitro conditions in the absence of biological factors (enzymes, cells) do not degrade [30]; therefore, the release of drugs from PHA carriers obeys the laws of chemical reactions and is independent of the state of the carrier material.

Table 3. Characteristics of PHA-based microparticles with deposited drugs

Composition of microparticles	Composition of emulsion	Drug concentration, %	*e*, %	Average diameter of microparticles, μm
PHB–dye	Water–oil	1	78.3 ± 4.4	5.8 ± 0.8
		5	65.6 ± 5.08	7.21 ± 0.7
		10	56.3 ± 3.5	8.34 ± 1.1
PHB–PEG-40–dye	Water–oil	1	64.0 ± 3.6	5.5 ± 0.8
PHB–PHV (10 mol %)	Water–oil	1	73.5 ± 5.2	4.3 ± 0.6
PHB–PHV (37 mol %)	Water–oil	1	69.3 ± 4.5	4.1 ± 0.9
PHB–Rubomycin	Water–oil	1	82.3 ± 6.4	5.67 ± 0.7
PHB–Rubomycin	Water–oil–water	1	65.2 ± 3.6	5.45 ± 0.4
		1	52.8 ± 3.2	8.9 ± 0.7
		5	57.5 ± 4.6	6.73 ± 0.9
PHB–Rifampicin	Water–oil–water	5	65.5 ± 5.1	7.86 ± 0.9
PHB–Vancomycin	Water–oil–water	5	70.1 ± 3.5	8.91 ± 1.3
PHB–Tienam	Water–oil–water	5	53.0 ± 4.5	6.43 ± 0.8

The experimental curves of drug release from microparticles into the medium are shown in Figs. 2–4. As is seen, all curves have a two-step pattern. At the first step (5–8 days), the concentration of the drug in the medium increased and, beginning from 6–9 days, it changed insignificantly.

As was shown for Hoffmann's violet dye, the release of the drug was affected by the composition of the polymer used to synthesize microparticles and to influence their characteristics. Thus, during the study of dye release from microparticles obtained from PHB, from PHB–PHV (10 mol %), and from PHB–PHV (37 mol %), 1.98 ± 0.29, 3.9 ± 0.18, and 5.17 ± 0.57% of the drug with respect to the incorporated amount of the drug were registered in the medium during the first day. Then (during the next six days), these differences were less defined: 7.85 ± 0.6, 10.0 ± 0.9, and 10.9 ± 0.6%, respectively. By the end of the experiment, the total release of the dye from the microparticles based on the copolymer was almost

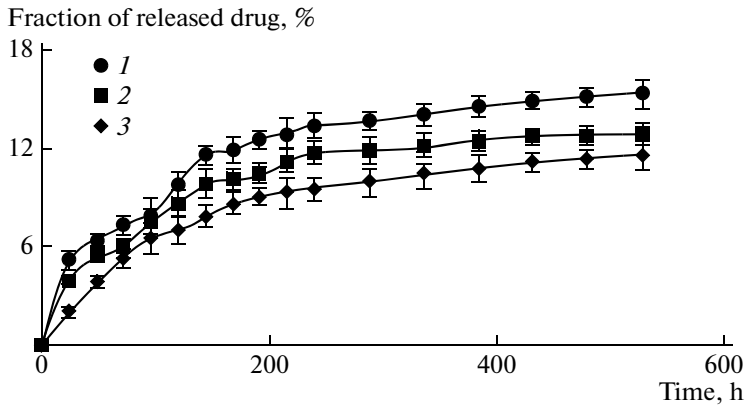


Fig. 2. Dynamics of release of Hoffmann's violet dye from (3) PHA microparticles and (*1, 2*) PHB–PHV copolymers containing (*2*) 10 and (*3*) 37 mol % HV units. The content of the dye is 1% with respect to the weight of the polymer matrix.

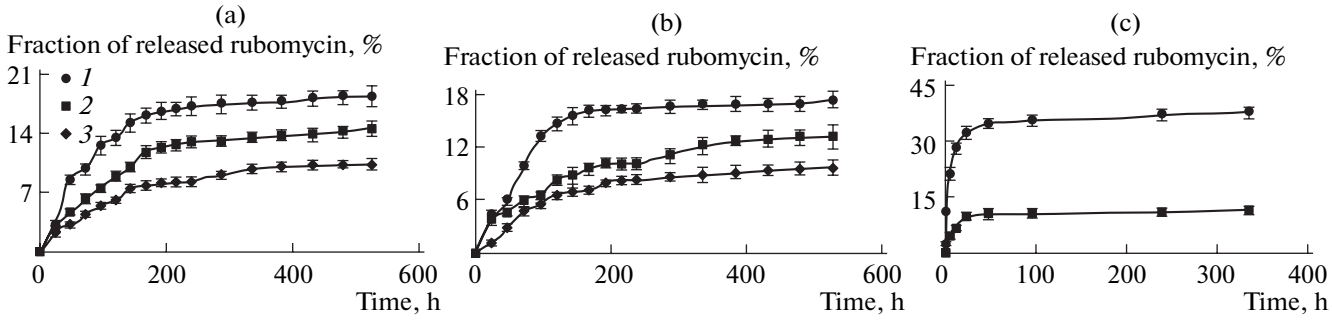


Fig. 3. Dynamics of rubomycin release from (a) PHB microparticles with different levels of antibiotic loading, (b) PHB microparticles of different diameters, and (c) PHB microparticles obtained with the addition of 20% PEG-40. (a) The contents of the antibiotic are (*1*) 1, (*2*) 5, and (*3*) 10% with respect to the weight of the polymer matrix; (b) the particle diameters are (*1*) 3.6, (2) 10.2, and (*3*) 15.6 μm; and (c) (*1*) PHB–PEG-40 and (*2*) PHB.

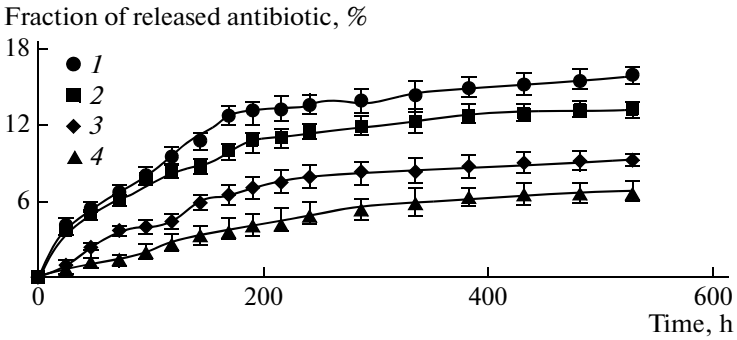


Fig. 4. Dynamics of antibiotic release from PHB microparticles: (*1*) tienam, (*2*) rubomycin, (*3*) rifampicin, and (*4*) vancomycin.

1.5 higher than that in the case of microparticles based on PHB (Fig. 2).

The kinetics of drug release was affected by the degree of loading of the matrix. As is seen from Fig. 3, the higher the level of incorporation of the drug into the matrix, the more pronounced the release of rubomycin from PHB microparticles. At maximum loading and minimum loading of the matrix, the levels of release of the antibiotic during the first day were 1.5 ± 0.3 and 3.6 ± 0.4% (with respect to the weight of the incorporated antibiotic), respectively. However, beginning from the fifth day, the curves reached a plateau. Then, the concentration of rubomycin in the model system remained practically unchanged. By the end of the experiment, the contents of the antibiotic in the medium were 9.5 ± 0.9, 13.6 ± 0.98, and 17.3 ± 0.8%, respectively, at initial levels of loading of microparticles of 1, 5, and 10% (with respect to the weight of the polymer carrier).

The kinetics of rubomycin release from microparticles having different diameters but containing the same amounts of the antibiotic is presented in Fig. 3b. During the initial period (first–fourth days from the onset of the experiment), a gradual release of the drug was observed and, then, the release of the antibiotic remained practically unchanged (6th to 22nd days). The release of rubomycin into medium depended on the microparticle diameter: The smaller the particles, the higher the release. For microparticles 3.6 μm in diameter, the release of rubomycin was 18.3 ± 1.1%; for particles 10.2 and 15.6 μm in diameter, the levels of release of rubomycin were 14.5 ± 0.9 and 10.3 ± 0.6% with respect to the incorporated drug, respectively. In addition, the release of rubomycin into the medium depended on the surface structure of microparticles: The higher the porosity of particles, the higher the release. As is seen from Fig. 3c, during the first day, the release of rubomycin from porous particles (particles with pore diameters of 3.5–4 μm) was 28.4 ± 3.04%, whereas in the second variant (particles having the folded structure with small cavities), the release did not exceed 1.8 ± 0.06%. For 14 days, the release of rubomycin from porous particles was 49.03 ± 1.1%, while in the case of nonporous particles, the release of the antibiotic did not exceed 8.5 ± 0.26% with respect to the incorporated drug.

The dynamics of drug release from microparticles was affected by the molecular mass of the drug: The higher the molecular mass, the slower and more even the release of the drug (Fig. 4). Higher molecular mass antibiotics (rifampicin, vancomycin) featured similar curves. After a day, the levels of release of these drugs were 1.02 ± 0.08 and 0.57 ± 0.06%, respectively; by the

end of the experiment (22nd day), 8.15 ± 0.7 and 6.8 ± 0.5%, respectively. For tienam and rubomycin, which have lower molecular masses, during the first day, the values of release were 1.28 ± 0.1 and 1.20 ± 0.08% with respect to the weight of the drug incorporated into the matrix. By the end of the experiment, these values were 15.81 ± 1.1 and 13.06 ± 0.80%, respectively.

Note that the rate of antibiotic release may depend also on the chemical nature of the drug. Thus, for tienam, the antibiotic having a higher solubility in water, the release was higher than that in the case of rifampicin, for which release was almost seven times lower. A different picture was observed in the case of vancomycin and rubomycin. This result is apparently due to the fact that they contain functional groups capable of forming hydrogen bonds with the hydroxyl groups of the polymer. Similar data were obtained when the drugs diclofenac and gentamicin were deposited into the PHA polymer matrix [31, 32].

An examination of the dependences given in Figs. 2–4 shows that the release of deposited drugs from PHA microparticles occurs via diffusion. During the first period, the drugs are released from the surface structures of the carrier; this period lasts from 5 to 8 days, depending on the properties of the polymer matrix. The second segment of the curve (beginning from 6–9 days and up to the end of the experiment) corresponds to the release of the drug from the internal structures of the matrix, including release due to a slow and insignificant change in the molecular mass of the polymer. Thus, our experiments demonstrated that, during the studied period, M_w of the polymer used to prepare microparticles decreased from 54.6×10^4 to $(49.0–46.7) \times 10^4$. The drop of the molecular mass of microparticles on average was no higher than 10–15% with respect to the initial molecular mass of microparticles.

The two-step pattern of drug release from PHA microparticles can be described by the diffusion–kinetic equations advanced in [33]. The first step is characterized by the diffusion mechanism of drug release from the surface of the polymer matrix. The second step is associated with the release of the drug from the internal structures of the matrix and the onset of hydrolytic processes in the microporous medium and is characterized by the zero-order reaction:

$$\frac{\partial c_s}{\partial_t} = D_s\left(\frac{\partial^2 c_s}{\partial x^2}\right) + K, \quad (1)$$

where D_s is the effective diffusion coefficient (cm/s), K is the kinetic constant of the hydrolytic degradation of the polymer (s^{-1}), and c_s is the antibiotic concentration (%).

To exclude the linear contribution related to an insignificant reduction in the molecular mass of the polymer from the total concentration of the released antibiotic, we introduced the variable

$$G_t = c_s - Kt, \quad (2)$$

where G_t is the amount of antibiotic (%) released via the diffusion mechanism during time t.

Then, the diffusion–kinetic equation assumes the traditional form:

$$\frac{\partial c_s}{d_t} = D_s\left(\frac{\partial^2 c_s}{dx^2}\right). \quad (3)$$

If there is no concentration difference at the solid-particle/liquid-phase interface, the latter equation characterizes the mechanism of drug desorption from a microparticle.

At a small time of antibiotic desorption at the initial level $m_t/m_\infty \le 0.5$, the release of the drug may be described by Fick's equation:

$$\frac{m_t}{m_\infty} = 4\sqrt{\frac{D_t}{\pi h^2}}. \quad (4)$$

Taking into account that particles are spherical in shape, matrix thickness h may be replaced with particle diameter d. Then,

$$\frac{m_t}{m_\infty} = 4\sqrt{\frac{D_t}{\pi d^2}}, \quad (5)$$

where D_t is the diffusion coefficient, $\frac{m_t}{m_\infty}$ is the total release of the drug at time $t(m_t)$ and at finite time $t \to \infty$ (m_∞), d is the diameter of a matrix (microparticle), and t is time.

At the initial section of the drug release curve, the diffusion coefficient is

$$D_\beta = \frac{\pi d^2(\tan\beta)^2}{16}, \quad (6)$$

where $\tan\beta$ is the slope of the linear section of the kinetic curve in (m_t/m_∞)–$t^{0.5}$ coordinates.

The linear sections of kinetic curves plotted in semilogarithmic coordinates make it possible to calculate slope $\tan\alpha$ and then the diffusion coefficient:

$$D_\alpha = \frac{d^2(\tan\alpha)^2}{\pi^2}, \quad (7)$$

where $\tan\alpha$ is the slope of the kinetic curve in $\ln(1 - m_t/m_\infty)$ semilogarithmic coordinates.

The graphical solution of equations in (m_t/m_∞)–$(t)^{0.5}$ coordinates and in $\ln(1 - (m_t/m_\infty)$ semilogarithmic coordinates allowed quantitative determination of the diffusion coefficient for the drug in the polymer phase (Fig. 5).

Table 4 lists the diffusion coefficients for rubomycin in PHB microparticles. It is clear that there is a

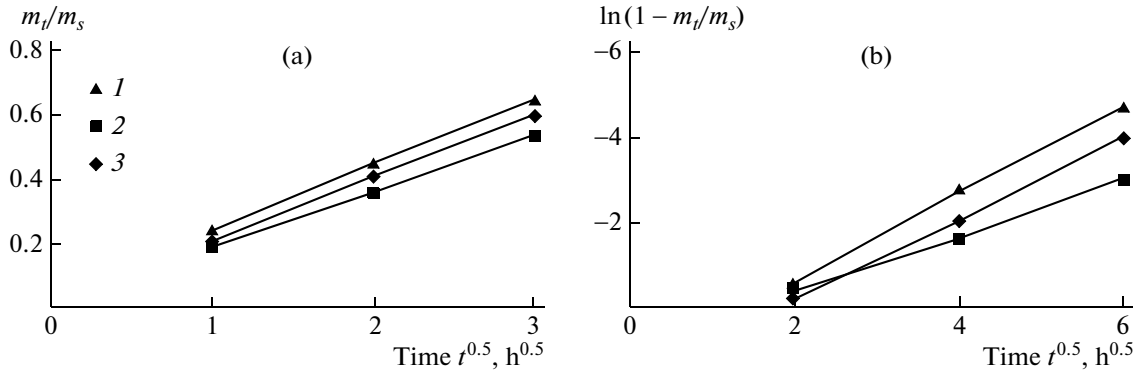


Fig. 5. (a) Initial and (b) final sections of the kinetic curve for rubomycin release from PHB microparticles. The microparticle diameters are (*1*) 15.6, (*2*) 3.6, and (*3*) 10.2 μm.

well-defined dependence of the diffusion coefficients on the microparticle sizes. Thus, at the first stage, the diffusion coefficient for microparticles 3.6 μm in diameter is an order of magnitude higher than that for large microparticles (Table 4, Eq. (6)). At the second stage (the curve reaches a plateau), the diffusion coefficient declines by an order of magnitude, regardless of the microparticle diameter.

The data described above suggest that the classical diffusion mechanism controls the kinetics of drug release from the PHB-based polymer matrix.

CONCLUSIONS

Our studies have ascertained factors affecting the properties of PHA-based microparticles: namely, the chemical composition of polymers, the type of the polymer system, and the technique of mixing. Variation in these parameters allows preparation of microparticles having various diameters and degrees of porosity suitable for drug deposition. The absence of sharp surges of the drug into the medium and the low rates of drug release from microparticles based on PHB and PHB–PHV copolymers make it possible to state that microparticles are useful for the deposition of drugs. In the case of microparticles with different diameters, the mathematical description of the mechanism controlling the release of drugs from the polymer matrix in the form of PHA-based microparticles has been derived. Further variation in the conditions of designing of PHA carriers and improvement of available techniques will make it possible to control the rate of drug release from microparticles and to begin experiments in vivo.

Table 4. Diffusion coefficients of rubomycin in PHB microparticles determining the initial and final stages of the diffusion process

Particle diameter, $d \times 10^{-4}$, cm	Diffusion coefficients $D \times 10^{-4}$, cm/s	
	at initial stage	at final stage
3.6	9.90	0.0260
10.2	0.64	0.0610
15.6	0.02	0.0067

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