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Biocompatible Systems for Controlled Delivery of Antiseptics for Topical Application

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Abstract. Controlled drug delivery is one of the frontier areas of science, which uses an interdisciplinary approach. The delivery systems offer numerous advantages over conventional dosage forms, such as improved efficacy and patient compatibility, reduced toxicity, and ease of use. Such systems often use micro- and nanoparticles as carriers for drugs, the prolonged effect of which is achieved due to the controlled slow release of the encapsulated drug. This study investigated the effects of encapsulation of various antiseptics (brilliant green, miramistin, and furacilin) and the chemical composition of the polymer on the yield, structure, size, drug release kinetics, and antibacterial activity of microparticles produced from resorbable polyesters of microbiological origin, polyhydroxyalkanoates. Microparticles with a 5.6–94.8 μm diameter were produced. The form of the active substance molecule has been found to be the most significant factor affecting the characteristics of polyhydroxyalkanoate microparticles. The surface structure of particles is rather determined by the chemical composition of the polymer, and the release kinetics to the model medium depends on the encapsulated drug. Microparticles based on PHAs loaded with brilliant green and furacilin showed antibacterial effects in *S. aureus* and *E. coli* cultures. The study demonstrated that microparticles with antiseptics encapsulated in them have potential as prolonged drug delivery systems and are of interest for further research.

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Биосовместимые системы для контролируемой доставки антисептиков для местного применения

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Аннотация. Технология контролируемой доставки лекарств является одной из передовых областей науки, включающей междисциплинарный научный подход. Эти системы доставки обладают многочисленными преимуществами по сравнению с обычными лекарственными формами, такими как повышенная эффективность и биосовместимость, сниженная токсичность и простота использования. В таких системах в качестве носителей лекарственных средств часто используют микро- и наночастицы, пролонгированное действие которых достигается за счет контролируемого медленного высвобождения инкапсулированного лекарственного средства. В данном исследовании изучалось влияние включения различных антисептиков (бриллиантовый зелёный, мирамистин и фурацилин), а также химического состава полимера на выход, структуру, размер, кинетику высвобождения лекарственных средств и антибактериальную активность микрочастиц, полученных из резорбируемых полиэфиров микробиологического происхождения, полигидроксиалканоатов. Было получено семейство микрочастиц диаметром 5,6–94,8 мкм. Установлено, что наиболее значимым фактором, влияющим на характеристики микрочастиц полигидроксиалканоатов, является форма молекулы активного вещества. Структура поверхности частиц больше зависит от химического состава полимера, а кинетики выхода в модельную среду – от инкапсулированного лекарства. Микрочастицы на основе ПГА с депонированными бриллиантовым зеленым и фурацилином показали антибактериальное действие в культурах

S. aureus и *E. coli*. Результаты продемонстрировали, что микрочастицы с депонированными антисептиками обладают потенциалом в качестве систем пролонгированной доставки и представляют интерес для дальнейших исследований.

Ключевые слова: полигидроксиалканоаты, системы доставки лекарств, контролируемая доставка, заживление ран, реконструктивные технологии, антисептики местного действия.

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Introduction

Over the past few decades, a considerable amount of research has been devoted to the development of nano- and micro-carriers using biocompatible and biodegradable materials for medical and biotechnological applications. Polymer microparticle systems have become an integral part of controlled and prolonged drug delivery, because they can be easily adapted to various administration methods (oral, topical, inhalation, etc.) (Campos et al., 2013; Anselmo, Mitragotri, 2014; Eke et al., 2014). In addition, such systems can transport several drugs simultaneously (González et al., 2020). The absence of infection is a prerequisite for successful complete tissue regeneration. With normal healing, the wound cleansing process lasts several days, but the development of an infection significantly increases this time. The combination of components with antibiotic, regenerative or anti-inflammatory properties and polymeric materials is a promising approach to meet all the requirements of next-generation bioactive healing systems (Zhang et al., 2013; Teo et al., 2017). A fast and effective wound healing process will significantly reduce the cost of medical care, wound care, and hospitalization, which will significantly improve patients' health-related quality of life (Zhang et al., 2018).

The materials used for encapsulation directly affect the particle properties, encapsulation efficiency, and the behaviour of microparticles in the body (Khem et al., 2016). In this context, the polymeric material and microencapsulation process are crucial factors that determine the efficiency and stability of such systems.

Bacterial polyester polyhydroxyalkanoates (PHAs) are currently among the major biodegradable polymers to develop controlled and prolonged drug delivery systems (Bonartsev et al., 2012; Liu et al., 2014; Lizarraga-Valderrama et al., 2016; Murueva et al., 2019). They are polyesters of (R)-3-hydroxy-alkane acids, which are accumulated intracellularly by various microorganisms as sources of carbon and energy. PHAs are particularly attractive for use in drug delivery because of their properties, such as optical activity, biocompatibility, nontoxicity, thermoplastic and biodegradable properties. The *in vivo* biodegradation of PHAs occurs via humoral and cellular pathways, involving macrophages and giant cells of foreign bodies, with the high activity of acid phosphatase, which correlates with the activity of the enzyme in the

blood serum of animals. The process of *in vivo* biodegradation of PHAs depends on the chemical structure of the polymer, the shape and place of implantation of the product. A decrease in the mass of polymer products implanted subcutaneously into muscle tissue and bone is accompanied by minor changes in the microstructure of the implants, without significant loss of strength, for a long time. PHAs, affected by macrophage cells, which are highly active in acid phosphatase, slowly degrade *in vivo* without a sharp loss of strength, ensuring long-term functioning of polymer products – from several months to a year or more. Polyhydroxyalkanoates have been extensively studied for use in tissue engineering and the development of medical devices (Lim et al., 2017; Koller, 2018). Particular attention should be paid to the biocompatibility of these polymers in terms of their potential in controlled release formulations (Imre, Pukánszky, 2013; Zhang et al., 2018).

By using modern encapsulation techniques, various substances can be entrapped in particles. Nevertheless, a review of the literature revealed a considerably smaller amount of data on encapsulation of pigments with bacteriostatic effect compared to the data regarding other bioactive substances/antiseptics. Chlorhexidine is one of the most common and best-studied antiseptic drug products encapsulated in polymer microparticles. Despite its effectiveness, it can cause allergic reactions ranging from skin symptoms to anaphylactic shock (Silvestri, McEnery-Stonelake, 2013; Toholka, Nixon, 2013).

Furacilin is another widely used antiseptic for encapsulation in polymer matrices. S. Yu. Zaitsev and others studied films with furacilin. Using a mixture of N-polyvinylpyrrolidone and polyvinyl alcohol and a mixture of copolymer of N-vinylpyrrolidone with maleic anhydride, the researchers produced transparent films with furacilin embedded into them. Repeatedly swollen gel films were tested as dressings for the treatment of burns and wounds. The (co) polymer films had antimicrobial properties and were able to absorb wound exudate. The film was easily separated from the wound surface. Such preparations can be used in human and veterinary medicine as burn dressings and other medical materials (Zaitsev et al., 2015). Also, in order to create biodegradable polymeric materials for the delivery of medicinal substances, A. A. Ol'khov and his colleagues studied the PHB-shungite–furacilin triple system, which turned out to be very promising: with an increase in the shungite content from 0 to 5 %, the rates of drug release from the composites decreased by a factor of 10. The supposed formation of PHB-furacilin–shungite associates leads to a significant increase in the strength of composite films (by a factor of 2–3) as compared with the initial and two-component films (Ol'khov et al., 2012).

The antimicrobial activity of miramistin was also studied. The article by Kamaeva S. S. described the development of medicinal films based on acrylamide, vinyl, and acrylic monomers with miramistin for the treatment of inflammatory diseases in gynaecology. The developed films showed a pronounced antimicrobial effect against Gram-positive and Gram-negative bacteria, *Treponema pallidum*, pathogenic fungi, some viruses, and protozoa (Kamaeva, Potselueva, 2010). Yu. G. Chernetskaya showed high antifungal activity of hydrogel polymer matrices containing miramistin against *Candida albicans*. A clinical study demonstrated that the applications of the miramistin hydrogel sheets 0.05 % produce a local bacteriostatic effect, which is confirmed by a pronounced decrease in the microbial number, and protect wounds from secondary infection (Chernetskaya et al., 2009).

The topical antiseptics effectively control the bacterial load, but they can also cause local cytotoxicity and wound drying and reduce

healing rate. Choosing the most effective and safe antiseptic is crucial to prevention of infection when treating various skin injuries. When a system with an antiseptic based on PHA microparticles is applied to damaged skin, the plastic fills the tissue defect and delivers the drug to the injured area.

The objective of this study was to create carriers for the controlled delivery of bioactive substances in the form of microparticles based on polyhydroxyalkanoates (PHAs), study their functional characteristics, and assess their *in vitro* efficiency for potential use in skin surgery.

Materials and methods

Two types of polyhydroxyalkanoates, which are biodegradable and biocompatible polymers, were used: poly(3-hydroxybutyrate) (P(3HB)) with a molecular weight of 1200 kDa and a copolymer of 3-hydroxybutyrate with 3-hydroxyvalerate (P(3HB)-co-P(3HV)), 1500 kDa, containing 11 % 3HV. Topical antiseptics, such as brilliant green, miramistin, and furacilin were chosen as model drugs for encapsulation (Table 1).

Microparticles were produced using an emulsion method based on evaporation of a solvent from a double emulsion. This method has recently been of great interest because of its ability to encapsulate and release hydrophilic or solid substances as drug delivery systems in cosmetics and food products (Iqbal et al., 2015). We added an aqueous solution of the drug with a content equal to 10 % of the polymer weight to a 2 % polymer solution dissolved in dichloromethane. The solutions were homogenized using Sonicator-S 3000 ultrasound bath made by Misonix Incor. (USA) at a power of 7W for 2 min. Then, we poured the resulting emulsion stepwise into 100 ml of a 1 % aqueous solution of polyvinyl alcohol stirred with a magnetic stirrer at 750 rpm. We left the resulting double emulsion for 24 h under constant mechanical stirring until the solvent completely evaporated. We then collected the microparticles by centrifugation (9,000 rpm for 5 min), washed them with distilled water and dried in a drying oven at 37 °C.

The microparticle yield was calculated as a percentage of the weight of the polymer used to produce these microparticles. Microparticle morphology was studied by scanning electron microscopy using TM4000 microscopes (Hitachi, Japan). Platinum sputtering of samples was performed in a K575XD Turbo unit (Emitech, UK).

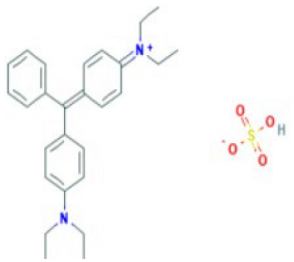
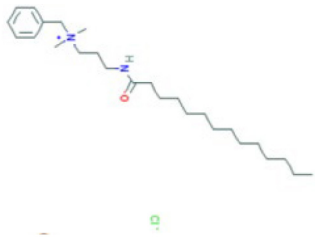
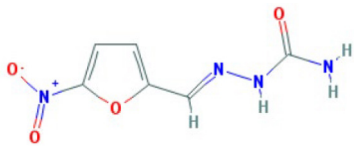
The size and distribution of polymer microparticles were studied using the Eclipse Ti-U light microscope (Nikon, Japan) by analysing 10 fields of view.

The electrokinetic potential (zeta potential), which is determined by the electrophoretic mobility of the particles in the suspension, was measured using Henry's equation on a Zetasizer Nano ZS particle analyser. Measurements were performed automatically according to the standard procedure recommended by the manufacturer.

The drug amount to be included in the polymer matrix was determined by spectrophotometry based on its initial and residual emulsion concentration at 625 nm (brilliant green), 260 nm (miramistin), and 367 nm (furacilin), using a Cary 60 UV–Vis spectrophotometer (Agilent Technologies, USA).

To study the kinetics of the drug release from the polymer matrix, microparticles produced from PHAs and loaded with drugs were sterilised by UV radiation for 3 hours and placed in sterile test tubes with caps containing 40 ml of a phosphate-buffer saline (pH 7.4) each. The tubes were incubated in a thermostat at 37 °C (n = 3). The samples were taken in 1; 2; 3; 6; 8; 24; 48; 72; 168; 240; 528; and 648 hours. Microparticles were precipitated by centrifugation (9,000 rpm for 5 min), and the amount of drug released into the medium was determined by analysing

Table 1. Drugs used for encapsulation in polymer matrices

Drug	Manufacturer	Formula	Molecular weight, g/mol	Mechanism of action
Brilliant green Bis-(p-diethylamino) triphenylanhydrocarbinol oxalate	Obnovlenie PFK, Russia		475.6	Scarcely studied
Miramistin Benzyldimethyl[3-(myristoylamino)propyl] ammonium chloride	LLC INFAMED K, Russia		457.2	It interacts with a lipid layer of microorganism membranes, causing their destruction and increasing permeability, induces cytolysis.
Furacilin Semicarbazone 5-nitrofurfural [(E)-[(5-nitrofuran-2-yl) methylidene]amino] urea	Irbit Chemical and Pharmaceutical Plant JSC, Russia		198.2	It restores 5-nitro groups of microbial flavoproteins with the formation of reactive amino derivatives that cause changes in proteins, causing the death of the cells of pathological microorganisms.

the supernatant liquid using a Cary 60 UV–Vis spectrophotometer (Agilent Technologies, USA). The encapsulation efficiency of the drug in the polymer matrix and drug release in a phosphate buffer were calculated using generally accepted formulas (Murueva et al., 2013).

The antibacterial activity of microparticles loaded with antiseptics was determined using the disk diffusion method and the minimum inhibitory concentration method against Gram-positive bacterium *Staphylococcus aureus* and Gram-negative bacterium *Escherichia coli*. The disk diffusion method is based on recording the diameter of the zone of growth inhibition of the studied microorganisms around the carrier of an antibacterial drug (paper disk).

To obtain a nutrient medium, Mueller-Hinton medium (BioRad, France) was diluted with distilled water (at the rate of 25 ml per cup) and heated until complete dissolution. Then, the prepared Petri dishes and nutrient medium were sterilised by autoclaving at 1 A and 121 °C for 3 hours. After cooling, the Petri dishes were filled with the molten medium so that the thickness of the agar layer in the dish was 4 mm on average and left at room temperature until completely solidified.

To determine the sensitivity of microorganisms to antiseptic preparations, we used an inoculum corresponding to a density of 0.5 according to the McFarland standard and containing about 1.5×10^8 CFU/ml. Inoculation was carried out with sterile cotton swabs with uniform stroke movements, turning the Petri dish at an angle of 30°. After 10 minutes, vertical wells 1 cm in diameter were made in the middle of the Petri dish in agar, and a suspension of microparticles in physiological saline and antiseptic solutions (300 µl) were transferred into them. Then the Petri dishes were left in a thermostat at 37 °C. Twenty-four hours later, the diameter of the culture inhibition zones was measured.

Results and discussion

Techniques based on double emulsions are commonly used for the encapsulation of both hydrophobic and hydrophilic drugs, cosmetics, foods, and other high value products (Iqbal et al., 2015).

The functional characteristics of microparticles based on P(3HB) and P(3HB)-co-P(3HV) produced by an emulsion method and loaded with various antiseptics for topical use that were studied here included size, yield, suspension stability, and drug release kinetics.

All microparticles produced from PHAs were spherical in shape. Small defects were observed on the surface of the particles (Fig. 1), indicating possible adhesion and aggregation of microparticles during the production process. There were no obvious differences associated with the different chemical composition of the particles. However, P(3HB)-based particles had a more regular spherical shape but were heterogeneous in size. The microphotographs showed the presence of large particles, but they only accounted for 1 % of the total number of microparticles. The irregular shape of large particles can be attributed to the formation of sufficiently large polymer droplets created by merging of microparticles during the production process.

Particles from P(3HB) with furacilin had the most deformed shape and the largest size. Microparticles loaded with brilliant green were homogeneous in shape and size.

The main characteristics of the empty microparticles, as well as those loaded with antiseptics are shown in Table 2. Initial loading with biologically active substances was performed at the polymer to antiseptic ratio of 9:1.

The average diameter of the microparticles with encapsulated antiseptics was about 50.5 µm, which was close to the average diameter of the non-loaded particles (67 µm). These results show

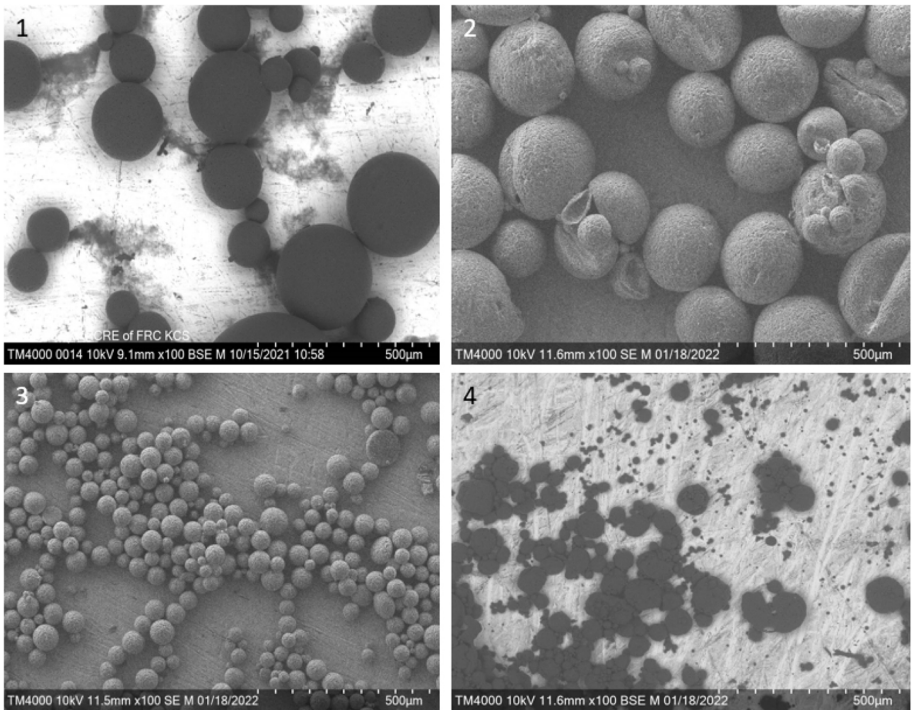


Fig. 1. SEM micrographs of microparticles from P(3HB) (1), P(3HB) with furacilin (2), P(3HB) with brilliant green (3), P(3HB) with miramistin (4). Marker 500 µm

Table 2. Characteristics of microparticles loaded with antiseptics

Microparticle composition	Average diameter, µm	Zeta potential, mV	Encapsulation efficiency, %	Yield, %
P(3HB) + brilliant green	61.25 ± 0.66	–27.30 ± 1.90	95.5	80.0
P(3HB) + miramistin	5.64 ± 0.17	–0.805 ± 0.40	82.4	82.5
P(3HB) + furacilin	94.81 ± 2.40	–29.10 ± 1.76	80.0	67.5
P(3HB)-co-P(3HV) + brilliant green	58.86 ± 1.34	–22.40 ± 0.66	98.8	81.5
P(3HB)-co-P(3HV) + miramistin	37.14 ± 3.59	–6.60 ± 1.20	92.0	76.5
P(3HB)-co-P(3HV) + furacilin	45.73 ± 0.08	–29.90 ± 4.01	96.7	93.5
P(3HB)	63.56 ± 1.67	–19.20 ± 0.95	-	92.0
P(3HB)-co-P(3HV)	70.80 ± 1.73	–27.50 ± 0.95	-	94.0

that loading with antiseptics does not significantly change the average diameter of the microparticles. Besides, no relationship was found between the chemical composition of the polymer and the average diameter of the microparticles.

In a study by E.V. Grekhneva et al., the authors microencapsulated furacilin into water-soluble polymers of natural (sodium alginate and guar gum) and synthetic (polyvinyl alcohol and polyvinylpyrrolidone) origin. Furacilin microcapsules made of synthetic polymers formed 4 to 10 µm agglomerates, which indicated that the system was not monodisperse (Grekhneva et al., 2017).

The study of the electrokinetic potential of the polymer drug delivery systems showed that microparticles made of P(3HB) and copolymer P(3HB-co-3HB) with encapsulated miramistin had the lowest values of ζ-potential, indicating a tendency of particles to rapid agglomeration.

In another work, cross-linked chitosan microspheres with different contents of miramistin were produced by spray drying. The particle size ranged from 2.4 ± 0.6 to 3.1 ± 1.0 μm. Drug incorporation and encapsulation efficiency were about 30.8 % and 92.0 %, respectively. The release profile of miramistin *in vitro* showed that 80 % (wt.) of the encapsulated miramistin was released from chitosan microspheres after 2–4 hours (Grib, 2014).

Microparticles of calcium pectinate were also prepared by spray drying. Kulikouskaya V. I. developed a technique enabling encapsulation of up to 30 wt.% miramistin. In normal saline, the yield of the substance was 2/3 of the encapsulated amount after 48 h (Kulikouskaya et al., 2015).

The microparticles made of P(3HB) and P(3HB)-co-P(3HV) with furacilin had the highest value of ζ-potential, which confirmed their stability. Encapsulation of antiseptics increases the electrokinetic potential and, hence, enhances the stability of microparticles. A possible relationship between the average diameter and zeta potential was not revealed. Moreover, there was no relationship between ζ-potential and the chemical composition of the polymer.

In addition to the size and stability of the produced systems, an important criterion for practical application is the outflow of the drug product from the microspheres. The antiseptic was released into the model medium at a high rate, increasing stepwise in a month. On the first day of observation, the average release of the drug was 16 % (P(3HB) with brilliant green) and 11 % (P(3HB) with furacilin). After that, the drug concentration in the medium increased by an average of 2.5 % per day (Fig. 2). The maximum release of antiseptics was 34.6 % from particles loaded with brilliant green and 25.7 % from particles loaded with furacilin. No burst release was observed, indicating the high quality of the delivery system produced. The study by Kosenko et al. addressed the mechanisms for the release of furacilin from PHB membranes. The authors found that the release occurs simultaneously by diffusion and kinetic mechanisms by zero-order reactions, and the diffusion coefficients depend on the concentration of the drug (Kosenko et al., 2007). The study by Ol'khov et al. demonstrated the possibility of using polyamide-PHB mixtures as matrices for the long-term release of furacilin

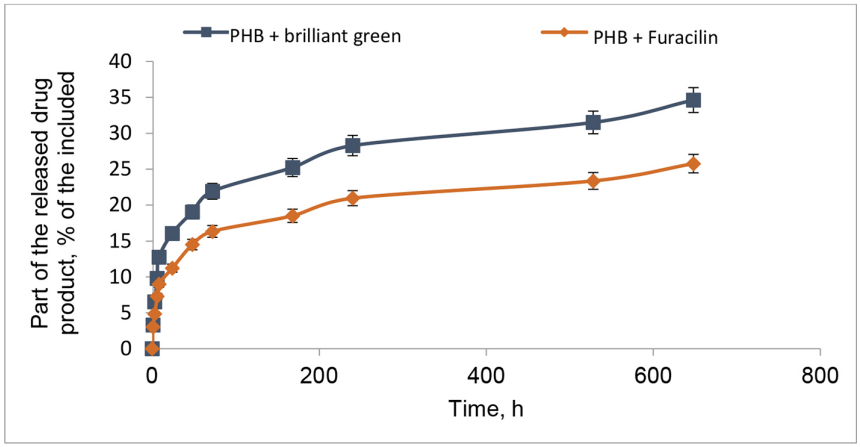


Fig. 2. Curves of antiseptic release from P(3HB)-microparticles

with a constant release rate for more than 1 month. The results obtained show the suitability of embedding furacilin in polymeric matrices for sustained release (Ol'khov et al., 2018).

Verification of the antibacterial activity of the engineered particles was carried out in cultures of *S. aureus* and *E. coli*, which represent the most common Gram-positive and Gram-negative bacteria in the human body. The results of the antibacterial evaluation are shown in Table 3 and Fig. 3–5.

The study of the effectiveness of the PHA-brilliant green system against *S. aureus* and *E. coli* grown on agar demonstrated a low antibacterial effect compared to the control – the initial form of the antiseptic, a 1 % alcohol solution of the drug. The smaller zones of growth inhibition in *E. coli* culture compared to *S. aureus* culture are associated with the lower effectiveness of brilliant green against Gram-negative microorganisms.

Particles loaded with furacilin produced a low antibacterial effect as well. That could be caused by the slow release of the antiseptic from the polymer matrix: about 2 mg on Day 1. Grehneva et al. studied the antimicrobial activity of furacilin encapsulated in matrices of polyvinyl alcohol, polyvinylpyrrolidone, sodium alginate, and guar gum. The authors noted that the antimicrobial activity of the antiseptic in PVA and PVP matrices was slightly lower than the activity of the initial solution against *E. coli*, *P. aeruginosa*, *S. aureus*, and others. One exception was *Candida albicans*, whose inhibition by the encapsulated drug was greater by a factor of two (Grehneva et al., 2017).

In addition to the experiments described above, the activity of P(3HB) and P(3HB)-co-P(3HV) microparticles non-loaded with antiseptics (Fig. 5) and saline used to prepare the suspension of microparticles was also tested as the control. In both cases, no growth inhibition of the bacterial growth was observed, which showed that neither the chemical composition of the polymer nor the medium used to prepare the suspension affected the effectiveness of the encapsulated formulations of brilliant green and furacilin.

Conclusions

The current study showed that loading of the antiseptics into PHA microparticles resulted in adequate values of encapsulation efficiency, drug release, and stability in the model medium. Brilliant green and furacilin demonstrated high

Table 3. Diameters of growth inhibition zones of *S. aureus* and *E. coli* cultures

Sample	Inhibition zone diameter, mm	
	S. aureus	*E. coli*
Saline	-	-
P(3HB) MP	-	-
P(3HB)-co-P(3HV) MP	-	-
Brilliant green	6.50	5.50
P(3HB) with brilliant green MP	3.25	2.50
P(3HB)-co-P(3HV) with brilliant green MP	3.50	2.50
Furacilin	7.00	6.50
P(3HB) with furacilin MP	2.50	2.00
P(3HB)-co-P(3HV) with furacilin MP	2.55	2.25

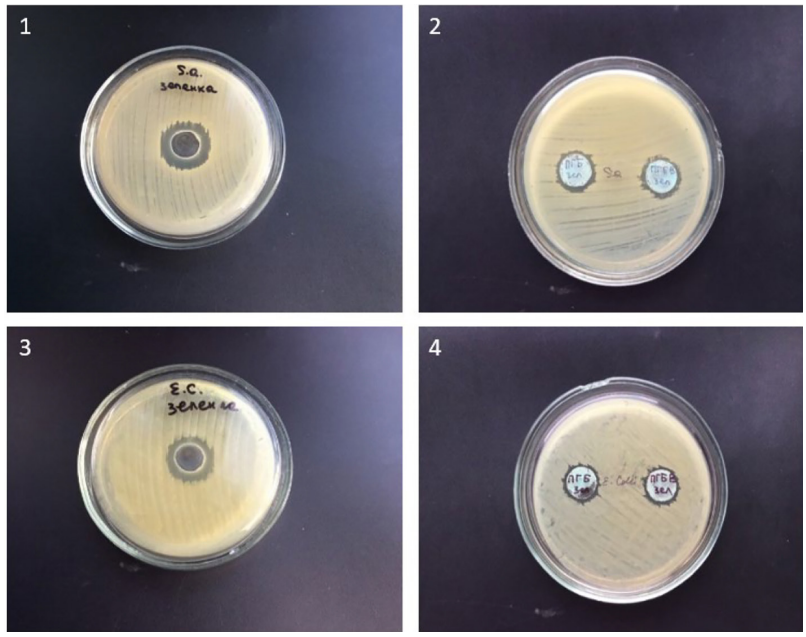


Fig. 3. Zones of growth inhibition of *Staphylococcus aureus* (1, 2) and *Escherichia coli* (3, 4) after the introduction of brilliant green and P(3HB) and P(3HB)-co-P(3HV) microparticles with encapsulated brilliant green: 1 – Effect of brilliant green on *Staphylococcus aureus*, 2 – P(3HB) and P(3HB)-co-P(3HV) microparticles with encapsulated brilliant green, 3 – Effect of brilliant green on *Escherichia coli*, 4 – P(3HB) and P(3HB)-co-P(3HV) microparticles with encapsulated brilliant green

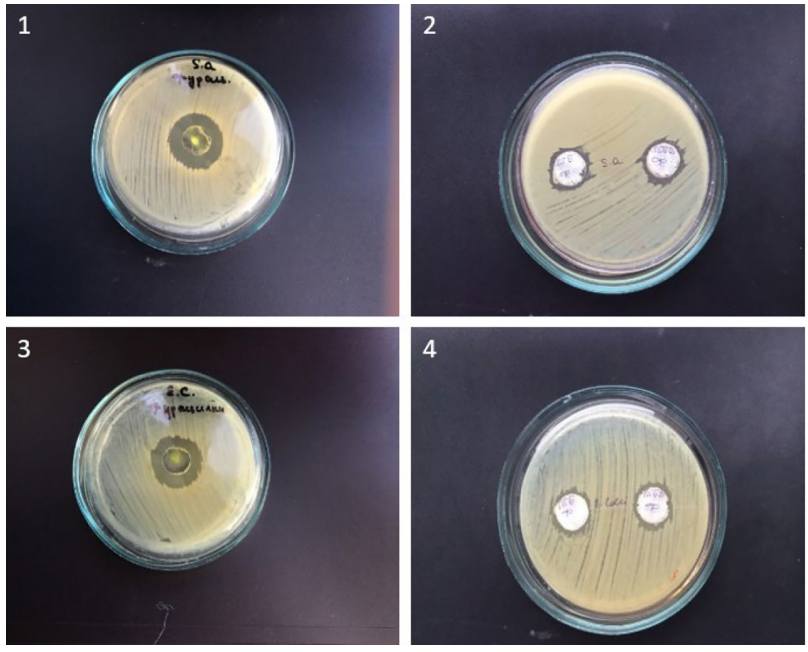


Fig. 4. Zones of growth inhibition of *Staphylococcus aureus* (1, 2) and *Escherichia coli* (3, 4) after the introduction of P(3HB) and P(3HB)-co-P(3HV) microparticles with encapsulated furacilin: 1 – Effect of furacilin on *Staphylococcus aureus*, 2 – P(3HB) and P(3HB)-co-P(3HV) microparticles with encapsulated furacilin, 3 – Effect of furacilin on *Escherichia coli*, 4 – P(3HB) and P(3HB)-co-P(3HV) microparticles with encapsulated furacilin

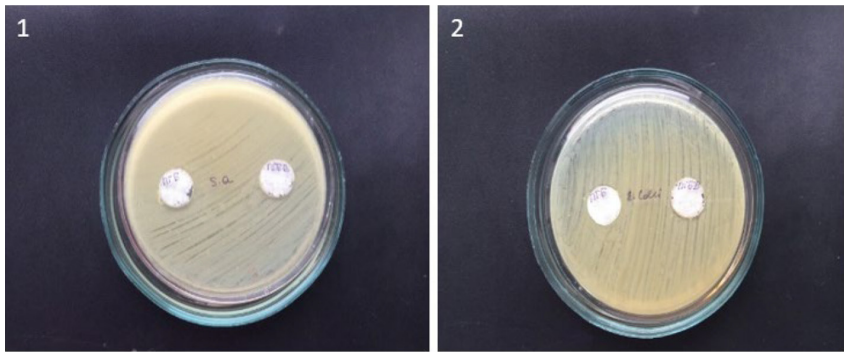


Fig. 5. Zones of growth inhibition of *Staphylococcus aureus* (1) and *Escherichia coli* (2) after the introduction of empty P(3HB) and P(3HB)-co-P(3HV) microparticles

efficiency of encapsulation in polymer matrices, while the encapsulation of miramistin was lower. That was attributed to the structure of the miramistin molecule and its effect on laying of polymer chains, as well as the nature of mixing of the product with the matrix material. The system for controlled delivery of antiseptic drugs produced in the present study showed better effectiveness compared to available pharmaceutical forms. In general, the study showed satisfactory antibacterial effect and stability of the produced pharmaceutical microparticles in the model medium *in vitro*.

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