Development and characterization of ceftriaxone-loaded P3HB-based microparticles for drug delivery

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ABSTRACT

In this study, polymer-based microparticles are used to improve the therapeutic properties of ceftriaxone (CEF) and render them safer. Poly-3-hydroxybutyrate (P3HB) and poly-3-hydroxybutyrate/polyethylene glycol (P3HB-PEG)-based microparticles were prepared by two methods: a double emulsification technique and spray-drying. The microparticles were characterized in terms of size and zeta potential, morphology, total drug loading and drug release. The microparticles had spherical shapes with diameters of a size range from 0.74 to 1.55 μm (emulsification technique) and from 3.84 to 6.51 μm (spray-drying); CEF encapsulation efficiency was around 63% and 49% for these methods respectively. The CEF release from microparticles obtained by spray-drying reached 100% after 150h, while for microparticles obtained by emulsification technique the total release of CEF did not exceed 34% after 312 h. The release profiles could be best explained by Zero order kinetics model, Higuchi and Korsmeyer-Peppas models, as the plots showed high linearity. Antibacterial activity of the microparticles was evaluated against gram-positive and gram-negative bacterial strains. In general, CEF encapsulation in polymeric microparticles preserves the therapeutic efficacy of the CEF and provides its prolonged effect.

KEYWORDS: Ceftriaxone; P3HB-microparticles; emulsification technique; spray-drying; drug release; microbiological evaluation

INTRODUCTION

Scientific research and development of innovative drug delivery systems is a rapidly developing area worldwide. This trend is going to be strengthened in the future, as the cost of health care requires reducing costs and improving the effectiveness of existing dosage forms. Currently, we observe the crisis of antibiotic therapy not only due to a large number of resistant microorganisms, but also due to the lack of drugs that do not have resistance of one or other pathogens. Moreover, bacteria appeared that are resistant to all existing antibiotics. In the past, the pharmaceutical industry solved the problem of resistance by producing a new, more effective antibiotic. However, today there are no fundamentally new classes of antibiotics that are acceptable for clinical use and the development of new drugs usually takes about 10-15 years.[1] Therefore, the interest of the pharmaceutical sciences is gradually shifting from the discovery of new chemicals to the optimization of their routes of administration and delivery.

Thus, one of the drawbacks of classical antimicrobial therapy is that many intracellular bacteria are at rest or still, deactivating, and dramatically changing the permeability of cell membranes, which affects the sensitivity to antibacterial agents. Therefore, such bacteria can persist for a long time.[2] In addition, the most severe complications of antibiotic therapy are anaphylactic shock, allergic reactions, neurotoxic phenomena, dysbiosis, and liver damage.[3] The use of antibacterial drugs in large doses to inhibit the vital activity of microorganisms and overcome resistance also leads to delayed and late serious depression of immunological system, which leads to the development of systemic diseases including the formation of malignant tumors.

To overcome these problems various approaches are developed including the use of immobilized biologically active agents, such as fullerenes, carbon nanotubes, graphite, nanodiamonds, graphene oxide, metal oxides' nanoparticles, and metalloids, which may be used for bacterial infections treatment.[4,5] The mechanism by which nanostructured materials inactivate bacteria is complex and depends on intrinsic properties materials, for example, composition and surface modification, the nature of the target microorganisms, and the characteristics of the environment in which cell-materials interactions take place.[6]

Another popular approach to increase the effectiveness of antibiotic therapy is the use of antibacterial drugs in the form of microcarriers and nanocarriers.[7-10] By placing antibiotics in carriers we can expect improved deliver)' to infected cells, increased bioavailability of drugs with poor absorption characteristics, prolonged drug residence time, targeted transport of therapeutic agents to specific organs, reduced toxicity and stability.[11-13] A significant portion of antibiotics during the first 6-8 h is mostly removed from the body. The use of microparticles allows extending the elimination time.[12,14]

Ceftriaxone (CEF) is a cephalosporin antibiotic of third generation that is effective mostly against gram-positive and gram-negative bacteria. CEF is reportedly hygroscopic, sensitive to humidity, heat and light, and oxidizing agents; CEF aqueous solution is reportedly unstable. In solution, the optimal pH for CEF stability is 7.5 giving more than 6h without significant degradation at 37 °C, however degradation is faster at lower or higher pH.[15] CEF is poorly absorbed through mucosal membranes,[16-18] which indicates that in this pharmaceutical form it is intended only for intravenous, intramuscular or subcutaneous injections, and this limits its therapeutic use in other modes of administration.[19]

The use of the encapsulated form of CEF in the form of microcarriers and nanocarriers will increase the stability and bioavailability of the drug, provide a reduction in undesirable systemic effects and also allow for oral, nasal, and inhalative administration into the body.[20-22]

The literature describes examples of delivery systems for cephalosporin based on various materials. Thus, Kumar et al.[23] demonstrated the efficacy of CEF-loaded solid lipid nanoparticles for gram-positive and gram-negative bacterial strains. In another article, the authors noted the pronounced antibacterial effect of conjugates of silver nanoparticles with CEF in the culture of various pathogenic strains of bacteria.[24] In addition, examples of prolonged forms of CEF in the form of conjugates based on poly(styrene-alt-maleic)anhydride,[25] PEGylated microscopic lipospheres,[21] and chitosan nanoparticles[26] are given.

Among the huge variety of polymers, used to produce microparticles and nanoparticles for drug delivery, polyhydroxyalkanoales (PHA) are widely used in controlled release applications, including the encapsulation of different antimicrobial drugs.[27-34] This class of polyester attracts attention due to the presence of unique properties – natural origin, true biodegradation and biocompatibility. PHAs are thermoplastic, have less effect on pH values of tissues and have a longer in vivo degradation period, which allows them to be used for the development of prolonged drug delivery systems. Varying the conditions for PHA synthesis, as well as the production of composite and block copolymer materials with hydrophilic components makes it possible to design microcarriers and nanocarriers suitable for drug delivery systems.[35-37]

The key role in the formation of micro- and nanoparticles is played by the choice of their production technique. Currently, such carriers can be prepared using a variety of methods: electrohydrodynamic techniques,[38-41] microfluidic method,[42,43] coacervation[44-45]; polymerization of monomers,[46] emulsification of solutions (two- and three-component emulsions with evaporation or diffusion of the solvent);[47-49] spray drying of solutions[50-52] etc. With regard to the preparation of PHA-based microparticles and nanoparticles, the emulsion method is the most adapted from those, listed above and recently the application of the spray drying method has become topical.

It should be noted that spray drying is a popular way of depositing drugs in various polymer carriers, but with respect to PHAs this method has not been properly developed. Single examples of the use of spray drying for producing microparticles based on polyhydroxybutyrate (P3HB) loaded with analgesic drug – paracetamol.[53] However, publications on the preparation of PHA-based antibiotic-loaded microparticles by spray drying method have rarely been seen until now.

Earlier, we showed examples of the successful encapsulation of various antibacterial drugs in microparticles from P3HB and its copolymers with 3-hydroxyvalerate using the emulsion method.[29,54] For the first time, the possibility of encapsulating an antitumor drug in a P3HB microparticle was demonstrated using the spray drying method.[55,56] The dependency of microparticle characteristics (yield. average diameter, zeta potential) on the parameters of the production process (inlet temperature, polymer solution feed rate, and polymer solution concentration) has been established.[57]

Thus, the main objective of our study was the development of a delivery system for CEF as polymer microparticles. Microparticles from P3HB and its blend with polyethylene glycol (PEG) were obtained by the spray-drying technique and the emulsion method. Furthermore, we evaluated the effects of these methods and chemical composition on the properties of the microparticles and CEF release in vitro. Besides, microbiological evaluation of CEF-loaded РИА microparticles was also investigated in vitro.

MATERIALS AND METHODS

Materials

P3HB with low molecular weight was produced at tl Institute of Biophysics of the Siberian Branch of the Russian Academy of Sciences (SB RAS) by the microbial fermentation process. The register mark of material is "Bioplastotan™." PEG 35,000 Da was purchased Sigma-Aldrich USA, CEF – Farm-Center (Russia).

Preparation of microparticles by emulsion method

Microparticles were prepared by the solvent evaporation technique using (oil/water) emulsions. The emulsion contained 0.1 g P3HB or P3HB/PEG (50:5 in 10 ml of dichloromethane and 100 ml 0.5% PVA. The obtained emulsions were mechanical stirred at 24000 rpm during 5min (IKA Ultra-Turrax T25 digital high-performance homogenizer Germany). All emulsions were continuously mixed mechanically for 24h until the solvent complete evaporated. Microparticles were collected by centrifuging (at 10,000 rpm, for 5min), rinsed four times distilled water and lyophilic dryer in an Alpha 1-2 1 plus (Christ, Germany).

The described above method was also used for loading CEF into microparticles. The CEF was dissolved in 1 ml of distillated water and added the solution of 0.1 g P3HB or P3HB/PEG (50:50) dichloromethane and the

resulting emulsion was sonicated at 6W for 2min (Misonix 3000. USA). Then obtained emulsions were added to 0.5% (w/v) PVA solution al stirring at 24.000 rpm (IKA Ultra-Turrax T25 digital high-performance homogenizer. Germany). Centrifugation and washing conditions were similar to those described earlier.

Preparation of microparticles by spray-drying

Spray-dried microparticles were prepared from P3HB or P3HB/PEG (50:50) solutions in dichloromethane (400 mg, 40 ml) using Buchi B-290 Spray dryer (BUCHI Laboratory Equipment, Switzerland, Elawil). In brief, a polymer solution was sprayed through a nozzle (diameter of 0.7 mm) at a feed rate 1.5ml/min at the inlet temperature 75°C. The value of the aspirator (current of argon) was supported at the maximum gas flow rate 35 m^3/h.

CEF-loaded microparticles were prepared by spray drying of water/oil-emulsions. In this regard, an aqueous solution of GEE (80mg/ml) was added to the solution of the polymer and those emulsions were homogenized using sonication at a power of 6W for 2min The obtained homogeneous emulsions were continuously stirred (700rpm) and sprayed at the described above parameters.

Characterization of microparticles

Morphological analysis

To study the morphology of microparticles surface, the scanning electron microscopy of samples was performed. S-5500 (Hitachi. Tokyo, Japan) in the Center of the common use, Krasnoyarsk Scientific Center, Siberian Branch of Russian Academy of Sciences. The samples were sputter-coated with platinum using an electrical potential of 2.0kV at 25 mA for 6min with a sputter coaler K550X (Emitech, Quorum Technologies Ltd., UK).

Process yield (%)

The processing yields were defined as the percentage of the weight of microparticles (Wm) compared to the weight of polymer (Wp) in the initial solution as shown in Equation (1):

$$Yield(\%) = \frac{W_m}{W_p} \times 100 \quad (1)$$

Measurement of the particle size and zeta potential

About 5mg of each sample was suspended in bidistilled water and sonicated at 6W for 1 min. The size distribution and polydispersity index (PdI) of microparticles were determined by the first measuring of Brownian motion of particles using the dynamic light scattering method on the Zetasizer Nano ZS (Malvern, Worcestershire, UK). The average particle size measurements were studied in triplicates for all samples. The surface charge of microparticles was characterized by value of zeta potential, which was determined by the electrophoretic mobility of the particles in the suspensions.

Drug encapsulation efficiency

The amount of drug loaded in the polymeric microparticles was determined by spectrophotometric analysis. The CEF-loaded MPSD were dissolved in dichloromethane and water was then added in a ratio 1:1. The resulting emulsion was mechanically stirred on a shaker to extract the CEF into water. An aqueous phase containing CEF was taken and optical density

measurements were made. CEF encapsulation efficiency of MPEM was measured according to the procedure described previously.[54]

The quantity of CEF loaded into microparticles was determined on a UV-Vis spectrophotometer Cary 60 (Agilent Technologies, Selangor, Malaysia) by measuring the UV-Vis absorbance at 240nm using pre-built calibration graphs. The experiment was carried out in triplicates. The encapsulation efficiency (EE) was defined as the percentage of the drug weight in microparticles (Wm) compared to the initial weight of drug (Wi) as shown in Equation (2):

$$EE(\%) = \frac{W_m}{W_i} \times 100 \quad (2)$$

In vitro CEF release studies

The controlled drug release from CEF-loaded microparticles was carried out in vitro. The microparticles were initially sterilized by UV radiation for 40 min and placed in a sterile centrifuge tubes, containing 10 ml of phosphate-buffered saline (PBS, pH 7.4). Those centrifuge tubes were exposed to thermostat at 37 °C (n=3). Microparticles were precipitated by centrifugation (10,000 rpm, 10 min). One milliliter of supernatant was withdrawn from the centrifuge tube to observe the change in CEF concentration by UVVis spectroscopy (Agilent Technologies, Malaysia), and volume was replenished with fresh PBS, the dilution was taken into account in the calculations. The amount of CEF in the supernatant was determined at 240 nm. Triplicate measurements were performed for all samples.

The data obtained from in vitro experiments were fitted to various mathematical models to assess the CEF release kinetics.[58]

Zero-order kinetic model

$$Q_t = Q_0 + K_0 \times t, \quad (1)$$

where Q_t is amount of drug dissolved in time t, Q_0 is initial amount of drug in the solution, and K_0 is zero-order release constant.

First-order kinetic model

$$\log Q_t = \log Q_0 + \frac{K_1 + t}{2.303}, \quad (2)$$

where Q_t is amount of drug dissolved in time t, Q_0 is initial amount of drug in the solution, and K1 is first-order release constant.

Higuchi model

The model relates cumulative drug release versus square root of time as shown in Equation (3).

$$Q = K_H \times \sqrt{t} \quad (3)$$

Hixson–Crowell model

This model relates cube root of drug percentage remaining in microparticles versus time. As given by Equation (4).

$$Q_0^{1/3} = Q_t^{1/3} + K_S \times t \quad (4)$$

Korsmeyer–Peppas model

This model relates exponentially the drug release to the elapsed time. The equation is given as Equation (5).

$$\frac{Q_t}{Q_\infty} = K_k \times t^n \quad (5)$$

Microbiological efficiency evaluation

The antibacterial activity of CEF-loaded microparticles was determined using the disc diffusion test for grampositive bacteria Staphylococcus aureus and gramnegative bacteria Escherichia coli. This test is based on the diffusion of an antibacterial drug from the carrier into a dense nutrient medium and inhibition of the growth zone of the culture.[59] Susceptibility disk of CEF was used as the control (with a drug content of 0.03 mg, BioRad, France). The concentration of encapsulated CEF when microparticles were introduced into the cell culture as a suspension was 0.3 mg. The Mueller-Hinton medium (BioRad, France) was diluted in distilled water (25 ml per dish) and heated until completely dissolved. The nutrient medium was then sterilized by autoclaving at 1A and 121 °C for 15 min. Petri dishes were filled with medium on a horizontal surface so that the thickness of the agar layer in the dish was on average 4 mm, and left at room temperature until completely solidified.

To determine the sensitivity of the microorganisms, an inoculum corresponding to a density of 0.5 according to the McFarland standard and containing about 1.5×10^8 CFU/ml was used. The inoculation was performed with sterile cotton swabs with uniform strokes in aseptic conditions. After 15 min, in the middle of the Petri dish, vertical wells of 15mm in diameter were made in agar and the microparticles suspensions in physiological saline in a volume of 100 μl were dropped into them. The application of the disks was performed on agar without well formation using sterile tweezers. Upon completion, Petri dishes were left in a thermostat at 37C. After a day, the diameters of the culture growth retardation zones were measured.

RESULTS AND DISCUSSION

In this study, PHAs-based microparticles are used to improve the therapeutic properties of CEF and render antibiotic safer. Microparticles were prepared by two methods, with a double emulsification technique and with a spray-drying (abbreviations are presented in Table 1).

Characterization of microparticles

The size of microparticles and PdI were determined using dynamic light scattering by analyzer of Zetasizer Nano ZS. Results revealed that microparticles had a size range from 0.74 to 1.55 mm (emulsion method) and from 3.84 to 6.51 mm (spray-dried method); a negative zeta potential ranging from –17.8 to –28.0mV and from –37.5 to –95.7 mV, respectively (Table 2). It is advisable to assume that the observed difference in zeta-potentials is probably due to the adsorption on the surface of the microparticles of polyvinyl alcohol, which is used in the emulsion method and absent in the spray drying method. A similar effect was mentioned in the work of Francis et al.[60] the nonionic

surfactants such as PVA are known to strongly adhere on the microsphere surface by anchoring the hydrophobic tail into the polymer only when it is hydrophobic, leaving the polar head protruded on the surface. Thus, with an increase in the concentration of polyvinyl alcohol from 0.5% to 1%, the zeta potential of the P3HB microparticle decreased from –34mV to –13 mV.[60]

It was found that the addition of PEG leads to a decrease in process yield, an increase in average diameter and zeta-potential of MPEM. However, the presence of PEG in MPSD resulted in a decrease of size and an increase of zeta-potential. Thus, CEF loading did not significantly affect size characteristics and process yield of MPs. The encapsulation efficiency of CEF was about 60% and 50% for MPEM and MPSD, respectively.

The results of encapsulation efficiency correlate with those presented in the literature. Thus, encapsulation efficiency is found to depend on the nature of polymer and drug, amount of drug loaded, preparation/loading techniques. In the study of Kumar et al.[23] lipid nanoparticles with CEF were obtained by the emulsion method and it was shown that encapsulation efficiency of CEF increased with increasing lecithin concentrations and at higher PVA values (EE about 71%–77%). While encapsulating CEF into lipospheres EE ranged from 29% to 60%.[21]

Figure 1 shows the SEM images of CEF-loaded MPs. It was found, that the addition of PEG had important influence on the surface morphology and microstructure of MPs. Therefore, PCSD had a smooth surface, while the surface of the PPCSD was rough with the presence of small pores. Consequently, the addition of PEG as surfactant most likely led to a decrease in the amorphization of substances in the spray-drying process. A similar effect of the PEG used in spray drying was noted in the review of Paudel et al.[61] Moreover, PPCEM were also characterized by a change in the structure upon the addition of PEG. However, in this case PEG was dissolved in the process of microparticles preparation, leading to the formation of deformed particles. According to Li et al.[62] PEG influenced the formation of pores on the surface of microparticles, but only with the correct ratio PHBPEG. When the ratio of PEG was excessively high, the erosion and dissolving effect were so strong that it was impossible to integrate microparticles.[62] On the whole, from the micrographs of Figure 1 it could be seen that the MPSD were spherical in shape and significantly larger in size, than the MPEM.

Study of the CEF release from MPs in vitro

CEF release profiles from MPEM and MPSD are presented in Figure 2. The mean amount of CEF released from PCSD and PPCSD was 51.3% and 39.1%, respectively, in the first 6 h and all the amount of drug from these formulations was released at ~150 h (Figure 2(a)).

Otherwise, CEF release from PCEM and PPCEM microparticles was seen retardant - the effect compared with PCSD and PPCSD (Figure 2(a)). In the first 6 h 9.4% and 2.6% of the CEF were released from the PPCEM and PCEM, respectively. About 15% of drug was released within 48 h for PPCEM, while 4% was released in the same period from PCEM. It is obvious that the drug release rate was increased when PEG was introduced into P3HB solution at emulsification technique. Likely, incorporation of PEG increased the release rate due to its high hydrophilicity and water solubility.[62-64] As soluble filler, dissolution of PEG upon contact with a release medium would provide more channels for drug diffusion, inducing more drug molecules to be released. After 312 h, 34% and 10% of CEF were released from the PPCEM and PCEM microparticles, respectively.

Earlier scientific literature reports that in the absence of biologic factors (enzymes, cells) the P3HB does not hydrolyze into carbon chains.[65] This is due to the very high crystallinity and hydrophobic nature of polymer. Therefore, the rate of drug diffusion was substantially higher than that of polymer degradation, so the drug release profiles are more dependent on drug diffusion rather than on polymer degradation.

The difference in the total release of CEF from MPEM and MPSD is most likely due to the fact that the drug molecules are differently encapsulated in the particles. MPEM had a relatively low total drug release, which could be explained by the hydrophobicity of P3HB and dense packing of polymer chains in the transformation process of microdroplets into microparticles, as a result, most of molecules of CEF being inside the particles.

At the same time, it was found that the increase of CEF release rates from PCSD and PPCSD can be related to the fact that the part of CEF solidifies separately during co-spray drying polymers. The remaining part of CEF may most probably be located near the surface. Therefore, drug molecules close to the surface leave the matrix very easily. Most likely, the drug release from PCSD and PPCSD in the first 3–6h is associated with solubilization of free CEF. The next phase of CEF release (48–312 h) was dominated by the diffusion mechanisms associated with the drug release from the surface structures of microparticles.

The data obtained from in vitro CEF release were fitted to different mathematical models, that is, Zero-order, First-order, Higuchi, Hixson-Crowell, Korsmeyer-Pappas, to predict the kinetics and drug release mechanism.

The release constant and regression coefficient (R2) values obtained from the mathematical models are shown in Table 2 and Figure 2(b, c). The data obtained shows that the formulations PCEM, PPCEM, and PPCSD follow Zero-order kinetics with R2 values of 0.922 and 0.925, 0.832, respectively. As shown, the formulations did not follow First-order kinetics. The data obtained for the formulations PCEM, PPCEM, and PPCSD best fit Higuchi model as indicated by the correlation coefficient, that is, 0.982, 0.963, and 0.894, respectively, indicating the CEF release from formulation followed Fickian diffusion. Low R2 values in all samples in Hixson–Crowell model indicate the absence of polymer erosion. The value of release exponent “n” obtained by applying Korsmeyer–Pappas equation for all formulations was <0.5 indicating CEF is released through Fickian diffusion from these formulations. Thus, the release profiles could be best explained by Zero-order, Higuchi and Korsmeyer-Peppas models.

In this way, these results confirm that it is possible to prolong the drug release by varying the method of preparation and chemical composition of MPs. Microbiological evaluation In modern scientific literature, several approaches are used to evaluate the antibacterial efficacy of the developed drugs. Among the most common are the following: colony count method in bacterial broth suspensions[66] and disc diffusion susceptibility test.[67] In our work, we showed the possibility of successful loading of CEF in P3HB microparticles using various technologies. The antibacterial activity of CEF-loaded microparticles and empty particles against various bacterial strains including *E. coli* and *S. aureus* was investigated using the disk diffusion test with the zones of inhibition (ZOI) to be measured (Figures 3 and 4). The antibacterial activity was compared against susceptibility disk of CEF.

The antibacterial efficiency of free CEF was higher against *E. coli* in comparison to *S. aureus*, which is consistent with these minimum inhibitory concentrations (MIC). It has been reported that the MIC of CEF against *E. coli* and *S. aureus* is 0.03–0.12 μg/ml and 1–8 μg/ml, respectively.[68] Herewith, samples obtained by spray-drying showed higher antibacterial activity in comparison to disk (Figure 4). Presumably, this effect is associated with the prolonged effect of the loaded CEF. Based on the literature data, the encapsulation of biologically active substances can lead to improvement in their functional properties.[23,69] The average diameter of ZOI caused by PCSD and PPCSD groups against *E. coli* were 26.5 ± 0.2 and 23.3 ± 0.1mm and was comparable to those against *S. aureus* (26.7 ± 0.2, 22.0 ± 0.3 mm). In contrast, the only sample obtained by the emulsification technique, PPCEM insignificantly suppressed growth of the more sensitive *E. coli*, while the remaining samples did not inhibit both *E. coli* and *S. aureus* (Figures 3 and 4). Probably this is due to insufficient concentration of released CEF (less than the MIC) from PCEM and PPCEM for the suppression of the growth of colonies after a short time of incubation.

In the study of Attama et al.[21] small inhibition zones were observed for liposphere batches formulated with 10, 20, 30, and 40%w/w PEG 4000 and 1%w/w CEF probably because the concentration of drug contained in this batch was low to yield concentrations equal to or above the MIC to cause significant inhibition.

It is also worth noting that there have been some case studies of antibiotic-loaded PH3B, while their applications for cephalosporins cannot be easily found. For instance, Vilos et al.[30] developed ceftiofur-loaded PHBV microparticles, which showed a slight bacterial inhibition during the first 5 h and a delay in the onset of the bacterial exponential growth *E. coli*. As a control, the activity of empty P3HB microparticles and the physiological saline, used for suspending the microparticles, was checked. In both cases, no growth inhibition was observed in the analyzed cultures, which excludes the possibility of the influence of the chemical composition of the polymer and the medium used for suspending on the results of the efficacy of the encapsulated form of CEF. The results obtained correlate with the data presented in the paper by Hema et al.[70] where the researchers confirmed the absence of antibacterial activity of empty films based on P(3HB), P(3HB-co-3HV) (6 mol%) and P (3HBco-4HB) (70 mol%) with respect to *E. coli* and *S. aureus*.

CONCLUSION

The outcome of this study is the successful development of CEF-loaded PHAs-based microparicles and their complex characterization. P3HB and P3HB-PEG microparticles were prepared by double emulsification technique and spray-drying. The encapsulation efficiency of CEF was about 60% and 50% for MPEM and MPSD, respectively. It was shown that the surfactants used in the emulsion method affect on the electrophoretic activity of the microparticles. Therefore, the zeta potential of MPEM was lower than that of MPSD by an average of three times. The addition of PEG to the P3HB solutions had important influence on the surface morphologies and microstructures of microparticles; however, the effect on drug release rate was clearly expressed only for MPEM. The total release of CEF from MPEM and MPSD had significant difference, which is most likely related to the localization of the drug molecules in the microparticles. Therefore, the CEF release of MPSD reached 100% after 150 h, while for MPEM the total release of CEF did not exceed 34% for the entire observation period. The release profiles could be best explained by Zero-order, Higuchi, and Korsmeyer-Peppas models, as the plots showed high linearity.

The bactericidal effect of MPEM and MPSD CEF-loaded was investigated in cultures of *E. coli* and *S. aureus* by disk diffusion test. MPSD with CEF showed higher antibacterial activity (ZOI over 22.0mm) in comparison to MPEM (ZOI not more than 5.1 mm). Thus, using various methods, the possibility of loading CEF in P3HB-carriers with satisfactory indicators of the encapsulation efficiency, drug release, and preservation of therapeutic activity in vitro is shown, which allows concluding that this class of polymers is promising for the development of long-acting dosage forms.

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REFERENCES

[1] Zhang, Y.; Chan, H. F.; Leong, K. W. Advanced Materials and Processing for Drug Delivery: The past and the Future. Adv. Drug Deliv. Rev. 2013, 65, 104–120. DOI: 10.1016/j.addr.2012.10.003.

[2] Lamprecht, A. Nanotherapeutics Drug Delivery Concepts in Nanoscience; Pan Stanford Publishing: Singapore, 2009; pp 199–220.

[3] Wright, J.; Paauw, D. S. Complications of Antibiotic Therapy. Med. Clin. North Am. 2013, 97, 667–679. DOI: 10.1016/j.mcna.2013.02.006.

[4] Matharu, R. K.; Porwal, H.; Ciric, L.; Edirisinghe, M. The Effect of Graphene–Poly(Methyl Methacrylate) Fibres on Microbial Growth. Interface Focus 2018, 8, 20170058. DOI: 10.1098/rsfs.2017.0058.

[5] Matharu, R. K.; Charani, Z.; Ciric, L.; Illangakoon, U. E.; Edirisinghe, M. Antimicrobial Activity of Tellurium-Loaded Polymeric Fiber Meshes. J. Appl. Polym. Sci. 2018, 135, A46368. DOI: 10.1002/app. 46368.

[6] Al-Jumaili, A.; Alancherry, S.; Bazaka, K.; Jacob, M. V. Review on the Antimicrobial Properties of Carbon Nanostructures. Materials 2017, 10, 1066. DOI: 10.3390/ma10091066.

[7] Kataoka, K.; Kabanov, A. Polymeric Micelles in Biology and Pharmaceutics; Elsevier: Amsterdam, 1999; pp 342.

[8] Re, M. I. Microencapsulation by Spray Drying. Drying Technol. 1998, 16, 1195–1236. DOI: 10.1080/07373939808917460.

[9] Harsha, N. S. In Vitro and in Vivo Evaluation of Nanoparticles Prepared by Nano Spray Drying for Stomach Mucoadhesive Drug Delivery. Drying Technol. 2015, 33, 1199–1209. DOI: 10.1080/07373937.2014.995305.

[10] Deshmukh, R. K.; Wagh, P. S.; Naik, J. B. Solvent Evaporation and Spray Drying Technique for Microand Nano Spheres/Particles Preparation: A Review. Drying Technol. 2016, 34, 1758–1772. DOI: 10.1080/07373937.2016.1232271.

[11] Raval, P.; Naik, D. R.; Patel, P. S. Spray-Dried Cefixime Encapsulated Poly(Lactide-Coglycolide) Microparticles: Characterization and Evaluation of in Vitro Release Kinetics with Antibacterial Activity. Drying Technol. 2012, 30, 865–872. DOI: 10.1080/07373937.2012.673526.

[12] Xie, S.; Tao, Y.; Pan, Y.; Qu, W.; Cheng, G.; Huang, L.; Chen, D.; Wang, X.; Liu, Z.; Yuan, Z. Biodegradable Nanoparticles for Intracellular Delivery of Antimicrobial Agents. J. Controlled Release 2014, 187, 101–117. DOI: 10.1016/j.jconrel.2014.05.034.

[13] Hamzehloo, M.; Karimi, J.; Eghbali, N.; Mirzakhani, M.; Aghapoor, K.; Reza, H. D. A New Blend of Polymeric Encapsulation of Azithromycin by Spray-Drying with a pH Responsive in Drug Release. Drying Technol. 2017, 35, 1688–1695. DOI: 10.1080/07373937.2016.1269807.

[14] Zazo, H.; Colino, C. I.; Lanao, J. M. Current Applications of Nanoparticles in Infectious Diseases. J. Controlled Release. 2016, 224, 86–102. DOI: 10.1016/j.jconrel.2016.01.008.

[15] Gaudin, K.; Langlois, M.-H.; Kauss, T.; Phoeung, T.; Arrachart, S.; Demartini, A. M.; Gaziello, F.; Ashley, E.; Gomes, M.; White, N. In Vitro Ceftriaxone Stability at Newborns' Rectal pH Assessed by UV and HPLC Methods. Pharm. Anal. Acta 2015, 6, 1–5. DOI: http://dx.doi.org/10.4172/2153-2435.1000393.

[16] Manimekalai, P.; Dhanalakshmi, R.; Manavalan, R. Preparation and Characterization of Ceftiaxone Sodium Encapsulated Chitosan Nanoparticles. Int. J. App. Pharm. 2017, 9, 10–15. DOI: http://dx.doi.org/10.22159/ijap.2017v9i6.16317.

[17] Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. Experimental and Computational Approaches to Estimate Solubility and Permeability in Drug Discovery and Development Settings. Adv. Drug Deliv. Rev. 1997, 23, 3–26. DOI: 10.1016/S0169-409X(00)00129-0.

[18] Shiffman, M. L.; Keith, F. B.; Moore, E. W. Pathogenesis of Ceftriaxone Associated Biliary Sludge. In Vitro Studies of Calcium-Ceftriaxone Binding and Solubility. Gastroenterology 1990, 99, 1772–1778. DOI: 10.1016/0016-5085(90)90486-K.

[19] Kauss, T.; Marchivie, M.; Phoeung, T.; Gaubert, A.; Desire, A.; Tonelli, G.; Boyer, C.; Langlois, M.-H.; Cartwright, A.; Gomes, M.; et al. Preformulation Studies of Ceftriaxone for Pediatric Non-Parenteral Administration as an Alternative to Existing Injectable 'Formulations. Eur. J. Pharm. Sci. 2017,104, 382–392. DOI: 10.1016/j.ejps.2017.04.010.

[20] Ofokansi, K. C.; Adikwu, M. U.; Okore, V. C. Preparation and Evaluation of mucin-gelatin mucoadhesive microspheres for rectal delivery of ceftriaxone sodium. Drug Dev. Ind. Pharm. 2007, 33, 691–700. DOI: 10.1080/03639040701360876.

[21] Attama, A.; Okafor, C. E.; Builders, P. F.; Okorie, O. Formulation and in Vitro Evaluation of a PEGylated Microscopic Lipospheres Delivery System for Ceftriaxone Sodium. Drug Deliv. 2009, 16, 448–457. DOI: 10.3109/10717540903334959.

[22] Patel, N.; Lalwani, D.; Gollmer, S.; Injeti, E.; Sari, Y.; Nesamony, J. Development and Evaluation of a Calcium Alginate Based Oral Ceftriaxone Sodium Formulation. Prog. Biomater. 2016, 5, 117–133.DOI: 10.1007/s40204-016-0051-9.

[23] Kumar, S.; Bhanjana, G.; Kumar, A.; Taneja, K.; Dilbaghi, N.; Kim, K.-H. Synthesis and Optimization of Ceftriaxone-Loaded Solid Lipid Nanocarriers. Chem. Phys. Lipids. 2016, 200, 126–132. DOI: 10.1016/j.chemphyslip.2016.09.002.

[24] Harshiny, M.; Matheswaran, M.; Arthanareeswaran, G.; Kumaran, S.; Rajasree, S. Enhancement of Antibacterial Properties of Silver Nanoparticles–Ceftriaxone Conjugate through Mukia Maderaspatana Leaf Extract Mediated Synthesis. Ecotox. Environ. Safe 2015, 121, 135–141. DOI: 10.1016/j.ecoenv.2015.04.041.
[25] Najafi Moghadam, P.; Azaryan, E.; Zeynizade, B. Investigation of Poly(Styrene-Alt-Maleic Anhydride) Copolymer for Controlled Drug Delivery of Ceftriaxone Antibiotic. J. Macromol. Sci. Pure Appl. Chem. 2010, 47, 839–848. DOI: 10.1080/10601325.2010.492265.
[26] Zaki, N. M.; Hafez, M. M. Enhanced antibacterial effect of ceftriaxone sodium-loaded chitosan nanoparticles against intracellular Salmonella Typhimurium. AAPS Pharm. Sci. Tech. 2012, 13, 411–421. DOI: 10.1208/s12249-012-9758-7.
[27] Li, H.; Chang, J. Preparation, characterization and in vitro release of gentamicin from PHBV/wollastonite composite microspheres. J. Control. Release 2005,107, 463–473. DOI: 10.1016/j.jconrel.2005.05.019.
[28] Wang, Y.; Wang, X.; Wei, K.; Zhao, N.; Zhang, S.; Chen, J. Fabrication, Characterization and Long-Term in Vitro Release of Hydrophilic Drug Using PHBV/HA Composite Microspheres. Mater. Lett. 2007, 61, 1071–1076. DOI: 10.1016/j.matlet.2006.06.062.
[29] Goreva, A. V.; Shishatskaya, E. I.; Volova, T. G.; Sinskey, A. J. Characterization of Polymeric Microparticles Based on Resorbable Polyesters of Oxyalkanoic Acids as a Platform for Deposition and Delivery of Drugs. Polym. Sci. Ser. A 2012, 54, 94–105. DOI: 10.1134/S0965545X12020022.
[30] Vilos, C.; Constandil, L.; Herrera, N.; Solar, P.; Escobar-Fica, J.; Vel asquez, L. A. Ceftiofur-Loaded PHBV Microparticles: A Potential Formulation for a Long-Acting Antibiotic to Treat Animal Infections. Electron. J. Biotechnol. 2012, 15. DOI: 10.2225/vol15-issue4-fulltext-2.
[31] Rodriguez-Contreras, A.; Canal, C.; Calafell-Monfort, M.; Ginebra, M.; Julio-Moran, G.; Marques-Calvo, M. Methods for the Preparation of Doxycycline-Loaded PHB Micro- and Nano-Spheres. Eur. Polym. J. 2013, 49, 3501–3511. DOI: 10.1016/j.eurpolymj.2013.08.010.
[32] Shershneva, A. M.; Murueva, A. V.; Shishatskaya, E. I.; Volova, T. G. Study of Electrokinetic Potential of Drug Micro-Carriers Prepared from Resorbable Polymers Bioplastotan. Biophysics 2014, 59, 561–567. DOI: 10.1134/S000635091404023X.
[33] Chen, Q.; Li, W.; Goudouri, O.-M.; Ding, Y.; Cabanas-Polo, S.; Boccaccini, A. R. Electrophoretic Deposition of Antibiotic Loaded PHBV Microsphere-Alginate Composite Coating with Controlled Delivery Potential. Colloids Surf. B: Biointerfaces 2015, 130, 199–206. DOI: 10.1016/j.colsurfb.2015.04.009.
[34] Nigmatullin, R.; Thomas, P.; Lukasiewicz, B.; Puthussery, H.; Roy, I. Polyhydroxyalkanoates, a Family of Natural Polymers, and Their Applications in Drug Delivery. J. Chem. Technol. Biotechnol. 2015,90, 1209–1221. DOI: 10.1002/jctb.4685.
[35] Rai, R.; Keshavarz, T.; Roether, J.; Boccaccini, A.; Roy, I. Medium Chain Length Polyhydroxyalkanoates, Promising New Biomedical Materials for the Future. Mater. Sci. Eng. R 2011, 72, 29–47. DOI: 10.1016/j.mser.2010.11.002.
[36] Huerta-Angeles, G.; Brandejsov, M.; Nigmatullin,R.; Kopecka, K.; Vagnerova, H.; Smejkalova, D.; Roy, I.; Velebny, V. Synthesis of Graft Copolymers Based on Hyaluronan and Poly(3-Hydroxyalkanoates). Carbohydr. Polym. 2017, 171, 220–228. DOI: 10.1016/j.carbpol.2017.05.011.
[37] Volova, T.; Vinogradova, O.; Zhila, N.; Kiselev, E.; Peterson, I.; Vasil'ev, A.; Sukovatyi, A.; Shishatskaya, E. Physicochemical Properties of Multicomponent Polyhydroxyalkanoates: Novel Aspects. Polym. Sci. Ser. A 2017, 59, 98–106. DOI: 10.1134/S0965545X17010163.
[38] Reardon, P.; Parhizkar, M.; Harker, A.; Browning, R.; Vassileva, V.; Stride, E.; Pedley, B.; Edirisinghe, M.; Knowles, J. Electrohydrodynamic Fabrication of Core–Shell PLGA Nanoparticles with Controlled Release of Cisplatin for Enhanced Cancer Treatment. Int. J. Nanomed. 2017, 12, 3913–3926. DOI: 10.2147/IJN.S134833.
[39] Parhizkar, M.; Reardon, P.; Knowles, J.; Browning, R.; Stride, E.; Barbara, P.; Harker, A.; Edirisinghe, M. Electrohydrodynamic Encapsulation of Cisplatin in Poly(Lactic-Co-Glycolic Acid) Nanoparticles for Controlled Drug Delivery. Nanomed. Nanotechnol. Biol. Med. 2016, 12, 1919–1929. DOI: 10.1016/j.nano.2016.05.005
[40] Zhao, D.; Li, J.; Suen, W.; Chang, M.-W.; Huang, J. Preparation and Characterization of Ganoderma Lucidum Spores-Loaded Alginate Microspheres by Electrospraying. Mater. Sci. Eng. C 2016, 62, 835–842. DOI: 10.1016/j.msec.2016.02.020.
[41] Shams, T.; Parhizkar, M.; Illangakoon, U. E.; Orlu, M.; Edirisinghe, M. Core/Shell Microencapsulation of Indomethacin/Paracetamol by Co-Axial Electrohydrodynamic Atomization. Mater. Des. 2017, 136, 204–213. DOI: 10.1016/j.matdes.2017.09.052.
[42] Kucuk, I.; Edirisinghe, M. Microfluidic Preparation of Polymer Nanospheres. J. Nanopart. Res. 2014, 16, 2626. DOI: 10.1007/s11051-014-2626-5.
[43] Gunduz, O.; Ahmad, Z.; Stride, E.; Edirisinghe, M. A Device for the Fabrication of Multifunctional Particles from Microbubble Suspensions. Mater. Sci. Eng. C 2012, 32, 1005–1010. DOI: 10.1016/j.msec.2012.01.018.
[44] Chirio, D.; Gallarate, M.; Peira, E.; Battaglia, L.; Serpe, L.; Trotta, M. Formulation of Curcumin-Loaded Solid Lipid Nanoparticles Produced by Fatty Acids Coacervation Technique. J. Microencapsul. 2011, 28, 537–548. DOI: 10.3109/02652048.2011590615.
[45] Yan, C.; Zhang, W. Coacervation Processes. In Microencapsulation in the Food Industry, Part III: Process Technologies in Microencapsulation; Gaonkar, A.G., Vasisht, N., Khare, A.R., Sobel, R., Eds;. Academic Press: San Diego, 2014; pp. 125–137.

[46] Kim, J.-W.; Suh, K.-D. Monodisperse Polymer Particles Synthesized by Seeded Polymerization Techniques. J. Ind. Eng. Chem. 2008, 14, 1–9. DOI:10.1016/j.jiec.2007.06.001.
[47] Palamoor, M.; Jablonski, M. Comparative Study on Diffusion and Evaporation Emulsionmethods Used to Load Hydrophilic Drugs in Poly(Ortho Ester) Nanoparticle Emulsions. Powder Technol. 2014, 253, 53–62. DOI: 10.1016/j.powtec.2013.11.014.
[48] Vilos, C.; Velasquez, L. Therapeutic Strategies Based on Polymeric Microparticles. J. Biomed. Biotechnol. 2012, 2012, 1–9. DOI: http://dx.doi.org/10.1155/2012/672760.
[49] Waghulde, M.; Jitendra, N. Comparative Study of Encapsulated Vildagliptin Microparticles Produced by Spray Drying and Solvent Evaporation Technique. Drying Technol. 2017, 35, 1644–1654. DOI: 10.1080/07373937.2016.1273230.
[50] Islam, M.; Langrish, T.; Chiou, D. Particle Crystallization during Spray Drying in Humid Air. J. Food Eng. 2010, 99, 55–62. DOI: 10.1016/j.jfoodeng.2010.01.037.
[51] Yoshii, H.; Neoh, T. L.; Furuta, T.; Ohkawara, M. Encapsulation of Proteins by Spray Drying and Crystal Transformation Method. Drying Technol. 2008, 26, 1308–1312. DOI: 10.1080/07373930802330888.
[52] Sosnik, A.; Seremeta, K. P. Advantages and Challenges of the spray-drying technology for the production of pure drug particles and drug-loaded polymeric carriers. Adv. Colloid Interface Sci. 2015, 223, 40–54. DOI: 10.1016/j.cis.2015.05.003.
[53] Re, M.-I. Formulating Drug Delivery Systems by Spray Drying. Drying Technol. 2006, 24, 433–446. DOI: 10.1080/07373930600611877.
[54] Murueva, A. V.; Shishatskaya, E. I.; Kuzmina, A. M.; Volova, T. G.; Sinskey, A. J. Microparticles Prepared from Biodegradable Polyhydroxyalkanoates as Matrix for Encapsulation of Cytostatic Drug. J. Mater. Sci. Mater. Med. 2013, 24, 1905–1915. DOI: 10.1007/s10856-013-4941-2.
[55] Murueva, A. V.; Shershneva, A. M. Production of Resorbable Microparticles Loaded with a Cytostatic Drug Using the Spray-Drying Method and Investigation of Their Properties. J. Sib. Fed. Univ. Biol. 2016, 9, 75–87. DOI: http://elib.sfu-kras.ru/bitstream/handle/2311/20183/07_Murueva.pdf?sequencej1.
[56] Shershneva, A.; Murueva, A.; Nikolaeva, E.; Shishatskaya, E.; Volova, T. Novel Spray-Dried PHA Microparticles for Antitumor Drug Release. Drying Technol. 2018, 36, 1387–1398. DOI: 10.1080/07373937.2017.1407940.
[57] Shershneva, A. M.; Shishatskaya, E. I. Construction of Microparticles Based on Resorbable Polymers Bioplastotan Using Spray Drying Method. J. Sib. Fed. Univ. Biol. 2014, 7, 195–208. DOI: http://elib.sfukras.ru/bitstream/handle/2311/13406/07_Shershneva.pdf?sequence =1.
[58] Eltayeb, M.; Stride, E.; Edirisinghe, M.; Harker, A. Electrosprayed Nanoparticle Delivery System for Controlled Release. Mater. Sci. Eng. C 2016, 66, 138–146. DOI: 10.1016/j.msec.2016.04.001.
[59] Cavalieri, S. J. Manual of Antimicrobial Susceptibility Testing; American Society for Microbiology: Washington, DC, 2009; pp 241
[60] Francis, L.; Meng, D.; Knowles, J.; Keshavarz, T.; Boccaccini, A.; Roy, I. Controlled Delivery of Gentamicin Using Poly(3-Hydroxybutyrate) Microspheres. Int. J. Mol. Sci. 2011, 12, 4294–4314. DOI: 10.3390/ijms12074294.
[61] Paudel, A.; Worku, Z. A.; Meeus, J.; Guns, S.; Van den Mooter, G. Manufacturing of Solid Dispersions of Poorly Water Soluble Drugs by Spray Drying: Formulation and Process Considerations. Int. J. Pharm. 2013, 453, 253–284. DOI: 10.1016/j.ijpharm.2012.07.015.
[62] Li, F.; Tian, F.; Liu, C.-J.; Zhao, Y.-L. Preparation and Characterization of Improved Microspheres Containing Bovine Serum Albumin. J. Appl. Polym. Sci. 2009, 114, 818–825. DOI: 10.1002/app.30478.
[63] Parra, D. F.; Fusaro, J.; Gaboardi, F.; Rosa, D. S. Influence of Poly(Ethylene Glycol) on the Thermal, Mechanical, Morphological, Physical-Chemical and Biodegradation Properties of Poly (3-Hydroxybutyrate). Polym. Degrad. Stab. 2006, 91, 1954–1959. DOI: 10.1016/j.polymdegradstab.2006.02.008.
[64] Rodrigues, J. A. F. R.; Parra, D.; Lugao, A. V. Crystallization on Films of PHB/PEG Blends. J. Therm. Anal. Calorim. 2005, 79, 379–381. DOI: 10.1007/s10973-005-0069-z.
[65] Kose, G. T.; Kenar, H.; Hasırci, N.; Hasırci, V.Macroporous Poly(3-Hydroxybutyrate-Co-3-Hydroxyvalerate) Matrices for Bone Tissue Engineering. Biomaterials 2003, 24, 1949–1958. DOI: 10.1016/S0142-9612(02)00613-0.
[66] Xu, Z.; Mahalingam, S.; Basnett, P.; Raimi-Abraham, B.; Roy, I.; Craig, D.; Edirisinghe, M. Making Nonwoven Fibrous Poly(e-Caprolactone) Constructs for Antimicrobial and Tissue Engineering Applications by Pressurized Melt Gyration. Macromol. Mater. Eng. 2016, 301, 922–934. DOI:10.1002/mame.201600116.
[67] Fernandes, J. G.; Correia, D. M.; Botelho, G.; Padrao,J.; Dourado, F.; Ribeiro, C.; Lanceros-Mendez, S.; Sencadas, V. PHB-PEO Electrospun Fiber Membranes Containing Chlorhexidine for Drug Delivery Applications. Polym. Test 2014, 34, 64–71. DOI: 10.1016/j.polymertesting.2013.12.007.
[68] Labinskaya, A. S.; Volina, E. G. General and Sanitary Microbiology with Microbiological Research Techniques; Binom. Laboratoriya znanij: Moscow, 2010; pp. 1080. (in Russia)
[69] Mahalingam, S.; Xu, Z.; Edirisinghe, M. Antibacterial Activity and Biosensing of PVA-Lysozyme Microbubbles Formed by Pressurized Gyration. Langmuir 2015, 31, 9771–9780. DOI: 10.1021/acs.langmuir.5b02005.
[70] Hema, R.; Ng, P. N.; Amirul, A. A. Green Nanobiocomposite: Reinforcement Effect of Montmorillonite Clays on Physical and Biological Advancement of Various Polyhydroxyalkanoates. Polym. Bull. 2013, 70, 755–771. DOI: 10.1007/s00289-012-0822-y.

Table 1. Samples composition and characterization of the obtained microparticles

Samples	Process yield (%)	Particle size (urn)	Pdl	Zeta -potential (mV)	Encapsulation efficiency (%)
Emulsification technique					
P_{EM} P3HB	725	0.74±18S2	0.187 ±0.063	-178 ±0.3	-
PP_{EM} P3HB-PEG	39.0	155 ±0.05	0.167±0.037	-28.0 ±0.4	-
PC_{EM} P3HB-CEF	71.4	089 ±0.02	0250 ±0.003	-223 ±0.6	66.0
PPC_{EM} P3HB-PEG-CEF	268	155 ±0.01	0297 ±0.027	-25.0 ±0.1	60.5
Spray-drying					
P_{SD} P3HB	332	651 ±0.47	0211 ±0.058	-95.7 ±0.6	-
PP_{SD} P3HB-PEG	51.1	4.06 ±0.38	0318 ±0.192	-375 ±2.2	-
PCs_{D} P3HB-CEF	85.7	421 ±0.70	0260 ±0.118	-48.0 ±1.4	50.6
PPC_{SD} P3HB-PEG-CEF	345	384 ±0.27	0224-0.058	-38.7 ±1.2	47.0

Table 2. Release parameters of CEF from microparticles in PBS *in vitro.*

	Zero-order		First- order		Higuchi		Hixson-Qowell		Korsmeyer-Pappas	
Samples	R^2	$K_0 \cdot h^{-1}$	R^2	$K_1 \cdot h^{-1}$	R^2	$K_H \cdot h^{-0.5}$	R^2	$K_H C^{(h-1/3)}$	n	R^2
PC_{EM}	0.922	0.027	0.671	0.006	0.982	0.539	0.766	0.003	0.017	0.920
PPC_{EM}	0.925	0.099	0.605	0.006	0.963	1.900	0.705	0.005	0.062	0.935
PC_{SD}	0.699	0.295	0.463	0.003	0.766	4.377	0.494	0.004	0.161	0.936
PPC_{SD}	0.832	0.302	0.565	0.004	0.894	5.012	0.621	0.005	0.173	0.967

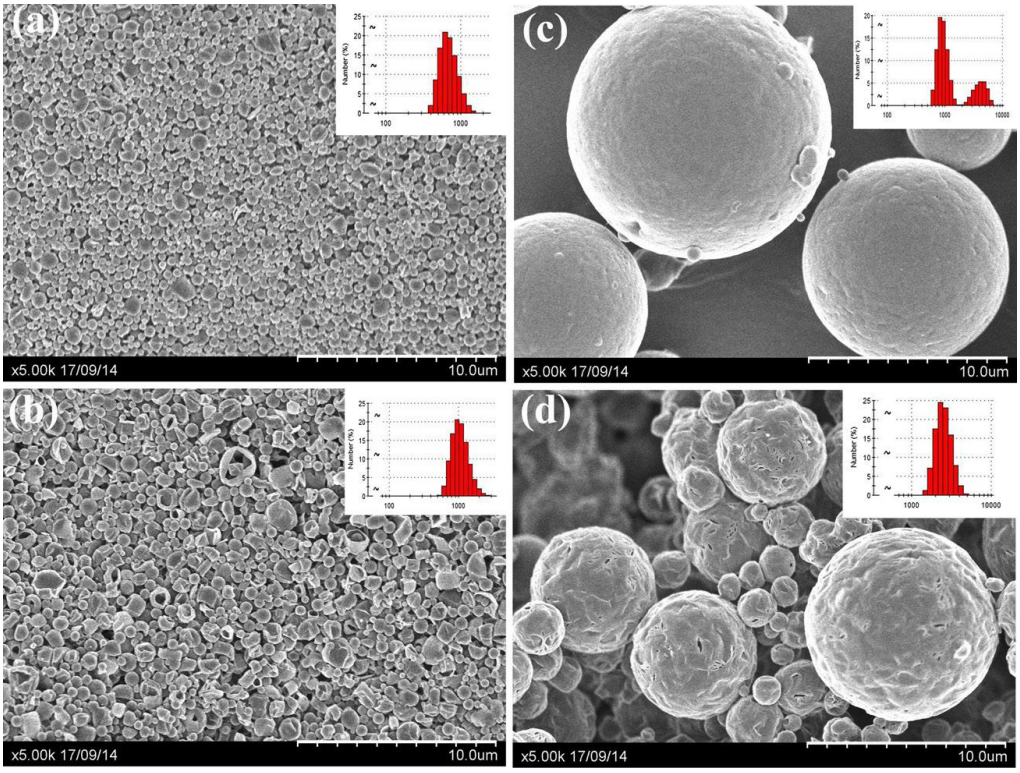

Figure 1. SEM images and size distribution of microparticles with CEF: (a) microparticles of poly-3-hydroxybutyrate prepared by emulsification technique; (b) microparticles of poly-3-hydroxybutyrate/PEG prepared by emulsification technique; (c) microparticles of poly-3-hydroxybutyrate prepared by spray-drying; (d) microparticles of poly-3-hydroxybutyrate/PEG prepared by spray-drying. Bar represents 10 μm.

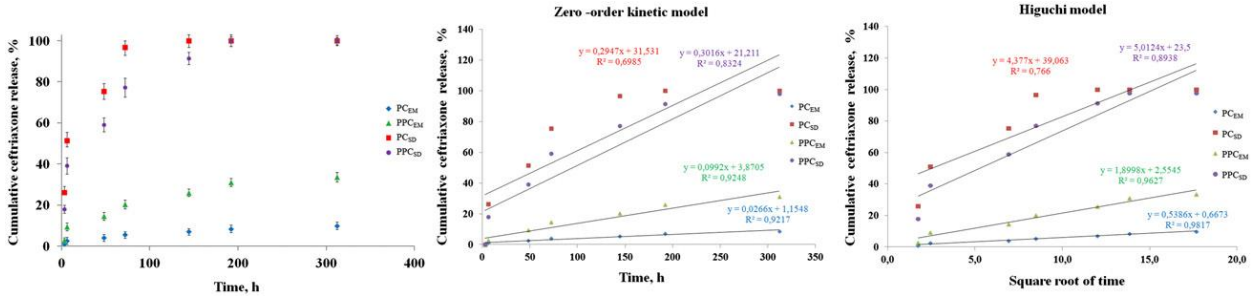

Figure 2. Release assays (a), kinetic profiles of CEF loaded in microparticles of (♦) PCEM, (▲) PPCEM, (■) PCSD, (●) PPCSD; (b) Mechanism of CEF release by Zero-order model; (c) Mechanism of CEF release by Higuchi model.

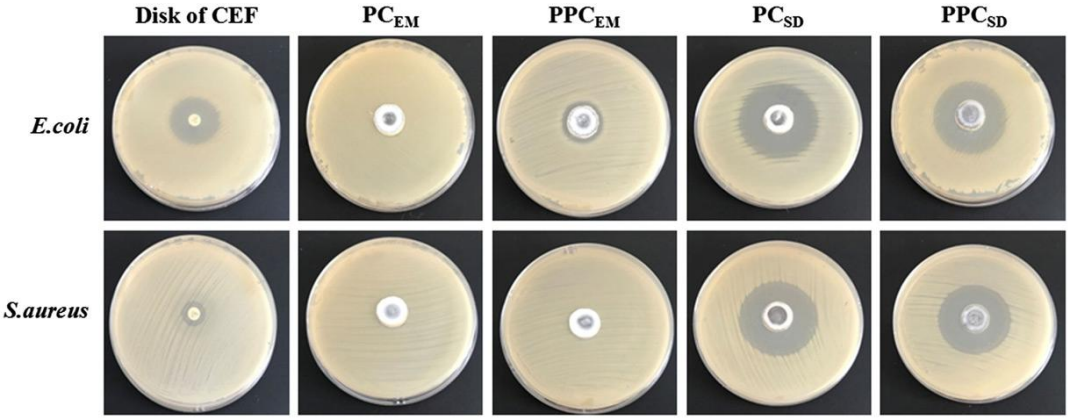

Figure 3. Images of antibacterial activity of microparticles against *E. coli* and *S. aureus*.

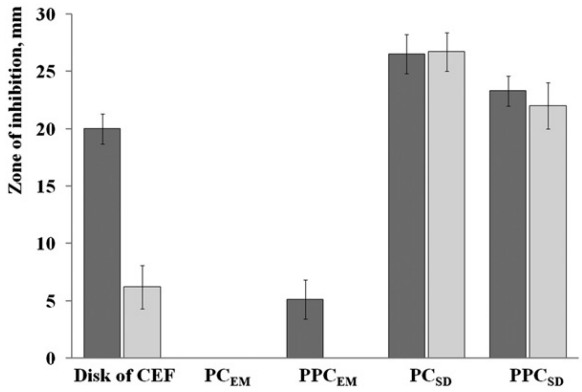

Figure 4. Antibacterial activity of microparticles against *E. coli* and *S. aureus*. Diameter of inhibition zones in mm.