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Methods for the preparation of doxycycline-loaded phb micro- and nano-spheres

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ABSTRACT

Natural and synthetic biodegradable polymers have been investigated for controlled drug release. Poly(3-hydroxybutyrate) can be produced by bacteria and is remarkable for this application due to its excellent biocompatibility and biodegradability. The objective of this work was to study different drug-entrapment and emulsification methods for the obtaining of doxycycline-loaded PHB micro- and nano-spheres. The micro-/nano-particles were prepared by polymer precipitation via dialysis, simple emulsion (*O*/*W*) or multiple emulsion ($W_1/O/W_2$) applying solvent evaporation in the last two cases. This was carried out either by ultrasonication, dripping and/or high speed stirring. Different processing conditions were varied in order to evaluate their influence on morphology, size, and drug entrapment capabilities. The highest drug loading was obtained by single emulsion with high speed stirring. In the case of multiple emulsion, the combination of ultrasound with high speed stirring resulted in the most elevate process yield and drug loading capability.

1. Introduction

Poly(3-hydroxybutyrate) (PHB) is a biopolyester from the family of Polyhydroxyalkanoates which is produced by microorganisms from renewable resources [23]. Among natural and synthetic biodegradable polymers, PHB is found to be remarkable for its applications in drug delivery due to its excellent biocompatibility and degradability [21].It is also important that PHB is free from degradation related toxic products (e.g. monomers, stabilizers, polymerization initiators, emulsifiers). The biopolymer production via microbial fermentation avoids the presence of toxic products from the synthetic polymerization process [11], while the hydrolytic degradation of PHB leads to obtain the monomer D-3-hydroxybutyric acid. This acid is a normal constituent of blood and is one of the three ketone bodies which are produced endogenously by the process known as ketogenesis [19]. Other advantages of PHB, when compared with chemically produced polymers such as polyglycolic acid (PGA), polylactic acid (PLA), and poly(lactide-co-glycolide) (PLGA), which are mostly well known as biologically degradable drug carriers with good retarding characteristics, excellent biocompatibility, and propensity to biodegradation under different environmental conditions [4,5]. In addition, the controllable retarding properties of drug delivery systems based on PHB can be modulated by variations in processing and molecular mass of the polymer and copolymer composition [10,5].

There are very few papers reporting on the incorporation and subsequent release of therapeutic agents with PHB systems. Mora-Huertas et al. [15] reported a summary of the methods for the formation of polymeric capsules formation for drug delivery, but not all methods can be

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applied for PHB. The use of PHB in drug delivery systems (DDS) has been reported in recent years, and emulsion followed by solvent evaporation has been the most frequently used microencapsulation technique [3,15,12]; Mora-[20,22]. Although some studies report low entrapment efficiency (EE) [3], the double emulsion (water-in-oil-in-water, $W_1/O/W_2$) solvent evaporation is an advantageous technique because it provides higher protection for the substance to be encapsulated. Nevertheless, it is possible to increase the encapsulation capability of the system by modifying the double emulsion preparation conditions [7,9,2,1]. Also, Errico et al. [5] recently reported a new technique for PHB drug-loaded capsules via dialysis. It is the aim of this work to evaluate the suitability and compare the efficiency of these three techniques in order to produce antibiotic-loaded PHB capsules.

Doxycycline (DOXY) is a well-known broad-spectrum antibiotic, which is effective against both Gram-positive and Gram-negative bacteria, protozoa, and various anaerobes. As a tetracycline analog, it can work as a bacteriostatic which is capable of inhibiting the bacterial protein synthesis at the ribosomal sites. DOXY presents a long half-life, high lipid solubility, and very good oral absorption. After being introduced in the clinical practice in 1967, DOXY has been frequently used in treating destructive periodontal diseases such as juvenile periodontitis and acute periodontal abscesses. It is used against periodontal infection and for enhancing bone regeneration after periodontal diseases. Additionally, it is also utilized to prevent bacterial infection related to septic arthritis. However, there are some concerns over possible side effects such as gastro-intestinal disturbance, esophageal erosion, and photosensitivity when administrated orally. Therefore, in order to reach the infection deep inside affected tissues with an effective drug concentration and to circumvent the systemic side effects, controlled local delivery of DOXY is desirable [6]. A possible route to achieve it is by encapsulating DOXY within a biodegradable matrix. Therefore, the aim of the present work is to achieve the micro- and/or nano-size particles for the first time with the system of PHB/DOXY. In this study, the evaluation of different drug-entrapment and emulsification methods was studied in order to obtain optimal DOXY-loaded PHB micro- and nano-particles. The micro-/nano-spheres were prepared by: (a) Polymer precipitation via dialysis; (b) simple emulsion (O/W); or (c) multiple emulsion ($W_1/O/W_2$) applying solvent evaporation in the last two cases. This was carried out either by ultrasonication, dripping and/or high speed stirring. Different processing conditions were also varied in order to evaluate their influence on morphology, size, and drug entrapment capabilities (drug loading, encapsulation efficiency and method efficiency).

2. Methods

2.1. Materials

PHB from microbial fermentation with *Cupriavidus necator* and glycerol as carbon source (M_w 313 KDa and polydispersity index of 4.15) was purchased from Institute of Biotechnology and Biochemical Engineering (Graz University of Technology). The used antibiotic was doxycycline hyclate (DOXY) (Sigma–Aldrich) (doxycycline hydrochloride hemiethanolate hemihydrate, $C_{22}H_{24}N_2O_8 \cdot HCl \cdot 0.5H_2O \cdot 0.5C_2H_6O$, M_W 1025.89 Da), which is freely soluble in water (1:1–10 w/w) [18]. Fig. 1 shows the chemical structure of both, biopolymer (Fig. 1a) and antibiotic (Fig. 1b). Chemical products such as dimethyl sulfoxide (DMSO), trifluoroethanol 99% (TFE) and dichloromethane (DCM) were purchased from Sigma Chemical Co. and used without further purification. The gelling agent was bovine gelatin (Gel) and the surfactants used were Pluronic-F127, poly(vinyl alcohol) (PVA) and sodium taurocholate (TAU) (Sigma Chemical Co.).The utilizad dialysis bags were porous cellulose membranes with a molecular mass cut off of 12,000.

2.2. PHB-based particles

Different methods were applied in this study to prepare DOXY-containing micro- and nano-particles: (a) precipitation via dialysis; (b) O/W or (c) $W_1/O/W_2$ emulsions with solvent evaporation. Three different routes were used to obtain the emulsions, namely ultrasound, dripping, and high speed stirring. Data relevant to individual experiments are summarized in Table 1, whereas the general procedures are described in detail in the following paragraphs.

2.2.1. PHB-particle production by precipitation via dialysis

The method followed was previously reported by Errico et al. [5]. For unloaded particles, an amount of 10 mg of PHB dissolved in 9 mL of TFE and 1 mL of DMSO was introduced in the dialysis membrane. To prepare DOXY-loaded particles, 10 mg of PHB dissolved in 9.6 mL of TFE with different amounts of Pluronic-F127 solution in TFE (10 mg/mL) and DOXY dissolved in different amounts of DMSO were used. The resulting solutions were stirred at room temperature for 20 min, then dialyzed against 1 L of distilled water for 3 h and then distilled water exchange at intervals of 3–4 h during 24 h.

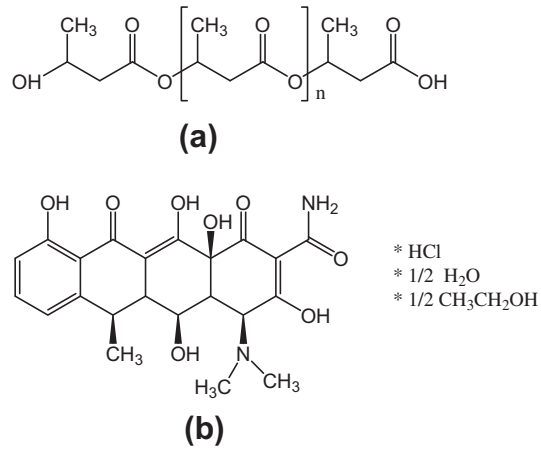

Fig. 1. Chemical structure of PHB and DOXY.