Materials Science and Engineering C 60 (2016) 511–517

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Marbofloxacin-encapsulated microparticles provide sustained drug release for treatment of veterinary diseases

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ARTICLE INFO

Article history:
Received 24 August 2015
Received in revised form 3 November 2015
Accepted 3 December 2015
Available online 4 December 2015

Keywords:
Marbofloxacin
Microparticle
Sustained drug release
Veterinary disease

ABSTRACT

Fluoroquinolone antibiotics with concentration-dependent killing effects and a well-established broad spectrum of activity are used commonly to treat infectious diseases caused by bacteria. However, frequent and excessive administration of these antibiotics is a serious problem, and leads to increased number of drug-resistant bacteria. Thus, there is an urgent need for novel fluoroquinolone antibiotic formulations that minimize the risk of resistance while maximizing their efficacy. In this study, we developed intramuscularly injectable polymeric microparticles (MPs) that encapsulated with marbofloxacin (MAR) and were composed of poly(D,L-lactide-co-glycolic acid) (PLGA) and poloxamer (POL). MAR-encapsulated MP (MAR-MP) had a spherical shape with particle size ranging from 80 μm to 120 μm. Drug loading efficiency varied from 55 to 85% (w/w) at increasing amount of hydrophilic agent, POL. Drug release from MAR-MP demonstrated a significant and sustained increase at increased ratios of POL to PLGA. These results indicate that MAR-MP is an improved drug delivery carrier for fluoroquinolone antibiotics, which can reduce the number of doses needed and sustain a high release rate of MAR for 2–3 days. As a novel and highly effective drug delivery platform, MAR-MP has great potential for use in a broad range of applications for the treatment of various veterinary diseases.

1. Introduction

Antibiotics are critical therapeutic agents used for the treatment of bacterial infection in humans and animals. While commercially available antibiotics have been developed and utilized in veterinary medicine, there are many problems associated with their use such as rapid release at disease site [1,2]. Antibiotics are generally administered through intravenous, intramuscular, and subcutaneous injection routes for animals. However, administration through these routs is time- and labor-intensive because multiple injections of a conventional formulation are often required to maintain drug concentration in the body. In addition, animals may become fractious and may not cooperate after multiple days of parenteral therapy [3].

Resistant bacterial strains appear owing to the misuse of an antibiotic, resulting in a reduction of its therapeutic efficacy [4,5]. Therefore, a long acting antibiotic formulation that can reduce the frequency of administration and total dose should be beneficial in terms of convenience for animals, and to minimize the emergence of antibiotic resistance and side effects due to misuse [6].

Marbofloxacin (MAR), a third generation fluoroquinolone antibiotic, has been extensively used to treat infectious diseases in animals. MAR can affect various Gram-positive and Gram-negative strains and mycoplasma that easily infect the dermis, respiratory system, mammary gland, and urinary tract in dogs, pigs, and cows [7,8]. MAR exhibits concentration and time dependent bactericidal activity against Gram-negative and positive bacteria, respectively [9]. However, injection times are frequent and the dose is high because of its short in vivo half-life [10,11]. To overcome these limitations, we developed MAR-encapsulated microparticles (MAR-MPs) as a sustained drug release carrier.

A number of studies have reported the development of biodegradable and biocompatible carrier systems for sustained release of drug after administration to animals. Polymeric sustained drug release formulations modulate the release pattern of drug by regulating the decomposition of the polymer in the body. Poly(D,L-lactide-co-glycolic acid) (PLGA) is an attractive biodegradable polymer for development of carrier systems. There are many methods for manufacturing PLGA

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http://dx.doi.org/10.1016/j.msec.2015.12.004
0928-4931/

micro-/nano-sized drug carriers, including phase separation, spray drying, and solvent evaporation [12]. Among these, the solvent evaporation technique, classified as single (oil-in-water, O/W) emulsion or double (water-in-oil-in-water, W/O/W) emulsion methods, has been used most commonly [13]. However, PLGA particles generated by the solvent evaporation method often have limitations such as low encapsulation efficiency and poor sustained release after initial administration. Therefore, development of a novel formulation is needed to increase drug loading and realize sustained release to achieve therapeutic efficacy [14]. The release of drug should be slow enough to obtain controlled release for a prolonged drug effect to maintain therapeutic effect [3]. Here, we developed MAR-encapsulated MP (MAR-MP) for sustained drug release. In the MAR-MP system, sustained release of MAR was achieved by poloxamer (POL) degradation as the step. Next, in the second step, PLGA degradation maintains the controlled release of the drug, hence, MAR-MP can be used as a potential antibiotic carrier in veterinary medicine for sustained drug release.

2. Materials and methods

2.1. Materials

PLGA (Resomer® RG 504 H, MW 30,000, 0.45–0.60 dl/g, lactide:glycolide = 50:50) was purchased from Evonik Industries (Essen, Germany). Lutrol® F 127 (poloxamer 407, POL) was purchased from BASF (Monheim, Germany). Marbofloxacin (MAR) was supplied by Zhejiang Guobang Pharmaceutical Co. Ltd. (Hangzhou Bay, China). Dichloromethane (DCM), acetone, and poly(vinyl alcohol) (PVA, MW 30,000–70,000, 87–90% hydrolyzed) were purchased from Sigma-Aldrich (St. Louis. Mo. USA). Chloroform was purchased from Burdick & Jackson (Muskegon, MI, USA). Phosphate-buffered saline (PBS) tablets were purchased from AMRESCO Inc. (Solon, OH, USA). Müeller-Hinton agar was supplied by Merck (Darmstadt, Germany). Standardized antibiotic disks were obtained from the Mast Group (Merseyside, UK).

2.2. Preparation of MAR-MP

MAR-MP was prepared as a sustained drug release carrier by using the solid-in-oil-in-water (S/O/W) self-emulsifying emulsion method (SEEM). The overall scheme to prepare MAR-MP is shown in Fig. 1, and the composition of the MAR-MP is shown in Table 1. Briefly, PLGA (500 mg) and POL (0, 20, 50, and 100 mg) were dissolved in 4 ml of a mixed solvent (acetone/DCM). MAR (250–2000 mg) was added to the polymer solution. The mixture was sonicated with an ultrasonic probe (Sonic Dismembrator Model 500, Thermo Fisher Scientific Inc., Waltham, MA, USA) for 30 s at 100 W to suspend the solid-state drug within the polymer solution. The resulting S/O suspension was added to 50 ml of PVA solution (5% at 20 °C) by using a syringe at an input rate of 0.1 ml/s, and the mixture was simultaneously homogenized (Ultra Turrax T8, IKA®, Staufen, Germany) at 8000 rpm for 40 s to obtain the S/O/W emulsion. The emulsion was then stirred using a magnetic stirrer at 800 rpm for 10 min and percolated through a filter paper (Whatman®, Grade 40, USA). The collected MAR-MPs were washed three times with distilled water to remove residual solvent and PVA, and then exposed to air at 37 °C for 10 h. The MAR-MPs were then incubated in a vacuum oven (Jeio Tech Co. Ltd., Seoul, Korea) at 35 °C for 96 h to remove the residual solvent inside the particles.

2.3. Morphology of the MAR-MP

Morphologies and shapes of MAR-MP were observed by using a microscope (Olympus®, Japan) and by scanning electron microscopy (FE-SEM, Tescan Mira 3, Tescan USA, Warrendale, PA, USA). We

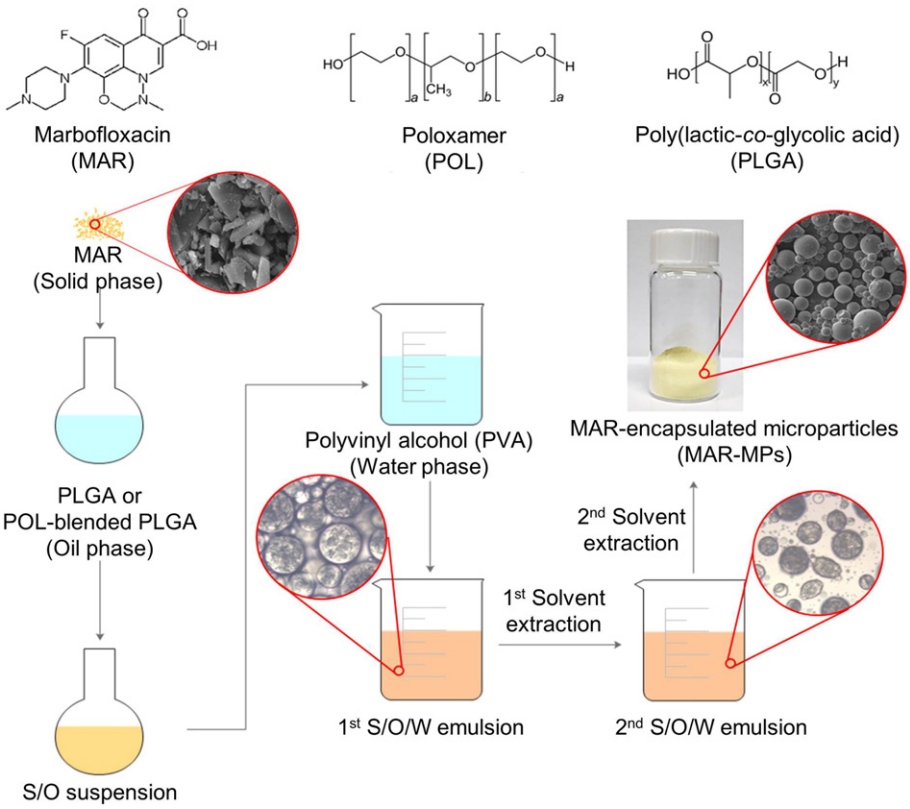

Fig. 1. Schematic illustration of the preparation procedure for MAR-MP by the S/O/W solvent extraction evaporation method.