Available online at www.sciencedirect.com
ScienceDirect
European Journal of Pharmaceutics and Biopharmaceutics 69 (2008) 750–759
European Journal of Pharmaceutics and Biopharmaceutics
www.elsevier.com/locate/ejpb

Research paper

The effects of cryoprotectants on the freeze-drying of ibuprofen-loaded solid lipid microparticles (SLM)

Lijuan Zhang, Lei Liu, Yu Qian *, Yun Chen

School of Chemical Engineering, South China University of Technology, Guangdong, PR China

Received 31 May 2007; accepted in revised form 6 December 2007
Available online 15 December 2007

Abstract

The effects of cryoprotectants on the diameter and the entrapment efficiency of ibuprofen-loaded solid lipid microparticles (SLM) during the freeze-drying process were investigated extensively. The SLM were prepared by the emulsion–congealing technique in which a glycerol behenate was used as the lipid matrix for the SLM and a soybean lecithin/bile salt used as the stabilizer. Also, trehalose, glucose, mannitol, and sucrose were chosen as the cryoprotectants. Trehalose and glucose proved to be the most effective in preventing particles aggregation and in inhibiting leakage from drug-loaded particles during the SLM freeze-drying process. The most suitable concentrations were proved to be 15% and 5% (wt), respectively.

Keywords: Solid lipid microparticles; Lyophilisation; Ibuprofen; Cryoprotectant

1. Introduction

During the last few decades, an increasing attention has been paid to the sustained release of various drugs [1–5]. Solid lipid microparticles (SLM) are micro- or nano-scale drug carrier systems composed of fatty acid, glyceride, fatty alcohol and solid wax. SLM are suitable as the carriers of lipophilic drugs [6–8]. SLM are characterized by their better bio-compatibility as compared to competing polymeric microparticles. Moreover, they are excellent in controlling and sustaining drug release efficiencies.

Ibuprofen is a well-known non-steroidal, anti-inflammatory drug which has been widely used for the treatments of inflammations, a variety of pains, and some rheumatism. However, it is limited by a short biological half-life time (1.5–2 h) [9] that leads to a short duration of action. In order to overcome the shortcoming, multiple intakes of ibuprofen are required to maintain the effective concentration in human bodies which potentially lead to the occurrences of some side effects [10]. A possible approach to resolve this problem is the application of SLM carrying ibuprofen [1].

In order to produce different pharmaceutical formulations which ensure the satisfaction of the specific needs related to storage, packing, and transportation, solid powder of SLM needs to be made by a drying process. Freeze-drying technology appears as one of the most suitable methods to stabilize and facilitate the handling of colloidal systems. It is widely used for drying pharmaceutical systems, e.g. liposomes [11–13], drug-loaded polymer microspheres [14,15], and solid lipid nanoparticles (SLN) [16]. At the same time, the freeze-drying of the drug-free and drug-loaded SLM was studied, and the diameters of both types of solid SLM were reported to increase as a result of drying [16].

Zimmermann et al. [17] investigated the protective efficiency of cryoprotectants, the freezing velocities, and the thermal treatments during the SLM freeze-drying process and optimized the freeze-drying process parameters. Schwarz and Mehnert [18] investigated the influence of differ-

* Corresponding author. School of Chemical Engineering, South China University of Technology, The Research Center for Process System Engineering, 381 Wushan Road, Guangzhou, Guangdong 510640, PR China. Tel.: +86 20 87112046; fax: +86 20 87113046.
E-mail address: ceyuqian@scut.edu.cn (Y. Qian).

0939-6411/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejpb.2007.12.003

ent cryoprotectants and different redispersion methods on the diameters and poly-dispersities of SLM. These studies presented a comprehensive understanding of the influence of various parameters on the freeze-drying process and promoted the application of the lyophilisation technique in liposome drying.

In addition to above-mentioned contributions, we present a new approach for the development of strategies aiming at lowering of drug toxicity and increasing drug's storage time as well as reducing the costs of drug preservation. The study reported in this paper is focused on the development of a new SLM. The lyophilisation of ibuprofen SLM has been investigated. And in particular, the effects of freeze-drying on the particle size, the entrapment efficiency and the role of cryoprotectants were examined.

2. Materials and methods

2.1. Materials

Ibuprofen was provided by Shanghai Yuanji Chemicals Co. Bile salt was produced by Guangdong Huankai Biology Co. (China); glycerol behenate by Gattefosse (France); glucose, mannitol, and soybean lecithin by Shanghai Boao Biology Ltd. (China); trehalose with two coordinated water molecules by Guangxi Jiewoli Biology Ltd. (China), sucrose by Guangzhou Chemical Reagent Co. (China). Water was double distilled with quart still. All agents were of analytical grade.

2.2. The preparation of SLM

The emulsion–congealing technique was employed to prepare SLM. The SLM consist of 10% lipid, 3% stabilizer (wt), 10% ibuprofen (wt, relative to lipid only), and water as the rest. Soybean lecithin and bile salt were mixed together at the ratio of 2:1 as the stabilizer. The emulsion–congealing process is achieved as follows [7]: the lipid mixed with drug is first melted at 85 °C, and then emulsified into an aqueous phase in which the stabilizer is added. The emulsion is stirred at 300 rpm using a magnetic blender for 5 min, and subsequently treated with a high shear dispersing emulsifier (M200, Shanghai Fuluke Liquid Machinery Co., China) at 8000 rpm for 10 min. Finally, the emulsion is cooled down in a water bath at 5 °C and the lipid crystallizes.

2.3. Freeze-drying process

Trehalose, glucose, mannitol, and sucrose were selected, respectively, as the cryoprotectants for the freeze-drying process of ibuprofen-loaded SLM. Freeze-drying was performed in an ALPHA1-2 freeze-drier (CHRIST, Germany) equipped with the condenser operating at −50 °C and a chamber with cooled shelves. The freeze-drying process lasted for 24 h to allow for a complete solidification. Prior to drying, 50 ml liquid SLM with the cryoprotectants were frozen to −25 °C forming a 10 mm thick layer on a stainless steel tray in a refrigerator. The freeze-drying was conducted at a pressure of 5.0 Pa.

2.4. Freeze–thaw process and reconstruction of freeze-dried samples

After thawing been freeze-dried, the trays with SLM were placed into a desiccator at room temperature. Prior to use, purified water was added into the dried SLM to make the final volume to be 50 ml. Then an ultrasonic disperser was used for 5 min to ensure a complete dispersion of SLM. The rehydration was performed at room temperature.

2.5. Morphology and particle size analysis

The morphology of the particles was determined using an optical microscope (E200, Nikon, Japan). The volume based particle sizes of the formulations were analyzed using photon correlation spectroscopy (PCS) (Mastersize-2000, Malvern Instruments, UK). The PCS analysis yielded the mean diameter of the particles (Z-average). The particle size distribution was described by the monodispersity index δ, Eq. (1)

$$\delta = \frac{D_{90} - D_{10}}{D_{50}} \tag{1}$$

where D_{90}, D_{50}, and D_{10} refer to the particle sizes when cumulative total distributions were 90%, 50%, and 10%, respectively. The smaller the δ value is, the better the monodispersity is. The samples were diluted with distilled water to reach a suitable concentration. All measurements were repeated three times.

2.6. Determination of drug entrapment efficiency

The SLM were centrifuged for 20 min at 10,000 rpm (TGL-20M, Hunan Saitexiangyi Centrifuge Ltd., China). The drug contents in the supernatant after centrifugation were measured spectrophotometrically (751-GW spectrophotometer, HP Analytical Apparatus Ltd., Shanghai, China) at a wavelength of 222 nm. Prior to the measurement, the samples were filtered using a 0.22 μm pore size membrane. One milliliter of aliquot was withdrawn from the filtrate and added to a 25 ml volumetric flask. The flask was then filled with distilled water to make the final volume to be 25 ml. The drug entrapment efficiency in the microparticles was calculated using Eq. (2)

$$\text{Entrapment efficiency} = \left(\frac{W_{\text{initial drug}} - W_{\text{free drug}}}{W_{\text{initial drug}}}\right) \times 100\% \tag{2}$$

where $W_{\text{initial drug}}$ is the weight of drug added to the system, while $W_{\text{free drug}}$ is the analyzed weight of free drug in the aqueous phase.