

The Preparation of Silk Fibroin Drug-loading Microspheres

Rui-Juan Xie*, Hai-Yan Wu, Jian-Mei Xu, Qi-Ming Deng

*School of Material Engineering, Soochow University,
Campus Box 64, No.178 East GanJiang Road, Suzhou, Jiangsu, 215021, P.R. China*

Abstract: This paper deals with the development of a kind of water-in-oil-in-water (w/o/w) multiple-emulsification method to fabricate the SF drug-loading microspheres. The silk fibroin (SF) microspheres were prepared by using SF as vehicle and dexamethasone sodium phosphate (DSP) as drug model. The surface morphology was observed by SEM, the particle diameter and its distribution were observed with Laser particle sizer. The structure was studied by X-ray diffraction and Fourier transform infrared. The properties of drug release were assessed in vitro. The results showed the average size of microspheres varied from 6.53 μm to 68.60 μm . The structure of SF drug-loading microspheres had an obviously change compared with pure SF; and the change is the appearance of silk I and silk II structures; and its molecular conformation was β -sheet. The average drug-loading varied from 5.32 % to 9.01 %, and the average loading varied from 56.43 % to 99.07 %. The drug-loading and loading efficiency increased with the increase of SF concentration. The drug-loading and loading efficiency differed when the treated organic solvent differed, their order is: isopropanol<acetone<ethanol. Drug release of SF drug-loading microspheres: there was a slow release effect when the concentration of SF was 3% or 6%. Moreover, in the experimental condition, there was an obvious burst release of the drug when the SF concentration was 9 % or 12 % or when the treated solvent was ethanol.

Keywords: silk fibroin, microspheres, preparation, structure, drug-loading, controlled release

1. Introduction

In recent years, the encapsulated technology on the polymer drug-loading microspheres was actively applied in the medical field; especially natural macromolecule materials received much more attentions for their unique biocompatibility [1-6]. Among them, silk fibroin (SF) had received many researchers' interesting for its good biocompatibility, convenient preparation and rich sources. Former preparation of drug-loading microspheres all used chemical cross-linking agent, SF, SF and chitosan or sodium alginate as vehicle [7-10]. Chemical cross-linking agent had a potential threat to human. There are researches on the immobilized enzyme using SF as vehicle; due to the small quantity of the immobilized enzyme, the encapsulation was easy [8]. In general composite vehicles were used to raise the drug loading [9,10]. In this paper no chemical cross-linking agents were used, and the vehicle had only one component, i.e. SF; using dexamethasone sodium phosphate (DSP) as drug model. The silk fibroin drug-loading microspheres were prepared by

improved water-in-oil-in-water (w/o/w) multiple-emulsification to enhance the drug loading and loading efficiency. The surface morphology, particle size and distribution of the size were observed by SEM and Laser particle sizer. The structure was studied by X-ray diffraction and Fourier transform infrared methods. The properties of drug release were also studied by in vitro.

2. Materials and methods

2.1 Materials

Cocoon shells of *Bombyx mori*, isopropanol (Changzhou wuwei reagent Co., Ltd. AR), acetone and ethanol (Shanghai reagent Co., AR), DSP (Tianjin Tian yao Pharmaceuticals Co., Ltd.), CaCl_2 (Shanghai Meixing Chemical Engineering CO., LTD., AR), Disodium hydrogen phosphate (Sinopharm Chemical Reagent Co. Ltds, AR), Potassium dihydrogen phosphate (Shanghai chemical reagent Co., Ltd., AR), Span-80 and liquid paraffin (Tianjin damao chemical reagent Co., CP) et al.

* Corresponding author's email: xieruijuan@suda.edu.cn

2.2 Preparation of SF drug-loading microspheres

After degumming, the Cocoon shells of *Bombyx mori* were dissolved in the mixed solution of C_aCl_2 : H_2O : $C_2H_5OH=1:8:2$ (molar ratio) at $72\pm 2^\circ C$, SF solution was obtained by dialyzing and filtering [7]. Liquid paraffin which included a certain amount of Span-80 was heated to $37^\circ C$. Then a mixture of SF and DSP was dropped into oil phase slowly (the mass ratio of DSP-to-SF was 1:10), the mixture emulsion was emulsified for 20 min with stirring at 720 ± 20 rpm. After adding organic solvent (the volume ratio of organic solvent to-SF was 4:1) and stirring for a certain time, the mixture emulsion was centrifuged at 3000 rpm; and then removed the supernatant. Adding a certain amount of organic solvent, then the mixture was stored at $4^\circ C$ for a certain time, and then centrifuged. The mixture emulsion was washed twice with isopropanol and once with deionized water. Then the SF microspheres were obtained under vacuum freeze-drying (USA, VIRTIS GENESIS 25-LE freeze-drier) at $-40^\circ C$.

The effects of the SF concentration and organic solvent on the properties of drug-loading microspheres were investigated. The concentration of SF was 3 %, 6 %, 9 % and 12 % respectively. The organic solvent was isopropanol, acetone and ethanol respectively.

2.3 Morphology

Morphology of the microspheres was observed by using SEM (Japan, S-570). The microspheres particle size and its distribution were measured by Laser particle sizer (Zhuhai, LS800).

2.4 Structure of the SF drug-loading microspheres

X-Ray Diffraction (XRD): X-ray diffraction was performed by using X'Pert PRO MRD polycrystalline diffractometer (Holland, PANalytical Company) with CuK_α radiation from a source operated at 40 kV and 40 mA. The diffraction intensity curves with 2θ from 5° to 45° were obtained.

Fourier Transform Infrared (FTIR): Samples were prepared with KBr disk. FTIR spectra were obtained

with NICOLET-5700 FT-IR (USA, Thermo Electron Corporation) and the wave number ranged from 400 cm^{-1} to 4000 cm^{-1} .

2.5 Drug-loading and loading efficiency of microspheres

Samples of 50 mg dried drug-loading microspheres were added into the tube of phosphate buffer saline (PBS, pH 7.4); let the volume of the buffer solution be V . Oscillated the tube at $37^\circ C$ for 12 h, and then centrifuged the mixture at 3000 rpm for 20 min. Then took a certain amount of supernatant to test its absorbance by using ultraviolet spectrophotometer (UV-2550, Japan Shimadzu), and absorbance A_1 was detected. Moreover, the residual solution was carefully removed. And then the same volume of PBS was added, and centrifuged the tube after oscillating it at $37^\circ C$ for 6h, and absorbance A_2 was detected by the same method. By measurement the absorbances A of different DSP concentrations C ($\mu\text{g/ml}$) could be obtained, and then the regressive equation between A and C could be established as follows:

$$A=0.02704C+0.01263. \quad (1)$$

And the weight of the drug in the microspheres W_1 could be calculated from the DSP concentration C_1 and C_2 in the supernatant according to the following equation:

$$W_1=(C_1 \times n_1 + C_2 \times n_2) \times V, \quad (2)$$

Where n_1 , n_2 were respectively the diluted times of the samples that were used to test the absorbance. Thus from equation (1) concentration C_1 and C_2 could be calculated according to A_1 and A_2 respectively; W_1 could then be obtained by equation (2). Thus the Drug-loading and loading efficiency could be obtained as follows:

$$\text{Drug - loading (\%)} = \frac{W_1}{W_2} \times 100\% .$$

$$\text{Loading efficiency (\%)} = \frac{W_1}{W_3} \times 100\% .$$

Where W_2 is the total weight of microspheres, W_3 is the input weight of drug (DSP).