Journal of Pharmaceutical Sciences xxx (2016) 1–9

Contents lists available at ScienceDirect

Journal of Pharmaceutical Sciences

journal homepage: www.jpharmsci.org

Research Article

Preparation of a Novel Form of Gelatin With a Three-Dimensional Ordered Macroporous Structure to Regulate the Release of Poorly Water-Soluble Drugs

Jie Xu, Zongzhe Zhao, Yanna Hao, Ying Zhao, Yang Qiu, Jie Jiang, Tong Yu, Peng Ji, Ying Liu, Chao Wu*

Pharmacy School, Liaoning Medical University, 40 Songpo Road, Linghe District, Jinzhou, Liaoning Province 121000, China

ARTICLE INFO

Article history:
Received 10 October 2015
Revised 14 December 2015
Accepted 22 December 2015

Keywords:
biomaterials
polymeric drug carrier
nanotechnology
materials science
bioavailability
gelatin
three-dimensional ordered macroporous
fenofibrate
sustained release
biodegradability

ABSTRACT

In this study, a novel three-dimensional ordered macroporous gelatin (3DOMG) was fabricated as a carrier for increasing the solubility of poorly water-soluble drugs, offering sustained release and a high oral bioavailability. Polymethyl methacrylate nanospheres (257 nm) were used as a colloidal plastic framework to synthesize 3DOMG. Fenofibrate (FNB) was selected as a model drug and loaded onto 3DOMG by the adsorption equilibrium method. Detailed characterization showed that the FNB absorbed onto 3DOMG was in a microcrystalline state. A fluorescence experiment and the prepared drug microcrystal network gave further information on the physical state of the drug. A degradation experiment proved that 3DOMG was readily biodegradable. *In vitro* release testing showed that 3DOMG increased the dissolution rate of FNB and produced a sustained release. An *in vivo* pharmacokinetic study confirmed that 3DOMG improved the oral bioavailability compared with that of commercial sustained-release capsules. These findings confirm that 3DOMG can be regarded as a promising carrier for an oral drug delivery system.

Introduction

Biopharmaceutics Classification System class II drugs have low solubility and high permeability, and the dissolution process limits their extent and speed of absorption following oral administration.[1] It has been reported that approximately 40% of all new chemical entities are poorly soluble in water or water insoluble.[2] Therefore, how to improve the solubility of poorly water-soluble drugs has attracted a great deal of attention. Porous materials possess several attractive features that include an ordered nanoscale porous structure, a large specific surface area, high adsorption capacity, and adjustable pore size.[3] All these features make porous materials suitable for improving the solubility of poorly water-soluble drugs. Nanopores reduce the particle size of adsorbed drugs to increase specific surface area of drug particles. According to the Noyes-Whitney equation, this would increase the drug dissolution rate. Furthermore, porous materials can inhibit drug recrystallization to be in an amorphous or microcrystalline form.[4] Therefore porous materials can markedly improve the solubility of poorly water-soluble drugs.

At present, studies of porous materials mainly concentrate on mesoporous and macroporous materials. The diameter of mesoporous materials ranges from 2 to 50 nm and the most widely used mesoporous materials include mesoporous alumina,[5] mesoporous TiO_2,[6] mesoporous carbon,[7,8] and mesoporous silica.[9,10] Compared with mesoporous materials, macroporous materials have a larger pore size, which allows higher drug loading. Meanwhile, the dissolution rate is accelerated with the increase in pore size in the nanoscale range. But when the pore size exceeds an upper limit, the dissolution rate will reduce.[11] Therefore macroporous materials are clearly superior to mesoporous materials as insoluble drug carriers.

Hu et al.[12] exploited three-dimensional (3D) ordered macroporous silica as matrix to increase the dissolution rate and reduce the gastric irritation produced by indomethacin. Zhang et al.[13] increased the dissolution rate and oral bioavailability of valsartan

* *Correspondence to:* Chao Wu (Telephone: +86-0416-4673439; Fax: +86-0416-4673439)
E-mail address: wuchao27@126.com (C. Wu).

http://dx.doi.org/10.1016/j.xphs.2015.12.026
0022-3549/

by using hierarchical porous carbon monoliths with three-dimensionally ordered macropores. However, inorganic macroporous materials have an unpredictable biological safety and are nonbiodegradable. This markedly limits the application of inorganic macroporous materials and therefore it is necessary to construct biodegradable macroporous materials. Currently, the shrinkage and collapse of the macroporous frame structure during the preparation of natural organic macroporous materials is inevitable. However, Xie et al.[14] have developed a three-dimensionally ordered macroporous chitosan matrix as a drug carrier, with an added silicon source to avoid collapse of the chitosan macroporous structure that led to degradation problems. Furthermore, current studies on macroporous materials mostly involve fast release leading to a burst release effect and fluctuation of blood drug concentrations. A peak and valley phenomenon is seen after repeated administration. Although the coating method can be used to regulate drug release,[15,16] the process is complex and time-consuming. Therefore, it is more meaningful to develop a new biodegradable macroporous material to improve the water solubility of poorly water-soluble drugs and produce a sustained-release effect.

Gelatin, a natural biopolymer obtained from the partial hydrolysis of collagens, has generally recognized safe status at the US Food and Drug Administration. It is widely considered as a superior drug delivery material. In this study, we have shown that gelatin is able to form a 3D ordered macroporous structure. Moreover, 3DOMG not only avoids collapse of the gelatin macroporous structure but also produces a sustained- release effect. We investigated whether 3DOMG, as a poorly water-soluble drug carrier, has great potential for application in oral drug delivery systems.

Materials and Methods

Materials

Fenofibrate was provided by the Dalian Meilun Company (Dalian, China) with a purity >99%. Methyl methacrylate (MMA) and gelatin were obtained from Sinopharm Chemical Reagent Company, Ltd. (Shanghai, China). Potassium persulfate, tetrahydrofurane, acetic acid, and anhydrous ethanol were purchased from Tianjin Yongsheng Fine Chemical Company, Ltd. (Tianjin, China). Fluorescein isothiocyanate (FITC) and Rhodamine B were purchased from Beijing Dingguo Changsheng Biotechnology Company, Ltd. (Beijing, China). Trypsin was provided by GENView (Beijing, China). Dichloromethane was obtained from Tianjin Guangfu Fine Chemical Company, Ltd. (Tianjin, China). Commercial sustained-release capsules were produced by Ethypharm Pharmaceutical Company, Ltd. (Shanghai, China) and deionized water was used in all experiments.

Synthesis of 3DOMG

Step 1

The polymethyl methacrylate (PMMA) nanospheres were prepared by an emulsifier-free emulsion polymerization method,[17,18] which involved the addition of 80 mL H_2O and 3.7 mL MMA (with removal of the polymerization inhibitor) to a three-necked flask reactor. The reaction was performed at 80°C for 20 min with stirring at 600 rpm/min under an N_2 atmosphere. Then, 20 mL $K_2S_2O_8$ (4.9 mmol/L) aqueous solution was added to the above solution and stirring was continued for 4-5 h. The synthesized emulsion was cooled to room temperature and centrifuged at 10,000 rpm for 10 min. Finally, the isolated precipitate was washed with water 3 times and dried.

Step 2

Ten grams of gelatin was dissolved in 100 mL water with stirring at 80°C in a water bath for 1 h. The PMMA nanospheres prepared in step 1 were layered on the bottom of the filler and compacted. Then, 10% aqueous gelatin solution was filtered and allowed to gel at 4°C. The mixture was dried and then soaked twice (at an interval of 12 h) in tetrahydrofuran to remove the template. Finally, washing with anhydrous ethanol was carried out 3 times, followed by drying at 50°C in a vacuum oven to give 3DOMG.

Drug Loading Procedure

FNB, a lipid-regulating drug, clinically used to prevent hypercholesterolemia and hypertriglyceridemia,[19,20] was selected as a model drug. The drug was loaded onto 3DOMG by the adsorption equilibrium method. The loading capacity of macroporous materials is dependent on their concentration. The same amount of 3DOMG was soaked in different concentrations of FNB dichloromethane solution for 2-4 h. The loading procedure was performed in closed containers in the dark at room temperature. The supernatant was removed by centrifugation at 6000 rpm and, finally, the drug-loaded sample (3DOMG-FNB) was dried in a vacuum (as far as possible avoided residual solvent). The above procedure was repeated several times in order to fill the pores with FNB. The loading capacity was determined by ultraviolet spectrophotometry (UV) (UV-2000; Unico, Dayton, NJ).

Characterization of 3DOMG and 3DOMG-FNB

The structure and morphology of 3DOMG and PMMA nanospheres were examined by SEM (JEOL JSM-7001F, operated at 20 kV). The drug crystallinity was examined using a differential scanning calorimeter (DSC) (DSC-60, Shimadzu, Japan) at a constant heating rate of 10°C/min from 50°C to 300°C under a constant nitrogen flow of 150 mL/min. A powder X-ray diffractometer (PXRD) (Rigaku Geigerflex XRD, Company, Japan, Cu-Ka radiation, 30 kV and 30 mA Philips) was used to further investigate the physical state of FNB in 3DOMG. The step size was 0.02°, the scanning rate was 4°/min, and the range (2θ) was from 3° to 60°. Fourier transform infrared (FTIR) spectra were recorded using an FT-IR spectrometer over the range 400-4000 cm^{-1} (Bruker IFS 55, Switzerland) and the KBr pellet technique.

Investigation of the Existing Drug State

Fluorescence Microscopy

The purpose of the experiment was to investigate the uniformity of adsorption and dispersion of the drug in carrier nanochannels. FITC was used to label 3DOMG framework and Rhodamine B was loaded onto the nanopores to simulate FNB.[21] FITC-labeled 3DOMG (FITC-3DOMG) was prepared as follows: 2 mg FITC was dissolved in 1 mL deionized water and the FITC solution was added to 10% aqueous gelatin solution with gentle stirring. Then, the mixed solution was used to prepare 3DOMG. In this experiment, Rhodamine B was used instead of FNB as a model drug. An appropriate amount of FITC-3DOMG was soaked in 1 mg/mL Rhodamine B ethanol solution for 4-5 h to achieve adsorption equilibrium. After centrifugation, the precipitate was dried in a vacuum for fluorescence microscopy observation. The whole process was protected from the light environment.

Preparation of Drug Microcrystal Network

In order to study the existing state of FNB in the nanopores, we removed the 3DOMG framework after loading the drug to obtain the FNB microcrystal network.[22] The preparation process was as