Design of Lipid Matrix Particles for Fenofibrate: Effect of Polymorphism of Glycerol Monostearate on Drug Incorporation and Release

DENGNING XIA,[1,2] FUDE CUI,[2] YONG GAN,[3] HUILING MU,[1] MINGSHI YANG[1]

[1]Department of Pharmacy, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen 2100, Denmark
[2]Department of Pharmaceutics, School of Pharmaceutical Science, Shenyang Pharmaceutical University, Shenyang 110016, China
[3]Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China

Received 30 July 2013; revised 1 December 2013; accepted 4 December 2013

Published online 20 December 2013 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jps.23830

ABSTRACT: The effect of polymorphism of glycerol monostearate (GMS) on drug incorporation and release from lipid matrix particles (LMPs) was investigated using fenofibrate as a model drug. X-ray powder diffraction and differential scanning calorimetry were used to study the polymorphism change of GMS and the drug incorporation in GMS matrix. When medium-chain triglycerides (MCT) was absent, melted GMS was frozen to α-form of GMS with drug molecularly dispersed, whereas β-form of GMS was formed with part of drug crystallized out when the ratio of GMS/MCT in the lipid matrix was 2:1 (w/w). For LMP composed of GMS/MCT (2:1, w/w) prepared, GMS was in α-form when the particles were in nanometer range, whereas GMS was in β-form when lipid particles were in micrometer range. The model drug was molecularly dispread in α-form lipid nanoparticles, whereas part of drug was expulsed out from microparticles because of the denser crystalline packing than α-form of GMS, and caused a faster drug release from lipid microparticles than that from nanoparticles. During the storage, the transformation of GMS from α-form into the more stable β-form promoted drug expulsion and caused drug precipitation. In conclusion, the polymorphism of GMS is an important factor determining particle stability, drug incorporation, and the release of the drug from LMP. Critical attention should be paid on the investigation as well as control of the lipid polymorphism when formulating lipid-based matrix particles. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:697–705, 2014
Keywords: polymorphism; lipids; drug-excipient interaction; stability; dissolution; glycerol monostearate; fenofibrate; lipid matrix particles; drug incorporation

INTRODUCTION

Lipid matrix particles (LMPs), such as solid lipid nanoparticles and oil-loaded solid lipid nanoparticles, also described as nanostructured lipid carriers (NLC), have attracted considerable attention in the last two decades for improving oral absorption of drugs. This is especially the case for the drugs with high lipophilicity.[1,2] The oral absorption of lipophilic drugs can be greatly improved by the solubilization ability of digested lipids, avoiding first-pass drug metabolism via lymphatic drug transport and the interaction with enterocyte-based transport process.[2,3]

For the production of LMP, hot melt emulsification method is often adopted because of its simple procedure. The lipids and drug are first melted, and then dispersed in hot water containing surfactants to form an emulsion, and finally cooled down to solidify the melted lipid particles. During this process, the supercooled melts of lipid-containing drugs are usually first formed and subsequent crystallization of lipids takes place during cooling.[4,5] However, some undesired consequences of the crystallization of the solid lipid may occur, including particle growth, unpredictable gelation, expelling of incorporated drugs, and unexpected dynamic polymorphic transitions of the lipid particles.[6–9] The introduction of liquid lipid, such as medium-chain triglycerides (MCT), in the formulation to produce NLC may circumvent some of the aforementioned limitations, for example, the liquid lipid can cause imperfectly structured solid lipid matrix or structureless solid matrix, which can accommodate more drug than highly crystalized solid lipid.[10,11] However, this kind of structureless matrix is still thermodynamically unstable; phase separation between liquid and solid lipids still exists in NLC if the crystallization tendency of solid lipid is high. The crystallization tendency of amorphous solid lipids potentially has great effect on the drug incorporation, the structure of lipid particles, and further drug release from these lipid particles; however, less attention has been paid to this during formulation study.

In this study, the effect of polymorphism of solid lipid on some quality attributes, that is, the drug incorporation and the drug release profiles of LMPs were investigated. Glycerol monostearate (GMS), a commonly used solid lipid, and a liquid lipid MCT were used as the model lipids. Fenofibrate was used as the model drug, which has a log *P* value of 5.2 and a aqueous solubility of 0.29 μg/mL at 37°C.[12] X-ray powder diffraction (XRPD) and differential scanning calorimetry (DSC) were used to study the effect of MCT on the polymorphism of GMS and the drug incorporation into the lipid particles. The effect of GMS polymorphism on the drug release from the lipid particles with different particles sizes and the stability of lipid particles were also studied.

Correspondence to: Mingshi Yang (Telephone: +45-35336141; Fax: +45-35306030; E-mail: mingshi.yang@sund.ku.dk); Fude Cui (Telephone: +86-24-23986353; Fax: +86-24-23986353; E-mail: cuifude@163.com)
Dengning Xia's present address is Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China.

Journal of Pharmaceutical Sciences, Vol. 103, 697–705 (2014)

MATERIALS

Fenofibrate (purity: minimum 99%) was purchased from Sigma–Aldrich (Munich, Germany). GMS (40–55), sodium dodecyl sulfate (SDS) and MCT (Miglyol 812) were obtained from Unikem (Copenhagen, Denmark), Poloxamer 407 (Lutrol® F127) was obtained from BASF Aktiengesellschaft (Ludwigshafen, Germany). All organic solvents were of HPLC grade (VWR, Rødovre, Denmark). Purified water was obtained from a Millipore Milli-Q ultrapure water purification system (Billerica, Massachusetts).

METHODS

Preparation of LMPs

Fenofibrate-loaded LMPs were prepared by melting probe sonication method. GMS was used as the solid lipid, and MCT was used as the liquid lipid material. Weighed lipid mixture of GMS/MCT (1.25 g/0.625 g) and drug (0.125 g) were melted at 75°C–80°C for 5 min using a water bath. The melted phase was dispersed into 20 mL of aqueous solutions containing 3% Poloxamer 407 and 0.1% SDS (w/v) under magnetic stirring at the same temperature to form an o/w emulsion. Then, the o/w emulsion was treated with an ultrasonic probe (50/60 KHz; 230 V; Chemical Instruments AB, Sollentuna, Sweden) at 80 W for different time lengths. The process parameters are shown in Table 1. The probe with a tip diameter of 6 mm was immersed 15 mm in depth in the liquid, resulting in the wave traveling downwards and reflecting upwards. The period of ultrasound burst was set to 2 s with a pause of 2 s between two ultrasound bursts. After probe sonication, the sample was cooled down to room temperature to obtain the LMP suspension. The microparticles were prepared as the same process with nanoparticles without sonication.

Particles Size Determination of LMPs

Particles sizes (z-average) of fenofibrate-loaded LMPs were determined using Zetasizer (Nano-ZS; Malvern instruments, Worcestershire, United Kingdom). The measurements were performed at 25°C with a scattering angle of 173°. The obtained LMP suspensions were diluted 25 times using Milli-Q water (Millipore) before determination. Each sample was measured in triplicate in 2 h after preparation. The particle size of microparticles was roughly observed by optical microscope, and the diameter of 40 particles was measured at 40× magnification.

Morphology of LMPs

The morphology of LMPs was studied using transmission electron microscopy (TEM). After diluting the samples 50-fold with purified water, a drop of the suspension was immediately spread on a copper grid, and the excess liquid was removed with filter paper. The samples were dried under room temperature for 10 min, and observed using a transmission electron microscope (JEM-1230; JEOL, Tokyo, Japan). The samples were also observed using a light microscope (Axiolab; Carl Zeiss, Göttingen, Germany) under a magnification of 40× (objective lens) at 20 ± 1°C, and the micrographs were obtained using a digital camera (Deltapix, Måløv, Denmark) controlled with the Deltapix software (version 1.6; Deltapix).

Drug Entrapment Efficiency

The drug entrapment efficiency (EE), which corresponds to the amount of drug that can be incorporated in the LMPs, either inside the particles or adsorbed at the surface, was determined indirectly by measuring the concentration of the free drug in the aqueous phase of the LMP suspensions. The free drug in the LMP suspensions was separated from LMP using ultrafiltration centrifugation method. Centrifugal filter (Amicon® Ultra-0.5, Millipore, County Cork, Ireland) with a 50-kDa molecular weight cutoff was used. Half milliliter of LMP suspension was accurately taken and placed into the upper chamber of the centrifuge filter, which was centrifuged at 3400g for 15 min at room temperature. Drug concentration in the ultrafiltrate was determined using a HPLC method (HP 1100 series; Agilent Technologies, Santa Clara, California), on an Agilent Extend C_{18} column (3.5 μm, 150 × 4.6 mm^2; Agilent Technologies, Santa Clara, California). The mobile phase was composed of methanol and water (80:20, v/v), and used at a flow rate of 1.0 mL/min. The detection was performed at 288 nm using UV–Vis detector. Half milliliter LMP suspension was accurately taken and placed into 5 mL volumetric flask and dissolved with 4.5 mL acetone. The drug amount was determined using the same HPLC method to obtain the total drug amount in suspension. The drug EE was calculated by the following equation ($n = 3$):

$$\mathrm{EE}(\%) = \frac{W_{\mathrm{total}} - W_{\mathrm{free}}}{W_{\mathrm{total}}} \times 100 \quad (1)$$

where W_{free} is the free drug in the supernatant and W_{total} is the total drug amount in suspension.

Drug Release Rate

The drug release from the LMPs was studied using a paddle method. Five hundred milliliter of aqueous solution containing 0.5% of SDS was used as the dissolution medium to guarantee sink condition. The temperature was maintained at 37 ± 0.5°C, and the paddle speed was 100 rpm. Two milliliter of LMP suspension was dispersed in the dissolution medium. Samples of 0.4 mL were withdrawn at 5, 15, 30, 60, 120, 240, 360, and 480 min and centrifuged at 5600g for 3 min using a centrifugal

Table 1. Lipid Particles with Different Particles Sizes and Corresponding Process Parameters ($n = 3$)

Formulations	Process Parameters			Particle Size	PDI	EE (%)
	Lipids and Stabilizers	Sonication Time	Sonication Intensity			
Nano-LMP-1	Solid content: 10%	10 min	80%	107 ± 9 nm	0.214 ± 0.043	99.5 ± 0.2
Nano-LMP-2	GMS/MCT(2:1, w/w)	20 s	80%	355 ± 20 nm	0.243 ± 0.021	99.0 ± 0.3
Micro-LMP	0.1% SDS + 3% P407	No sonication		~20 μm[a]	–	97.3 ± 0.5

[a]Roughly observed by an optical microscope.