International Journal of Pharmaceutics 495 (2015) 302–311

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Supercritical fluid precipitation of ketoprofen in novel structured lipid carriers for enhanced mucosal delivery – a comparison with solid lipid particles

V.S.S. Gonçalves[a,b,c], A.A. Matias[a,b], S. Rodríguez-Rojo[c], I.D. Nogueira[d], C.M.M. Duarte[a,b,*]

[a] Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Av. da República, 2780-157, Oeiras, Portugal
[b] iBET, Instituto de Biologia Experimental e Tecnológica, Apartado 12, 2780-901, Oeiras, Portugal
[c] Escuela de Ingenierías Industriales, Universidad de Valladolid, C/Dr. Mergelina s/n 47011 Valladolid, Spain
[d] Universidade Técnica de Lisboa, Instituto Superior Técnico, Instituto de Ciências e Engenharia de Materiais e Superficies, P-1096, Lisboa, Portugal

ARTICLE INFO

Article history:
Received 19 May 2015
Received in revised form 5 August 2015
Accepted 8 August 2015
Available online 13 August 2015

Keywords:
Structured lipid carriers
Glyceryl monooleate
Particles from gas saturated solutions® (PGSS®)
Supercritical fluids
Ketoprofen
Mucosal drug delivery

ABSTRACT

Structured lipid carriers based on mixture of solid lipids with liquid lipids are the second generation of solid lipid particles, offering the advantage of improved drug loading capacity and higher storage stability. In this study, structured lipid carriers were successfully prepared for the first time by precipitation from gas saturated solutions. Glyceryl monooleate (GMO), a liquid glycerolipid, was selected in this work to be incorporated into three solid glycerolipids with hydrophilic-lipophilic balance (HLB) ranging from 1 to 13, namely Gelucire 43/01™, Geleol™ and Gelucire 50/13™. In general, microparticles with a irregular porous morphology and a wide particle size distribution were obtained. The HLB of the individual glycerolipids might be a relevant parameter to take into account during the processing of solid:liquid lipid blends. As expected, the addition of a liquid lipid into a solid lipid matrix led to increased stability of the lipid carriers, with no significant modifications in their melting enthalpy after 6 months of storage. Additionally, Gelucire 43/01™:GMO particles were produced with different mass ratios and loaded with ketoprofen. The drug loading capacity of the structured lipid carriers increased as the GMO content in the particles increased, achieving a maximum encapsulation efficiency of 97% for the 3:1 mass ratio. Moreover, structured lipid carriers presented an immediate release of ketoprofen from its matrix with higher permeation through a mucous-membrane model, while solid lipid particles present a controlled release of the drug with less permeation capacity.

1. Introduction

Solid lipid particles (SLP's) are carriers often used as drug delivery systems since the beginning of 1990s, being composed of lipids that are solid at body temperature (Pardeike et al., 2009). Despite their excellent tolerability, controlled release and protection from degradation of drugs, they present some limitations, such as low drug loading capacity and drug leakage after storage (Almeida and Souto, 2007; Müller et al., 2000; Souto et al., 2004). It is known that during the production of solid lipid carriers, particularly those composed of highly pure lipids, the particles crystallize in metastable polymorphic forms of low thermodynamic stability. Usually, active compounds are incorporated in the imperfections of this crystal structure, between the fatty acid chains. Thus, the more perfect is the crystal structure formed, less drug is incorporated (Muchow et al., 2008; Siekmann and Westesen, 1994). Moreover, during storage, the number of imperfections in this structure is reduced due to the formation of low-energy modifications resulting in a more ordered structure, hence leading to the expulsion of the active compound (Himawan et al., 2006; Pardeike et al., 2009; Teeranachaideekul et al., 2007). To overcome this problem, a second generation of lipid particles whose matrix is composed of solid and liquid lipids have been developed (Hu et al., 2006; Jenning et al., 2000; Müller et al., 2002; Pardeike et al., 2009). This binary mixture, known as structured lipid carriers, is still solid at room and body temperature. It enables the formation of more imperfections

* Corresponding author at: Nutraceuticals and Delivery, Instituto de Biologia Experimental e Tecnológica, Apartado 12, 2781-901 Oeiras, Portugal. Tel.: +351 214469727; fax: +351 214421161.
E-mail address: cduarte@ibet.pt (C.M.M. Duarte).

http://dx.doi.org/10.1016/j.ijpharm.2015.08.026
0378-5173/

in the crystal structure, leading to higher drug entrapment and minimum drug leakage during storage (Muchow et al., 2008). Furthermore, drugs are mostly soluble in liquid lipids than in solid ones, being these structured carriers attractive alternatives for transdermal and mucosal delivery of active compounds(Shen et al., 2010; Teeranachaideekul et al., 2008, 2007).

Glyceryl monooleate (GMO) is a polar amphiphilic waxy lipid with a Hydrophilic-Lipophilic Balance (HLB) of 3, that has been recently used as carrier for drug delivery systems, being non-toxic, biocompatible and biodegradable (Ganem-Quintanar et al., 2000). This water insoluble surfactant is capable to form sequential lyotropic liquid crystalline structures under certain circumstances. As the temperature and water content increase, GMO enters the cubic phase region which is capable to simultaneously accommodate lipophilic, hydrophilic and amphiphilic active compounds with distinct molecular weights (Ganem-Quintanar et al., 2000; Garg et al., 2007; Nielsen et al., 1998). This highly stable and robust mesophase is insensitive to salts and solvents, being appropriate for the incorporation of sensitive molecules such as peptides (Moebus et al., 2012). Moreover, upon hydration, the matrix of GMO swells and earns higher viscosity, thus acquiring mucoadhesive properties (Nielsen et al., 1998). This, coupled to its ability to increase the bioavailability of drugs by inhibiting the P-glycoprotein and by promoting their lymphatic transport, makes GMO an interesting alternative to polymeric carriers for the mucosal release of active compounds (Bansal et al., 2009; Hauss et al., 1998). Usually, this lipid carrier is applied in aqueous formulations, however is highly desirable to work with alternative formulations (non-aqueous and solid) to promote higher chemical stability of drugs and thus lower rates of oxidation or hydrolysis (Mahlin et al., 2005; Moebus et al., 2012).

Despite the fact that supercritical fluid precipitation techniques, more precisely Particles from Gas Saturated Solutions (PGSS®), are being increasingly applied in the production of solid lipid particles, the production of structured carriers composed of solid and liquid lipids has not yet been explored(García-González et al., 2010; Pestieau et al., 2015; Sampaio de Sousa et al., 2007; Weidner et al., 1995). Thus, the objective of this work was to produce a novel carrier system based on a binary mixture of solid and liquid lipids, through PGSS®, in order to obtain particles with improved features for mucosal delivery of drugs. PGSS® technique consists of dissolving $scCO_2$ in the melted mixture of lipids, causing a depression of their viscosities and melting points, being possible to produce particles at mild processing conditions (Nunes and Duarte, 2011). The expansion of this CO_2-saturated solution to atmospheric pressure through a nozzle, causes the atomization and precipitation of particles with complete expansion of carbon dioxide (Knez et al., 2011; Weidner, 2009). GMO was chosen as the liquid lipid in order to confer mucoadhesive properties to lipid particles. Three different carriers constituted by mixtures of glycerides, namely Gelucire 43/01™ (HLB 1), Gelucire 50/13™ (HLB 13) and Geleol™ (HLB 3), were used as the solid lipid matrix due to their capacity to provide controlled release of drugs(Fraile et al., 2013; Rodríguez-Rojo et al., 2013; Sampaio de Sousa et al., 2007). The influence of temperature and pressure of the PGSS® process on the physical properties of the precipitated particles was investigated. Furthermore, it was studied whether or not the incorporation of a liquid lipid increases the stability of the particles during storage by verifying the melting enthalpy variation during aging at room temperature (Choy et al., 2005). Further experiments were performed with the solid lipid Gelucire 43/01™, namely the production of particles with different solid lipid:liquid lipid mass ratios and also the development of ketoprofen-loaded structured lipid particles. The particles obtained were characterized considering their size, morphological and thermal properties as well as their drug release/permeation behavior.

2. Materials and methods

2.1. Materials

Gelucire 43/01™, Gelucire 50/13™, Geleol™ and Peceol™ (GMO) were kindly supplied by Gattefossé (France). Ketoprofen (≥98% purity) was purchased from Sigma–Aldrich (Steinheim, Germany). CO_2 (99.95 and 99.998 mol% purity) was delivered by Air Liquide (Portugal). Ethanol (96%) was purchased from AGA (Portugal). All the chemicals were used without further purification.

2.2. Melting point measurements

The melting point depression of the lipid carriers in the presence of compressed CO_2 was determined within this work using a visual method previously described (Sampaio de Sousa et al., 2006). Briefly, the lipid or mixture of lipids were placed inside a glass (1 cm^3), which was then inserted in a stainless steel high-pressure visual cell with an internal volume of approximately 5 cm^3. After, CO_2 was pumped using a Haskel pump (model 29723-71) into the cell until the desired pressure was reached. The pressure in the cell was measured with a pressure transducer Digibar II calibrated between 0 and 25 MPa (accuracy: 0.15%). The temperature was then gradually increased until it was possible to visually observe the complete melting of the lipid or mixture of lipids. The heating system was composed of a heating cable (Horst), a controller (Ero Electronic LMS) and a high accuracy thermometer (Omega HH 501 AT, 0.1%). Measurements were performed in a pressure range up to 19 MPa. In order to confirm the reproducibility of the results some points were repeated resulting in maximum deviations of 0.2 K.

2.3. Particles from gas saturated solutions (PGSS®)

Structured lipid particles unloaded and loaded with ketoprofen were produced through PGSS® process. The schematic representation of the equipment (FAME UNIT, Separex, France, 2010) used to produce the particles is represented in Fig. 1 and was previously described (Gonçalves et al., 2015; Rodríguez-Rojo et al., 2013).

Carbon dioxide was fed by a pneumatic pump (29723-71, Haskel International Inc., CA, USA) to a 50 cm^3 electrically thermostated high-pressure stirred vessel, containing the mixture of lipids with and without the drug to be processed, until the desired working pressure was reached. After 30 min of stirring (150 rpm), the mixture was depressurized by an automated depressurization valve and atomized through a two-fluid nozzle (d = 250 μm) to a cyclone, where it was externally mixed with compressed air (0.7 MPa). The particles were finally recovered into a collector vessel of 18 L at atmospheric pressure.

The operating conditions (T and P) were chosen according to the measurements of melting point depression of the mixtures in the presence of compressed CO_2 and varied in order to see their effect on the particles' morphology and size.

2.4. Particles characterization

2.4.1. Particle size, particle size distribution and morphology analysis

Particle size and morphology were analyzed visually by FE-SEM (Field Emission Scanning Electron Microscopy) JEOL 7001F at 10–15 kV. Before analysis, particles were covered with approximately 300 Å of a gold-platinum film with a sputter-coater in argon atmosphere (Polaron). Particle size and particle size distribution (PSD) were determined by Laser Diffraction (Malvern Mastersizer 2000). Particles were dispersed in distilled water and measurement was carried out after a gentle rotation of the particles suspension