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Liquid crystalline phase as a probe for crystal engineering of lactose: Carrier for pulmonary drug delivery

Sharvil S. Patil [a,*], Kakasaheb R. Mahadik [a], Anant R. Paradkar [b]

[a] Bharati Vidyapeeth University, Poona College of Pharmacy, Department of Pharmaceutics, Erandwane, Pune 411038, India
[b] Centre for Pharmaceutical Engineering Science, University of Bradford, Bradford BD7 1DP, UK

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ABSTRACT

The current work was undertaken to assess suitability of liquid crystalline phase for engineering of lactose crystals and their utility as a carrier in dry powder inhalation formulations. Saturated lactose solution was poured in molten glyceryl monooleate which subsequently transformed into gel. The gel microstructure was analyzed by PPL microscopy and SAXS. Lactose particles recovered from gels after 48 h were analyzed for polymorphism using techniques such as FTIR, XRD, DSC and TGA. Particle size, morphology and aerosolisation properties of prepared lactose were analyzed using Anderson cascade impactor. *In situ* seeding followed by growth of lactose crystals took place in gels with cubic microstructure as revealed by PPL microscopy and SAXS. Elongated (size ~ 71 μm) lactose particles with smooth surface containing mixture of α and β-lactose was recovered from gel, however percentage of α-lactose was more as compared to β-lactose. The aerosolisation parameters such as RD, ED, %FPF and % recovery of lactose recovered from gel (LPL) were found to be comparable to Respitose® ML001. Thus LC phase (cubic) can be used for engineering of lactose crystals so as to obtain particles with smooth surface, high elongation ratio and further they can be used as carrier in DPI formulations.

1. Introduction

Nowadays, particle engineering techniques have gained prime importance owing to their role in drug delivery process. Drug delivery processes are comprised of administration of drug, its release and transport to the site of action. Pulmonary drug delivery is one of the areas wherein particle engineering plays a vital role. Formulation scientists are continuously working in the area of particle engineering so as improve traditional techniques such as milling and advanced techniques including spray drying, spray freeze drying and supercritical fluid technology. Some of these techniques are harsh whereas some are quite promising (Shoyele and Cawthorne, 2006). Thus searching newer methods for particle engineering is one of the thrust areas of formulation scientists.

Lyotropic liquid crystalline (LC) phases as a drug delivery system have attracted most of the researchers owing to their unique properties including their structural resemblance to human membranes, large surface area and high solubilization capacities (Patil et al., 2013). Besides drug delivery, LC phases have been used for crystallization of membrane proteins (Caffrey, 2003). Glycerol monooleate (GMO), a polar lipid is one of the metabolites of triglycerides (Patton, 1979). It has been reported to form variety of LC phases including reverse isotropic micellar solution (L_2), lamellar (La), inverted type (reverse) hexagonal (H_{II}), and cubic (V_2) having different physical properties and hence being explored as a drug delivery systems including biomolecules (Chernik, 2000; Larsson, 1983; Qiu and Caffrey, 2000). However, to the best of our knowledge utility of LC phases as one of the particle engineering techniques has not yet been assessed.

Dry powder inhaler (DPI) is one of the drug delivery systems used to deliver the drug by pulmonary route. DPIs present advantages such as propellant-free, portable and easy to operate. Further these are low-cost devices and improve stability of the formulation due to dry state (Carpenter et al., 1997; Prime et al., 1997). The majority of DPI formulations consist of a powder mixture of coarse carrier particles and micronized drug particles having aerodynamic particle diameters in the range of 1–5 μm (Iida et al., 2003). The improvement in dose accuracy and minimization of dose variability is achieved by the use of carrier particles which when mixed with drug improve its flowability (Schiavone et al., 2004). According to the literature, carrier particles having size in the range of 63–90 μm, smooth surface and high elongation ratios are desirable for delivering the drug to the site of action (Zeng

* Corresponding author at: Department of Pharmaceutics, Bharati Vidyapeeth University, Poona College of Pharmacy, Erandwane, Maharashtra, India. Tel.: +91 20 25437237; fax: +91 20 25439383.
E-mail address: sharvilpatil25@gmail.com (S.S. Patil).

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et al., 1999, 2000). Particulate interactions within drug and carrier play vital role with respect to the drug delivery by DPIs. The physical forces of interactions such as van der Waals forces, electrostatic charges, capillary forces and mechanical interlocking dominate the interparticulate interactions (both cohesion and adhesion). Further particle size, shape, surface roughness, the intensity and duration of drug-carrier mixing, contamination of carrier particles and relative humidity (RH) govern magnitude of these forces (Telko and Hickey, 2005; de Boer et al., 2003). For example, the improvement in surface smoothness of lactose carrier particles improved respirable fraction of salbutamol sulfate from the Rotahaler® (Ganderton, 1992) which was attributed to the reduction in adhesion forces between the drug and carrier particles upon smoothing carrier surface. The literature states that generally the respirable drug fraction increases with the use of carrier particles having small size (Kaialy et al., 2012a; Young et al., 2011; Le et al., 2012). Higher amounts of fines in the carrier particles (Islam et al., 2004; Kaialy et al., 2011) or more surface roughness of the same (Kaialy et al., 2012b) have shown higher amounts of drug deposition on lower airway regions. Carrier particles with more elongated shape and rough surface have shown reduction in drug–carrier adhesion (Kaialy et al., 2012c; Podczeck et al., 1996). Further better drug content homogeneity within DPI formulations was observed for carriers having rough surface (Kaialy et al., 2012c, 2012b; Flament et al., 2004). The flowability of carrier particles has shown to affect the performance of DPI formulations. The amount of drug loss or deposition of drug on the throat has been observed with carrier particles with poor flowability (Kaialy et al., 2012b). Additionally, high drug loss and low drug emission upon aerosolisation was observed in case of carrier particles with high bulk and tap densities (Kaialy et al., 2012c). Reports on effect of particle size of the carrier on drug deposition are also well documented (French et al., 1996; Steckel and Muller, 1997).

Lactose is most commonly used carrier particles in the formulation of DPI owing to its well investigated toxicity profile, physical and chemical stability along with its compatibility with drug and most importantly its broad availability at low price (Pilcer and Amighi, 2010). Lactose, 4-(b-D-galactosido-)-D-glucose can exist as a single hydrated form, α-lactose monohydrate (Lα·H_2O) and three dehydrated forms, β-lactose (Lβ), stable anhydrous α-lactose (Lα_S) and unstable hygroscopic anhydrous α-lactose (Lα_H) (Kirk et al., 2007). The crystal habit of lactose is greatly influenced by the recrystallization conditions. It is reported that recrystallization of lactose above 93.5 °C yields β-lactose while below this temperature α-lactose monohydrate (α-LM) is obtained. Out of the above mentioned forms of lactose, α-LM is most widely used as pharmaceutical excipient (Kibbe, 2000).

Different methods have been employed for carrying out crystallization of lactose which includes seeding technique (Liang et al., 1991), precipitation of lactose using anti-solvent such as ethanol (Bund and Pandit, 2007), acetone (Larhrib et al., 2003), methanol (Leviton, 1949), DMSO (Dincer et al., 1999), ultrasound assisted antisolvent crystallization (Dhumal et al., 2008; Bund and Pandit, 2007a) and carbopol gel (Zeng et al., 2001). Besides simple milling (Inhalation grades available with DFE Pharma, Germany) crystallization techniques involving use of antisolvent, ultrasound and carbopol gels have presented lactose which can be used as a carrier in DPI. Further engineering of lactose particles in order to improve dispersibility so as obtain higher FPF and lower ED has been reported in literature which includes mechanofusion of lactose with magnesium or sucrose stearate (Kumon et al., 2006, 2008), wet-smoothing using solvent and magnesium stearate (Ferrari et al., 2004; Young et al., 2002), surface erosion using high-speed elliptical-rotor-type mixer (Iida et al., 2004), surface dissolution using aqueous-ethanol solution or by means of temperature (El-Sabawi et al., 2006), coating of lactose particles with magnesium stearate or HPMC (Iida et al., 2005).

It was observed that elongated lactose crystals with smooth surface could be produced using anti-solvent ethanol. However at the same time increased number of primary nuclei lead to generation of small crystals. Ultrasound has been reported to produce lactose crystals with desired quality; however erosion of ultrasonic probe may contaminate the product (Hansson, 1980). Lactose crystallized from carbopol gel resulted in large regular crystals with smooth surface and improved fine particle fraction (FPF).

In the present work, suitability of LC phase for engineering of lactose particles was assessed. Gel consisting of cubic phase is used for crystallization of α-LM. The gel microstructure was analyzed by plane polarized light (PPL) microscopy and small angle X ray scattering (SAXS). The lactose particles harvested from gel were characterized by Fourier Transform Infra Red spectroscopy (FTIR), X-ray diffraction analysis (XRD), Differential scanning Calorimetry (DSC), Thermogravimetric analysis (TGA) and Scanning Electron Microscopy (SEM). The aerosolisation properties of the particles were determined by using salbutamol sulfate as a model drug and compared to marketed lactose particles (Respitose® ML001) used for DPI formulation.

2. Materials and methods

2.1. Materials

Glyceryl monooleate (GMO, Rylo MG 19 Pharma), Salbutamol sulfate (SS) was a gift sample from Danisco culture, Denmark and Cipla Ltd. Mumbai, India respectively. Lactose monohydrate (Respitose® ML001) was purchased from DFE Pharma, Germany.

2.2. Method

Glyceryl monooleate (GMO) was used for the preparation of gel. To describe in brief, saturated solution of lactose (20%w/v) in deionised water was prepared. GMO was melted. The molten GMO was diluted with lactose solution at 70 °C so as to obtain 35%v/v aqueous mixture of GMO. The dispersion transformed into translucent gel upon cooling. The gel became turbid when kept undisturbed for two days (48 h) indicating crystallization of lactose (LPL). The crystals so formed were recovered by addition of isopropyl alcohol which acts as a solvent for GMO and antisolvent for lactose. The suspension was filtered to obtain lactose crystals which were washed with 60% (v/v) ethanol followed by 100% (v/v) ethanol. The crystals were allowed to dry overnight before drying in vacuum oven at 70 °C for 3 h. In order to assess reproducibility, the method was repeated twice and the lactose collected was analyzed further.

2.3. Characterization

2.3.1. Plane polarized light microscopy

Polarized light microscopy was used to confirm the type of mesophase formed by GMO in the gel form after addition of lactose (Rosevear, 1954). Hydrated gel was transferred to a specially fabricated glass tube (internal diameter 0.5 cm) and examined for presence or absence of birefringence under a microscope at 25 ± 0.5 °C with a $\lambda/4$ plate oriented at 45° to the polarizer axes under 40× magnification (Nikon Eclipse E 600, Nikon Instech Co., Japan).

2.3.2. Small angle X-ray scattering (SAXS)

In order to confirm the type of mesophase formed during crystallization of lactose, SAXS experiments were performed on a Bruker Nanostar with rotating Cu anode and pinhole geometry using a