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Particle engineering of materials for oral inhalation by dry powder inhalers. I—Particles of sugar excipients (trehalose and raffinose) for protein delivery

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ABSTRACT

The pulmonary route of delivery offers a potential alternative to parenteral administration of peptides and proteins. Protection of protein structure is essential in both processing and storage of the final formulation. Sugars, such as trehalose and raffinose, have been employed to act as protein stabilisers. Optimisation of the aerodynamic characteristics of microparticles in dry powder inhaler formulations is critical to ensure optimum deposition of the formulation into the respiratory tract.

In the present study we examine the adaptation to hydrophilic materials, specifically the disaccharide, trehalose and the trisaccharide, raffinose, of a previously reported spray drying process for producing nanoporous microparticles (NPMPs). We also investigate the feasibility of incorporating a model protein, lysozyme, into these sugar-based NPMPs.

While spray drying raffinose or trehalose from aqueous solution or ethanol:water solutions resulted in non-porous microspheres, spray drying from a methanol:n-butyl acetate mixed solvent system resulted in microparticles which appeared to consist of an agglomeration of individual nanoparticles, i.e. nanoporous/nanoparticulate microparticles.

NPMPs of trehalose and raffinose were amorphous, with glass transition temperatures (Tgs) that were sufficiently high (124 °C and ~120 °C for trehalose and raffinose, respectively) to suggest good physical stability at room temperature and good potential to act as protein carriers and/or stabilisers.

NPMPs demonstrated improved aerosolisation properties compared to spray dried non-porous particles. The successful incorporation of lysozyme into these NPMPs at a sugar to protein weight ratio of 1:4 demonstrated the potential of these systems to act as carriers for peptide or protein drugs which could be delivered via the pulmonary route.

1. Introduction

Protein and peptide drugs are generally poorly absorbed across biological membranes. The majority of proteins and peptides are thus formulated as parenterals. The parenteral route has several disadvantages however, including patient discomfort, potential high cost and the risk of needle-stick injuries (Patton, 1997; Shoyele and Cawthorne, 2006). Less invasive and more patient-acceptable routes have been sought, and the pulmonary route has emerged as a particularly interesting and viable alternative. Advantages of the pulmonary route include a very large absorptive surface area (~80–140 m^2), decreased metabolism and efflux transporter activity compared to the oral route, avoidance of first pass effect and potential for rapid onset of action (Adjei and Gupta, 1997; Patton et al., 2004; Scheuch et al., 2006).

Numerous studies have shown the feasibility of the pulmonary route for the systemic delivery of proteins and peptides. The first protein for systemic delivery via inhalation to be marketed was insulin (Exubera®), for the treatment of type 1 and type 2 diabetics. The formulation was a dry powder of recombinant human insulin (60%) spray-dried with excipients (mannitol, glycine, sodium citrate, and sodium hydroxide) to produce a glassy amorphous material (White et al., 2005). Other proteins/peptides currently in development for pulmonary delivery include interleukin-1 for asthma, interferons for multiple sclerosis or hepatitis and calcitonin for osteoporosis (Mansell, 2007).

Where the protein/peptide to be used for pulmonary delivery is a low dose, high potency material, it may be desirable, for dry powder inhalation, to formulate with a carrier material (inert excipient) to increase the volume of powder loaded and delivered from the dry powder inhaler (DPI) device.

Protection of protein structure is critical in both processing and storage of the final formulation. The use of excipients to stabilise proteins during drying was initially adopted from freeze-drying data, and has shown considerable success also in spray-drying

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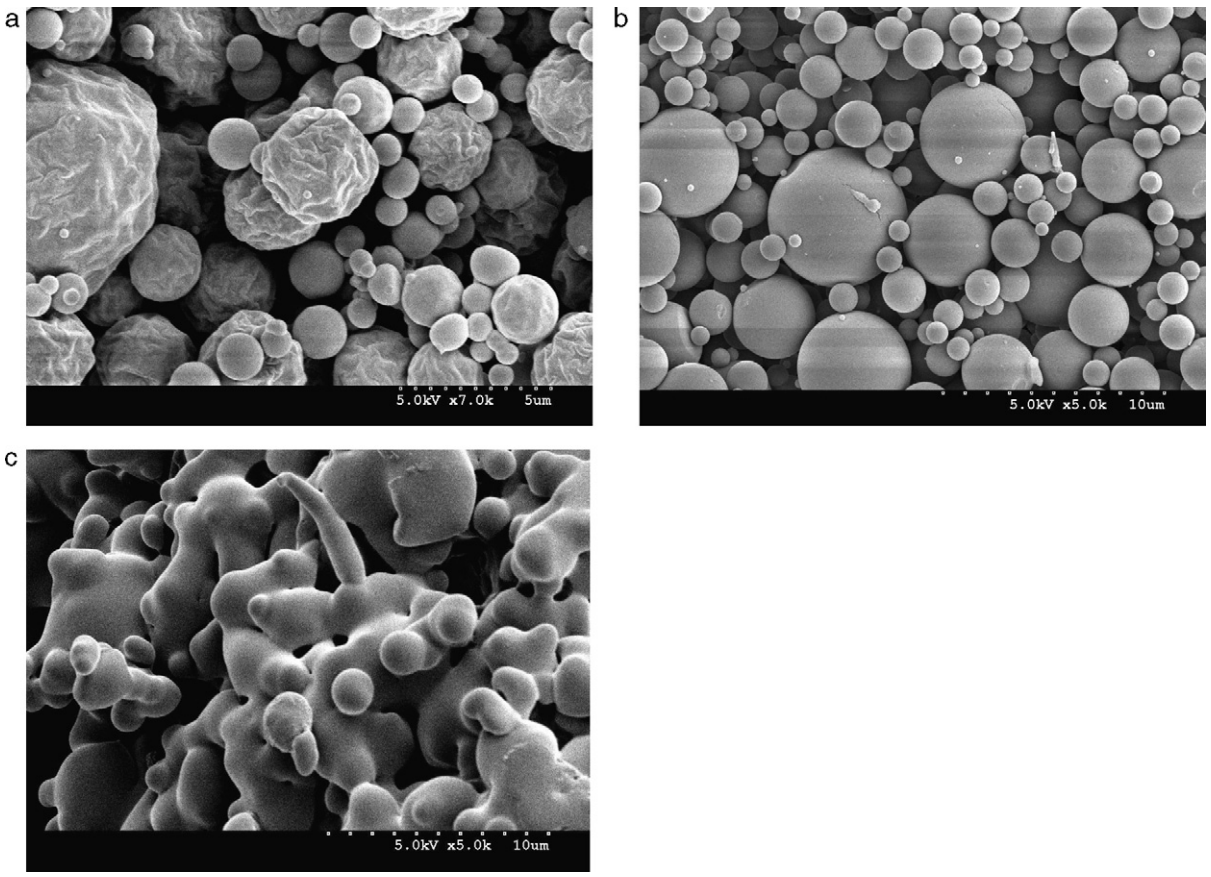

Fig. 1. SE micrographs of (a) trehalose spray dried from a 2% (w/v) aqueous solution, with inlet temperature of spray dryer at 130 °C; (b) raffinose spray dried from a 2.5% (w/v) aqueous solution, with inlet temperature of spray dryer at 130 °C; (c) fused particles of trehalose spray-dried as a 0.5% (w/v) solution from a solvent comprising 80% ethanol/20% water, 10% of total solid content ammonium carbonate; inlet temperature 85 °C.

experiments (Lee, 2002). Sugars (particularly disaccharides), polyols, amino acids or organic salts have been used to protect native protein structure during drying processes, including spray-drying, and also on storage. Two hypotheses are described for the mechanism of stabilisation – the glassy immobilisation hypothesis (Franks et al., 1991) and the water replacement hypothesis (Carpenter and Crowe, 1988, 1989). The glassy stabilisation hypothesis states that stabilisation is achieved by formation of an amorphous glass during drying. In the glassy state, structural changes are significantly delayed and only minor molecular motions for example rotational motions of side chains and vibrations occur (Lee, 2002). Nektar Therapeutics Pulmosol® technology makes use of glass stabilisation of spray-dried microparticles and forms the basis of the Exubera® insulin spray-dried product. In the water replacement hypothesis, stabilisation is attributed to the formation of H-bonding between the sensitive component and the excipient, when water is removed, thus maintaining the structural integrity of proteins, and membranes. An amorphous state may be important to allow maximal H-bonding between the excipient and protein (Arakawa et al., 2001).

Lactose, sucrose, trehalose and raffinose have been investigated as examples of stabilising sugars in spray-drying and other process drying experiments and against specific experimentally induced stresses (for example liquid–solid interfacial stress) (Lee, 2002; Liao et al., 2002; Wendorf et al., 2004). Sucrose, trehalose and raffinose are non-reducing sugars and as such have the advantage that they will not undergo the Maillard browning reaction with proteins. These sugars, which tend to form amorphous glasses on spray-drying, have been shown to stabilise proteins both during processing and also on storage below the glass transition temperature (Tg) of the formulation (Lee, 2002).

We have previously reported on the use of a spray drying method to produce nanoporous microparticles (NPMPs) of two hydrophobic materials with low aqueous solubility, bendroflumethiazide (Healy et al., 2008) and later budesonide (Nolan et al., 2009). The NPMPs prepared were shown to have advantageous micromeritic and aerosolisation properties for pulmonary drug delivery.

In this paper we explore the feasibility of producing nanoporous microparticles of the hydrophilic excipient materials, trehalose and raffinose with a view to ascertaining if it is possible to produce NPMPs of these excipients which could then be developed as carriers and stabilisers for incorporated therapeutic proteins. We also undertake a preliminary investigation of the effect of including a model protein, lysozyme, in these NPMPs, to see if composite particles can be produced while still retaining the porous morphology of the systems.

2. Materials and methods

2.1. Materials

D-(+)-trehalose dihydrate, D-raffinose pentahydrate and lysozyme (~50,000 units/mg) raw materials were purchased from Sigma, Ireland. Freeze dried lysozyme was prepared as previously described by Chin et al. (1994) and Bromberg and Klibanov (1995). Methanol (MeOH) was purchased from Lab Scan Analytical Sciences, Ireland, while n-butyl acetate (BA) was purchased from