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Development of a solid dosage platform for the oral delivery of bilayer vesicles

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ABSTRACT

Within this work, we develop vesicles incorporating sub-unit antigens as solid dosage forms suitable for the oral delivery of vaccines. Using a combination of trehalose, dextran and mannitol, freeze-dried oral disintegrating tablets were formed which upon rehydration release bilayer vesicles incorporating antigen. Initial studies focused on the optimisation of the freeze-dry cycle and subsequently excipient content was optimised by testing tablet hardness, disintegration time and moisture content. The use of 10% mannitol and 10% dextran produced durable tablets which offered strong resistance to mechanical damage yet appropriate disintegration times and dispersed to release niosomes-entrapping antigen. From these studies, we have formulated a bilayer vesicle vaccine delivery system as rapid disintegrating tablets and capsules.

1. Introduction

The oral route as a means of drug delivery and immunisation offers a range of advantages including ease of administration, reduced need for trained personnel to administer vaccines and generally an increased convenience and compliance. Furthermore, in the case of vaccines, given that the mucosal sites are often the primary access point for human pathogens, oral vaccination can enhance mucosal immunity and promote strong resistance against many pathogens. In the development of oral vaccines, a range of delivery systems have been considered including liposomes and non-ionic surfactant vesicle carriers, such as niosomes or bilosomes. These systems are employed to encapsulate/associate vaccine antigens and thus provide protection and targeting within the gastro-intestinal tract. In previous studies from our group, we have shown that liposomes (Perrie et al., 2002) were able to protect and deliver DNA vaccines orally and non-ionic vesicles (with and without the addition of bile salts) were able to protect and deliver sub-unit antigens to within the target site of the Peyer's Patches (Wilkhu et al., 2013, 2014). Furthermore, these bilayer vaccines incorporating recombinant HA were able to reduce median temperature differential change and promote a reduction in viral cell load in an influenza challenge study (Wilkhu et al., 2013).

In the development of an oral vaccine, generally the dosage form has been in a liquid format. However in terms of shelf stability, storage and distribution, vaccines in a liquid dosage form are not ideal and development of a stable solid dosage vaccine platform is required. Solid dosage forms such as tablets and capsules are the most commonly adopted oral delivery system, offering high patient compliance and easy storage. In addition, options such as orally disintegrating tablets are useful for paediatrics, geriatrics, patients who may struggle with swallowing (after stroke or renal failure patients) or patients with dysphasia (Lindgren and Janzon, 1991; Wilson et al., 1987; Gupta, 2010). Such solid dosage forms also offer a cost effective way to carry out bulk immunisation, as tablets can be distributed worldwide without the use of trained personnel.

To format liposomes into a dry format, freeze drying has been widely used as a standard method. Freeze drying of liposomes is used to prevent hydrolysis and physical degradation of the phospholipids within the vesicles during extended storage (Van Winden, 2003; Bridges and Taylor, 2001). However, the process of freezing and resultant dehydration of the formulation can exert stress onto the vesicles thus affecting the integrity of the vesicles; freezing may result in ice formation thus disrupting the bilayers and result in phase transition changes (Stark et al., 2010). Furthermore, upon dehydration, an increase in solute concentration can occur which may cause bilayer fractures, subsequently leading to vesicle aggregation, changes in vesicle size, and loss of entrapped antigen/material (Crowe et al., 1985; Crowe et al., 1986; Stark et al., 2010).

The associated problems with freeze drying of bilayer vesicles can be minimised by the inclusion of cryo- and lyoprotectants (which

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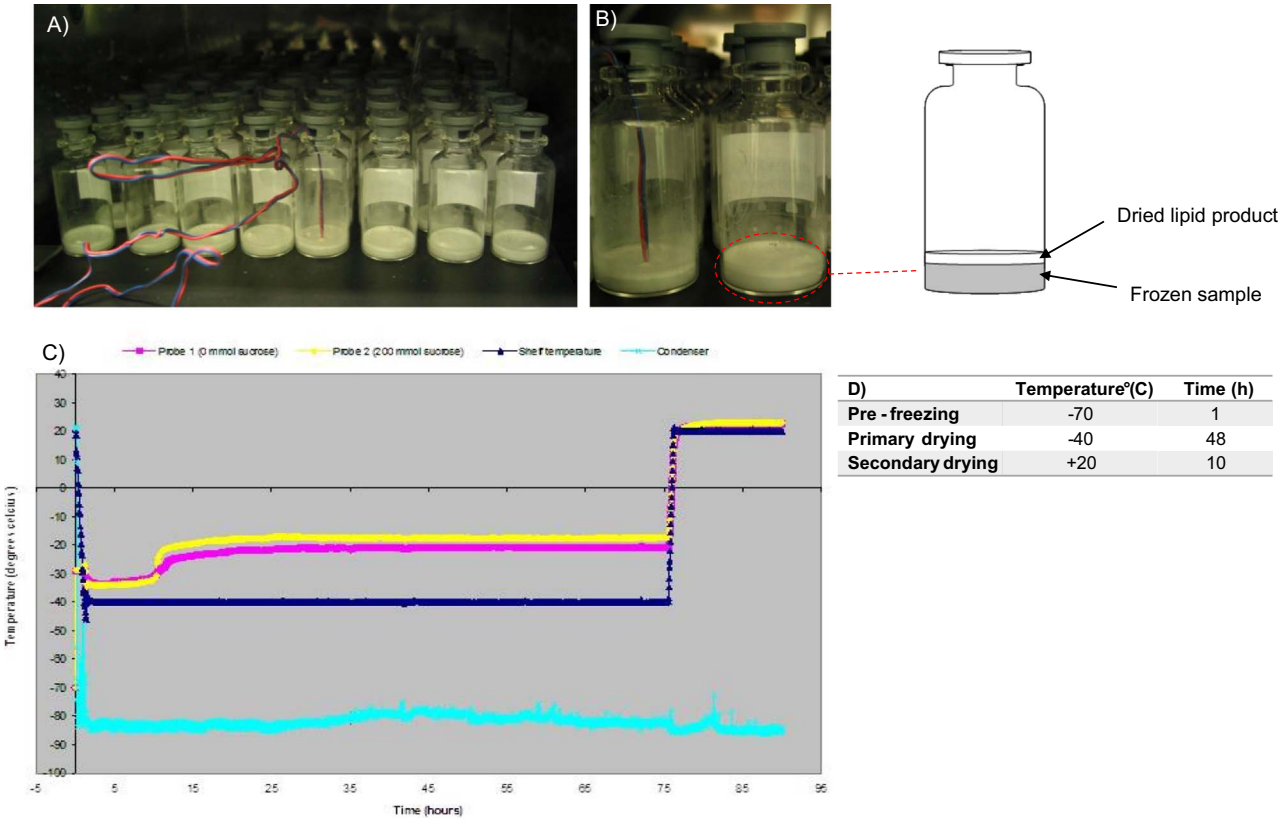

D)	Temperature°(C)	Time (h)
Pre - freezing	-70	1
Primary drying	-40	48
Secondary drying	+20	10

Fig. 1. Samples undergoing lyophilisation where: A) Samples are in process during a freeze drying cycle. B) Sublimation taking place within a vial containing the vesicle formulation. Temperature probes are constantly monitoring internal temperature over the time period of the lyophilisation cycle. C) Key stages during a freeze drying cycle taken using a thermocouple placed inside vesicle suspension vials with and without sucrose. D) Final optimised parameters for lyophilisation of vesicles with condenser temperature set at − 75 °C.

include disaccharide carbohydrate sugars) within the formulation prior to freeze drying. Protectants such as trehalose or sucrose are characterised by their non-eutectic nature, thus protecting the vesicles by forming an amorphous matrix around the vesicles. The addition of trehalose to a vesicle suspension inhibits vesicle fusion and aggregation during the freezing process (Abdelwahed et al., 2006; Van Winden et al., 1997). The mechanism of action of such cryoprotectants has been examined by Crowe et al. (1996) where they found that the sugar molecules are able to interact with the head groups of the phospholipids, thus, preventing membrane disruption by counteracting fusion (Crowe et al., 1996). In addition to using cryoprotectants to stablise the suspensions, other excipients have also been known to aid in the lyophilisation process, offering protection of the product. This includes bulking agents such as hydroxyethyl starch, trehalose, mannitol, lactose, and glycine. These are used when the concentration of product is low. Stabilisers such as sucrose, lactose, trehalose and mannitol can be used to offer protection through the freezing stages and isotonicity modifiers (e.g. mannitol, sucrose and glycerol) can be used to control isotonicity. As can be seen, a range of studies have considered the production of bilayer vesicles in a dried format; however, as noted by Tan et al. (2013), to aid their clinical translation, research into converting bilayer vesicles into convenient oral drug delivery systems is required. Therefore, the aim of this work was to exploit such excipients to formulate an oral vaccine solid dosage platform which, upon rehydration or ingestion, releases the vesicles containing the antigen. These vesicles should also maintain their integrity, potency and function as they would in a liquid dosage form.

2. Materials and methods

To form the vesicles, the surfactants monopalmitoyl glycerol (MPG; Larodan AG, Sweden), synthetic cholesterol (Chol), dicetyl phosphate (DCP) (Sigma-Aldrich, UK) were used as this formulation has previously been shown to act as a successful vaccine delivery system orally (Wilkhu et al., 2013, 2014). The buffers were made up of sodium bicarbonate (Sigma-Aldrich, UK) at pH 7.6, where hydrochloric acid and sodium hydroxide (NaOH) (Sigma-Aldrich, UK) were used for pH adjustments. For the antigen, a recombinant H3N2 sub-unit protein (Immune Tech, USA) was used.

2.1. Preparation of vesicles for lyophilisation

A 5:4:1 M ratio of MPG, CHO and DCP was weighed and placed into a 10 mL glass beaker. 25 mM sodium bicarbonate (pH 7.6) formed the aqueous phase and was placed in a heated water bath for 10 min at 30–35 °C. Whilst the aqueous buffer was preheated, the beaker containing the lipids was placed into a hot oil bath (120–125 °C) and melted for 10 min with occasional mixing. The beaker containing the molten mixture was removed from the oil bath and the buffered stock solution was immediately added and homogenised at 8000 rpm at 30–35 °C. After homogenising for 10 min, the homogenisation speed was reduced to 4000 rpm to act as a mixer and dextran (Sigma Aldrich, UK) was added into the solution for a minute followed by mannitol (Sigma Aldrich, UK) for another minute of mixing. A 400 mM trehalose solution was prepared and then added at a 1:1 ratio of vesicle mixture: trehalose in a bijoux tube which formed the mould for the tablets and were then pre-frozen in the − 70 °C freezer until the freeze drying cycle was ready to begin.

3. Lyophilisation cycle

Lyophilisation was performed with the Virtis Advantage (Bio Pharma) freeze dryer. The freeze drying protocol was set for primary drying to occur at − 40 °C for 48 h with a secondary drying cycle set at