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A comprehensive production method of self-cryoprotected nano-liposome powders

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ABSTRACT

This study provided a convenient approach for large scale production of hydrogenated soya phosphatidylcholine nano-liposome powders using beclometasone dipropionate as a model drug and sucrose as proliposome carrier. Fluid-bed coating was employed to manufacture proliposomes by coating sucrose with the phospholipid (5%, 10%, 15% and 20% weight gains), followed by hydration, size reduction using high pressure homogenization, and freeze-drying to yield stable nano-vesicles. High pressure homogenization was compared with probe-sonication in terms of liposome size, zeta potential and drug entrapment. Furthermore, the effect of freeze-drying on vesicle properties generated using both size reduction methods was evaluated. Results have shown that high-pressure homogenization followed by freeze-drying and rehydration tended to yield liposomes smaller than the corresponding vesicles downsized via probe-sonication, and all size measurements were in the range of 72.64–152.50 nm, indicating that freeze-drying was appropriate, regardless of the size reduction technique. The liposomes, regardless of size reduction technique and freeze drying had slightly negative zeta potential values or were almost neutral in surface charge. The entrapment efficiency of BDP in homogenized liposomes was found to increase following freeze-drying, hence the drug entrapment efficiency values in rehydrated liposomes were 64.9%, 57%, 69.5% and 64.5% for 5%, 10%, 15% and 20% weight gains respectively. In this study, we have reported a reliable production method of nano-liposomes based on widely applicable industrial technologies such as fluid-bed coating, high pressure homogenization and freeze-drying. Moreover, sucrose played a dual role as a carrier in the proliposome formulations and as a cryoprotectant during freeze-drying.

1. Introduction

Liposomes are microscopic phospholipid vesicles which were used as artificial membrane models to mimic simple cell systems for the investigation of transport functions and mechanisms, and study the permeation properties of small ions and molecules, and the adhesion and fusion kinetics of biological cells. Since they were reported by Bangham and co-workers liposomes have attracted a lot of interest as drug carriers (Wagner and Vorauer-Uhl, 2011). Liposomes have been recognized as highly promising delivery carriers and many liposome formulations have gained clinical approval (Chang and Yeh, 2012) and many have reached the cosmetics market (Rahimpour and Hamishehkar, 2012), (Carbone et al., 2013) and (Choi and Maibach, 2005). Doxil® and DaunoXome® were the first liposome-based products to be commercially available for clinical use (Allen and Cullis, 2013).

Many methods of liposome preparation on small laboratory scale level have been reported in the literature such as the reverse phase evaporation method (Patil and Jadhav, 2014). Sonication has been used widely for the production of nano-liposomes and nano-niosomes; however this approach is not applicable for large scale production and the use of titanium probe-sonicators may result in contamination of the liquid because of the titanium particles leaching from the probe into the preparation. Large scale production of liposomes has been proposed using solvent injection based methods such as improved supercritical reverse phase evaporation (ISCRPE) and depressurization of expanded solution into aqueous media (DESAM) (Meure et al., 2008). The inconsistency of the final product characteristics, low product yield, high cost and complexity of the methods are all obstacles hindering the

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development of new liposome products (Laouini et al., 2013). Micro-fluid methods for large-scale production of nano-liposomes have been proposed; however, this approach requires the design of special equipment and frequent replacement of the micro-engineered membranes (Laouini et al., 2013).

Proliposomes have been proposed as a stable alternative with ability to generate liposomes upon addition of aqueous phase prior to administration. They have been conventionally made by coating carbohydrate carrier particles with phospholipids within a rotary evaporator (Elhissi and Taylor, 2005). Rare examples are available on the production of proliposomes via fluidized bed in larger quantities. Previous attempts used insoluble multilayer core (Chen and Alli, 1987) or ionisable substrate (Katare et al., 1990), which may compromised the stability of the hydrated formulation and limited its use. More recently, Tantisripreecha et al. (2012) used a coating pan to apply lipids as a sub-coat for enteric coating of protein tablets. Moreover, hydration of proliposomes predominantly generates micro-liposomes and liposome aggregates (Elhissi and Taylor, 2005), which may limit their potential for use clinically. Thus, the design of a comprehensive approach to manufacture stable nano-liposomes in larger quantity is highly in need.

The aim of this study was to develop stable powdered phospholipid formulations that upon hydration can generate self-cryoprotected nano-liposomes. We have designed a production approach for liposomes based on manufacture of proliposomes via fluidized bed as an intermediate step. This was followed by hydration, high pressure homogenization and cryoprotected lyophilization, using the antiasthma steroid beclometasone dipropionate (BDP) as model drug.

2. Materials and methods

2.1. Materials

Sucrose (AnalaR® grade) was obtained from BDH Laboratory Supplies, UK. Hydrogenated soya phosphatidylcholine (HSPC) (Phospholipon® 90H) was a gift from Lipoid, Switzerland. Water, absolute ethanol, and methanol used for coating were all of HPLC grade and purchased from Fisher Scientific Ltd., UK. Beclometasone dipropionate (BDP) was of analytical grade and purchased from Sigma–Aldrich, USA.

2.2. Preparation of proliposomes

Sucrose carrier particles were sieved using a sieving set (Fisher Scientific, UK) and particles in the size range of 400–800 μm were collected for the preparation of proliposomes. HSPC (10.6 g) was dissolved in absolute ethanol (200 mL) mixed using a magnetic stirrer at 60 °C for 1 h to ensure complete lipid dissolution. BDP was incorporated to constitute 5 mole% of the lipid phase. Sucrose particles (40 g) were placed in the coating chamber of Strea-1Classic fluidized bed-dryer (GEA Pharma systems, Switzerland) and coating with the ethanolic HSPC solution was carried out at the rate of 2.1 mL/min and atomization pressure of 0.3 bar. The inlet air temperature was maintained at 33 ± 3 °C and the outlet temperature was approximately 35 ± 3 °C. It was necessary to maintain the room temperature below 20 °C to keep the temperature of the product relatively low during the coating process and avoid particle coalescence (i.e., agglomeration), since melting of lipids at high temperature may promote the adherence between the proliposome particles. In addition, low temperature may enhance the spreading of wet phospholipid on the sucrose carrier particles and is also highly desirable to provide better safety conditions during coating. Coating was completed within 2 h to obtain 5%, 10%, 15% and 20% weight gain from the original weight of sucrose used prior to the commencement of coating. Fig. 1 summarizes the manufacturing steps carried out for the production of proliposomes and liposomes.

2.3. Preparation of liposomes from proliposomes

Proliposome samples (150 mg) were hydrated using 3 mL of deionized water (65 °C) in glass vials followed by vortex mixing for 2 min. The vials were placed in a water bath (Büchi B480, Switzerland) at 60 °C for 15 min to ensure complete lipid hydration followed by further vortex mixing for 3 min. The liposomes were allowed to anneal for 2 h prior to further processing or analysis.

2.4. Scanning electron microscopy

The proliposomes were fastened on a carbon pads (Agar Scientific, UK) and coated with gold using a JFC-1200 Fine Coater (JEOL, Tokyo, Japan). A scanning electron microscope (Quanta 200, FEI Company, USA) was utilized to observe the samples at 30 kV to provide details of the surface characteristics as well as the morphology of the uncoated and lipid-coated sucrose particles.

2.5. Size reduction of liposomes using probe-sonication or high-pressure homogenization

The hydrated liposome formulations were converted into nano-liposomes via size reduction using the Nano DeBEE high pressure homogenizer (BEE International, Inc., MA, USA). Liposomes generated from proliposome were placed into the sample holder and homogenization took place at a pressure of 25,000 psi at 20 °C. All formulations underwent three cycles of homogenization in order to reduce particle size. The size of liposomes was measured using dynamic light scattering to ascertain the generation of nano-liposomes following the completed cycles of homogenization and in between the cycles. Size reduction of hydrated proliposomes was conducted using probe sonication (Sonics Vibra cell CV33, CT, USA) and the findings were compared to size analysed using high pressure homogenization. To minimize the risk of heat-induced sample degradation, the flask accommodating the sample was placed in an ice bath during probe-sonication at 50 W for 5 min.

2.6. Freeze-drying of liposome formulations

The homogenized liposome formulations were loaded into vials and kept in the freezer (−20 °C) overnight. The vials were then loaded into the freeze-drier (Edwards, Micro Modulyo, IL, USA). The unit was closed and the temperature of the system was lowered to −20 °C and vacuum of 1.5–2.0 mbar was applied for 8 h to obtain the dried liposomes.

2.7. Liposome size and zeta potential analysis

The laser diffraction instrument Mastersizer Hydro 2000 SM (Malvern Instruments Ltd., UK) was used to measure the size distribution of liposomes prior to size reduction. The median size and size distribution were calculated by the instrument's software as the volume median diameter (VMD; 50% undersize) and Span respectively, where Span = (90% undersize – 10% undersize)/VMD. Following size reduction, the size analysis of nano-liposomes was carried out via dynamic light scattering using Zetasizer Nano ZS (Malvern Instruments Ltd., UK).

2.8. Entrapment efficiency of BDP in liposomes

The entrapment efficiency of BDP in sonicated or homogenized liposome vesicles was determined by adapting the method