https://doi.org/10.31925/farmacia.2026.4.9 **ORIGINAL ARTICLE**

DRYING TECHNIQUE IMPACT ON LIPOSOMAL MICROPARTICLES: A COMPARATIVE STUDY OF FREEZE-DRIED AND SPRAY DRIED FORMULATION FOR LUNG DELIVERY

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Abstract

The drying process plays a critical role in determining the physicochemical and aerosolization properties of pulmonary drug delivery systems. This study compares freeze-drying (FD) and spray-drying (SD) for the preparation of liposomal microparticles intended for inhalation. Beclomethasone dipropionate-loaded liposomes were prepared by dissolving the lipophilic components (soy phosphatidylcholine, SPC, and Cholesterol, CH; total 100 mg) in absolute ethanol (120 mg) at 70°C. The aqueous trehalose solution was then added, resulting in phospholipid:carrier mass ratios of 1:5 and 1:10 (w/w). FD produced a greater yield compared to SD, but vesicle size was reduced in SD. Zeta potential values were similar across all formulations, while FD vesicles retained higher entrapment efficiency. X-ray diffraction confirmed that both methods yielded less crystalline particles. SEM images revealed that FD particles were irregular, whereas SD particles were spherical. Aerosolization studies demonstrated superior performance of SD formulations, with higher emitted dose, improved Carr's index, and lower MMAD. Under the studied conditions, SD formulations showed superior *in vitro* aerosolization performance, whereas FD achieved higher production yield and entrapment efficiency.

Rezumat

Procesul de uscare joacă un rol esențial în determinarea proprietăților fizico-chimice și de aerosolizare ale sistemelor de administrare pulmonară a medicamentelor. Prezentul studiu compară liofilizarea (FD) și uscarea prin pulverizare (SD) în prepararea microparticulelor lipozomale destinate inhalării. Lipozomii încărcați cu dipropionat de beclometazonă au fost preparați prin dizolvarea componentelor lipofile (fosfatidilcolină din soia, SPC, și colesterol, CH; total 100 mg) în etanol absolut (120 mg) la 70°C. S-a adăugat apoi soluția apoasă de trehaloză, rezultând rapoarte de masă fosfolipid:suport de 1:5 și 1:10 (g/g). FD a produs un randament mai mare comparativ cu SD, dar dimensiunea veziculelor a fost redusă în cazul SD. Valorile potențialului zeta au fost similare pentru toate formulările, în timp ce veziculele FD au păstrat o eficiență de încapsulare mai mare. Difracția cu raze X a confirmat că ambele metode au produs particule mai puțin cristaline. Imaginile SEM au relevat că particulele FD erau neregulate, în timp ce particulele SD erau sferice. Studiile de aerosolizare au demonstrat performanța superioară a formulărilor SD, cu o doză emisă mai mare, un indice Carr îmbunătățit și un MMAD mai mic. În condițiile studiate, formulările SD au prezentat performanțe superioare de aerosolizare *in vitro*, în timp ce FD a obținut un randament de producție și o eficiență de captare mai mari.

Keywords: liposomal microparticles, spray drying, freeze drying, pulmonary delivery

Introduction

In the pharmaceutical field, pulmonary delivery of drugs has long been intriguing for the treatment of not only respiratory diseases, but also systemic diseases. The key extraordinary characteristics of inhalation therapy encompass excessive focusing on efficiency, low drug dose, and big alveolar surface place for drug absorption. In addition, the pulmonary route bypasses first-pass metabolism, which increases the therapeutic efficacy of drugs (1). Both liquid and solid formulations are used for inhalation treatments, the former being delivered through nebulisers and pressurised metered-dose inhalers and the latter through dry powder inhalers (DPIs) (2). Due to their stability and sterility for long-term use, inhalable dry powders are now emerging as the most desirable formulation (3). However, an efficient drug deposition must be ensured to make pulmonary delivery effective (4). Despite these advantages, dry powder formulation for pulmonary delivery can be difficult due to limitations on aerodynamic size and increased tolerance capacity of the lung compared to an oral route of drug delivery (5).

Furthermore, it was shown that different physicochemical characteristics of dry powder, such as particle size distribution, particle shape, surface roughness, interparticle force, and solid state, have a substantial impact on aerosolization, deposition, and clearance along the respiratory tract, therefore determining DPI performance. Hence, the production of dry powder for inhalation is difficult, especially within the

most required aerodynamic particle size range of 1 - 5 µm (6). Several approaches have been investigated to produce this ideal aerodynamic size range, such as milling, spray-drying, freeze-drying, spray freeze-drying, and supercritical fluid technology (7). Out of all these mentioned techniques, freeze-drying and spray-drying are primarily used in pharmaceutical companies to prepare dry powder for inhalation (8).

Liposomes are a possible carrier for active pharmaceutical ingredients (APIs) (9). Liposomes are small spherical vesicles composed of bilayer membranes, created by the rearrangement of lipids in an aqueous medium. Liposomes are flexible delivery carriers; hydrophilic compounds can be enclosed in the aqueous centre of the vesicles while hydrophobic compounds may have their location in the lipid bilayer (10). However, in spite of being an attractive and versatile delivery system, adaptation of liposomes for pulmonary delivery is still fraught with challenges, including *in vivo* instability and formulation problems, which lead to batch-to-batch variation (11). A solution for overcoming these challenges is to treat the liposomes by converting them to a solid-state. Conversion of liposomes to dry form has been widely investigated (12), and found to enhance their storage stability, since the removal of the aqueous environment allows to produce a stable powder that can be reconstituted back to liposomes (13).

Spray drying is a well-established method – low-cost, fast-acting, and easily scalable (14). In addition to this, the powder particle can be designed to achieve various diverse particle properties (size, porosity) during spray drying (15). Freeze-drying (*i.e.*, lyophilization), which is used to reduce the water content in long-term stable dry powder liposomes, is the usual method for long-time stability (16).

To stabilize liposomal membranes, the stabilizer should be in an excess amount with respect to lipids (17). Trehalose, a non-reducing disaccharide composed of two glucose units, is often present in high concentrations in organisms that can withstand desiccation (18). Due to the flexibility of its structure, trehalose improves the interaction between sugar and liposome phospholipid heads (19). Additionally, trehalose has low hygroscopicity and high glass transition temperature, which are important properties for improving the storage stability of lyophilized liposomal powders (20).

This work aims to compare spray drying and freeze-drying techniques for the preparation of liposomal microparticles using trehalose as a cryoprotectant for pulmonary drug delivery *via* a dry powder inhaler. The study seeks to investigate the impact of these drying techniques on various formulation factors such as the yield of production, particle size, zeta potential, crystallinity, morphology, entrapment efficiency, and aerosolization. Moreover, this research will also attempt to investigate the effect of trehalose in maintaining the stability of liposomes during drying and on the drug entrapment and lung deposition.

Materials and Methods

The study utilized a range of materials obtained from different suppliers, listed in Table I.

Table I

Materials and their details that are used in the study

Ingredients	Purity	Grade	Cat. No.	Suppliers
Beclomethasone dipropionate	≥ 98%	Pharmaceutical grade	B3023	Sigma-Aldrich, UK
Cholesterol	≥ 99%	Analytical grade	C8667	Sigma-Aldrich, UK
Trehalose dihydrate	≥ 99%	Analytical grade	T9449	Sigma-Aldrich, UK
Phosphate buffer saline (PBS) tablets	pH 7.4	Analytical grade	P4417	Sigma-Aldrich, UK
Soya phosphatidylcholine	≥ 90%	Pharmaceutical grade	PL90G	Lipoid, Switzerland
Deionized water	Milli-Q water purification system	Analytical grade	-	Millipore, USA

Preparation of liposomal-based microparticles using spray and freeze-drying methods

Beclomethasone dipropionate (BDP) was incorporated into the lipid phase. Soy phosphatidylcholine (SPC) and cholesterol (CH) were used at a 1:1 molar ratio, with a total lipid amount of 100 mg *per* batch. BDP (10 mg) was co-dissolved with SPC and CH in absolute ethanol (120 mg) at 70°C under continuous magnetic stirring (600 rpm) until a clear solution was obtained. Trehalose was dissolved separately in deionized water and used as a stabilizing carrier. The formulation ratio was defined as total lipid:trehalose (w/w) at 1:5 and 1:10, corresponding to 500 mg and 1000 mg trehalose, respectively. The aqueous phase was added dropwise to the ethanolic lipid solution under vortex mixing (2000 rpm) for 5 min to facilitate vesicle formation, followed by continuous stirring at 600 rpm for 30 min at room temperature. (21)

Ethanol was removed by continuous magnetic stirring at 40°C for 2 h to allow solvent evaporation, resulting in the formation of a liposomal dispersion. The final volume of the dispersion was adjusted to 10 mL with deionized water. The final dispersion contained a total lipid concentration of 10 mg/mL and a BDP concentration of 1 mg/mL. Depending on the formulation, trehalose concentration was 50 mg/mL for the 1:5 lipid-to-trehalose ratio and 100 mg/mL for the 1:10 ratio (Table II).

The prepared liposomal dispersions were subsequently subjected to either spray-drying or freeze-drying as described below.

Spray-drying

The spray-drying process was performed using a Büchi B-290 Mini Spray Dryer under controlled conditions. The liposomal suspensions were fed into the dryer at a pump rate of 10%, corresponding to approximately 3 - 5 mL/min. Drying was performed at an inlet temperature of 120 - 130°C, resulting in an outlet temperature of 75 ± 2°C. The aspirator airflow was maintained at 600 L/h (100%), and the atomization process was performed *via* the use of a two-fluid nozzle with a size of 0.7 mm, with the atomizing pressure being maintained at 0.6 bar. The solid content in the dispersion feed varied from 6 to 11% (w/v), depending on the lipid-to-trehalose ratio. The formed microparticles were collected through the cyclone separator and were quickly transferred to a tightly sealed container and placed in a desiccator for future examination (21).

Freeze-drying

The prepared liposomal dispersions were first frozen at -20°C for 24 hours in order to attain full solidification and then deep-frozen at -80°C for 6 hours. Lyophilization was done by using the bench-top freeze-dryer (Büchi, Switzerland). The first stage of drying was conducted at a temperature of -40°C under a chamber pressure of around 0.1 mbar for 24 hours to allow the sublimation of the ice. The second stage of drying was conducted at 20°C and a reduced pressure of around 0.01 mbar for 6 hours. Upon completion of the freeze-drying cycle, the resulting dry powders were collected and stored in sealed glass containers within a desiccator at room temperature until further characterization (22).

Table II

Composition of liposomal formulations before and after drying

Component	BD (pre-dried liposomes)	SD1 (1:5)	SD2 (1:10)	FD1 (1:5)	FD2 (1:10)
BDP (mg)	10	10	10	10	10
SPC (mg)	50	50	50	50	50
CH (mg)	50	50	50	50	50
Trehalose (mg)	0	500	1000	500	1000
Ethanol (mg)	120	120	120	120	120
Water (mL)	ad 10	ad 10	ad 10	ad 10	ad 10
Drying method	None	Spray drying	Spray drying	Freeze drying	Freeze drying

BD: pre-dried liposomal dispersion; BDP: beclomethasone dipropionate.

Evaluation of liposome-loaded microparticles

Determination of Product Yield. The yield (Y) of spray-dried (SD) and freeze-dried (FD) powders was calculated as:

$$Y\ (\%) = \frac{M1}{M2} \times 100 \quad (1)$$

where M1 is the mass of the obtained powder, and M2 is the total mass of solids initially used.

Reconstituted liposomal vesicle size and zeta potential measurement. Dried powder formulations were reconstituted by dispersing 10 mg of powder in 1 mL of deionized water, followed by vortex mixing for 2 min and gentle magnetic stirring for 10 min at 25 ± 2°C to ensure complete hydration of the liposomal vesicles. The size and size distribution of the reconstituted vesicles were determined using laser diffraction (Mastersizer 2000, Malvern Instruments Ltd., UK). The results were expressed as volume median diameter (VMD) and Span, where Span was calculated as:

$$Span = \frac{D90 - D10}{D50} \quad (2)$$

The zeta potential of the reconstituted liposomes was measured using a Zetasizer Nano series (Malvern Instruments Ltd., UK). Briefly, the hydrated dispersion was gently shaken, and 70 µL of the sample was transferred into a polystyrene latex cell. Measurements were performed at 25°C with an equilibration time of 2 min. All measurements were carried out in triplicate (21).

Encapsulation efficiency and drug loading studies. An accurately weighed 10 mg sample of dried powder was reconstituted in 1 mL aqueous medium and allowed to hydrate, followed by annealing at room temperature for approximately 1 h.

To achieve separation of entrapped and unentrapped drug, deuterium oxide (D_2O) was used as the dispersion medium due to its higher density compared to water. The dispersions were centrifuged for 90 minutes at 13,000 rpm using a bench centrifuge (Labnet Spectrafuge 24D, UK). Under these conditions, the free crystals of BDP were expected to sediment to the bottom of the tubes, while the encapsulated drug remained in the supernatant, allowing for separation of free and entrapped drug fractions. However, this separation approach was not formally validated in terms of recovery or selectivity. The entrapped portion was separated from the unentrapped drug and dissolved in methanol for analysis. The total amount of drug present was determined after disrupting the liposomes using methanol.

Entrapment Efficiency (EE%) was determined by using the equation given below:

$$EE\ (\%) = \frac{\text{Total drug loading} - \text{Unentraped drug}}{\text{Total drug loading}} \text{ x } 100 \quad (3)$$

The entrapment efficiency (EE%) was calculated according to the formula below. The amount of drug loaded was indicated as a percentage of the drug *per* total weight of the powder sample.

HPLC analysis of beclomethasone dipropionate. The amount of BDP was analyzed using a validated HPLC method. The procedure was adapted from the previous method described (23, 24).

To obtain a calibration curve, 5 mg of BDP was dissolved in 100 mL of methanol, which resulted in a stock solution concentration of 50 µg/mL. An aliquot ranging from 1 - 10 mL of the stock solution was pipetted to a 10 mL volumetric flask and diluted to volume using methanol to produce a standard solution with concentrations ranging from 5 - 50 µg/mL.

Sample preparation involved placing the liposome dispersion in a 10 mL volumetric flask, adding 1 mL of methanol for vesicle disruption and drug solubilization, and then diluting to volume with deionized water. The samples were filtered using a 0.45 µm membrane filter before injection into the system.

The HPLC analysis was carried out by using the C18 column (150 mm x 4.6 mm, 5 µm; Waters Ltd., UK). The solvent system was methanol-water (75:25 v/v), pumped at a flow rate of 1.7 mL/min. The detection was done at 239 nm, the temperature of the column was kept at 40°C, and the sample injection volume was 20 µL. The method was previously validated and demonstrated acceptable linearity, precision, and accuracy, with suitable limits of detection and quantification for the analysis of beclomethasone dipropionate, as reported in the cited references (23, 24).

Powder X-ray Diffraction Studies. The structural properties of the dried powders were analysed by means of X-ray powder diffraction (XRD) using the Equinox 2000 diffractometer (Inel, France) with a diffracted beam monochromator and Cu radiation. The samples of powder were uniformly placed on glass plates, forming a circle with a diameter of about 4 cm and a thickness of 1 mm. The powder surface was gently pressed and smoothed using a glass slide before measurement. Diffraction intensity was recorded as a function of the 2θ angle over a total scan time of 20 min. The X-ray generator was operated at 32 kV and 28 mA.

The results from the diffractograms were interpreted on a qualitative basis by comparison between the diffraction patterns for the dried samples and the raw materials, with special emphasis on beclomethasone dipropionate and trehalose. Peaks that appeared to be sharp and intense were taken to indicate that there was crystal content present, while the presence of halos that were either broad or weak peak intensities indicated an amorphous nature after the drying processes. Changes in peak intensity and shape were therefore used to assess how spray-drying and freeze-drying affected the physical properties of the formulations.

Powder Morphology studies. The microparticle morphology was evaluated *via* Scanning Electron Microscopy (SEM) analysis (Quanta-200, FEI, USA). The microparticles were placed on aluminium stubs using double-sided carbon adhesive tape. Before imaging, samples were coated with a thin layer of gold using a JFC-1200 Fine Coater (JEOL, Tokyo, Japan) under vacuum to improve conductivity.

Gold coating was performed for approximately 2 min, resulting in an estimated coating thickness of 10 - 20 nm, under a vacuum of approximately 0.05 - 0.1 mbar. SEM images were obtained at an accelerating voltage of 20 kV at various magnifications (400×, 600×, 2000×, 4000×, and 6000×). Images were captured and processed using the instrument software.

Powder Bulk density and flowability studies. Bulk and tapped densities of the dried powders were determined using a tapped density tester (Erweka GmbH, Germany). Due to the limited quantity of spray-dried and freeze-dried liposomal formulations, a scaled-down method was employed.

An accurately weighed 1.0 g sample of powder was gently introduced into a 10 mL graduated cylinder without applying any compaction, and the initial unsettled volume (V_0) was recorded to calculate the bulk density. The cylinder was then subjected to tapping using the instrument. The sample was tapped for 100 taps initially, followed by additional tapping until no further significant change in volume was observed. The final tapped volume (Vf) was recorded.

Bulk density (ρ_bulk) and tapped density (ρ_tapped) were calculated using the following equations:

$$\rho\_bulk = \frac{m}{V^0} \quad (4)$$

$$\rho\_tapped = \frac{m}{Vf} \quad (5)$$

Here, m is the mass of the powder, V_0 is the initial volume, and Vf is the final tapped volume.

The flowability of the powders was evaluated using Carr's Index (CI), calculated as:

$$\text{Carr's Index}(\%) = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \text{ x100} \quad (6)$$

Powder aerodynamic property studies. The aerosolization characteristics and *in vitro* deposition profile of powders containing beclomethasone dipropionate (BDP) were evaluated using a Next Generation Impactor (NGI) (Copley Scientific, Nottingham, UK) equipped with a mouthpiece adaptor, induction port, and pre-separator. The NGI was connected with the Copley TPK 2000 critical flow controller and the Copley HCP5 vacuum pump, while the volume flow rate was determined and regulated for each experiment using a Copley DFM 2000 flow meter. The collection surfaces were coated prior to each experiment with 1% w/v silicon oil solution in n-hexane to avoid bouncing and re-entrainment of particles. In each trial, five capsules (size 3, Qualicaps, Spain)

were separately filled with 10 mg powder to get a final loaded dose of 50 mg. The capsules were inserted sequentially into a Miat® Monodose inhaler (Miat S.P.A., Milan, Italy), and the inhaler was tightly fitted into the induction port. The impactor was operated at an airflow rate of 45 L/min for 5.3 s, corresponding to a sampled air volume of 4 L. Under these conditions, the manufacturer-stated effective cut-off aerodynamic diameters were 9.1, 5.2, 3.3, 1.9, 1.1, 0.6, and 0.4 µm for stages 1 to 7, respectively.

After actuation, the capsules, inhaler device, mouthpiece adaptor, induction port, pre-separator, stages 1 - 7, and micro-orifice collector (MOC) were disassembled, and each component was washed separately using a mixture of deionized water and methanol (7.5:2.5, v/v) to recover deposited drug (21).

The resulting washings were collected and analysed for BDP content by the validated HPLC method described above. The recovered dose (RD) was defined as the total amount of drug recovered from all components. Emitted dose (ED) was calculated as the fraction of RD leaving the capsules and inhaler, and fine particle fraction (FPF) was defined as the fraction of RD with an aerodynamic diameter of ≤ 5.2 µm. MMAD was determined from the plot of cumulative percentage undersize versus effective cut-off diameter. All experiments were performed in triplicate. Drug recovery was assessed from all NGI components collectively as the recovered dose (RD), and the mass balance was considered acceptable when the recovered dose was within acceptable experimental variability of the loaded dose.

Statistical analysis

Statistical analysis was performed using SPSS statistical software (version 23, IBM Corp., Armonk, NY, USA) at $p < 0.05$. All experiments were conducted using three independent batches ($n = 3$), and results are presented as mean ± standard deviation (SD). Prior to analysis, data were assessed for normality and homogeneity of variance to confirm the assumptions for parametric testing.

For comparisons involving the dried formulations (SD1, SD2, FD1, and FD2), a two-way analysis of variance (two-way ANOVA) was applied to evaluate the effects of drying method (spray-drying *vs.* freeze-drying), lipid-to-trehalose ratio (1:5 *vs.* 1:10), and their interaction. When significant differences were observed, appropriate post hoc multiple-comparison tests were performed.

For comparisons between the pre-dried liposomal dispersion (BD) and dried formulations, one-way ANOVA was applied, followed by Duncan's multiple range test for pairwise comparisons at $p < 0.05$.

Results and Discussion

Influences of drying process on the powder production yield

Production yield is a key parameter that reflects process efficiency and directly affects the cost of the final product (25). Because formulations with identical formulation compositions were subjected to different drying techniques, while the processing conditions were specific to each method, the difference in production yield is attributed to both the drying technique and the lipid-to-carrier ratio. Post hoc comparisons were performed using Duncan's multiple range test to identify significant differences between formulations.

Freeze-drying consistently produced higher yields (FD1, FD2) compared to spray drying (SD1, SD2) ($p < 0.05$), with a significant main effect of drying method, while the lipid-to-carrier ratio also showed a significant effect. Minor product loss during freeze-drying may occur due to suspension adherence to the glass surfaces of the apparatus (8), but this effect was limited. Conversely, the relatively low yield from spray drying was mainly due to adherence of droplets or powders to the drying chamber walls and the limited efficiency of the cyclone in collecting fine particles (26). As shown in Figure 1, yield increased with higher carrier-to-phospholipid ratios in both processes. Lower yields in FD1 and SD1, compared to FD2 and SD2, may be explained by the greater lipophilic phase content in these formulations, which enhanced adhesion to drying surfaces.

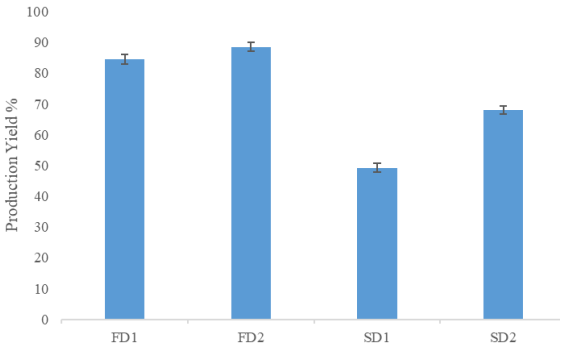

Figure 1.
Percentage of production yield of different ratios of liposomal microparticles after spray-drying and freeze-drying process

Impact of drying process on the size, span, and surface charge of liposomal vesicles

The particle size and size distribution of the reconstituted dispersion (Table III) decreased significantly ($p < 0.05$) after drying compared to pre-dried liposomes (BD), with significant effects of both drying method and trehalose ratio, while their interaction was not statistically significant. It should be noted that the reported size values were obtained using

laser diffraction and therefore represent the size of the reconstituted dispersion, including aggregates, rather than individual liposomal vesicles. Increasing trehalose ratios further reduced the measured particle size in both FD and SD formulations, likely due to reduced aggregation resulting from the cryoprotective effect of trehalose (27). Polydispersity also decreased significantly ($p < 0.05$), indicating improved homogeneity upon rehydration, consistent with previous findings (28). Trehalose, therefore, enhanced size uniformity, consistent with results reported by Sepúlveda *et al*. (2021) (17). Since disaccharides are recognized for their effectiveness as stabilizing agents for liposomes (29).

The observed changes after drying may be associated with vesicle fusion or agglomeration of vesicles during the dehydration and rehydration process, which is quite common in liposomes. This process may cause an increase in size when reconstitution occurs. However, the reduction in the size and polydispersity seen in this study shows that trehalose counteracts this effect through its ability to stabilize the lipid bilayer. Nonetheless, it is important to state that the result obtained by using laser diffraction represents the estimated average size of the dispersion, and additional testing methods, like dynamic light scattering (DLS) and transmission electron microscopy (TEM), would have been needed to verify the size and structure of the vesicles (9).

Zeta potential (surface charge) was markedly reduced ($p < 0.05$), and the effect increased as trehalose content increased, where surface charge became slightly negative for FD2 vesicles. Even though the zeta potentials were close to neutral, the values obtained were uniform among all the samples, suggesting that there was no significant change in surface properties after the drying process. This behaviour may be attributed to the presence of trehalose, which can partially mask the surface charge upon reconstitution (29), indicating that no clear difference in surface characteristics between drying methods could be established based on zeta potential values (30)**.** This reduction may reflect lower charge density on enlarged vesicles, a trend consistent with the findings of Elhissi *et al*. (2010) (28). The relatively neutral zeta potential values observed after drying may indicate limited electrostatic stabilization, which can influence vesicle aggregation behaviour during storage (30). Reduced surface charge may decrease interparticle repulsion, potentially contributing to the formation of larger aggregates upon reconstitution. This may partially explain the relationship between zeta potential and particle size observed in this study.

Table III

The size, span, and surface charge of liposomal vesicles before and after the drying process. The information is presented as Mean ± SD (n = 3)

Evaluation parameter	BD Mean ± SD	SD1 Mean ± SD	SD2 Mean ± SD	FD1 Mean ± SD	FD2 Mean ± SD
Particle size of reconstituted dispersion (µm)	4.91 ± 0.17	3.73 ± 0.15	3.22 ± 0.20	3.95 ± 0.13	3.25 ± 0.22
Span	2.54 ± 0.02	2.06 ± 0.005	2.11 ± 0.02	1.67 ± 0.03	2.25 ± 0.06
Zeta potential (mV)	3.49 ± 0.19	2.1 ± 0.23	2.4 ± 0.25	0.63 ± 0.13	- 0.97 ± 0.13

Entrapment efficiency of liposomal vesicles before and after the drying process

Entrapment efficiency (EE) decreased significantly ($p < 0.05$) after both drying processes compared to pre-dried liposomes (BD) (Figure 2). Two-way ANOVA indicated a significant effect of drying method on EE ($p < 0.05$), while the effect of trehalose ratio and the interaction term were not statistically significant. This reduction may be attributed to process-induced effects during drying and rehydration, including lipid bilayer disruption, vesicle fusion or aggregation, and possible drug leakage. These effects are commonly associated with dehydration–rehydration stress in liposomal systems (31, 32). In addition, a potential contributing factor may be the differential loss of formulation components during drying. Due to their adhesive and surface-active nature, phospholipids may preferentially adhere to equipment surfaces, leading to a relative reduction in lipid content in the recovered powder. This could reduce the effective phospholipid-to-drug ratio upon reconstitution and therefore decrease entrapment capacity. However, this effect was not directly quantified in the present study.

Freeze-dried formulations (FD1, FD2) showed significantly higher EE than spray-dried formulations (SD1, SD2) ($p < 0.05$). The lower EE observed in spray-dried formulations may be associated with greater thermal and mechanical stresses during atomization and drying, which can promote drug leakage or structural destabilization (33). Furthermore, increasing the trehalose ratio improved EE in both drying methods, likely due to stabilization of the lipid bilayer and inhibition of membrane fusion, although differences between trehalose concentrations were not statistically significant ($p > 0.05$). These findings agree with Sepúlveda *et al*. (2021), who reported trehalose as an effective stabilizer of drug-loaded liposomes (17). The protective effect of trehalose may be explained by its interaction with phospholipid bilayers through hydrogen bonding and vitrification. According to the water replacement theory, trehalose can replace water molecules around phospholipid head groups, helping to preserve

membrane integrity during dehydration (34). However, the extent of stabilization depends on the sugar-to-lipid ratio and processing conditions. In addition, vitrification contributes to the formation of a glassy matrix that reduces molecular mobility and limits vesicle fusion during drying and rehydration. Compared to other commonly used stabilizers such as sucrose or mannitol, trehalose is often considered more effective due to its higher glass transition temperature and lower tendency to crystallize, which enhances the stability of dried liposomal systems.

Moreover, beclomethasone dipropionate, being a lipophilic corticosteroid, may be sensitive to thermal and processing stresses, particularly during spray drying. Such conditions could contribute to drug leakage or degradation, thereby influencing the observed reduction in entrapment efficiency. However, drug stability was not directly evaluated in this study and should be investigated in future work using appropriate analytical methods.

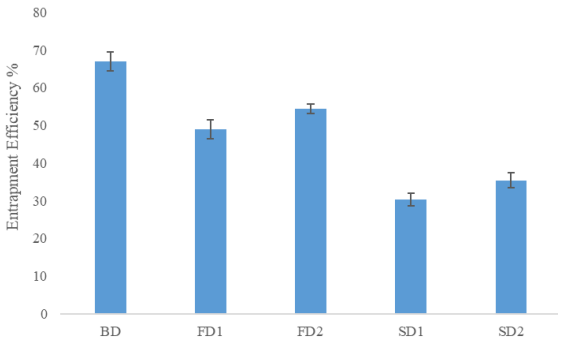

Figure 2.
Percentage of entrapped drug in liposomal vesicles before and after the drying process using various lipid-to-trehalose ratios

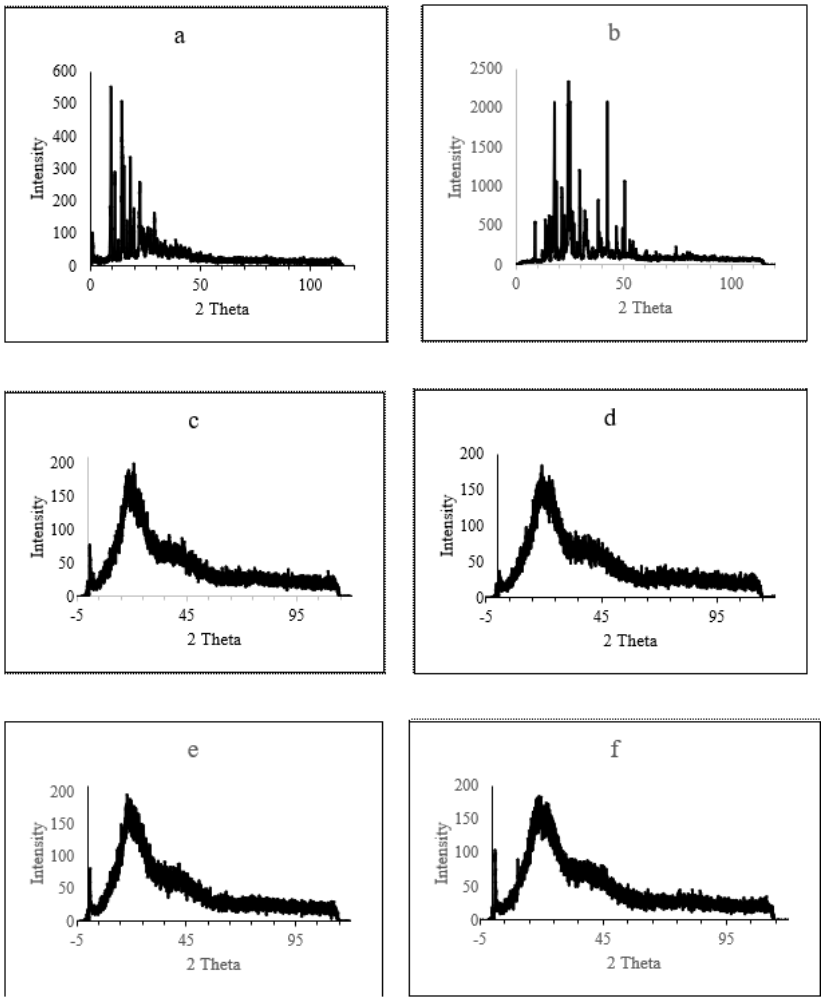

Figure 3.
The X-ray diffraction images of (a) Pure drug; (b) Trehalose alone; (c) FD1; (d) FD2; (e) SD1; (f) SD2

X-Ray Diffraction studies for spray-dried and freeze-dried microparticles

X-ray diffraction (XRD) patterns demonstrated a reduction in crystallinity, with both drying methods (Figure 3), yielding predominantly amorphous phases rather than fully amorphous structures. The pure trehalose showed distinct diffraction peaks, which were an indication of crystallinity, whereas the dried powders

presented low diffraction peaks as well as lower crystallinity compared to the pure drug (Figure 3a) and trehalose (Figure 3b). Both SD (Figure 3e, 3f) and FD (Figure 3c, 3d), regardless of trehalose ratio, yielded powders with reduced crystallinity and predominantly amorphous characteristics. These results are consistent with previous research conducted by Giovannelli *et al*. (2021) (35) and Osanlóo *et al*. (2023) (36), which have shown that drying processes lead to amorphization or decreased crystallinity, which may increase the dispersibility and hydration of powders in the lungs (37). The observed reduction in crystallinity may be associated with the formation of a partially amorphous matrix during drying, which can influence powder properties, stability, and performance (38).

The presence of a partially amorphous matrix might also be attributed to the glass transition property of trehalose, which may stabilize the lipid matrix and reduce molecular mobility (39, 40). Although this might improve physical stability, as suggested in the literature, amorphous materials tend to be highly hygroscopic and thus susceptible to recrystallization when exposed to humid environments.

Moisture content is also reported in the literature to be an important factor affecting the stability of dried liposomal powders. Increased moisture uptake may lead to plasticization of amorphous systems, a reduction in glass transition temperature, and agglomeration or recrystallization, all of which can negatively affect the aerosolization and stability properties of the powder. However, residual moisture content and storage stability were not evaluated in the present study; therefore, these interpretations are based on literature reports.

Morphological studies of spray-dried and freeze-dried microparticles

SEM images showed that the spray-dried powders were mostly spherical with smooth surfaces; however, in some cases, they exhibited concave or porous structures on their surfaces (Figures 4c, 4d). This kind of morphology is usually associated with fast-drying conditions rather than stabilizer concentration (41, 42).

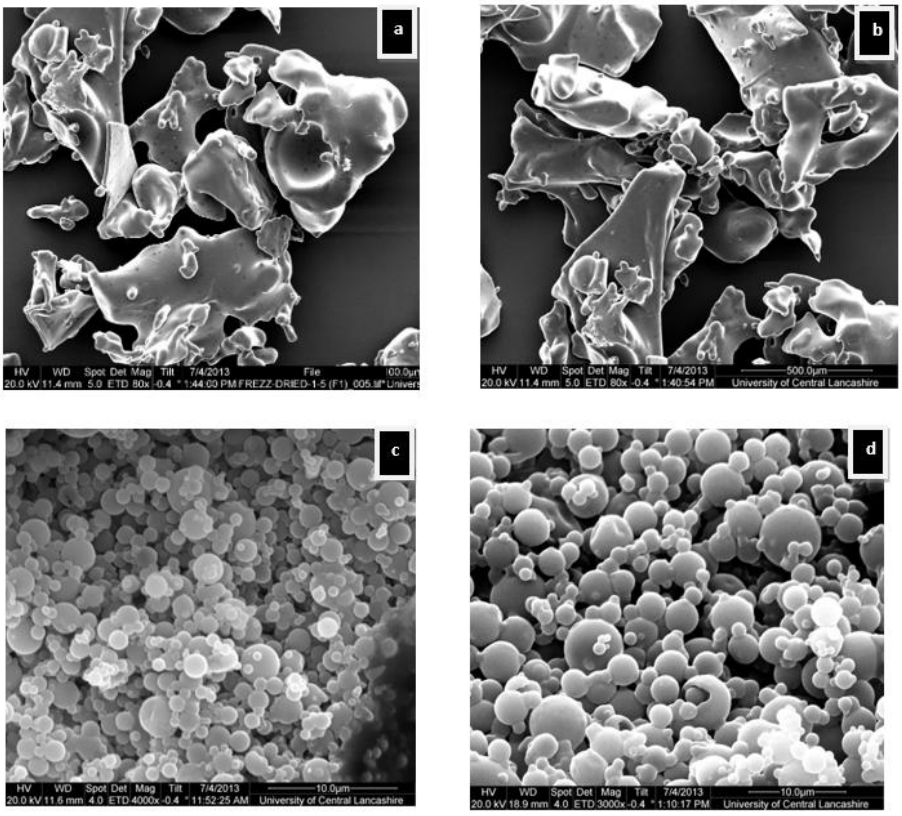

Figure 4.
Scanning electron microscopy image of (a) FD1; (b) FD2; (c) SD1; (d) SD2

On the other hand, the freeze-dried powders had elongated particles with a non-uniform shape (Figures 4a, 4b). The formation of such irregular shapes could be explained by the nature of the freeze-drying process involving freezing and sublimation steps (32, 43). This finding supports earlier comparative studies (42, 44). Nevertheless, Kabra and Lahoti (2023) (8) found contrary results, suggesting that the morphology of particles could be dependent on the formulation components.

Additionally, differences in particle shape and surface properties may influence the physical properties of the powder, where spherical particles are associated with increased flowability and dispersibility, compared to irregular ones, which exhibit greater inter-particle interaction (45). In addition, the density of particles, the roughness of their surfaces, and the forces of cohesion between them are key factors influencing aerosolization efficacy. Generally, particles with low density and spherical shape show a higher tendency to be de-agglomerated and dispersed, while irregular particles can have larger cohesive strength (46). These differences can be attributed to the distinct processing conditions of each technique. Spray drying involves rapid solvent evaporation, producing spherical particles with improved aerosolization but potentially lower entrapment efficiency due to thermal and mechanical stresses. In contrast, freeze-drying preserves liposomal structure under mild conditions, resulting in higher entrapment efficiency but less favourable particle morphology and aerosolization performance.

In vitro aerosolization deposition performance of spray-dried and freeze-dried microparticles

Aerosolization studies using NGI revealed that capsule retention losses were significantly higher ($p < 0.05$) in freeze-dried powders compared to spray-dried powders. Statistical analysis indicated a significant effect of drying method on aerosolization parameters ($p < 0.05$). Consequently, the emitted dose (ED) was higher for SD formulations, likely due to their spherical morphology and improved flow properties, as indicated by lower Carr's Index values.

ED values ranged from 85.83% to 94.1% across formulations, indicating efficient emission of the powder from the device (45). Fine particle fraction (FPF) was significantly higher ($p < 0.05$) in SD formulations (Table IV). This improvement is consistent with their smaller size, smoother morphology, and improved flow properties (as reflected by Carr's Index), compared to the larger, irregular particles with higher Carr's Index values produced by freeze-drying. These results confirm previous studies, which suggest that the shape and flowability of particles affect their aerosolization properties (45, 47). These results align with the SEM observations (Figure 4) and Carr's Index values reported in Table IV. Mass median aerodynamic diameter (MMAD) was lower in SD microparticles, further supporting their superior deposition performance. The promising aerosolization properties of SD2, in particular, may be attributed to reduced agglomeration and improved flowability, from lower phospholipid ratios and improved powder flowability (Table IV). It should be noted that parameters such as ED, FPF, and MMAD are *in vitro* indicators of aerosolization performance and do not directly predict *in vivo* or clinical outcomes; therefore, clinical performance was not assessed in this study.

Table IV

The Carr's index, Mass median aerodynamic diameter (MMAD), Emitted dose (ED%), Fine particle fraction (FPF%) of spray-dried and freeze-dried microparticles. The information is presented as Mean ± SD (n = 3)

Evaluation parameter	SD1 Mean ± SD	SD2 Mean ± SD	FD1 Mean ± SD	FD2 Mean ± SD
Carr's index	20.85 ± 1.34	18.61 ± 1.43	29.94 ± 1.26	27.40 ± 1.84
MMAD (µm)	3.40 ± 0.09	3.10 ± 0.09	5.10 ± 1.21	4.78 ± 1.15
ED (%)	91.93 ± 1.34	94.10 ± 1.65	85.83 ± 2.10	87.76 ± 0.61
FPF (%)	41.83 ± 0.83	47.40 ± 1.01	27.70 ± 2.33	32.43 ± 3.0

The EE, FPF, and MMAD values obtained in this study are comparable to those reported for similar liposomal or corticosteroid-based dry powder inhaler systems, which typically exhibit EE values in a moderate-to-high range and MMAD values within the respirable size range (1 - 5 µm). The improved aerosolisation performance observed for spray-dried formulations is consistent with previous findings, further supporting the suitability of this approach for pulmonary delivery.

Based on the emitted dose and fine particle fraction values obtained, the estimated fine particle dose falls within a range that is broadly comparable to typical inhaled doses of beclomethasone dipropionate used in dry powder inhaler systems. However, it should be noted that the long-term safety and biocompatibility of SPC/cholesterol-based liposomal carriers require further *in vivo* evaluation before clinical application.

Aerodynamic performance of dry powder inhalers depends on several factors, including the characteristics of particles and air flow, apart from the inhaler device used. In the present study, only one inhaler was used to deliver the drug, which was a drawback because device-specific resistance and dispersion significantly affect aerosolization (46). Moreover, deposition in the lungs depends on other factors such as airways' shape, mucociliary clearance, and dissolution rates, none of which were considered in the current study (48).

Limitations of the study

This study has certain limitations. Only a limited number of formulations in specific drying conditions have been analyzed. Furthermore, the absence of formulations without trehalose should be noted, as

preliminary observations indicated that lipid-based formulations without a stabilizing carrier adhered strongly to the spray dryer surfaces, preventing effective powder recovery. This limitation may affect the generalizability of the findings. The size analysis of the vesicles was done using laser diffraction, which evaluates the dispersion of the vesicles in general rather than the vesicles in particular; therefore, another method, such as DLS or TEM, could provide more precise structural analysis. In addition, the centrifugation-based separation method used for entrapment efficiency determination was not formally validated in terms of recovery or selectivity, which may influence the accuracy of the reported EE values.

Furthermore, studies on stability, moisture effects, and *in vivo* evaluation have not been carried out. In addition, only one inhaler was used for the aerosolization test, and the density or interparticle bonding forces of particles were not measured directly. Further research is needed to investigate these issues.

From the perspective of production, the use of spray drying can result in certain difficulties, related to losses that can occur during the process, a lack of reproducibility, and control of operating conditions during scaling up. Freeze-drying is a more reproducible method, but it requires a significant amount of time, expense, and energy. The observed differences in production yield and variability between spray-dried and freeze-dried formulations highlight the importance of controlling key process parameters, such as feed composition, inlet temperature, atomisation conditions, and drying rate, to ensure reproducibility and consistent product quality during industrial scale-up.

Conclusions

The results of this study showed the superior and inferior aspects of each drying method with regard to production yield, particle properties, drug encapsulation efficiency, stability, and aerosol performance. Liposome preparations generated using freeze-drying had comparatively better entrapment efficacy compared to those obtained *via* spray-drying, which was due to lower thermal and mechanical stresses during processing. However, the generated particles were larger and had a non-uniform structure, which affected their poor aerodynamic properties. In contrast, spray-dried particles were smaller, spherical, and exhibited better dispersability, thus contributing to better aerosolization efficiency at the tested conditions. Despite all the aforementioned benefits, the yield obtained by this method was comparatively lower, with a higher loss of drug being noted.

Trehalose contributed to the stabilization of liposomes during the drying processes, minimizing agglomeration and supporting vesicle integrity and drug encapsulation within the tested ratios. It also influenced the morphology and performance of the dried microparticles.

Under the studied conditions, spray-dried formulations showed superior *in vitro* aerosolisation performance, whereas freeze-dried formulations achieved higher production yield and entrapment efficiency. However, these findings are limited to the tested formulations, and no conclusion can be drawn regarding overall superiority, particularly as stability was not evaluated.

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Availability of data and materials

The data generated in the present study are included in the figures and/or tables of this article.

Ethics approval and consent to participate

Not applicable.

AI use disclosure

During the preparation of this manuscript, the author used ChatGPT for editing assistance and language improvement, specifically to improve spelling, grammar, word choice, readability, and clarity. The author reviewed and revised all outputs from these tools and takes full responsibility for the final content of the work.

Conflict of interest

The authors declare no conflict of interest.

References

1. Newman SP. Delivering drugs to the lungs: The history of repurposing in the treatment of respiratory diseases. Adv Drug Deliv Rev. 2018;133:5-18.
2. Jain H, Bairagi A, Srivastava S, Singh SB, Mehra NK. Recent advances in the development of microparticles for pulmonary administration. Drug Discov Today. 2020;25(10):1865-1872.
3. de Boer AH, Hagedoorn P, Hoppentocht M, Buttini F, Grasmeijer F, Frijlink HW, et al. Dry powder inhalation: past, present and future. Expert Opin Drug Deliv. 2017;14(4):499-512.
4. Newman SP. Drug delivery to the lungs: challenges and opportunities. Ther Deliv. 2017;8(8):647-661.
5. Muralidharan P, Malapit M, Mallory E, Hayes D, Mansour HM. Inhalable nanoparticulate powders for

respiratory delivery. Nanomedicine. 2015;11(5):1189-1199.
6. Kwon YB, Kang JH, Han CS, Kim DW, Park CW. The Effect of Particle Size and Surface Roughness of Spray-Dried Bosentan Microparticles on Aerodynamic Performance for Dry Powder Inhalation. Pharmaceutics. 2020;12(8):765.
7. Ioannou Sartzi M, Drettas D, Stramarkou M, Krokida M. A Comprehensive Review of the Latest Trends in Spray Freeze Drying and Comparative Insights with Conventional Technologies. Pharmaceutics. 2024;16(12):1533.
8. Kabra V, Lahoti S. Comparison between spray drying and freeze-drying techniques for the preparation of microparticles for delivery via a dry powder inhaler to treat cystic fibrosis. J Res Pharm. 2023;27(6):2443-2451.
9. Giordani S, Marassi V, Zattoni A, Roda B, Reschiglian P. Liposomes characterization for market approval as pharmaceutical products: Analytical methods, guidelines and standardized protocols. J Pharm Biomed Anal. 2023;236:115751.
10. Liu P, Chen G, Zhang J. A Review of Liposomes as a Drug Delivery System: Current Status of Approved Products, Regulatory Environments, and Future Perspectives. Molecules. 2022;27(4):1372.
11. Lutta A, Knopp MM, Tollemeto M, Pedersen GK, Schmidt ST, Grohganz H, et al. The interplay between trehalose and dextran as spray drying precursors for cationic liposomes. Int J Pharm. 2024;652:123798.
12. Thakur A, Xu Y, Cano-Garcia G, Feng S, Rose F, Gerde P, et al. Optimizing dry powder inhaler formulations for pulmonary immunization. Front Drug Deliv. 2022;2:973599.
13. Barbălată CI, Porfire AS, Ambrus R, Mukhtar M, Farkas Á, Tomuță I, et al. Process development of inhalation powders containing simvastatin loaded liposomes. J Liposome Res. 2024;34(3):421-434.
14. Fan C, Eedara BB, Sinha S, Uddin MKM, Doyle C, Banu S, et al. Triple combination dry powder formulation for tuberculosis treatment. Int J Pharm. 2024;654:123984.
15. Aekwattanaphol N, Das SC, Khadka P, Nakpheng T, Bintang MAKM, Srichana T, et al. Development of a proliposomal dry powder inhaler for tuberculosis. Int J Pharm. 2024;664:124608.
16. Kannan V, Balabathula P, Thoma LA, Wood GC. Effect of sucrose as a lyoprotectant on the integrity of paclitaxel-loaded liposomes during lyophilization. J Liposome Res. 2015;25(4):270-278.
17. Sepúlveda CT, Alemán A, Zapata JE, Montero MP, Gómez-Guillén MC. Characterization and storage stability of spray dried soy-rapeseed lecithin/trehalose liposomes loaded with a tilapia viscera hydrolysate. Innov Food Sci Emerg Technol. 2021;71:102708.
18. Crowe LM, Womersley C, Crowe JH, Reid D, Appel L, Rudolph A, et al. Prevention of fusion and leakage in freeze-dried liposomes by carbohydrates. Biochim Biophys Acta. 1986;861:131-140.
19. Khan I, Edes K, Alsaadi I, Al-Khaial MQ, Bnyan R, Khan SA, et al. Investigation of Spray Drying Parameters to Formulate Novel Spray-Dried Proliposome Powder Formulations Followed by Their Aerosolization Performance. Pharmaceutics. 2024;16(12):1541.
20. Khatib I, Chow MYT, Ruan J, Cipolla D, Chan HK. Modeling of a spray drying method to produce ciprofloxacin nanocrystals inside the liposomes utilizing a response surface methodology: Box-Behnken experimental design. Int J Pharm. 2021;597:120277.
21. Omer HK. Spray-dried bioadhesive formulations for pulmonary delivery [PhD thesis]. University of Central Lancashire; 2014.
22. Franzé S, Selmin F, Samaritani E, Minghetti P, Cilurzo F. Lyophilization of Liposomal Formulations: Still Necessary, Still Challenging. Pharmaceutics. 2018;10(3):139.
23. Batavia R, Taylor KM, Craig DQ, Thomas M. The measurement of beclomethasone dipropionate entrapment in liposomes: a comparison of a microscope and an HPLC method. Int J Pharm. 2001;212(1):109-119.
24. Khan I, Yousaf S, Subramanian S, Korale O, Alhnan MA, Ahmed W, et al. Proliposome powders prepared using a slurry method for the generation of beclometasone dipropionate liposomes. Int J Pharm. 2015;496(2):342-350.
25. Balci-Torun F, Ozdemir F. Encapsulation of strawberry flavour and physicochemical characterization of the encapsulated powders. Powder Technol. 2021;380:602-612.
26. Maa YF, Nguyen PA, Sit K, Hsu CC. Spray-drying performance of a bench-top spray dryer for protein aerosol powder preparation. Biotechnol Bioeng. 1998;60(3):301-309.
27. Lee S, Lee J, Choi WY. Characterization and Evaluation of Freeze-dried Liposomes Loaded with Ascorbyl Palmitate Enabling Anti-Aging Therapy of the Skin. Bull Korean Chem Soc. 2007;28(1):99-102.
28. Elhissi AMA, Islam MA, Arafat B, Taylor M, Ahmed W. Development and characterization of freeze-dried liposomes containing two anti-asthma drugs. IET Micro Nano Lett. 2010;5(3):184-188.
29. Andreana I, Bincoletto V, Manzoli M, Rodà F, Giarraputo V, Milla P, et al. Freeze drying of nanoparticles and liposomes. Materials. 2023;16(3):1212.
30. Honary S, Zahir F. Effect of zeta potential on the properties of nano-drug delivery systems - A review (Part 1). Trop J Pharm Res. 2013;12(2):255-264.
31. Karn PR, Cho W, Park HJ, Park JS, Hwang SJ. Characterization and stability studies of a novel liposomal cyclosporin A prepared using the supercritical fluid method: comparison with the modified conventional Bangham method. Int J Nanomedicine. 2013;8:365-377.
32. Chennakesavulu S, Mishra A, Sudheer A, Sowmya C, Reddy CS, Bhargav E, et al. Pulmonary delivery of liposomal dry powder inhaler formulation for effective treatment of idiopathic pulmonary fibrosis. Asian J Pharm Sci. 2018;13(1):91-100.
33. Valková V, Ďúranová H, Falcimaigne-Cordin A, Rossi C, Nadaud F, Nesterenko A, et al. Impact of Freeze- and Spray-Drying Microencapsulation Techniques on β-Glucan Powder Biological

Activity: A Comparative Study. Foods. 2022;11(15):2267.
34. Crowe JH, Crowe LM, Carpenter JF, Aurell Wistrom C. Stabilization of dry phospholipid bilayers and proteins by sugars. Biochem J. 1987;242(1):1-10.
35. Giovannelli L, Milanesi A, Ugazio E, Fracchia L, Segale L. Effect of Methyl–β–Cyclodextrin and Trehalose on the Freeze–Drying and Spray–Drying of Sericin for Cosmetic Purposes. Pharmaceuticals. 2021;14(3):262.
36. Osanlóo DT, Fransson J, Bergenståhl B, Millqvist-Fureby A. Effects of drying methods on physical properties and morphology of trehalose/mannitol mixtures. Dry Technol. 2023;41(4):503-522.
37. Hancock BC, Shamblin SL, Zografi G. Molecular mobility of amorphous pharmaceutical solids below their glass transition temperatures. Pharm Res. 1995;12(6):799-806.
38. Hancock BC, Zografi G. Characteristics and Significance of the Amorphous State in Pharmaceutical Systems. J Pharm Sci. 1997;86(1):1-12.
39. Kafetzis KN, Papalamprou N, McNulty E, Thong KX, Sato Y, Mironov A, et al. The Effect of Cryoprotectants and Storage Conditions on the Transfection Efficiency, Stability, and Safety of Lipid-Based Nanoparticles for mRNA and DNA Delivery. Adv Healthc Mater. 2023;12(18):e2203022.
40. Liu XH, Song HP, Tao LL, Zhai Z, Huang JX, Cheng YX. Trehalose-loaded LNPs enhance mRNA stability and bridge in vitro in vivo efficacy gap. NPJ Vaccines. 2025;10:201.
41. da Silva Júnior ME, Araújo MVRL, Martins ACS, dos Santos Lima M, da Silva FLH, Converti A, et al. Microencapsulation by spray- and freeze-drying. Sci Rep. 2023;13(1):15222.
42. Papoutsis K, Golding JB, Vuong Q, Pristijono P, Stathopoulos CE, Scarlett CJ, et al. Encapsulation of Citrus By-Product Extracts by Spray-Drying and Freeze-Drying Using Combinations of Maltodextrin with Soybean Protein and ι-Carrageenan. Foods. 2018;7(7):115.
43. Chen C, Chi YJ, Xu W. Comparisons on the Functional Properties and Antioxidant Activity of Spray-Dried and Freeze-Dried Egg White Protein Hydrolysate. Food Bioproc Technol. 2012;5(6):2342-2352.
44. Ballesteros LF, Ramirez MJ, Orrego CE, Teixeira JA, Mussatto SI. Encapsulation of antioxidant phenolic compounds extracted from spent coffee grounds by freeze-drying and spray-drying using different coating materials. Food Chem. 2017;237:623-631.
45. Telko MJ, Hickey AJ. Dry powder inhaler formulation. Respir Care. 2005;50(9):1209-1227.
46. Islam N, Cleary MJ. Developing an efficient and reliable dry powder inhaler for pulmonary drug delivery-a review for multidisciplinary researchers. Med Eng Phys. 2012;34(4):409-427.
47. Adi H, Traini D, Chan HK, Young PM. The influence of drug morphology on aerosolisation efficiency of dry powder inhaler formulations. J Pharm Sci. 2008;97(7):2780-2788.
48. Labiris NR, Dolovich MB. Pulmonary drug delivery. Part I: physiological factors affecting therapeutic effectiveness of aerosolized medications. Br J Clin Pharmacol. 2003;56(6):588-599.