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RESEARCH PAPER

Lyophilization of Unit Dose Pharmaceutical Dosage Forms

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ABSTRACT

A lyophilization process for a pharmaceutical unit dosage form was developed which comprised a container closed with an impermeable membrane pierced with one or more holes through which the material in the container can be lyophilized. The hole or holes in the membrane have to be sufficiently large to allow water vapor to escape but small to ensure that the material is kept within the container. Lyophilization from sealed, perforated, unit-dose package has shown to be feasible. The technique offers a novel convenient means of lyophilizing nonsterile products in their primary pack and increases the potential for the development of lyophilized formulations for nonparenteral applications.

Key Words: Freeze-drying; Lyophilization; Lyophilized dosage form; Nasal insert.

INTRODUCTION

Lyophilization is a widely used technique for the stabilization and preservation of heat labile substances and its use in the preservation of microorganism, food stuffs, biological products, and pharmaceuticals is well documented.[1–8] Lyophilization is the term given to the process whereby water is sublimed from frozen solutions, generally under reduced pressure, leaving a dry porous mass of approximately the same size and shape as the original frozen mass. A typical freeze-drying cycle essentially consists of three stages: freezing, primary drying, and secondary drying. In the first stage, the material is cooled until it is completely frozen. This has the effect, at least in part, of separating the water from the solutes. The second stage, which is usually accomplished under vacuum and by supplying heat to the product, involves removal of most of the water by sublimation of the ice in the product. The last stage involves the removal of sorbed water and is normally carried out at elevated product temperature to

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achieve efficient water removal. Since freeze-drying takes place at a lower temperature than spray-drying it is normally considered to be less destructive, especially to protein products.[9] In addition, control of sterility and foreign particulate matter is relatively straightforward in lyophilization, and accurate, clean dosing into final product containers is simple. Because of the porous nature of freeze-dried products, rehydration is fast and complete. Hence, albeit inherently expensive from the viewpoints of plant cost and energy consumption, lyophilization is often the processing method of choice for protein products.

LYOPHILIZED PHARMACEUTICAL FORMULATIONS AND DOSAGE FORMS

The development of a suitable formulation and freeze-drying cycle for a pharmaceutical dosage form requires knowledge of some basic properties such as the eutectic temperature, if one exists, the effect of temperature on drug solubility, the degree of super cooling of the material during freezing, the heat transfer properties of the frozen product, and equipment design and capability. The desired characteristics that can be achieved, by the proper formulation of the solution to be freeze-dried and by employing an optimum freeze-drying cycle, include an intact cake with sufficient strength, uniform color, and rapid reconstitution containing stable active ingredient.

The last decade has seen the emergence of some interesting ideas such as soft ice and supporting matrix technology in the lyophilization of pharmaceuticals.[10] Some solutes may be unstable if in solution for too long at temperatures above zero and may also be poorly soluble and difficult to maintain in stable suspension for the time needed for freezing. In such situations, soft ice technology may be useful in which a homogeneous solution of the main constituents is progressively frozen in a scraped surface heat exchanger and extruded as a soft ice sherbet into a refrigerated vessel where it is mixed with those powdered ingredients which were not compatible with the original solution. To this flow can be added a second sherbet coming from another line or other streams of low-temperature-stable emulsions, oils, and aromatics. The whole mass is then mixed as a plastic, cold paste and finally extruded into appropriate molds, of the conventional drug tablet type, which are then brought to low temperatures for final hardening. The frozen product is then lyophilized and when reconstituted, it gives an instantaneous metastable mix which is sufficiently durable for immediate therapeutic use.

Similarly, the supporting matrix concept is particularly useful in the freeze-drying of highly dilute solutions of very active biologicals such as human hormones and vaccine antigens. Such solutions are difficult to dry due to a lack of structure and during sublimation the frozen mass disintegrates under vacuum and is partially lost in the vapor stream. Although such blow out can be overcome by the use of bulking agents such as mannitol, lactose, sucrose, trehalose, dextran 40, and povidone, these excipients must not interfere with the biological activity of the active compound. An alternative is to adsorb the solute from solution onto a neutral porous material which can be handled as a granular solid and may be readily frozen and dried. Porous materials such as sintered glass, ceramics, aluminum and zirconium oxides, expanded polymers, or porous natural supports (chitin, chitosan, cellulose) can be used for this purpose. The support can be shaped as threads, beads, plates, and granules, and manufactured by machining moulding or extrusion as a dry, inert matrix carrying the active substance. At the time of reconstitution, the support material is washed by the excess suitable solvent, and the active substance released from the support.

Recently, freeze-drying technology has been applied to the manufacture of unit dose, fast dissolving dosage forms,[11,12] such as rapidly disintegrating tablets,[13] and lyophilized dry emulsion (LDE) tablets[14] for the oral delivery of drugs. Freeze-dried tablets may be of benefit to patients who find it difficult to swallow conventional tablets or hard gelatin capsules and therefore tend to be noncompliant. This applies to pediatric and geriatric patients, but also to the bedridden and to some active working, patients who are busy or traveling and have no ready access to water. Such problems can be mitigated by fast dissolving tablets, and the high bioavailability of drugs from freeze-dried tablets may be greater than from conventional tablets.[12,15] Lyophilization has also been used for the manufacture of a pessary in a unit dose applicator.[16] Similarly, lyophilization has been used to produce a lyophilized nasal unit dose delivery system.[17–22]

An attempt has also been made to combine extrusion and freeze-drying technologies to produce particles containing active ingredient.[23] Protein inhalation powders, prepared by spray freeze-drying, have superior aerosol performance as they

are light and porous and have better aerodynamic properties than powders prepared by spray drying only.[24]

Recently, there has been an increasing interest in the possibility of administering pharmaceutically active peptides via the nasal route, e.g., in the form of a nasal ointment or gel, or for greater accuracy of dosing, in the form of a liquid nasal spray. The concept of a solid nasal insert for peptide drug delivery may give improved results, e.g., in terms of patient compliance (e.g., facilitated self-application), achievable accuracy of dosing/bioavailability when compared with a liquid nasal spray.[25]

Active agents such as peptides which are unsuitable for oral delivery because they are degraded in the gut may, however, be absorbed across the buccal and/or nasal mucosae, tissue sites which represent alternative routes of administration. Conventional freeze-drying, however, generally results in a low bulk density solid with poor flow characteristics which is difficult to administer reproducibly to such mucosae. Thus, it would be useful to have a lyophilized formulation in which the active was stable and which could expedite delivery to mucosal surfaces.

In conventional freeze-drying of a pharmaceutical, glass vials are partially filled with a solution of the substance to be lyophilized and placed in the freeze dryer. The partially stoppered vials remain open throughout the drying process to allow escape of water vapor from the frozen solution and on completion of lyophilization cycle, a plug of dry material is left in the vial. However, in certain pharmaceutical applications, for example, freeze-dried tablets for oral administration, it may be desirable to produce the freeze-dried product in a packaged form, ready for administration. Kearney et al.[26] disclosed a manufacturing process in which the solution precursor of the dosage form was filled into and freeze dried in the depressions of a blister film (Zydis technology RP Scherer). Following drying, a plastic sheet is placed over the depressions and the blisters sealed. However, the freeze-dried plugs are usually fragile and tend to disintegrate depending on the solid content of the plug and the freeze-dried residue in an open blister pack is easily disturbed by static or air currents, or contaminated during handling operations prior to film sealing.

This article describes lyophilization of a pharmaceutical unit dosage form which may mitigate some of the problems described above. The process comprises a container closed with an impermeable membrane pierced with one or more holes through which the material in the container can be lyophilized. The hole or holes in the membrane have to be sufficiently large to allow efficient lyophilization but small enough to ensure that the material is retained intact and protected within the container.

MATERIALS

D(-)Mannitol (GPR) was purchased from BDH. Hydroxypropyl methyl cellulose (Methocel) of different viscosity grades was obtained as a gift from Dow Chemical Company, USA. Different pore size membrane filters were purchased from Whatman and the dialysis membrane was purchased from Medicell Ltd.

METHOD

Apparatus

A laboratory Modulyo Freeze Dryer (Edwards) was used to freeze dry the samples. Throughout, unless stated otherwise, all solutions were quench cooled in liquid nitrogen before drying.

Preparation of Methocel Solutions Containing Mannitol

Solutions of Methocel K and mannitol were prepared by dissolving mannitol in one-third of the required volume of distilled water and then sprinkling Methocel powder into this solution with constant agitation by either magnetic follower or high shear stirrer. When the Methocel particles were thoroughly wetted and evenly dispersed, the remaining volume of water was added to make 100 g of solution and stirring continued until free from lumps. These solutions were then left standing at 4°C overnight to ensure complete hydration and to allow entrapped air to separate.

Lyophilization of Mannitol and Polymer Solutions Through Different Pore Size Membranes

One milliliter each of samples I to IV (Table 1) were filled in 10-mL glass vials, frozen, placed in the freeze dryer, and lyophilized for 24 hr at a temperature of −55°C and a chamber pressure of 0.08 mbar. Vial containing samples II to IV were sealed with an

Table 1. The relationship between hole diameter and rate of water loss during freeze drying process for 24 hr at −55°C condenser temperature and 0.08 mbar chamber pressure.

Vial (individual sample)	Hole diameter (microns)	Formulation (% w/w)	Hole area (mm^2)	24 hr weight loss (%)
I	Open container	Mannitol (10)	132.67	90.00
II	Sealed container	Carbopol (1.5), methocel (1.5), and mannitol (5)	0	0
III	300	Mannitol (10)	0.071	71.00
IV	550	Carbopol (1.5), Methocel (1.5), and Mannitol (5)	0.238	90.00

Table 2. The relationship between the rate of water loss and the number of holes in the impermeable membrane seal during freeze drying of a solution containing Methocel F50 Prem LV (1.5% w/w) and carbopol 934P (1.5% w/v) for 24 hr at −55°C condenser temperature and 0.078 mbar pressure.

Vial (individual sample)	Hole numbers[a] (area, mm^2)	24-hr weight loss (%)
I	Open	92.00
II	5 (0.166)	92.50
III	10 (0.332)	93.00
IV	15 (0.498)	93.00
V	20 (0.664)	92.00
VI	25 (0.830)	92.00
VII	Sealed	0.00

[a]0.46-mm diameter holes formed in membrane, total hole area indicated.

impermeable membrane (polythene sheet) with the help of cyanoacrylate adhesive. Holes of 300 and 550 μm were pierced in these membranes for samples III and IV using a needle.

In another series of experiments, 1 mL 10% mannitol solution was filled into the vials and sealed with various pore size filter membranes filters (0.0029, 0.2, 0.45, 0.8, 1.2, and 5 μm). These samples were lyophilized as described above and the percentage weight loss calculated.

To study the relationship between the rate of water loss and number of holes in the impermeable membrane, 2 mL 1.5% Methocel F50 Prem LV and 1.5% Carbopol 934P solutions were placed in each of seven vials I to VII (Table 2). Vial I was left open. Each remaining vial was covered with a polythene membrane approximately 80 μm thick. The polythene membrane was held in place by a rim of rubber plug, which formed a sealing engagement with the vial mouth. Each plug had a hole bored through leaving a portion of the sheet exposed.

Holes were made in the exposed polyethylene membrane in the vials by piercing with a 460-μm needle. Five holes were made in the membrane of vial II, 10 in vial III, 15 in vial IV, and 20 and 25 holes in vials V and VI, respectively. These samples were lyophilized as described above. The percentage weight loss (water) was calculated.

Drying Kinetics of Different Solutions

A quantity of 0.4 mL each of 1.5 and 10% w/w mannitol and 1.5% w/w Methocel K4MP and K100LVP solutions were filled into 30, 0.5-mL capacity, snap seal polypropylene microfuge tubes (Life Sciences International UK Ltd.) of internal diameter 6 mm. These 30 tubes were grouped into three sets of 10 tubes. The mouths of one set were left open, in the second set, the lid was snap sealed, and in the third set, the lid was snap sealed but with one 600-μm hole bored through the lid. After prefreezing in liquid nitrogen the tube contents were lyophilized for 2, 4, 6, 12, and 24 hr. This experiment was carried out in triplicate.

Similarly, different fill volumes (0.1, 0.2, and 0.4 mL) of 1.5% w/w K4MP solution were filled into the microfuge tubes, grouped as described above, and the contents lyophilized for 2, 4, 6, and 12 hr.

RESULTS AND DISCUSSION

The results of these lyophilization studies are shown in Figs. 1–3 and Tables 1–3. Figure 1 depicts the effect of pore size on weight loss (%) relative to an open vial. Weight loss through a dialysis membrane (pore size 0.0029 μm) was negligible. However, it can be seen that there was no significant difference in weight loss (about 70%) through different membranes of pore size 0.2–5 μm during 24 hr lyophilization. The weight loss through these various pore size

Table 3. Compilation of % vapor rate loss and r^2 values during freeze-drying (fill volume = 0.4 mL and $n = 3$).

	Open		1 hole (600 μm)	
Formulation (% w/w)	Slope (% weight loss per hour)	r^2	Slope (% weight loss per hour)	r^2
Mannitol (1.5)	8.1295	0.9631	8.124	0.9915
Mannitol (10)	7.6185	0.9952	7.5295	0.9867
K100LVP (1.5)	8.2193	0.9298	8.3528	0.9292
K4MP (1.5)	7.9721	0.8689	8.2128	0.9692

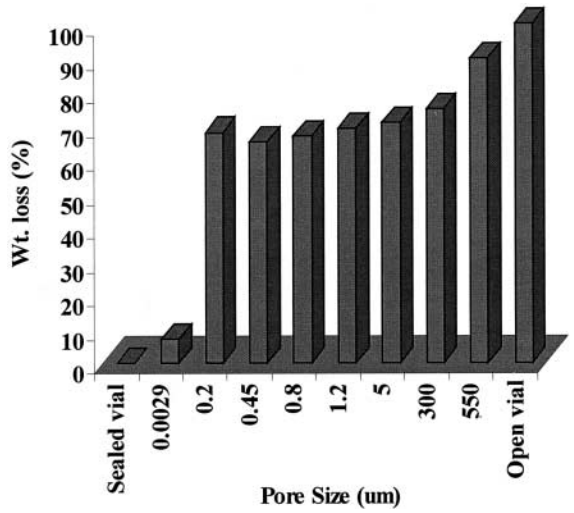

Figure 1. Effect of pore size on weight loss (%) relative to an open vial during lyophilization of 1 mL 10% solution for 24 hr.

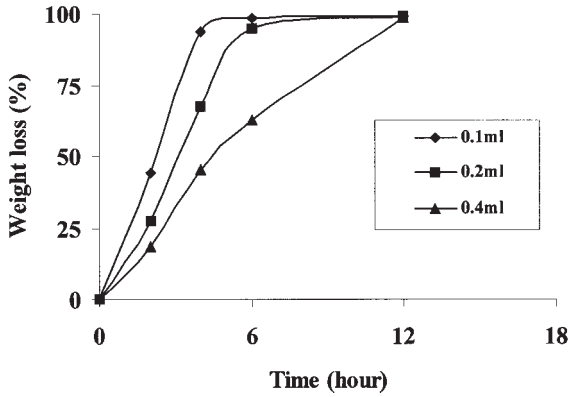

Figure 2. Effect of fill volume on drying rate during lyophilization of 1.5% K4MP solution through a single hole of 600 μm ($n = 3$).

membranes and through an impermeable membrane with a single 300-μm hole suggested that the overall resistance to vapor flow was similar. This was surprising, since considerable effort has been devoted to the design of efficient stoppers for lyophilization vials to optimize pathways for the removal of water vapor.[27–34] However, comparing a 300-μm hole with a 550-μm hole, there was respectively 71 and 90% water loss compared with an open vial after 24 hr indicating that under the conditions used here the threshold for unimpeded water vapor loss was close to the 550-μm diameter hole (Fig. 1 and Table 1).

The relationship between the rate of weight (water) loss during freeze-drying and the number of holes (surface area) present in the impermeable membrane is shown in Table 2. It is evident that the single hole in the seal of vial II allowed drying as efficiently as from an open vial. Further increase in the number of holes (vials III–VI) gave no increase in drying rate.

The drying rate (% water vapor loss per hour) through a single hole (diameter of 600 μm) and open container (6.5-mm aperture) was essentially the same (Table 3). In other words, under the conditions used (vials size and fill volume), the drying kinetics of different formulations as studied through a single hole and open containers (Table 3) was nearly the same. The fill volume, however, had an appreciable effect on drying rate (Fig. 2).

The water loss (sublimation) from product during lyophilization involves heat and mass transfer. Heat transfer takes place by direct conduction from the shelf to the vial via points of direct contact and radiant heat transfer.[35] Heat transfer also occurs by means of gas molecules in the chamber, and this source increases with chamber pressure so that a higher chamber pressure shortens the drying time. Chamber pressure also has a significant effect on the resistance to conductive heat transfer under the vial where there is poor contact between the shelf and the vial. However, the benefits of high chamber pressure must be balanced by the requirement for a vacuum sufficient to permit sublimation.

The sublimation rate is also limited by mass transfer. Pikal suggested that mass transfer (water vapor flow) is impeded by various mass transfer

barriers: the dried layer found above the drying front, the stopper opening, and chamber to condenser pathways.[35,36] The dried product resistance is an important controlling factor of drying at a fixed temperature. The rate of freezing influences ice crystal size in the frozen mass which determines the size of the resulting pores in the dried material and the resistance in the dried layer is lower at large pore size.[37]

In this study it was observed that weight loss (sublimation) was dependent on hole size (area) up to a certain level (Fig. 1 and Table 1). However, weight loss was similar to that from an open vial when pierced hole diameter reached 550 μm (Fig. 1 and Table 3). Under the conditions used here, a hole diameter of 500–600 μm appeared to be the cut-off size for the efficient passage of water vapor. This argument was further supported by the fact that no weight loss difference was observed when freeze-drying was carried out through different numbers of 460-μm diameter holes (Table 2).

In a previous study, sublimation rates (weight loss of ice) from vials through capillaries of different size (2.71, 2.17, or 1.15 mm diameter) were found to be equal to that from open vials.[38] However, Ybema et al.[38] suggested that during the temperature ramp from −40 to 40°C, primary drying temperature, sublimation rate from the vial with the smallest restriction was slowest. This phenomenon might be explained by a slightly higher pressure in a partially closed vial than in an open vial. The narrower the restriction, the higher the vapor pressure in the vial during sublimation and consequently, the sublimation at any particular temperature. As a result, the temperature difference ($\Delta T = T_{shelf} - T_{subl}$), the driving force for heat transfer, is slightly reduced, which results in lower heat flow to the vial. At the beginning of the ramp, ΔT is small because of the low shelf temperature and the vapor pressure effect on sublimation relatively large. However, when the shelf approaches its maximum temperature, the vapor pressure effect is negligible compared to ΔT.[38] This would be the case in our study, since drying was performed at a fixed shelf temperature (20°C).

In the present study, sublimation rate from the frozen solution would depend on the resistance to vapor flow of the dried solute layer as well as that due to the pierced hole area at the container mouth. The dried cake is generally regarded as an assembly of minute capillaries formed by sublimation of ice crystals. If the fill height and solid content are high, the resistance to escape of water vapor is high and melt back or collapse during the lyophilization (Fig. 3) can occur even from an open container. If the total effective area of the pores/hole in the vial seal is greater than that of the cake capillaries, then drying rate is unaffected by the seal.

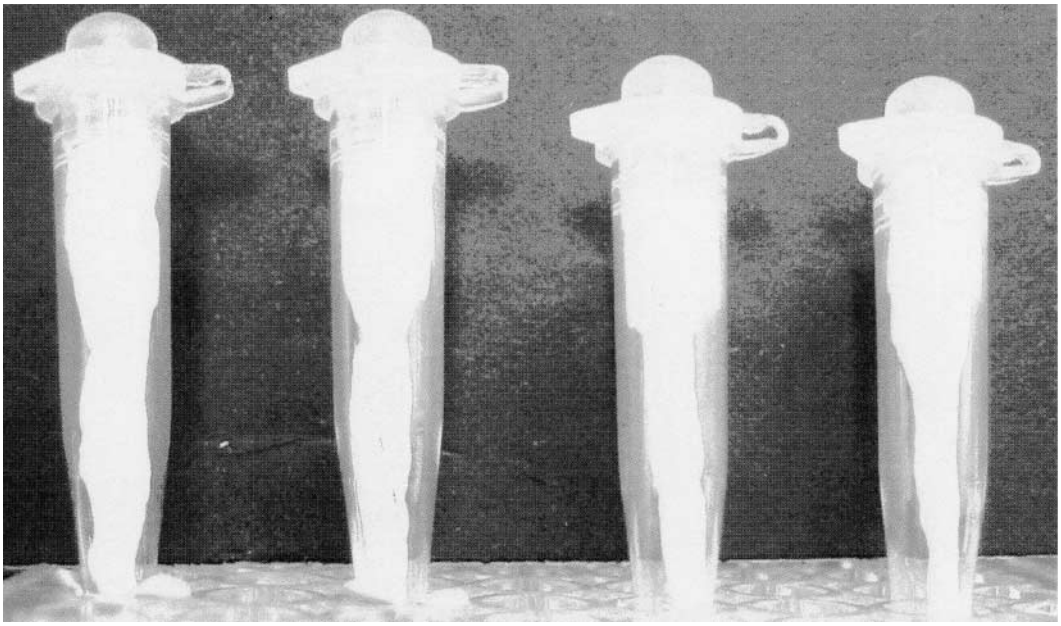

Figure 3. An example of typical meltback occurring at high solids content and elongated cake depth, observed during lyophilization from open microfuge tubes (subsequently resealed).

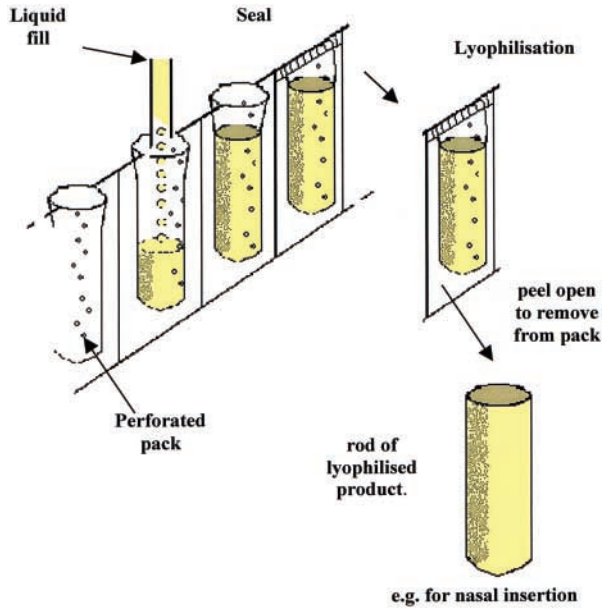

Figure 4. Illustrations of some proposed packs for manufacture of unit dose lyophilized dosage units (based on strip pack).

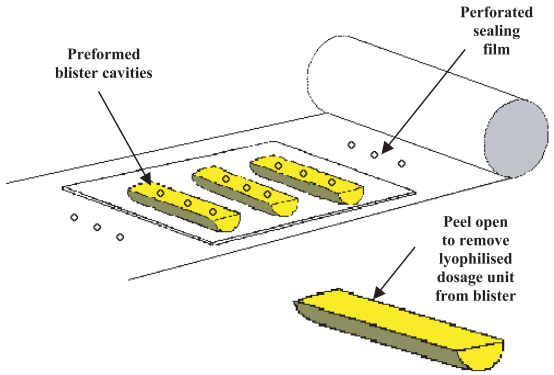

Figure 5. Illustrations of some proposed packs for manufacture of unit dose lyophilized dosage units (based on blister pack).

Under the conditions used here (vial size and fill volume), significant drying rates were achieved. Based on these studies it should be possible to design perforated containers (such as those in Figs. 4 and 5) which could be filled and sealed prior to lyophilization to offer product protection from contamination prior to freezing. Such designs would offer the prospect of a more effective process for the manufacture of unit-dose, nonsterile, lyophilized preparations.

CONCLUSION

Lyophilization from sealed, perforated, unit-dose packages was shown to be feasible. The results indicated that a novel convenient means of lyophilizing nonsterile products in their primary packaging would be possible which would increase the development potential of freeze-dried nonparenteral formulations.

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