

Why, What and How? Understanding the freeze drying process

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The advantages of freeze drying

Freeze drying, also known as 'lyophilisation' is a method of processing a liquid product into a dry solid product.

With freeze-drying, heat-sensitive drugs and biologicals can be dried at low temperatures. The avoidance of high temperatures helps to reduce the extent of decomposition or loss of activity in biological products, and can also circumvent the alteration of taste and sensory qualities in foods. Freeze-dried products have a very high surface area, which enables them to be reconstituted quickly and easily with the re-introduction of the solvent (usually water). This is particularly valuable in the case of emergency vaccines and antibodies, which need to be solubilised and administered as quickly as possible.

Foods (see figure 1) benefit from being freeze dried as their sensory qualities such as colour, size, smell and taste are only minimally affected in comparison to other drying processes.

Freeze drying is also more compatible with the production of particle-low pharmaceuticals, in comparison to dry powder filling. Solutions can be sterile filtered immediately before being transferred to vials and freeze-dried. Freeze drying can increase a product's viable storage time at more economical and practical temperatures. Freeze drying a vaccine, for example, can increase product lifetime from just a few hours/days to several months/years, which has clear advantages; reducing the importance and criticality of forecasting product demand, storage facility requirements, transport logistics and associated costs of these. In short, freeze-drying offers a range of advantages to alternative methods.

The freeze drying process

Freeze drying involves removing the water from a material, a process which involves three stages. First,

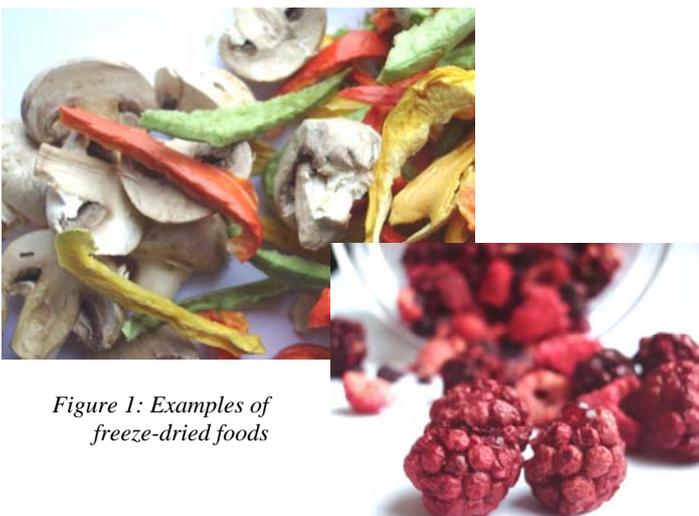


Figure 1: Examples of freeze-dried foods



Figure 2: Freeze drying in vials

the product is frozen to a defined temperature, then the free ice is removed during "primary drying" by sublimation under vacuum. Finally, in "secondary drying", typically at a higher temperature and lower pressure, much of the remaining (unfrozen) water may be desorbed under vacuum.

A typical freeze-dried product will occupy the same volume as the original sample, even if it was initially a liquid. This "cake" as it is usually known may be fragile and should be easy to reconstitute. Many freeze-dried products are therefore hygroscopic and for preservation need to be kept in a closed container. Freeze-drying in the pharmaceutical and biotech industries is commonly carried out in vials (figure 2) but it may be carried out in a number of other formats, for example ampoules, trays (known as bulk drying). In laboratories, material is sometimes freeze dried in flasks, attached to small-scale manifold dryers. Newer innovations in the field include drying in blister packs and syringes.

Critical temperatures for freeze drying

For successful and robust lyophilisation, the behaviour of the frozen product needs to be characterised prior to freeze drying. Parameters such as collapse temperature (T_c), glass transition in the frozen state (T_g'), eutectic temperature (T_{eu}), molecular mobility, knowledge of excipient and active characteristics (e.g. an amorphous or crystalline material) need to be understood for the development of a rational and economical freeze drying cycle. The resulting end product should have an acceptable cake structure (see figure 5), good rehydration time, and retention of active viability with sufficient stability at the required temperature; the exact requirements will vary with the end product and market.

The collapse temperature (T_c) can be defined as the point at which an amorphous material in the frozen state when subjected to a vacuum can no longer maintain its

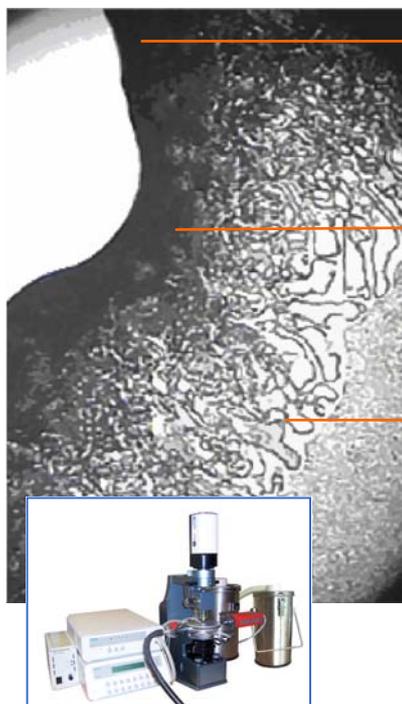
structure as the frozen solvent is removed during drying. A collapsed product often has a poor re-hydration time, low retention of active viability and high residual water level, as well as a shrunken, cosmetically unacceptable appearance (see figure 5).

The glass transition temperature in the frozen state (T_g') is the point at which an amorphous material shows signs of softening indicating a possible loss of structure may occur. T_c and T_g' values can be quite close, though T_g' is generally lower than the equivalent T_c . A 1°C increase in shelf temperature during primary drying can lead to a 13% decrease in drying time; therefore measurement of the T_c can lead to a significant saving of both time and money, whilst still retaining a good end product.

The eutectic temperature (T_{eu}) is the point at or above which a frozen eutectic solid becomes a liquid which would boil as the solvent is removed.

T_c and T_{eu} can be measured by freeze drying microscopy (FDM). Here at Biopharma Technology Ltd (BTL) we use our purpose-designed Lyostat2 instrument. This is essentially a micro-freeze dryer and enables analysis of a formulation with only $2\mu\text{l}$ of material.

The material is placed on the cryo-stage under a microscope, the sample is held between two slides with a spacer in between to control the thickness of material. The sample is freeze dried under controlled conditions and its behaviour observed at different temperatures. Most products are at least partially amorphous and need to be dried below their collapse point; therefore, measurement of this is important for defining the maximum allowable product temperature during primary drying. Figure 3 shows how the collapse of a sample can be observed by FDM. The collapse point for a chosen product will depend on the individual behaviour and relative proportions of active and excipients. A freeze-dried pharmaceutical or biotech product will usually consist of a number of components, including the active ingredient, bulking agents, protective agents, thermal stabilisers, pH adjusters and possibly also buffer salts and surfactants, each of which may contribute to the 'character' of the formulation to a different extent.



Product is drying well at -45°C

Temperature raised to -38.7°C and sample shows initial signs of collapse

Temperature at -19°C and sample has completely collapsed

Figure 3: Screen-shot from a Lyostat2 freeze-drying microscope as a sample is tested, with explanation of results. Inset: Lyostat2 instrument

A complex material can provide many challenges and often additional information on the frozen product is required. This can be provided by analysis of both the thermal events and impedance changes within the frozen product; this can be conducted with the Lyotherm2 instrument using about 6ml of material. Exothermic or endothermic changes can be observed as a solution freezes, melts or softens using differential thermal analysis (DTA). Impedance ($Z\sin\phi$) analysis can detect possible structural changes/molecular mobility which can contribute significant information on sample behaviour (see figure 4).

Once a product has been analysed and critical points determined, an assessment can be made as to whether the formulation is suitable for freeze drying and additionally what processing options are important for efficient and economical freeze drying.

Why shorten your freeze drying cycle?

Generally freeze drying production equipment does not go lower than -70°C and this in itself provides a limitation on how low a temperature a product can be commercially freeze dried. Another important factor is the time, energy and consequently cost. A product with a low freeze drying tem-

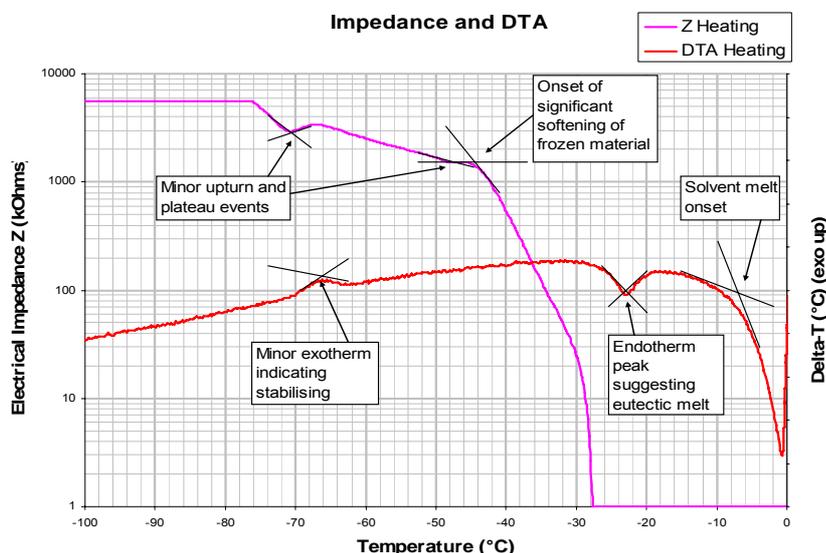


Figure 4: Data returned by BTL's Lyotherm2 instrument, demonstrating changes within the structure of a frozen formulation. Inset: The Lyotherm2 instrument



perature will have a much longer run time, a higher energy cost and lower product output per unit time, therefore reducing a run from 7 days to 48 hours clearly has significant advantages.

Basics of formulation development

Changing the formulation to increase the T_c , could result in significant savings. Table 1 shows that the T_c for sucrose is -31°C , whilst dextran (70KDa) is -11°C , crystalline mannitol (which often requires annealing, which comprises heating and cooling of a frozen solution to encourage solute crystallisation) has T_{eu} of -1.4°C . Therefore if the formulation can be changed and different excipient(s) substituted for other material(s) with a higher critical temperature then an improvement in the product critical temperature can be achieved. It is, however, essential to understand the reason certain excipients are used and the role they play e.g. protective agents, thermal stabilisers, bulking agents, maintenance of a predominantly amorphous or crystalline product, all of which may be critical in maintaining product viability. A good example is mannitol; crystalline mannitol can help to give a product a good cosmetically acceptable cake and improve the T_c , however not all products respond well to the presence of a purely crystalline material, and as with any excipients there can be many complex interactions to consider that will vary with each product.

In summary it is important to understand your product, the excipients involved and how they behave when frozen and dried from the same solution when developing a formulation for freeze drying.

Process changes

A simple way to reduce the drying time is to reduce the fill depth (providing the process allows). During the freeze drying process, the top part of the cake will dry first, the drying front will move down from the top to the bottom of the material, the depth of the dried layer increases the resistance to drying as the water vapour needs to travel through an ever increasing thickness of the dried cake (figure 6). Therefore the higher the fill depth, the longer the drying cycle; this is not necessarily

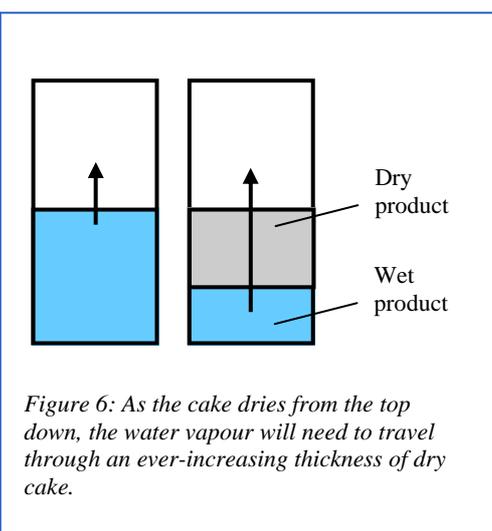


Figure 6: As the cake dries from the top down, the water vapour will need to travel through an ever-increasing thickness of dry cake.



Figure 5: Examples of freeze dried product. Right: successful freeze-drying. Product has dried with good structure. Above: examples of unsuccessful freeze-drying. Above left, product has undergone eutectic melt; above centre, product has suffered a slight collapse during primary drying, resulting in shrinkage; and above right, product has undergone complete collapse. Once a product has been analysed and its critical temperatures determined, such costly problems can be avoided.

proportional (a doubling in the fill depth could increase the drying time by more than three times). Using a vial with a larger diameter should enable the same volume and formulation to be dried in a shorter time; due to the decrease in fill depth, although this factor will need to be weighed against the number of vials per batch, product throughput, packaging and customer requirements.

One of the other factors to consider is the long term storage stability of the product, which is linked to the moisture level. Here the ingress of moisture from the stopper could be significant; especially in low solid dose products; hence the type of stopper chosen and stopper treatment could effect the long term product stability.

Further information

Here at BTL we have over eleven years experience with over 550 different formulations having undergone analysis / formulation development / cycle development in our laboratories. For more information on the above topics please visit our website :

www.lyophilizationtechnology.com

Excipients	Collapse temperature $T^\circ\text{C}$	Eutectic temperature $T^\circ\text{C}$
Sucrose	-31	-
Dextran (70KDa)	-11	-
Trehalose	-28	-
Glucose	-41	-
Lactose	-32	-
NaCl	-	-21
Mannitol (crystalline)	-	-1.4
Calcium Chloride	-	-53
Potassium Chloride	-	-11

Table 1: Critical temperatures of some common excipients. The exact values quoted in literature will vary depending on whether the onset, mid- or end- point has been chosen as the point of reference.