Lyophilisation: Art or Science?

Dr Kevin Ward at Biopharma Technology Ltd outlines some of the principles of lyophilisation and how scientists are now moving to a more rational, analytical approach for greater success and efficiency in the development and production of freeze-dried products

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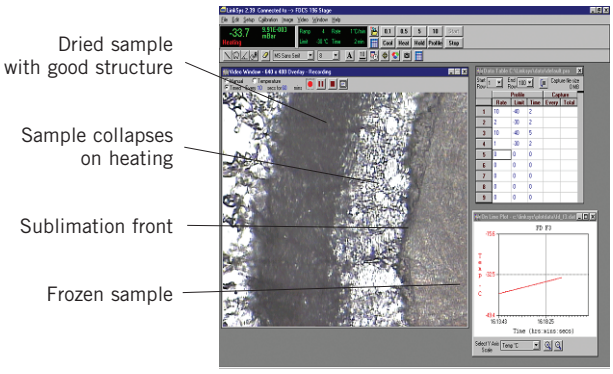
Freeze-drying (lyophilisation) has been employed on an industrial scale for many decades as a means of increasing the shelf-life of a wide range of materials. It is now well established throughout many industries and applied to various products and specimens, including pharmaceuticals, vaccines, diagnostics, biological materials, body tissue, foodstuffs, whole organisms and even whole animals. However, despite its wide and increasing usage, it is apparent that many industries, scientists and process operators still regard the technology as something of an art, and possibly to a greater extent in the pharmaceutical industry than elsewhere. It is easy to see why this myth persists – after all, it is a complex process in terms of the physics involved, while pharmaceutical-grade freeze-dryers are themselves often sophisticated pieces of machinery, built to operate as a freezer, a vacuum vessel and an autoclave (for sterilisation), with complex and secure control systems, safety features and data readouts. Further to this, materials that are freeze-dried are often composed of multiple ingredients, which may give variable and unpredictable freezing and drying behaviour. Therefore, there are several variables to consider as part of the process, many of which are interdependent.

For new products that are developed with a view to lyophilisation (freeze-drying), it is now becoming more widely recognised that the formulation process and the lyophilisation cycle development process may be carried out at the laboratory scale, with scale-up and technology transfer issues taken into account during this early stage. How might this be achieved? One of the most important factors to understand is how each product will respond to the various stages, with arguably the most 'stressful' stages (from the point of view of the product) being freezing and sublimation. This article describes some of the basic features of the lyophilisation process, and how the development of analytical procedures, such as freeze-drying microscopy (FDM), thermal analysis and electrical impedance analysis, are allowing formulation and process development to be approached on an integrated, scientific basis.

HOW DOES FREEZE-DRYING WORK?

The process works by initially freezing a material, then removing the solvent (typically water, as shall be considered here) under vacuum. The main drying step (traditionally referred to as 'primary drying') involves the removal of ice from

Visuals of freeze-drying microscopy



the frozen material, chiefly by sublimation (that is, a direct phase change from ice to vapour without passing through the liquid state). However, not all the water molecules in the starting material may form part of the ice structure, but instead will remain as individual (or clusters of) molecules termed 'unfrozen' water (for example within salt crystals, in the 'hydration shells' of proteins, or otherwise 'associated' within the structure). As such, these molecules will not sublime, but some of them may be removed (intentionally or otherwise) by evaporation and desorption. Additional further drying – traditionally known as 'secondary drying' – involves the removal of unfrozen water, with a view to enhancing the shelf-life of the final product.

MINIMISING PROCESSING DEFECTS IN FREEZE-DRIED PRODUCTS

Different materials freeze in different ways, depending on a number of factors, including the nature of the individual components and their effect on each other, the speed of cooling and the cleanliness of the material (filtered solutions, for example, will often have different ice formation patterns to unfiltered solutions). However, it is the behaviour of the solutes themselves that is particularly important in freeze-drying, since once the ice is removed during the sublimation process, it is these solutes that will become the dried product.

Few materials will crystallise in as predictable a manner as sodium chloride, for example, which adopts what is termed 'eutectic' behaviour (that is, it freezes at the same temperature and the same concentration irrespective of the initial concentration). Therefore, although reducing the temperature to just below 0°C should be sufficient to convert the freezable water to ice in such a solution (and indeed it may look frozen to the naked eye), the spaces within the ice crystal structure will be occupied by a eutectic liquid, which will boil under vacuum. To achieve full solidity, the solution must be cooled to below its eutectic temperature (T_{eu}), so that the eutectic liquid solidifies fully and is maintained in this state throughout the drying process.

Most materials that are currently freeze-dried are non-crystalline, so will solidify to give an amorphous glass containing regions of unfrozen water. This is typically much more difficult to remove than the water in a frozen eutectic system, since it requires not only sublimation, but also higher levels of evaporation and desorption than would be necessary as when compared to those required in an eutectic material. In contrast to eutectic materials, solutions containing solely amorphous components will not contain an eutectic liquid between the ice crystals; instead, they will comprise a glassy structure which changes from being 'flexible' to 'rigid' at their characteristic glass transition temperature (T_g').

Once fully solidified, a vacuum is applied to the material in order to initiate the sublimation process. The subliming vapour is then condensed and 'trapped' as ice on the product condenser (sometimes referred to as the 'vapour trap'), which is held at a lower temperature than the drying material. Control of product temperature during this stage is possible by adopting an appropriate combination of temperature and pressure conditions

within the drying chamber. Heat input is required to provide sufficient energy to the drying material to convert ice to vapour (approximately 2,800 Joules for every one gram of ice), yet it is also important not to provide more energy to the material than it is able to 'lose' through sublimation, otherwise the temperature of the material will rise. This can lead to physical defects in the final product if a crystalline material rises above T_{eu} or an amorphous material rises above its collapse temperature (T_c), which will be at or some way above T_g'. The collapse temperature may be defined as the temperature at which the material softens to the point of undergoing viscous flow; a phenomenon that may be closely linked with unacceptable product appearance, incomplete drying, poor stability and difficulty in reconstitution.

DETERMINING EUTECTIC, GLASS TRANSITION AND COLLAPSE TEMPERATURES

Glass transitions have been traditionally determined by thermal analysis. However, the thermal change in a frozen material when cooled or warmed through T_g' can be subtle and difficult to determine. Therefore, alternative methods such as electrical resistance or impedance analysis may be used in conjunction with thermal methods, in order to provide a more complete picture of frozen state behaviour, including identification of temperatures at which frozen solutes undergo relaxation and mobility changes.

Collapse temperatures can really only be identified using freeze-drying microscopy (FDM), since the collapse event only occurs in a material that is subjected to freeze-drying and therefore can only be determined under freeze-drying conditions. A freeze-drying microscope is essentially a 'micro-freeze-dryer' that allows the intrinsic freeze-drying characteristics of a small volume of material (around 2µl) to be identified.

Used in combination, thermal analysis, electrical impedance (or resistance) analysis and FDM provide a comprehensive means of formulation characterisation in terms of frozen state and freeze-drying behaviour and critical temperatures, from which it is possible to determine whether a material is practically freeze-dryable without further refinement of the formulation, and to begin the design and development of a lyophilisation cycle for a given product. In addition to the information taken from such methods, further factors such as the product container type and dimensions, fill depth (or volume), solute density and level of particulate content should be taken into consideration.

Many real formulations contain a mixture of crystallising and amorphous components, which can make for complex behaviour and heterogeneity in the final product, sometimes with the amorphous and crystalline components forming separate phases within the frozen structure. In terms of minimising visible product defects, arguably the most prudent approach when freeze-drying such materials is to maintain the product temperature below the lowest 'critical temperature' of the identified solute phases (so T_{eu} for a crystalline phase or T_g'/T_c for an amorphous phase).

In practical terms, when developing a lyophilisation cycle that is efficient yet safe for a product, a safety margin is often adopted, whereby the product temperature is maintained at some temperature below that of its critical temperature – typically between 3°C and 7°C below, depending on the perceived intra- and inter-batch variability upon scale-

up. During cycle development, product temperature is often measured by inserting thermocouples or resistance thermometers into samples of product in the freeze-dryer, which provide an indication of what the product itself is experiencing during the process. This information can then be employed to refine processing conditions in the subsequent freeze-drying cycle, or indeed in the present cycle if the control system allows parameters to be changed 'on the fly'.

Once the sublimation process is complete (the endpoint of which may be judged using a number of available techniques), the final part of the process – secondary drying – involves removal of the unfrozen water in order to achieve the target moisture content identified to provide sufficient shelf stability under the expected storage conditions. However, care often needs to be taken with heat-sensitive materials such as biologically active proteins, which may undergo undesirable reactions (for example degradation) or interactions (for example aggregation) if inappropriate conditions are employed even towards the end of the freeze-drying process.

A MORE RELIABLY ACCEPTABLE FINAL PRODUCT

Generally, one looks for the final freeze-dried product to fulfil a number of set criteria, including maintenance of activity, cosmetic acceptability, good reconstitution characteristics, appropriate moisture content and good shelf stability. Due to the complex interrelationship between the numerous product and process variables, some developmental work may be required to achieve these criteria. However, the advent of new technologies, particularly FDM, thermal analysis and more recently electrical impedance (Zsinφ) analysis, together with more sophisticated and flexible control systems on research scale freeze-dryers, is enabling such work to be approached on a more rational basis. This is achieved by allowing a greater understanding of how products respond to freezing and drying, enabling development scientists to rapidly refine conditions accordingly, without the need for months of trial and error experimentation. ◆

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