Understanding the Physical Properties of Freeze-Dried Materials

A review of how, by understanding the scientific principles and physical properties involved in lyophilisation, it is possible to design an optimal, cost-effective process that results in a high quality product with long-term stability.

Over the years, scientists have strived to understand and apply scientific principles to the formulation and process development of freeze-dried products. As opposed to using a 'trial and error' approach, modern formulation and process development scientists have the information and tools necessary to design an optimal formulation and lyophilisation cycle using a scientifically based empirical approach. By understanding the scientific principles and physical properties of the formulation and the lyophilisation process, the development scientist can design a product that is produced in the most cost-effective manner and in the least amount of time, and that is of the highest quality and has adequate long-term stability. Additionally, understanding the scientific principles and physical characteristics in this way better allows the development scientist to troubleshoot and correct problematic formulations and/or lyophilisation cycles.

PHYSICAL PROPERTIES OF MATERIALS

The absolute first step in designing a formulation for a lyophilised product and an optimised cycle for freeze-drying it is to get a good understanding of the physical properties of the materials involved, and an understanding of how these will affect the thermal characteristics of the overall formulated product.

Key terms that are routinely used in lyophilisation science are 'eutectic mixture' and 'glassy phase'. Their definitions are as follows:

- **Eutectic mixture**: A solid mixture of two or more crystalline materials that are in such close, intimate contact that they melt as one pure substance.

- **Glassy phase**: A rigid solid in which the molecules are in complete disarray, with no order to their arrangement whatsoever. This is also known as an amorphous phase.

The location of these different phases in the freeze-dried matrix is shown in Figure 1.

![Figure 1: The different phases in the freeze-dried matrix](image)

The top of Figure 1 represents the initial solution or slurry – that is, the initially formulated product at some temperature above freezing. As the temperature of the product is decreased, represented by the down arrow, one of two different things can happen. At a certain low temperature, either water will nucleate to form pure ice crystals, or the solubility of one of the additives will be exceeded, and it will crystallise or precipitate out of solution. In most cases, except for highly concentrated formulations, it is the ice crystals that form initially during cool down. After pure ice nucleation, all of the other additives in the formulation are concentrated into the interstitial space, or the areas surrounding the ice crystals. As the temperature of the product is reduced further, by proceeding down through Figure 1, the ice crystals continue to grow by pulling water from the interstitial space. As water is removed to grow the ice crystals, the concentration of the other additives remaining in the interstitial space will become super-concentrated. Towards the bottom of Figure 1, the temperature and concentration of the interstitial space reach...
a point where it can no longer remain a solution, and one of
four different things can happen, as shown in the figure.
The first event that can occur, if the solute is crystalline, is
that a eutectic will form. This eutectic comprises the
crystalline solute itself and crystalline ice (water from the
interstitial space). Since the solute crystals and ice crystals
have formed a eutectic, this new crystalline solid will have
one melting point and melt like a pure substance, even
though it comprises two different materials.

If the solute in the interstitial space is an amorphous
material and will not form crystals, the material will
solidify and form a glass. As is characteristic of this glassy
phase, the material has formed a solid; however, there is
no order to the system and the molecules are randomly
oriented. As shown at the bottom of Figure 1, there are
two types of glass that can be formed during freezing: a
stable glass and a metastable glass. A stable glass is a
glassy phase that will always remain amorphous
regardless of how it is treated or formulated. A
metastable glass is a glassy phase that can be made to
crystallise into a eutectic under the right conditions. It is
extremely important to ensure that formulation/process
development scientists do not try to freeze-dry a system
that contains a metastable glass. The term ‘metastable’
technically means unstable, and can make products
difficult to freeze-dry and/or cause substantial stability
problems in the long run. Products that are known to
form metastable glasses during the freezing phase of
freeze-drying will need to undergo a process known as
annealing; this will be described in more detail below.

Although it is rare, occasionally a lyotropic liquid
crystalline phase may form in the interstitial space as a
result of freeze concentration of surface-active
components. Little work has been done on studying
these systems with regard to freeze-drying behaviour –
and so only crystalline and amorphous systems will be
discussed in depth here.

CRITICAL TEMPERATURES IN FREEZE-DRYING

As discussed above, the interstitial space between the ice
crystals in a frozen matrix contains all of the active
ingredients and excipients in the formulation that have
been forced together after the nucleation and subsequent
growth of pure ice crystals. The different phases that
form upon additional cooling (eutectics, stable glasses,
metastable glasses, and so on) all have characteristic
temperatures associated with them where they will go

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from a rigid solid to a flowing mass. It is the critical temperatures associated with these different phases that represent, in most cases, the maximum allowable product temperature during primary drying. The key terms used to describe these temperatures are as follows:

- Eutectic melting temperature ($T_e$): the temperature at which the solid, crystalline, binary eutectic melts
- Glass transition temperature of the maximally frozen concentrate ($T_g'$): the temperature at which the rigid, amorphous glass goes through a change in viscosity to form a flowing mass. There is a fixed water content of this mass that affects this specific transition temperature. In the case of amorphous phases, water acts as a plasticising agent
- Collapse temperature ($T_c$): the temperature at which an amorphous phase or a partially amorphous phase physically loses its structure during drying

In the freeze-drying process, primary drying starts when a vacuum is applied to the closed chamber and pure, frozen ice begins to sublime (that is, be converted directly from a solid to a gas). It is the pure, frozen ice in the ice channels surrounding the interstitial space that is being removed during primary drying, and during the removal of these ice channels the critical temperatures described above become extremely important. In the frozen state, the solid ice in the ice channels helps to provide physical support to the system. Essentially, ice is acting as the scaffolding on which the other components are suspended. When ice is removed through sublimation during primary drying, the remaining components in the interstitial space must have enough strength to support their own weight since the ice scaffolding is being removed. If the remaining solids cannot support their own weight, they will collapse in upon themselves, resulting in a non-porous mass at the bottom of the container. The critical temperatures ($T_e$, $T_g'$) represent the temperatures at which the different phases go from being a rigid solid capable of supporting its own weight, to a flowing mass, which will collapse when ice is removed during primary drying. The product temperature must thus remain below the $T_g'$, $T_e$ or $T_c$ during primary drying in order for the interstitial space to remain a solid phase capable of supporting its own weight after the ice is removed.

While the development scientist will want to keep the product temperature below the critical temperature during primary drying, they do not want to automatically keep the temperature at an extremely low value that is well below the critical value. The reason for this is that the vapour pressure of ice – and hence the sublimation rate – is extremely temperature-dependent (see Figure 2).

As observed in Figure 2, as the temperature of the ice is increased, the vapour pressure of the ice is increased, and this translates into a faster sublimation rate. It should be noted, however, that ice temperature and
vapour pressure do not have a direct relationship. In fact, the vapour pressure of the ice is a logarithmic function of ice temperature. This means that if freeze-drying is being conducted in conditions where the product temperature is kept well below its critical value, the development scientist can be assured that the product won’t lose structure; however, the primary drying will need to continue for an extremely long time to allow for all of the frozen ice to sublime. This example highlights the second reason why it is imperative for the development scientist to characterise the formulation prior to beginning pilot studies in the freeze-dryer. In addition to maintaining the porous, rigid solid when all of the ice is gone, as described above, characterising the thermal properties of the formulation allows the development scientist to design an optimised cycle that safely dries the product in the least time – which can significantly reduce manufacturing costs.

In light of this, the development scientist needs to keep in mind that all excipients are not created equal, and that some excipients that are commonly added to solution products can impart very poor thermal properties to a freeze-dried product, making it very difficult to freeze-dry and/or stabilise. Table 1 shows a list of some excipients that have been used in previous injectable products and their associated critical temperatures.

As observed in Table 1, some excipients can significantly depress the thermal properties of a formulation that is to be freeze-dried. For example, adding certain salts can lower the Te of the formulation to the point that it cannot be freeze-dried using conventional equipment. This is where it becomes important for the development scientist to think about what they are adding to their formulation from a standpoint of not only how it will affect the stability of the product, but also how it will affect the thermal properties. Many excipients having ‘good’ thermal properties can be substituted for those demonstrating ‘poor’ thermal properties (for example, substituting Dextran for sucrose as a bulking agent).

### Table 1: Excipients used in injectable products and their associated critical temperatures

<table>
<thead>
<tr>
<th>Excipient</th>
<th>Eutectic melting temperature (Te), °C</th>
<th>Excipient</th>
<th>Glass transition temperature (Tg'), °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dibasic sodium phosphate</td>
<td>-0.5</td>
<td>Dextran</td>
<td>-9.0</td>
</tr>
<tr>
<td>Mannitol</td>
<td>-1.0</td>
<td>Gelatin</td>
<td>-10.0</td>
</tr>
<tr>
<td>Citric acid</td>
<td>-12.2</td>
<td>Ficoll</td>
<td>-19.5</td>
</tr>
<tr>
<td>Sodium acetate</td>
<td>-18.0</td>
<td>PVP</td>
<td>-23.0</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>-21.5</td>
<td>Trehalose</td>
<td>-29.5</td>
</tr>
<tr>
<td>Sodium iodide</td>
<td>-31.5</td>
<td>Lactose</td>
<td>-32.0</td>
</tr>
<tr>
<td>Magnesium chloride</td>
<td>-33.6</td>
<td>Sucrose</td>
<td>-32.0</td>
</tr>
<tr>
<td>Calcium chloride</td>
<td>-51.0</td>
<td>Glucose</td>
<td>-43.0</td>
</tr>
<tr>
<td>-</td>
<td></td>
<td>Fructose</td>
<td>-46.0</td>
</tr>
</tbody>
</table>

As mentioned above, sometimes the interstitial space can contain a metastable glass – that is, an amorphous phase that formed during freezing. The molecules comprising the metastable glass should have crystallised and formed a eutectic during freezing; however, for several reasons, they did not. The process of annealing allows the development scientist to ensure that the metastable glass has been converted to a crystalline eutectic prior to starting primary drying. As stated above, metastable glasses can be difficult to freeze-dry as they may suppress the thermal properties of the product; in addition, they may cause problems with the long term stability of the product, which can be affected if and when the metastable glass crystallises over time.

If the development scientist determines that a metastable glass has formed during freezing, then they will need to force the metastable glass to crystallise into a eutectic. This is done by warming the sample temperature above the Tg’, and then holding it for a certain amount of time; this will be determined through thermal analysis studies and pilot studies, which are beyond the scope of this paper. By raising the temperature above the Tg’, the metastable glass will become fluidised and begin to flow. By holding the product under these conditions, the molecules now have time to orient themselves into a crystalline lattice, which forms a binary eutectic with ice. After

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annealing is complete, the product is re-cooled to its freezing temperature, the vacuum is initiated and primary drying begins. Now, instead of dealing with the Tg of the metastable glass, the thermal properties of the formulation are based on a Te, which generally occurs at a higher temperature than the critical temperature of a metastable glass. The product temperature can now be taken to a higher temperature without risk of losing the physical structure, and can be dried much faster since the sublimation rate will be higher for ice at warmer temperatures. It should be noted here that glycine and mannitol – which are typical bulking agents – do have the potential to form metastable glasses during freezing, especially when combined with excipients that act as stable glasses.

DESIGNING A SECONDARY DRYING PROTOCOL

The approach to designing a secondary drying protocol is also based on the physical properties of the formulation. In the case of crystalline eutectics, there is no room for unfrozen water to become entrapped within the lattice of a crystalline solid. Therefore, completely crystalline systems are composed mostly of frozen ice that is either located in the ice channels or is part of the binary eutectic in the interstitial space that has formed with the other crystalline species. At the end of primary drying, the completely crystalline product is greater than 99 per cent dry as the ice is easily sublimed and carried to the condenser. These types of system can be dried very aggressively in secondary drying. In the case of amorphous phases, anywhere from 10 to 40 per cent of the total water in the product can become embedded inside the glassy amorphous phase itself. In order to get the unfrozen water out of these types of system, the water must first diffuse out of the glass prior to vapourising and going to the condenser. These types of system therefore require care when designing the secondary drying protocol, as collapse can occur if the drying rate is too aggressive.

Reference

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