Critical Factors in the Design and Optimisation of Lyophilisation Processes

The large number of variables at play in the lyophilisation process necessitates that it will remain as much an art as a science; however, as the science becomes better understood, the art can be analysed, categorised and repeated with greater accuracy.

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Development of a freeze-dried drug is a typically lengthy and multi-faceted process, starting in the development laboratory and culminating in the production facility that supplies the commercial product to the marketplace. Lyophilisation cycles that are ‘good enough’ for early clinical batches are often never optimised for commercial production. Although existing processes are adequately validated to fulfil regulatory requirements, normal variations that are likely to occur are not planned for or built into acceptable conditions for production of the drug. Because the processes were not fully characterised or understood from the beginning, a design space that allows for deviations within the validated parameters does not exist. The FDA states that:

Many manufacturers file (in applications) their normal lyophilisation cycles and validate the lyophilisation process based on these cycles. Unfortunately, such data would be of little value to substantiate shorter or longer cycles. (1)

The same holds true for countless parameters, from formulation to storage temperature of the final product. With planning and forethought, steps can be taken in early development that will ultimately streamline the process and produce a more robust regulatory submission.

Ideally, freeze-dryers (lyophilisers) used during development will be built on similar principles and relatively scaled in size to the larger dryers used for GMP production batches. Comparable parameters to evaluate include location, size, and ice capturing capabilities of the condenser, and ratio of shelf surface area to size of the opening leading to the condenser. Even if similarity of design is not evident, characterising each dryer used will facilitate optimal scale-up.

MAXIMUM SUPPORTABLE SUBLIMATION RATE

A fundamentally important measurement is the maximum supportable sublimation rate for a dryer, with upper boundaries influenced by the heating/cooling power of the shelves and gas flow in the dryer. A basic measurement can be made using ice slab testing, as described by Searles (2).

By (a) loading a dryer with a known mass of water, (b) finding the maximum shelf temperature that can maintain a selected chamber pressure, and (c) measuring the weight of water sublimed during a measured time span at these conditions, the average maximum supportable.
sublimation rate for a particular dryer can be determined. To further characterise the dryer, the temperature of the ice at set shelf temperatures can be measured, while slowly increasing the chamber pressure. After plotting chamber pressure versus the associated previously determined sublimination rate, the corresponding ice temperatures can be added. The line along which a specific product temperature lies can then be selected, and this – along with previously determined dryer limitations – creates the boundaries of a safe zone within which sublimation can occur (see Figure 1). Operating at more aggressive conditions can lead to choked flow, where the velocity of vapour flow in the chamber exceeds the maximum possible flow rate in the tube connecting the chamber to the condenser. This causes the chamber pressure to rise, in turn increasing the product temperature and causing cake collapse.

Alternatively, dynamic measurement of water vapour concentration and velocity can be determined through the use of a relatively new technique, tunable diode laser absorption spectroscopy (TDLAS). A TDLAS unit can be installed externally between the product chamber and condenser, with the laser light focused through an installed site glass (see Figure 2), thus eliminating worries about product contact and any changes to sublimation rate that could be caused by adding complexity to the flow path.

Cycles may be controlled by other variables, and it is important to know how and where these variables are measured. For example, if control for shelf temperature is determined by shelf fluid readings, a sensor placed in the flow path immediately after the heat exchanger will read differently than further downstream. Dryer design and materials may also influence the translation of temperature from shelf fluid to shelf surface. Shelf mapping with thermocouples affixed to the shelf surface will characterise this relationship for each dryer. Chamber pressure control may be determined by readings from a capacitance manometer, which measures absolute pressure, or by a gauge based on gas thermal conductivity that measures relative vacuum (for example, Pirani). During active sublimation, the perceived pressure measured by each of the gauges would differ, thus leading to different control conditions for the cycle.

FORMULATIONS

In addition to the active ingredient, most lyophilised formulations require the addition of one or more excipients to act as bulking agents to create an acceptable cake, or stabilisers to protect the drug substance during freezing, drying, and/or storage in the dry state. It is important to choose commonly known excipients that generate the highest possible collapse temperature during lyophilisaton, and create rugged cakes with rapid reconstitution times and the appropriate finished product of crystalline or amorphous structure. The final formulation should generally contain 5 to 30 per cent solids, with a target of 10 to 15 per cent. When screening multiple excipients for a formulation, statistical modelling (experiment design and data evaluation) can be a time saver. For six or fewer variables, a simple factorial design can be used (that is, four trials to test four variables). For more than six variables, multi-factorial style designs are more appropriate (for example, Plackett-Burman (3), MANOVA (4)).

Thermal characterisation of formulations can determine the maximum allowable product temperature during primary and secondary drying. The liquid formulation can be assayed by differential scanning calorimetry (DSC), or other thermal or electrical analytical techniques, to find the transition temperature in the frozen state (Tg') (see Note 1) and also characterised by freeze dry microscopy to define the actual collapse temperature (Tc). For secondary drying temperatures, drug substance stability data as a function of temperature is helpful, and DSC can be used...
to find the transition temperature of a formulation in the
dried form ($T_g$). Length of secondary drying can be
determined by analytically evaluating the dried form at
different humidity levels on accelerated stability testing
to determine the optimal final water content. In order to
evaluate finished product formulations, analysis over
time on accelerated stability should be performed to
monitor hydrolytic stability as well as potential
different polymorphic changes that might influence increasing

crystallisation or changes in the $T_g$. Tools to be used
include DSC, near infrared (NIR) spectroscopy, Fourier
transform infrared (FTIR) spectroscopy and X-ray
powder diffraction (XRPD).

Dryer capacity and surface area of each shelf should be used
to determine the appropriate vial size for the dosage form.
In general, fill volume should not exceed one-third the
overflow capacity of the vial to allow for expansion during
freezing and to hinder possible loss through vacuum
expulsion. Tubing vials are usually preferred over moulded
because the flatter bottom maximises contact with the shelf
surface, thus optimising conductive heat transfer. Rubber
closures should be evaluated in tandem with the desired
vial to ensure seal integrity and to ensure stoppers do not
stick to the dryer shelf once they are depressed and the
shelves are lifted. Stoppers should be chosen that
demonstrate low water transmission during storage (<1%)
and no adsorption of potential oil vapour from the dryer.

**CYCLE DEVELOPMENT**

Factors to consider during cycle development include the
rate of initial cooling and freezing, the potential benefits
of annealing (see Note 2), the combination of shelf
temperature and chamber pressure that will yield the
desired product temperature, the maximum sublimation
rate the dryer will support for the formulation, the
desired final water (residual moisture) content, and vial
headspace pressure in the final product. For each variable,
in addition to establishing specifications, surrounding
ranges should be established to create a design space that
will encompass safe conditions for product processing
(that is, maintaining product quality specifications).

Shelf temperature/chamber pressure conditions that will produce the desired product temperature during primary
drying can be determined in the development dryer. To
begin, a chamber pressure that is 10 to 30 per cent of the
vapour pressure of ice at the maximum allowable
temperature for the formulation is chosen, in combination
with a shelf temperature 10°C warmer than the
formulation collapse temperature. Adjustments can then
be made to shelf temperature and/or chamber pressure to
pinpoint the exact product temperature desired.

If sublimation rate characterisation for a dryer, as discussed
previously, has been performed, the information can be
used to define boundaries that can be tested for safe
sublimation conditions for a specific product.

Alternatively, the safe zone can be determined
mathematically and with computer modelling based on
product resistance, which can be calculated from
sublimation rates and product temperature profiles (5,6).

In addition to establishing a range of drying conditions
that will yield acceptable product, samples processed with
variations in formulation, annealing, cooling rate, final
water content and so on can be placed on accelerated
stability and tested to establish alert and action limits
around the specifications.

**SCALE-UP FROM LAB TO GMP**

Beginning with the initial design of small laboratory-scale
batches, it is best to mimic the process a product will
experience in the GMP environment to the fullest extent
possible. There are a multitude of variables that should be
addressed when scaling up to GMP production, in order
to determine how efficiently a process will translate from
the lab, as well as potential product impact caused by
increasing batch size.

Before GMP production begins, a scaled version of all
planned production activities should be performed in the
laboratory to test for product contact material
compatibility, hold times, light sensitivity, product
stability in solution, product impact during/after
filtration, in-process testing and lyophilisation cycle times.
Special attention should be directed to surface-to-volume
ratios when testing materials, and to timing delays that
may be expected during in-process testing.

In the GMP dryer, longer time frames and the impact
of a larger thermal load (due to larger quantities of glass
containers and rubber closures) necessitates the

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**Figure 2:** TDLAS water vapour mass flow
monitor that optically measures water vapour
concentration and velocity to determine
the water vapour removal rate from the
product. Results are integrated to predict
the total amount of water removed

Source: Kessler W, Physical Sciences Inc
running of at least one demonstration batch. The batch does not need to be full-sized, but the dryer should be at least 10 per cent full (at least one full shelf). If API is limited, it is possible to seed product vials in a matrix of well-characterised placebo vials. The placebo must demonstrate similar thermal characteristics, and create similar product resistance as it dries.

Analytical results obtained from extensive sampling from the lab and demonstration batches will provide assurance that both the process and formulation are robust.

**FINAL VALIDATION OF COMMERCIAL BATCHES**

The regulatory submission for a drug product can be as restrictive as allowing for only one size batch in a single lyophiliser, or it can contain data to support multiple volumes of formulation, multiple sized dryer loads, and multiple allowable dryers to be used. Not every combination requires testing if a well-planned matrix approach is designed. Figure 3 outlines four such possibilities, progressing left to right from most to least restrictive. If such an approach is attempted, a protocol must be in place before testing begins, and must contain the following:

- Pre-determined acceptance criteria for the process and product
- Batch load range
- Data to support dryer equivalency
- Endpoints for primary and secondary drying, and how they are determined
- Sampling plan design
- Analytical testing to be performed (content uniformity, potency, particulates, reconstitution time, moisture content, and so on)

In addition to specified parameters, it is recommended to also incorporate planned interventions in the studies to explore worst-case scenarios that might occasionally be encountered, such as short-term power outages, extended hold times after cycle completion, and so on.

**CONCLUSION**

The incredible number of variables at play in the lyophilisation process means that freeze drying will remain as much an art as a science. However, as the science becomes better understood, the art can be analysed, categorised and repeated with greater accuracy. It is worth the extra initial effort so that surprises at the end will be kept to a minimum and so that each batch will be released with greater confidence that it contains quality product.

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**Notes**

1. Glass transition temperature is the temperature below which there is a large increase in the viscosity of an amorphous solute where the mobility of the formulation becomes rigid (‘glassy’). Above the glass transition temperature, the formulation becomes ‘rubbery’ as viscosity decreases. It is desirable to maintain product temperature during primary drying below the glass transition temperature.

2. Annealing can reduce the time a formulation remains in primary drying by creating larger ice channels throughout the cake structure. The larger channels allow the solvent to sublime at a faster rate through the cake structure.

**References**

1. FDA Guide to Inspections of Lyophilisation of Parenterals, July 1993