A focus on PAT in freeze-drying

Overview

- Freeze-drying – Process steps and questions?
- QbD in Freeze-Drying
- Freezing Stage
  - Critical process parameters: solidification end point, eutectic formation, glass formation
  - PAT in the freezing Stage (development & production)
- Primary Drying Stage
  - Critical process parameters: collapse temperature, drying rate, drying end point
  - PAT in the 1ry drying Stage (development & production)
- LyoDEA – A new PAT

Problems with lyophilizing biologicals

- Biologicals are labile, complex, often multi-domain macromolecules
- Freeze drying can strip stabilising water from proteins
- requiring careful excipient choice to maintain activity
- Freeze-concentration may induce unfolding and aggregation
- Specific electrolyte balance required to control the weak forces
- Final product storage stability may be problematic (cold chain)
- Instability on reconstitution
- May require chilling during dispensing to minimise degradation
- May suffer from aggregation and denaturation
- May lose active material by non specific adsorption to glass/metal/plastics

So process optimisation is a key issue for manufacturers whose processes include freeze drying.

QbD in Freeze Drying

- Identify Critical Quality Attributes for the freeze dried product
  - Stability, Reconstitution time, cake structure, mechanical strength
- Determine critical thermal properties of the product
  - Eutectic temperatures, glass transition (Tg) and collapse temperature (Tc)
- Develop & implement in-line PAT tools to monitor the process
  - Freezing onset, freezing rate, amount of ice formation, solidification end point
  - Primary drying rate and end point
- Use DoE the to establish the Design Space for safe operation
  - Assess the impact of product, formulation, container and dryer
  - Develop models for each stage of the process to assess the impact of changes
  - Risk assess the impact of excursion outside of this space (product & Equipment failure)

Process Analytical Technologies

- A system for designing, analyzing, and controlling manufacturing through timely measurements (i.e. during processing) of critical quality and performance attributes of raw and in-process materials and processes with the goal of ensuring final product quality

In the PAT framework (FDA September 2004) these tools can be categorized according to the following:

- Multivariate tools for design, data acquisition and analysis
- Process analyzers
- Process control tools
- Continuous improvement and knowledge management tools


PAT in production : The expectations are high

| Global load monitoring | As freeze drying is dependent upon heat and mass thermal transfer, some sublimation may lead to control. It may be necessary to rely on individual lot measurement to control the whole load. |
| Loading/unloading | Compatibility with automatic loading/unloading devices. The placement and removal of vials must be unimpaired. |
| CIP & stoppering | Compatibility with cleaning in place (CIP)/stoppering devices. No leads should compromise the movement of shelves, CIP ramps, or nozzles. |
| Aseptic handling compliance | Compliance with aseptic handling. There should be no source of contamination within the materials or during positioning. |
| Steam sterilization | Placement of the device should not induce freeze-dryer leakage. It should also support a level of inoculation viscosity, and measurement should be independent of equipment build size. |
| Integration | Simple integration into an industrial environment. The device should be installed and continue functioning during freeze-drying, and the data acquisition signal should be compatible with 21CFR/Scada/recorders. |
Comparison of PATs for Production

<table>
<thead>
<tr>
<th>Device Factor</th>
<th>NIBSC</th>
<th>Uni Air</th>
<th>Lab</th>
<th>Freeze Dryer</th>
<th>Super Critical Fluid Extraction</th>
<th>Solvent Recycling</th>
<th>Lyophilisation Chamber</th>
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Drying rate & End Point

Freeze drying cycle design

- Process design achieved by multiple cycles to establish high&low operational limits
- Repeatability established by consistency of batches and process trend monitoring

FREEZING STAGE

The Desired state?
(Freezing onset, freezing rate, amount of ice formation, solidification end point)

End of freezing stage: Product temperature stabilises

Freezing is a critical step

- Super-cooling & nucleation, induction
  - Ice nucleation is a random process can impact homogeneity of product
  - Slower freezing gives rise to bigger ice crystals and permits faster sublimation. JA Searles et al / Pharm Sci 90; 860-71 (2001)
  - Rapid freezing may be needed for labile products (Åkerblom et al Infusions Therapie 1992; 19:283-287)
  - Annealing (raising the temperature during freezer stage) may improve ice crystal growth. JA Searles et al / Pharm Sci 90; 872-87 (2001)

PAT: What do you need to measure and at what scale

Product scales
1: Microscopic: Molecular dynamics in the unfrozen phase (relevance to collapse temperature)
2: Mesoscopic: Ice crystals and connectivity (relevance to drying rates)
3: Macroscopic: Ice formation from the base (impact on scale 2). Temperature differences across the ice layer, changing ratio of ice layer and dry layer during drying.
4: Macroscopic II: design of vial (size, wall thickness, base characteristics), Impact of vial dimensions in relation to fill height, clustering of vials

Engineering scale
1: size of shelf, separation of shelves, edge effects
2: loading of drier, condenser capacity, coolant capacity, dimensions of ducting between the chamber and condenser (choke flow) etc.
Impact of formulation on critical temperatures

When freezing: Ice formation followed by crystallization of excipients/drug and/or formation of the amorphous state

- Characteristics of excipient may define whether it is a crystallising excipient (mannitol) or a glass forming excipient (sucrose)
- Freeze to well below the critical temperatures (eutectic) and hold to ensure complete solidification (But for how long?)
- Formulation changes (e.g. mixtures) may result in marked changes in critical temperature (But are off-line measurements representative?)
- Crystallisable excipients may require annealing (But at what temperature and for how long?)

PAT for laboratory studies: Critical temperatures Tm, Te, Tg

Lyotherm2 — integrated electrical Impedance ($Z\sin\phi$) and DTA designed to measure glass transition ($T_g$), eutectic ($T_{eu}$) and melting ($T_m$) temperatures relevant to freeze-drying formulations

PAT in freezing stage limited to Temperature Measurement

- Thermocouple
  - positioned bottom-centre in the vial
  - less robust (difficult to handle, sterility problems)
  - Used mainly in laboratory scale

LyoDEA Brief Description

- The system connects via a junction box to 5 individual LyoDEA™ test vials positioned around the freeze-drier shelf.
- Frequency scans (10 Hz – 1 MHz) of the LyoDEA™ test vial impedance were recorded every 1-5 minutes throughout a freeze-drying cycle (20 s for each spectrum)
- The LyoDEA™ measurement and control software saves the spectra from each time point

Applications

- Formulation variables
  - Freezing rates/end points
  - Eutectic crystallization
  - Glass transition
  - Structural relaxation
- Process variables
  - Temperature
  - Annealing — ice growth rates
  - Drying rates
  - Primary drying end points

LyoDEA response surface

Impedance Modelling

- CPE explains the interfacial impedance of the glass wall of the vial.
- Resistance element records conductivity of ions
- Capacitor element defines dielectric properties of the product.
- The circuit element R was shown to be a sensitive indicator of the phase behavior of the solution, i.e. ice formation and solute crystallization during the freezing cycle.

Effect of Sucrose on mannitol crystallization

Mannitol crystallization suppressed with the inclusion of sucrose in the solution.

Product Characterization – Ice formation

- Peak profile records freezing step (B-D) which progress through 2 discrete stages: solidification (B-C) and equilibration (C-D). Time duration for the former increase with the fill height while the latter remain broadly unchanged as it is related to thermal coefficient of the vial base.

Completion of Annealing (Maltodextrin 10% w/v)

- The capacitance of the formulation changes minimally while the resistance changes significantly and plateaus at 3-4 h.
- After 3h annealing hold time, both the capacitance and drying time changes insignificantly.

Predictive control of the primary drying time

- Increase in the capacitance correlates well with the decrease in the primary drying time.
- Changes in the Product capacitance during annealing may be predictive of the reduction in primary drying time.
- For every ~2% increase in capacitance the primary drying decreases by ~8%.

Product Characterization: $T_d$ determination by LyoDEA

Aqueous solution of Maltodextrin 10% w/v by LyoDEA on heating at 1 C min$^{-1}$
Measurement of Fragility of frozen solution (Maltodextrin 10% w/v)

- Below Tg, the changes in product resistance follows Arrhenius trend with $E_a \approx 20 \text{ KJ.Mol}^{-1}$.
- Above Tg, VTF function models the resistance profile.
- The fragility of the glassy matrix calculated from VTF results and slope of resistance was recorded to be $\approx 0.7$, suggesting a fragile glass.

$$y = 2.4865x + 5.9707$$
$$R^2 = 0.9914$$

VTF Fit to describe the above Tg' resistance

- Above Tg, the temperature dependence of the product resistance follows the Vogel-Tammann-Fulcher function.
- The curvature of the resistance plot decreases following annealing
- which relates to the increased strength of the glassy material.

Fragility of different solutions

<table>
<thead>
<tr>
<th>Solution details</th>
<th>Fragility</th>
</tr>
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<tbody>
<tr>
<td>Maltodextrin 10% w/v</td>
<td>0.7</td>
</tr>
<tr>
<td>Lysozyme 4.5% w/v</td>
<td>0.8</td>
</tr>
<tr>
<td>Lysozyme 4.5% + trehalose 1.5% w/v</td>
<td>0.6</td>
</tr>
</tbody>
</table>

- The higher the fragility number the more fragile the glass
- A fingerprint for stability – reproducibility?

Precision of these numbers in relation to relevance requires validation

Pre-heated Lysozyme 4.5% w/v (pre-aggregated)

- Lysozyme solution
- Pre-heated Lysozyme solution

Effect of Sucrose on mannitol crystallization

Mannitol crystallization suppressed with the inclusion of sucrose in the solution.

PRIMARY DRYING STAGE

The Desired state?
Fast drying rate, without compromising product quality, or operating at limits of equipment
**Design Space for Primary Drying**

- The aim is to achieve an acceptable drying rate, without compromising product quality, operating the equipment at (or beyond) the limits of its capability.
- Lab scale instruments for screening formulations and process conditions to optimise drying profiles (Microbalance).
- PAT and “intelligent” freeze drying software has allowed in-process monitoring, and interactive control of the cycle.

**PAT in Primary Drying**

Methodologies for Production Scale

- Pressure rise method (Tang et al Pharm Res 2005, 22; 685-700).
- Soft sensor probes (Barresi et al Int J Refrigeration 2009, 32; 1003-14).

...have enabled critical process parameters (drying rate) to be monitored and used to drive cycle progression and method optimisation.

**Primary Drying Modelling: Heat and Mass Transfer**

\[ \frac{dm}{dt} = \frac{A_p (P_i - P_c)}{R_{ps}} \]

\[ dq/dt = \Delta H_i \frac{dm}{dt} \]

\[ \frac{dq}{dt} = A_p \Delta T \frac{dm}{dt} \]

**Design Space**

- Lower chamber pressures \( P_i \) increases the driving force for sublimation.
- Effect seen for a constant ice vapour pressure, \( P_c \).
- i.e. A constant product temperature \( T_{sp} \).
- Linear increase in rate with decreasing chamber pressure.

**Design Space**

- Increases sublimation rates greater rate of heating \((dq/dt)\),

\[ \frac{dm}{dt} = -\Delta H_i \frac{dq}{dt} \]

...which, for a constant product temperature, can only come from increasing the shelf temperature \( T_{sp} \).

\[ \frac{dq}{dt} = A_p \Delta T \frac{dm}{dt} \]
**Design Space**

\[
\frac{d q}{dt} \propto K_{ch} \quad T_w \propto \frac{d q}{dt} \quad P_c \propto T_p \quad \text{dm/dt} \propto (P_c - P_v)
\]

- Higher chamber pressures also increases the rate of heat transfer by increasing the thermal conductivity of the gas in the gap between the shelf and the bottom of the vial (K_{ch}).
- This in effect increases the product temperature (T_p), which increases the ice vapor pressure, increasing driving force for flow of vapor in the chamber.

![Diagram showing Design Space](image)

**Design Space – Failure Modes**

There are two failure points resulting from:

(i) **Formulation**

(ii) **Equipment**

![Diagram showing Design Space Failure Modes](image)

**Design Space**

There are two failure points resulting from:

(i) **Formulation**

(ii) **Equipment**

Formulation Limits:
- If the product exceeds its critical temperature at which the viscosity of the unfrozen matrix is too low to support its weight then it collapses (e.g., -25°C).

![Diagram showing Design Space Failure Points](image)

**Design Space**

There are two failure points resulting from:

(i) **Formulation**

(ii) **Equipment**

Equipment Limits:
- Condenser capacity to trap ice and maintain its temperature.
- Shelf coolant capacity to maintain its temperature.

![Diagram showing Design Space Equipment Limit](image)

**Design Space Choked Flow**

With aggressive drying, the sublimation rate is eventually suppressed by the maximum volume of water vapor which could traverse from chamber to condenser in unit time.

\[
P_1 = P_c
\]

![Diagram showing Design Space Choked Flow](image)
**Design Space Choked flow**

Similar effects have been observed with the physical spacing of the shelves in a stack can also pose a resistance to the increasing sublimative flow, with pockets of greater chamber pressure building up between narrowly separated shelves and limiting the effective drying rate.

**Design Space**

Complete the DoE!!

Using a range of chamber pressures and shelf temperatures to establish the limits of the equipment.

The design space is shown by the yellow triangle

Operate at the apex of the triangle to drive process efficiencies

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**Pharmaceutical Quality by Design**

**Product Characterization**: phase behaviour, temperature

- The $T_{sub}$ showed a good correlation with the product temperature during product cooling (A), freezing (B) and thawing (C).
- Provided there is no change in phase, then a linear correlation exists between Log F and temperature (A, C-D)
- Use LyoDEA response to drive the process

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**Lactose 3%**

* In some cases the maximum in the derivative corresponds to the point at which the ice front has receded to 50% of the height of the product (in the case of lactose)
Sucrose 3%

- In other cases, the maximum in the derivative corresponds to the point at which the external ice front has receded to 100% of the height of the product (in the case of sucrose).
- Can these observations be used to indicate/inform the user about the flatness of the drying front?
- In all cases, the approach of the derivative to a value of zero indicates the end of the primary drying process.

End Point Determination

- Impedance measurement data from sucrose 2.5% w/v were analyzed for the determination of primary drying end point.
- Time slice of the imaginary capacitance at 1 kHz showed a sharp decline as the ice sublimation was complete.

Results: Defining the End of Primary Drying

LyoDEA offers a non-invasive measurement of primary drying time which is in good agreement with the thermocouple.

Shelf temperature distribution: Spatial mapping

- The temperature variation measurement during freezing stage.
- Thermocouple measurements of vials filled with oil.
- Gray scale shows minimum-maximum during freezing.
- $\Delta T \approx 1$-2°C across shelf can affect ice formation (already stochastic) and impact drying time.
- 1°C increase in 1°C drying T can shorten drying time by ~13%.

Impact of Spatial Temperature Map on Ice Formation

- Ice crystallization rates can impact the amount of ice and the particle size.

<table>
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<tr>
<th>F Rate</th>
<th>Ice Crystal Size</th>
<th>$R_p$</th>
<th>Unfrozen Fraction</th>
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<tbody>
<tr>
<td>Fast</td>
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<tr>
<td>Slow</td>
<td>Large</td>
<td>Low</td>
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Leading to variations in:

1. drying rates (because of the impact on the resistance to vapour flow, $R_p$)
2. concentration on solutes in the unfrozen fraction, which impacts $T_f$ which impacts the primary drying temperature.

LyoDEA Spatial mapping: Primary drying times

1. Primary drying time distribution across the shelf identifies three distinct spatial regions characteristic of thermal variations in the shelf.
2. Edge effects – may extend across three vials around the periphery of the shelf.
Conventionally measured by cryo-microscopic images

Microscopic images may not account for increase in the vapor pressure at sublimation front, following increased resistance to vapor flow during the later stage of primary drying; potentially vulnerable to collapse.

Collapse measurement within the real conditions may provide such information.

Lactose 10% collapse: 2nd derivative

the capacitance profile of collapse free product (LEFT) was seen to be fairly uniform, un-like the collapsed product (RIGHT)

Acknowledgements

• Paul Matejtschuk (NIBSC)
• Tim McCoy (Genzyme)
• Sohail Arshad (Bahauddin Zakariya University, Multan, Pakistan)
• Evgeny Polgalov (DMU)
• Irina Ermolina (DMU)

LyoDEA References