DEVELOPMENT AND OPTIMIZATION OF LYOPHILIZATION CYCLE

Sandeep kumar¹, Namrata Gautam², Dr. Md. Akram Minhaj¹³, Sana⁴, Sapna Kapoor⁵

¹Research Fellow, Department of Pharmacy, IEC Group of Institution, Greater Noida, UP, India.
³Assistant Professor & Head, Department of Pharmacy, IEC Group of Institution, Gr. Noida.
²,⁴,⁵Assistant Professor, Department of Pharmacy, IEC Group of Institution, Gr. Noida.

ABSTRACT
Development and optimization of Lyophilization cycle or freeze drying cycle is a trial and Error method which is not well known and understood properly. This technology is developed for individual pharmaceutical products for preservation and to improve the stability of product during its storage condition. This technique is applicable for thermo labile substances. Development and optimization of lyophilization cycle require scientific knowledge and experience in this field. Development and optimization of Lyophilization cycle for most of the products depends upon some justified simple rules based on well accepted scientific principles. The aims and objectives of this review article are to study all the critical parameters and to develop knowledge on which Lyophilization cycle depends. In the present review article, importance of controlled freezing, control of ice nucleation, Glass transition temperature, Eutectic temperature, Significance of collapse temperature during primary drying, and impact of freezing on primary drying and rest of the lyophilization cycle is elaborated.

KEYWORDS: Lyophilization, Eutectic temperature, Collapse temperature, Primary drying.

INTRODUCTION
Lyophilization or freeze drying is the method of drying any pharmaceutical or food products in the frozen state. The product in solution form is first frozen to ice by applying very low temperature and then removing this ice from the pharmaceutical products by applying low temperature drying in low pressure. Freeze drying works on the principle of sublimation in which liquid state is converted in the solid state below the triple point of water.
For freeze drying? \[^{[1]}\]
1. To stabilize highly degradable protein drugs.
2. To stabilize heat sensitive and chemically unstable solutions.
3. Low particulate contamination.
4. Low temperature drying process.
5. Compatible with aseptic/sterile processing.
6. To get amorphous form of drug desirable for solubility in freeze drying.

Desirable freeze drying characteristics \[^{[4]}\]
1. Intact cake of products.
2. Sufficient strength in terms of assay pH.
3. Uniform color of products.
5. Sufficient porosity of finally dried products.

Advantages
1. Heat labile costly drug product can be stabilized in dry form.
2. Increases shelf life of product during storage condition.
Disadvantages of lyophilization Technology
1. Very costly technology increases the cost of drug product.
2. Require monitoring of each and every step of lyophilization and time consuming process.

Formulation Development of Aqueous Lyophilized powder for injection
1. Dissolving the drug (API) and excipients in a suitable solvent, generally water.
2. Sterilizing the bulk solution by passing it via a bacteria proof filter membrane generally 0.22um PVDF (polyvinylidine fluoride) filter membrane or 0.22um PES (polyethylene sulfone) filter membrane or any other filter membrane required by specific product.
3. Filling into individual glass vials.
4. Stoppering with coated rubber stopper.
5. Space should be provided between the vial and stopper so that water vapour may escape out of the vials.
6. Freezing the solution by placing the stoppered vials on the shelves in the lyophilizer.
7. Applying vacuum to the chamber and heating the chamber to sublime the ice.

Lyophilizer

Fig: A benchtop freeze dryer, front view[14].
Fig: A typical freeze drying cycle showing shelf temperature, product temperature on different time under pressure in mbar.

Steps involved in Lyophilization cycle
1. Freezing
2. Primary drying
3. Secondary drying

A. Freezing
Freezing is a process of converting the liquid solution to the solid state in which water is converted to ice form by applying low temperature. During the freezing stage solute gets concentrated in between the ice crystals called the freeze concentrate. Vacuum is applied to get the desired temperature of -40 to -45°C in freezing.

Critical Parameter on which Freezing Depends
Cooling Rate - One practical approach for the stabilization of pharmaceutical product is to minimize the specific surface area of ice by growing large ice crystals. This can be done by controlling the rate of super cooling. Generally higher supercooling rate results in larger ice specific surface area that form smaller ice crystals. Cooling rate of 2-3°C/min causes the higher nucleation of the ice crystal that makes compact ice crystals. A moderate cooling rate of 1°C/min is good for freezing drug product in lyophilizer. This cooling rate yields moderate supercooling with moderate ice specific surface area and reasonably fast freezing rate which is best for both formulation prone to phase separation and in those phase separation is not an issue. This also maintains the homogeniety between the vials on shelves in lyophilizer. This usually produces uniform ice structure in vials.
Annealing - This is a process of holding drug solution at a temperature above the final freezing temperature for a defined period of time to crystallize the potentially crystalline components (usually crystalline bulking agents) in the formulation during freezing state. This vial breakage can be prevented by slow freezing or by avoiding temperature lower than -25°C until bulking agent is completely crystallized as in case of mannitol (bulking agent). Annealing temperature should be in between Tg of amorphous phase →Tg’ eutectic of bulking agent. Annealing time is dependent on ratio and property of bulking agent used.\(^\text{[1]}\)

A high mass ratio of crystalline bulking agent to the drug (≥80%) crystallizes much faster than lower ratio (≤50%). For mannitol and glycine annealing temperature →-20 -25°C. If fill depth is 1cm or more→2hr hold time is required. Annealing condition can be studied by DSC and X-ray diffraction in frozen state to evaluate development of crystalinity.\(^\text{[7]}\)

Primary Drying

It is the process of drying to remove ice from the frozen product by the process of sublimation under high vacumme this forms a dried cake of drug product. Solute must form the rigid structure to support its weight after ice removal. During this step product must be maintained below its collapse temperature to produce acceptable drug product.

Collapse Temperature (Tc) - it is that temperature above which the product collapses (product degradation) in the primary drying phase the product is always kept 2-3°C below the collapse temperature to prevent collapse of drug product.

Product Temperature is controlled by following factors

A. Chamber pressure

B. Target product temperature

Note: Change in product temperature during primary drying may influence appearance of final drug product. Damage once occurred during primary drying is irreversible and can never be repaired.

A. Chamber Pressure (Pc) - Chamber pressure control can be done by applying high vacumme in millitorr, this improves the rate of sublimation. Pc should be well below the ice vapour pressure to allow a high sublimation rate. Sublimation rate is mass of ice sublimed (gm)/time(hr)\(^\text{[2]}\)

\[
\frac{dm}{dt} = P_{ice} - Pc/R_p + R_s
\]

\(\frac{dm}{dt}\)- rate of sublimation
Pice-vapour pressure of ice interface

Pc- chamber vapour pressure

Rp- product resistance

Rs- Stopper resistance

Sublimation rate = pressure difference between the pressure at ice interface and pressure at chamber partial pressure. Smallest chamber pressure - gives highest sublimation rate.

Note: very low chamber pressure may cause contamination of product with volatile stopper component or pump oil & also cause larger heterogeneity in heat transfer.

Chamber Pressure (Pc) = 0.29*10(0.019*Tp)

Pc - chamber pressure

Tp - product temperature (°C)

B. Target Product Temperature (Tp)

In the lyophilization cycle product temperature is always kept below the collapse temperature as the product degradation may occure above this temperature. Temperature difference b/w the product temperature (Tp) and the collapse temperature (Tc) is called the product safety margin. 1°C rise in product temperature reduces the primary drying by 13% on an average. For optimum drying product temperature is kept 2-3°C lower than collapse temperature.\[3\]

Overloading of lyophilizer is avoided as it may cause loss in chamber pressure and that will increase the product temperature suddenly. Product temperature is higher at front sides and back and colder at the interior on the shelf due to additional heat transfer from the door and chamber wall to the vials placed at edges. This heterogeniety can be prevented by placing empty vials at the edges of shelf or by placing Aluminium foil at the outside of the acrylic door so that constant temperature be maintained in between the vials on the shelf in lyophilizer.

Rate of heat transfer from shelf to the vials \[1\]

\[
\frac{dq}{dt} = 3600*Av*Kv*(Ts -Tp)
\]

\(\frac{dq}{dt}\) = rate of heat transfer

Av = outer area of vial bottom

Kv = coefficient of heat transfer

Ts = shelf temperature in °C

Tp = product temperature

3600= arises from heat flow unit from cal/hr/vial
Calculation of shelf temperature

\[ \frac{dq}{dt} = \frac{dH_s}{dt} \frac{dm}{dt} \]  \hspace{1cm} \text{equation 4}

Combining equation 3 & 4

\[ T_s = T_p + \frac{1}{AV} \frac{dq}{dt} \left( \frac{1}{K_v} + \frac{L_{ice}}{K_l} \right) \]  \hspace{1cm} \text{equation 5}

d\( H_s \) = heat of ice sublimation (cal/gm)

\( L_{ice} \) = ice thickness

\( K_l \) = thermal conductivity of ice

\( L_{ice} \) = (account for temperature difference across the frozen layer)

\( \frac{dq}{dt} \) = heat transfer rate

As protein drug product may also contains stabilizers like sucrose or trehalose that get highly viscous after freeze concentrate that prevent protein denaturation upto some extent and prevent preparation from degradation. Formulation which contains both stabilizer and crystalline bulking agent has the collapse temperature close to Eutectic temperature. \[^3\]

Correlation between the unfolding half life and viscosity of the product

\[ T_{1/2} = A \times nq \]

Where A= proportionality constant

n = system viscosity

q = coupling constant

**Overloading of lyophilizer** - To prevent overloading of lyophilizer person should not allow shelf temperature above which mass flow exceeds 1kg/hr/m\(^2\) which is maximum load for a typical lyophilizer.

**Primary Drying Endpoint** - various observation determine the endpoint of primary drying as at the end point there is no ice present in the vials a dry cake observed with no free water, product temperature increases rapidly to shelf temperature no water vapour observed from lyophilization chamber when some sensor are placed.

**C. Secondary Drying**

When the primary drying completes there is no free water left in the product but 5-20% of water still left in the form of bound water or residual water in the dried solid cake. In the secondary drying water is removed by method of desorption. The objective of secondary drying is to reduce the water level in the final product upto the level of 1-2% or as required
for stability of particular lyophilized product. In secondary drying shelf temperature should be increased slowly as the product may collapse if fast temperature ramping is done. Generally a ramp rate of 0.1-0.15°C/min is safe for amorphous products. For amorphous product generally higher temperature is applied for longer duration of time to remove absorbed water from the product. In secondary drying temperature should be in the range of 10-50°C but it vary from product to product. Moisture content present in finally dried lyophilized cake is measured by [1]

A. Karl Fischer Titration
B. Thermo Gravimetric Analysis
C. Near IR spectroscopy

**Container closure system used in Lyophilization Technology**

**Vials** - for small lyophilized powder for injection generally tubular vials are used as it has greater heat transfer by contact conductivity, greater Kc value and these vials are clear transparent vial of lyo grade. For larger volume lyophilized powder for injection molded vials are used as these vials can tolerate higher vaccume pressure and heat transfer from the shelf. Type I glass is used. [10]

**Rubber Stopper** - coated rubber stopper are generally used in lyophilization as they are non volatile in nature and prevent the drug product from contamination as in high vaccume pressure or by high heat volatile oil from rubber stopper may contaminate the product. This coating may be of Teflon, sulfur or other material compatible with the product. [10]

**Example of Lyophilization cycle**

**Step 1 Freezing**

<table>
<thead>
<tr>
<th>Thermal Treatment Steps</th>
<th>Temp (in °C)</th>
<th>Time (in min)</th>
<th>Ramp/Hold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step 1</td>
<td>5</td>
<td>30</td>
<td>H</td>
</tr>
<tr>
<td>Step 2</td>
<td>-40</td>
<td>60</td>
<td>R</td>
</tr>
<tr>
<td>Step 3</td>
<td>-40</td>
<td>300</td>
<td>H</td>
</tr>
<tr>
<td>Step 4</td>
<td>0</td>
<td>0</td>
<td>R</td>
</tr>
<tr>
<td>Step 5</td>
<td>0</td>
<td>0</td>
<td>R</td>
</tr>
<tr>
<td>Step 6</td>
<td>0</td>
<td>0</td>
<td>R</td>
</tr>
<tr>
<td>Step 7</td>
<td>0</td>
<td>0</td>
<td>R</td>
</tr>
<tr>
<td>Freeze temp</td>
<td>- 45 °C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Additional Freeze</td>
<td>0 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Condenser set point</td>
<td>- 45 °C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vacuum set point</td>
<td>400 Torr</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Step 2 Primary Drying and Secondary Drying Process Steps

<table>
<thead>
<tr>
<th>Primary Drying Steps</th>
<th>Temp (in °C)</th>
<th>Time (in min)</th>
<th>Vac (m Torr)</th>
<th>Ramp/Hold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step 1</td>
<td>-30</td>
<td>60</td>
<td>200</td>
<td>R</td>
</tr>
<tr>
<td>Step 2</td>
<td>-30</td>
<td>60</td>
<td>50</td>
<td>H</td>
</tr>
<tr>
<td>Step 3</td>
<td>-25</td>
<td>60</td>
<td>50</td>
<td>R</td>
</tr>
<tr>
<td>Step 4</td>
<td>-25</td>
<td>580</td>
<td>10</td>
<td>H</td>
</tr>
<tr>
<td>Step 5</td>
<td>-25</td>
<td>1200</td>
<td>10</td>
<td>H</td>
</tr>
<tr>
<td>Step 6</td>
<td>-25</td>
<td>1200</td>
<td>10</td>
<td>H</td>
</tr>
<tr>
<td>Step 7</td>
<td>-25</td>
<td>1200</td>
<td>10</td>
<td>H</td>
</tr>
<tr>
<td>Step 8</td>
<td>8</td>
<td>120</td>
<td>10</td>
<td>R</td>
</tr>
<tr>
<td>Step 9</td>
<td>8</td>
<td>600</td>
<td>10</td>
<td>H</td>
</tr>
<tr>
<td>Step 10</td>
<td>30</td>
<td>60</td>
<td>10</td>
<td>R</td>
</tr>
<tr>
<td>Step 11</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>H</td>
</tr>
<tr>
<td>Post Heat</td>
<td>30</td>
<td>240</td>
<td>50</td>
<td></td>
</tr>
</tbody>
</table>

Graph of lyophilization cycle representing the drying of solution on different temperature and chamber pressure with respect to time.

**Issues Related to Improper Lyophilization Cycle**

A. Melting of product
B. Shrunken freeze dried plug
C. Collapse product
D. Fly off
E. Cracking and breakage of vials
REFERENCES


