ABSTRACT

Lyophilization also known as freeze drying is a process in which water is removed from a product after it was frozen and placed under a vacuum, allowing the ice to change directly from solid to vapor without passing through a liquid phase. The process consists of three separate, unique, and interdependent processes; freezing, primary drying (sublimation), and secondary drying (desorption). The objective of study is Gemcitabine different formulations used to Optimization of Lyophilization cycles and the main focus is to minimize consistently drying times, while maintaining constant Gemcitabine product quality.

Methods: The various formulations were studied for thermal events by DSC technique, Impedance & DTA analysis. These parameters were determined on sample solution using Lyotherm2 analysis. By collaborated observations from Impedance, DTA of drug solution and set vacuum data from the Operation manual of Freeze dryer, lyophilization parameters for Gemcitabine for Injection were developed.

Results: The data obtained from Impedance & DTA analysis of drug solution was used to determine set temperature to be used during freezing, primary drying and secondary drying set temperatures. The set vacuum for the chamber during the primary drying and secondary drying was determined from the Operation manual of Martin Christ Gefriertrocknungsanlagen GmbH Freeze dryer. The optimized cycle was about 57 hrs used for better productivity. Evaluated the lyophilized product for water content and assay. Pharmaceutical equivalence study was conducted for Gemcitabine for Injection with innovator product (Gemzar).

Conclusion: Finally concluded that the present developed lyo cycle was used to execute the Gemcitabine for Injection product.

Keywords: Gemcitabine Hydrochloride, Lyophilization cycle, Impedance, DTA analysis, Poor thermal stability, Freezing, Primary drying, Secondary drying.

INTRODUCTION

The chemical name for Gemcitabine Hydrochloride is 2′-deoxy-2′, 2′-difluorocytidine monohydrate (R-isomer). Molecular formula is C9H11F2N3O4.HCl. Gemcitabine Hydrochloride is a white or almost white powder. It is soluble in water, slightly soluble in methanol, and practically insoluble in alcohol and polar organic solvents. PKa is 3.6. pH of the solution is between 2.0 and 3.0. Gemcitabine bulk solution has poor thermal stability, hence the product was lyophilized. The input drug substance and excipients, Mannitol and Sodium acetate are White (or) white to off white in color. Hence the product obtained is white to off white lyophilized powder. Unit operations involved in manufacturing of Gemcitabine for injection are Bulk Solution preparation, Sterilization, Filling, Lyophilization, Visual inspection & packing.

Of all pharmaceutical unit operations, the highest manufacturing costs arise from drying processes. Lyophilization is the most expensive of all drying operations, both in capital investment and in operation expenses. In this context the main focus in Optimization of Lyophilization (process development) is to minimize consistently drying times, while maintaining constant product quality [1]. Obviously the primary drying step should be carried out at the highest temperature possible, which is limited by the so-called “maximum allowable temperature”. This temperature indicates the eutectic temperature for a solute that crystallizes during freezing or the “collapse temperature” for a system that remains amorphous. Therefore process control means control of the product temperature vs. time profile during lyophilization [2]. To reach this goal the balance between heat and mass transfer which determines the product temperature must be more or less equal. Heat and mass transfer are also key issues during scale-up of lyophilization processes. Differences in (1) the degree of super cooling between laboratory, pilot and manufacturing plants, (2) heat transfer owing to differences in dryer design, and (3) the efficiency in the condenser or refrigerator system can result in substantial heterogeneity in sublimation rates and/or desorption rates and hence variation in drying time.

For optimum process control an indicator for the end of primary drying is needed, because an increase in shelf temperature before all vials have completed primary drying carries a high risk of collapse of the product. Some well-established methods for distinguishing between primary and secondary drying are available, such as commonly-used thermocouples, the pressure rise test, manometric temperature measurements or comparative pressure measurement. New investigations in modern process control have also been made. Near-infrared spectroscopy, mass spectrometry and a remote electrode system are some examples representing a growing research area.

MATERIALS AND METHODS

Materials

Gemcitabine Hydrochloride was generously supplied as a gift sample by Dr.Reddy’s Laboratories Limited, (Hyderabad, India). Mannitol supplied by Merck, (Hyderabad, India). All other chemicals were of analytical reagent grade and were used as received.

Methods

Preformulation [3, 4, 5, 6 & 7]

Standard calibration curve of Gemcitabine Hcl

A stock solution of (2 ppm) of standard drug was prepared, later required dilutions were made with diluent. The standard chromatogram of Gemcitabine Hydrochloride showed in Figure and plotted graphically the calibration curve.

Compatibility studies

In the present study the thermal events were recorded on Differential scanning instrument used model DSC Q 1000 V 9.4, Build 287. Modulated DSC method is a technique used to determine small heat flows resulting from various physical transitions of formulation components.

DSC conditions:

Temperature: Equilibrate at -70.0°C
Nitrogen flow: 50ml/min
Heating Qty: 5°C/min
Sample Qty: 20 µl
Pan: Hermetically sealed Aluminum pan
Sampler: Auto Sampler.

Preparation of Gemcitabine for injection [8]

Weigh accurately Mannitol and Sodium acetate anhydrous for batch size and dissolved in 80% of water for injection. Mix the solution until to get clear solution by using mechanical stirrer. Add and dissolve Gemcitabine hydrochloride (40mg/mL) concentration in the above solution and mix until to get uniform solution. The pH of the bulk solution was adjusted with diluted sodium hydroxide and diluted Hydrochloric acid and the volume made up to required volumes.

Lyotherm2 Analysis [9, 10, 11]

Lyotherm2 is a thermal analyzer containing cells for measuring both solution impedance (ZSinΦ) against temperature, and exothermic or endothermic changes observed as a solution freezes, melts or softens using differential thermal analysis (DTA). Gemcitabine vial sample was analyzed using lyotherm2.

DTA analysis using the Lyotherm2

Differential thermal analysis was carried out on the formulations to below -150.0°C according to standard procedure. The temperature of the sample together with the temperature of the reference material (AnaR water) was measured at 5-second intervals during cooling and warming. The data was exported directly to Microsoft Excel for graph plotting and interpretation of the warming profile of each analysis carried out to determine the temperatures of the significant events, which could be attributed to thermal changes occurring in the solutions.

Impedance analysis using the Lyotherm2

Impedance analysis was carried out for the formulations to below -150.0°C according to standard procedure. Impedance of the formulation was measured at 5secs intervals during cooling and warming. The data was exported directly to Microsoft Excel for graph plotting and interpretation of the warming profile of the analysis carried out to determine the temperatures of the significant events, which could be attributed to thermal changes occurring within the formulations.

Vapour pressure [12, 13]

Vapour pressure is the pressure of a vapor in equilibrium with its non-vapor phases. All liquids and solids have a tendency to evaporate to a gaseous form, and all gases have a tendency to condense back into their original form (either liquid or solid). Ice vapor pressure data provides minimum vapor pressure needed over ice at set temperature, to prevent melt back of ice. Ice vapor pressure data of Gemcitabine gases have a tendency to condense back into their original form (either liquid or solid).

Lyophilization cycle development [14, 15, 16, 17, 18, 19]

Based on the observations from the Lyotherm2 Analysis data and Vapor pressure data set the freezing, primary drying and secondary drying parameters for lyophilization cycle development.

Freezing

Minimum freezing temperature of the product should be set based on Lyotherm2 Analysis data and the chamber shall be at atmospheric pressure. The freezing step shall comprise of four set points:

Temperature for beginning of ice formation
Temperature for stabilization of frozen material
Temperature for completion of freezing
And temperature for extra freeze step to ensure that the product reaches a temperature at least 35°C.

Primary drying

The shelf temperature in primary drying shall be ramp up from at optimize temperature in six steps. The chamber vacuum would be set at optimized range. This vacuum is derived from ice vapour pressure data at optimized temperature, which is the minimum temperature required for freezing the product.

Secondary drying

Secondary drying of the product shall be done at set temperature based on Lyotherm2 Analysis data. The vacuum of the chamber at secondary drying shall be increased or reduced. The minimum duration of secondary drying shall be 360 minutes. This step is provided as a process control to ensure adequate drying.

Water content

After completion of lyophilization cycle, five vials were taken from each corner of bottom, middle, top shelf and sampling point was taken from middle of each bottom, middle, top shelf and collected samples were analyzed for water content analysis.

Assay of Gemcitabine

Weigh accurately 57.0 mg of Gemcitabine Hydrochloride Working Standard into a 250 ml volumetric flask, dissolve and dilute to volume with water and mix.

Chromatographic Conditions

Mobile phase: Dissolved 13.8 g of monobasic sodium phosphate and 2.5ml of phosphoric acid in 1000ml of water. (Note: pH of the solution is between 2.4 and 2.6)

Column: C4 4.6 mm x 250 mm column, 5µ (Zorabax or equivalent)
Flow rate: 1.2 ml / minute.
Wavelength: 275 nm.
Temperature: Ambient.
Load: 20 µl.
Run time: 20 minutes

Calculate Gemcitabine assay % =

\[
\frac{250}{1} \times \frac{100}{100} = 250
\]

Where,

At = Peak area due to Gemcitabine in Sample preparation,
As = Peak area due to Gemcitabine in Standard preparation,
Sw = Weight of Gemcitabine Hydrochloride Working Standard taken in mg,
P = Purity of Gemcitabine Hydrochloride Working Standard used.
LC = Label claim

RESULTS & DISCUSSION

Standard calibration curve of Gemcitabine Hcl

The absorbance of different concentrations of solution was measured at respective wave length of maximum absorbance, using UV detector. Absorbance values were plotted against respective concentration to obtain standard calibration curve. The standard chromatogram of Gemcitabine HCl was shown in figure 1.
Compatibility studies

There was no significant difference in the drug-excipients mixture, which confirmed the absence of any chemical interaction between the drugs and other excipients. The interpretation was shown graphically in figure 2.

Preparation of Gemcitabine for injection

Prepared Gemcitabine for injection bulk solution with optimized API and excipients concentrations. The ingredients concentrations were described in table 1.

Lyotherm2 Analysis

Gemcitabine for Injection bulk solution 25mL solution was filled into vial and sample was analyzed using Lyotherm2.

DTA analysis using Lyotherm2

Prepared Gemcitabine for injection bulk solution with optimized API and excipients concentrations. The ingredients concentrations were described in table 1. The Gemcitabine bulk solution was analyzed for thermal attributes. Analytical results revealed that minimum temperature of -30°C is required to freeze the Gemcitabine bulk solution. The data obtained from DTA analysis of drug solution was used to determine set temperatures to be used during freezing, primary drying and for secondary drying. Results were shown graphically in figure 2 and observations were mentioned in table 2.

Impedance analysis using the Lyotherm2

The Gemcitabine bulk solution was analyzed for impedance. Analytical results revealed that minimum temperature of -30°C is required to freeze the Gemcitabine bulk solution. The data obtained from DTA analysis of drug solution was used to determine set temperatures to be used during freezing, primary drying and for secondary drying. Results were shown graphically in figure 3 and observations were mentioned in table 3.

Ice vapor pressure data was collected from Operation manual of Martin Christ Freeze dryer is tabulated in table -6.25. From this data during sublimation minimum vacuum of 0.37 mbar is necessary at a product temperature of -30°C. Hence vacuum set point at start of primary drying can be 0.37 mbar. The pressure data was shown in table 4.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Ingredients</th>
<th>Concentration in solution mg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Gemcitabine Hydrochloride equivalent to Gemcitabine 40mg</td>
<td>45.5408 mg / mL</td>
</tr>
<tr>
<td>2</td>
<td>Mannitol (Pyrogen free)</td>
<td>40 mg / mL</td>
</tr>
<tr>
<td>3</td>
<td>Sodium acetate anhydrous</td>
<td>2.5 mg / mL</td>
</tr>
<tr>
<td>4</td>
<td>Water for Injection</td>
<td>q.s to 1mL</td>
</tr>
</tbody>
</table>

Fig. 1: Standard chromatograph of Gemcitabine Hcl

Fig. 2: Gemcitabine for injection thermal events by DSC
Table 2: Observations of DTA Thermo-gram

<table>
<thead>
<tr>
<th>Type of analysis</th>
<th>Temperature</th>
<th>Description of events/ general comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>DTA</td>
<td>From -68.0°C to -50.0°C</td>
<td>Exothermic (peak at -61.0°C) indicative of a stabilization / rearrangement within the frozen material.</td>
</tr>
<tr>
<td></td>
<td>From -50°C to -30°C</td>
<td>DTA shows that it is a frozen material.</td>
</tr>
<tr>
<td></td>
<td>From -30°C to -21.0°C</td>
<td>Endothermic (peak at -26.0°C) indicative of a stabilization within the frozen material</td>
</tr>
<tr>
<td></td>
<td>From -20.0°C to -5.0°C</td>
<td>Further formation of ice</td>
</tr>
<tr>
<td></td>
<td>-5.0°C</td>
<td>Possible start of ice formation</td>
</tr>
<tr>
<td></td>
<td>-1.0°C</td>
<td>Ice Melt</td>
</tr>
</tbody>
</table>

Table 3: Observations of Impedance (Zsinφ) analysis

<table>
<thead>
<tr>
<th>Type of analysis</th>
<th>Temperature</th>
<th>Description of events/ general comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Impedance</td>
<td>-74.0°C</td>
<td>Increase in impedance curve indicative of a stabilization event</td>
</tr>
<tr>
<td>(Zsinφ)</td>
<td>-72.0°C</td>
<td>Decrease in impedance curve indicative of a softening event</td>
</tr>
<tr>
<td>DTA</td>
<td>-36.0°C</td>
<td>Decrease in impedance gradient indicative of a softening event</td>
</tr>
</tbody>
</table>

Fig. 3: Impedance and DTA analysis results for Gemcitabine solution Vapour pressure

Table 4: Ice vapor pressure data

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Vacuum (mbar)</th>
<th>Temperature (°C)</th>
<th>Vacuum (mbar)</th>
<th>Temperature (°C)</th>
<th>Vacuum (mbar)</th>
<th>Temperature (°C)</th>
<th>Vacuum (mbar)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6.110</td>
<td>-16</td>
<td>1.510</td>
<td>-34</td>
<td>0.250</td>
<td>-54</td>
<td>0.024</td>
</tr>
<tr>
<td>-1</td>
<td>5.620</td>
<td>-17</td>
<td>1.370</td>
<td>-35</td>
<td>0.220</td>
<td>-55</td>
<td>0.021</td>
</tr>
<tr>
<td>-2</td>
<td>5.170</td>
<td>-18</td>
<td>1.250</td>
<td>-36</td>
<td>0.200</td>
<td>-56</td>
<td>0.018</td>
</tr>
<tr>
<td>-3</td>
<td>4.760</td>
<td>-19</td>
<td>1.140</td>
<td>-37</td>
<td>0.180</td>
<td>-57</td>
<td>0.016</td>
</tr>
<tr>
<td>-4</td>
<td>4.370</td>
<td>-20</td>
<td>1.030</td>
<td>-38</td>
<td>0.160</td>
<td>-58</td>
<td>0.014</td>
</tr>
<tr>
<td>-5</td>
<td>4.020</td>
<td>-21</td>
<td>0.940</td>
<td>-39</td>
<td>0.140</td>
<td>-59</td>
<td>0.012</td>
</tr>
<tr>
<td>-6</td>
<td>3.690</td>
<td>-22</td>
<td>0.850</td>
<td>-40</td>
<td>0.120</td>
<td>-60</td>
<td>0.011</td>
</tr>
<tr>
<td>-7</td>
<td>3.380</td>
<td>-23</td>
<td>0.770</td>
<td>-41</td>
<td>0.110</td>
<td>-61</td>
<td>0.009</td>
</tr>
<tr>
<td>-8</td>
<td>3.010</td>
<td>-24</td>
<td>0.700</td>
<td>-46</td>
<td>0.060</td>
<td>-62</td>
<td>0.008</td>
</tr>
<tr>
<td>-9</td>
<td>2.840</td>
<td>-25</td>
<td>0.630</td>
<td>-47</td>
<td>0.055</td>
<td>-63</td>
<td>0.007</td>
</tr>
<tr>
<td>-10</td>
<td>2.560</td>
<td>-28</td>
<td>0.470</td>
<td>-48</td>
<td>0.050</td>
<td>-64</td>
<td>0.006</td>
</tr>
<tr>
<td>-11</td>
<td>2.380</td>
<td>-29</td>
<td>0.420</td>
<td>-49</td>
<td>0.045</td>
<td>-65</td>
<td>0.0054</td>
</tr>
<tr>
<td>-12</td>
<td>2.170</td>
<td>-30</td>
<td>0.370</td>
<td>-50</td>
<td>0.040</td>
<td>-66</td>
<td>0.0047</td>
</tr>
<tr>
<td>-13</td>
<td>1.980</td>
<td>-31</td>
<td>0.340</td>
<td>-51</td>
<td>0.035</td>
<td>-67</td>
<td>0.0047</td>
</tr>
<tr>
<td>-14</td>
<td>1.810</td>
<td>-32</td>
<td>0.310</td>
<td>-52</td>
<td>0.030</td>
<td>-68</td>
<td>0.0035</td>
</tr>
<tr>
<td>-15</td>
<td>1.650</td>
<td>-33</td>
<td>0.280</td>
<td>-53</td>
<td>0.025</td>
<td>-69</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Lyophilization cycle development

By corroborated observations from Impedance & DTA of drug solution and Ice vapor pressure data, the proposed cycle parameters are summarized. Graphically represented the lyo Rx value (%) vs. cumulative time (mins) in figure 3.

Freezing

Minimum freezing temperature of product was 30°C and the Chamber vacuum shall be at atmospheric pressure. The product temperature of less than -30°C shall be attained by gradually cooling the shelf from room temperature to -40°C. The freezing step shall comprise of four set points:
- 5°C: For beginning of ice formation.
- 25°C: For stabilization of frozen material.
- 40°C: For completion of freezing.
- 42°C: Extra freeze step to ensure that the product reaches a temperature at least -35°C.

The above process steps would ensure that the product is at least 5°C below its freezing points of -30°C.

**Primary drying**

The shelf temperature in primary drying shall be ramped up from -40°C to +40°C in six steps. The heating rates during early steps of this ramp up would be kept below 3°C per hour. This ensures a gradual heating of the product via the vacuum would be maintained (< 0.37 millibar) such that melt back during the process does not happen.

The chamber vacuum would be set at 0.37 millibar. During ramp up of shelf temperature from -40°C to +40°C, the chamber vacuum would further be reduced from 0.37 millibar to 0.035 millibar (less than 1/10th of 0.37 millibar). This is done gradually by reducing the vacuum in a programmed manner from 0.37 millibar, to 0.25 millibar, 0.2 millibar and then to 0.035 millibar. This reduction in vacuum to less than 1/10th of 0.37 millibar is necessary, because at end of primary drying when pressure increase test is performed, the chamber vacuum does not increase beyond 0.37 millibar. This ensures that product does not melt back during the process. Consistent with reduction in vacuum in a programmed manner, from 0.37 millibar to 0.035 millibar ramp up of shelf temperature is sequenced as: -40°C to +20°C (< 3°C/hr), +20°C to +30°C (< 10°C/hr) this is to mobilize vapour, +30°C to +35°C (<1°C/hr) and +35°C to + 40°C (<3°C/hr).

**Secondary drying**

Secondary drying of the product done at +40°C. The vacuum of the chamber further reduced from 0.035 millibar to less than 1/18th of 0.035 millibar. During secondary drying there was rapid loss of free moisture from the product. Hence, a low chamber vacuum would ensure that moisture is removed efficiently. Further, during pressure increase test in secondary drying stage the chamber vacuum would not increase to 0.035 millibar. This ensures that no melt back of drug product would occur due to increase of chamber pressure during pressure increase testing in secondary drying.

The minimum duration of secondary drying shall be 360 minutes. Subsequently to which the cycle has a 2 hours step at 40°C. This step is provided as a process control to ensure adequate drying. When pressure increase test fails, the 2 hours step was repeated at vacuum of 0.0019 millibar, and shelf temperature of +40°C.

Graph representing the Shelf & Lyo-product temperature (°C) vs. Cumulative time (min) of Gemcitabine for Injection in figure 5.

**Water content**

Water content of the Gemcitabine for injection USP 200 mg vials varied from 0.23% w/w to 0.46% w/w which is within the limit of NMT 5.00% w/w. Based on observations it can be concluded that drying of the product is uniform & satisfactory. Hence the Lyophilization parameters can be used for further batches. The results were shown in table 5.

**Assay of Gemcitabine**

The recommended method for assay of Gemcitabine was optimized. The method was found suitable for assay analysis. The results are found satisfactory. Hence the recommended test method is suitable for analysis of assay of finished product. The results were shown in table 6.
The freezing step in the primary drying and secondary drying set temperatures. The set temperatures were optimized using a freeze drying microbalance technique, Int. J. Pharm. 2002; 246: 16-22.


11. A guide to freeze drying for the laboratory, LABCONCO, An industry service publication; 1-17