Using DSC, the Method for tablet formulation:

G) Drying at T

Freeze drying is a critical step of tablet fabrication. It can cause aggregation of the protein and loss of activity. There is a need for a dosage form that would prolong the local residence time of the protein. It causes an increase in intraocular pressure (IOP), which leads to optic nerve damage and subsequent visual impairments. Bevacizumab, a monoclonal antibody against VEGF-A, has shown potential to control scarring when injected into the subconjunctiva following GFS. But it clears very rapidly and multiple injections are required.

H) Drying at T

There is a need for a dosage form that would prolong the local residence time of the protein. Bevacizumab has been developed as a solid implant.

An in vivo study showed that this formulation is biologically active and prolongs the survival of the bleb in a rabbit scarring model.

Figure 1: Diagrammatic representation showing the channel made during GFS and placement of the tablet in the bleb. (From www.gescact)

Figure 2: Anti-scarring effect of bevacizumab tablet in a rabbit scarring model.

To characterise the thermal properties of the excipients and formulation that are important in optimisation of freeze drying of a solid implantable dosage form of bevacizumab.

AIM

To characterise the thermal properties of the excipients and formulation that are important in optimisation of freeze drying of a solid implantable dosage form of bevacizumab.

TABLES AND METHODS

Tablet fabrication:

• Bevacizumab tablet formulation was prepared as described in Figure 2.
• Freeze drying is a critical step of tablet fabrication. It can cause aggregation of the protein and loss of activity.
• There can be collapse of cake which causes increased moisture retention in the lyophilized product and may lead to protein degradation (1).

Thermal transitions critical for freeze drying:

Tg - Glass Transition Temperature: This is the temperature at which the freeze concentrate becomes so concentrated that it increases in viscosity and vitifies (2).

Method for detection: Differential Scanning Calorimetry (DSC) (Q2000, TA instruments)

Experimental Method: Equilibrate sample at -60°C and increase the temperature up to 100°C at a rate of 10°C/min.

Method for tablet formulation: Equilibrate at -70°C, modulate ±0.23°C every 60 seconds and then ramp at 1.5°C/min to 25°C.

Tc - Collapse Temperature: Collapse is the microscopic or macroscopic changes in structure of a dehydrated material as a result of environmental stress. The temperature at which this occurs is called the collapse temperature. It is usually the maximum allowable product temperature for an amorphous solute system during primary drying (3,4).

Method for detection: Freezing Drying Microscopy (FDM) (Linkam)

Figure 3: FDM at NIBSC

Figure 4: Bevacizumab tablet fabrication steps

Figure 5: DSC thermogram of Bevacizumab

Figure 6: DSC thermogram of Hyaluronic acid pharmaceutical formulation

Figure 7: DSC thermogram of Bevacizumab pharmaceutical formulation

A clear Tg is seen at -29°C. It may suggest that the thermal behavior of the formulation is dominated by turbulence.

Figure 8: DSC thermogram of Bevacizumab tablet formulation

Table 1: Thermal events observed in FDM and DSC

<table>
<thead>
<tr>
<th>Sample name</th>
<th>Collapse temperature Tc (°C)</th>
<th>Transition observed in DSC (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bevacizumab, in its pharmaceutical formulation</td>
<td>28.15 ± 0.76</td>
<td>Exotherm at -29.0°C and endotherm at 24.0°C</td>
</tr>
<tr>
<td>Hyaluronic acid in pharmaceutical formulation</td>
<td>40.15 ± 0.47</td>
<td>Endotherm at -26.0°C and exotherm at 25.0°C</td>
</tr>
<tr>
<td>Bevacizumab in water</td>
<td>-24.0 ± 0.29</td>
<td>Not Detected</td>
</tr>
<tr>
<td>Bevacizumab in PBS</td>
<td>-28.3 ± 0.15</td>
<td>Not Detected</td>
</tr>
<tr>
<td>Bevacizumab tablet formulation</td>
<td>-34 ± 1.41</td>
<td>Endotherm at -23.8°C and exotherm at 25.0°C</td>
</tr>
<tr>
<td></td>
<td>40.3 ± 0.56</td>
<td></td>
</tr>
</tbody>
</table>

CONCLUSIONS

- Using DSC, the Tg of Bevacizumab was determined to be -28°C in its pharmaceutical formulation.
- Both DSC and FDM were shown to give useful information on the critical formulation temperature of bevacizumab, the values by FDM were generally slightly lower. Collapse is assessed subjectively from captured images, Tc is also an event spanning several °C and can be assigned in different ways (Tentem, midpoint etc).
- By studying the protein in different formulations the impact of different excipients on the critical temperature can be assessed. This information is critical when optimising the freeze drying of such preparations.