Thermal characterisation of protein-based sterile ocular implants

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BACKGROUND

- Glaucoma is the leading cause of irreversible blindness worldwide.
- It causes an increase in intraocular pressure (IOP), which leads to optic nerve damage and subsequent irreversible blindness.
- Glaucoma filtration surgery (GFS) is a procedure that creates a fistula to allow the outflow of aqueous humor, thus delaying the progression of the disease (Figure 1).
- Scarring prevents flow and leads to surgical failure.
- There is no licensed treatment to control scarring after GFS.
- Bevacizumab, a humanised monoclonal antibody against VEGF- A, has shown the potential to control scarring when injected into the subconjunctiva following GFS. But it clears very rapidly and multiple injections are required.
- 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.

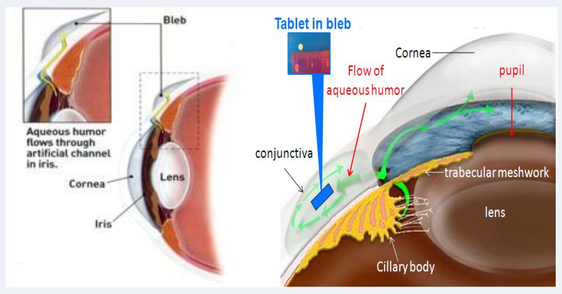


Figure 1:Diagrammatic representation showing the channel made during GFS and placement of the tablet in the bleb. (From www.gcot.net)

Bevacizumab tablet 

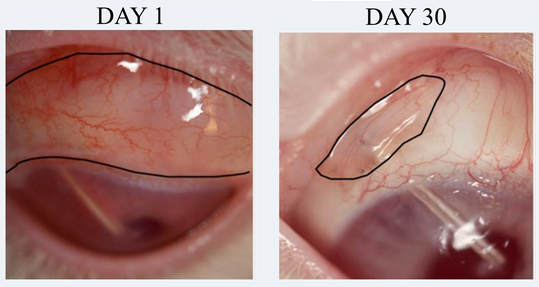


Figure 2. Anti-scarring effect of bevacizumab tablet in a rabbit scarring model.

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.

MATERIALS 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:

- **T_g'- Glass Transition Temperature:** This is the temperature at which the freeze concentrate becomes so concentrated that it increases in viscosity and vitrifies (2).
- **Method for detection:** Differential Scanning Calorimetry (DSC) (Q2000, TA instruments)
- **Experimental Method**: Equilibrate sample at -60^{0}C and increase the temperature up to 10^{0}C at a rate of 10^{0}C/min.
- **Method for tablet formulation:** Equilibrate at -70^{0}C, modulate ± 0.23 ^{0}C every 60 seconds and then ramp at 1.5 ^{0}C/min to 25^{0}C

- **T_c – 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-** Freeze Drying Microscopy (FDM) (Linkam)

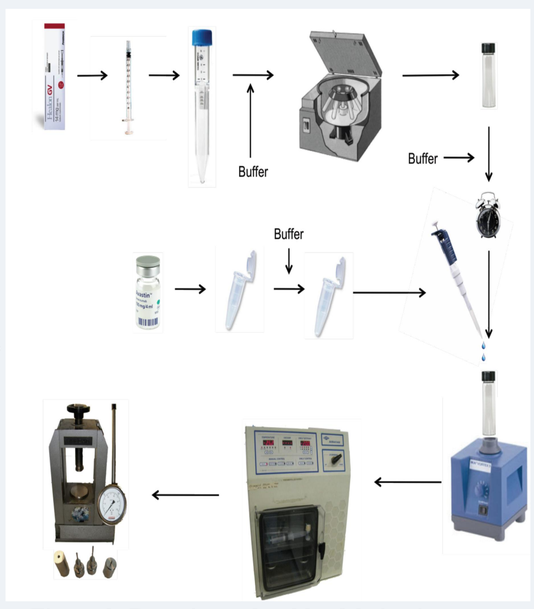


Figure 2: Bevacizumab tablet fabrication steps

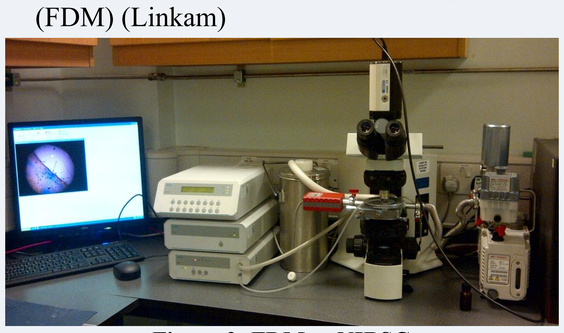


Figure 3: FDM at NIBSC

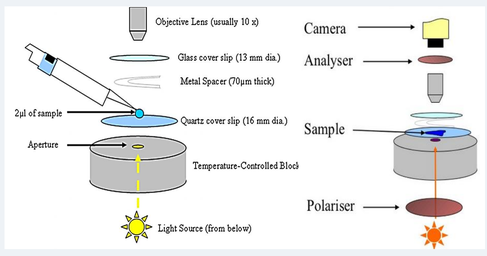
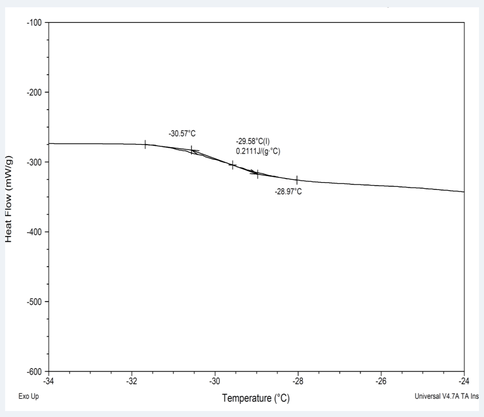


Figure 4: Diagrammatic representation showing FDM sample prep and use of plane polarised light. (From www.biopharma.co.uk)

RESULTS AND DISCUSSION

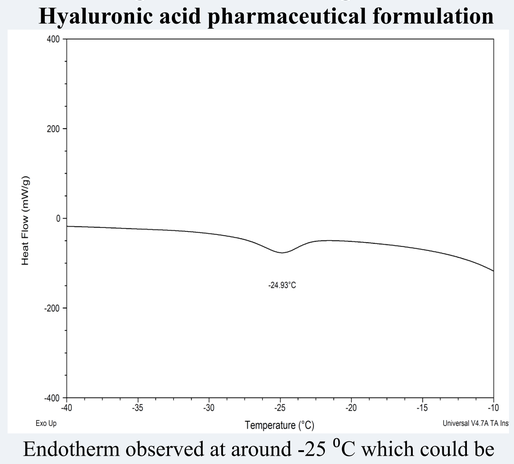
Characterisation of excipients and formulation using DSC

Figure 5: DSC thermogram of Trehalose



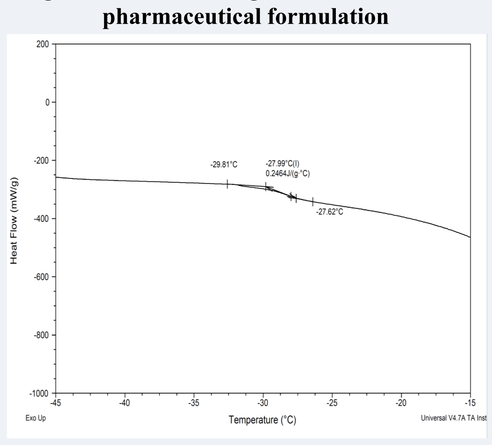
T_g' -29.58^{0}C

Figure 6: DSC thermogram of Hyaluronic acid pharmaceutical formulation



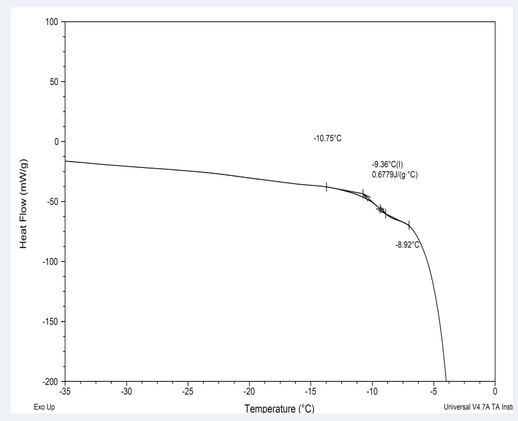
Endotherm observed at around -25 ^{0}C which could be a eutectic melt due to the presence of salts in the commercial product.

Figure 7: DSC thermogram of Bevacizumab pharmaceutical formulation



A clear T_g' is seen at -28^{0}C. It may suggest that the thermal behavior of the formulation is dominated by trehalose.

Figure 8: DSC thermogram of Bevacizumab tablet formulation



A T_g' is seen at -9 ^{0}C. The tablet formulation, being a complex mixture, shows a difference in behavior as compared to the individual excipients.

Characterisation of excipients and formulation using FDM

Figure 9: Representative FDM images of Bevacizumab tablet formulation

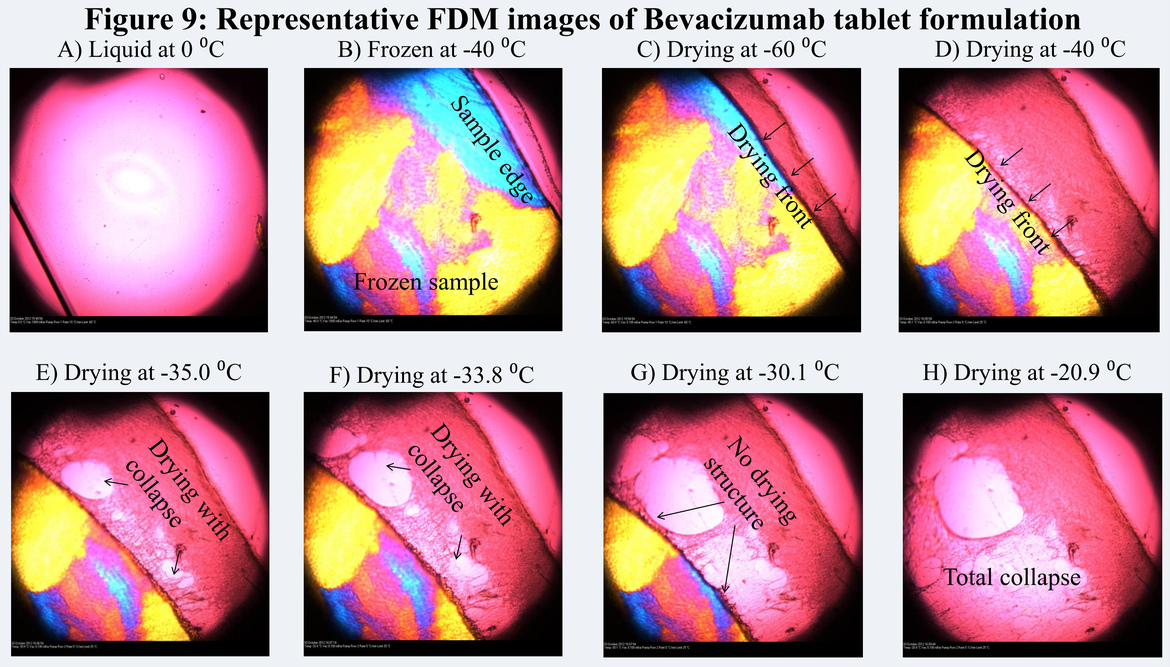


Table 1: Thermal events observed in FDM and DSC

Sample name	Collapse temperature $T_{c\ (onset)}$ (N=2) ^{0}C	Transition observed in DSC (N=2) ^{0}C
Bevacizumab, in its pharmaceutical formulation	-36.05 ± 0.77	T_g'-28.12 ± 0.17
Hyaluronic acid, in its pharmaceutical formulation	-29.15 ± 1.48	Endotherm at -24.60 ± 0.47
Bevacizumab in water	-12.45 ± 1.76	Not Detected
Bevacizumab in PBS	-28.15 ± 0.35	Endotherm at -23.84 ± 0.13
Bevacizumab tablet formulation	-34 ± 1.41	T_g' -9.48 ± 0.16

CONCLUSIONS

- Using DSC, the T_g' of Bevacizumab was determined to be -28 ^{0}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, Tg′ is also an event spanning several ^{0}C and can be assigned in different ways (Tonset , 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

References:
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