Optimisation of Lyophilisation Process Development in the Biopharmaceutical Industry
An Application of the Lyotherm3 Frozen State Solution Analyser

Introduction

Optimisation of the lyophilisation process starts with a deep understanding of the Active Product Ingredient (API) being used and its interaction with excipients. Knowledge of phase transitions occurring in the frozen state directly leads to efficiency and promotes stability during and after the lyophilisation process. One of the most powerful methods used to find these phase transitions is Zsinφ analysis, performed using the Lyotherm3.

The Lyotherm3 identifies the temperature that transitions occur at using variations in Zsinφ, a function of electrical impedance, and supplements this with differential thermal analysis (DTA). As Zsinφ is a metric of molecular mobility this is sensitive enough to identify protein folding and other small reorientation events.

As a study on this effect, an analysis of a substitute API, human serum albumin (HSA), was analysed, adding the excipient sucrose as a cryo-/lyo-protectant. HSA is a sample protein that has a serum half-life of 20 days without preservation and a molecular mass of 66.5 kDa – in the range of other potential biopharmaceutical APIs. A very low concentration was used to demonstrate the sensitivity of the Zsinφ technique.

Methodology

The sample solution was prepared with 199.7mg sucrose and 100µl HSA, and diluted into 20ml Analar water, an equivalent of 1% w/v sucrose and 0.5% v/v HSA. Two 3ml samples of this solution were pipetted into two stainless steel cuvettes and used to immerse the Zsinφ and sample temperature probes. A 3ml sample of Analar water was also pipetted into a stainless-steel cuvette and used to immerse the reference temperature probe.

The cuvettes were then placed into the cuvette recesses in the thermal block, and the thermal block lowered into 750ml of liquid nitrogen. Data sets were logged every 3 seconds during the experiment. When both probes reached below -100°C the integrated heating was turned on. Over the next 40 minutes the sample warmed and the Zsinφ and DTA were logged against sample temperature.

Results and Discussion

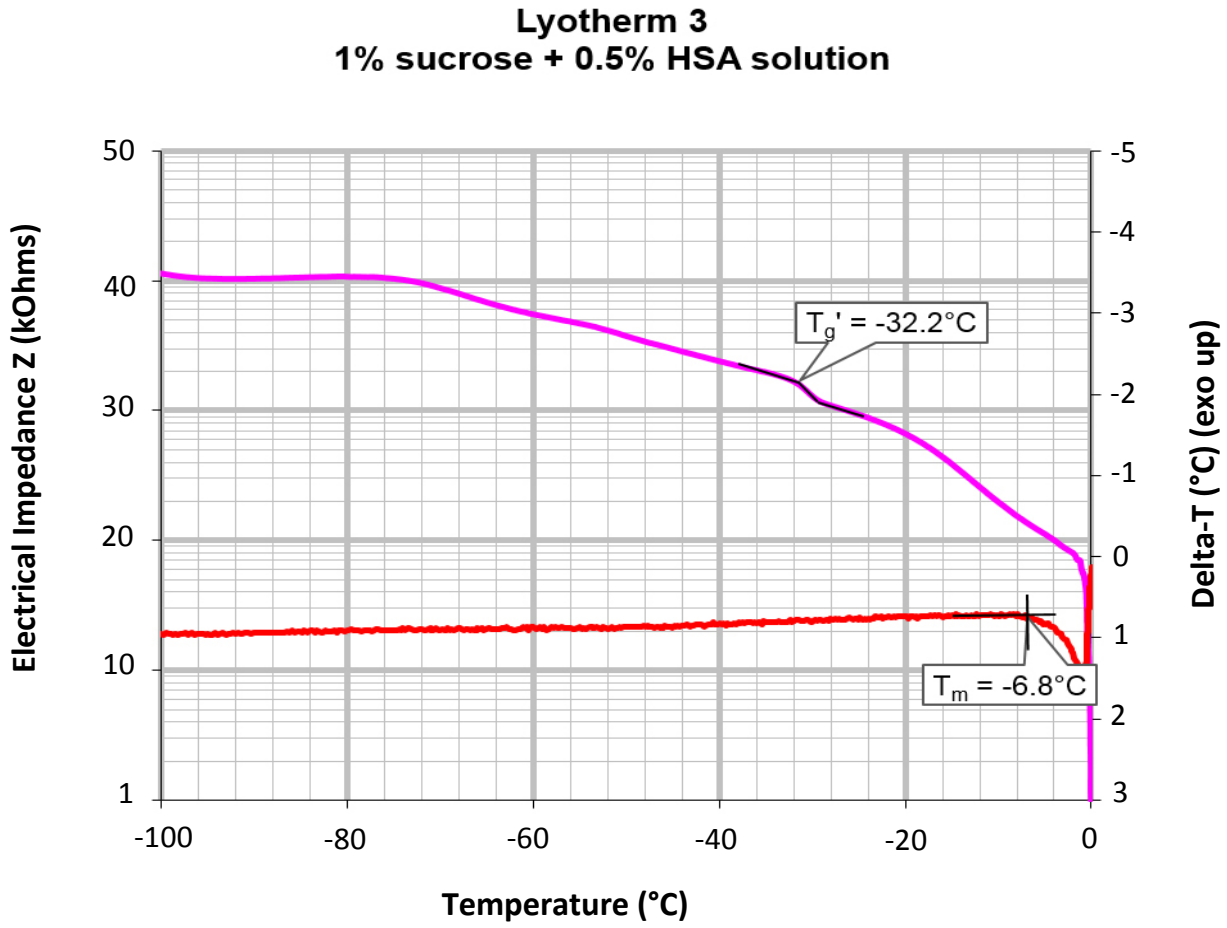

Figure 1: 1% sucrose and 0.5% HSA substitute biopharmaceutical solution

In Figure 1 there are two critical events that can be seen in the two analytical methods used. Using Zsinφ there is the clear signature of a frozen state glass transition (T_g') with an onset at -32.2°C. In DTA there is also the onset of the melt of the bulk ice at -6.8°C. Of the two, the glass transition could be a damaging transition and the preferable primary drying temperature would be -34°C to -39°C (giving a standard 2 - 7°C safety margin).

Conclusion

With such low concentrations of solute the evident glass transition is an example of the sensitivity of the Zsinφ technique, with the subtle isothermal reorientation that the glass transition represents not being visible on the DTA curve. The melt of the bulk ice crystals is also picked up in both Zsinφ and DTA.

Using the Lyotherm to investigate events that could compromise the integrity and activity of the post-lyophilised cake before the process begins can help optimise cycle time and efficiency. This can substantially reduce overheads and improve the reliability of process engineering efforts.

References

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2) Kevin Ward and Paul Matejtschuk (2010) The use of microscopy, thermal analysis and impedance measurements to establish critical formulation parameters for freeze-drying cycle development, In: L. Rey & J. May (eds.) Freeze-Drying / Lyophilization of Pharmaceuticals and Biological Products (3rd Edition), Informa Healthcare (New York / London), pp.112-135 (ISBN-13: 9781439825754)