Use of Dynamic Mechanical Analysis and Dynamic Scanning Calorimetry To Determine Critical Temperature Parameters for the Stabilization of Freeze-dried Biologicals

Kiran Malik, Chinwe Duru, Paul Matejtschuk
Standardization Science, NIBSC, A Centre of the Health Protection Agency,
Blanche Lane, Potters Bar, EN6 3QG, UK. email: kiran.malik@nibsc.hpa.org.uk

INTRODUCTION

Dynamic Mechanical Analysis (DMA) is a thermal technique used to measure the mechanical changes which occur in a product as temperature is raised or lowered in a controlled environment. In particular, in the application at NIBSC, the glass transition temperature (the temperature at which a rigid glass changes to a more mobile state) of a biological formulation to be freeze dried can be studied in order to better predict suitable freeze drying conditions. During primary drying, this temperature should not be exceeded in the product. Differential Scanning Calorimetry (DSC) is often used but can be unrevealing for complex formulations (Gearing et al 2010) . In this study we compared the use of DSC (Kett et al 2005) and DMA for the determination of glass transition (Tg') values for formulants commonly used in freeze drying. The influence of changes in formulation on the Tg' value has also been studied by sequential modification of each component to assess its impact. Selection of formulation to achieve a raised Tg' value is important for optimisation of freeze drying processes, as a rise of a few degrees may save many hours in drying time.

METHODS

All materials were prepared at NIBSC as lyophilized preparations and reconstituted with 1ml distilled water just before analysis.

Plasma (06/158)
5% Fetal calf serum in tissue culture medium (RPMI)
Factor IX formulated in 50mM Tris HCl, 150mM NaCl, 0.1% trehalose, 0.5% HSA (SS-071)

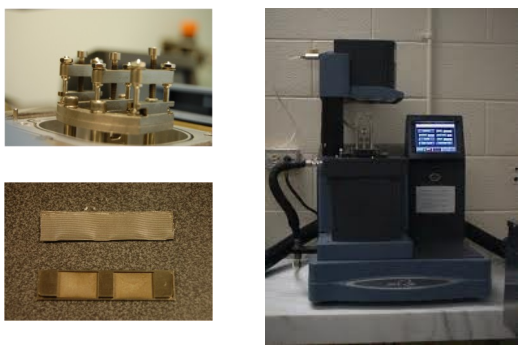
Multi-component excipient formulations (SS-155)
0.9% NaCl
0.9% NaCl and 10mg/ml human serum albumin
0.9% NaCl and 50mg/ml human serum albumin
0.9% NaCl and 10mg/ml trehalose
0.9% NaCl and 20mg/ml trehalose
0.9% NaCl, 20mg/ml trehalose, 10mg/ml human serum albumin
0.9% NaCl, 20mg/ml trehalose, 50mg/ml human serum albumin

DMA

Q800 DMA (TA Instruments, Elstree, UK) fitted with a liquid nitrogen cooling accessory was used for the analyses. Samples were loaded into the DMA using a steel pocket with a filter paper wick. The solution of interest was pipetted evenly to soak the wick. The lid was then placed over the steel pocket, and inserted into the DMA using the dual cantilever mode. Once an initial temperature of –70°C was achieved, a clamping torque of 6-7 in-lbs was applied.

DMA method used:

Method Log:
1: Motor drive: Off
2: Data storage: Off
3: Initial temperature: -70.00°C
4: Motor drive: On
5: Isothermal for 5.00 min
6: Data storage: On
7: Ramp 1.00°C/min to 20°C
8: End of method



DMA images. Top left: Dual cantilever clamp with fixed and moveable clamps, Bottom left: Sample holder for use with DMA-GCA (liquid sample placed inside on filter paper 'wick') and powder samples. Right: DMA instrument with furnace lid open

The storage modulus and Tan δ values were recorded and plotted against temperature for 1Hz, 5Hz, 10Hz. Analysis of profiles was performed using Universal Analysis software.

DSC

Solutions were loaded in 80µL aliquots into large volume sealed pans (TA instruments part number 900825902). Samples were analysed using StdFeb02 method on Q2000 calorimeter (TA instruments) using modulated DSC. Programmable heating and cooling rates were applied using a Refrigerated Cooling System (RCS-90). Profiles were expressed as heat flow and reverse heat flow(W/g) and the profiles analysed using Universal Analysis software.

DSC method used:
Method Log:
1: Isothermal for 2.00 min
2: Ramp 10.00°C/min to -70.00°C
3: Modulate +/- 0.23°C every 60 seconds
4: Isothermal for 8.00 min
5: Ramp 1.50°C/min to 25.00°C

References:
[1] J. Gearing, K.P Malik, P. Matejtschuk (2010) Use of dynamic Mechanical Analysis to determine critical transition temperatures in frozen biomaterials intended for lyophilisation. *Cryobiology* 61 (27-32)
[2] V.Kett, D. McMahon, K. Ward. (2005) Thermoanalytical techniques for the investigation of the freeze drying process and freeze-dried products. *Current Pharmaceutical Biotechnology*, **6** (3), 239-250

RESULTS

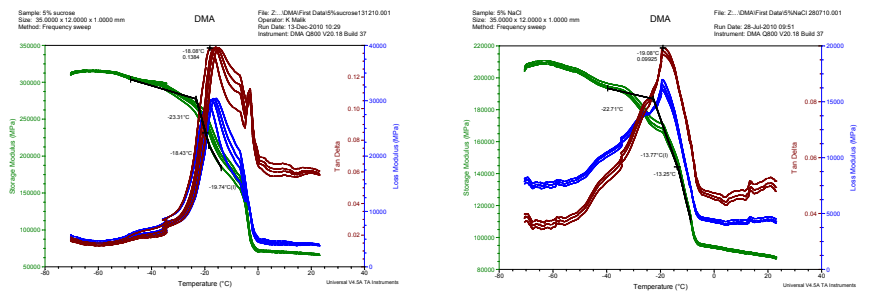


Figure 1a and b : DMA for sucrose at 1, 5, 10 Hz showing a shift in Tan δ Max with frequency. DMA for NaCl showed no significant shift in Tan δ Max with frequency

Sample	DMA Tan δ Max 1Hz (°C)	mDSC main transition (°C)
Plasma	-10	Not detected
5% Foetal Calf serum, tissue culture medium (RPMI)	-14 (shoulder -28.5)	-28 (eutectic)
Factor IX, 50mM tris HCl, 150mM NaCl, 0.1% trehalose, 0.5% HSA	-12(shoulder -27)	-26 (eutectic), -35 (weak Tg)

Table 1: Summary table of thermal properties of the biological reference materials studied (All values are means of three determinations)

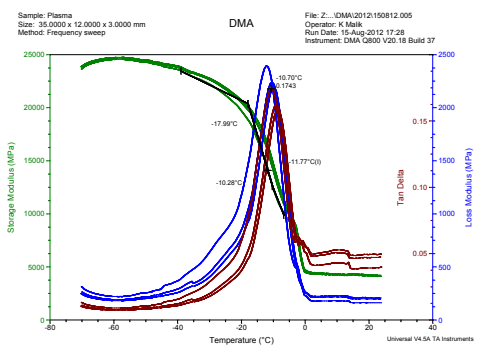


Figure 2: DMA of plasma at 1, 5, 10Hz. (Tan δ Max – 10°C 1Hz)

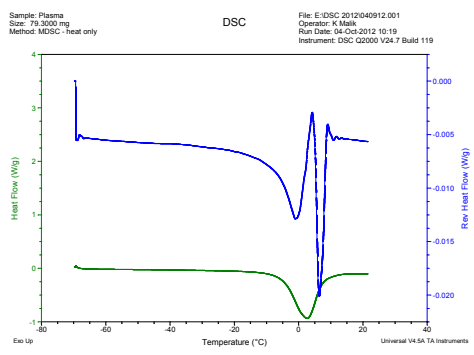


Figure 3: DSC of plasma. No transition detected

Sample	DMA Tan δ Max (°C)	mDSC main transition (°C)
0.9% NaCl	-24	-23 eutectic
0.9% NaCl, 10mg/ml HSA	-22	-25 eutectic
0.9% NaCl, 50mg/ml HSA	-7	-32 (Tg'), no eutectic
0.9% NaCl, 10mg/ml trehalose	-15	-41 (weak Tg'), -25
0.9% NaCl, 20mg/ml trehalose	-24	-23 eutectic
0.9% NaCl, 20mg/ml trehalose, 10mg/ml HSA	-14	-25 (weak Tg')
0.9% NaCl, 20mg/ml trehalose, 50mg/ml HSA	-10	-41 (weak Tg')

Table 2: Thermal properties of multi-component study samples

CONLUSIONS

DSC and DMA were used to determine transition temperatures for a range of commonly freeze dried biological materials.

Several DMA frequencies were studied, as for simpler systems it is possible to distinguish between glass transitions (Tg') and crystalline melts. Phase morphology changes such as Tg' are frequency dependent, whereas melts or crystallisation events are not.

Data presented in the poster indicated that Tg' were detected in multi-component samples more readily by DMA than by DSC although the transition values are usually at higher temperatures. The effects of adding of excipients was studied in terms of their influence on Tg' and crystallinity using both DMA & DSC.

DMA is a useful alternative technique when DSC is unrevealing for biological mater. We are investigating its use to predict shelf temperature during primary drying along with freeze drying microscopy. Both these methods tend to give higher critical temperatures than DSC.

ACKNOWLEDGEMENTS

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