APPLICATION NOTE DSC

ANALYSIS OF FORMULATIONS FOR FREEZE DRYING

1. Introduction

Differential Scanning Calorimetry (DSC) is arguably the preferred method of thermal analysis for freeze drying. DSC measures the heat flow in and out of a sample over a temperature range compared against a reference pan which is usually empty. Traditionally heat flow is plotted against time but, in order to make it easier to interpret it is often plotted against temperature (see figures 1-2 below). DSC provides useful data for storage applications for a variety of materials as well as the limit temperature for use of materials in a range of applications. These data include: melting (Tm, Teu), freezing, crystallisation and thermal transitions such as the glass transition (Tg'). They play a critical part in product development in a variety of industries such as fine chemicals, pharmaceuticals and biotechnology.

Freeze drying (lyophilisation) of products should be performed below the critical temperature of the formulation to reduce the risk of damage or defects occurring during the process. It is important to ensure that any amorphous components which can crystallise have fully crystallised to increase the critical temperature of the formulation. For example, when mannitol is heated above both its glass transition temperatures the product will crystallise.

2. Methods

Five formulations were prepared as per Table 1. All chemicals were sourced from Sigma Aldrich.

Formulation	Ingredient	Concentration	mg/ml
1	Mannitol	5%	50.49
2	Mannitol	2.5%	25.24
	Glycine	2.5%	25.55
3	Glycine	5%	50.42
4	Histidine	5%	50.38
5	Sucrose	0.4%	20.82
	Tris (HCl)	10mMol	1.13

20µL aliquots of each sample were analysed using a TA instruments Q100 modulated DSC (mDSC) with auto-sampler and with a Linkam DSC450 stage with LNP96 and T96 control units. The samples were analysed using the profiles in Table 2. DSC450 used steps 1-2 and mDSC sed steps 1-3.

Table 2 Analysis profile for both mDSC⋆ and DSC450.

Step	Ramping rate °C/minute	Limit temperature °C	Hold Time (minutes)
1	20	-70	5
2	5	20	0
3⋆	Modulations @ 3°C/minute (mDSC only)		

3. Results

MDSC analysis of amorphous mannitol showed a single crystallisation event on the Non Reversing Heat Flow line at -26.5°C and a peak at -23.5° C and a single melting event for ice at -2.6°C. DSC450 analysis of amorphous mannitol showed a single crystallisation event at -25.3°C and a peak at -24.2°C and a single eutectic melting event for mannitol at -3.0°C.

Table 3: Mannitol summary

	Method	mDSC	DSC450
A	Crystallisation Onset (°C)	-26.5	-25.3
	Crystallisation Peak (°C)	-23.5	-24.2
B	Eutectic Melt (°C)	-2.6	-3.0

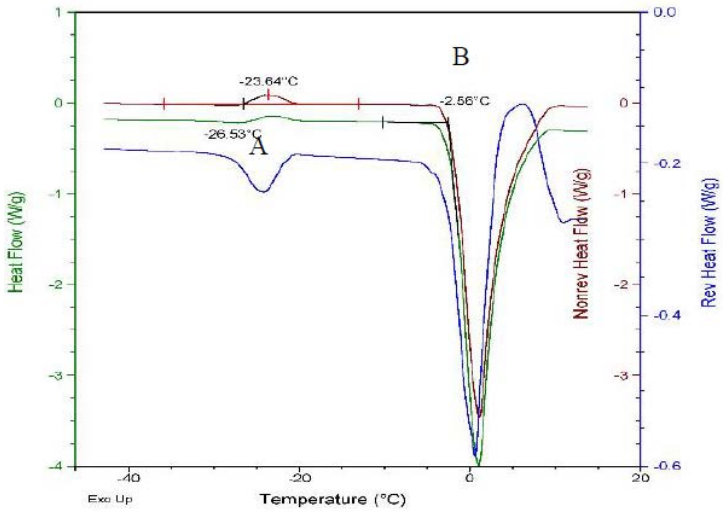

Figure 1:5% mannitol analysed by mDSC showing crystallisation (A) and eutectic melt of mannitol (B).

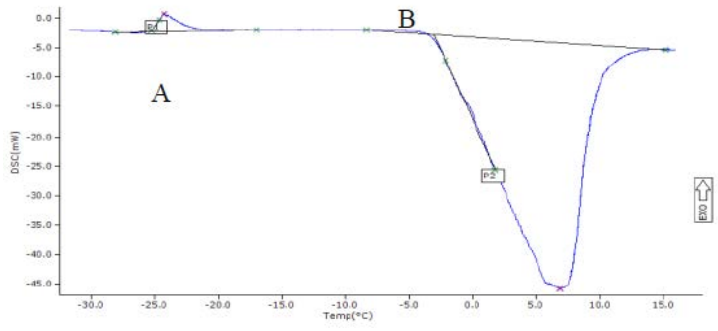

Figure 2: 5% mannitol analysed by DSC450 stage showing cystallisation (A) and eutectic melt of mannitol (B).

MDSC data for formulation 2 shows at -46.3°C the onset of the glass transition of glycine is observed.

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This MDSC data for formulation 2 shows at -46.3°C the onset of the glass transition of glycine is observed. This mobility change facilitates the crystallisation of glycine at -43.8°C. As the sample is heated a second glass transition, attributed to mannitol, is observed at -34.8°C which enables the crystallisation of mannitol at -34.1°C. DSC450 data for Formulation 2: The first crystallisation is observed at -43.7°C and a second crystallisation is observed at -33.5°C no evident glass transitions were observed using the DSC stage.

Table 4: Mannitol and glycine summary

Method	mDSC	DSC450
Glass Transition Onset (°C)	-46.3	Not observed
Glass Transition Mid (°C)	-43.3	Not observed
Crystallisation Onset (°C)	-43.8	-43.7
Crystallisation Peak (°C)	-40.1	-38.8
Glass Transition Onset (°C)	-34.8	Not Observed
Glass Transition Mid (°C)	-33.3	Not Observed
Crystallisation Onset (°C)	-34.1	-33.5
Crystallisation Peak (°C)	-30.9	-31.4
Eutectic Melt (°C)	-7.4	-6.5

MDSC of 5% glycine showed a melt around -4.1°C while DSC analysis supports this showing a melt around -4.9°C. No crystallisation events are observed in either method.

Table 5: Glycine summary

Method	mDSC	DSC450
Eutectic melt (°C)	-4.1	-4.9

MDSC of formulation 4 (figure 2) showed one glass transition of histidine at -47.9°C with a midpoint at -45.7°C. A second glass transition is seen starting at -30.6°C with a midpoint at -29.1°C before a crystallisation starting at -26.7°C with a peak at -20.6°C. DSC450 (figure 1) shows a glass transition starting at -49.8°C with a midpoint at -48.3° C. A crystallisation is observed starting at -29.3°C with a peak at -24.6°C.

Table 6: Histidine summary.

	Method	mDSC	DSC450
A	Glass Transition Onset (°C)		
	Glass Transition Mid (°C)		
B	Glass Transition Onset (°C)		
	Glass Transition Mid (°C)		
C	Crystallisation Onset (°C)		
	Crystallisation Peak (°C)		

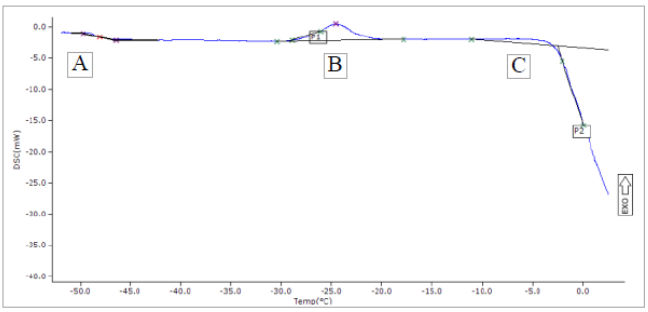

Figure 3: DSC450 analysis of 5% histidine – showing Tg' (A), crystallisation (B) and eutectic melt (C).

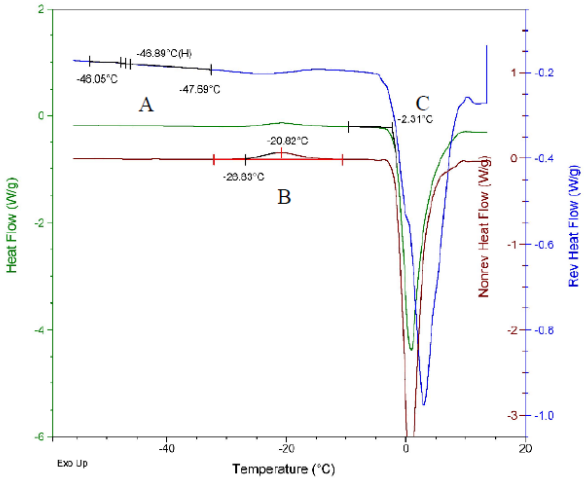

Figure 4: mDSC analysis of 5% histidine - showing Tg' (A), crystallisation (B) and eutectic melt (C).

MDSC of formulation 5 shows a glass transition starting at –36.2°C with a midpoint at –34.1°C and the onset of melt at -1.8°C. Instead, DSC450 shows the onset of melt at -1.9°C with no glass transition visible.

Table 7: Sucrose tris summary

Method	mDSC	DSC450
Glass Transition Onset (°C)	-36.2	Not observed
Glass Transition Mid (°C)	-34.1	Not observed

4. Discussion

For samples with overlapping events (histidine, mannitol, glycine) mDSC was able to separate the glass transition from the crystallisation whereas the DSC stage could only do this in the case of resolved events. Both methods showed good levels of agreement with the temperatures of the events observed. The onset temperatures were typically within a few points of a degree (max 2.6°C, min 0.1°C). Typical analysis time for the two methods was 72 minutes for mDSC and 25 minutes for DSC450. Both instruments showed analogous data for each transition however, the ability of the mDSC to separate the Reversible and Non-Reversible transitions meant that additional transitions were identified that could not be observed by DSC alone.

5. Conclusion

DSC450 is a useful tool to determine critical events for frozen samples quickly and cheaply although some sensitivity may be compromised. The ability of mDSC to be able to resolve overlapping peaks is important for freeze drying development but the cost and analysis time can be the limiting factor for routine and high throughput analysis.

6. References

[1] R. V. N. P. A. e. a. Cavatur, "Crystallization Behavior of Mannitol in Frozen Aqueous Solutions," vol. 19, p. 894, 2002.