



Development of a freeze-drying protocol for model protein Lactate Dehydrogenase

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OBJECTIVES

1. To use a range of thermal and analytical techniques to evaluate the applicability of a range of excipient formulations containing lactate dehydrogenase (LDH)
2. To determine whether any correlation exists between the key parameters determined by these methods with respect to freeze-drying behavior and final product qualities

INTRODUCTION

Freeze-drying (lyophilization) is a process in which a solvent, usually water, is frozen and then removed by sublimation. Freeze-drying is often used in the manufacture of therapeutic proteins due to the sensitivity of the active ingredient. However there is a risk of protein degradation in-process and during storage¹. Excipients are added to formulations to prevent processing defects. These typically provide thermal stability, bulking properties and activity conservation. Examples of excipients include disaccharides, amino acids, polymers and non-ionic surfactants².

MATERIALS

Dextran (mw 9500Da) glucose, mannitol, sucrose and trehalose were obtained from Sigma (analytical grade). LDH (also Sigma) was obtained as an ammonium sulfate suspension and dialyzed prior to formulation. The LDH activity assay was based on the spectrophotometric method of Henry, et al³ (ThermoTrace, Australia). Dextran and mannitol were used as excipients at 2%w/v, either alone or in combination with 1%w/v glucose, sucrose or trehalose. The solutions were analyzed using modulated temperature DSC (MTDSC), Freezing resistance analysis (FRA), differential thermal analysis (DTA) and freeze drying microscopy (FDM). Freeze dried products were analyzed using MTDSC and thermogravimetric analysis (TGA). Reconstituted samples were analyzed for retained protein activity (details below).

METHODOLOGY

Freeze-drying
 1ml portions of the solutions were dispensed into freeze-drying vials, stoppered and freeze dried using a VirTis Genesis 12EL freeze-dryer. The solutions were frozen to -40°C and held for 1hr. The pressure was decreased to 200mT, and shelves heated gently to 10°C and held for 10hrs. Pressure was reduced to 100mT, shelves set to -10°C, held for 11hrs. Pressure was further reduced to 50mT and temperature increased to 20°C, held for 15hrs.

MTDSC

Samples of solutions were analyzed using MTDSC (TA Instruments DSC2920). Samples were cooled at 2°C/min from 20°C to -60°C and reheated at the same rate. The modulation period was ±0.4°C over 60s. Freeze-dried products were also analyzed using MTDSC over the range 20-200°C (same parameters as previously).

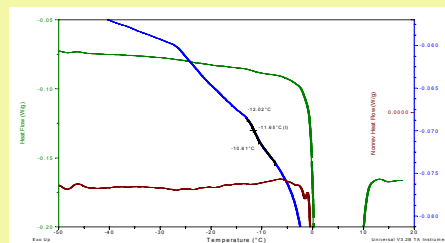


Figure 1 MTDSC of 2%w/v dextran solution showing Tg'

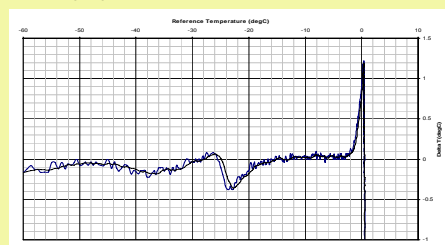


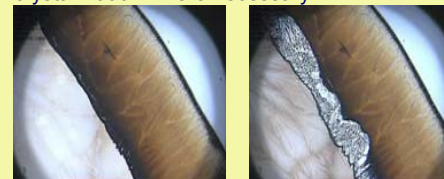
Figure 2 DTA of 2% mannitol-1% glucose (w/v) showing thermal events

FRA and DTA

FRA and DTA analyses were performed as follows (using a Biopharma Technology Ltd *Lyotherm*). Samples were cooled using liquid nitrogen to 150°C, before being placed in a pre-warmed heating block which was supplied with a constant heat input until the temperature reached 0°C, monitoring sample and reference temperatures (together with resistance for FRA). Analytical grade water was used as the DTA reference material.

FDM

Freezing and freeze-drying behaviors of solutions were analyzed using a Biopharma Technology Ltd *Lyostat2* FDM). Samples of solutions (1-2µl) were cooled to -20°C or below, before the pressure was reduced and sample temperature adjusted in order to observe collapse of sample while drying, with annealing used to encourage solute crystallization where necessary.



Figures 3a & b showing 2%w/v dextran at -11.4°C and -9.1°C respectively

Excipients	Collapse range (°C)	FRA events (°C)	DTA events (°C)
Mannitol	-11.2 to -9.3	-10.0	-26, -11
Mannitol-glucose	-42.0 to -26.4	-15	-26, -15
Mannitol-sucrose	-20.0 to -16.7	-16	-26, -20
Mannitol-trehalose	-18.5 to -15.9	-17	-28, -24
Dextran	-12.4 to -10.9	-28	-30
Dextran-glucose	-18.9 to -12.8	-13	-20, -8
Dextran-sucrose	-16.5 to -14.5	-7	-15, -7
Dextran-trehalose	-14.2 to -6.2	-15	-20, -12

Table 1 showing a range of solution properties for the various formulations

RESULTS

The sub-ambient Tg' of the solutions was clearly observed using MTDSC (Figure 1) and other thermal events were analyzed using DTA (Figure 2). These generally correlated with collapse events observed using FDM (Figures 3a & b). A range of pre-lyophilization properties for some of the mixtures used here are shown in the Table 1.

The protein activity assay indicated that dextran and mannitol by themselves do not protect LDH. However, including sugars had a lyoprotective effect (Figure 4).

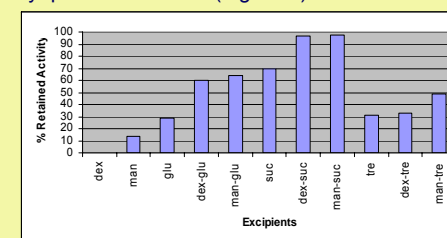


Figure 4 Effect of inclusion of low mw weight saccharides to dextran and mannitol on maintained protein activity

CONCLUSIONS

1. There was a general correlation between Tg' and collapse range for many of the solutions studied, with some differences that we attribute to a dependence on the mechanical strength of the excipients used.
 2. Excipients with favorable thermal or bulk properties did not necessarily maintain protein activity.
 3. However, these data indicate that formulations containing sucrose performed the best overall.
- Statistical analysis and rationalization of these and other properties for a range of formulations is ongoing.

REFERENCES

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3. Henry RJ et al. (1960) *Am. J.Clin.Path.* 34:381-383.