



## Rationalising the freeze-drying of protein formulations: Comparison of a series of pre- and post- lyophilisation properties

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### INTRODUCTION

Freeze-drying (lyophilisation) is a unit operation in which a solvent, usually water, is frozen and then sublimed in a vacuum. It is commonly used in the pharmaceutical industry when there are stability issues with the active ingredient in solution, as is often true for proteins<sup>1</sup>. In order to prevent processing defects during freeze-drying, active ingredients are formulated with excipients, which may serve specific functions, such as providing bulk properties, thermal stability and activity preservation to the product. Many groups of molecules have been shown to perform these functions, including disaccharides, amino acids, polymers and non-ionic surfactants<sup>2</sup>. The aim of the present study was to evaluate the pre- and post- lyophilisation properties of a large series of protein-exci-pient combinations and to evaluate them on the basis of retained protein activity and other qualitative and quantitative analyses.

### MATERIALS AND METHODS

Dextran (mw 9500kDa) glucose, mannitol, sucrose and trehalose were obtained as analytical grade from Sigma. Lactate dehydrogenase (LDH, also Sigma) was obtained as an ammonium sulphate suspension and dialyzed prior to formulation. The LDH activity assay kit was based on the spectrophotometric method of Henry, et al<sup>3</sup> and was obtained from 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 MTDSC, DTA and freeze drying microscopy (details below).

Aliquots (1ml) of the solutions were dispensed into freeze-drying vials, partially 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, and shelves lowered to -10°C and held for 11hrs. Pressure was further reduced to 50mT and temperature increased to 20°C and held for 15hrs.

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. Freezing resistance analysis and DTA were carried out using a *BTL Lyotherm* (Biopharma Technology Ltd)

Freeze-drying behaviour of the samples was observed using a freeze-drying microscope (*BTL Lyostat2*, Biopharma Technology Ltd., UK). Samples of solution (1-2ul) sandwiched between cover slips 75um apart were cooled to -20°C or below, before pressure was reduced and sample temperature adjusted in order to observe eutectic melting or collapse of the sample while drying, with annealing used to encourage solute crystallisation where necessary.

Freeze dried products were analyzed using MTDSC (same modulation as previously) and TGA over the range 20-200°C. Reconstituted samples were analyzed for retained protein activity.

### RESULTS

Thermal events in the frozen solutions were clearly observed using MTDSC (Figure 1). These generally correlated with collapse events observed using FDM (Figures 2a & b). Dry state transitions were also yielded by MTDSC (Figure 3).

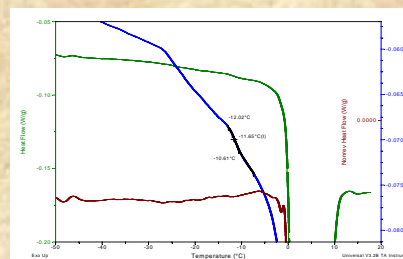


Figure 1 MTDSC of 2%w/v dextran solution showing Tg'



Figure 2a & b Dextran at -11.4°C and -9.1°C respectively

A range of pre- and post- lyophilisation properties for some of the mixtures used here are shown in the Table below.

Excipients	Collapse range (°C)	Product Tg (°C)	Water content %
Mannitol	-11.2 to -9.3	107.39	0.46
Mannitol-glucose	-42.0 to -26.4	82.43	2.28
Mannitol-sucrose	-20.0 to -16.7	119.42	2.5
Mannitol-trehalose	-18.5 to -15.9	99.01	1.90
Dextran	-12.4 to -10.9	112.01	4.83
Dextran-glucose	-18.9 to -12.8	103.02	2.94
Dextran-sucrose	-16.5 to -14.5	102.77	3.94
Dextran-trehalose	-14.2 to -6.2	136.05	3.98

The results of the protein activity assay indicated that dextran and mannitol by themselves do not protect LDH. However, including sugars had a lyoprotective effect (Figure 4) with sucrose performing the best in both cases.

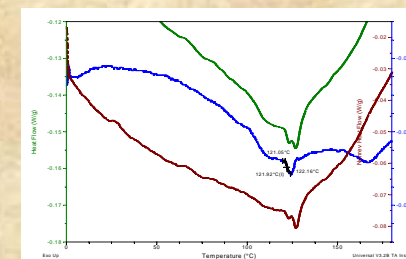


Figure 3 MTDSC of freeze-dried dextran (2%w/v) showing Tg

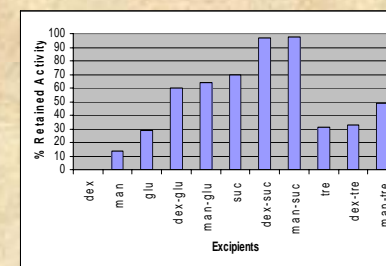


Figure 4 Effect of Inclusion of low mw saccharides to dextran and mannitol on maintained LDH activity

### CONCLUSIONS

Initial results confirm expectations that while excipients with favourable thermal or bulk properties impart certain qualities to a formulation, they do not necessarily maintain protein activity. Statistical analysis and rationalisation of these and other pre- and post- lyophilisation properties for a range of formulations is currently ongoing.

### REFERENCES

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