An investigation using thermal and microscopic analysis to determine an optimal freeze-drying protocol for lyophilized proteins

Debra L. M. McMahon[1,2], Duncan Q. M. Craig[1], Vicky L. Kett[1], Kevin R. Ward[2]
[1]School of Pharmacy, Queen's University Belfast, 97 Lisburn Road, Belfast, UK
[2]Biopharma Technology Ltd, Biopharma House, Winnall Valley Road, Winchester, UK

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[1]. 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[2]. The aim of this study was to evaluate the pre- and post- lyophilisation properties of a large series of protein-excipient combinations using different analysis methods. These success of these mixtures were evaluated 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 sulfate suspension and dialyzed prior to formulation. The LDH activity assay kit was based on the spectrophotometric method of Henry, et al[3] 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).

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, and shelves lowered to -10°C, held for 11hrs. Pressure was further reduced to 50mT and temperature increased to 20°C, 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 of samples were analyzed using a Biopharma Technology Ltd *Lyotherm*. Experiments were performed as follows with distilled water as the reference liquid. Samples were cooled using liquid nitrogen to 150°C. Then they were placed in a pre-warmed heating block were it was supplied with a constant heat input until the temperature reached 0°C.

Freezing and freeze-drying behavior of samples of solution (1-2ul), were observed using a *Lyostat2*(Biopharma Technology Ltd). Experiments were performed by cooling samples to -20°C or below, then 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.

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

The collapse range of the solutions are clearly observed using FDM (Figures 1a & b). These generally correlated with Tg' and other thermal events in the frozen solutions observed using MTDSC (Figure 1) and DTA (Figure 3).

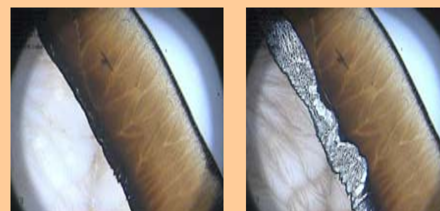

Figure 1a & b Dextran at -11.4°C and -9.1°C respectively

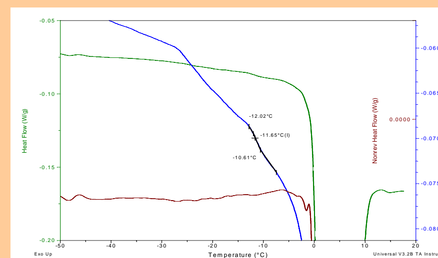

Figure 2 MTDSC of 2%w/v dextran solution showing Tg'

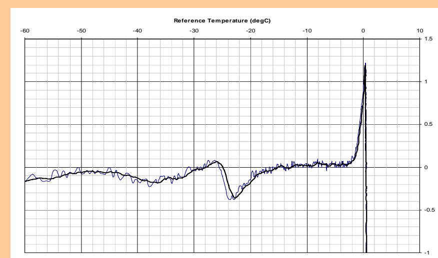

Figure 3 DTA of 2% mannitol-1% glucose (w/v) showing thermal events

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.

A range of pre-lyophilisation properties for some of the excipient combinations used here are shown in the Table below.

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

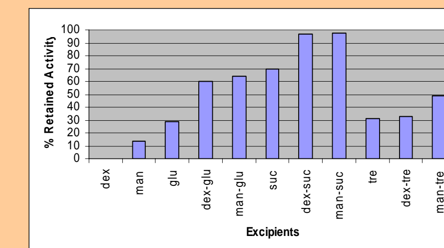

Figure 4 Effect of Inclusion of low mw saccharides to dextran and mannitol on maintained LDH activity

CONCLUSIONS

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

REFERENCES

1: MJ Pikal. *BioPharm*, 3(8):18-27 (1990).
2: JF Carpenter, MJ Pikal, et al. *Pharm Res.* 14(8):969-975 (1997).
3: RJ Henry et al *Am. J.Clin.Path.* 34:381-383 (1960)