APPLICATION NOTE

Investigating the Effect of Glycerol on the Mechanical Properties of Freeze-Dried Model Biotherapeutic Formulations

biopharma group
intelligent freeze drying

Introduction

Freeze-drying (lyophilisation) is a common method of stabilization for protein-based therapeutics and vaccines. While lyophilisation is considered more gentle than other drying methods, proteins are often formulated with amorphous saccharides or polymers, which can serve not only to prevent denaturation during processing but also as a rigid matrix that provides some physical stabilization in the dried state. However, crystalline bulking agents such as mannitol are also commonly included within freeze-dried formulations to produce an aesthetically pleasing cake with good structure and appearance.

Glycerol can act as a plasticiser, reducing the glass transitions temperature of formulations when present in the frozen and dried state, and has been observed to impede the freeze-drying process in several cases. One example of this is in polymerase chain reaction (PCR) reagents used for rapid medical diagnostic tests; here, individual components of a 'Mastermix' are typically supplied as concentrates in glycerol-rich solutions where, even after mixing individual components, the glycerol still persists in the Mastermix itself. There is, however, some evidence that low levels of glycerol can act as a lyoprotectant when employed in combination with other excipients, hence its inclusion in the present study.

The MicroPress is an instrument that can quantitively determine the strength and physical characteristics of freeze-dried cakes in-situ. With a number of selectable parameters and methods, the MicroPress will be able to analyse your freeze-dried cake structure. This allows for fast and effective batch screening to be applied to your products, especially when time and personnel costs are at such a premium; with a standard method the analysis can be completed in less than one minute per freeze-dried cake. With just a small number of samples, the MicroPress is able to provide pivotal data as to whether a product is likely to withstand the rigours of handling and shipping from manufacturing site to the point of use. While the MicroPress analysis will leave a small indentation on the cake surface, the cakes can still be used for other types of analysis such as Karl-Fischer and DSC to provide yet more information.

Materials & Methods

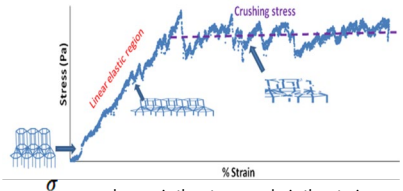
Sixty-four different formulations (Table 1) containing different concentrations of excipients were freeze-dried using a conservative freeze-drying cycle. The mechanical properties and robustness of the resulting freeze-dried cakes were probed using the MicroPress (Fig. 1), which uses a linear actuator to gently compress the cake while a load cell measures the force applied.

Table 1: Formulations and possible combinations of excipients explored during a conservative freeze-drying cycle

Formulations	BSA (%w/v)	Lysozyme (%w/v)	Mannitol (%, w/v)	Dextran (%, w/v)	Sucrose (%, w/v)	Glycerol (%, w/v)
1 to 16	1	n/a	0, 1, 2, 3	n/a	0, 1, 2, 3	0, 0.2, 0.4, 0.6
17 to 32	1	n/a	n/a	0, 1, 2, 3	0, 1, 2, 3	0, 0.2, 0.4, 0.6
33 to 48	n/a	1	0, 1, 2, 3	n/a	0, 1, 2, 3	0, 0.2, 0.4, 0.6
49 to 64	n/a	1	n/a	0, 1, 2, 3	0, 1, 2, 3	0, 0.2, 0.4, 0.6

Fig. 1: MicroPress (inset showing indenter in vial)

Fig. 2: Example of a stress/strain profile



$E = \frac{\sigma}{\varepsilon}$ where σ is the stress and ε is the strain, and E is the Young's /

Freeze-dried Cake Formulations

A total of 64 formulations were freeze-dried, half of which contained 1%, (w/v) BSA, and the other half containing lysozyme at the same concentration in 6mM phosphate buffer at pH 7. These model proteins were combined with mannitol, dextran, sucrose and glycerol at different concentrations as shown in Table 1. Of the 64 formulations, 38 collapsed during the conservative freeze-drying cycle, with primary drying conducted at a shelf temperature of -45°C until ice sublimation was deemed complete. Formulations that contained 0.2 % (w/v) glycerol or higher underwent collapse except for the BSA formulations containing mannitol higher than 2% (w/v) and sucrose lower than 1% (w/v), and two lysozyme formulations that contained dextran at 2% (w/v) and 3% (w/v), sucrose at 0% (w/v) and 1% (w/v), and glycerol at 0.2% (w/v).

Fig. 3: 1 % BSA, 2% Mannitol, and 1 % Sucrose increasing concentration of Glycerol

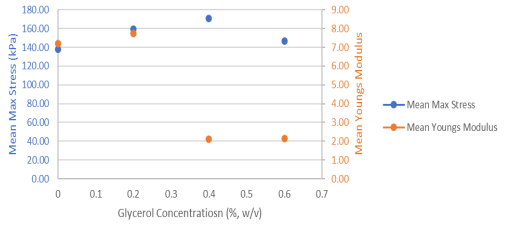
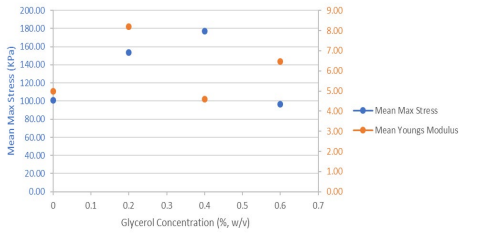


Fig. 4: 1 % BSA, and 3% Mannitol, increasing concentration of Glycerol



MicroPress Results

The mean max stress and Young's Modulus of two formulations with increasing concentrations of glycerol present are shown in Fig. 3 & 4. As the glycerol content increases, the max stress of formulations increases until 0.4% glycerol is achieved; however, with 0.6% glycerol, a decrease in max stress is observed. Conversely, in formulations containing 1% BSA, 2% Mannitol, and 1% Sucrose, the Young's Modulus decreased at >0.2% glycerol, indicating lower stiffness (greater elasticity). The mean max stress of several formulations with 0% and 0.2% glycerol present were compared as shown in Fig. 5. The inclusion of 0.2% glycerol in all of the formulations increased the mean max stress of the formulations after freeze-drying, provided they did not collapse during the freeze-drying process itself. When comparing the shape of the stress-strain curves of the formulations, it was observed that the inclusion of glycerol resulted in a smoother curve, suggesting lack of micro-fractures during compression of the cake structure as shown in Fig. 6.

Fig. 5: Mean max Stress of Formulations with and without glycerol present

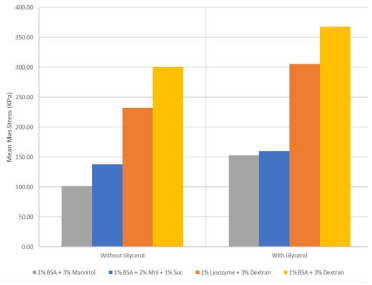
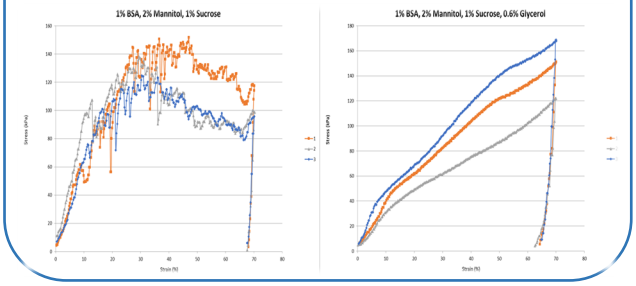


Fig 6: Stress Strain Profiles of 1% BSA, 2% Mannitol, 1% Sucrose, with/without 0.6% glycerol present



Conclusion

The influence of a number of excipients in varying proportions on the mechanical properties of freeze-dried formulations of two model proteins was investigated by MicroPress. MicroPress was able to identify the strength and stiffness clearly and quantitatively (Young's Modulus) of the cakes. This process allows for a much more exact result and therefore a greater certainty as to whether lyophilised cakes will remain robust throughout transport and handling. It was found glycerol can have a positive impact on the mechanical properties of the final freeze-dried cakes, provided they do not undergo collapse during the lyophilisation process itself, even though the additional of glycerol typically has a negative impact on the thermal properties of the frozen formulations during the freeze-drying process.