APPLICATION NOTE

MICROPRESS

HOW ROBUST ARE YOUR CAKES?

Rationale

The use of freeze-drying (Lyophilisation) for labile drugs has been a staple in pharmaceutical world for over 40 years. However, this comes with several challenges, one of which is to produce strong and durable FD cakes that can withstand downstream processing after freeze drying and during transport. Qualitative analysis has been used on freeze dried cakes, to identify whether the cake appearance is stable; but this is a subjective test.

The MicroPress is an instrument which can quantitively determine the strength and physical characteristics of freeze-dried cakes in-situ. With set parameters and analysis 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.

MicroPress is able to provide pivotal data as to whether or not the products produced will be able to survive the potentially destructive process of shipping from manufacturing site to clinical sites, or even to your local pharmacy. 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. A small batch will be needed to provide quantitative data on how likely, or not, the freeze-dried product will survive transport.

Dextran and mannitol, two very different excipients can be used as bulking agents or thermal stabilisers in freeze dried formulations. Post freeze drying, dextran and mannitol both produce a white cake, and look similar in structure, likely passing visual inspection after production by quality control. However, freeze dried cakes containing these excipients may have very different physical characteristics which could affect the appearance of the cakes after transportation, and also their rate of reconstitution. Examples of freeze-dried mannitol and dextran cakes are shown in Figure 1.

Figure 1 Lyophilised Cakes. Left Mannitol, Right Dextran

Method

The sample solutions were prepared as reported in Table 1, by weight and diluted into 100ml AnalaR water. All chemicals sourced from Sigma Aldrich. 6 ml vials were used with a 2 ml fill in each.

Table 1 Concentrations of the starting solutions

Sample	Concentration	Formulation
Mannitol	10mg/ml	1
	20mg/ml	2
	30mg/ml	3
Dextran	10mg/ml	4
	20mg/ml	5
	30mg/ml	6

These vials were freeze dried with the method shown in Table 2. Thermal treatment aAll samples were analysed using the same set of parameters allows for all the samples to be frozen and for crystal size to increase before moving on to the primary drying step. During the primary drying stage the pressure is reduced to encourage sublimation of the ice to dry the product more quickly. All the samples were placed on the same tray in the freeze-dryer, to control the variables seen during the drying process.

Table 2 Recipe used on VirTis Freeze-dryer

Thermal Treatment				
Step	Temp	Time	Vacuum	R/H
1	20	5	Off	H
2	-40	120	Off	R
3	-40	120	Off	H
		Drying		
Step	Temp	Time	Vacuum	R/H
1	-4	600	100	H
2	0	80	100	R
3	0	2700	100	H
4	20	40	50	R
5	20	720	50	H

All samples were analysed using the same set of parameters on the MicroPress; with a user-friendly software design the parameters are easily set and can be changed as required.The parameters from this experiment can be seen in Table 3.

Table 3 The stages and the corresponding speeds

Stage	Velocity (mm/s)
Extend	10
Seek	0.1
Compress	0.05
Decompress	0.05
Home	-

The Extend stage moves the indenter at a velocity of 10mm/s to within 5mm of the estimated cake height. The Seek phase finds the top of the cake; as soon as force is detected the Compress stage starts and the force applied to the cakes is then recorded.

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Results

As can be seen from Figure 2, 3% dextran shows elastic properties, which is demonstrated in the Figure 2 by the curve returning from 40% back to 18% strain during the decompression stage. Figure 3 depicts the results from the strongest 3% mannitol cake, which shows a very different graph to 3% dextran. Figure 3 shows a brittle cake which cannot withstand much stress before the cake breaks.

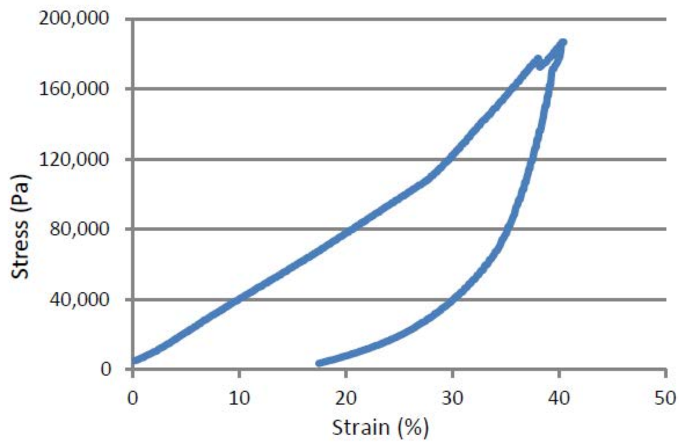

Figure 2 Graph of 3% dextran obtained using the MicroPress

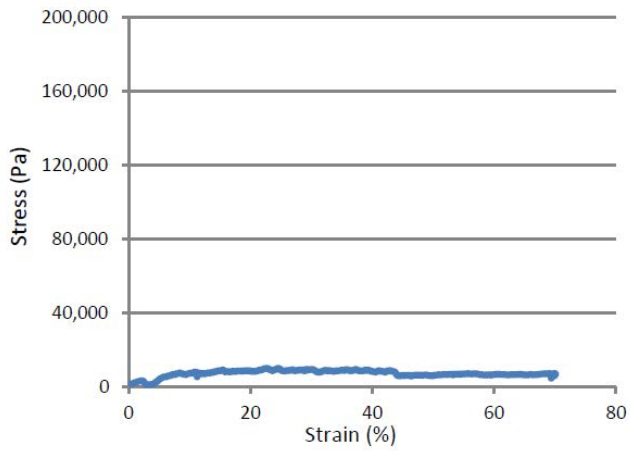

Figure 3 Graph of 3% Mannitol obtained using the MicroPress

From 1% Mannitol to 2% Mannitol there is a near ten-fold increase in the Youngs Modulus (E) from the weakest to the strongest as shown in Figure 4. This increase in strength does not continue up to 3% from 2% Mannitol, the average Youngs Modulus of the 3% Mannitol cakes decreases. However, there is an increase in the standard deviation therefore the variation between the samples is higher.

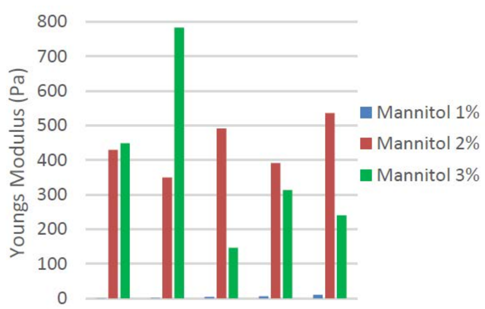

Figure 4 Average Youngs Modulus of mannitol at three concentrations, 1% (blue), 2% (orange) and 3% (green)

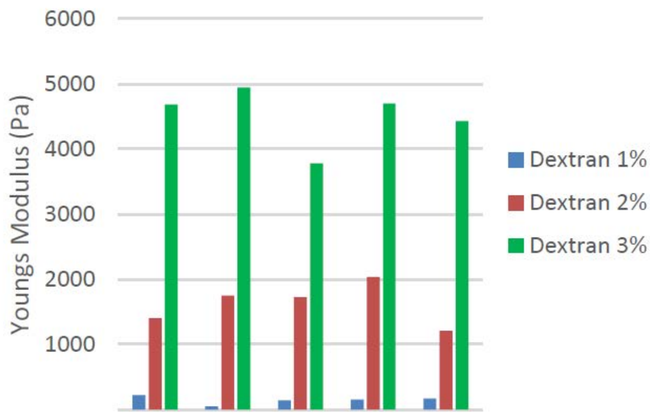

Figure 5 Average Youngs Moduluis of dextran at three concentrations, 1% (blue), 2% (red) and 3% (green)

Figure 5 the results from the increasing the concentration of dextran produces a much stronger cake than mannitol did, seen previously. From 1% Dextran to 3% Dextran there is an increase of 107% in the Youngs modulus of the cakes, therefore the amount of force needed for deformation to occur increases. The Max. stress which could not be determined due to the overall strength of the cake exceeding the limits of the MicroPress. However, this can be corrected with a smaller diameter indenter allowing for more force to be applied, before the max stress of the cakes can be reached.

Table 4 Average of the results from 5 samples of each formulation

Excipient	Mean E (Pa)	SD E	Mean Max Stress (Pa)	SD Max Stress
Dextran 1%	137.4	89.4	13745	4627.5
Dextran 2%	3626.3	1060.6	178419	18600.5
Dextran 3%	4506.5	445.0	187017	431.8
Mannitol 1%	4.6	4.2	360	51.2
Mannitol 2%	439.5	74.8	6417	990.8
Mannitol 3%	386.7	247.8	6009	2585.8

Discussion

It can be seen from the results that the MicroPress is able to determine the Youngs Modulus of the cakes and the max stress the cakes are able to withstand before failure. It can be seen that the better-looking cake, Mannitol, has weaker properties than the Dextran. The Youngs Modulus and Max stress also correlate to the concentration of the excipients within the cakes. The higher the concentration the stronger the cakes will be. The relative lack of robustness and strength of mannitol may be related to a number of factors including pore size and polymorphism.

Conclusion

MicroPress has clearly and quantitatively identified the strength and physical properties of the cakes. This process allows for a much more exact result for the strength of cakes and therefore a greater certainty lyophilised cakes will stay robust throughout transport and handling. These results confirm that mannitol is much weaker than dextran and therefore using mannitol as an excipient to increase cake strength may not be advisable. Good appearance doesn't mean strength.