Application note:
How does Micro Collapse affect Lyophilised Cake Strength?

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Introduction

During freeze drying, products should be maintained below their critical temperatures, such as glass transition ($T_{g'}$), eutectic melt (T_{eu}), and collapse (T_c), to ensure a safe and robust cycle and to reduce the risk of detrimental defects exhibited in the product post freeze drying. Defects can include, but are not limited to, physical collapse or micro collapse within the product, loss of activity and a high moisture content.

Degree of micro collapse is one of the most challenging defects to quantify with many formulations failing at the first cycle due to loss of activity of the drug substance.

The *MicroPress* is an innovative instrument fully developed by Biopharma to quantify in-situ microscopic defects within low density materials and, specifically, in all freeze-dried products. For example, mannitol is often used in formulations as a bulking agent and has a high critical temperature which can mask collapse of products with a significantly lower critical temperature. Glucose has a critical temperature around -41°C and, when combined with mannitol, collapses if dried above the critical temperature.

However, due to the bulking nature of mannitol, the mixture has a good appearance once freeze dried despite possible structural defects. The degree of micro collapse and the effect of cake structure can be determined by changing the concentration of glucose whilst keeping the concentration of mannitol constant. Often the cycles used to freeze-dry this solution would not allow the glucose to freeze dry sufficiently due to the high temperature and therefore collapse may occur. In contrast, the mannitol would dry a sufficient amount to keep the cake structure. The resulting, seemingly unnoticeable, micro collapse however, would affect resuspension as well as stability and activity over the time of the drug substance itself.

Method

The sample solutions were prepared as reported in Table 1. All chemicals sourced from Sigma Aldrich. 6 ml vials were used with a 2 ml fill.

Sample	Concentration	Formulation
Glucose **Mannitol**	5 mg/ml 20mg/ml	1
Glucose **Mannitol**	10mg/ml 20mg/ml	2
Glucose **Mannitol**	20mg/ml 20mg/ml	3

Table 1 Concentrations of the starting solutions

These vials were freeze dried with the method shown in Table 2. Thermal treatment allows for all the samples to be frozen and for crystal size to increase allowing for the samples to go onto the next stage, drying.
During the drying stage the pressure is reduced to allow for sublimation of the ice to occur and dry the product. All the samples were placed on the same tray in the freeze-dryer, to control the variables seen during the drying process.

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	0	40	50	R
5	20	720	50	H

Table 2 Recipe used on VirTis Freeze-dryer

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 to suit any requirements. The parameters set can be seen in Table 3.

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

Table 3 The stages and the corresponding speeds

The **Extend** stage has a velocity of 10mm/s to within 5mm of the estimated cake height. The **Seek** phase find the top of the cake, as soon as force is felt the **Compress** stage starts and the force applied to the cakes is then recorded.

Results

Table 5 below show the results obtained from the formulations analysed. Formulation 1 had the highest Young's modulus so is therefore the strongest cake.

Formulation	Mean (kPa)	SDE	Mean Max Stress (kPa)	SD Max Stress
1	0.969	0.186	11.565	3.236
2	0.507	0.205	8.315	2.213
3	0.085	0.109	2.797	0.220

Table 4 strength results obtained for the 3 formulations

The average Young's modulus for formulation 1 was 0.969 kPa, the strongest of formulation 1 can be seen in orange in Figure 1. This cake had a Young's modulus of 1.246 kPa and max stress of 17.600 kPa. The other two traces in Figure 1, represent Formulation 2 (Grey) and Formulation (Blue). Formulation 2 has a Young's modulus of 0.473 kPa and a max stress of 7.110 kPa. At first glance, the blue trace seems to have a higher max stress than the grey trace, which is should be stronger due to less micro collapse within the sample is due to the cake scaffold-like lattice being broken down for formulation 3 (Blue) and therefore the indenter is compressing compacted material. The true max stress can be seen at around 51% strain where there is a plateau (A) before the stress starts to steadily increase. Formulation 3 had a Young's modulus of 0.017 kPa and a max stress of 2.660 kPa, the weakest of all the cakes analysed.

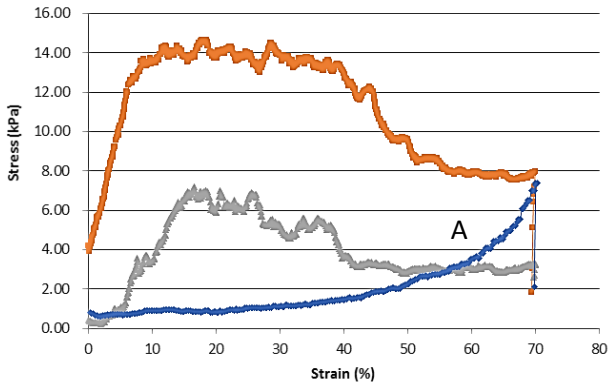

Figure 1: Graph of three formulations being analysed using the MicroPress. Formulation 1, orange, Formulation 2, Grey, and Formulation 3, Blue

Discussion

Table 4, shows the data obtained during this experiment. The higher the concentration of glucose within the solution of mannitol/glucose samples the weaker the cake will be. Classically mannitol is used as a bulking agent and it is known to have a positive impact on the critical temperature of many formulations. This can however, mask some of the critical temperatures that a formulation might have. Glucose has a collapse temperature of -41.0°C and, when lyophilised with mannitol collapses onto the mannitol scaffold. The cake appears to be strong with a good appearance to the eye however, when analysed with SEM or a similar technique the lattice appears 'wetter' with wider pores.

When analysed with the *MicroPress* the effect of increasing the concentration of glucose on the strength of the material is apparent. The more glucose within the cake which collapses weakens the material. When analysed using freeze drying microscopy (FDM) or Differential Scanning Calorimetry (DSC), the samples tend only to show the crystallisation and melting of mannitol which, being such a powerful evert, masks the glass transition of glucose.

The critical temperature analysis only reveals the melting of mannitol, so when the product is lyophilised maintaining the sample below -10°C, the glucose collapses within the bulk of the material. Therefore, due to the low critical temperature of the glucose the mannitol is usually able to dry well under standard primary drying conditions, while the glucose is not. Moreover, when the concentration of the glucose is increased within the formulations the degree of micro-collapse within the cake increases, thus producing a weaker cake. During storage or shipment materials with micro-collapse are more prone to damage or reduced activity.

Conclusions

Traditionally the quality of lyophilised samples are determined by a number of qualitative techniques including; visual assessment, reconstitution time, physical strength compared to a reference, moisture content. However, this is a subjective analysis and quality of the data can depend on the experience of the operator. Biopharma has developed this indentation technique and applied it to lyophilised cakes in order to reduce subjectivity and to provide qualitative data on lyophilised products to determine whether product defects are present within the samples

Knowledge of the critical temperatures of the individual constituents within the sample is equally as important as the overall critical temperature of the formulation as lyophilising products at a seemingly safe temperature can lead to weak cakes due to the structure weakening during lyophilisation and micro collapse affecting cake strength.

Once the material has been lyophilised the *MicroPress* can be used to test the physical characteristics of the resulting cakes and whether they are within the required parameters regarding the overall strength. The cakes analysed on the *MicroPress* can then also go on to be tested for moisture content using Karl-Fischer as well as the mDSC if the glass transition temperature or melting point temperatures is required.

Once the initial traces for lyophilised samples are determined the formulation can be altered to include other excipients which may affect the strength of the lyophilised cake. As seen in Table 4, increasing the concentration of the materials does not necessarily increase the strength of the cake. So, if issues are seen with breakdown or crumbling of the cake during shipping slight alterations or the inclusion or exclusion of one or more excipients may be beneficial.

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