Determining the Physical Robustness in Post-Lyo Cakes

New stabilisation methods such as lyophilisation need to be considered and tested for the stabilisation of biotherapeutics to ensure greater product quality and time efficiency

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Dr Kevin R Ward, Joshua Robinson, and Thomas B Codd at Biopharma Group

Lyophilisation, also known as freeze drying, has long been the preferred method for the stabilisation of labile drugs, biotherapeutics, and vaccines, considered a more gentle process next to traditional drying methods (such as spray, oven, and fluidised bed drying). Lyophilisation also offers an opportunity to produce material with low moisture content and high surface area, allowing the possibility of long-term stability at ambient temperatures and rapid reconstitution prior to use. However, lyophilised products can undergo physical breakup during transportation and handling due to their low density, sometimes becoming fragmented and powdery, which, in turn, impacts end-user perception of product quality as well as the time taken for reconstitution.

Standard quantitative tests for critical quality attributes (CQAs), such as residual moisture (or solvent) levels, activity, thermal properties, and stability, are numerous, and most manufacturers would agree that the cosmetic appearance of the product is also important. However, in the past, there has been no method to quantify this aspect. Techniques such as scanning electron microscopy can contribute an idea of morphology at the microscopic level, while gas adsorption methods even further provide an estimate of specific surface area and mean pore diameter of a lyophile; however, it can legitimately be argued that the sample preparation process itself, for either of these measurements, can lead to changes in the morphology of the material under test. Rheometers and tensile testing devices on the market are generally designed for application to less flexible and higher-density materials than lyophiles, and with some degree of sample preparation required.

In one study, a customised testing device comprising a load cell, linear actuator, indenter, and control software was developed in order to measure the mechanical properties of freeze-dried materials *in situ*, thus circumventing the need for sampling. Vials of mannitol, sucrose, trehalose, dextran, model proteins, and various combinations of these components were lyophilised under different processing conditions (temperatures, ramp rates, chamber pressures) and from a series of starting concentrations to provide a realistic range of samples for testing the sensitivity of the device and the repeatability of measurements.

Materials and Methods

A range of excipients, excipient mixtures, and model protein formulations at a series of concentrations and molecular ratios were lyophilised under a number of different freezing and sublimation conditions in glass vials, using a VirTis AdVantage EL or Genesis 25EL freeze dryer (SP Scientific). A device was built using a standard load cell and linear actuator combined with a customised indenter and control software. The resulting instrument is able to provide a 1g force at 0.01mm steps into freeze-dried materials. Samples were subjected to stress-strain measurements in situ (without the need to remove them from the vials). Young's modulus was taken as the gradient of the plot of stress vs strain in the linear elastic region, and the failure point defined at the point of the gradient suddenly changed to zero, indicating crushing (see **Figure 1**). All tests were carried out

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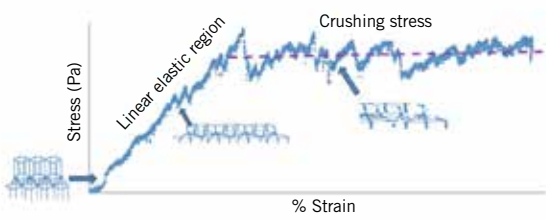
within three minutes in order to limit atmospheric moisture uptake by the lyophilised materials, which can alter their mechanical properties by plasticisation (1).

Results and Discussion

Experimental data indicate that excipients freeze-dried under identical conditions from the same starting concentration of 5% (w/v) can display markedly different mechanical properties (see **Figure 2**), even when the outward appearance of the cakes is similar with no obvious visible defects. Freezing conditions have a pronounced effect on the properties of resulting mannitol cakes, which may be related to ice crystal size (affecting porosity), but



$E=\sigma/\varepsilon$ Where σ is the stress and ε is the strain, and E is the Young's/elastic modulus

Figure 1: Example of a stress/strain profile

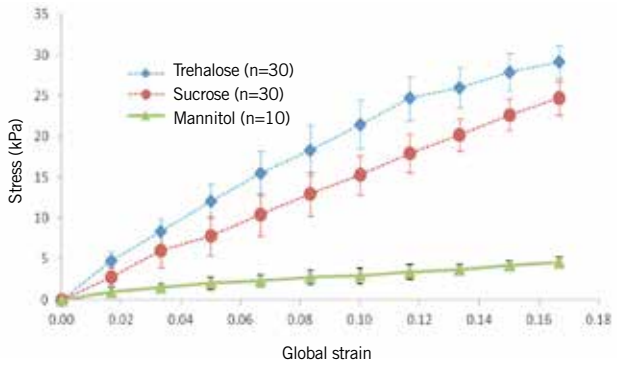


Figure 2: Mean maximum stress-strain curves for excipients processed using identical conditions; freeze-drying runs were carried out in triplicate for trehalose and sucrose (n=10 per run), single run for mannitol

> *Excipients that appear to have similar cake-forming properties have markedly different inherent Young's modulus values*

also possibly to inherent properties related to polymorph type that can result from the application of different cooling rates and/or the application of annealing during the cycle; this is supported by evidence from X-ray diffractograms that show that different polymorphs had been created; this method appears to have sufficient sensitivity to detect subtle differences in mechanical properties between neighbouring vials of identical material from a single lyophiliser shelf – indicating when cycle conditions may need to be optimised to reduce intra-batch variability. Results also demonstrated that both Young's modulus and strength increase near linearly with density, which may assist in the optimisation of the starting concentration vs fill volume for a particular formulation (2).

Data demonstrate that the customised instrument is sufficiently sensitive to detect statistically significant differences in the mechanical properties of single ingredients when lyophilised individually and that excipients that appear to have similar cake-forming properties have markedly different inherent Young's modulus and strength values.

Sizable differences were detected for samples of mannitol, where different freezing conditions were employed in the lyophilisation cycle, indicating that not only is the mean pore diameter hugely influential on the mechanical properties of the resulting lyophile, but also that amorphous/crystalline behaviour and possibly even polymorphism could have a measurable impact.

With further optimisation of the instrument parameters, differences were even detected in the mechanical properties of lyophiles in vials taken from multiple locations across a single lyophiliser shelf. Therefore, this method could represent a valuable addition to the existing array of techniques available to provide quantitative measurement of lyophile CQAs.

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Dr Kevin R Ward joined **Biopharma** in March 2000. As a graduate in Applied Chemistry, Kevin received his PhD for investigations into the freeze-drying of pharmaceutical formulations and drug/vaccine delivery systems. Prior to joining the team, Kevin worked at Pfizer Central Research and as a Research Fellow in vaccine development at Aston University, UK. Kevin lectures on freeze drying extensively, as part of BioPharma's freeze drying training courses and as a regular on the conference circuit, as well as having been an industrial supervisor to PhD students.

Joshua Robinson joined **Biopharma** in January 2018 as Instrument R&D Engineering Assistant, which comprised a balance of customer instrument builds/validations/installations and new product development. Joshua completed his degree in Chemistry at the University of Plymouth, UK, where his final year project was in synthetic organic chemistry. Having spent some of his childhood in South Africa and China, he has always been very keen to travel and experience new cultures and places, which stands him in good stead for some of the customer site visits in far-flung places.