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Research paper

Humidity induced collapse in freeze dried cakes: A direct visualization study using DVS

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ABSTRACT

Maintaining low moisture content is seen as crucial to sustaining long term stability in freeze dried (FD) cakes as higher moisture could lead to cake collapse, degradation and a loss of biological potency. Using a combination of gravimetric data and video images captured from a Dynamic Vapour Sorption instrument the onset humidity Collapse Point (RH_{cp}), the humidity onset Crystallisation (RH_c) and onset Glass Transition (RH_g) points for a series of freeze dried cakes at 10, 25 and 40 °C have been determined. The moisture sorption behavior with respect to cake collapse and other morphological phase transitions are reported for a two freeze drying excipients and one product formulation; sucrose, trehalose (both 5% w/w) and an influenza antigen (A/Wisconsin/15/2009 H3N2 NYMCX-183, formulated with 1.1% w/w sucrose). Stability maps for all three formulations tested were reported as a function of %RH and temperature using the methods described in this work, thus the direct visualization of collapse behavior for any FD cake can now be standardized and routinely determined, facilitating the formulation of FD products with improved stability and storage performance.

1. Introduction

Lyophilisation, otherwise known as freeze-drying (FD), is a drying process in which water is removed from a frozen solution through sublimation at low pressures and temperatures resulting in the formation of a solid porous cake. The process consists of three stages: initial solution freezing, primary drying and secondary drying. Most biologics are inherently unstable in liquid formulations and therefore freeze drying is able to offer greater stability and shelf life via a solid state final product form [1,2]. In recent years almost 50% of marketed biopharmaceutical products have needed to be lyophilised and this is expected to continue to increase [3]. Biologic formulations which are FD usually contain an excipient to help increase stability and mechanical properties of the freeze dried cakes. Amorphous sucrose and trehalose are some of the most commonly used excipients which not only help stabilize proteins but can also improve both processing and storage performance [4,5].

The output of the FD process are highly hygroscopic porous cakes which, though superior in terms of stability compared to liquid based formulations, can still present challenges in regards to long term moisture uptake. The rise of moisture over time in storage for low dry mass presents a challenge for the industry. Matejtschuk et al. [6] conducted a study which looked at moisture ingress exchange in vials versus ampoules for products at elevated temperatures. The stoppers had been baked and the products had a modest dry mass of less than 23 mg. Their study showed that moisture content rose in vials over time with the worst effect seen in vials where a lower dry mass was contained. Similar studies have also showed how moisture ingress in vials can occur during storage [7–11]. Newer freeze dried products with small fill volumes and highly active therapeutics are especially susceptible as the actual amount of water needed to drive instability scales down directly with mass of freeze dried cake.

The glass transition temperature (T_g) is a critical physical property of all amorphous solids and is the temperature at which the brittle glassy solid transitions into that of a liquid like rubbery state [12]. In addition, the T_g of amorphous solids can also be reduced by the presence of plasticising molecules dissolved in the amorphous solid such as water. So as the moisture levels increase in the amorphous materials their T_gs are reduced due to plasticisation. Indeed if the temperature or the moisture content is raised sufficiently, the glassy solid will revert to be a viscous fluid. In the case of amorphous freeze dried cakes, such decreases in the T_g thereby facilitate potential complete cake collapse back to a liquid solution [13,14]. Materials that have been freeze dried are generally found to be amorphous solids which are metastable glasses in which structural transitions can occur from the glassy to rubber states [15]. Previous studies have investigated cake collapse as a

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function of temperature by heating the cake until the dry FD cake exhibited a visual loss in structure. Tsourouflis et al. [16] suggested that this phenomena is related to viscosity decreases, and that the loss of cake structure could be achieved by a combination of water content and temperature. They observed that the collapse of freeze-dried materials occurred above a specific dry collapse temperature, T_c, over time (not to be confused with when in the frozen state). These collapse temperatures were high at low water contents but decreased as moisture content rose. Gerschenson et al. [17] investigated the effect of moisture content and temperature on the collapse of FD tomato juice cakes. They showed that high temperatures and high moisture contents induced cake collapse and subsequent loss of volatiles. A compressive mechanical study by To and Flink [18,19] further showed the time-dependant relationship with respect to T_g and T_c. They also found that water content has a crucial role in 'collapse', and a 1% difference in moisture content is sufficient to change T_c by 5 °C in most samples studied. Despite these studies, direct visual determinations of freeze dried cake collapse as a function of both temperature and humidity have not been previously reported and is the subject of this work.

Dynamic Vapor Sorption (DVS) is a gravimetric technique which measures water sorption uptake by solid state samples such as freeze dried cakes. As the water vapor concentration increases the sample absorbs more water vapour and the sample mass increases monotonically. Since its development in the early 1990s [20], DVS has become a major research tool for virtually all pharmaceutical laboratories around the world interested in studying the effects of water sorption on solid state dosage forms. Previous studies on amorphous solid state dosage forms involving DVS have solely focused their attention on the onset crystallization point and T_g in regards to humidity and temperature. For example, Burnett et al. looked into the critical relative humidity for various moisture induced phase transitions (glass transition and crystallization) in spray-dried lactose as well as looking into moisture induced crystallization kinetics [21,22]. They showed that increasing temperature and humidity led to lowering of crystallization induction and the associated glass transition. Other studies have reported the use of DVS in examining the moisture sorption uptake behaviour of spray-dried amorphous sucrose and trehalose [23,24]. The industrially important accurate quantification of low amorphous contents in crystalline solids has also been achieved with DVS using a range of experimental protocols [25–27].

In this paper the use of real time video imaging camera has been combined with DVS to visually observe the key cake transitions including cake collapse at elevated relative humidity's for series of temperatures. A standardized protocol will be presented in this paper to highlight the critical RH%, for a specific temperature, at which freeze dried cakes collapse; the humidity induced collapse point- RH_{CP}. Humidity induced collapse can be described as a physicochemical event in which increasing water uptake leads to a decrease in viscosity/modulus above the environment T_g conditions and eventual subsequent loss of structure [9]. In such circumstances the cakes starts to shrink and eventually revert back to its liquid state and a complete loss of the physical cake structure occurs. Using the real-time video footage, along with frame by frame image analysis, here are presented for the first time detailed and comprehensive visual images with an associated analysis into collapse behaviour of common freeze dried excipients and biologic formulations.

2. Materials and methods

2.1. Materials and lyophilisation cycle

Sucrose (Sigma: S5016-1 kg, Dorset, UK) and Trehalose (Cargill 16400, Surrey, UK) were used to make the 5% (w/w) solutions with purified deionized water. As a model biological material we chose a lysed Influenza antigen preparation (A/Wisconsin/15/2009 H3N2 NYMCX-183, nominal 50 ugμg/mL antigen in six-salt PBS pH 7.4 formulated with 1.1% w/w sucrose), of the type used in immunodiffusion. 5 mL Type I screw capped vials (41.5 × 18 mm i.d. Adelphi Tubes, Haywards Heath, UK) were filled with the excipient solutions and influenza antigen to a fill-volume of 1 mL using an automated multi-pipette stream (Eppendorf, UK). All 3 solutions were freeze dried separately using a Virtis Advantage Plus (SP Scientific, USA). The freeze drying cycle run for the excipients and influenza antigen are show in Tables 1 and 2, respectively. After the cycle had finished the vials were backfilled with nitrogen gas before stoppering down on the 14 mm diameter igloo halobutyl stoppers (Adelphi Group, Haywards Heath, UK).

Table 1
Freeze drying cycle run for 5% (w/v) sucrose and trehalose.

Step	Duration (min)	Temperature (°C)	Hold/ ramp	Vacuum (mTorr)
1 (Precooling)	30	4	H	Atmospheric
2 (Ramp to Freeze)	270	−50	R	Atmospheric
3 (Hold Frozen)	240	−50	H	Atmospheric
4 (Apply Vacuum)	60	−50	H	150
5 (Apply Vacuum)	60	−50	H	70
6 (Ramp to Primary Drying)	50	−35	R	70
7 (Primary Drying Hold)	1200	−35	H	70
8 (Primary Drying Hold)	1200	−35	H	70
9 (Ramp to Secondary Drying)	1200	25	R	70
10 (Secondary Drying Hold)	960	25	H	20
Release Vacuum to dry nitrogen and remove product	n/a	25	n/a	Atmospheric

2.2. DVS sorption profiles

The sorption behaviour of the FD samples were measured using a DVS Advantage (Surface Measurement Systems, London, UK) that had been fitted with a 5 megapixel video camera underneath the sample holder. The instrument is able to simultaneously measure the uptake/loss of water vapour gravimetrically and capture video images. Freeze dried samples weighing approximately 10–20 mg were cut carefully from the FD cakes and placed on a quartz sample holder (see Fig. 1). The experiment was repeated for a fresh sample at 15, 25 and 40 °C with a 2 cycle humidity ramp (ramping from 0% to 90% RH then back down again to 0% twice), whilst real-time video camera recorded all events or changes in phases induced by humidity changes. The humidity steps increased by 1% increments at a rate of 12% per hour and

Table 2
Freeze drying cycle run (2 days) of Influenza Antigen[a] (A/Wisconsin/15/2009).

Step	Duration (min)	Temperature (°C)	Hold/ ramp	Vacuum (mTorr)
1 (Precooling)	10	20	H	Atmospheric
2 (Ramp Freeze)	90	−50	R	Atmospheric
3 (Hold Frozen)	240	−50	H	Atmospheric
4 (Apply Vacuum)	60	−50	H	150
5 (Apply Vacuum)	60	−50	H	20
6 (Ramp to Primary Drying)	50	−40	R	20
7 (Primary Drying Hold)	1200	−40	H	20
8 (Ramp to Secondary Drying)	600	15	R	20
9 (Secondary Drying Hold)	560	15	H	20
Release Vacuum to dry nitrogen and remove product	n/a	15	n/a	Atmospheric

[a] Note: Flu Antigen lyophilised in screw capped glass vials with a cycle designed to give an elevated moisture content to allow collapse of the cake to be more easily measured.