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Research paper

Structural attributes of model protein formulations prepared by rapid freeze-drying cycles in a microscale heating stage

Björn-Hendrik Peters *, Ferdinand Molnár, Jarkko Ketolainen

University of Eastern Finland, School of Pharmacy, Kuopio, Finland

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ABSTRACT

Downscaled freeze-drying was demonstrated to be a valuable alternative for formulation development and optimization. Although the pore structure is known to exert a major influence on the freeze-drying cycle, little is known about the ones of microscale preparations. This study describes morphology evaluation methods for lysozyme formulations prepared in one microscale processing option and the assessment of fundamental product quality criteria. Scanning electron microscopy (SEM) revealed cooling rate dependent pore size variations at the nucleation site which diminished as the rate increased. Micro-X-ray computed tomography (μ-CT) showed that porosity generally increased in the sample from bottom to top, the pore size fractions shifted toward larger pores in elevated sample levels, and horizontal homogeneity was found throughout each sample with minor deviations in the bottom region. Furthermore, the event of microcollapse could be identified and quantified. Low residual moisture was achieved repeatedly and the procedure did not influence the post freeze-drying bioactivity. This microscale heating stage is a valuable option to reduce overall cycle times and cost, and to prepare freeze-drying formulations with high reproducibility. The mapping tools permit a quick but detailed insight into the structural features resulting from the process environment and processing conditions.

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1. Introduction

Freeze-drying, also known as lyophilization, is a widely used process for handling biopharmaceuticals to ensure the maintenance of product stability and quality criteria during shipment and storage [1]. Designing a suitable product often entails a trial and error approach requiring substantial quantities of active pharmaceutical ingredient (API) and time to prepare candidate formulations. In this respect, trial and error are both expensive and inefficient. Adopting a rational formulation design based on scientific knowledge or utilizing external expertise could be a better approach but still involves a considerable number of choices as found from published recommendations [2,3]. Instead, reducing the needed amount of API by downscaling and simultaneously increasing the development efficiency by adopting a design of experiment (DoE) approach have been claimed as beneficial [4,5]. Nonetheless, the processing periods remain in the range of 15+ hours and as a result, the bottleneck of overall process cycle time must be addressed. In return the best candidate formulation may be quickly identified or formulation problems can be dealt with immediately. Even though in some cases adequate quantities of API are available, process duration remains a major delay factor. Freeze-drying cycle acceleration is often associated with optimization of the primary drying phase since one can shorten this phase by 13% per 1 °C elevated drying temperature [6]. This represents a significant increase in efficiency especially at the scaled up production levels. Be that as it may, the individual process optimization of each candidate formulation would not be resource efficient. Several studies have described outsourcing and shortening of the freezing step by directly placing formulation containers into varying cooling agents [7–9]. Unfortunately, freezing in a cooling bath differs drastically from shelf freezing and the morphological features, which are known to influence the sublimation rate [10], may well change in this situation. It is further important to appreciate the morphological design space of the process equipment,

Abbreviations: 2D, two-dimensional; 3D, three-dimensional; API, active pharmaceutical ingredient; DoE, design of experiment; DSC, differential scanning calorimetry; FDA, Food and Drug Administration; FDM, freeze-drying microscope/microscopy; Fr. I., fragmentation index; KF, Karl Fischer titration; K_v, heat transfer coefficient; NDCD, National Drug Code Directory; RGB, red–green–blue; RH, relative humidity; ROI, region of interest; R_p, resistance to vapor flow; SEM, scanning electron microscopy; T_c, collapse temperature; T_n, nucleation temperature; μ-CT, micro-X-ray computed tomography.

* Corresponding author. University of Eastern Finland, School of Pharmacy, P.O. Box 1627, Yliopistonranta 1C, FI-70211 Kuopio, Finland. Tel.: +358 50 594 455 3.

E-mail address: bjorn.peters@uef.fi (B.-H. Peters).

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meaning here the relation of cycle input parameters and the resulting lyophilized cake morphology. Micro-X-ray computed tomography (μ-CT) can be valuable in this respect: it has a major benefit of not applying force to the fragile cake structure, thus being a non-destructive technique [11]. One common factor in all of the above described approaches is that they facilitate laboratory scale or pilot scale freeze-dryers and do not utilize a microscale freeze-drying cell. In 1994, a basic model of the current freeze-drying microscope (FDM) heating stage was introduced to provide a convenient means of direct observation of freezing and freeze-drying processes [12]. The FDM is regularly used to assess a formulation's collapse temperature (T_c), a critical process factor in freeze-drying cycle optimization. The T_c is arguably superior to the glass transition temperature of the maximal concentrated solution which is determined by differential scanning calorimetry (DSC) [13]. In addition, it should be noted that the T_c depends on the total solid content, the formulation, and weakly the nucleation temperature (T_n) and is best described as a range in which collapse occurs [14]. This knowledge has also practical implications e.g. for freeze-drying performed at the edge of the T_c. Here the phenomenon of microcollapse, leading to increased porosity, permitted higher vapor flow rates and thus a significant reduction of the primary drying time was achieved while the macroscopically elegant cake structure was maintained [15]. The cycle optimization described in Ref. [15] was also performed at pilot scale, limiting its usefulness for application in early developmental stages. Subsequently, there have been significant improvements made to the equipment e.g. utilizing silver as primary heating element material to provide high thermal conductivity, a prerequisite for efficient heat transfer, and addition of a liquid nitrogen pump to enlarge the operational temperature range. With regard to the temperature controlled shelf of tray freeze-dryers, the microscale heating stage yields the potential to be a realistic scaled down version: the silver heating element replaces the tray and liquid nitrogen replaces the cooling fluid. This study describes a time saving approach for microscale freeze-drying utilizing a FDM heating stage. The morphology of the dried cakes was studied by scanning electron microscopy (SEM) and μ-CT to provide insights into the formation of pores. In order to evaluate the potential for rapid biopharmaceutical formulation development, a model protein with biological activity was examined. Sucrose and trehalose were chosen as lyoprotectants both representing the most commonly used disaccharides [16]. Furthermore, mannitol was identified as the most frequent additive according to the freeze-drying formulations listed in the National Drug Code Directory (NDCD) from 1992 to 2012 published by the Food and Drug Administration (FDA). The residual moisture content, a critical factor with respect to protein stability, was introduced as a secondary end point and assessed by coulometric Karl Fischer titration (KF).

2. Materials and methods

2.1. Materials and formulations

Hen-Egg White Lysozyme was obtained from Dalian Greensnow Egg Products Development Co., Ltd. (Jinggang Industrial Area, Dalian City, China). Mannitol, sucrose, trehalose dihydrate, naproxen and methanol were purchased from Sigma Aldrich (St. Louis, MO, USA). Water was produced immediately before use by filtration in a Milli-Q-Gradient station equipped with a Millipak Express 0.22 μm filter (EMD Millipore, Billerica, MA, USA). The model formulations and their corresponding drying temperatures and processing times are summarized in Table 1.

2.2. Freeze-drying equipment

Freeze-drying was performed with a THMS350V heating stage (Linkam Scientific Instruments Ltd., Guildford, Surrey, UK). The heating stage was supplemented with a liquid nitrogen pump LNP94/2, a temperature controller TMS 94 (both Linkam Scientific), a vacuum pump E2M1.5 and a Pirani gauge (both Edwards Group Ltd., Crawley, West Sussex, UK). The equipment was controlled via the Linksys32 (Linkam Scientific) software package. The silver heating element's thermocouple was calibrated as described before [14] with the addition of water and naproxen for melting points of 0.0 °C and 156.1 °C, respectively. The melting point of naproxen was determined with DSC 823e (Mettler Toledo, Columbus, OH, USA).

2.3. Freeze-drying process – SEM samples

About 5 μl of silicon oil was spread between the silver heating block and a cover glass #1 with a diameter of 15 mm (Menzel GmbH, Braunschweig, Germany) to ensure close contact. The sample height was set by a 70 μm aluminum spacer (Biopharma Technology Ltd., Winchester, Hampshire, UK). A sample droplet volume of 0.3 μl and the surrounding spacer were placed directly onto the cover glass followed by a second cover glass on top. Each formulation was frozen at cooling rates of 5 °C, 10 °C and 20 °C/min down to −50 °C and held for 8 min with pressure reduction to 50 mTorr (approximately 6.67 Pa) starting after 4 min of holding time. The drying temperature was approached at a heating rate of 5 °C/min. Drying temperatures for all formulations described in Table 1 have been defined by applying a safety regimen of approximately

Table 1
Freeze-drying protocols for SEM (0.3 μl) and μ-CT, KF and bioactivity (200 μl) assessment.

	Target drying temperature (°C)[a]	Drying time (min)	Total processing time (min)[b]
Mannitol (1.10% w/V) + Lysozyme (2.00% w/V)			
SEM	−23	15	57.5; 61; 68
μ-CT, KF, bioactivity	−23	40	111.4
Sucrose (2.08% w/V) + Lysozyme (2.00% w/V)			
SEM	−28	20	62.5; 66; 73
μCT, KF, bioactivity	−28	75	146.4
Trehalose (2.08% w/V) + Lysozyme (2.00% w/V)			
SEM	−24	15	57.5; 61; 68
μ-CT, KF, bioactivity	−24	75	146.4

All samples were frozen down to −50 °C and held for 8 min. Cooling rates (°C/min) were 10 for μ-CT, KF, and bioactivity, and 5, 10, and 20 for SEM samples. The pressure during the freeze-drying process was held at 50 mTorr.

[a] The target drying temperature was determined in a preliminary T_c assessment by FDM and the introduction of a 5 °C safety range.

[b] The total processing time includes the beginning of the freezing process until the release of the sample from the heating stage.

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