Journal of Pharmaceutical Sciences 105 (2016) 40–49

Contents lists available at ScienceDirect

Journal of Pharmaceutical Sciences

journal homepage: www.jpharmsci.org

Pharmaceutical Biotechnology

Freeze-Drying Above the Glass Transition Temperature in Amorphous Protein Formulations While Maintaining Product Quality and Improving Process Efficiency

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ARTICLE INFO

Article history:
Received 3 June 2015
Revised 31 August 2015
Accepted 5 October 2015
Available online 18 November 2015

Keywords:
freeze-drying
lyophilization
protein formulation
thermal analysis
glass transition
stability
processing
drying

ABSTRACT

This study explored the ability to conduct primary drying during lyophilization at product temperatures above the glass transition temperature of the maximally freeze-concentrated solution (T_g') in amorphous formulations for four proteins from three different classes. Drying above T_g' resulted in significant reductions in lyophilization cycle time. At higher protein concentrations, formulations freeze dried above T_g' but below the collapse temperature yielded pharmaceutically acceptable cakes. However, using an immunoglobulin G type 4 monoclonal antibody as an example, we found that as protein concentration decreased, minor extents of collapse were observed in formulations dried at higher temperatures. No other impacts to product quality, physical stability, or chemical stability were observed in this study among the different drying conditions for the different proteins. Drying amorphous formulations above T_g', particularly high protein concentration formulations, is a viable means to achieve significant time and cost savings in freeze-drying processes.

Introduction

Freeze-drying, or lyophilization, is a unit operation commonly used to stabilize protein formulations to achieve longer shelf life, compared with solution formulations, during product development and commercial distribution. In the last decade, more than 40% of marketed biotherapeutics were freeze dried,[1] and with new complex molecular formats being developed (e.g., bispecific molecules, fusion proteins, antibody drug conjugates), this percentage is expected to further increase. However, the processes of freezing and drying could each be detrimental to protein stability. Thus, optimization of the formulation composition so that the protein is stable against the stresses imposed by freeze-drying is a key element of drug product development.[2-4] Additionally, optimization of the freeze-drying process is equally critical to minimize processing time for increased throughput and also to reduce production costs.[5] A typical freeze-drying process consists of three steps: (1) freezing wherein water is converted into ice; (2) primary drying wherein ice is removed by sublimation; and (3) secondary drying wherein unfrozen water is removed by desorption. Typically, at the end of secondary drying the residual water content is less than 1%. Of these three steps, primary drying is often the longest step and hence optimization of this step is usually the main focus in industry.

During primary drying, the product temperature needs to be maintained 2°C–3°C below the maximum allowable temperature, which for an amorphous matrix is the glass transition temperature of maximally freeze-concentrated solution (T_g') or the collapse temperature (T_c).[6] Drying above T_g' or T_c may result in cake collapse that could further impact product quality attributes such as residual water content, reconstitution time, and protein stability.[7-9] Usually, T_c is within 1°C–2°C of T_g', and T_g' and T_c may be used interchangeably.[10] However, for higher concentration protein formulations, visible collapse may not always be observed when drying above T_g' as the difference between T_g' and the T_c may increase as protein concentration increases.[11]

Recent publications have shown that even with total collapse, protein stability either improves or is not significantly different compared with that in the uncollapsed cake.[12-14] However, cake

Abbreviations used: HPSEC, high-performance size-exclusion chromatography; IgG1, immunoglobulin G type 1; IgG4, immunoglobulin G type 4; mAb, monoclonal antibody; MFI, microflow imaging; SbVP, subvisible particle; T_c, collapse temperature determined by freeze-dry microscopy; T_g', glass transition temperature of the maximally freeze-concentrated solution; T_s, shelf temperature.

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http://dx.doi.org/10.1002/jps.24705
0022-3549/

appearance is a potential critical quality attribute for a lyophilized product and hence included in product specifications for lot release and stability. In general, improvement in process efficiency at the expense of product appearance may not be desired. Thus, although drying above the T_c may not be practical from an appearance perspective, drying above T_g' is a very practical approach to reduce freeze-drying cycle time without impacting product quality,[11] especially for high concentration protein formulations. Furthermore, for existing processes where primary drying occurs at product temperatures below T_g', data supporting maintained product quality if product temperature exceed T_g' during primary drying is invaluable in the event of any temperature and pressure excursions that may occur during production.

On the basis of the Fox equation and the relatively high T_g' values of proteins, the T_g' of a protein-containing amorphous formulation is expected to increase with protein concentration assuming an otherwise constant formulation composition.[15,16]

However, understanding the effect that increasing protein concentration may have on T_c, and the extent to which T_c may differ from T_g', is critical in defining the maximum allowable product temperature during primary drying. Because every 1°C increase in product temperature during primary drying may result in approximately 13% reduction in primary drying time,[6] operating at the highest allowable product temperature is highly desired. Thus, thermal characterization of the formulation by differential scanning calorimetry (DSC) for T_g' and freeze-dry microscopy (FDM) for Tc is critical for both product quality and process efficiency.

In this work, both product quality and process performance were evaluated when conducting primary drying at product temperatures above and below T_g' for four different proteins [two immunoglobulin G type 1 (IgG1) monoclonal antibodies, an immunoglobulin G type 4 (IgG4) monoclonal antibody (mAb), and a fusion protein] in an amorphous formulation matrix. Different primary drying conditions were systematically evaluated, and stability studies were performed to show there was no impact to product quality both upon lyophilization and also during long-term storage. The relationship between T_g' and T_c as a function of protein concentration is presented for the various molecules, indicating the potential to exceed product temperature above T_g' during primary drying, particularly at higher protein concentrations, to achieve significant reduction in lyophilization cycle time.

Materials and Methods

Materials

The proteins evaluated in this work were produced and purified at MedImmune (Gaithersburg, Maryland) using proprietary methods. mAb A and mAb B are IgG1 monoclonal antibodies, and mAb C is an IgG4 mAb. Protein X (or "Pro X") is a fusion protein with a molecular weight of approximately 90 kDa. Each of these molecules was formulated in a buffered solution with a disaccharide and polysorbate 80 as excipients. mAb B contained an amino acid as an additional excipient. Formulations contained excipients expected to remain amorphous upon lyophilization, and no components expected to crystallize were included. All excipients used were multicompendial grade. For thermal analysis, mAb A and mAb C samples were prepared in their formulation buffer at concentrations ranging between 1 and 100 mg/mL. For lyophilization, mAb A was prepared at 100 mg/mL, and mAb C was prepared at three different concentrations: 5, 25, and 100 mg/mL with the levels of all other formulation components remaining the same. mAb B and Pro X were prepared at 50 mg/mL for thermal analysis and lyophilization. Type 1 glass tubing vials (3-, 5-, and 20-mL), and chlorobutyl, single vent lyophilization stoppers were used. Both vials and stoppers were obtained from West Pharmaceutical Services, Inc. (Exton, Pennsylvania).

Lyophilization

For their individual lyophilization cycles, the proteins were filled into vials at the following conditions:

- mAb A and mAb C: 3-mL vial, 1.1 mL fill
- mAb B: 5-mL vial, 2.7-mL fill
- Pro X: 20-mL vial, 5.5-mL fill

Vials were partially stoppered with 13 mm (for 3-mL vials) or 20 mm (for 5-mL and 20-mL vials) lyophilization stoppers. Freeze-drying was performed using a Virtis Genesis 35 EL freeze dryer (SP Scientific, Stone Ridge, New York) or Millrock PDQ24XS-S freeze dryer (Millrock Technology, Kingston, New York). Vials were loaded onto the freeze dryer at a shelf temperature (T_s) of 20°C. After loading the shelves, they were cooled to 5°C and held for 30 min. The shelves were then cooled to −5°C and held for 15 min before cooling to −40°C. All molecules except mAb C underwent an annealing step at −16°C for 120 min before bringing back to −40°C. The annealing time and temperature was selected based on process optimization activities leading to batch homogeneity in terms of drying and product quality. After holding at −40° for 2 h, the T_s was raised to the desired primary drying T_s (see *Results* section) where primary drying occurred at a chamber pressure of 100 mTorr. Chamber pressure was controlled using the Capacitance Manometer gauge. End of primary drying was determined by comparative pressure measurement wherein the Pirani gauge pressure measurement converges with that of the Capacitance Manometer chamber pressure.[17] Secondary drying was performed at 40°C for 6 h while maintaining the pressure at 100 mTorr. Product temperature was monitored during the freeze-drying process using thermocouples placed at the bottom center of selected vials. The variability in product temperature as measured by the thermocouples was ±0.5°C.[18] With the following two exceptions, all T_s ramps throughout the cycle were conducted at 0.5°C/min: (1) for mAb A, the ramp rate from −40°C to the primary drying T_s was 0.1°C/min, and (2) all ramp rates during secondary drying to 40°C were 0.1°C/min.

Differential Scanning Calorimetry

A Q2000 series differential scanning calorimeter from TA Instruments (New Castle, Delaware) was used for glass transition temperature measurements. For T_g' measurements, 20 μL of liquid sample was added into an aluminum pan and sealed hermetically. An empty pan and lid was used as the reference. Liquid samples were frozen to −60°C at a rate of 5°C/min and then heated to 25°C at a rate of 5°C/min. The T_g' values were determined using Universal Analysis software and reported as the midpoint of the glass transition.[19-21] For T_g determination of the lyophilized cakes, approximately 5 mg of sample were sealed into an aluminum pan and analyzed in modulated DSC mode. Pans were heated at 5°C/min through the glass transition with a modulation period of 80 s and amplitude ±0.5°C.

Freeze-Dry Microscopy

All liquid samples were tested using an Olympus BX50 microscope with a Linkam FDCS 196 stage. Images were recorded using a QImaging camera attachment. A sample volume of 5-10 μL was placed between two cover slips on the FDM stage. The samples were cooled and frozen to −40°C. All samples, with the exception of