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# Aggressive conditions during primary drying as a contemporary approach to optimise freeze-drying cycles of biopharmaceuticals

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ABSTRACT

Freeze-drying is the method of choice to dry formulations with biopharmaceutical drugs, to enhance protein stability. This is usually done below the glass transition temperature of maximally freeze-concentrated solutions ($T_g'$), to avoid protein aggregation, preserve protein activity, and obtain pharmaceutically 'elegant' cakes. Unfortunately, this is a lengthy and energy-consuming process. However, it was recently shown that drying above $T_g'$ or even above the collapse temperature ($T_c$) is not necessarily detrimental for stability of biopharmaceuticals, and hence provides an attractive option for freeze-drying cycle optimisation. The goal of the present study was to optimise the freeze-drying cycle for a model IgG monoclonal antibody (20 mg/mL) in sucrose and sucrose/glycine formulations, by reducing primary drying time. To study the impact of shelf temperature ($T_s$) and chamber pressure on product temperature ($T_p$), one conventional and five aggressive cycles were tested. Aggressive conditions during primary drying were achieved by increasing $T_s$ from −20 °C (conventional cycle) to 30 °C, with chamber pressure set to 0.1 mbar, 0.2 mbar or 0.3 mbar. These combinations of $T_s$ and chamber pressure resulted in $T_p$ well above $T_g'$, and in some cases, even above $T_c$, without causing macrocollapse. Other critical quality attributes of the products were also within the expected ranges, such as reconstitution time and residual water content. Physical stability was tested using size exclusion chromatography, dynamic light scattering, and micro-flow imaging. All of the lyophilised samples were exposed to stress and the intended storage conditions, with no impacts on the product seen. These data show that implementation of aggressive conditions for the investigated formulations is possible and can significantly contribute to the reduction of primary drying times by up to 54% (from 48 to 22 h) in comparison to conventional freeze-drying.

## 1. Introduction

Nowadays, biopharmaceuticals represent the fastest growing area of the pharmaceutical market and constitute a multi-billion dollar industry throughout the world. Protein-based therapeutic drugs are widely used to treat several diseases, such as chronic viral hepatitis, rheumatoid arthritis, psoriasis, Crohn's disease, and different types of cancers (Tovey and Lallemand, 2011; Awotwe-Otoo et al., 2012). Nevertheless, the development of such therapeutic drugs is very challenging and requires great effort and expertise.

Although liquid formulations are the preferred dosage form for injectable protein therapeutics, this form is not always feasible given the susceptibility of proteins to denaturation and aggregation. For protein molecules that are not stable in aqueous media, freeze-drying represents the method of choice in the manufacture of stable biopharmaceutical drug products. As chemical and physical degradation rates are significantly reduced in the dried state, this provides acceptable shelf life for commercial drug products (Johnson et al., 2010). Over the last decade, about 50% of approved biopharmaceutical products have been produced in lyophilised forms, and with the rise in new and highly complex molecular formats, such as bispecific and tetravalent molecules, antibody drug conjugates and fusion proteins, the number of freeze-dried products is expected to grow (Kasper and Friess, 2011).

In general a lyophilisation cycle consists of the three main steps: (i) freezing, where the solution is transformed into ice and freeze-concentrated solution is formed; (ii) primary drying, where the frozen solvent from the product is removed by sublimation; and (iii) secondary

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drying, where the unfrozen water is removed by desorption. The target lyophilisation process must deliver a product with acceptable quality attributes, such as low residual water content, short reconstitution time, and retention of potency, as well as providing pharmaceutical 'elegance'. As lyophilisation is a costly and time-consuming process from an operational and financial point of view, it needs to be quick, reproducible, scalable and robust (Tang and Pikal, 2004).

The most time and energy extensive stage in freeze-drying processes is the primary drying, which therefore contributes crucially to the total costs of freeze-drying (Depaz et al., 2016). Additionally, the macrocollapse phenomenon can occur during the primary drying stage, and thus the selection of the process parameters is of great importance. Due to the relatively high residual moisture content at the end of primary drying, and therefore low glass transition temperature ($T_g$), the occurrence of collapse may also be triggered at the beginning of secondary drying if the shelf temperature is too rapidly increased (Tang and Pikal, 2004). Essentially, primary drying is a function of shelf temperature ($T_s$) and chamber pressure. Therefore, the appropriate selection of these two parameters can shorten this phase.

When optimising the primary drying step, the critical formulation parameters and the critical process parameters both need to be taken into account. The most critical formulation parameter is collapse temperature ($T_c$). $T_c$ represents the maximum allowable product temperature during the primary drying to avoid collapse, and therefore to provide an acceptable appearance for the final product (Pikal and Shah, 1990). $T_c$ is closely related to the $T_g'$ for the amorphous form, or the eutectic temperature ($T_{eu}$) for the crystalline form. $T_g'$ is defined as a thermally reversible transition at which the frozen viscous solution changes to a glass state (Remmele et al., 2012; Duddu and Monte, 1997). Different types of excipients can be used in protein formulations to increase $T_g'$ and $T_c$. As a consequence, a higher product temperature ($T_p$) can be achieved during the primary drying (Chang and Patro, 2004).

In more recent years, the theory of conventional drying has been used, which states that $T_p$ during primary drying should be kept 2 °C to 3 °C below $T_g'$ to avoid macrocollapse and to maintain the physical stability of the protein. However, it was recently shown that drying above $T_g'$, or even above $T_c$ (which is described as aggressive drying), is not necessarily detrimental to the stability of biopharmaceuticals (Depaz et al., 2016; Lewis et al., 2010). This thus provides an attractive option for optimisation of the freeze-drying cycle.

Appropriately designed formulations are a prerequisite for implementation of aggressive freeze-drying cycles. Such formulations need to contain amorphous stabilisers that act as cryoprotectants and lyoprotectants. Most commonly, sucrose or trehalose is used here, as well as crystalline bulking agents, such as glycine or mannitol (Wang et al., 2004). Bulking agents provide the cake with a stronger structure and help to maintain the physical stability of the protein, even when $T_c$ of the amorphous part is exceeded.

When primary drying is conducted in the area above $T_g'$, the viscosity of the amorphous phase decreases (Chatterjee et al., 2005), which causes collapse onto the supporting matrix of the crystalline bulking agents (Johnson et al., 2002). Thus, the occurrence of collapse at the macroscopic level is prevented. On the other hand, small scale collapse can be induced, which is known as microcollapse (Schersch et al., 2010). The microcollapse phenomenon leads to increased pore size of the drying material, which increases the sublimation rate and reduces the primary drying time. Therefore, drying in a microcollapse region represents a promising approach for optimisation of the freeze-drying cycle. In contrast, macrocollapse can adversely affect the course of the primary drying, as the sublimation rate is restricted, although it has been reported that even when macrocollapse occurs, the stability of the protein might not be compromised (Schersch et al., 2012).

In the present study, primary drying was carried out under conventional ($T_p < T_g'$) and aggressive ($T_p > T_g'$ or $T_c$) conditions, and the effects on two different formulations of a model IgG monoclonal antibody were evaluated. The goal was to investigate primary drying above $T_g'$ or $T_c$, to reduce the primary drying time while maintaining the quality attributes of the model biopharmaceutical drug product. The freeze-dried products were evaluated after each freeze-drying cycle, and stability studies were also performed under stressed and intended conditions.

## 2. Materials and methods

### 2.1. Materials

The model IgG monoclonal antibody used in this study was obtained from Lek d.d., Slovenia, as a 40 mg/mL formulation in 10 mM succinate buffer (pH 5.0) and 4% (w/v) sucrose. The protein solution was stored at −80 °C, and was thawed overnight at room temperature prior to use. The sucrose used was defined as suitable for biopharmaceutical production (lyoprotectant; ≥99.5% purity; D+; Merck, Germany), and the glycine as suitable for use as an excipient (bulking agent; > 99% purity; Merck, Germany). Polysorbate 20 (a surfactant) and sodium hydroxide (a buffering agent) were from Merck, Germany. Succinic acid (a buffering agent) was from Sigma-Aldrich, Germany.

The buffered solutions of the model IgG monoclonal antibody were prepared in advance, with sucrose as the stabiliser. Ultra-pure water was obtained from a Milli-Q purification system (A10 Advantage; Milipore Corporation, Bedford, MA, USA).

### 2.2. Methods

#### 2.2.1. Sample preparation

The water solution containing the monoclonal antibody (20 mg/mL), 6% (w/v) sucrose or sucrose/glycine (2:1; i.e., 4% [w/v] sucrose, 2% [w/v] glycine), 10 mM succinate buffer (pH 5) and 0.02% (w/v) polysorbate 20 was first filtered through 0.22-μm filters (Corning Filtration Systems, Sigma-Aldrich, USA). Then 8.5 mL of each solution was aliquoted into 20 mL vials (Ompi, Piombino Dese, Italy). Blank solutions were prepared by the same procedure.

#### 2.2.2. Freeze-drying procedure

Freeze-drying was carried out in a pilot freeze-dryer (Epsilon 2-6D; Christ, Osterode am Harz, Germany), equipped with a capacitance manometer (722B Baratron; mks, USA). The vials containing the solutions were partially stoppered with 13 mm rubber stoppers, and distributed on the tray that was placed on a shelf in the freeze-dryer. To ensure the maximum heat transfer to the product, the bottom of the tray was removed. To optimise the freeze-drying cycle, one conventional and five aggressive cycles were designed by varying $T_s$ and chamber pressure during the primary drying. The $T_p$ was monitored with calibrated thermocouple probes, positioned within the vials. The graphic display of the process parameters, primary drying time and $T_p$ was provided by the Christ LPC-32 (LSC) SCADA software. The primary drying time was determined indirectly by monitoring the Tp, and the criteria was based on the fact that approaching of $T_p$ to $T_s$ is an indication of the end of primary drying. As an end point the offset point was selected. For accurate determination of primary drying time the recorded cyclical data were used.

The freeze-drying cycles were run with a row of placebo vials around the edge of the shelf. At the beginning of each cycle, the shelves were cooled to 5 °C. The freezing procedure was kept constant throughout the cycles, with the shelf temperature ramped to −40 °C at 0.5 °C/min, where it was held for 4.5 h.

For the conventional primary drying, the shelves were then heated at a rate of 0.25 °C/min to reach −20 °C, and the chamber pressure was decreased to 0.1 mbar. For the aggressive cycles, the shelf temperature in the primary drying was significantly higher (15, 20 or 30 °C; ramp rate 0.5 °C/min), and different chamber pressures were used (0.1, 0.2 or 0.3 mbar).