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Research paper

Effect of temperature ramp rate during the primary drying process on the properties of amorphous-based lyophilized cake, Part 2: Successful lyophilization by adopting a fast ramp rate during primary drying in protein formulations

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ABSTRACT

In the lyophilization process for injections, the shelf temperature (T_s) and chamber pressure (P_c) have mainly been investigated to optimize the primary drying process. The objective of this study was to show that lyophilization of protein formulations can be achieved by adopting a fast ramp rate of T_s in the beginning of the primary drying process. Bovine serum albumin was used as the model protein, and seven different lyophilized formulations obtained were stored at elevated temperature. We found that although acceptable cake appearance was confirmed by the fast ramp cycle, all formulations of lyophilized cakes obtained by the slow ramp cycle severely collapsed (macrocollapse). It is thought that the collapse in the slow ramp cycle occurred during the shelf ramp in the beginning of primary drying and that insufficient removal of water from the dried matrix caused viscous flow (product collapse). Regarding storage stability, moisture-induced degradation was confirmed in some of the formulations prepared by the slow ramp cycle, whereas all lyophilized BSA formulations prepared by the fast ramp cycle were stable. Thus, the results indicate that the ramp rate appears to be one of the critical operational parameters required to establish a successful lyophilization cycle.

1. Introduction

Among the top-10 pharmaceutical drugs sold in 2017, six products were biopharmaceuticals and mainly therapeutic proteins such as antibodies [1]. In the production of protein pharmaceuticals, lyophilization is frequently performed to stabilize the active ingredients, and various therapeutic proteins have now been launched to market as lyophilized products. Thus, lyophilization is expected to be essential technology for the development of injections, even if formulation development is shifted from conventional low molecular drugs to large molecular drugs.

It is generally known that lyophilization is a time-consuming and expensive process. In the ideal lyophilization process, acceptable cake with low residual moisture is stably manufactured, and the lyophilization time is as short as possible. Great effort has been expended to optimize the primary drying conditions [2,3] because primary drying significantly affects final product quality, and it is the longest of the drying times in the entire lyophilization process (freezing, primary drying and secondary drying). Maintaining a higher product temperature (T_p) during primary drying, which is often called an “aggressive cycle”, is desirable to shorten the process time; however, lyophilization may fail due to collapse or meltback when the T_p remarkably exceeds the critical temperature. Therefore, mainly shelf temperature (T_s) and chamber pressure (P_c), which are related to T_p, have been investigated [2–6], as have freezing conditions such as cooling rate, freezing temperature and annealing [7–10]. Although the effects of these manufacturing parameters on the properties and quality of lyophilized cakes have been reported by a large number of authors so far, there has been no systematic investigation of the ramp rate of T_s at the beginning of primary drying. We evaluated the effect of the ramp rate on the properties of lyophilized cake in our previous study and confirmed that the ramp rate has a significant effect on the appearance and properties of amorphous-based lyophilized cake [11]. Specifically, acceptable cake appearance was obtained by a fast ramp cycle, whereas a slow ramp cycle led to collapse during the primary drying process. We also confirmed that the effect of the ramp rate was remarkable in a high T_s

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cycle, whereas that of the ramp rate was negligible in a low T_s cycle. In addition, lyophilized cakes were successfully prepared in a high T_s cycle only when the fast ramp rate was used. These findings indicated that a faster ramp rate led to successful, aggressive lyophilization, and it was expected to significantly reduce the process time. However, these earlier experiments were performed using a single placebo formulation containing mainly 10% trehalose, and we did not clarify whether the above phenomena occur in other lyophilized formulations. Therefore, there was a need to demonstrate these phenomena in other active formulations (e.g., protein formulations).

In general, formulation researchers and engineers associated with injections recognize that product collapse during the primary drying process should be avoided because poor product appearance and longer reconstitution time are commercial disadvantages [12,13]. Also, collapse results in higher residual moisture, and moisture-induced instability of the lyophilized product may occur [14–16]. With regard to lyophilized protein, the effect of collapse on quality and stability has been investigated for quite some time. Recently published works have mainly reported that structural collapse during the lyophilization process has no negative impact on the stability of protein. For example, Wang et al. reported on the storage stability of lyophilized recombinant Factor VIII and α-amylase, and they concluded that collapse is not necessarily detrimental to the long-term stability of freeze-dried proteins [17]. A similar conclusion was arrived at by several authors studying other lyophilized proteins [18,19]. Also, it was reported that collapsed cakes containing protein are more stable than non-collapsed cakes [20–22]. Additionally, Schersch et al. found that lyophilized protein collapsing during the lyophilization process was more stable than protein collapsing during storage at an elevated temperature [23]. In contrast, evident negative effects of collapse on the storage stability of protein were reported by several authors [24,25]. Thus, the correlation between protein stability and collapse is a complicated issue, and it is difficult to predict stability. For these reasons, it would be of interest to learn whether the fast ramp cycle results in superior stability compared with the slow ramp cycle in protein formulations or whether stability is comparable between the two cycles.

The aim of study was to show that successful lyophilization can be achieved by adopting the fast ramp rate in protein formulations. In the present study, bovine serum albumin (BSA) was used as the model protein and was prepared in seven different formulations. The formulated protein solutions were initially lyophilized by two different cycles (fast and slow ramp cycles) and evaluated to determine collapse of the protein formulation occurred due to the slow ramp rate. Subsequently, a stability evaluation of the lyophilized products at elevated temperature was conducted, and the effect of the ramp rate on stability was investigated.

2. Materials and methods

2.1. Materials

All of the chemicals used in this study were obtained from the following commercial venders: Lyophilized powder of BSA (Fraction V) containing no other ingredients (Product #A2153, purity ≥ 96% by agarose gel electrophoresis; Sigma-Aldrich, St. Louis, MO); D-(+)-trehalose dihydrate (Hayashibara, Okayama, Japan); sucrose, L-arginine, L-arginine hydrochloride and citric acid monohydrate (EMD Millipore, Billerica, MA); Tween 80 (Croda, Edison, NJ); disodium hydrogen phosphate dodecahydrate, sodium dihydrogen phosphate dihydrate (Wako Pure Chemical, Osaka, Japan); and Hydranal® Coulomat AG and Hydranal® Coulomat CG (both, Sigma Aldrich).

2.2. Sample preparation and lyophilization conditions

BSA is widely used in various research fields, and its structure is well characterized [26]. For this reason, BSA was selected as the model protein in the present study. Among commercial lyophilized protein pharmaceuticals, the active ingredient is frequently formulated with non-reducing disaccharide, amino acids and a small amount of surfactant in a weak acidic or neutral pH solution [27]. Therefore, seven different formulations were prepared as described in Table 1. After preparation, the BSA solutions were filtered (0.22-m PVDF filters, Merck Millipore, Tullagreen, Ireland) to remove insoluble BSA aggregates.

A lyophilizer (S20NS, Nissan Edwards, Osaka, Japan) was used for lyophilization of the samples. The sample solution (2.2 mL) was filled in a 5-mL Type 1 glass vial (BB vial 23 × 43VIST; outside diameter, 23 mm; inside diameter, 20 mm; Daiwa Special Glass Co., Ltd., Osaka, Japan) that was partially stoppered with a 20-mm gray butyl rubber stopper (V10-F8, D713, RB2-40; Daikyo Seiko, Tokyo, Japan). The filled vials were loaded onto the shelf of a lyophilizer in a hexagonal arrangement, and thermocouples were placed in the bottom center in representative center vials (formulation 7 [F7]) to monitor the product temperature.

Lyophilization was performed at different ramp rate cycles as follows. (1) Shelf cooling was performed from the ambient temperature to

Table 1
Overview of formulation, composition and sample assignment in the present study.

Sample	Lyophilization cycle[a]	Composition[b]					
		BSA (mg/mL)	Suc (mg/mL)	Tre (mg/mL)	Arg (mM)	PS80 (mg/mL)	Buffer
F1F	FRC	1	100	–	–	0.1	pH 5.5, 10 mM CB
F1S	SRC	1	100	–	–	0.1	pH 5.5, 10 mM CB
F2F	FRC	5	100	–	–	0.1	pH 5.5, 10 mM CB
F2S	SRC	5	100	–	–	0.1	pH 5.5, 10 mM CB
F3F	FRC	25	100	–	–	0.1	pH 5.5, 10 mM CB
F3S	SRC	25	100	–	–	0.1	pH 5.5, 10 mM CB
F4F	FRC	1	100	–	100	0.1	pH 5.5, 10 mM CB
F4S	SRC	1	100	–	100	0.1	pH 5.5, 10 mM CB
F5F	FRC	1	100	–	–	0.1	pH 7.2, 10 mM PB
F5S	SRC	1	100	–	–	0.1	pH 7.2, 10 mM PB
F6F	FRC	1	–	100	–	0.1	pH 5.5, 10 mM CB
F6S	SRC	1	–	100	–	0.1	pH 5.5, 10 mM CB
F7F	FRC	1	–	100	100	0.1	pH 5.5, 10 mM CB
F7S	SRC	1	–	100	100	0.1	pH 5.5, 10 mM CB
F7P[c]	–	–	–	100	100	0.1	pH 5.5, 10 mM CB

[a] FRC, fast ramp cycle; SRC, slow ramp cycle.
[b] BSA, bovine serum albumin; Suc, sucrose; Tre, Trehalose; Arg, L-arginine; PS80, Polysorbate 80 (Tween80); CB, citrate buffer; PB, phosphate buffer.
[c] Placebo formulation of F7 was used in our previous study [11].