SINGLE USE SUPPORT.

PIONEERING BIOPHARMA

How Controlled Freezing becomes Reality

Impact of Ice Front Growth Speed on Scalability of Freezing Protein Solutions

September 2022 | Roland Jenewein, Michael Breitrainer

Abstract

Being able to control the freezing behavior of bulk drug substance is the ultimate goal in Biopharma manufacturing. It opens doors to process reproducibility, consistent quality of the final drug product by maintaining uniform conditions for the biopharmaceuticals during freezing, and consequently for frozen storage and shipment. This simplifies commercial bulk production, particularly when manufacturing different drug substances with various product characteristics.

As one of the most essential parameters in terms of achieving control over freezing & thawing bulk, the freezing rate has been considered and evaluated in different tests. Using the ice front growth speed as a leverage had a significant impact on controllability and, as a result, on protein quality.

Introduction

In Biopharma manufacturing, substances are being stored, frozen and transferred in bulk day in day out. The operation of different production and fill & finish locations is a given fact, and the industry requires a logistics process that is capable of dealing with the challenges associated with remote production sites.

Usually, high-purity bulk protein solution is produced as per demand and independent of time from the final protein drug. At times, the various steps are executed at different locations. While on the one hand this segregation allows for a conversion of bulk solution according to market demand, it also leads to storage as well as transportation requirements for the bulk solution.[1, 2]

The most crucial aim is to transport the active pharmaceutical ingredient, either highly sensitive proteins, mRNA, mABs, ADCs or other biopharmaceutical structures, from A to B at the highest quality possible. The final quality is highly dependent on the process step of freezing & thawing and relies on a reliable cold chain all throughout storage and shipment of frozen BDS. During freezing, the product is exposed to stress that leads to a loss of protein activity.

Different approaches favoring cost-effectiveness, speed of performance, quality of freezing, suitability for cold chain temperature, robustness of packaging, etc. have led to different options for manufacturers. The urge to amplify scalable production options leads to a phasing out of conventional freezing procedures that utilize cryovessels or static freezers. Single-use systems are a trend, offering the best solutions for the industry.[3]

What is still perceived as an unsolved issue due to lack of real-world evidence is the fact that freezing can be controlled, thus allowing for full scalability of frozen storage & shipment.

But how to control freezing?

Minatovicz et al. have defined the homogeneity score of frozen bulk, the degree of cryoconcentration and the freezing rate as the most influential parameters. The latter showed to have significant impact on the stability of other proteins during bulk freezing.[4]

A study performed by Single Use Support and the Department of Bioprocess Technology at the Technical University of Vienna has investigated the impact and outcome of the freezing rate or ice front growth speed, to be more precise.

Considerations before Freezing Bulk

Some of the most important and challenging aspects that need to be considered in the freezing of drug products are the following:

Cryoconcentration

The most fundamental concern when designing freeze/thaw procedures is the phenomenon of cryoconcentration. During the freezing process, the ice formation excludes solutes (including protein) from the growing ice crystal.

As shown in Figure 1 (A), the solutes concentrate towards the center and bottom during freezing.[4]

This concentration effect leads to other effects that induce the denaturation of the protein. Previous studies have shown that cryoconcentration correlates with a slow freezing process, allowing proteins to aggregate in the center and water to freeze in its most natural state around proteins. For example, the high protein concentration itself leads to denaturation through protein aggregation.[1, 2]

(A) STATIC FREEZER
„slow freezing“
dark blue region visible at the bottom center

(B) PLATE FREEZER
„fast freezing“
more uniform coloring

Figure 1: Comparison slow (A) vs. fast freezing (B): Increased cryoconcentration at slow freezing

Freezing Rate

The freeze-path length, which is the distance from the edge of the primary packaging to its center, is a major aspect of how to limit cryoconcentration.

The freezing rate showed to have significant impact on the protein stability during bulk freezing [4]. In most cases, a low freezing rate causes the ice to form slowly enough for the proteins to be pushed along the ice rather than being trapped by the ice front of bags (see Figure 1 (A)). Additionally, longer path lengths from the surface to the center restrict heat transfer and result in slower freezing rates.

Cryoconcentration usually occurs when the freezing process is slow. However, the process of thawing also impacts the degradation of proteins, if they are exposed to slow uncontrolled changes in their physical state. In general, uncontrolled freezing of multiple bags can create variations in freezing patterns and subsequent concentration gradients similar to the one observed in bottles.[5]

Scalability

The aim is to achieve consistent freezing kinetics throughout all different scales, i.e. batch sizes. Freezing must be controlled and independent of the bag size, not only to ensure scalability for freezing & thawing but also to prevent cryoconcentration and the related decrease of the drug, e.g. in terms of cell viability.

Reproducibility of freezing at different scales must be granted in order to guarantee scalable freezing. Single Use Support has already demonstrated that due to very similar freezing curves, scalable freezing is possible both at lab scale and large scale by applying their plate-based freeze & thaw platforms (see also Figure 2).[6]

There is no doubt that various parameters have an impact on scalable freezing, such as the behavior of biologics during freezing along with their solubility and viscosity, but also the equipment's performance for full loads vs. partial loads, setpoint time and others. As long as the freezing kinetics remain under control, nothing will affect scalability under specific conditions[6].

Figure 2: Scalable freezing with single-use bags from 1L to 50L.

Controlled Freezing

Freezing needs to be closely controlled as it can be both harmful and beneficial to the active pharmaceutical ingredient (API). Neither a too-slow nor too-fast freezing process has demonstrated ideal conditions for the protein products. Different freeze studies have highlighted the necessity of examining which freeze rates work best for new systems or products being introduced into a facility. [5]

The rate at which the bulk drug product is frozen is only one of the factors affecting protein recovery.[4] Container dimensions play another important role in API recovery. The freeze distance is the distance from the edge of a container to its center, and thus the use of scaled-up or -down models to describe API stability in bulk-freeze containers is not always advisable as it may not be accurate.[4,7]

Figure 3: Equipment used in the experiments: RoSS® shell with bag

Experimental Methodology

Study Hypothesis

Single Use Support, in collaboration with the Department of Bioprocess Technology at the Technical University of Vienna, have conducted a study and examined the following predefined hypothesis:

The ice front growth speed is independent of scale and can be used for scale-up.

Measurement setup & plan

In a series of experiments, the study's goal was to prove that the ice front growth speed is independent of scale and can therefore be used for scale-up.

The medium used for the experiments was Laccase enzyme, which is commonly used for mimicking valuable drug substances. For the experiments, Laccase was filled into single-use bags of four different sizes, ranging from 2L to 20L.

All experiments were conducted with Single Use Support products and technologies: RoSS shells (see Figure 3) were used as containers to surround the single-use bags. While RoSS.pFTU Lab Scale (LA) (see Figure 4) was used for freezing bags of 2L and 5L, RoSS.pFTU Large Scale (LS) (see Figure 5) was used for freezing bags of 10L and 20L.

Figure 4: Equipment used in the experiments: RoSS.pFTU Lab Scale

Figure 5: Equipment used in the experiments: RoSS.pFTU Large Scale

The experimental conditions are identifiable from the freezing curves shown in Figure 4. In total, 11 different freezing runs were scheduled and conducted one after another over a period of 5 days.

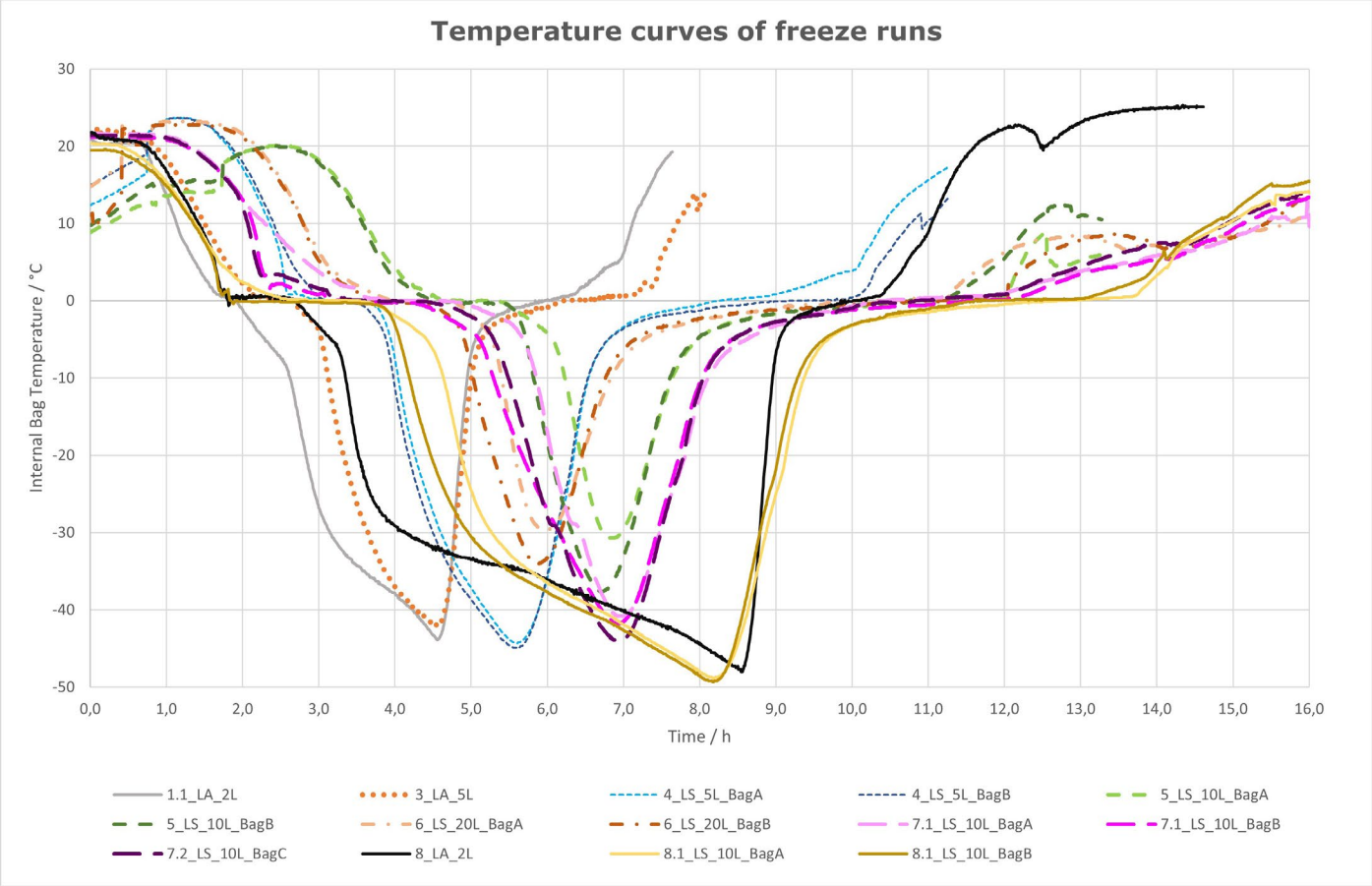

Figure 6: Temperature curves of freeze runs

The ice front growth speed (IFGS) is defined as freeze path length (FPL) in cm devided by phase change duration (PCD) in min.

$$\mathbf{IFGS} = \frac{FPL}{PCD} \; \mathbf{/\ cm/min}$$

Results and Discussion

Impact of ice front growth speed

The ice front growth speeds measured ranged from 0.02 up to 0.05 cm/min (see Table 1). **There was no trend to be observed regarding a correlation of the ice front growth speed and the bag size.** All bags with 2L volume had an ice front growth speed ranging from 0.020 cm/min to 0.034 cm/min, whereas bags with 20L volume had a speed ranging from 0.028 cm/min to 0.035 cm/min.

The resulting average values per single-use bag size were very similar:

- 0.028 cm/min with 2L bags
- 0.044 cm/min with 5L bags
- 0.026 cm/min with 10L bags
- 0.030 cm/min with 20L bags

Table 1: Ice front growth speed per ID freeze run

ID	for units: Volume / L	Time / h	IFGS / cm/min
1.1_LA_2L	2	8.5	0.034
3_LA_5L	5	8.5	0.050
4_LS_5L_BagA	5	8.5	0.038
5_LS_10L_BagA	10	8.5	0.035
6_LS_20L_BagA	20	8.5	0.028
6_LS_20L_BagB	20	8.5	0.035
7.1_LS_10L_BagA	10	10.5	0.025
7.1_LS_10L_BagB	10	10.5	0.023
7_LA_2L	2	10.5	0.031
8.1_LS_10L_BagA	10	12.5	0.022
8_LA_2L	2	12.5	0.020

Freezing could be controlled to the extent of enabling a constant ice front growth rate by time of freezing. The faster the single use bags were frozen, the higher the ice front growth speed. The average ice front growth speed decreased when freezing within a longer timeframe:

- 8.5 hours of freezing resulted in an average IFGS of **0.036 cm/min**
- 10.5 hours of freezing resulted in an average IFGS of **0.026 cm/min**
- 12.5 hours of freezing resulted in an average IFGS of **0.021 cm/min**

Effect of ice front growth speed on protein activity

The tests have shown that the ice front growth speed correlates with the decrease in protein activity. The threshold of protein activity decrease was very low, proving that a controlled ice front growth speed at least has **no negative effect on protein activity**.

The test results confirm literary sources, which claim that controlling the ice front growth speed will subsequently also control protein quality as well as protein activity decrease.[4]

Discussion

Of all the parameters impacting a drug substance's freezing behavior, the **ice front growth speed** has shown to have the biggest impact. Study results have proven the following:

- **Being able to control a drug substance's ice front growth speed at a fast-freezing velocity enables similar freezing kinetics for all sizes of single-use bags.**
- **Being able to control freezing independent of single-use bag size by implication also controls the degree of protein activity decrease.**
- **Furthermore, it is to be assumed that the use of different plate-based freezers utilizing the same freezing technology, RoSS.pFTU Lab scale for smaller and RoSS.pFTU Large Scale for larger volumes, provides control of protein activity or, more specifically, prevents protein degradation.**

Conforming with literary sources, the study results prove that controlled ice front growth speed makes scalable freezing possible (see Figure 5). Performed with plate-based freeze & thaw platform RoSS.pFTU in combination with the RoSS shell protecting any single-use bag on the inside, the doors to scalable freezing experiences, from clinical studies to commercial bulk drug substance production, are pushed wide open.

Further studies that were performed at Single Use Support have reconfirmed scalability. The freezing curves below confirm the independence of ice front growth speed from the type of freezing platform (LabScale or LargeScale) and the height of the water column (minimum and maximum load), as long as the customized freezing recipe per drug substance is correctly implemented. [8]

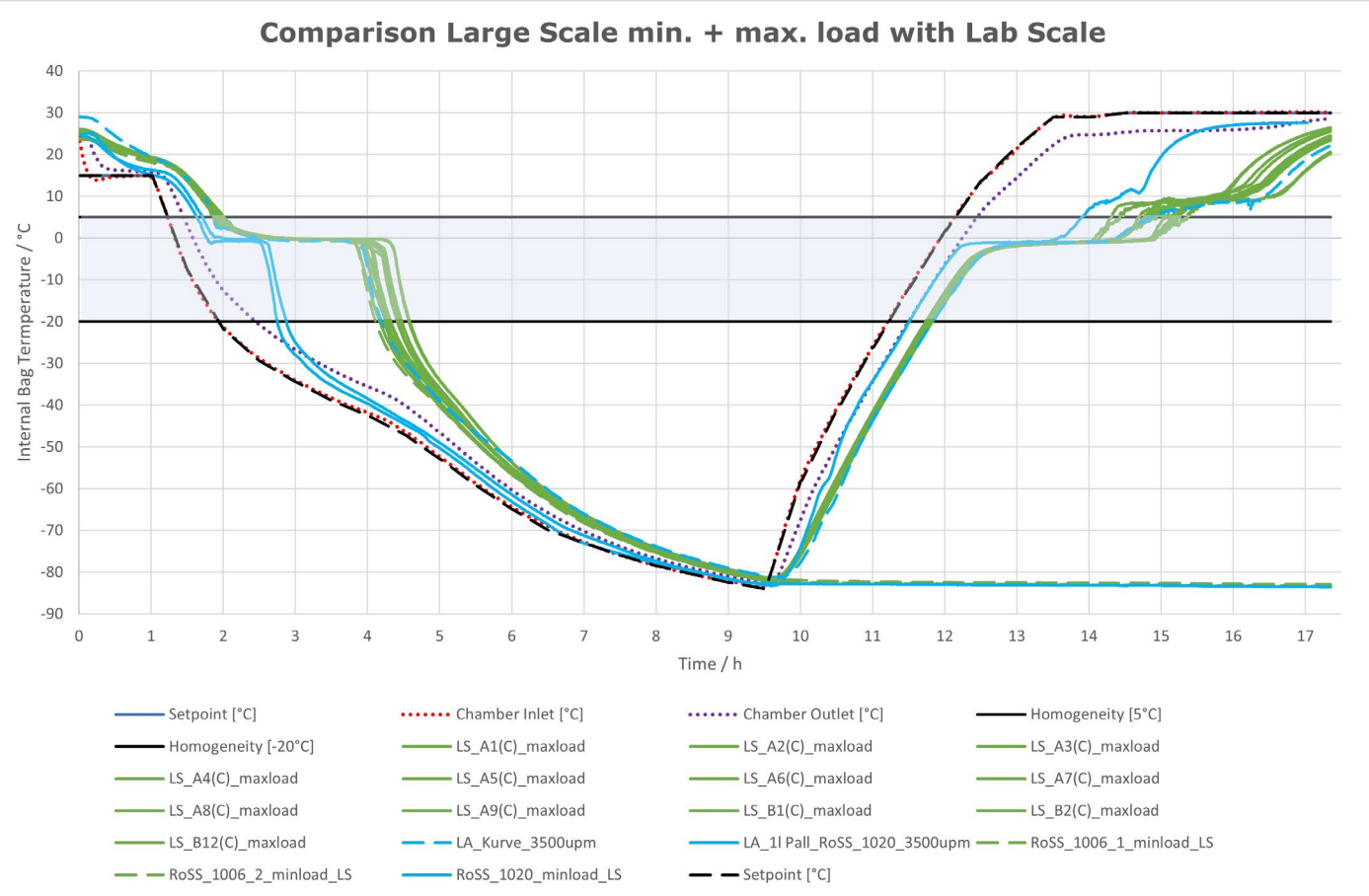

Figure 7: Freezing curves confirming scalability through consistent ice front growth speed:
• Blue curves show results from RoSS_1020 to protect 1L bags frozen in both RoSS.pFTU Lab Scale and LargeScale;
• Green curves show results from RoSS_1006 to protect 10L bags frozen in both RoSS.pFTU LabScale and LargeScale.

Conclusion

Pharmaceutical manufacturers know the characteristics of their drug products by heart. They know how they behave under different circumstances. They know which conditions are beneficial and which are counterproductive. Emerging technologies, such as mRNA vaccines, AAVs or autologous and allogeneic cell and gene therapies, are very sensitive to denaturation and therefore have to be frozen in a highly controlled manner. If neither the highest quality is ensured nor the most reliable technologies are used, there is a risk of negatively impacting the product at the very last stage of production.

This has turned controlled freezing into an increasingly important topic. There is a growing need to maintain uniform or at least similar conditions for freezing, frozen storage and thawing of a drug substance; and there is a growing need of being able to control the freezing and thawing process in order to open doors to enhanced process reproducibility that is both scalable and of high quality. In this way standardized cGMP cold chain process steps can be established in pharmaceutical manufacturing and applied for various products. Cell, gene and gene-modified therapies, but also enveloped vector viruses, for example, require slow and regulated freezing to minimize cell damage and to secure cell viability. Product-based freezing recipes that deploy desired temperature setpoints and consequently desired ice front growth speed, ensure control over the optimal freezing performance for different products.

Providing pharmaceutical manufacturers with a platform that allows for controlled freezing & thawing is a true improvement benefitting the entire industry.

The new insights from the study conducted by Single Use Support and the Department of Bioprocess Technology at the Technical University of Vienna pave the way towards freezing in a controlled and scalable environment by controlling the ice front growth speed throughout different scales, and by reaching high product homogeneity during freezing as well as maintaining highest protein activity.

Acknowledgement

Single Use Support thanks the Department of Bioprocess Technology at the Technical University of Vienna for the ongoing collaboration and the scientific support in gathering expertise and achieving breakthrough results.

References

1. *Singh, S. et al.: Large-Scale Freezing of Biologics; A Practitioner's Review, Part 2: Practical Advice, Bioprocess Int. Vol.7 Issue 10, 2009, P.1., 2009.*
2. *Zippelius, R.: Untersuchungen zum Einfrier- und Auftauverhalten pharmazeutischer Humanproteinlösungen im Großmaßstab. Dissertation 2002, LMU München: Fakultät für Chemie und Pharmazie. Available at: https://edoc.ub.uni-muenchen.de/687/1/Zippelius_Ralf.pdf*
3. *Single Use Support.: Protecting single-use bags to foster their progress, 2021. Available at: Webb, S.D. et al., Freezing bio-pharmaceuticals using common techniques — and the magnitude of bulk-scale freeze-concentration, Biopharm Int. 15 (May) (2002) 22–34)*
4. *Minatovicz, B. et al.: Freeze-concentration of solutes during bulk freezing and its impact on protein stability, Journal of Drug Delivery Science and Technology Vol.58, 101703. 2020. P.1.*
5. *Webb, S.D. et al., Freezing biopharmaceuticals using common techniques — and the magnitude of bulk-scale freeze-concentration, Biopharm Int. 15 (May) (2002) 22–34. Available at: https://cdn.sanity.io/files/0vv8moc6/biopharn/cb3b8ffa70456f-80b72eeb3ef7cac1c29333540a.pdf/article-22913.pdf*
6. *Single Use Support: Scalability Guide, 2021. Available at: https://www.susupport.com/datasheet/freeze-thaw-scalability-guide*
7. *Goldstein, A.: Freeze Bulk Bags: A Case Study in Disposables Implementation, Biopharm International, Vol. 2009 Supplement, Issue 8. 2009. Available at: https://www.biopharminternational.com/view/freeze-bulk-bags-case-study-disposables-implementation*
8. *Single Use Support: Scalability Studies, 2022. Data on file.*

Abbreviations

Abbr.	***Meaning***
AAV	*Adeno-associated virus*
ADC	*Antibody-drug conjugate*
API	*Active Pharmaceutical Ingredient*
BDS	*Bulk Drug Substance*
cGMP	*Current Good Manufacturing Practice*
FPL	*Freeze path length*
ID	*Identification freezing runs*
LA	*Lab Scale (smaller RoSS.pFTU)*
LS	*Large Scale (larger RoSS.pFTU)*
LDH	*L-lactate dehydrogenase*
mAB	*Monoclonal Antibody*
mRNA	*Messenger ribonucleic acid*
PCD	*Phase change duration*
RoSS	*Protective shell for single-use bags. Abbrev: Robust Storage and Shipping*
RoSS.pFTU	*Robust Storage and Shipping plate Freeze-Thaw Unit*