



A Comparison of Freeze Concentration

in Celsius-Paks and a Conventional Freeze-Thaw System

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Sartorius Stedim Biotech is the world leader in controlled Freeze-Thaw technology

Executive Summary

The stability of biopharmaceuticals during storage in the frozen state is dependent upon the appropriate choice of formulation. Changes in the proportion of any component can alter the stability outcome. Because the process of freezing can affect local concentrations as discussed below, the development of a process capable of maintaining the chosen formulation during freezing and thawing is a necessity. The design of such a process also requires a small scale model capable of providing data which will be equally applicable at all process scales as well as a production scale process capable of maintaining the formulation homogeneity.

Conventional large-scale freezing methods are susceptible to fluctuations in product and formulation concentration throughout the processing volume due to the phenomenon of bulk scale freeze concentration. This phenomenon occurs when solutions are frozen in a manner such that the growth of ice through the solution volume occurs slowly enough to permit solutes to diffuse away from the ice surface and for convection to occur within the unfrozen portion of the solution. A combination of this convection, along with the properties of diffusion and crystallization lead to changes in the concentrations of both

excipients and protein differentially throughout the storage volume. Different portions of the solution can consequently end up frozen in conditions radically different from those initially present in the liquid. An additional complication of freeze-thaw process development is that scale down models of conventional freezing are typically not predictive of production scale results due to scaling issues. This leads to different storage conditions at each scale. Final storage temperature alone is not sufficient to establish the similarity of storage conditions between scales.

Sartorius Stedim Biotech's controlled freeze and thaw systems have been designed to minimize bulk scale freeze concentration and provide a scalable method to maintain homogeneous frozen storage conditions for biopharmaceuticals over the entire range of process scales. In this study, the freeze concentration effect has been investigated in Celsius controlled freeze-thaw systems as well as a conventional bottle type freezing process. The results demonstrate the superiority of the Celsius system for maintaining the intended formulation conditions throughout the product volume.

Introduction

Bulk scale freeze concentration is the differential movement of solutes throughout a volume of solution as it undergoes the freezing process. It can result in significant migration of solutes to certain areas of a container while depleting solute concentrations in the remaining areas. These inconsistencies remain until the solution is thawed and homogenized.

This study was undertaken to better understand the differences in freeze concentration behavior of Sartorius Stedim Biotech's production scale Celsius freeze thaw system and a conventional freezing system, consisting of a carboy and a laboratory freezer, on an equivalent product volume. In addition, scalability was examined in the Celsius freeze thaw system. Each system was frozen and the frozen solution was then removed from the container and completely dissected. The frozen portions of the solution were then melted and measured for protein concentration.

Materials and Methods

Definitions

Last Point to Freeze (LPTF): The point within a container that is the last to freeze.

Freezing Path Length: The distance over which ice grows within a volume of solution. Measured from the container wall to the LPTF. Dependent upon container geometry.

Nominal Freeze Time (NFT): Time required for the temperature at a thermocouple at the LPTF to change from +3°C to -5°C.

Effective Freeze Time (EFT): Time required for the temperature at a thermocouple at the LPTF to change from +10°C to -30°C.

Freeze Front Velocity (FFV): Average speed (mm/hr) at which ice grows through a volume of solution. This is calculated as the Freezing Path Length/NFT.

Formulated Protein Solution

The solution used for this study was a model protein solution containing Bovine Serum Albumin (BSA). BSA has a molecular weight of ~65 kDa. This molecular weight makes BSA a good model protein as it is an intermediate size which provides for an intermediate diffusion coefficient. 2 g/L of protein was formulated with in a 50 mM Citrate Buffer with 1% sucrose at a pH of 6.4. The BSA, sodium citrate (Enzyme Grade BP327-1) and citric acid (ACS Grade A940-1) were obtained from Fisher Scientific. The sucrose was obtained from Mallinckrodt (Part # 7723).

Containers

The Celsius system utilizes Celsius-Paks of various volumes as product containers. For this study a 16.6 L Celsius-Pak, and an 8.3 L Celsius-Pak were used. The Celsius-Pak is constructed of 360 µm Stedim 71 film. Stedim 71 film is a multilayer composite film constructed with a product contact layer of Medical Grade Ethylene Vinyl Acetate (EVAM®). The film contains a gas barrier layer constructed of Ethylene Vinyl Alcohol (EVOH). The Celsius-Paks are obtained from Sartorius Stedim Biotech: Part #DB-00016-1 and #DB-00008-2 respectively.

The carboys used for this study were 20 L and 10 L nominal fill volume containers constructed of fluorinated high-density polyethylene (FLPE). The carboys are Nalgene Part # 2097-0050 and # 2097-0020 respectively.

Container Filling

Each of the Celsius-Paks was filled with the nominal volume of solution appropriate to that size of Celsius-Pak, specifically 16.6 and 8.3 L. The 20 L carboy was filled with 16.6 L of solution and the 10 L carboy was filled with 8.3 L of solution.

Freezing

Each of the containers was frozen in a manner consistent with its usual use in freezing processes. The Celsius-Paks were frozen in an FT-16 (the 16.6 L Pak) and FT-100 (the 8.3 L Pak) controlled freeze thaw module, though they each could have been frozen in either apparatus. The standard profile was used in each case, and these are designed for equivalent thermal treatment between devices and scales. The temperature was recorded with a standard Celsius Product RTD using the Celsius-Pak's integrated thermowell allowing for non-product contact temperature monitoring. The FT16 was frozen using a Sartorius Stedim Biotech CryoPilot-B. A CU-5000 temperature control unit (TCU) was used to freeze the FT-100.

The carboys were frozen in a standard -80°C laboratory freezer (Revco model #ULT1386-5-D34). Each carboy was frozen separately so that no additional heat load was present. Each carboy was placed in the bottom of the freezer with only one shelf remaining at the top of the freezer. The temperature in the carboys was recorded at the last point to freeze using a thermocouple inserted through the cap. The thermocouple temperature was measured using Sartorius Stedim Biotech's CryoPilot software for data logging.

Container Dissection

Each container was dissected in a unique pattern due to the differences in geometry between them. The frozen Celsius-Pak has a slab-like geometry while the carboys are cylindrical. Although the two containers produce different frozen solution geometries, they were both dissected to very similar resolutions. Each of the containers was first cut away from the frozen solution. The Celsius-Paks were removed using a razor blade while the carboy walls were cut away using a table saw. Each of the frozen solutions was then dissected using a modified band saw. The band saw was modified by the inclusion of a larger work surface as well as an apparatus to clean the blade of residual sugar and liquid from cutting. This method reduces carryover contamination as each sample is cut away sequentially. An example of the dissection method will be given for each type of container.

Celsius-Pak Dissection

In 16.6 L and 8.3 L sizes, the Celsius-Pak conforms to a rectangular slab shape when frozen and has a thickness of 84 mm. The center of this 84 mm was the area of greatest interest for this study as it is the last area to freeze as heat is removed from the two large faces of the Celsius-Pak. In this case, the 16.6 L dissection method will be given as an example of dissection procedure of this type of geometry.

Figure 1 shows the geometry of the 16.6 L Celsius-Pak and the way in which this shape was divided into three planes from front to back with the highest resolution on the central plane of the Celsius-Pak. Each of the planes of the Celsius-Pak was then dissected into different patterns; the central pattern is shown in Figure 2. The sample sizes represented by each color in Figure 2 are documented in Table 1. The complete dissection yielded 202 samples for analysis. The 8.3 L Celsius-Pak was also dissected into three slabs, and similarly the cutting resolution was increased where appropriate.

Table 1: Legend for Figure 2

Sample Volume (mL)	Number of Samples
146	50 (not shown)
39	24
13	18
9.9	64
4.4	36

Carboy Dissection

The cylindrical geometry of the carboys led to a significantly different dissection scheme than the Celsius-Paks. The 20 L carboy will be illustrated here as an example of the dissection of both carboys.

The frozen solution was first cut out of the carboy and then the edges were removed to obtain a cuboid central core from the original cylinder. The four sides of the cylinder were each split into three pieces from top to bottom and analyzed with the remaining samples. The central core was then cut into eight slices from top to bottom. The slices were of varying thickness and different regions were analyzed at different resolution depending upon their location. Figure 3 highlights all details of the 20 L carboy dissection pattern.

The carboys and Celsius-Paks were split into similar resolution samples with the smallest sample in both cases being about 5 mL.

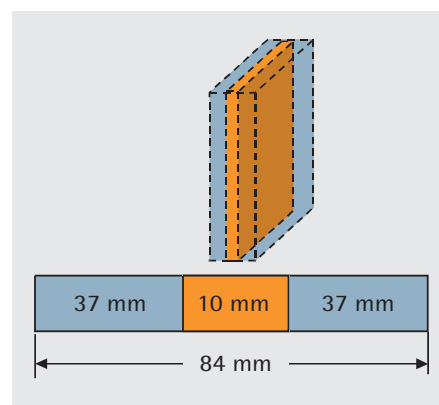


Figure 1: 16.6 L Celsius-Pak: initial separation of planes

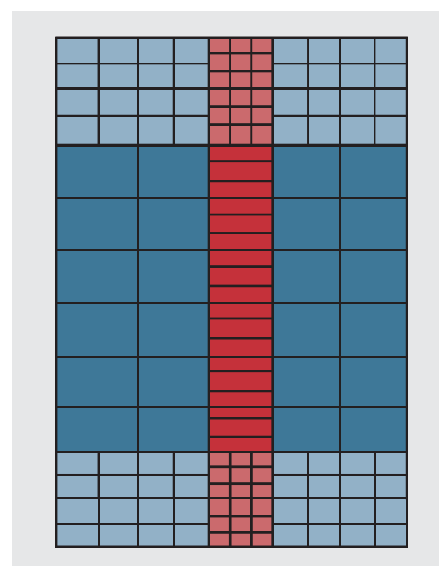


Figure 2: Celsius-Pak Central Plane Dissection Pattern

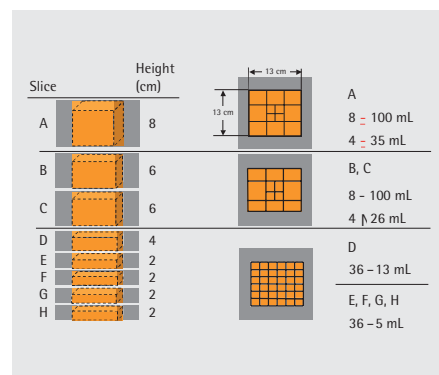


Figure 3: 16.6 L in 20 L carboy Dissection Pattern

Sample Analysis

Each sample from the containers was melted at 2–8°C in a refrigerator. Each solution was homogenized by gentle mixing, sampled, diluted as necessary and analyzed for protein concentration using a Hitachi 6000 series HPLC or Spectronic Genesys 5 spectrophotometer.

In the HPLC, because no separation of solutes was necessary, no column was used with the HPLC. Sample volume was set to 100 µL, and a mobile phase of 18 MΩ deionized water was used. A flow rate of 1 mL/min was found to produce fast reliable results. A diode array detector was used to determine protein concentration by absorbance at 280 nm. Each sample was run in triplicate. Eight standards were run in triplicate before each set of samples.

In the spectrophotometer, samples were diluted as necessary and absorbance was measured at 280 nm.

Results and Discussion

Freeze Time Comparison

The two types of containers were each frozen in their respective freezing apparatus and the resultant freeze temperature data were then analyzed using Sartorius Stedim Biotech’s standard measurement parameters.¹ A comparison of the different freezing results is shown in Table 2 below. Figure 5 offers a graphical comparison of the containers and their different freeze processes. It is clear from the table and figure that the conventional freezing process takes many times longer than the Celsius controlled freezing process. Freezing 16.6 L using controlled freeze-thaw technology is 10 times faster than using conventional techniques.

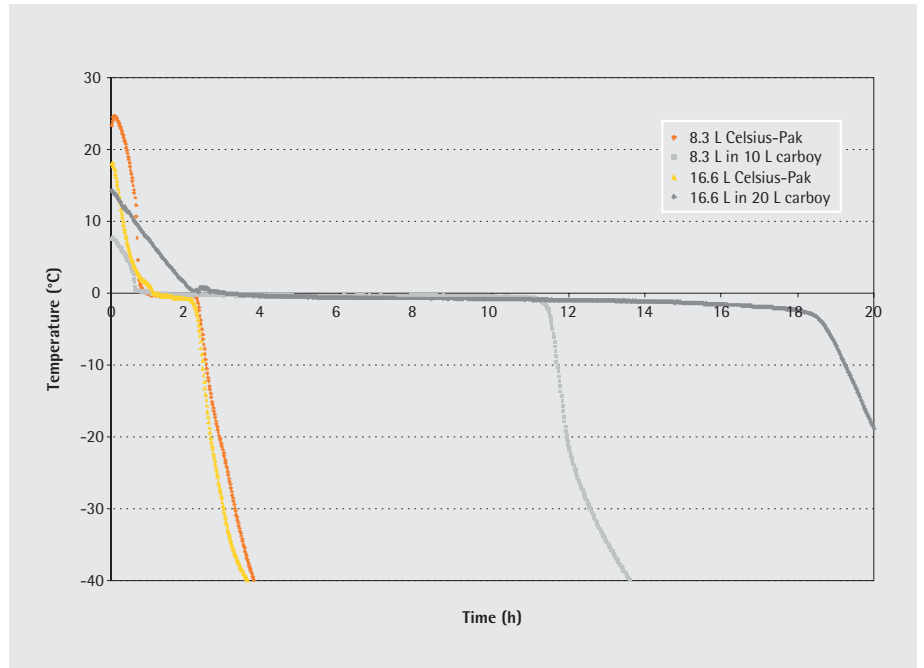


Figure 3: Graphical Comparison of Celsius and Conventional Freeze Times

Table 2: Comparison of Freeze Parameters

Container	NFT (hrs)	EFT (hrs)	FFV (mm/hr)
Celsius-Pak (16.6 L)	1.6	2	24
Celsius-Pak (8.3 L)	1.6	2	24
Carboy 20 L	17	21	11
Carboy 10 L	11	12	11

Freeze Concentration Comparison

The differences in freeze times and the growth of the freeze front within the different containers lead to differences in the bulk scale freeze concentration present in each container. The Celsius-Paks show a very consistent concentration within the volume in which no samples were found with a concentration factor greater than four and at most, 1.2% of samples were found to contain a concentration factor greater than two. Thus at least 98.8% of the solution volume within either of the the Celsius-Paks is frozen with a freeze concentration level of 2 or below.

Figure 4 below shows the concentration throughout the central plane of the 16.6 L Celsius-Pak using the same concentration scale as the other concentration figures. Figures 5 and 7 illustrate the same types of differences in freeze concentration between a Celsius-Pak (Figure 5) and a carboy (Figure 7) at the 8.3 L volume level.

The central plane was confirmed to be the plane with the highest concentration in the Celsius-Pak, but it can be seen to be very close to uniform in concentration, with only a limited region of moderate freeze concentration.

Figure 6 represents a plane through the center of the 20 L carboy showing the freeze concentration present in the carboy on the same concentration scale as the Celsius-Paks in the previous figures. The extent of freeze concentration is quite different. It can be seen that the area of highest concentration of solutes is near the bottom in the center of the carboy. The sample with the highest freeze concentration measured within the 20 L carboy showed a concentration more than 9 times the initial protein concentration.

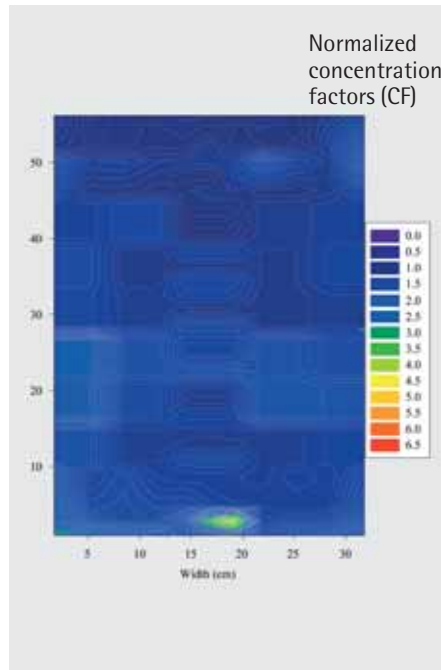


Figure 4: Freeze concentration in 16.6 L Celsius-Pak - central plane

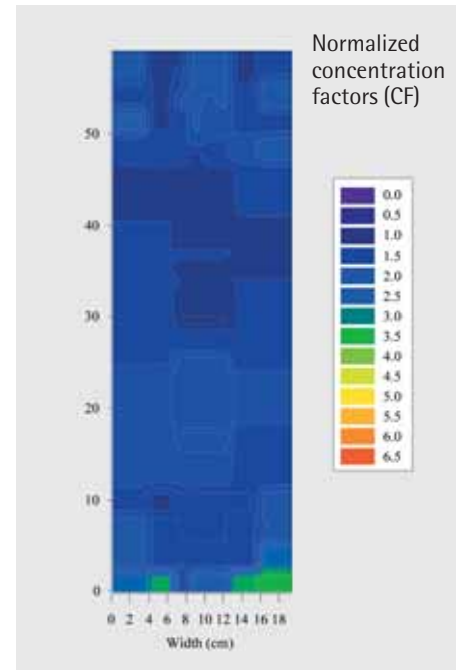


Figure 5: Freeze concentration in 8.3 L Celsius-Pak - central plane

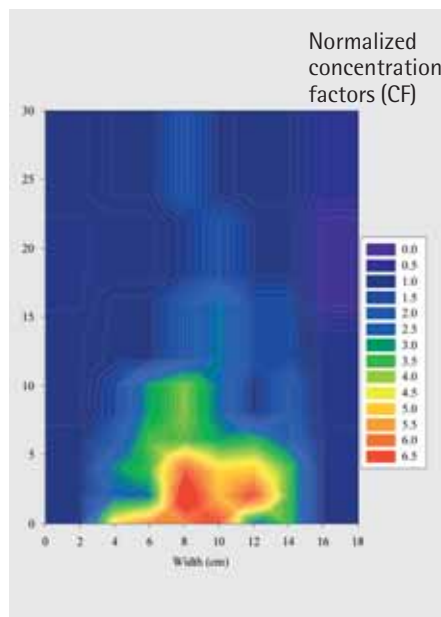


Figure 6: 16.6 L in a 20 L carboy - freeze concentration through a central plane

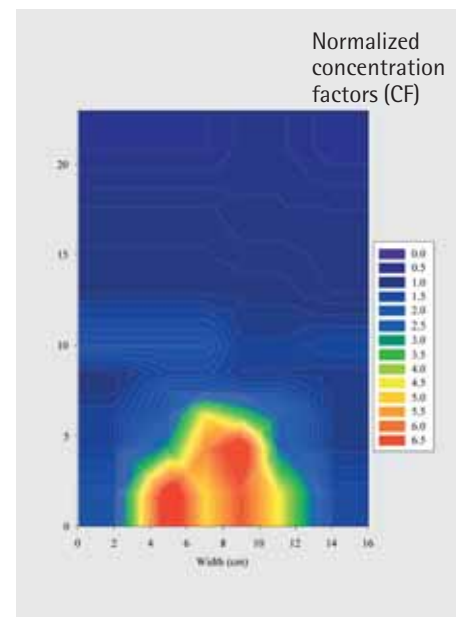


Figure 7: 8.3 L in a 10 L carboy - freeze concentration through a central plane

Figure 8 below shows a cumulative comparison based upon freeze concentration levels and solution volumes between every sample measured in the Celsius-Paks and the carboys. This chart illustrates the differences between the two freezing methods. The amount of volume within the carboys which experienced elevated concentration factors was quite high. The figure also includes the freeze concentration measured in the S³ laboratory scale Celsius Freeze/thaw unit for process development work with both Celsius-Pak sizes available at that scale. The freeze concentration can be seen to overlay well with that seen in the production scale Celsius equipment.

Table 3 below provides a numerical comparison of the freeze concentration recorded during each freezing processes. The proportion of protein stored in the freeze concentrated portion of the volume is quite large in the carboy as compared to the Celsius-Pak. The table shows that although 30% of the solution volume within 16.6 L in a carboy would be stored at a concentration greater than 2 times the intended concentration, only 3% of the protein within an equivalent Celsius-Pak would experience such conditions. By mass, the Celsius-Pak results in 1% of the protein being held over a CF of 2, while a conventional carboy holds ten times this quantity at a CF of greater than 2. Note also that 0% of the protein by mass is held over a freeze concentration of 5 in the Celsius-Pak, but a total of 7% of the protein is exposed to these concentrations in a carboy, and that in the carboy, 1% of the volume holds 7% of the protein.

This illustrates that the highly concentrated portions of the carboy contain about 10 times the protein of an equivalent volume Celsius-Pak. This data illustrates that the magnitude of redistribution of protein throughout a container can be quite large and produce conditions never investigated with the initial formulation. This redistribution subjects notable quantities of the protein to unintended conditions of both protein and excipient concentrations which are different from their original distribution.

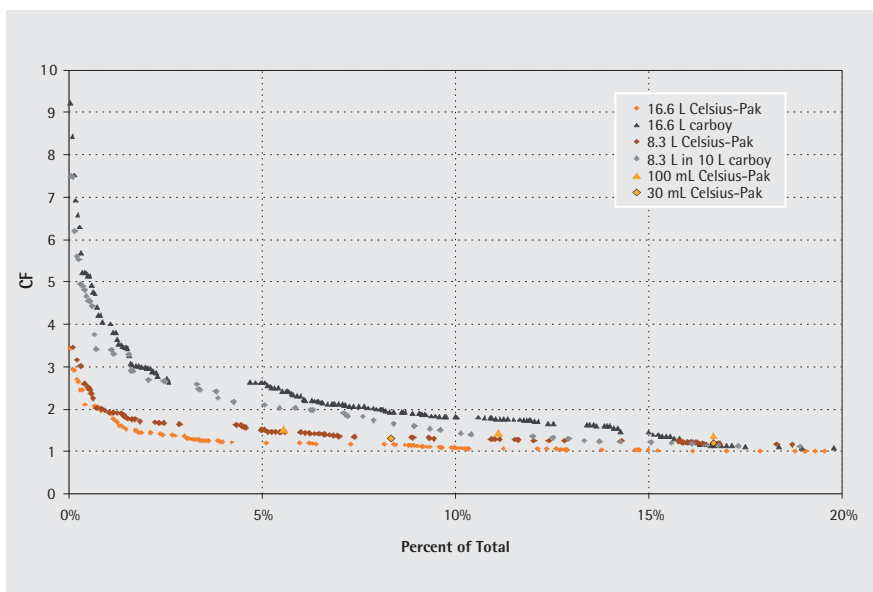


Figure 8: Cumulative comparison by Volume of Freeze Concentration within Celsius-Paks and Carboys

Table 3: Numerical comparison of freezing methods by percentage

	% by Volume		% by Protein Mass	
	16.6 L in a (20 L) carboy	16.6 L Celsius-Pak	16.6 L in a (20 L) carboy	16.6 L Celsius-Pak
Concentration Factor > 2	10%	1%	30%	3%
Concentration Factor > 5	1%	0%	7%	0%

It is also important to note that these results represent a best-case scenario for the conventional freezing method. The carboy in this study was frozen in a -80°C freezer with no other material inside. If more material were frozen at the same time in a conventional freezer the refrigeration system would be less able to handle the heat load and the resulting freeze times would be even longer; resulting in even more extensive freeze concentration. The Celsius system, however, provides for equivalent freezing conditions over a very wide range of volumes. The 16.6 L Celsius-Pak frozen in this experiment therefore experienced the same freezing conditions which would be experienced by any number of Celsius-Paks frozen in any volume Celsius system. The constant freeze concentration profiles available over the full range of laboratory and production volumes provide an advantage only available elsewhere in Sartorius Stedim Biotech's stainless steel CryoVessel based systems.

Conclusions

Sartorius Stedim Biotech's Celsius system is capable of mitigating the bulk scale freeze concentration inherent in the other freezing methods available for equivalent processing volume. The Celsius system shows a large degree of improvement in storage conditions in comparison to conventional methods.

References

¹ AN-100-01 CryoVessel Freeze Parameters

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