



Extent of Freeze Concentration in S³ Celsius-Paks

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

Executive Summary

Sartorius Stedim Biotech has developed the Celsius S³ system for freezing and thawing small volumes of biopharmaceutical products and modeling the freeze-thaw profile of the 100 L production-scale system. Sartorius Stedim Biotech Celsius technology minimizes freeze concentration by controlling the rate of freezing. Freeze concentration has been recognized as a potential freezing stress for biopharmaceuticals since the redistribution of solutes within the remaining unfrozen solution can lead to product concentration, aggregation, precipitation and pH shift. The goal of this study is to experimentally characterize the extent of product and buffer freeze concentration in S³ Celsius-Paks. With a maximum concentration factor of 1.8, the results indicate that the freeze concentration observed with Celsius is low compared to uncontrolled freezing method, for which a 12 times concentration increase was observed in the maximally freeze-concentrated zone. Sartorius Stedim Biotech's Celsius technology provides the product concentration homogeneity inside the solidified matrix favorable for maintaining product stability and quality.

Introduction

Freeze concentration has been recognized as a potential freezing stress for biopharmaceuticals and occurs at two levels. At the microscopic scale, when water molecules crystallize, an unavoidable dehydration of the amorphous phase occurs, called amorphous phase freeze concentration. Although highly concentrated, the amorphous phase quickly reaches its frozen glass transition temperature T_g' below which the high viscosity prevents molecular interactions leading to product loss.

At the macroscopic scale, a bulk-scale or progressive freeze concentration may result from the back-diffusion of solutes from the solidification front to the remaining unfrozen solution¹. This redistribution of solutes in front of the advancing surface of ice can lead to product and solute concentration in the liquid phase for extended period of time and may be detrimental to product stability. To limit the concentration of solutes in the liquid phase, Sartorius Stedim Biotech has developed the Celsius technology: a system for controlling the freezing and thawing rate at manufacturing-scale as well as at the laboratory scale². With the Celsius system, the ice crystal growth rate in the direction of the heat flux is sufficient to prevent the back-diffusion of solutes from inter-crystalline space into the liquid bulk, thus minimizing the bulk-scale freeze concentration³. The Celsius technology uses pre-sterilized single-use containers called Celsius-Paks. The Celsius S³ Freeze-Thaw Module is the bench-top system specifically designed for Scale-up, Scale-down and Stability studies using small quantity of product⁴. This note characterizes the extent of bulk-scale freeze concentration inside 30 mL Celsius-Paks for a model solution representative of the composition of a process intermediate or a bulk drug substance.

Materials and Methods

A solution of 1 mg/mL BSA in 50 mM Citrate buffer, pH 6.40, 150 mM NaCl, 1.89% (w/w) sucrose was filled into one 30 mL Celsius-Pak and then frozen along with nine 30 mL Celsius-Paks filled with deionized (DI) water, using the set point profile shown in Table 1, specially developed by Sartorius Stedim Biotech to model large scale freezing in Celsius using 30 mL Celsius-Paks.

Table 1: "Standard" 30 mL Celsius-Paks freezing set point profile

Time (min)	Temperature set point (°C)
0	-15
40	-70
45	-15
120	-20
210	-30
50	-40

The product temperature was monitored using a thermocouple introduced in the Celsius-Pak thermowell at the Last Point To Freeze (LPTF)⁵. The temperature of one of the DI water filled Celsius-Paks was also monitored. After controlled freezing, the frozen Celsius-Paks were removed from the freeze-thaw module. Then the Celsius-Pak containing the protein solution was cut into 21 (3 rows × 7 columns) square samples. Sample dimensions were approximately 12 × 20 mm (width × height). The samples were thawed in test tubes at room temperature and analyzed as follow:

Total Protein Concentration:

The absorbance was measured at 280 nm on undiluted samples using a small volume measurement cell. Protein concentration was then determined using a calibration curve and the data normalized with respect to the starting concentration.

Conductivity:

Using a daily-calibrated probe with a 12.88 mS/cm standard, conductivity measurements were made on 20–80 times diluted samples. The salt concentration was then determined using a calibration curve and the data normalized with respect to the starting concentration.

Results and Discussion

After taking temperature measurements at the LPTF, the data were converted to quantitative measures of the freezing for analysis. The duration of the phase change plateau is defined by the Nominal Freeze Time (or NFT) as the time required for the temperature at the LPTF to change from +3°C to -5°C. Average freeze front velocity (FFV) is defined by the ratio of the characteristic length for ice growth (42 mm in Celsius system) to the total fusion time as measured by the NFT.

Graph 1 shows the product temperature profiles obtained with the freezing set point profile defined in Table 1. A small freeze point depression is visible for the protein solution with the phase change plateau located at approximately -1°C compared to 0°C for the DI water. The freeze performances are reported in Table 2.

Table 2: Table of results for the freezing process of 30 mL Celsius-Paks filled with 1 mg/mL BSA, 50 mM Citrate, pH 6.4, 150 mM NaCl, 1.89% Sucrose (TC#1) and 30 mL Celsius-Paks filled with deionized water (TC#2).

	TC#1	TC#2
NFT hr	2.72	2.75
FFV mm/hr	15.44	15.27

For the same freezing set point profile, the NFTs of DI water and protein solution are similar and indicate that controlled freezing takes place inside the S³ unit, no matter what the solutes are.

Graph 2 shows the BSA and NaCl concentration distributions inside a Celsius-Pak for the freezing set point profile defined in Table 1. A mass balances performed on the melted samples indicated that more than 93% of the protein and the salt were recovered in the melted samples. Maximum and minimum concentration factors obtained for the protein and the salt are reported in Table 3.

Table 3: Range of protein and salt freeze concentration factors.

Range of protein freeze concentration: [BSA]/[BSA] ₀ (min-max)	0.50-1.74
Range of salt freeze concentration: [Salt]/[Salt] ₀ (min-max)	0.43-1.79

The data indicate that the freeze concentration is more pronounced in the bottom center of the bag and the maximum concentration factor is about 1.8. This is 6.5 times lower than the one obtained in a 1 L bottle placed in a freezer¹, for which a 12 times concentration increase was observed in the maximally freeze concentrated zone.

In general, a bell shape tendency is observed with a strong correlation between salt and protein concentration factors. That correlation may seem surprising because of the lower diffusion coefficient of the protein compared with that of ions. However, dual sided heat fluxes in the Celsius S³ in conjunction with the geometry of the bag create circular convection fluxes in the Celsius-Pak leading to higher solute concentrations in the bottom center of the bag. Aggregation is strongly related to protein concentration and with a maximum freeze concentration factor of 1.8, Celsius technology minimizes that potential freezing stress.

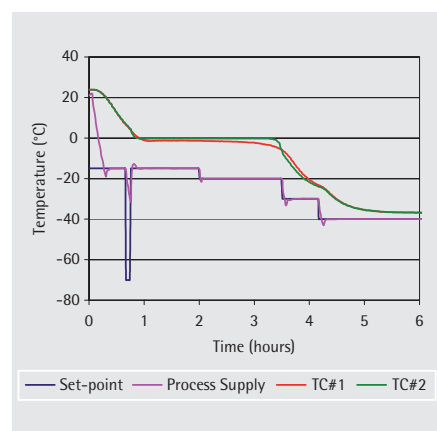
Conclusion

This study demonstrates a high correspondence between protein and salt concentrations. The maximum and minimum concentration factors observed are 1.79 and 0.43 times the initial concentration with the maximally freeze-concentrated zone in the bottom center of the bag. Aggregation is strongly related to protein concentration and with that range of freeze concentration, Celsius technology minimizes one of the potential freezing stresses.

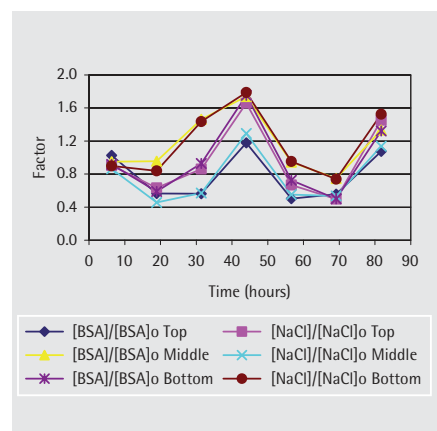
References

- S.D. Webb, J.N. Webb, T.G. Hughes, D.F. Sesin, A.C. Kincaid, "Freezing Bulk-Scale Biopharmaceuticals Using Common Techniques and the Magnitude of Freeze Concentration," *Biopharm* 15(5), 22-34 (2002).
- R. Wisniewski, "Controlled Freeze | Thaw for Biopharmaceuticals," *Gen. Eng. News* 4(23), 1-4 (2003).
- R. Wisniewski, "Developing Large-Scale Cryopreservation Systems for Biopharmaceutical Products," *BioPharm* 11(6), 50-56 (1998).
- N. Voute, T. Dooley, G. Péron, E. Lee, "Disposable Technology for Controlled Freeze-Thaw of Biopharmaceuticals at Manufacturing Scale," *BioProcess Int.*, October supplement, 40-43 (2004).

⁵ G. Péron, "Introduction to the Celsius S³," Sartorius Stedim Biotech AN200-4 (2004).



Graph 1: Temperature profiles for the freezing of a 30 mL Celsius-Pak filled with 1 mg/mL BSA, 50mM Citrate, pH 6.4, 150 mM NaCl, 1.89% Sucrose (TC#1) and a 30mL Celsius-Pak filled with water (TC#2).



Graph 2: BSA and NaCl concentration distributions in the direction perpendicular to the heat exchange plates.

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