



Large-Scale Freezing of Biopharmaceutical Drug Product

A Study of Sartorius Stedim Biotech' Scale-Down Approach Using Formulation Buffer
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Sartorius Stedim Biotech is the world leader in controlled Freeze-Thaw technology

Executive Summary

The purpose of this study is to examine how well Sartorius Stedim Biotech' CryoWedge™ models freezing in a large-scale freeze-thaw vessel, or CryoVessel™. A glycine- sucrose- phosphate formulation was selected as a representative solution for the freeze-thaw studies. Samples were drawn from the center of the CryoWedge and from the corresponding center of the wedge in the CryoVessel during the freezing process. Samples were subjected to conductivity and pH assays to examine the distribution of solutes. Results indicate that Sartorius Stedim Biotech's CryoWedge scale-down system models the CryoVessel freezing temperature profile well, and that freezing in a CryoWedge provides cryoconcentration conditions that are equal to or greater than the cryoconcentration effects measured for the CryoVessel. Near uniformity of the distribution of solutes during freezing was achieved for greater than 99% of the volume. No changes in pH were observed. The results further confirm that Sartorius Stedim Biotech' CryoFin™ technology provides favorable conditions for freezing protein products.

Scope

Sartorius Stedim Biotech has developed large-scale freeze-thaw technology for the biopharmaceutical and industries. The company has developed a scale-down device, the CryoWedge, for modeling the effects of freeze-thaw at the laboratory bench. The CryoWedge represents a cut-away "pie" section of the CryoVessel. (See Figures 1 and 2)

Purpose

The purpose of this study is to examine how well the CryoWedge models freezing in a large-scale freeze-thaw vessel, or CryoVessel. Cooling rates are compared and cryoconcentration effects are examined as a test for possible changes that may effect protein product.

Procedure

Freezing studies were performed using 350 mL of a 2% glycine-1% sucrose-10 mM potassium phosphate – 0.01% Tween 20 at pH 7.85 solution in a CryoWedge (see Figure 1). 17 liters of the same buffer solution was frozen in a CryoVessel (see Figure 3). Samples of buffer were taken during the freezing runs from the center of the CryoWedge, and from the top, middle, and bottom of the corresponding center compartment of the CryoVessel. Samples were subjected to conductivity and pH measurements to examine cryoconcentration effects.

Results

Temperature data and conductivity readings were obtained while freezing a glycine-sucrose-phosphate buffer in a 17 L CryoVessel and a 12 inch (305 mm) diameter CryoWedge filled with 350 mL. The temperature data is illustrated in Figure 4 and the conductivity and pH data are illustrated in Figure 5.

Temperature data indicate that 17 liters of formulation buffer in the CryoVessel solidified at the top (the last point to freeze) at approximately 2.75 hours (165 minutes), as determined by an inflection point and a downward slope in the curve (see "CryoVessel Top Temp") and by visual verification through inspection from the CryoVessel top ports. A temperature profile for the silicone oil was programmed for the CryoWedge to match the 2.75 hour freezing time as measured for the top thermocouple of the CryoVessel in order to achieve modeling of freezing toward the top of the CryoVessel.

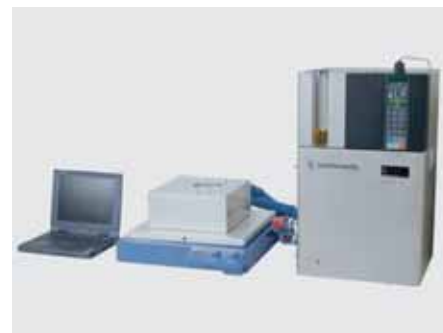


Figure 1. Scale-Down System with 20" CryoWedge

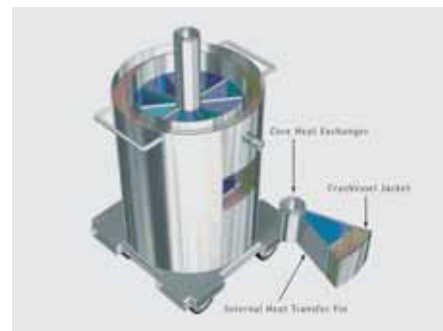


Figure 2. CryoWedge Scale-Down Concept



Figure 3. 20 L CryoVessel with Heat Exchanger

A review of the temperature curve for the CryoWedge (see "CryoWedge Temp" in Figure 4) indicates that solidification of formulation buffer as temperature is measured at the center of the CryoWedge, matched well with the cooling curve for the CryoVessel. A slight variation between the overlay of the CryoWedge and the CryoVessel top cooling curve may be due to the removal of 35 mL of sample (10% of the total volume of 350 mL) from the CryoWedge.

Conductivity measurements of samples taken during the freezing of the 17 liter CryoVessel indicate a starting conductivity of 2.16 milli-Siemens/cm prior to freezing, and with a peak conductivity of 3.05 mS/cm taken from a slurry at the top measuring approximately 1 cm in diameter. This represents 1.4 times the starting conductivity.

Note that a sample of the slurry which consists of a mixture of ice and liquid did not include drawing of ice crystal sample, and therefore produced the highest concentration of solutes. The next highest conductivity sample was 2.8 mS obtained from the middle sample at 150 minutes from the start of freezing.

Conductivity measurements of samples taken during the freezing of 350 mL of formulation buffer in the 12 inch CryoWedge indicate a starting conductivity of 2.16 mS/cm and a peak conductivity of 4.5 mS/cm obtained from 2 mL of solution at the end of the freezing, taken from an approximately 1 cm x 0.75 cm diameter opening in the ice, at approximately 165 minutes from the start of freezing. This represents 2.1 times the starting conductivity value. All samples were subject to pH measurement. No change in pH was observed.

Results indicate that in both the CryoVessel and the CryoWedge the peak levels of conductivity occurred only at the end of the freezing process representing less than 1% of the total volume of solution in the CryoWedge and in the 17 liter CryoVessel. See Calculations below.

Conclusions

Results indicate that the CryoWedge models the CryoVessel freezing temperature profile well, and the CryoWedge meets a worst case acceptance criteria of providing cryoconcentration conditions that are equal to or greater than the cryoconcentration effects measured for the CryoVessel. This result further confirms that the CryoFin technology provides excellent conditions for controlled freezing. One should note that higher levels of ionic solutes measured by conductivity in the CryoWedge and CryoVessel occurs in less than one percent of

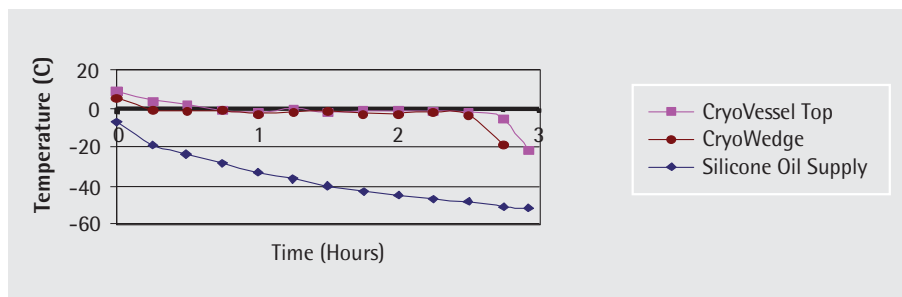


Figure 4. Freezing Glycine-Sucrose-Phosphate Buffer: CryoWedge and CryoVessel Temperature vs. Time

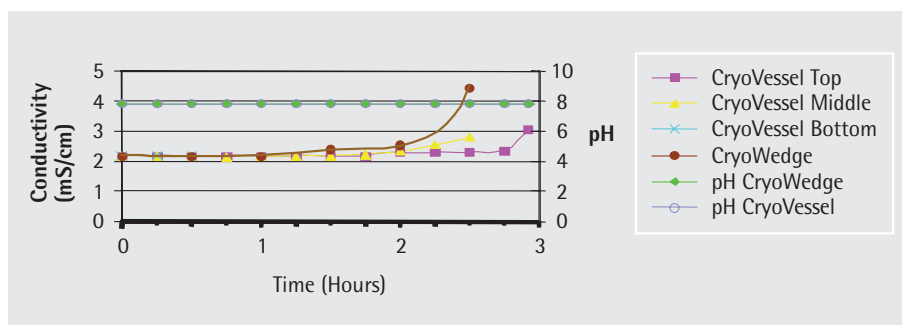


Figure 5. Freezing Glycine-Sucrose-Phosphate Buffer: CryoWedge and CryoVessel Conductivity vs. Time

the volume. Near uniformity of the distribution of solutes during freezing was achieved for greater than 99% of the volume. pH did not change in any of the samples also indicating a favorable environment for freezing protein products.

Calculations

Percent Volume Calculation of the buffer in the CryoWedge at peak conductivity: sample volume/total volume = 2 mL/350 mL = ~0.57%.

Percent Volume Calculation of the buffer in the CryoVessel volume at peak conductivity: $\pi R^2 \times \text{height of liquid} \times 6 \text{ wedges or fins in CryoVessel} = \sim 141 \text{ mLs}$, 141 mLs/17,000 mLs = 0.83%.

Further Reading

Wisniewski, R., *Principles of Large-Scale Cryopreservation of Cells, Microorganisms, Protein Solutions, and Biological Products (Chapter 2), and Large Scale Cryopreservation: Development for Freezing and Thawing of Large Volumes of Cell Suspensions, Protein Solutions and Biological Products (Chapter 3)*, Cryopreservation: Applications in Pharmaceuticals and Biotechnology, Drug Manufacturing Technology Series, vol. 5, edited by K.E. Avis and C.M. Wagner, Interpharm Press, Englewood, Colorado, 1999. ISBN: 1-57491-090-6

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