



Process development for freezing biological solutions

and biopharmaceutical formulations in the CryoVessels: scale up and scale down development using the CryoWedge

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

Executive Summary

Freezing and thawing biological solutions and biopharmaceutical formulations using Sartorius Stedim Biotech' portable freeze-thaw vessels, or CryoVessels, has many advantages such as controlled freezing and thawing rate, thorough distribution of solutes in the frozen product, consistent freezing conditions across the vessel volume, ease of handling of bulk product, and sterility of operations and containment.

Process development of freezing in CryoVessels may not always be possible at production scale if the available quantity of product during the development stage is limited. Sartorius Stedim Biotech' CryoPilot scale-down system provides a process development tool for simulating production scale freezing and thawing. Due to the symmetry of the CryoVessel internal heat exchanger design, the processing conditions that occur at each segment between two fins are identical within the CryoVessel.

The CryoWedge module represents a slice of volume between two fins located in the middle of the CryoVessel. See Figure 4. Since heat transfer during freezing in the CryoVessel occurs from the directly cooled surfaces of external jacket and central heat exchanger, these cooled surfaces also exist in the

CryoWedge with preservation of dimensional similarity within the segment. As in the CryoVessel the heat transfer fluid is recirculated through the segments of jacket and central heat exchanger of the CryoWedge. Additional heat transfer surfaces are the solid fins and their geometry is also preserved. The heat transfer characteristics of these elements are also preserved since the same types of construction materials are used and local material thickness ensures thermal simulation of phenomena occurring in the CryoVessel. Therefore, the side heat transfer surfaces in the CryoWedge represent the side heat transfer surfaces which exist in each symmetrical segment of the CryoVessel. To simulate freezing in the middle of the CryoVessel, the effects of the CryoWedge bottom and top needed to be minimized.

Minimizing the stainless steel bottom effect has been accomplished by utilizing a very thin plate for the bottom construction, thus minimizing the heat conduction effects from the cooled surfaces through this plate. Minimizing of the top effect has been accomplished by removing the top cover far from the product surface and by applying very thin plates connecting the directly cooled walls and solid fins with the module top plate. These thin plates conduct little heat and the gas phase cooling above the product surface is minimized. The heat gains from the cover are minimized as well. As a result the heat transfer occurs between the directly cooled surfaces and solid fins and the product, while there is no significant cooling of the gas phase above the product surface, e.g. there is no strong product surface freezing because of the presence of cold gas. There is also no significant temperature gradient (warming) from the cover towards the surfaces which are in contact with the product. The interior of CryoWedge module is thermally insulated to minimize effects of the environment. The resulting design of the CryoWedge module provides good simulation of the conditions inside the CryoVessel during product freezing and thawing.

The experiment design involves selection of a product volume to be processed in the CryoWedge. The product needs not only to cover the chamber bottom but also certain product depth is required to develop adequate contact area between the cooled surfaces and the product. The recommended product depth



Figure 1. CryoPilot with 20" CryoWedge



Figure 2. CryoVessel Concept



Figure 3. 20 L CryoVessel with Heat Exchanger

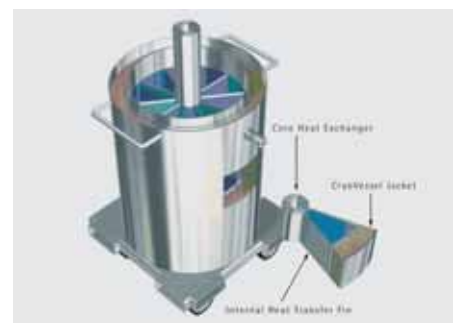


Figure 4. CryoWedge

is about 90 % of the actual fin height and this is also the optimum depth considering freezing phenomena in the CryoWedge. (See Table 1) At low product levels the results of freezing in the CryoWedge may not closely represent the phenomena occurring during freezing in the CryoVessel (for example, reduction of product volume may cause more pronounced liquid undercooling prior to freezing).

The freezing process shall be conducted using the temperature change conditions of the cooled surfaces similar to the conditions occurring in the production scale CryoVessel. This is accomplished by selection of a temperature profile for the CryoVessel and applying it to the CryoWedge. A controlled temperature profile applied to the cooled surfaces is important to maintain the controlled growth of dendritic ice crystals which are essential to product preservation within the frozen volume (Ref.: Wisniewski R., BioPharm, June 1998, pp. 50-60). The controlled temperature change rate can be accomplished by use of a computer-controlled (CryoPilot software) recirculating chiller that delivers heat transfer fluid to the CryoWedge precisely following the pre-programmed temperature profile. The CryoWedge modules match the corresponding CryoVessel geometries (Table 1).

The CryoPilot program does have certain vessel characteristics already built-in, e.g. the temperature profile that is produced in the CryoWedge may not reach beyond the envelope which is possible to accomplish using the corresponding CryoVessel design. The CryoPilot software permits programming linear, quasi-nonlinear (multiple segments) and nonlinear temperature profiles. The temperature profiles for freezing water in a CryoWedge and the CryoVessel is shown in Figure 5.

Thawing in the CryoWedge is accomplished by use of pre-programmed temperature profile of the recirculating heat transfer fluid with controlled maximum temperature of the heating fluid. The maximum temperature of the heat transfer fluid is typically equal to the maximum allowable product temperature at the CryoVessel wall during the process. Since a rapid thawing process is desirable from a production standpoint, the thawing procedure may use this maximum temperature from the beginning of the thawing step. The transparent CryoWedge cover permits observation of freezing progression. The CryoWedge is equipped with multiple thermocouples to monitor freezing and thawing temperature profiles at various points. Withdrawal of liquid samples is possible. The module can be autoclaved and may provide sterile product containment conditions.

Table 1. CryoWedge Scale-down Modules

| CryoWedge | Scale-up CryoVessel | CryoVessel Diameter | CryoWedge Nominal Buffer Fill Volume |
|-----------|---------------------|---------------------|--------------------------------------|
| CW40 | 20 L, 40 L | 12" | 350 mL |
| CW125 | 60 L, 100 L, 125 L | 20" | 650 mL |
| CW200 | 200 L | 30" | 3 L |
| CW300 | 300 L | 34" | 4 L |

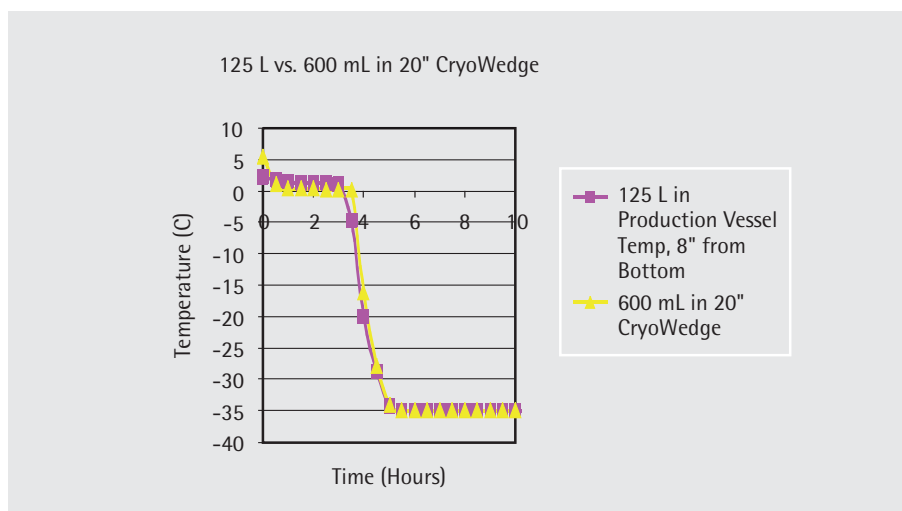


Figure 5. 125 L Vessel vs. 600 mL in 20\"/>

The user may consider the following approach to process development using the CryoWedge:

1. Program the temperature profile associated with rapid freezing in the corresponding CryoVessel design.
2. Conduct the freeze-thaw test (single and multiple – the multiple freeze-thaw test may involve up to five consecutive freezing and thawing steps) and determine effects on the product. If the results are satisfactory (no significant product degradation) the development may be concluded.
3. Further optimization of the freezing process involves use of pre-programmed temperature profile with linear temperature change in time. Single and multiple freeze-thaw tests need to be performed as previously.
4. Fine tuning of the process may involve use of complex temperature profiles to control spacing among the dendritic ice crystals. Steeper temperature decrease at the beginning of the process may produce smaller spacing between dendrites, while small temperature decrease may produce larger spacing. This initial period length may be as 20% to 30% of the overall freezing time. The subsequent rate of temperature decrease may be different from the initial value. The interdendritic spacing determines contact area between product

and ice. Empirical approach is recommended since the interdendritic spacing also depends on the concentration and composition of solutes.

The combination of the CryoWedge and CryoPilot Windows-based software provides an economical and powerful evaluative tool for conducting scale-down freezing and thawing experiments.

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 Publication No.: SL-1056-e07121
 Order No.: 85032-534-38