Introduction to the Celsius S^3 Controlled Freeze | Thaw System

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Freeze-Thaw Systems

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

Executive Summary

The Celsius S^3 is a laboratory freeze-thaw system specifically designed for development and stability studies. Freeze-thaw runs can be performed with as little as 30 mL of product using the same operating conditions as the 100 L production-scale system. The freezing and thawing conditions were compared at both scales. Excellent consistency of freeze-thaw product temperature profiles was observed. Thus, the Celsius S^3 system provides the capability to simulate production-scale freezing and thawing using disposable container with identical materials of construction.

Introduction

The Celsius S^3 controlled Freeze-Thaw system, developed by Sartorius Stedim Biotech, is a laboratory–scale tool specifically designed for Scale-up, Scale-down and Stability Studies.

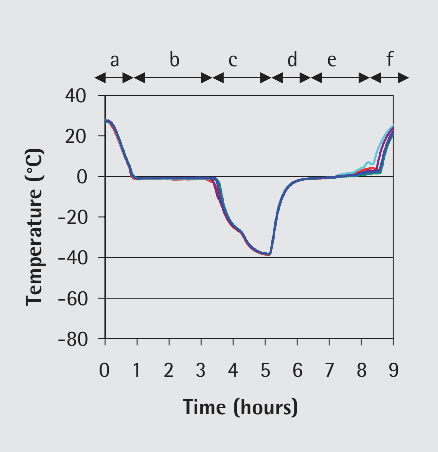
The system, presented in Figure 1, includes a freeze-thaw module: Celsius S^3 (a), a reciprocating mixer: Cryomixer Junior II (b), a temperature control unit: Cryopilot A or B (c), and a data acquisition system for temperature control and record (not shown). Liquid samples are filled in small Celsius-Paks, disposable containers available in two sizes, 30 mL or 100 mL. They are placed in the Celsius S^3 module between a pair of heat exchange plates within which circulates a heat transfer fluid. This setup reproduces the freezing and thawing conditions encountered at large-scale because the same freezing distance and the same material of construction as the production-scale Celsius-Paks are used. This configuration allows for a controlled freezing process based on bidirectional crystal growth along the general direction of the heat flow (1). Moreover, some Celsius-Pak are available with a thermowell that allows aseptic temperature measurements by a T-type thermocouple during freeze-thaw operations. The thermocouple tip is located 1 cm below the liquid level at the bag centerline known as the Last Point To Freeze (LPTF) of the container (2). Therefore, monitoring the temperature of the product and heat transfer fluid allows for documentation of the process uniformity, reproducibility and scalability.

Parameters to Monitor

There are three parameters to monitor during the freezing step. These variables are simple to measure since they are time-based parameters. The freezing of a product consists of three steps which are illustrated in Graph 1.

The first step is the cooling of the liquid (a) followed by a nucleation that starts at the product freezing point. It is important to note that no undercooling is detected. Undercooling is not reproducible and may affect the product quality. The second step is the removal of latent heat (b) which occurs at constant temperature (the phase change plateau). It is an important characteristic of the freezing profiles since the product quality is dependent on the freezing rate. The duration of the 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. The third and last freezing step is the cooling of the solidified bulk (c) to the end temperature. The second characteristic time measured at the LPTF is the Effective Freeze Time (or EFT). Practically, the EFT is defined as the time required for the temperature at the LPTF to change from +10°C to –30°C and is an additional measure of the similarity between freezing runs.

Figure 1: The Celsius S^3 system



Graph 1: Freeze and thaw process of ten 30 mL Celsius-Paks containing deionized water. Colored thin lines are the product temperature profiles inside the ten 30 mL Celsius-Paks.

Three steps also characterize the thaw. A first sep is the heating of the ice (d) up to the melting temperature at which starts the second step: the ice melting (e). A heating of the liquid (f) then follows the melting of the last piece of ice. The third parameter to monitor is the thaw time. The thaw time (or TT) is defined as the time that it takes for the frozen product to become completely liquid. Practically and to take into account different storage temperatures, the beginning of the thaw is when the START button is pressed on the CryoPilot software and the end of the thaw is defined as the time at which a depression is observed at the end of the ice melting phase.

Development of Set Point Profiles

These freezing and thawing parameters are useful to monitor freeze | thaw runs and help to reproduce product temperature curves obtained at a large-scale. Small-scale temperature set-point profiles have to be developed because of the differences in chiller cooling capacity and in cooling dynamics between the large and small-scales, as for the CryoFin product line, which uses reusable stainless steel vessels (3). Sartorius Stedim Biotech' application group has developed the Gold Standard Profiles (GSP) so that the product temperatures curves obtained by using the Celsius S³ system, either with the 30 mL or the 100 mL Celsius-Paks, match the ones obtained by using the Celsius production-scale system. Based on graph of large-scale LPTF product temperature curves, series of steps of 5°C or 10°C were draw and translated into a set of numerical instructions in the text format readable by the CryoPilot software. Once the freeze | thaw runs were completed, the data files were placed into a spreadsheet application along with data from the large-scale system. The data from both sources were plotted against each other and the parts of the set point profile that need modifications were determined in a graphical fashion. This procedure was repeated until both profiles from small and large-scale match each other to the desired degree.

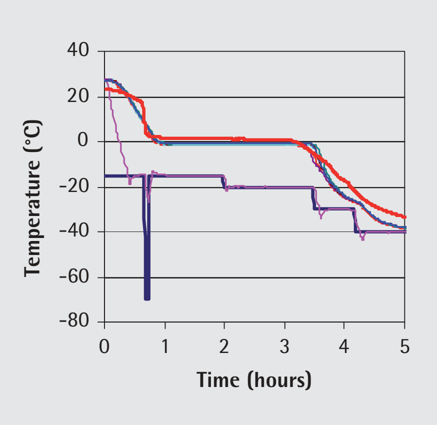
During the thawing step, the temperature measurement follows a different profile at each of the two scales. The position of the last piece of ice explains that difference observed between the two scales. Within the 30 mL Celsius-Pak, the last ice block is stuck to the thermowell. Thus, the temperature recorded during the entire period of ice melting is the melting temperature. Within the 16.6 L Celsius-Pak, the last piece of ice is stuck to the bottom of the container and the product temperature recorded at the LPTF is rapidly greater than the melting temperature. However, the depression observed is still caused by the melting of the last piece of ice which, at some point in the thawing process, rises to the top of the container.

Results and Discussion

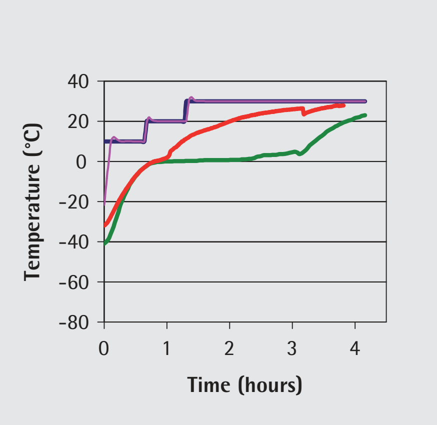
Graph 2 shows a comparison of the product temperature curves obtained for the freezing process at manufacturing scale (100 L) and at laboratory scale (30 mL). The temperature set point profile of the chiller is adjusted to take into account the differences in chiller cooling capacity and cooling dynamics, as well as changes in overall heat exchange efficiency between the large and the small-scale unit. This adjustment allows having the same freeze front progression. Thus, the nominal and effective freeze times obtained at large and small-scale are comparable within less than 6%. In addition, the ice crystal growth rate is similar at the two scales, allowing direct comparison of the micro-scale effects such as back-diffusion and crystal surface area on product stability. It was found critical to induce ice nucleation, on the small-scale unit, by a transient temperature drop to −70°C in order to minimize undercooling of the liquid phase. All ten Celsius-Paks freeze uniformly in the S³ unit.

Graph 3 shows a comparison of the product temperature curves obtained for the thawing process at production-scale (100 L) and at the small-scale (30 mL). Again, the temperature set point profile was adjusted so that the thawing times for both scales are comparable within less than 2%. The table below summarizes the results obtained at both scale:

Parameters	**Small-scale**	**Large-scale**	**Difference (%)**
NFT (h)	2.76	2.72	1.47
EFT (h)	3.95	3.73	5.90
TT (h)	3.48	3.44	1.16



Graph 2: Scalability of the freezing process from 30 mL to the 100 L scale. Red line is the temperature profile during the freezing step at the 100 L scale using six 16.6 L disposables; colored thin lines are the product temperature profiles inside the ten 30 mL disposable containers; pink line is the temperature of the heat transfer fluid entering the Celsius S^3; bold blue line is the set point profile of heat transfer fluid temperature.



Graph 3: Scalability of the thawing process from 30 mL to the 100 L scale. Red line is the temperature profile during the thawing step at the 100 L scale using six 16.6 L disposables; green line is the product temperature profiles inside the ten 30 mL disposable containers; pink line is the temperature of the heat transfer fluid entering the Celsius S^3; bold blue line is the set point profile of heat transfer fluid temperature in the Celsius S^3.

Conclusion

The optimized freeze-thaw conditions were reproduced at a reduced scale for process optimization and stability study. Thus, freeze-thaw performances with the Celsius S^3 system mimics with as little as 30 mL of product the performances obtained with the large-scale system using 100 L of product.

References

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(3) Matthew Olsen, "Working with and Developing Scalable Freeze-Thaw Profiles for Sartorius Stedim Biotech' CryoWedge System," Sartorius Stedim Biotech Application Note AN200-3.

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