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CryoVessel Freeze Characterization

Sartorius Stedim Biotech's Standard Freeze Analysis Techniques for Temperature Profile Analysis
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Freeze-Thaw Systems

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

Executive Summary
Sartorius Stedim Biotech defines several parameters for measuring and classifying the progress of freezing within CryoVessels which allow for meaningful analysis of freeze-thaw data. These variables can be used for comparison between CryoVessel runs as well as providing a basis of similarity for scaling up and down using Sartorius Stedim Biotech's CryoWedge small scale systems.

System Analysis
During process development the need to monitor and analyze CryoVessel system performance is a typical requirement. This is generally accomplished by mapping the temperature during processing throughout the CryoVessel using thermocouples or Resistance Temperature Devices (RTDs). Sartorius Stedim Biotech has developed some standard methodologies for gathering and analyzing that temperature data in an efficient manner.

CryoVessel processing is monitored in actual production situations by one RTD, known as the Product RTD. This RTD provides freezing data for one point within the CryoVessel, however, occasionally more comprehensive temperature monitoring and system analysis may be needed, for example during process development or validation. In this case, a number of T-type Thermocouples can be placed at various points within the CryoVessel. These additional thermocouples allow one to fully map the temperature within the CryoVessel, although necessitating non-sterile conditions.

As the CryoVessel freezes, the freeze front, the advancing surface of ice within the CryoVessel, moves through the CryoVessel in a characteristic manner. The location of thermocouples at strategically located points within the CryoVessel allows for monitoring of the freeze front's progress. The most important point for system analysis is known as the last point to freeze (LPTF) which always occurs in the geometric center of one of the wedges, at the liquid surface level. All CryoVessels freeze from the sides in and from the bottom up, ending at the surface center of the wedges due to the novel arrangement of their heat transfer surfaces.

Figure 1 shows a typical set of temperature profiles obtained from a CryoVessel fully outfitted with 9 thermocouples at various heights and distances from the heat transfer surfaces. In addition to these points, the graph contains traces from the Product RTD and the Heat Transfer Fluid (HTF) inlet and outlet temperatures. The variation between different thermocouple traces shown in Figure 1 is the result of their relative positions within the CryoVessel. The LPTF occurs at thermocouple A2 which is shown in red as the last thermocouple to register a temperature below 0°C. Each of the thermal profiles in Figure 1 can be divided into several segments based upon the processes occurring at that point in the system. There is an initial cooling phase during which all points quickly descend to the freezing point of the liquid within the CryoVessel (0°C for water).

This initial descent is followed by a plateau, caused by the liquid-solid phase transition, at the liquid's freezing point that extends until the latent heat of freezing for the liquid surrounding each thermocouple has been overcome. The third phase of each curve is the cooling of the frozen solution from the freezing point down to the system setpoint. These three phases are a characteristic of freezing curves produced by Sartorius Stedim Biotech technologies and the usefulness of our different measures of freezing is based upon the reproducibility of these curves at different points within CryoVessels.

The LPTF is the most important point for characterizing the freeze within the CryoVessel. It defines the longest freezing time experienced by material within the CryoVessel which therefore represents the longest time that any product in the CryoVessel is in contact with the liquid phase. Processes detrimental to the stability of the protein are more likely to occur while the protein is in the liquid phase. Because the LPTF represents the worst case for protein stability within Sartorius Stedim Biotech's CryoVessel Systems, it is used to correlate results between the CryoVessel and CryoWedge small scale systems. The LPTF occurs at the center of the CryoWedge as well, and if the thermocouples are located similarly within the large scale and pilot scale wedges then any run conducted at the large scale can be reproduced with the small scale system. In both systems, the standard thermocouple placement for measurement of the LPTF is in the geometric center of the wedge 0.5″ below the surface of the liquid.

The Product RTD is placed at the bottom of the CryoVessel thermowell, which is located very close to the bottom of the CryoVessel itself. Although it would be useful to gather data from the last point to freeze during actual production runs, it is impractical due to the various volumes to which CryoVessels are filled. Having the RTD near the bottom of the CryoVessel ensures that it will always be beneath the liquid surface, thereby maximizing the working volume range of the CryoVessel. As can be seen in Figure 1 the Product RTD point freezes long before the rest of the CryoVessel and is thus not indicative of the overall solidification time in the CryoVessel.

Process Descriptors
Data from the LPTF and Product RTD are converted to quantitative measures of the freeze progression in CryoVessels for system analysis. This quantitative data eases comparison between different runs as well as the determination of nominal process parameters and variability. This section describes the procedure for producing Sartorius Stedim Biotech's quantitative measures of freezing.

Figures 2, 3 and 4 display the temperature curves obtained from a thermocouple at the LPTF and the Product RTD for various CryoVessel sizes attached to different CryoTrols. The different shapes are characteristic for each of the CryoVessels when filled to capacity and are a result of the different wedge sizes and geometries. The two curves show that the Product RTD, near the bottom of the CryoVessel, freezes first, while the top of the CryoVessel freezes much later as described above. The three figures also show that the length of time required for freezing increases with CryoVessel size.

One important characteristic of temperature curves obtained from CryoVessels is the time required to overcome the latent heat of freezing which creates the plateau in the curve seen near 0°C. Sartorius Stedim Biotech defines the nominal freeze time (or NFT) as the time required for the product temperature at the LPTF to change from +5°C to –5°C. This value represents the time spent at the plateau which is the time required to overcome the latent heat of freezing along with a margin on each end to improve the consistency of the data. The margin about 0°C is added to account for slightly different freezing points of different solutions as well as slight differences in thermocouple placement between runs. The guidelines provided by Sartorius Stedim Biotech about the minimum time a CryoVessel takes to freeze were determined by measuring the temperature at this point.

The second characteristic time measured at the LPTF is the Effective Freeze Time (or EFT). The EFT is defined as the time required for the product temperature at the LPTF to change from +10°C to –30°C and is useful as an additional measure of the similarity between any two runs.

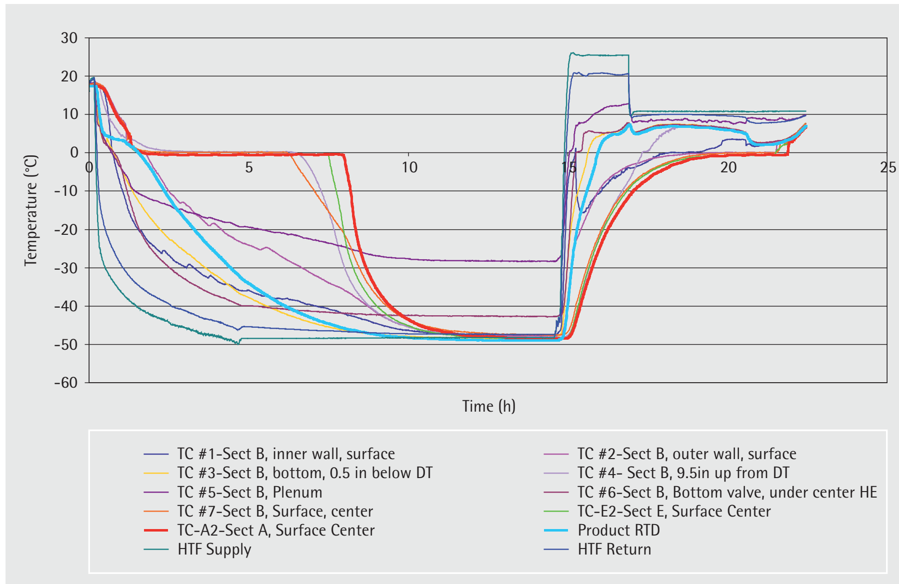

Figure 1: Typical Temperature Profiles from a 200 L CryoVessel

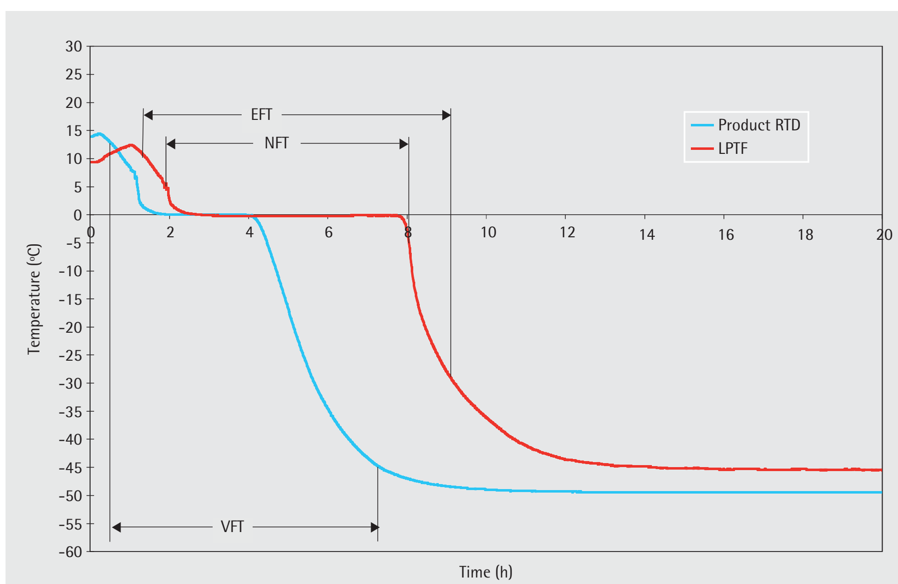

Figure 2: Characteristic Temperature Curves CV125 with CT200

The NFT and EFT provide useful data on the freezing in a CryoVessel, however, they are very difficult to monitor during day-to-day manufacturing operations due to sterility and GMP issues. Thus, the third freezing parameter used by Sartorius Stedim Biotech is calculated from Product RTD data. This measure is referred to as the Vessel Freeze Time (VFT) and is defined as the time required for the temperature at the Product RTD to change from +12°C to −45°C. Although there is still liquid in the CryoVessel when the Product RTD reads −45°C this data is still useful as it is the only data that customers obtain from within the CryoVessel during actual processing and so allows for troubleshooting and comparison to customer data. In addition it is important to remember that CryoVessel freeze times provided by Sartorius Stedim Biotech for use in process planning and process development are based upon data from the LPTF and not the Product RTD.

Table 1: Summary of Process Descriptors

Process Descriptor	Measurement Point	Measurement Range
NFT	LPTF	+5 to −5C
EFT	LPTF	+10 to −30C
VFT	PRODUCT RTD	+12 to −45C

Conclusions
Table 1 summarizes the three freeze parameters measured by Sartorius Stedim Biotech. Between these three measures of the freezing within a CryoVessel, any particular run in a CryoVessel can be well described. When all three are known, it is easy to compare different runs and determine their similarity. They are also useful during the development of profiles with a CryoPilot system to replicate conditions encountered at the large scale.

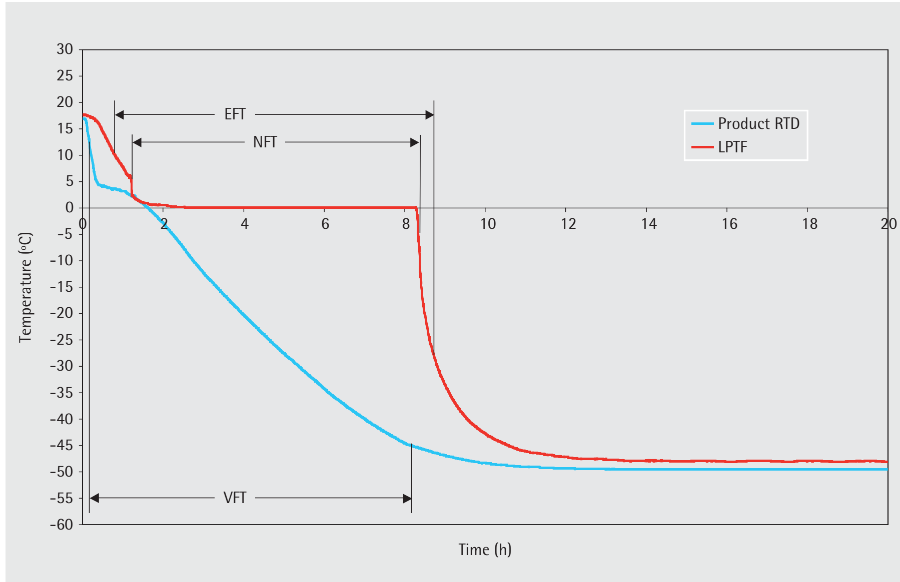

Figure 3: Characteristic Temperature Curves for CV200 with CT200

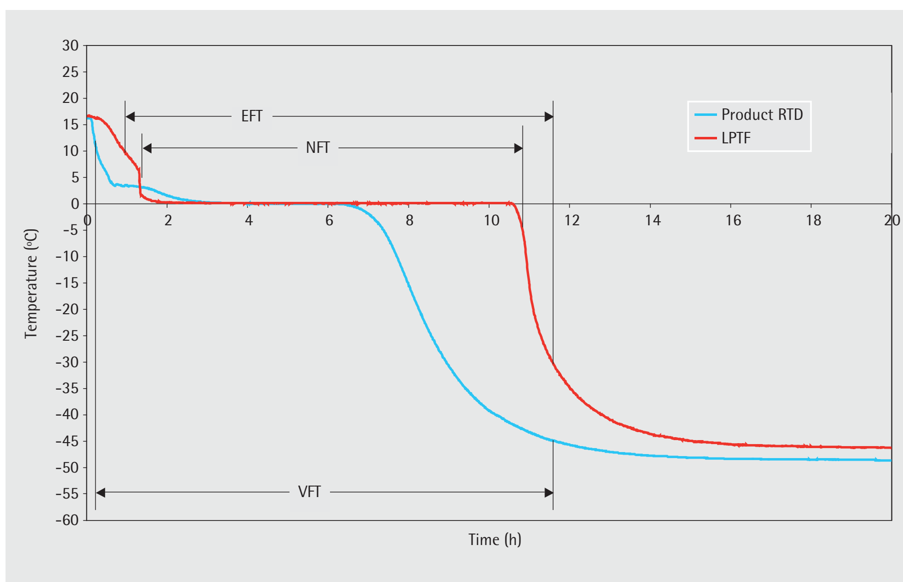

Figure 4: Characteristic Temperature Curves for CV300 with CT300

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