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Modelling Celsius® FFT | FFTp Freeze & Thaw Temperature Profiles in the Celsius® S³ Benchtop System

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Abstract

Celsius® S³ Benchtop System is a laboratory instrument that is designed to evaluate freeze & thaw processes of new drug candidates in 30 ml and 100 ml single-use bags to match the performances of the 100L-scale Celsius® CFT (Controlled-rate Freeze & Thaw) systems. This tool allows generation of a consistent samples library that is useful for evaluating stability, storage and shipping process steps and formulations to reproduce the exact same freeze & thaw conditions obtained at production scale.

In this study, the use of the Celsius® S³ Benchtop System to match typical freezing and thawing temperature profiles of two other production-scale Celsius® platforms offered by Sartorius Stedim Biotech, Celsius® FFT and Celsius® FFTp, has been evaluated. Celsius® S³ Benchtop System new freeze and thaw recipes have been developed to approximate typical performances that can be obtained using Celsius® FFT | FFTp in manufacturing. This offers to the end-users an efficient tool to investigate the behavior of the bulk drug substance while submitted to different freeze and thaw conditions and to support decision for the most suitable production-scale freeze & thaw platform.

Introduction

The Celsius® S³ Benchtop System is a tool to execute freeze & thaw process development and stability studies using minimal amount of product. Specifically designed to be scalable to the production-scale Celsius® CFT System, freeze & thaw runs are performed with as little as 30 mL of product matching the performances obtained at 100L-scale. The scalability concept of the Celsius® CFT platform comes from the use of the same freezing path length, the same material of construction of the single-use bags and the same heat transfer technology at both lab and production-scale. Freezing and thawing rates are controlled, freezing and thawing times are equivalent and the critical freeze & thaw process parameters have been characterized to be maintained throughout process scales.

Sartorius Stedim Biotech offers to the industry two other production-scale Celsius® platforms, Celsius® FFT and Celsius® FFTp. Celsius® FFT (Flexible Freeze & Thaw) and Celsius® FFTp (Flexible Freeze & Thaw for Plate Freezer) is a unique bag-in-shell system that replaces traditional freezing and thawing methods. The single-use containers are sterile, pre-assembled and ready-to-use for freezing and thawing biopharmaceutical solutions in commercially available equipment with the intent to leverage existing freeze & thaw infrastructure. Celsius® FFT can be used with any type of conventional freezers while Celsius® FFTp is specifically designed to be frozen in horizontal type plate freezers. Material of construction of the single-use containers is identical to the one used in the Celsius® CFT platform.

In horizontal plate freezers, Celsius® FFTp takes benefits of controlled-rate freeze & thaw process with fast and reproducible thermal cycling. Performances may depend upon the equipment and related manufacturer as well as to the operating conditions. No scale-down model is today proposed by the industry to match the results obtained in horizontal plate freezers.

Performances of Celsius® FFT on the other hand can be more diversified and more challenging to predict as the freezing and thawing methods are "uncontrolled" and coming from a wide range of possibilities (upright freezer, walk-in freezer, blast freezer, thawing in incubator, thawing at room temperature, thawing with or without shaking). Process conditions (freezing and thawing set point, product load) will also influence the overall production scale freezing and thawing times. Uncontrolled freezing and thawing methods using existing infrastructure are the easiest, quickest and least costly. However, freeze and thaw rates in Celsius® FFT can become slow depending on the selected methods, especially when large volume is required to be processed.

This may cause degradation of the active ingredient due to cryo-concentration. Certain protein formulations are robust enough to be processed at slow rates but consideration for lab-scale studies from a process perspective requires to obtain similar time–temperature profiles throughout all process scales to confirm the impact of the freeze and thaw rates on drug substances. In this case, scalable solutions on the basis of heat and mass transfer dimensions become critical and lab-scale controlled-rate freeze and thaw processes are required to approach Celsius® FFT performances.

Even though Celsius® S³ Benchtop System has not been designed to reproduce the results obtained at production-scale with Celsius® FFT|F FTp, special freezing and thawing temperature profiles can be developed to approximate those obtained with Celsius® FFT|F FTp. This would not be a truly scalable freeze & thaw process (freezing path length and heat transfer system different) but this will provide an approximation of the freeze & thaw conditions which will be experienced by the drug substances at production-scale in Celsius® FFT|F FTp.

Material and Methods

1. Selection of the Celsius® FFT|F FTp Temperature profile
Celsius® FFT and Celsius® FFTp production-scale performances have been selected from a wide range of in-house and end-user data to bracket the variety of freezing and thawing times that can be obtained from the different freeze and thaw methods used in the industry.

a. Freezing Temperature profile
4 different process conditions for freezing have been selected and described in Table 1 to be reproduced with the Celsius® S³ Benchtop System.

The following 3 process parameters from typical freezing curve (Figure 1) have been taken into account in order to develop the new temperature profiles in the Celsius® S³ Benchtop System:

- time to reach the freezing point (0°C)
- time to remove the latent heat or nominal freeze time (NFT): time required for the temperature to go from +3°C to -5°C
- time to complete freeze: time required for the temperature to get stable at the vicinity of the final freezer setpoint

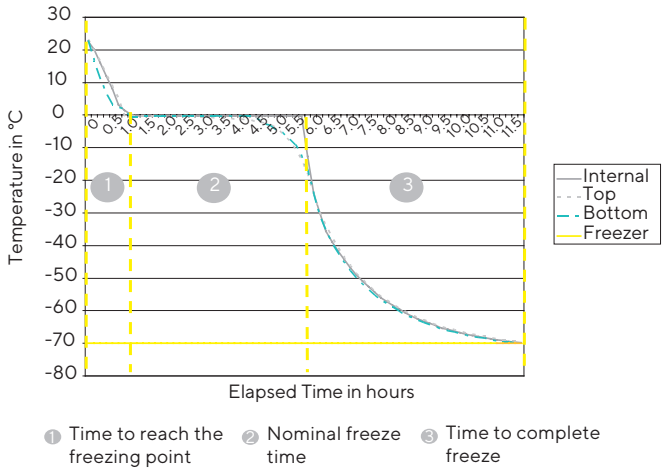

Figure 1: Celsius® FFT 2L typical freezing temperature profile

Celsius® FFT | F FTp Size	Freezer Type | setpoint	Loading pattern	Freezing parameters duration		Data Source
12L Celsius® FFT	Upright freezer | -70°C	5 × containers (1 × container per shelf)	time to freezing point:	~6.5 hr	end user
			nominal freeze time:	~17 hr	
			complete freeze time:	~50 hr	
12L Celsius® FFT	Cold room | -25°C	20 × containers stacked on shelf (4 × 5-high containers)	time to freezing point:	~6 hr	end user
			nominal freeze time:	~28 hr	
			complete freeze time:	~60 hr	
12L Celsius® FFT	Blast freezer | -80°C	9-high containers stacked	time to freezing point:	~3 hr	in-house SSB data
			nominal freeze time:	~3 hr	
			complete freeze time:	~11 hr	
12L Celsius® FFTp	Horizontal plate freezer | -70°C	10 × containers (2 per shelf)	time to freezing point:	~1.5 hr	end user
			nominal freeze time:	~2 hr	
			complete freeze time:	~7 hr	

Table 1: Celsius® FFT \| F FTp freezing conditions at production-scale selected for scale-down operation

b. Thawing Temperature Profile

5 different process conditions for thawing have been selected and described in Table 2 to be reproduced with the Celsius® S³ Benchtop System.

The following 3 process parameters from typical thawing curve have been taken into account in order to develop the new temperature profiles (Figure 2) in the Celsius® S³ Benchtop System:

- time to reach the melting point (0°C)
- time at the melting point (0°C): time required for the temperature to go from -5°C to +3°C
- time to complete thaw: time required for the temperature to get stable at the vicinity of the final thawing setpoint with absence of ice

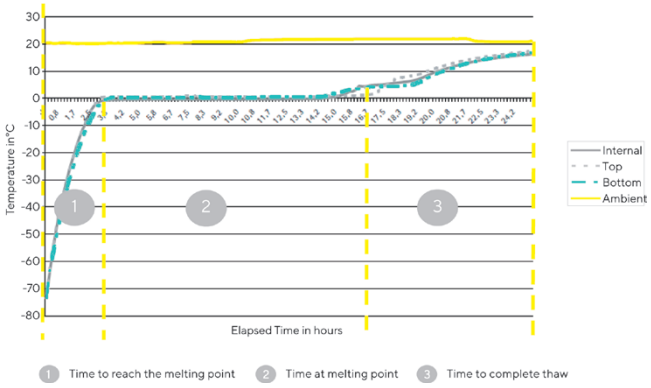

Figure 2: Celsius® FFT 2L typical thawing temperature profile

Celsius® FFT|FFTp Size	Thaw method | setpoint	Loading pattern	Thawing parameters duration		Data Source
12L Celsius® FFT	Flat table | RT	1 × container	time to melting point:	~6 hr	in-house SSB data
			time at melting point:	~28 hr	
			complete thaw time:	~40 hr	
12L Celsius® FFT	Flat table with forced air blowing | RT	1 × container	time to melting point:	~2 hr	in-house SSB data
			time at melting point:	~8 hr	
			complete thaw time:	~11 hr	
12L Celsius® FFT	Flat table with forced air blowing + 60 rpm agitation | RT	1 × container	time to melting point:	~1 hr	in-house SSB data
			time at melting point:	~3 hr	
			complete thaw time:	~5 hr	
12L Celsius® FFT	Blast Freezer | +22°C	9-high containers stacked	time to melting point:	~8 hr	in-house SSB data
			time at melting point:	~13 hr	
			complete thaw time:	~30 hr	
12L Celsius® FFTp	Horizontal plate freezer | +25°C	10 × containers (2 per shelf)	time to melting point:	~1.5 hr	end-user
			time at melting point:	~3 hr	
			complete thaw time:	~10 hr	

Table 2: Celsius® FFT|FFTp thawing conditions at production-scale selected for scale-down operation

2. Development of the new temperature profiles in Celsius® S³ Benchtop System

a. Loading samples for a freeze & thaw step

10x 100ml Celsius®-Paks were filled to nominal fill volume via a syringe. The sample solution was deionized water f or all experiments. Celsius®–Paks were placed in the heat exchange slots of the module of the Celsius® S³ Benchtop System. Thermo couples were placed into the thermowells of the bags to be monitored and secured.

The thermocouple tip-end was located at the last point to freeze meaning 1±0.5 cm below the liquid surface. The module was closed.

b. Creating new temperature profile

New temperature profiles need to be developed so that the product temperatures curves obtained by using the Celsius® S³ Benchtop System match the ones obtained by using the previously mentioned Celsius® FFT|FFTp production-scale performances described in table 1 and 2. Based on freezing and thawing curves from large-scale Celsius® FFT|FFTp product temperature curves, series of steps of X°C were drawn and translated into a set of numerical instructions in the text format readable by the CryoPilot software. CryoPilot software controls the chiller and the mixer by following a profile. A profile is a recipe with instructions to change the chiller temperature setpoint and the mixer mode setpoint (on| off) as a function of time.

Once the freeze & thaw runs were completed, the data files were placed into a spreadsheet application along with data from the large scale conditions. 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.

c. Running a freeze & thaw cycle

After having created and selected the desired profile, the CryoPilot software was started and used as is.

Results and Discussion

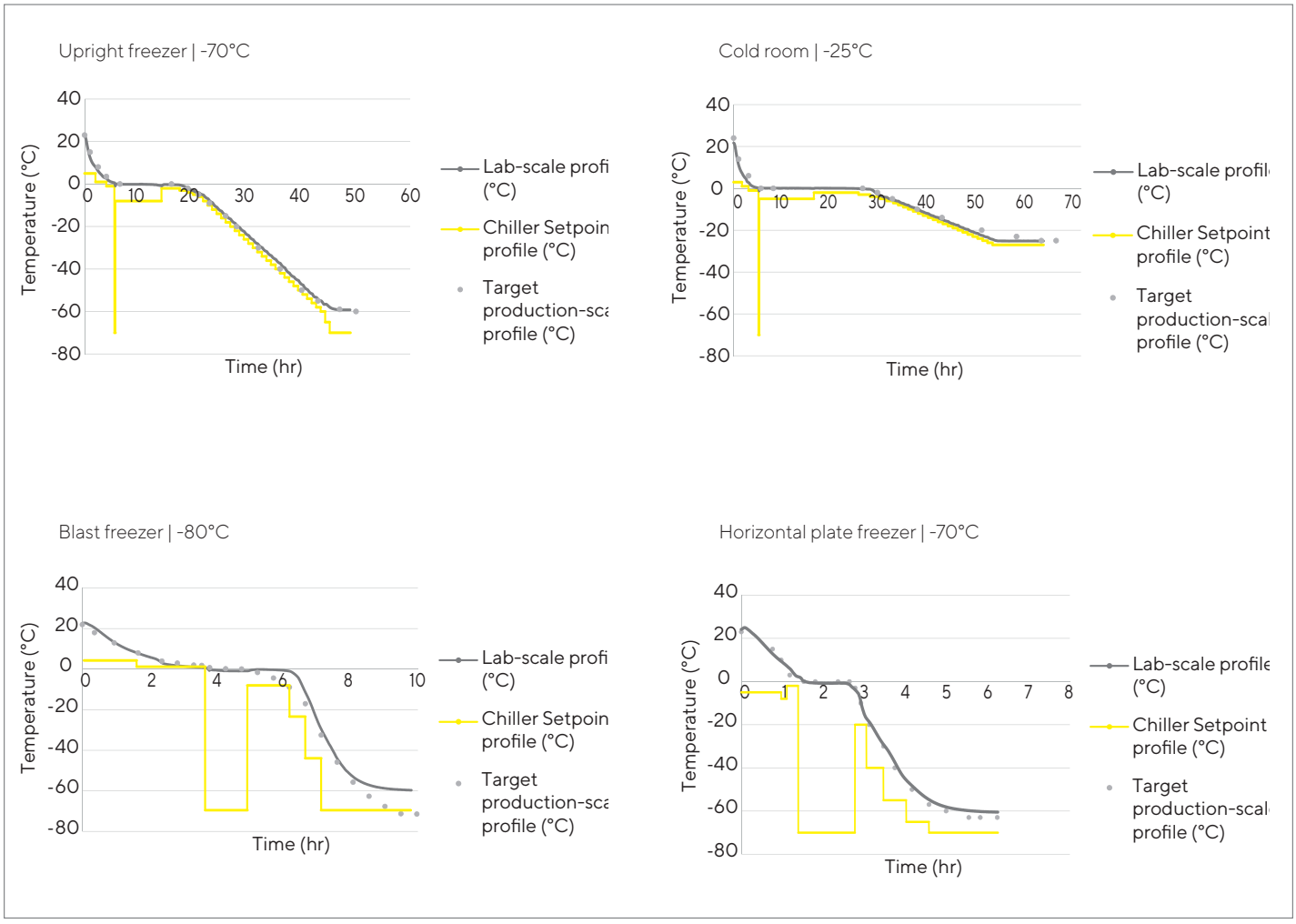

Figure 3: Superposition of production-scale and corresponding lab-scale freezing profiles generated with Celsius® S³ Benchtop System. The lab-scale profile represents the average of the temperature measurement made on the 10 × 100 ml Celsius®-Pak by the 10 thermocouples. The target production-scale profile has been obtained from the average (when applicable) of the temperature measurement made from temperature sensors located inside and | or at the surface of the Celsius® FFT and FFTp containers.

Profiles		Parameters	Celsius® FFT | FFTp freezing profile	Celsius® S³ Benchtop System freezing profile
Freezing	Upright freezer | -70°C	Time to freezing point	~6.5 hr	~ 5.5 hr
		Nominal freeze time	~17 hr	~ 18 hr
		Complete freeze time	~50 hr	~ 47 hr
	Cold room | -25°C	Time to freezing point	~ 6 hr	~ 5.5 hr
		Nominal freeze time	~ 28 hr	~ 29 hr
		Complete freeze time	~ 60 hr	~ 53 hr
	Blast freezer | -80°C	Time to freezing point	~ 3 hr	~ 4 hr
		Nominal freeze time	~ 3 hr	~ 4 hr
		Complete freeze time	~ 11 hr	~ 10 hr
	Horizontal plate freezer | -70°C	Time to freezing point	~ 1.5 hr	~ 2 hr
		Nominal freeze time	~ 2 hr	~ 2 hr
		Complete freeze time	~ 7 hr	~ 6.5 hr

Table 3a: Comparison of expected results and results obtained with scaled-down freezing models

Profiles		Parameters	Celsius® FFT | FFTp thawing profile	Celsius® S³ Benchtop System thawing profile
Thawing	Flat table | RT	Time to melting point	~ 6 hr	~ 5.5 hr
		Time at melting point	~ 28 hr	~ 34 hr
		Complete thawing time	~ 40 hr	~ 42 hr
	Flat table with forced air blowing | RT	Time to melting point	~ 2 hr	~ 2 hr
		Time at melting point	~ 8 hr	~ 9 hr
		Complete thawing time	~ 11 hr	~ 12 hr
	Flat table with forced air blowing + 60 rpm agitation | RT	Time to melting point	~ 1 hr	~ 1 hr
		Time at melting point	~ 3 hr	~ 4 hr
		Complete thawing time	~ 5 hr	~ 6 hr
	Blast Freezer | +22°C	Time to melting point	~ 8 hr	~ 7 hr
		Time at melting point	~ 13 hr	~ 13 hr
		Complete thawing time	~ 30 hr	~ 25 hr
	Horizontal plate freezer | +25°C	Time to melting point	~ 1.5 hr	~ 1.5 hr
		Time at melting point	~ 3 hr	~ 4 hr
		Complete thawing time	~ 10 hr	~ 8 hr

Table 3b: Comparison of expected results and results obtained with scaled-down thawing models

N.B.Celsius® S³ capacity limited to -60°C, even if set-point can be programmed at lower temperature.

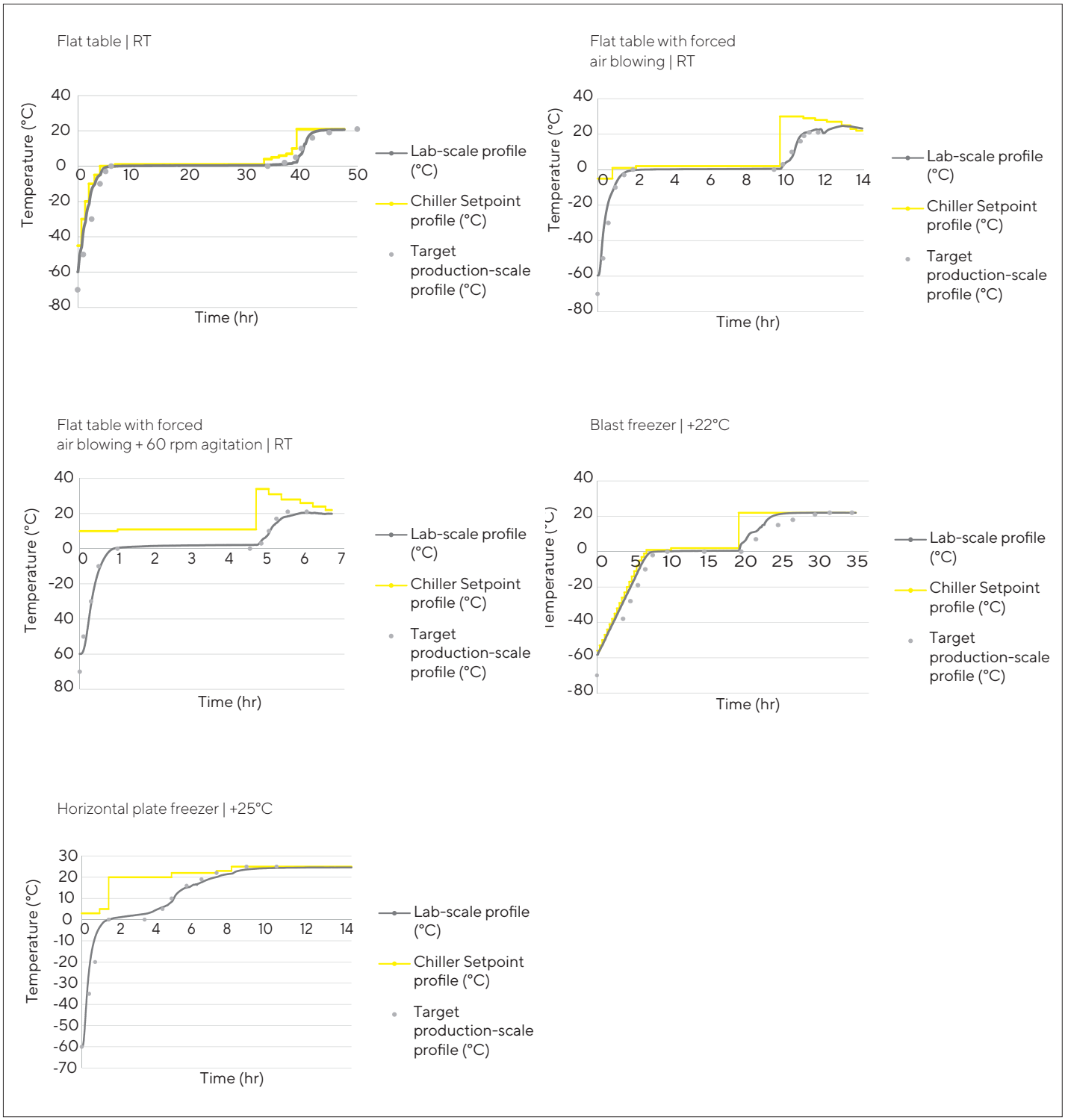

Figure 4: Superposition of production-scale and corresponding lab-scale thawing profiles generated with Celsius® S³ Benchtop System. The lab-scale profile represents the average of the temperature measurement made on the 10 × 100 ml Celsius®-Pak by the 10 thermocouples. The target production-scale profile has been obtained from the average (when applicable) of the temperature measurement made from temperature sensors located inside and | or at the surface of the Celsius® FFT and FFTp containers.

Celsius® FFT|F FTp production-scale temperature profiles have been screened and selected to bracket typical conditions as well as freeze & thaw methods generally used in manufacturing environment. These selected Celsius® FFT|F FTp temperature profiles represent an example only of performances that could be expected when using the same or close process-scale conditions.

As outlined in Table 3, the newly created lab-scale temperature profiles are approaching the critical freezing and thawing process parameters of the selected Celsius® FFT|F FTp temperature profiles. Figure 1 and 2 show the temperature curves obtained with the lab-scale temperature profiles and their respective targeted production-scale Celsius® FFT|F FTp temperature profiles.

The Celsius® S³ Benchtop System enables to control the chiller temperature between +45°C and -70°C with minimal 1°C possible temperature variation per step. This made possible to simulate long production-scale freezing or thawing processes that could eventually be obtained using Celsius® FFT|F FTp.

The lab-scale temperature profiles (Figure 3 and 4) obtained are close enough to their respective production-scale performances to satisfy the goal of this study.

Indeed, since the Celsius® FFT|F FTp production-scale performances may vary upon process conditions, equipment and methods, similar results rather than the exact same freezing and thawing time would be obtained by end-users when reproducing test parameters described in Table 1 and 2.

Although the scalability remains to be validated using proteins as a model, this work provides a mean to the end-user however to investigate the drug substance behavior at lab-scale as if it would be frozen and thawed at production-scale in Celsius® FFT|F FTp using different scenarios.

Coupled to the already available Celsius® CFT scalability feature, the Celsius® S³ Benchtop System allows to the end-user to explore the impact of freeze & thaw on proteins through the entire portfolio of Celsius® Scalable Solutions to ultimately select the most appropriate one at production-scale to preserve the product quality.

Conclusion

Celsius® S³ Benchtop System was demonstrated to be suitable for the reproduction of temperature profiles that can be obtained when using Celsius® FFT|FFTp containers at production-scale.

Approaching Celsius® FFT|F FTp Temperature Profiles for Smart and Early Process Decision with Celsius® S³ Benchtop System

Celsius® FFT

Celsius® S³ Benchtop System

Celsius® FFTp

Annex (Lab-scale freeze temperature profile)

Profile Upright freezer | -70°C

	Time (min)	Chiller Set Point (°C)	Mixer State	
START	0.00	5	OFF	
	120.00	1	OFF	
	240.00	-1	OFF	
	330.00	-70	OFF	
	340.00	-8	OFF	
	850.00	-2	OFF	
	1050.00	-3	OFF	
	1140.00	-4	OFF	
	1200.00	-5	OFF	
	1260.00	-6	OFF	
	1310.00	-8	OFF	
	1360.00	-10	OFF	
	1410.00	-12	OFF	
	1460.00	-14	OFF	
	1510.00	-16	OFF	
	1560.00	-18	OFF	
	1610.00	-20	OFF	
	1660.00	-22	OFF	
	1710.00	-24	OFF	
	1760.00	-26	OFF	
	1810.00	-28	OFF	
	1860.00	-30	OFF	
	1910.00	-32	OFF	
	1960.00	-34	OFF	
	2010.00	-36	OFF	
	2060.00	-38	OFF	
	2110.00	-40	OFF	
	2160.00	-42	OFF	
	2210.00	-44	OFF	
	2260.00	-46	OFF	
	2310.00	-48	OFF	
	2360.00	-50	OFF	
	2410.00	-52	OFF	
	2460.00	-54	OFF	
	2510.00	-56	OFF	
	2560.00	-58	OFF	
	2610.00	-60	OFF	
	2660.00	-65	OFF	
	2710.00	-70	OFF	STOP

Profile Cold room | -25°C

	Time (min)	Chiller Set Point (°C)	Mixer State	
START	0.00	3	OFF	
	100.00	1	OFF	
	180.00	-1	OFF	
	300.00	-70	OFF	
	310.00	-5	OFF	
	970.00	-2	OFF	
	1510.00	-3	OFF	
	1650.00	-4	OFF	
	1770.00	-5	OFF	
	1850.00	-6	OFF	
	1930.00	-7	OFF	
	1990.00	-8	OFF	
	2050.00	-9	OFF	
	2110.00	-10	OFF	
	2170.00	-11	OFF	
	2230.00	-12	OFF	
	2290.00	-13	OFF	
	2350.00	-14	OFF	
	2410.00	-15	OFF	
	2470.00	-16	OFF	
	2530.00	-17	OFF	
	2590.00	-18	OFF	
	2650.00	-19	OFF	
	2710.00	-20	OFF	
	2770.00	-21	OFF	
	2830.00	-22	OFF	
	2890.00	-23	OFF	
	2950.00	-24	OFF	
	3010.00	-25	OFF	
	3070.00	-26	OFF	
	3130.00	-27	OFF	
	3600.00	-27	OFF	STOP

Profile Blast freezer | -80°C

	Time (min)	Chiller Set Point (°C)	Mixer State	
START	0.00	2	OFF	
	100.00	-1	OFF	
	230.00	-70	OFF	
	320.00	-10	OFF	
	370.00	-25	OFF	
	410.00	-45	OFF	
	450.00	-70	OFF	
	600.00	-70	OFF	STOP

Horizontal plate freezer | -70°C

	Time (min)	Chiller Set Point (°C)	Mixer State	
START	0.00	-5	OFF	
	70.00	-8	OFF	
	80.00	-2	OFF	
	100.00	-70	OFF	
	200.00	-20	OFF	
	220.00	-40	OFF	
	250.00	-55	OFF	
	290.00	-65	OFF	
	330.00	-70	OFF	
	500.00	-70	OFF	STOP

Annex (Lab-scale thaw temperature profile)

Profile Flat Table | RT

	Time (min)	Chiller Set Point (°C)	Mixer State	
START	0.00	-45	OFF	
	40.00	-30	OFF	
	80.00	-20	OFF	
	120.00	-10	OFF	
	180.00	-5	OFF	
	240.00	0	OFF	
	400.00	1	OFF	
	2000.00	4	OFF	
	2080.00	5	OFF	
	2160.00	6	OFF	
	2240.00	7	OFF	
	2300.00	10	OFF	
	2350.00	21	OFF	STOP

Profile Blast freezer | +22°C

	Time (min)	Chiller Set Point (°C)	Mixer State	
START	0.00	-56	OFF	
	20.00	-53	OFF	
	40.00	-50	OFF	
	60.00	-47	OFF	
	80.00	-44	OFF	
	100.00	-41	OFF	
	120.00	-38	OFF	
	140.00	-35	OFF	
	160.00	-32	OFF	
	180.00	-29	OFF	
	200.00	-26	OFF	
	220.00	-23	OFF	
	240.00	-20	OFF	
	260.00	-17	OFF	
	280.00	-14	OFF	
	300.00	-11	OFF	
	320.00	-8	OFF	
	340.00	-5	OFF	
	360.00	-3	OFF	
	380.00	-1	OFF	
	400.00	1	OFF	
	600.00	2	OFF	
	1150.00	22	OFF	
	1500.00	22	OFF	STOP

Profile Flat Table with forced air blowing | RT

	Time (min)	Chiller Set Point (°C)	Mixer State	
START	0.00	-5	OFF	
	50.00	1	OFF	
	130.00	2	OFF	
	620.00	30	OFF	
	700.00	29	OFF	
	740.00	28	OFF	
	780.00	27	OFF	
	830.00	25	OFF	
	860.00	23	OFF	
	880.00	22	OFF	STOP

Profile Horizontal plate freezer | +25°C

	Time (min)	Chiller Set Point (°C)	Mixer State	
START	0.00	3	OFF	
	60.00	5	OFF	
	90.00	20	OFF	
	300.00	22	OFF	
	450.00	23	OFF	
	500.00	25	OFF	
	600.00	25	OFF	STOP

Profile Flat Table with forced air blowing +60 rpm agitation | RT

	Time (min)	Chiller Set Point (°C)	Mixer State	
START	0.00	10	ON	
	60.00	11	ON	
	280.00	34	ON	
	300.00	31	ON	
	320.00	28	ON	
	350.00	26	ON	
	370.00	24	ON	
	390.00	22	ON	
	400.00	22	OFF	STOP

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