PDA Journal
of Pharmaceutical Science and Technology

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RESEARCH

Impact of Uncontrolled vs Controlled Rate Freeze-Thaw Technologies on Process Performance and Product Quality

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ABSTRACT: Most biomolecules, owing to their marginal stability in liquid state, susceptibility to microbial growth, and tendency to foam upon storage/shipment in the liquid state, often require an alternate method of long-term storage. Cryopreservation is preferred, as it addresses most of these issues associated with liquid storage. However, the stability of the protein in the frozen state depends on the methodology of freezing/thawing and physico-chemical characteristics of the protein. A systematic study was undertaken to understand and evaluate the impact of freezing/thawing method on the process performance and product quality attributes using two freezing methods— conventional freezing in walk-in freezers and thawing in cold rooms using carboys as an uncontrolled rate method, and Celsius/CryoFin™ technologies as a controlled rate method. To assess the impact of freeze-thaw cycles on product quality, two types of proteins, a fusion protein and a peptibody (peptide fused to the Fc portion of the antibody), were used, employing appropriate stability-indicating assays. The results demonstrate superior process performance by the controlled rate freeze-thaw technology, both in terms of process times and cryoconcentration, compared to uncontrolled rate freeze thaw technology. Product impact studies indicate that the peptibody is sensitive to the method of freeze-thaw while the fusion protein is not and those that are sensitive to uncontrolled rate freeze-thaw processes can be effectively protected by controlled rate freeze-thaw technologies such as Celsius.

KEYWORDS: Freeze-thaw, Controlled rate freezing, Protein, Celsius-Pak, CryoVessel, Carboys, Cryoconcentration

Introduction

In order for biological molecules to manifest their full biological activity and therapeutic efficacy, it is imperative that they remain in their native conformation. This means each unit operation during manufacturing must proceed smoothly, ensuring preservation of the native structure of the molecule. The first challenge in fill/finish unit operations after the downstream purification process is the identification of the proper methodology that can preserve the integrity of the bulk drug substance during storage and shipment. Many biomolecules bear marginal shelf life in aqueous solutions because of their inherent physical and/or chemical instability. One common approach to enhance the storage stability of biopharmaceuticals is to store the bulk drug substance frozen. By storing frozen, the

rates of many of the common reactions leading to physical and/or chemical degradation are retarded. In addition to enhancement of product stability, frozen storage of the bio-product offers several advantages that include (1) minimization of the risk of microbial growth, (2) elimination of agitation and foaming during transport, and (3) flexibility for the manufacturing processes, such as introducing a hold step for pooling batches of intermediates and storing final bulk drug substance before fill/finish operations. Although frozen storage offers several advantages over liquid storage and is regarded as the safest and most reliable storage method, it is not free from risks. Cryoconcentration (1–4), ice-surface induced denaturation (5–7), and cold denaturation (8–10) are believed to be some common mechanisms by which biomolecules tend to undergo inactivation, and these phenomena are strongly influenced by the methodology of the freeze-thaw process employed (2).

Here, a systematic study was undertaken to understand the differences in the impact of controlled and uncontrolled freeze-thaw rates on the performance of the process and the product quality attributes. Conven-

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tional carboys were used for uncontrolled freeze-thaw rate study while CryoFin technology (11) and Celsius technology (12) were explored for controlled rate study. Two different types of biomolecules, a fusion protein and a peptibody, were used for the study. Additionally, process times for these freeze-thaw technologies were determined to assess their feasibility from an operational perspective in a manufacturing setting.

Materials and Methods

For uncontrolled freeze-thaw experiments, 10 L and 20 L regular and heavy-duty polycarbonate carboys with an 83B closure, obtained from Nalgene, were used. A −30 °C walk-in freezer to freeze filled carboys, a 2–8 °C cold room to thaw frozen carboys, a shaker (IKA HS 501 digital horizontal shaker 2527000) to provide agitation during thaw, and Temp-Tale® thermocouples to record temperature were used.

For controlled rate freeze-thaw experiments, two technologies, CryoFin™ technology for freezing and thawing of fusion protein solution and Celsius technology for freezing and thawing of peptibody solution, were used. For all controlled rate freeze-thaw experiments with fusion protein, a 200 L CryoVessel, obtained from Integrated Biosystems, CA, was used. All the runs identified as being conducted on a CT200 were actually performed on a CT300 tuned down to 75% capacity. A Eurotherm Chessel 4100G video recorder with 6 channels for data recording was attached to the CT300 and used for collecting data. A CU5000 refrigeration unit with a touch screen control was also used. An FT16 Celsius system, with 8.3 L Celsius-Paks, was obtained from Sartorius (Stedim Biosystems) and used for all controlled rate freeze-thaw experiments with peptibody solution.

A 25 mg/mL bovine serum albumin (BSA) solution containing mannitol, sucrose, and tris, a 25 mg/mL fusion protein solution containing mannitol, sucrose, and tris, a 30 mg/mL BSA solution containing arginine and potassium phosphate, and a 30 mg/mL peptibody solution containing arginine and potassium phosphate were used during the course of experiments, and all of these were prepared in-house.

Methods

Uncontrolled Rate Technology

Freezing in Carboys: Carboys were filled with BSA, fusion protein, or peptibody solution to the desired fill volume. The transfer of solutions into polycarbonate carboys was performed under the laminar flow hood using peristaltic pump and silicone tubing. All necessary precautions were taken to minimize exposure of protein solution to non-aseptic conditions. Temperature of the solution in the carboys was monitored by placing two Temptale® thermocouples, one at center of the carboy, which is presumed to be the last point to freeze (LPTF), and one at the top liquid surface, which is presumed to be the last point to thaw (LPTT). The hole in the cap through which the thermocouples were passed was sealed with paraffin wax. The carboys were placed on a stainless steel mesh rack to ensure even airflow at the base and were allowed to equilibrate in the −30 °C walk-in freezer.

Thawing in Carboys: Frozen carboys were statically thawed at 2–8 °C in a cold room, and completion of thaw was confirmed by visual inspection to ensure no residual ice was floating in the liquid. The fusion protein was also dynamically thawed on a shaker at 2–8 °C and was monitored at regular intervals to (i) ensure that the shaker was maintained at the set speed, (ii) check for potential carboy implosion, and (iii) check for completion of thaw in any of the carboys. Additional 2 h for freezing and 1 h for thawing were allowed in order to compensate for variations arising from differences in the capacities of freezers and number of carboys to be frozen and thawed. Once the freeze-thaw cycle was complete, data from thermocouples were downloaded (using TempTale® manager software from Sensitech, Inc.) and graphed to determine freezing and thawing times (both static and dynamic) in carboys. The freeze-thaw times determined from these experiments were utilized for performing the evaluation of impact of multiple freeze-thaw cycles on product quality. After completion of each thaw, the carboy was gently inverted or rolled horizontally inside the laminar flow hood to ensure product homogeneity before a ∼5 mL of sample was pulled out for analytical testing.

Controlled Rate Technology

CryoFin™: CryoVessel is a portable, jacketed, stainless steel freeze-thaw vessel used for cryopreservation of biopharmaceuticals, vaccines, blood products, and gene therapy products, offered in volumes ranging from 20 to 500 L. Volume compartmentalization results in reduction of freezing path lengths, creation of extended heat transfer surfaces to increase heat flux

and control of freezing rate, and represents a compact, dimensionally optimized containment design (11).

Development of a Freeze-Thaw Cycle: The development of the freeze-thaw cycle was performed in two stages. In the first set of studies, in addition to evaluation of the manufacturer's recommended freezing cycle, the time and temperature required to completely thaw the product while holding the product temperature below 8 °C was identified. The effect of mixing speed in revolutions per minute (rpm) on the thaw time and the degree of foaming was evaluated. Numerous thaw conditions in conjunction with the mixing speed ranging from 50 to 150 rpm were studied to identify a thaw cycle that is reasonably fast, holds the product below 8 °C, and produces a homogenous solution without generating foam.

All experiments were performed in a 200 L CryoVessel having either a 1-in. or 1/4-in. fin-to-wall gap. The temperature at various locations in the CryoVessel was monitored using a resistance temperature device (RTD) that fits in one thermowell (TW) and T-type thermocouples of various lengths. Mixing during thaw process was carried out using a CryoMixer-300HI set at a mixer speed of 45 rpm. All the runs were performed with fusion protein solution and tris/mannitol/sucrose buffer. Once the CryoVessel was fully wired for data acquisition, 200 L of solution was added to the CryoVessel and all the runs were conducted using the optimized freeze-thaw cycle using DC200 as the heat transfer fluid.

The CyroWedge is a scaled-down model of the CryoVessel that can be used to assess the impact of the freeze-thaw cycle on the product quality (1). The CryoWedge was filled with 3 L of 25 mg/mL fusion protein solution. The lid was covered and thermocouples were placed at three locations, each 2 cm below the liquid surface. The developed freeze-thaw cycle was then run and at the end of each freeze, a visual inspection was performed to verify that the product was completely frozen. The freezing program was exited and then the thawing profile was executed with mixer speed at 40 ± 1 rpm. Thermocouple TC #3 in the CryoWedge, which is analogous to the position of the top thermocouple in the 200 L tank (last point to freeze-thaw), was removed and the samples were pulled out aseptically under the laminar hood for analysis.

The impact of the process conditions of CryoFin technology on the integrity of the fused protein was stud-
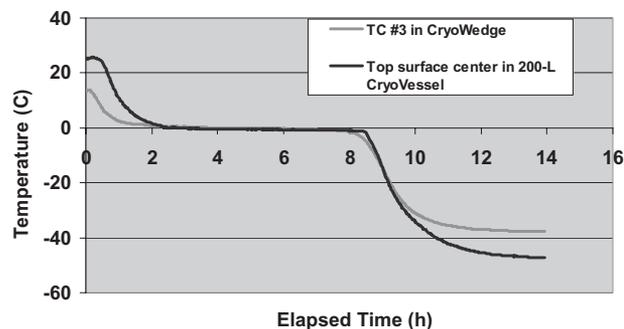

Figure 1

Overlay of product temperature profiles of CryoVessel (1-in. fin gap) and 30-in. CryoWedge.

ied in a CryoWedge, which mimics the internal configuration of the CryoVessel and requires only a few liters of material. However, the biggest challenge was the development of a stepwise freezing and thawing program on a small-scale unit that creates freezing and thawing conditions in a 30-in. CryoWedge comparable to what the products experience in the 200 L CryoVessel and generates a product temperature profile identical to 200 L CryoVessel with CryoTrol 200. The final optimized stepwise freeze-thaw conditions that resulted in a product temperature profile of a CryoWedge comparable to a CryoVessel with 1-in. fin clearance is depicted in Figure 1.

Celsius Technology

Celsius is a controlled rate freeze-thaw system offering a full line of disposable single-use bags (Celsius-Paks varying in volume from 200 mL to 16.6 L), control units, freeze-thaw modules, transfer carts, storage modules, and shipping units. Celsius systems are optimally scalable. The Celsius S³ Freeze-Thaw Module, the bench-top system, is specifically designed for development and stability studies and allows freeze-thaw runs with as little as 30 mL of product under the same conditions as the production scale system. The FT16 Celsius system, with a capacity of up to 16 L, offers a pilot-scale alternative and can easily be scaled up to the manufacturing scale FT 100 Celsius system, with a production capacity of up to 100 L (12).

In this study, an 8.3 L Celsius-Pak was filled with 5 kg of peptibody mimic solution, the space above the liquid was pressurized with 0.6 psi of air, and the bag was then loaded into the FT16 Celsius heat transfer unit. Another similar 8.3 L bag that was filled with

TABLE I
Freezing and Thawing Times in Polycarbonate Carboys

Subject Number	Material	Fill Volume in Carboy (L)	Freezing Time (h)	Shaker Speed during Thaw (rpm)	Thaw Time (h)
1	25 mg/mL protein solution	8 in 20 L	32	60	46
2	25 mg/mL protein solution	16 in 20 L	50	75	67
3	25 mg/mL protein solution	16 in 20 L	50	100	56
4	30 mg/mL protein solution	5 in 10 L	28	Static	100
5	30 mg/mL protein solution	8.5 in 10 L	29	Static	162
6	70 mg/mL protein solution	8.5 in 10 L	28	Static	156
7	100 mg/mL protein solution	8.5 in 10 L	38	Static	142.0
8	150 mg/mL protein solution	1.0 in 10 L	32	Static	84
9	150 mg/mL protein solution	8.5 in 10 L	42	Static	164

pure water was also loaded into the system to maximize the load in the system and represent the worst-case scenario for heat transfer efficiency. The doors were closed tightly and the bag was frozen. The bag was held at $-30\,°C$ after the completion of the freeze profile. The bag was taken out, the instrument defrosted by running the thaw cycle for ~15 min, and then the bag was reloaded into the system and subjected to the thaw profile.

Protein Distribution in Frozen State

An autoclaved, regular 10 L polycarbonate carboy was filled with 8.5 kg of peptibody mimic solution made up of BSA and the bulk frozen by placing on an open-wire shelf in the $-30\,°C$ walk-in freezer. The frozen carboy was taken out, its top chopped off using an electric saw, and the outer casing removed using a saw and a clipper. The frozen bulk was then transferred into a Nalgene tub and frozen samples from various locations were obtained by drilling using a Dewalt drill machine. The collected samples were labeled appropriately and left to thaw at $2–8\,°C$. The concentration of the BSA in these samples was then checked by UV-absorbance.

Similarly, an 8.3 L Celsius-Pak was filled with 8.3 kg of peptibody mimic solution made up of BSA, frozen to $-30\,°C$, taken out of the heat transfer unit, and stored in the $-30\,°C$ freezer until use. The bag was cut using a razor and frozen samples were collected from various locations using a Dewalt drill machine.

Results and Discussion

Initially, the impact of uncontrolled rate (using carboys) and controlled rate (using CryoFin and Celsius) on the

performance of the freeze-thaw process was evaluated by determining the freezing and thawing process times using mimic solutions. Once the processes were developed and optimized, the impact of process conditions on the integrity of the protein and the overall drug product quality attributes were evaluated with two different protein molecules at comparable concentrations by employing stability-indicating assays.

Impact of Uncontrolled and Controlled Rate Freeze-Thaw Technologies on Process Parameters

Uncontrolled Rate (Carboys)

The freezing time was defined as the time it takes to completely freeze protein solution to $-30\,°C$ in a carboy when stored in a $-30\,°C$ walk-in freezer. Using a mimic solution, freezing time was studied as a function of various fill volumes, protein concentration, and size of the container, the results of which are summarized in Table I. Results suggest that the freezing times are influenced by the fill volumes, size of the container, and protein concentration to some extent. Freezing times also vary with the variations in freezing rates, freezing temperatures, the environment—i.e., good manufacturing practice (GMP) vs non-GMP conditions—and the position and number of carboys in the room. Therefore, it is suggested that worst case be identified prior to setting the freezing times for manufacturing scale operations.

The thaw time was defined as the time taken to thaw the frozen bulk until no visual presence of ice could be observed. Keeping the thaw temperature of $2–8\,°C$ constant, the thaw time was also studied as a function of fill volumes, protein concentration, size of the con-

TABLE II
Freezing and Thawing Times in CryoVessel and FT16 Celsius Systems

Experimental Parameter	200 L Cryovessel (25 mg/mL fusion protein solution)	Pilot Scale Celsius FT16 (30 mg/mL peptibody mimic solution)
NFT (h)	N/A	1.74
EFT (h)	9	2.93
Freeze front velocity (mm/h)	20	24.1
Thaw time (h)	6–7	8.6

tainer, and mixing speed (rpm). For mixing, a horizontal shaker was preferred over the vertical roller racks because the horizontal shaker was found to be gentler and created less foam than the roller racks. Additionally, with a vertical roller rack, a risk of leaking is associated if the lid is not secured properly. The results of this study, which are summarized in Table I, clearly indicate a trend of decrease in thaw time with the increase in mixing speed and decrease in fill volumes, but no significant impact of protein concentration was observed. As carboys thaw faster when agitated on a shaker than when thawed statically, this methodology may be employed after ensuring protein stability in these conditions.

Controlled Rate (CryoFin Technology)

Freezing and thawing times using a controlled-rate CryoFin technology were determined using a 200 L CryoVessel. Freezing was characterized by determining the effective freeze time, that is, time for the temperature to drop from 10 °C to −30 °C at the LPTF, and the thaw time was determined as the time required to raise the temperature at the LPTT to 5 °C. The CryoVessel was challenged with the maximum working volume of 200 L of fusion protein solution, and effective freeze times and thaw times were determined using calibrated thermocouples. Using recorded product temperature profiles, it is concluded that it takes approximately 9 h to freeze to −30°C and 6–7 h to thaw to 5 °C, which are several folds faster than with the uncontrolled rate carboys (Table II). This faster freezing/thawing process at a uniform ice front velocity of ~20 mm/h is attributed to the design of the CryoVessel and the cooling system. CryoVessels, which are internally divided into compartments by fins, are able to achieve this optimal ice front velocity by virtue of a reduction in the freezing distance through the compartmentalization by fins and the ability of these fins to quickly exchange heat from the center and outer wall of the CryoVessel (11).

Controlled Rate (Celsius Technology)

A pilot-scale FT16 Celsius system was challenged with the maximum working volume of 16.6 L of mimic solution, and effective freeze times and thaw times were determined using calibrated thermocouples. The results, summarized in Table II, indicate the nominal freezing time (NFT) of the bulk to be only 1.74 h and the effective freezing time (EFT) of the bulk was found to be ~2.93 h, approximately 10-fold lower than what was observed in a carboy. Freezing front velocity, which was obtained as the ratio of the radius/path length of the container to the EFT, was found to be 24.1 mm/h in the case of Celsius system, consistent with that reported in published literature (2, 12). The smaller path length in a Celsius-Pak allows for faster freeze front velocities and hence smaller freezing times in comparison to a carboy. Thaw time, defined as the time taken for the temperature of solution to increase from −30 °C, was determined to be approximately 8.6 h in the Celsius system, which is several folds shorter than that in carboys. During thawing of frozen bulk, the Celsius systems provide agitation by rocking back and forth on tracks to hasten thawing and enhance mixing of thawed bulk.

The freezing and thawing times in a controlled-rate freeze-thaw system such as a CryoFin or Celsius system were found to be at least an order of magnitude shorter than in the case of a carboy. Shorter freezing and thawing times open up the possibility of achieving increased process efficiency with smaller turnaround times, which in turn can directly translate into improved quality and increased cost savings.

Cryoconcentration

One of the challenges with freezing protein solutions in containers at scale is the creation of significant amounts of cryoconcentration of solutes resulting from poor heat transfer (1–4). During the initial freez-

ing period, heat transfer will be adequate enough to allow the ice front to move faster and remove the latent heat of fusion as water starts converting into ice. However, as the conversion of water into ice increases, the resistance to heat transfer also increases due to the poor thermal conductivity of ice and decrease in temperature differential, leading to a decrease in ice front velocity and an increase in the diffusion rate of solutes/protein resulting in pockets rich in protein and excipients called *cryoconcentration* or *freeze concentration* (1). It can potentially have a deleterious affect on the integrity of the protein, the extent of which is dependent upon the methodology of freezing. Hence, the uncontrolled-rate freeze-thaw technology using carboys and controlled-rate technology using a Celsius system were evaluated for cryoconcentration and the extent of cryoconcentration was assessed by determining the protein distribution in the frozen state.

Cryoconcentration in Carboys

To measure the extent of cryoconcentration in a carboy, peptibody mimic solution was filled into a 10 L carboy at a fill weight of 8.5 kg and then frozen to -30 °C. Samples collected from various points of the frozen bulk (refer to Methods) were thawed, and the concentration of BSA in these samples was measured by UV absorbance. A new parameter, concentration factor (CF), was defined as follows:

$$\text{Concentration Factor (CF)} = C_{final}/C_{initial} \quad (1)$$

C_{final} = Concentration of BSA determined after thawing of the frozen sample

$C_{initial}$ = Concentration of BSA in the homogeneous solution before freezing

Figure 2 shows the CF observed in different sections of a single plane passing through the central cross section of the frozen bulk. As can be seen, the CF in the center is $\sim$3.7, in comparison to low values of $\sim$1 observed on the sides of the carboy. Because the ice front in a carboy moves from the sides toward the center, protein and other excipients are excluded and are concentrated toward the center, which is also the last point to freeze. These high values of CF suggest the presence of a protein solution that is highly concentrated ($\sim$120 mg/mL vs 30 mg/mL) in the center of the carboy.

TOP

1.0	3.7	1.1
0.8	3.2	0.5
0.8	2.6	1.0

LEFT / RIGHT

BOTTOM

Figure 2

Cryoconcentration in a vertical plane passing through the center of the frozen bulk when frozen to -30 °C in a regular 10-L PC carboy (the dotted line represents the plane across which cryoconcentration was measured and shown in figure).

Cryoconcentration in Celsius System

Figure 3 shows the CF for samples obtained from the front, center, and back faces of an 8.3 L Celsius-Pak filled with 8.3 kg of peptibody mimic solution and frozen to -30 °C in the FT16 Celsius unit. As shown, the highest CF was found to be approximately 1.40 in the bottom section in the center slice, whereas at every other point there is negligible cryoconcentration. Unidirectional higher freeze front velocity and reduced path length contribute to the uniform distribution of protein in the frozen state inside the Celsius-Pak. These results (Figures 2 and 3) together suggest that cryoconcentration is a significant phenomenon that occurs during the freezing of bulk in a carboy, whereas it is negligible when the bulk is frozen in a controlled rate system such as a Celsius unit. Although the cryoconcentration in CryoVessels was not measured during this study, previous work by Webb *et al.* (1)
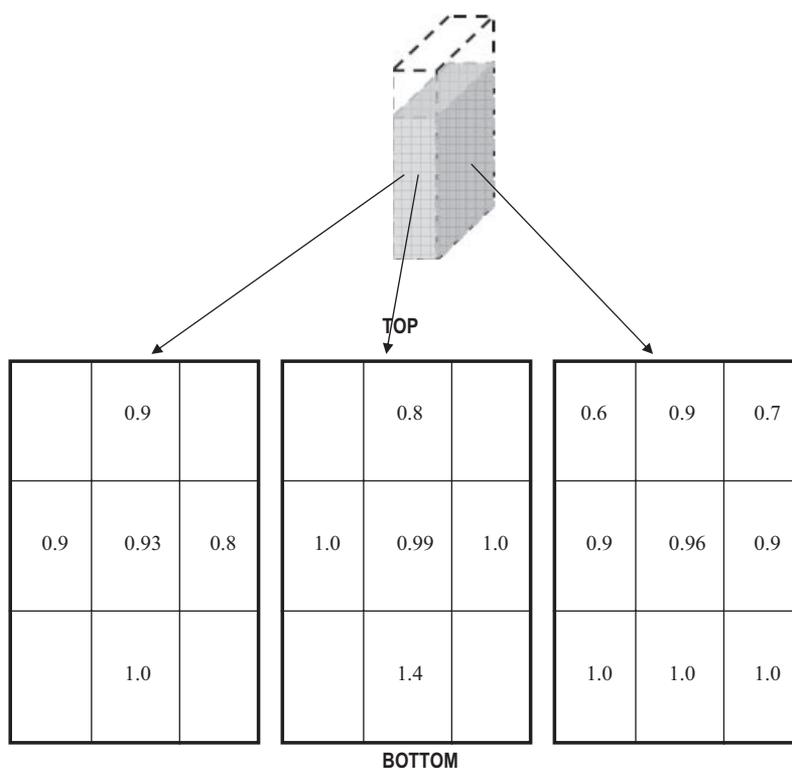
	0.9			0.8		0.6	0.9	0.7
0.9	0.93	0.8	1.0	0.99	1.0	0.9	0.96	0.9
	1.0			1.4		1.0	1.0	1.0

Figure 3

Cryoconcentration in an 8.3 L Celsius-Pak frozen in a pilot-scale FT16 Celsius unit.

showed negligible cryoconcentration at fast freezing rates, owing to the reduced freezing path length.

The impact of protein concentration on the extent of cryoconcentration may be significant and can affect product quality. To the best of our knowledge, no prior work exists on the measurement of the extent of cryoconcentration in carboys and in 8.3 L Celsius-Paks at a high protein concentration of 30 mg/mL. Results presented here add to the existing data available for various proteins at lower concentrations (<2 mg/mL) (1, 2). A systematic study to determine the impact of protein concentration, viscosity of solution, and the nature of excipients on the extent of cryoconcentration is necessary to understand the interplay of all these factors.

All results showed shorter freezing and thawing times, accompanied by minimal degrees of cryoconcentration, with the use of controlled-rate technologies such as CryoFin and Celsius, demonstrating their superior process performance compared to uncontrolled-rate freeze-thaw technology.

Impact of Uncontrolled and Controlled Rate Technologies on Product Quality—Case Studies of Two Proteins

As stated earlier, some proteins are cold-labile and undergo cold denaturation upon exposure to sub-zero temperatures, but others undergo denaturation during the freezing/thawing processes through two different mechanisms, either cryoconcentration and/or ice-liquid surface denaturation. However, some proteins tend to survive against the uncontrolled freezing- and thawing-related denaturation processes. These denaturation processes are believed to be influenced by the freezing rates, which in turn are influenced by the freezing/thawing methodologies. The objective of this study was to evaluate the product impact of uncontrolled- and controlled-rate consecutive freezing and thawing on the integrity of two types of protein, a fusion protein and a peptibody. The integrity of both the proteins against the multiple freeze-thaw cycle was evaluated using appropriate stability-indicating assays. The rationale behind studying multiple freeze-thaw cycles as opposed to a single freeze-thaw cycle was, firstly, the impact is not clearly apparent from a
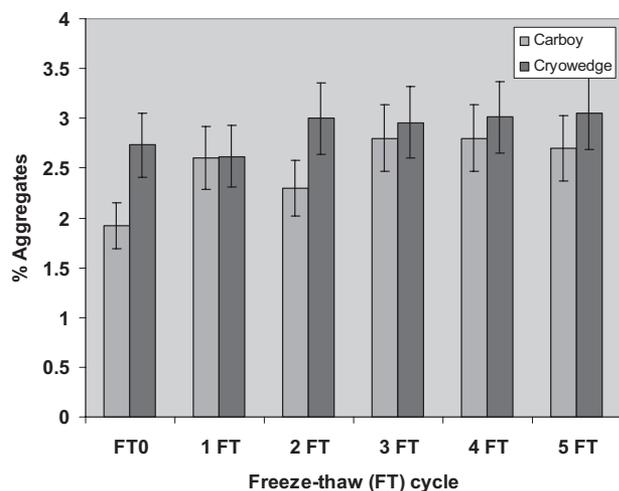
Figure 4

Results of stability-indicating assays of fusion protein as a function of number of freeze-thaw cycles in (a) carboy (uncontrolled freeze-thaw) and (b) CryoWedge (controlled freeze-thaw).

single cycle and, secondly, in practice the products are frozen and thawed multiple times for various reasons.

Case Study 1 (Fusion Protein)

Fusion protein solution, 25 mg/mL, was filled into carboys and subjected to multiple freezing and thawing cycles. Size exclusion-HPLC (high-performance liquid chromatography) was employed to analyze the samples taken at the end of each freeze-thaw cycle in a carboy and resolve the higher molecular weight species (aggregates) from the main component. The results, as shown in Figure 4, indicate a slight increase in the percentage of aggregates after the first freeze-thaw cycle in a carboy. However, no further increase in the percentage of aggregates, outside the assay variability, was observed for up to five consecutive freeze-thaw cycles. As no increasing trend in the percent aggregates with increase in the number of freeze-thaw cycles was observed, it is concluded that no significant impact of freeze-thaw operation was observed on product quality of the fusion protein when frozen and thawed in carboys. All results from other stability-indicating analytical tests also indicated no significant alterations in the integrity of the protein. When this material subjected to five freeze-thaw cycles was further processed to manufacture final dosage form, there was no change in the overall drug product quality attributes, demonstrating no adverse impact of uncontrolled five freeze-thaw cycles.

When the same fused protein solution was then subjected to five consecutive freeze-thaw cycles using an optimized freeze-thaw cycle in the CryoWedge, sizing results indicated no change in the integrity of the protein structure, and the quality of fusion protein product compared to the control, as depicted in the Figure 4. The side-by-side stability results of two technologies depicted in Figure 4 clearly demonstrates no impact of freezing methodology on the product quality, suggesting the robustness of the protein in relation to freezing-induced denaturation processes.

Case Study 2 (Peptibody)

When a peptibody solution was subjected to multiple freeze-thaw cycles under identical uncontrolled-rate conditions using 10 L carboys and its integrity and the drug product quality attributes were evaluated, an increase in the percent of aggregates with an increase in the number of freeze-thaw cycles was observed, as shown in Figure 5. Size exclusion HPLC results indicate a trend of increase in percentage of higher-order aggregates with the increase in the number of freeze-thaw cycles, with the maximum of 2.4% of aggregates at the end of five freeze-thaw cycles. Differences in the percentage of main peak compared to control were also observed with increasing scale (from 2 L to 10 L carboys; data not shown) of freeze-thaw, in agreement with the idea that the freezing-induced denaturation is a scale-dependent phenomenon in the case of uncon-
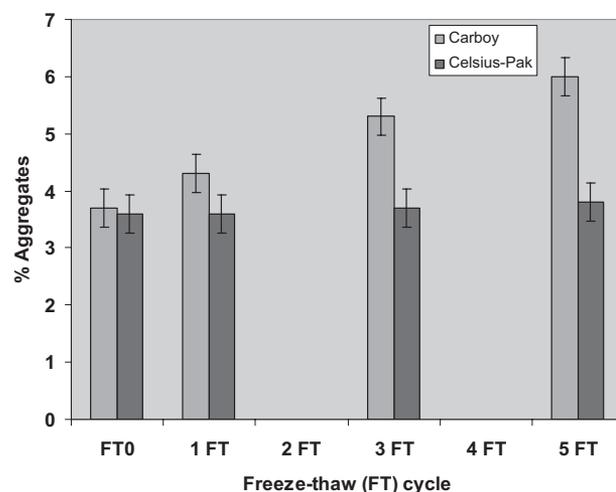

Figure 5

Results of stability-indicating assays of peptibody as a function of number of freeze-thaw cycles in (a) carboy (uncontrolled freeze-thaw, blue) and (b) Celsius-Pak (controlled freeze-thaw, maroon).

trolled rate freeze-thaw technology. On the other hand, product collected and analyzed from the Celsius-Pak after each freeze-thaw cycle did not show any significant change in percentage aggregates even after five freeze-thaw cycles, suggesting that the controlled-rate freeze-thaw technology is better at preserving product quality during freeze-thaw than a carboy.

The results of the above case studies clearly demonstrate that not all biomolecules are sensitive to freezing-induced denaturation phenomena and that advantage can be taken of their robustness against uncontrolled rate freeze-thaw technology such as walk-in freezers using either plastic or stainless steel containers, provided the material demand is not too high and initial capital investment in acquiring Celsius or Cryo-Fin technologies cannot be afforded. However, for those biomolecules that are sensitive to freezing-induced denaturation phenomena and for those that have a high material demand, use of controlled-rate technologies such as CryoFin or Celsius-Pak can prove to be quite beneficial, both from an efficiency and protection against denaturation point of view.

Conclusion

Controlled-rate technologies such as Celsius and Cryo-Fin prove to be much more efficient than the conventional uncontrolled-rate methods such as carboys, both from operations and process efficiency points of view, and also show minimal to negligible cryoconcentration. The results of product impact studies clearly demonstrate that some proteins are not sensitive to freezing rate-dependent denaturation phenomena, and conventional methods of cryopreservation such as freezing in walk-in freezers using carboys can be used for frozen storage/shipment. Those biomolecules that lose their integrity upon exposure to uncontrolled rate freeze-thaw methods can be effectively protected using controlled rate freeze-thaw technologies such as Celsius or CryoFin.

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