SINGLE USE SUPPORT.

Single-Use Technologies

Scalable, Modular Systems Bring Cost Efficiency to Bioprocessing

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GEN Genetic Engineering & Biotechnology News

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Introduction

The evolution of single-use technologies (SUTs) is among the most important developments of biomanufacturing. Large stainless-steel containers have long been used for storage and cooling of drug substances. However, there has been a shift toward SUT, which has enhanced manufacturing agility and flexibility. Establishment of single-use systems also has built a solid foundation upon which to improve production of several types of biopharmaceuticals, including mRNA, allogeneic cell therapies, and viral-vectored gene therapies. Especially for emerging modalities, single-use equipment is a logical choice. Manufacturers need to fill, freeze, and ship such therapies quickly. SUTs enable multimodal production, enhance throughput, and expedite changeover procedures. Disposable bioprocess systems also help companies to work flexibly, breaking up conventional batch sizes.

Single Use Support GmbH stands for customer-focused solutions surrounding single-use systems, seeking to improve pharmaceutical fluid management and cold-chain logistics, with the goal of minimizing product loss. Technologies around liquid transfer of biopharmaceuticals continues to evolve through the Austrian-based company's end-to-end process solutions consisting of sterile consumables such as single-use bioprocess containers, related assemblies, and robust storage and shipping shells for protection of bioprocess solutions. Our product portfolio is constantly growing with SUT platform systems for automated aseptic filling, controlled and scalable freezing, cold storage, shaking, and shipment of drug substances and products. It is essential to develop flexible end-to-end systems that can be applied with any type of single-use bag or bottle container. The vendor-agnostic approach to fluid management has helped this innovative company succeed.

Single Use Support is well known for being a speedy, agile, and trustworthy partner that provides cutting-edge solutions with short lead times. Proud to have already diminished the incidence of product loss for many customers globally to less than 0.001%, we are paving the way for SUTs to develop further in the coming years.

Fueled by the improved performance of SUTs, we are committed to preventing future bottlenecks for single-use systems. Our slogan, "Pioneering Biopharma," is manifested in our corporate DNA, and that sentiment will drive us to help advance the pharmaceutical industry.

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SINGLE USE SUPPORT.
PIONEERING BIOPHARMA

CASE STUDY

Reducing Product Loss:
Cost Efficiency

1 | The Situation

The customer, a global biotechnology company with a total turnover of > USD 5 billion, manufactures different drug substances (BDS) of highly valuable mABs in batch sizes ranging from 10L to 700L for clinical phases up to commercialized products. The BDS is frozen at < -85°C (-121°F), and shipped within Europe or on transatlantic routes over periods of max. 200 hours, and then stored, all the while being kept cool with dry ice. At the fill & finish site, the BDS is thawed to be used in the production of the drug product.

2 | The Problem

The biotech company planned to rely on single-use bioprocess containers as preferred primary packaging for BDS bulk filling, freezing, storing and shipping. This was due to various advantages of single-use bags like filling in a closed system, higher volumes, scalability and high storage density.

On the flipside, the biggest challenge of single-use bags was the high loss rate of **0.5% to 5%** due to their sensitivity at sub-zero temperatures. The product loss rate depends on the bag used, the product's clam shell, the process and the shipper/shipping route. Therefore, **the goal was to reduce the current product loss below 0.5%**.

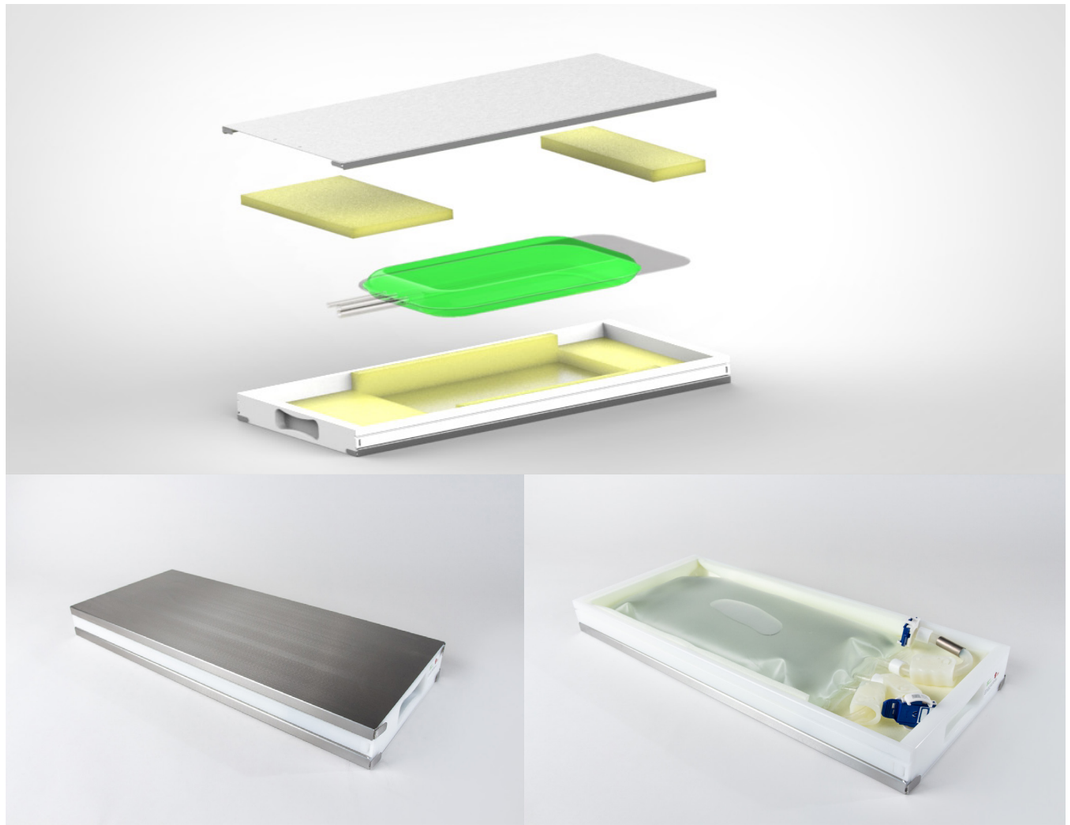
3 | The Solution

The biotechnology company compared available solutions to reduce the breakage rate of frozen and shipped single-use bags.

Representatives have reached out to Single Use Support for initial trials to set up a cold chain process and implement robust technologies around the brittle single-use bags and to consequentially reduce product loss.

The RoSS® (Robust Storage and Shipping) shell has been selected as a robust secondary packaging solution for the single-use bioprocess containers. The bag is covered by soft 3D foam, which provides a protective embedding of the single-use bag and absorbs about 8% of the bag's/liquid's expanding energy due to the density drop during freezing. The heavy-duty plastic and stainless-steel lids that make up the robust frame of the RoSS shell withstand even the lowest temperatures.

See more about RoSS® Shell.



RoSS® Shell: Robust Stoarge and Shipping

4 | The Result

Mapping and analyses of the cold chain process on-site have shown that bag breakages were caused by a combination of having in place many manual handling steps, the sensitivity of single-use bags at sub-zero temperatures and a missing robust secondary packaging. The customer validated the new process with RoSS shells over several weeks with more than 500 bags filled with frozen buffer media.

The implementation of RoSS with the effect of a much more robust secondary packaging has **diminished the risk of leakages or breakages to 0%**.

The single-use bag and its tubings adapt to perfectly fit into the 3D foam in the RoSS shell, which in turn maximizes the protective effect. The introduction of an end-to-end process for freezing and shipment based on RoSS has led to a decrease of product loss from up to 5% down towards 0%.

*sample of 500 bags

Given a batch volume of 500 x 10L bags per year with a filling volume of 8L at a value of USD 80,000 per bag, this mimics the situation at the biotech producing mAbs. An average product loss rate of 2.5% had resulted in a value loss of USD 1,000,000 per year previously.

Investing in **500 RoSS** shells that reduce product loss from 2.5% to 0% leads to **savings of approx. USD 700,000**. Higher initial purchase costs for RoSS shells pay off very soon. **ROI is achieved after 150 single-use bags or only 4 months**, since they are compensated through the diminished risk of product loss towards 0%.

COG and Economies of Scale for Advanced Therapies

By Angelo DePalma, PhD

Source: Coolpicture/Getty Images

Gene-based therapies are relatively new but the buzz surrounding them is deafening. Stakeholders are already thinking about cost of goods (COG), and bioprocessing is always a good place to look for COG improvements.

But despite their 22nd-century price tags, traditional viral vector production processes still rely on 20th-century technologies—essentially laboratory production methods which are subsequently industrialized as product volume requirements dictate.

A group led by Suzanne S. Farid, PhD, professor in the department of biochemical engineering, University College London, has recently completed a study of COG in the manufacture of lentiviral vectors. Farid compared viral vector production methods based on adherent cultures with suspension cells cultured in a single-use bioreactor. Her cost estimation tool was based on a whole-process economic model which was linked to optimization algorithms associating COG with various process conditions.

Farid concluded that suspension cultures in single-use bioreactors achieve an approximately 90% improvement in COG per dose, compared with more traditional methods based on adherent HEK293 culture in stackable, multi-layer vessels.

Her analysis also uncovered major contributors to COG according to production volume. For up to around 100 doses per year the most significant factors were labor and indirect costs. Above around 500 doses the relative contribution from indirect costs diminishes, and raw materials become the leading COG contributor.

For example, at the 100-dose level labor and indirect costs accounted for 35% and 40%, respectively, followed by raw materials and quality-related activities. Above 500 doses raw material costs begin to dominate. By the time the 1000-dose scale is reached these costs account for 40% of the total, and QC costs become almost negligible. Raw material costs dominated since they increase, more or less linearly, with scale while facility overheads are spread over more doses.

Trends in economies of scale

"The trends in economies of scale for viral vector processes are similar to those for mAbs with the dominant costs shifting from fixed costs at small scales to material costs at large scales," Farid tells GEN. "But of course the specific raw material cost drivers differ given that most viral vector processes still rely on transient transfection that is dependent on the supply of plasmid DNA, and many use lab-based methods. For example, for processes relying on multi-layer vessels, single-use components dominate given the large numbers of units required, while for processes relying on more scalable technologies, such as single-use bioreactors, the key material influencer shifts to the costly plasmid DNA required in transient transfection processes."

Yet one gets the feeling that COG considerations are different for gene therapies than for monoclonal antibody production, which for years seemed to be disconnected from normal economic forces.

"COG is a hot topic in cell and gene therapy given several notable failures attributed to manufacturing concerns, including high COG," Farid adds. "Given the relative infancy of the sector, the cost of manufacturing processes can represent a significant proportion of the selling price, and in that regard is not as mature as the mAb sector. Currently, viral vector costs represent a major component of the material manufacturing costs for gene-modified cell therapies such as CAR T-cell therapies, and these costs are even more pronounced for higher-dose products such as hematopoietic stem cell (HSC) therapies.

Given the pressures to reduce the price of these therapies (e.g., approx. $400k for CAR T therapies and $1.8M for HSC therapies), there is a lot of interest to drive down viral vector cost contributions to these gene-modified cell therapy costs."

With currently available production technology viral vectors represent anywhere from 15% to more than 50% of the COG of a gene-modified cell therapy. "For this reason this sector must shift away from lab-scale methods used nowadays to scalable alternatives. This will not only reduce costs but lead to more robust industrialized processes."

Farid's analysis illustrates how production scale and facility footprint change with different flowsheet options, degrees of process optimization, and market capture assumptions. "This helps determine whether processes will lead to practical facility sizes, and based on this the analysis can help prioritize R&D targets that will help with cost or space reduction."

CASE STUDY

Comparing Protective Shell Solutions: Bag-Independence

1 | The Situation

A multinational CDMO (contract development and manufacturing organization) with USD 5 billion turnover develops and manufactures pharmaceuticals that are outsourced from biotechnology companies. Based on requirements the CDMO takes full responsibility of manufacturing of drug substance, cell & gene therapies and more within the contracted period of time.

The CDMO's customers increasingly prefer to rely on single-use bags as primary packaging for their products during fluid management and cold chain transport. This allows for scalability, a storage density increased fourfold[1], easier handling, larger volumes of up to hundreds of liters, and improved options to work in a closed system without manual handling.

The CDMO's customers had different suppliers for single-use bags and protective clam shells, "company A" and "company B". The clam shell solutions of both providers were limited to only a few available types of single-use bioprocess containers. The CDMO therefore had to build its manufacturing plant based on different scenarios, resulting in inconsistent performance and insufficient product loss rates during cold chain logistics, which covered aspects such as aseptic filling, freezing to -80°C, and international cold chain shipment.

The CDMO was searching for solutions to increase process flexibility for different single-use bag & shell vendors whilst minimizing the product loss rate.

3 | The Solution

The CDMO compared available solutions to reduce the breakage rates of the frozen and shipped single-use bags, resulting in the following benchmark study.

	SINGLE USE SUPPORT: RoSS® shell system	COMPANY A: Clam shell provider only	COMPANY B: Single-use bag manufacturer with clam shell
Reduction of product loss	✓ 2	~	~
Bag-independence	✓	~	✗
Enables fast & controlled freezing	✓ 3	✗ a	~ b
Fully closed & tamper-evident system	✓	✗	✗ c
Robustness	✓	~	~
Confident handling	✓	✓	✓
Maximum storage density	✓ 1	✗ a	✓
Facilitates e2e process solutions	✓	~	~
Well validated	✓	✗	✓
Scalable	✓ 3	✗	✓
OPEX	✓	✓	✓

[a] *Bulky with slow freezing rates due to surrounding air*
[b] *Uncontrolled freezing when using blast/static freezer. Insufficient heat transfer for fast freezing when using plate freezer*
[c] *Tubings and bioprocess containers are exposed to environment and can be manipulated/risk of breakages*

The CDMO and Single Use Support have agreed to conduct first trials to set up a cold chain process and implement robust technologies around the brittle single-use bag and to consequentially reduce product loss.

As a first step, the RoSS (Robust Storage and Shipping) shell has been selected as a robust secondary packaging solution for both single-use bags in use. The bags are covered by soft 3D foam, which provides a protective embedding of the single-use bag and absorbs the expanding energy of the bag/liquid of about 8% due to the density drop during freezing.

The RoSS shell can be used for all available 2D bioprocess containers - all vendors, all sizes - which makes it a vendor-independent protective shell to unify the cold chain logistics process. See more about RoSS® Shell.

Above: Closed RoSS® Shell; right: RoSS® Shell offers the optimal solution for single-use bags from various manufacturers.

4 | The Result

The implementation of the RoSS shell has enabled the CDMO to use their single-use bioprocess container of choice:

From a myriad of possible options, the RoSS shell stands out as it

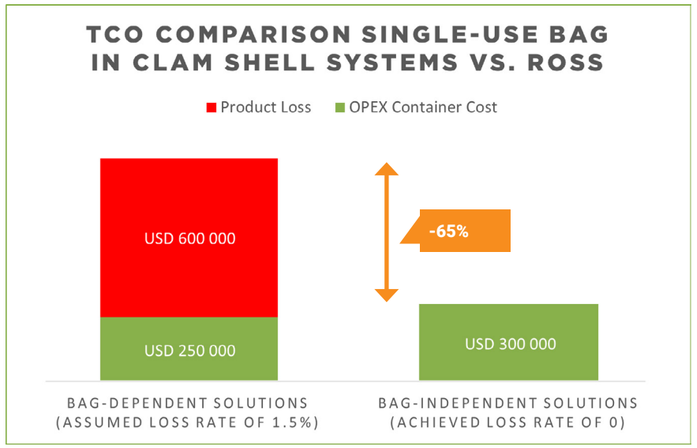
- helps to **streamline the liquid transfer process**
- is **compatible with all sizes and types of single-use bags**
- **optimizes** each full **cold chain management process** by enabling full flexibility
- **standardizes safe drug substance handling** in single-use systems **flexibility.**

In comparison to clam shell systems from other suppliers, RoSS shell has managed to achieve a minimization in product loss (breakages, leakages, contamination) to 0% over several batches of 500 bags[2].

Assumption

- Batch of 500 single-use bags
- Each single-use bag contains 8L
- Value/bag= USD 80,000

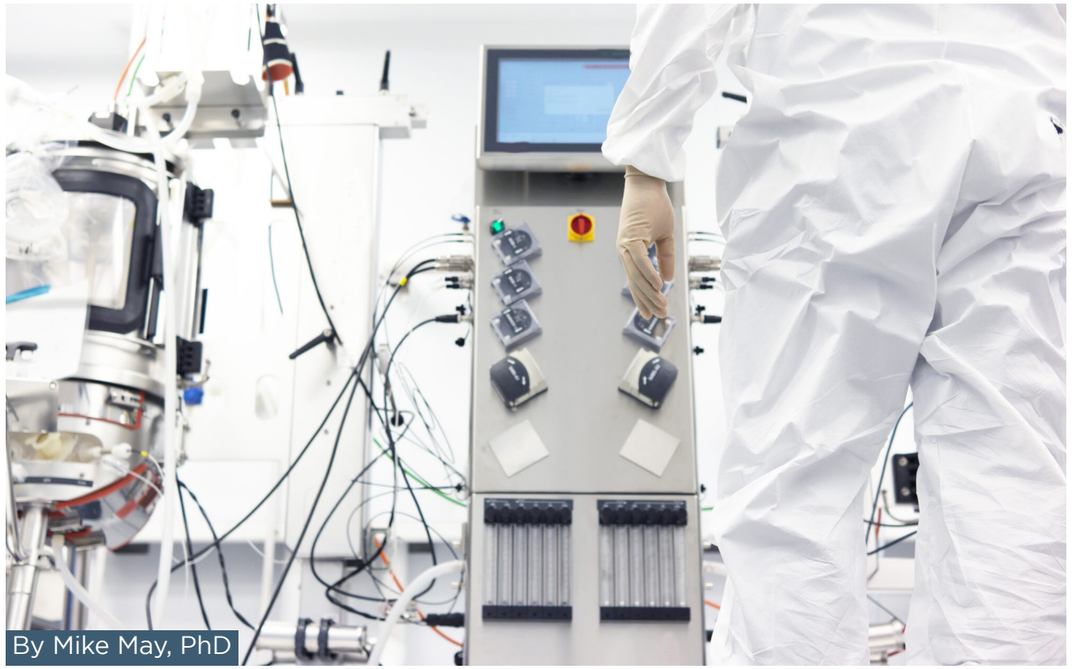
Given a batch volume of 500 x 10L bags per year with a filling volume of 8L, the total cost of ownership (TCO) of using a bag-independent RoSS shell with 0% product loss rate vs. a siloed clam shell system with 1.5% (assumed) product loss rate is illustrated below. **With the implementation of RoSS shells instead of other clam shell solutions, the CDMO managed to reduce its costs for chold chain logistics with single-use bags by more than 60%.** The fact that fewer process solutions are required reduces costs (capital expenditures) even more.



References

1. Single Use Support. Storage Density, 2022
2. Single Use Support. Case Study Reducing Product Loss Cost-Efficiency, 2022
3. Single Use Support. Scalability Guide, 2021

Countering Complexity with Bioprocessing Systems



By Mike May, PhD

Source: Reptile8488/Getty Images

Complexity might be the key word that describes today's bioprocessing. Philip Vanek, PhD, CTO at Gamma Biosciences in Menlo Park, CA, agrees. He says, "The biggest challenge I see facing the industry—notwithstanding current single-use supply chain challenges—is the increasing complexity of these emerging therapies."

For advanced therapies—such as ones based on cells, mRNA, or viruses—Vanek points out that one challenge is "their molecular and structural complexity, often linked hand in hand with the size of the therapeutic molecule." He adds that "size, complexity, and the requirement for sterility throughout the process has increased the demand for single-use technologies, automation, and scalable production platforms that—when combined—reduce risk and ultimately the cost of manufacturing."

That poses a collection of challenges for a bioprocessor to address. There are various ways to specifically approach those obstacles, but Vanek and his colleagues take a more general strategy.

"We believe that a systems-level perspective is necessary to solve the challenges faced by the advanced-therapy industry," Vanek explains. "We look beyond the unit operations of a manufacturing workflow, to understand the interconnectedness and knock-on effects of changes in any one step, and how those changes impact operations within the boundaries of a risk-focused regulated manufacturing environment."

Taking a systems-level approach, Vanek says, requires a "deep process-level understanding." With that, he adds, "we look for disruptive technologies that—when collectively enabled—add up to more than the sum of the parts." To put together such disruptive tools, Vanek and his colleagues "invest in these technologies to accelerate their development and market adoption."

As a result, he believes they "are playing a small but significant part in accelerating the availability of next-generation medicines for the patients so desperately awaiting them."

"We look beyond the unit operations of a manufacturing workflow, to understand the interconnectedness and knock-on effects of changes in any one step, and how those changes impact operations..."

CASE STUDY

Efficiency of Single-Use Technologies: Process Independence

Process-independent single-use technologies, like RoSS.pFTU, increase process flexibility.

1 | The Situation

With the implementation of RoSS® shells, a multinational CDMO has been able to standardize its fluid management process thanks to the protective shell's bag-independence, and regardless of size and type of single-use bag used.

RoSS – abbreviation for "robust storage and shipping":

- helps to streamline the liquid transfer process and
- optimizes each full cold chain management process by enabling full flexibility.[1]

The CDMO has now planned an extension of their manufacturing site and is searching for single-use technologies to implement a state-of-the-art manufacturing process.

2 | The Problem

The lack of prior infrastructure as well as process constraints required process flows that were conceptually thought through from scratch. It opened doors to optimize fluid handling functionalities that fostered operational and cost benefits in the long term. With having different single-use technologies in place, though, the CDMO had already planned to implement existing spare platform systems for their new facilities.

With the resulting balancing act between saving costs and achieving a process-efficient manufacturing setup, the company needed to find a way to limit the total cost of ownership, consisting of capital expenditures (CAPEX) upfront and operational expenditures (OPEX), while at the same time improving their biopharmaceutical fluid management's overall performance and minimizing product loss.

3 | The Solution

Until now, manufacturers were mostly dependent on specific suppliers. Lack of compatibilities of single-use bags and lack of interconnectedness among different platform systems from multiple suppliers has resulted in limited process flexibility, inhibited process flows, and slowed speed-to-market.

With RoSS technologies around biologics liquid transfer the customer is free to choose from any of the platform solutions that can be embedded in an end-to-end process covering aseptic fluid management and cold chain logistics in an automated manner. In fact, Single Use Support's end-to-end process does not exclude any types or sizes of single-use container – making the RoSS® shell an enabler to unify all single-use bioprocess containers and to standardize the process steps in biomanufacturing. Moreover, the robust protection reduces product loss towards 0% for single-use bags, saving the manufacturing company OPEX continuously.[2] Furthermore, the process-independent approach of RoSS technologies also decreases the need for single-use technologies that are solely designed to match with selected products.

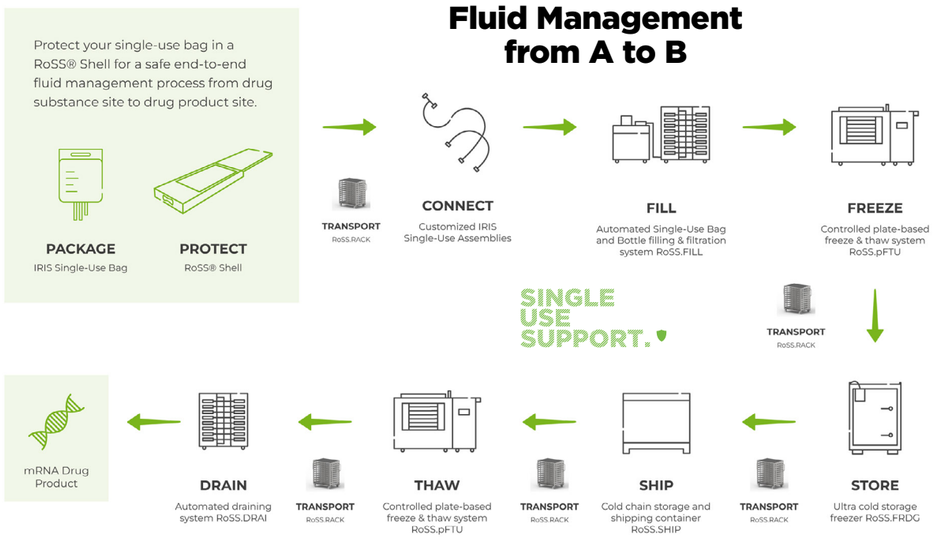
The implementation of RoSS technologies

- Reduces the number of platform systems required for filling, freezing and cold storage, leading to more storage space
- Offers a wider range of applicability
- Use of different primary packaging (any single-use bag, vial, bottle)[3]
- Use for cell and gene therapy manufacturing, bulk drug substances, and more
- Enables advanced process flexibility thanks to scalable solutions with transferable filling and freezing recipes
- Minimizes operational errors and product loss thanks to automatted workflows[4]
- Thus facilitates the implementation of a cGMP compliant reproducible and standardized manufacturing process

ADVANTAGES OVER SILOED PLATFORM SYSTEMS

- Reduced product loss
- Single-use bag-independent
- Bigger range of scalability from 10mL to 400L
- Improved efficiency through automation and RFID tracking

4 | The Result



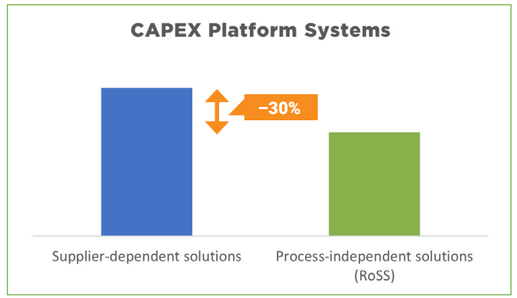
Less vendor-specific systems and more universally compatible technologies, like RoSS, **simplify the process flow thanks to a reduced need for different platform systems**. This drives further cost-efficiency due to reduced need of staff for manual handling, staff training, cleaning and validation.

Required number of single-use technologies depends on batch sizes but can be reduced even further through performances at increased speed.

-80% Aseptic filling of up to 300L with RoSS. FILL takes 1 hour, in contrast to other platform systems with customary 5 hours

-75% Freezing to -80°C with RoSS.pFTU takes approx. 6-8 hours, in contrast to other platform systems with customary 30 hours

The CDMO has reported **CAPEX savings of more than 30%** through the use of process-independent RoSS technologies for its facility



expansion – **adding to OPEX savings of more than 50% per year[2], primarily by reduced product loss**.

References

1. Advantages of single-use bag-independent shell solutions (susupport.com)
2. Reducing product loss in Biopharma to save costs (susupport.com)
3. Single Use Support, 2022. Case Study: Hybrid by primary packaging
4. How to reduce "Human Error" in Biopharma? (susupport.com)

WHITE PAPER

How Controlled Freezing Becomes Reality:

Impact of Ice Front Growth Speed on Scalability of Freezing Protein Solutions

By Roland Jenewein, Michael Breitrainer

Abstract

Being able to control the freezing behavior of bulk drug substance is the ultimate goal in biopharma manufacturing. It opens doors to process reproducibility, consistent quality of the final drug product by maintaining uniform conditions for the biopharmaceuticals during freezing, and consequently for frozen storage and shipment. This simplifies the commercial bulk production, particularly when manufacturing different drug substances with various product characteristics.

As one of the most essential parameters in terms of achieving control over freezing & thawing bulk, the freezing rate has been considered and evaluated in different tests. The leverage of the ice front growth speed had a significant impact on controllability and, as a result, on the protein quality.

Introduction

In Biopharma manufacturing substances are being stored, frozen and transferred in bulk day in day out. The operation of different production and fill & finish locations is a given fact, and the industry requires a logistics process that is capable of dealing with the challenges associated with remote production sites.

Usually, high-purity bulk protein solution is produced as per demand and independent of time from the final protein drug. At times, the various steps are executed at different locations. While on the one hand this segregation allows for a conversion of bulk solution according to market demand, it also leads to storage as well as transportation requirements for the bulk solution.[1,2]

The most crucial aim is to transport the active pharmaceutical ingredient, either highly sensitive proteins, mRNA, mABs, ADCs or other biopharmaceutical structures, from A to B at the highest quality possible. The final quality is highly dependent on the process step of freezing and thawing and relies on a reliable cold chain all throughout storage and shipment of frozen BDS. During freezing, the product is exposed to stress that leads to a loss of protein activity.

Different approaches favoring cost-effectiveness, speed of performance, quality of freezing, suitability for cold chain temperature, robustness of packaging, etc. have led to different options for manufacturers. The urge to amplify scalable production options leads to a phasing out of conventional freezing procedures that utilize cryovessels or static freezers. Single-use systems are a trend offering the best solutions for the industry.[3]

What is still perceived as an unsolved issue due to lack of real-world evidence is the fact that freezing can be controlled, thus allowing for full scalability of frozen storage & shipment.

Considerations before Freezing Bulk

Some of the most important and challenging aspects that need to be considered in the freezing of drug products are the following:

Cryoconcentration

The most fundamental concern when designing freeze/thaw procedures is the phenomenon of cryoconcentration. During the freezing process, the ice formation excludes solutes (including protein) from the growing ice crystal.

As shown in Figure 1A, the solutes concentrate towards the center and bottom during freezing.[4]

This concentration effect leads to other effects that induce the denaturation of the protein. Previous studies have shown that cryoconcentration correlates with a slow freezing process, allowing proteins to aggregate in the center and water to freeze in its most natural state around proteins. For example, the high protein concentration itself leads to denaturation through protein aggregation.[1,2]

Freezing Rate

The freeze-path length, which is the distance from the edge of the primary packaging to its center, is a major aspect of how to limit cryoconcentration.

But how to control freezing?

Minatovicz, B. et al. have defined the homogeneity score of the frozen bulk, the degree of cryoconcentration and the freezing rate as the most influential parameters. The latter showed to have significant impact on the stability of other proteins during bulk freezing.[4]

A study performed by Single Use Support and the department Bioprocess Technology at the Technical University of Vienna has investigated the impact and outcome of the freezing rate or ice front growth speed, to be more precise.

(A) STATIC FREEZER

"slow freezing" dark blue region visible at the bottom center

(B) PLATE FREEZER

"fast freezing" more uniform coloring

Figure 1: Comparison slow (A) vs. fast freezing (B): Increased cryoconcentration at slow freezing

The freezing rate showed to have significant impact on the protein stability during bulk freezing.[4] In most cases, a low freezing rate causes the ice to form slowly enough for the proteins to be pushed along the ice rather than being trapped by the ice front of bags (see Figure 1). Additionally, longer path lengths from the surface to the center restrict heat transfer and result in slower freezing rates.

Cryoconcentration usually occurs when the freezing process is slow. However, the process of thawing also impacts the degradation of proteins, if they are exposed to slow uncontrolled changes in their physical state. In general, uncontrolled freezing of multiple bags can create variations in freezing patterns and subsequent concentration gradients similar to that observed in bottles.[5]

Scalability

The aim is to achieve the same freezing kinetics throughout all different scales, i.e. batch sizes. Freezing must be controlled and independent from the bag size, not only to ensure scalability for freezing and thawing but also to prevent cryoconcentration and the related decrease of the drug e.g. cell viability.

Reproducibility of freezing at different scales must be granted in order to guarantee scalable freezing. Single Use Support has already demonstrated that due to very similar freezing curves, scalable freezing is possible both at lab scale and large scale by applying their plate-based freeze & thaw platforms (see also Figure 2).[6]

There is no doubt that various parameters have an impact on scalable freezing, such as the behavior of biologics during freezing along with their solubility and viscosity, but also the performance of the equipment for full loads vs. partial loads, setpoint time and others. As long as the freezing kinetics remain under control, nothing will affect scalability under specific conditions.[6]

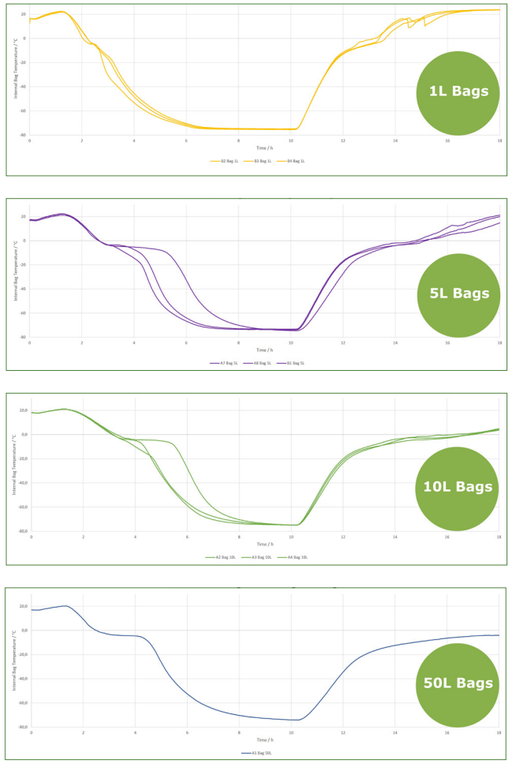


Figure 2: Scalable freezing with single-use bags from 1L to 50L.

Controlled Freezing

Freezing needs to be closely controlled as it can be both harmful and beneficial to the active pharmaceutical ingredient (API). Neither a too-slow nor too-fast freezing process has demonstrated ideal conditions for the protein products. Different freeze studies have highlighted the necessity of examining which freeze rates work best for new systems or products being introduced into a facility.[5]

The rate at which the bulk drug product is frozen is only one of the factors affecting protein recovery.[4] Container dimensions also play an important role in API recovery. The freeze distance is the distance from the edge of a container to its center, and thus the use of scaled-up or -down models to describe API stability in bulk-freeze containers is not always advisable as it may not be accurate. [4,7]

Figure 3: Equipment used in the experiments: (A) RoSS® shell with bag

Experimental Methodology

Study Hypothesis

Single Use Support, in collaboration with the Department of Bioprocess Technology of Technical University of Vienna, have conducted a study and examined the following predefined hypothesis:

The ice front growth speed is independent of the scale and can be used for scale-up.

Measurement setup & plan

In a series of experiments, the study's goal was to prove that the ice front growth speed is independent of scale and can therefore be used for scale-up.

The medium used for the experiments was Laccase enzyme, which is commonly used for mimicking valuable drug substances. For the experiments, Laccase was filled into single-use bags of four different sizes, ranging from 2L to 20L.

All experiments were conducted with Single Use Support products and technologies: RoSS shells (see Figure 3 A) from Single Use Support were used as containers surrounding the single-use bags. While RoSS.pFTU Lab Scale (LA), see Figure 3 B, was used for freezing bags of 2L and 5L, RoSS. pFTU Large Scale (LS), see Figure 3 C, was used for freezing bags of 10L and 20L.

Figure 3: Equipment used in the experiments: (B) RoSS.pFTU Lab Scale

Figure 3: Equipment used in the experiments: (C) RoSS.pFTU Large Scale

In total, 11 different freezing runs were scheduled and conducted one after another over a period of 5 days. The experimental conditions are identifiable from the freezing curves shown in Figure 4.

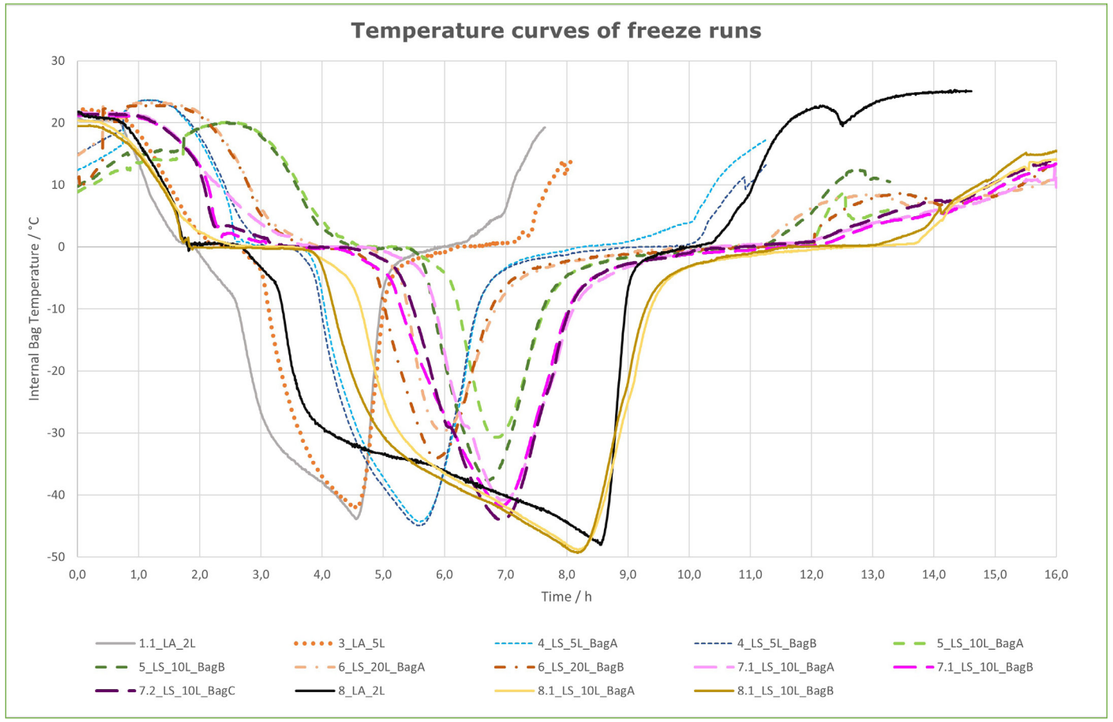


Figure 4: Temperature curves of freeze runs

The ice front growth speed (IFGS) is defined as freeze path length (FPL) in cm devided by phase change duration (PCD) in min.

$$\text{IFGS} = \frac{FPL}{PCD} \text{ / cm/min}$$

Results and Discussion

Impact of ice front growth speed

The measured ice front growth speed ranged from 0.02 cm/min up to 0.05 cm/min (see Table 1). **There was no trend to be observed regarding a correlation of the ice front growth speed and the bag size.** All bags with 2L volume had an ice front growth speed between 0.020 cm/min and 0.034 cm/min, whereas bags with 20L volume had a speed between 0.028 cm/min and 0.035 cm/min. The resulting average values per single-use bag size were very similar:

- 0.028 cm/min with 2L bags
- 0.044 cm/min with 5L bags
- 0.026 cm/min with 10L bags
- 0.030 cm/min with 20L bags

Table 1: Ice front growth speed per ID freeze run

ID	for units: Volume / L	Time / h	IFGS / cm/min
1.1_LA_2L	2	8.5	0.034
3_LA_5L	5	8.5	0.050
4_LS_5L_BagA	5	8.5	0.038
5_LS_10L_BagA	10	8.5	0.035
6_LS_20L_BagA	20	8.5	0.028
6_LS_20L_BagB	20	8.5	0.035
7.1_LS_10L_BagA	10	10.5	0.025
7.1_LS_10L_BagB	10	10.5	0.023
7_LA_2L	2	10.5	0.031
8.1_LS_10L_BagA	10	12.5	0.022
8_LA_2L	2	12.5	0.020

Freezing could be controlled to the extent of enabling a constant ice front growth rate by time of freezing. The faster the single use bags were frozen, the higher the ice front growth speed. The average ice front growth speed decreased when freezing within a longer timeframe:

- 8.5 hours of freezing resulted in an average IFGS of 0.036 cm/min
- 10.5 hours of freezing resulted in an average IFGS of 0.026 cm/min
- 12.5 hours of freezing resulted in an average IFGS of 0.021 cm/min

Effect of ice front growth speed on protein activity

The tests have shown that the ice front growth speed correlates with the decrease in protein activity. The threshold of protein activity decrease was very low, proving that a controlled ice front growth speed at least has **no negative effect on protein activity**.

The test results confirm literary sources, which claim that controlling the ice front growth speed will subsequently also control protein quality as well as protein activity decrease.[4]

Discussion

Of all the parameters impacting the freezing behavior of drug substance, the ice front growth speed has shown to have the biggest impact. Study results have proven the following:

- **Being able to control the ice front growth speed of drug substance at a fast-freezing velocity enables similar freezing kinetics for all sizes of single-use bags.**
- **Being able to control freezing independent of the single-use bag size by implication also controls the degree of protein activity decrease.**
- **Furthermore, it is to be assumed that the use of different plate-based freezers utilizing the same freezing technology, RoSS.pFTU Lab scale for smaller and RoSS.**
- **pFTU Large Scale for larger volumes, provides control of protein activity or, more specifically, prevent protein degradation.**

Conforming with literary sources, the study results prove that controlled ice front growth speed makes scalable freezing possible (see Figure 5). Performed with plate-based freeze & thaw platform RoSS.pFTU in combination with the RoSS shell protecting any single-use bag on the inside, the doors to scalable freezing experiences, from clinical studies to commercial bulk drug substance production, are pushed wide open.

Further studies that were performed at Single Use Support have reconfirmed scalability. The freezing curves below confirm the independence of ice front growth speed from the type of freezing platform (LabScale or LargeScale) and the height of the water column (minimum and maximum load), if the customized freezing recipe per drug substance is correctly implemented.[8]

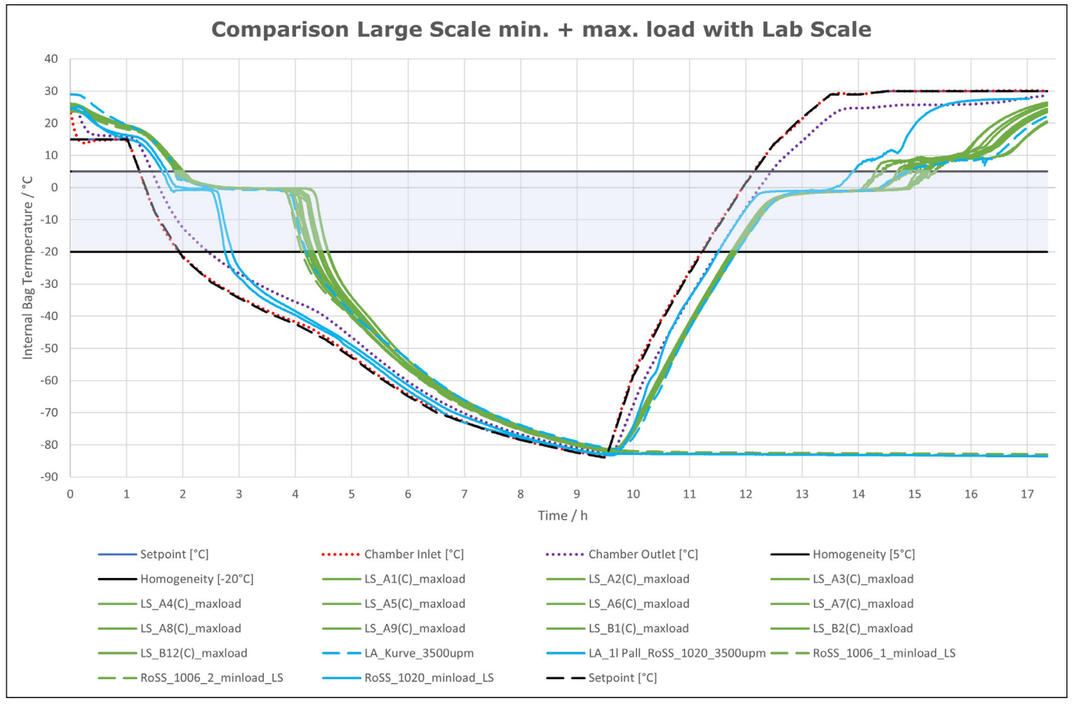


Figure 5: Freezing curves confirming scalability through consistent ice front growth speed:
• Blue curves show result from RoSS_1020 to protect 1L bags frozen in both RoSS.pFTU Lab Scale and LargeScale;
• Green curves show results from RoSS_1006 to protect 10L bags frozen in both RoSS.pFTU LabScale and LargeScale.

Conclusion

Pharmaceutical manufacturers know the characteristics of their drug products by heart. They know how they behave under different circumstances. They know which conditions are beneficial and which are counterproductive. Emerging technologies, such as mRNA vaccines, AAVs or autologous and allogeneic cell and gene therapies, are very sensitive to denaturation and therefore have to be frozen in a highly controlled manner. If neither the highest quality is ensured nor the most reliable technologies are used, there is a risk of negatively impacting the product at the very last stage of production.

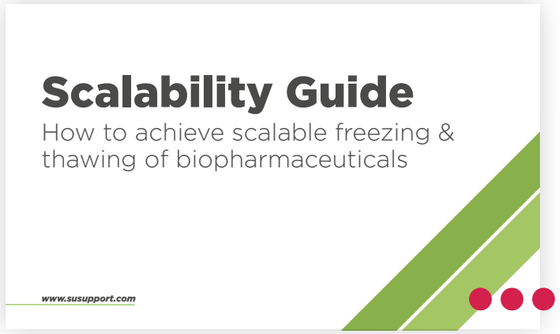
This has turned controlled freezing into an increasingly important topic. There is a growing need to maintain uniform or at least similar conditions for freezing, frozen storage and thawing of a drug substance; and there is a growing need of being able to control the freezing and thawing process in order to open doors to enhanced process reproducibility that is both scalable and of high quality. In this way standardized cGMP cold chain process steps can be established in pharmaceutical manufacturing and be applied for various products. Cell, gene and gene-modified therapies, but also enveloped vector viruses, for example, require slow and regulated freezing to minimize cell damage and to secure cell viability. Product-based freezing recipes that deploy desired temperature setpoints and consequently desired ice front growth speed, ensure control over the optimal freezing performance for different products.

Providing pharmaceutical manufacturers with a platform that allows for controlled freezing & thawing is a true improvement benefitting the entire industry.

The new insights from the study conducted by Single Use Support and the Department of Bioprocess Technology of Technical University of Vienna pave the way towards freezing in a controlled and scalable environment by controlling the ice front growth speed throughout different scales, and by reaching high product homogeneity during freezing as well as maintaining highest protein activity.

References

1. Singh, S. et al.: Large-Scale Freezing of Biologics; A Practitioner's Review, Part 2: Practical Advice, Bioprocess Int. Vol.7 Issue 10, 2009, P.1., 2009.
2. Zippelius, R.: Untersuchungen zum Einfrier- und Auftauverhalten pharmazeutischer Humanproteinlösungen im Großmaßstab. Dissertation 2002, LMU München: Fakultät für Chemie und Pharmazie. Available at: https://edoc.ub.uni-muenchen.de/687/1/Zippelius_Ralf.pdf
3. Single Use Support.: Protecting single-use bags to foster their progress, 2021. Available at: Webb, S.D. et al., Freezing biopharmaceuticals using common techniques — and the magnitude of bulk-scale freeze-concentration, Biopharm Int. 15 (May) (2002) 22–34)
4. Minatovicz, B. et al.: Freeze-concentration of solutes during bulk freezing and its impact on protein stability, Journal of Drug Delivery Science and Technology Vol.58, 101703. 2020. P.1.
5. Webb, S.D. et al., Freezing biopharmaceuticals using common techniques — and the magnitude of bulk-scale freeze-concentration, Biopharm Int. 15 (May) (2002) 22–34. Available at: https://cdn.sanity.io/files/0vv8moc6/biopharn/cb3b8ffa70456f80b72eeb3ef7cac1c29333540a.pdf/article-22913.pdf
6. Single Use Support: Scalability Guide, 2021. Available at: https://www.susupport.com/datasheet/freeze-thaw-scalability-guide
7. Goldstein, A.: Freeze Bulk Bags: A Case Study in Disposables Implementation, Biopharm International, Vol. 2009 Supplement, Issue 8. 2009. Available at: https://www.biopharminternational.com/view/freeze-bulk-bags-case-study-disposables-implementation
8. Single Use Support: Scalability Studies, 2022. Data on file.



How to enable scalable results regardless of the size of the single-use bags, the size of the freezing & thawing platform, or the number of single use bags you put into the freeze & thaw platforms for a freeze run.

Download the Guide

CASE STUDY

Protecting Bags, Bottles & Vials:
Hybrid for Primary Packaging

1 | The Situation

Offering a wide spectrum of biopharmaceutical manufacturing, a customer - a global manufacturing organization - transfers liquids in all common types of primary packagings:

Single-use bags: Single-use bioprocess containers that are available in different sizes ranging from 10 mL to 50 L as well as from different suppliers are the primary packaging for drug substance handling, trending throughout the industry.

Vials: Ideal for small yet highly valuable amounts of liquids. Sizes vary from 2R to 20R.

Bottles: Rigid containers are commonly used in pharmaceutical logistics, the reason for which can mainly be traced back to low costs. Filling volume per bottle ranges from few mL to 10 L.

2 | The Problem

Any primary packaging requires individual processes and platform systems for protection against product loss, filling-draining, freezing-thawing, storage and transportation.

Consequently, different processes and systems have led to different outcomes with variabilities in the process performance. The lack of standardized process solutions along with varying rates of product loss and inconsistent freezing behavior has resulted in process inefficiency:

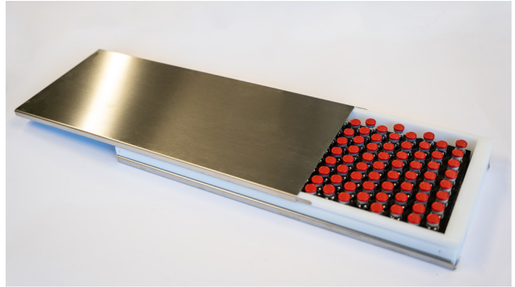
Due to the lack of robust protection, regular breakages of primary packaging have caused increased loss of product.

Drug substances have been filled into primary packaging manually – a potential threat for cross-contamination and human errors.

Freezing bulk drug substances in static freezers results in slow freezing, leading to cryoconcentration and limiting scalable freezing.

The manufacturer has therefore decided **to implement a harmonized process solution that can be used with different types of single-use primary packaging whilst at the same time minimzing the rate of product loss.**

3 | The Solution



With more than 200.000 RoSS® shells used globally, the novel technology has proven to effectively protect single-use bags. The RoSS® shell embeds any single-use bag, regardless of size and vendor, to reduce the rate of product loss towards 0%.[1]

The customer has implemented the RoSS technology (Robust Storage and Shipping) as it offers hybrid process solutions for the majority of primary packaging most frequently used for drug substance shipping purposes.

RoSS® shell for vials: The RoSS® shell offers optimal protection for substances in vials. Due to its design, which has been optimized for plate-based controlled freeze-thaw platforms, it has optimized freezing capabilities. Depending on quantity per batch, vials can either be placed in RoSS® shells for commercialized production or in RoSS.KSET for clinical studies or cell and gene therapies. The customer required a process solution to freeze and thaw 3,000 – 6,000 vials per batch and therefore opted for the RoSS® shell. With RoSS® as robust secondary packaging their closed system now benefits from the optimal heat transfer of plate-based platform systems around RoSS for controlled cryogenic applications.

RoSS for bottles: Converting the concept of RoSS® shells to bottles, the neck of the rigid containers - as a susceptible spot for leakages - is being protected with 3D foam. In spite of it being a closed and protected system, attached tubings allow operators to fill, drain and sample in a sterile manner. This allows for automated aseptic filling (with RoSS.FILL) and freezing in hybrid versions of Single Use Support's plate freezers that allow for both bags and bottles to be frozen at reproducible and recipe-driven temperature curves.

Any primary packaging can now be embedded in an end-to-end process for cold chain management of drug substances, which unifies biomanufacturing processes despite the use of different primary packagings. The principle of RoSS® is to protect the spots most vulnerable to breakage in order to reduce product loss, to establish process solutions and to improve the performance throughout each of the process steps, regardless of the primary packaging used.

4 | The Result

-60%

Required platform systems

Hybrid platform systems for bags, bottles and vials provide all-in-one solutions

-50%

Workforce

Less platform solutions require less training & less documentation; automation improves efficiency

0%

Reduced product loss

Occurrences of bag, bottle and vial breakages towards 0%[1]

Reference

1. Single Use Support. Case Study Reducing Product Loss Cost-Efficiency, 2022

Scaling up without product loss: The End-to-End Process for Liquid Transfer in Biopharma

View the Video