SINGLE USE SUPPORT.

PIONEERING BIOPHARMA

ADC Manufacturing

Navigating 5 overlooked challenges

What is an antibody-drug conjugate?

Antibody-drug conjugates (ADCs) represent a powerful application of bioconjugation that is transforming medicine and pharmaceutical science. For example, current ADC technologies are used in oncology to treat patients who have cancer with highly potent active pharmaceutical ingredients (HPAPIs).

ADCs work by attaching a monoclonal antibody (mAb) to a cytotoxic payload through a chemical linker, as illustrated in Figure 1. When administered, the ADC recognizes and binds to target antigens on the surface of cancer cells. The toxin is internalized into the cancer cell, where it is then released.

ADC manufacturing process

ADC manufacturing involves the production of mAbs, synthesis of payload and linker compounds, and conjugation of the mAb to the drug-linker. mAbs are produced through upstream bioprocessing with cell cultures and downstream process steps that include harvesting, purification, chromotography.

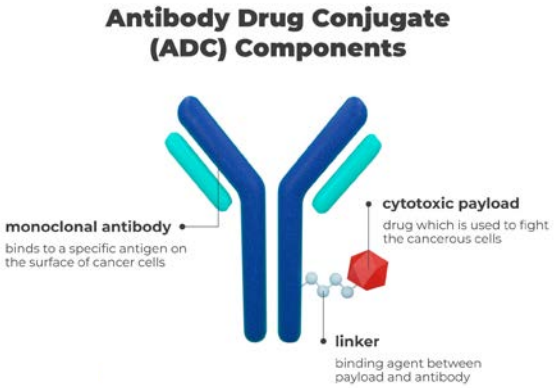

Figure 1: Components of an antibody-drug conjugate

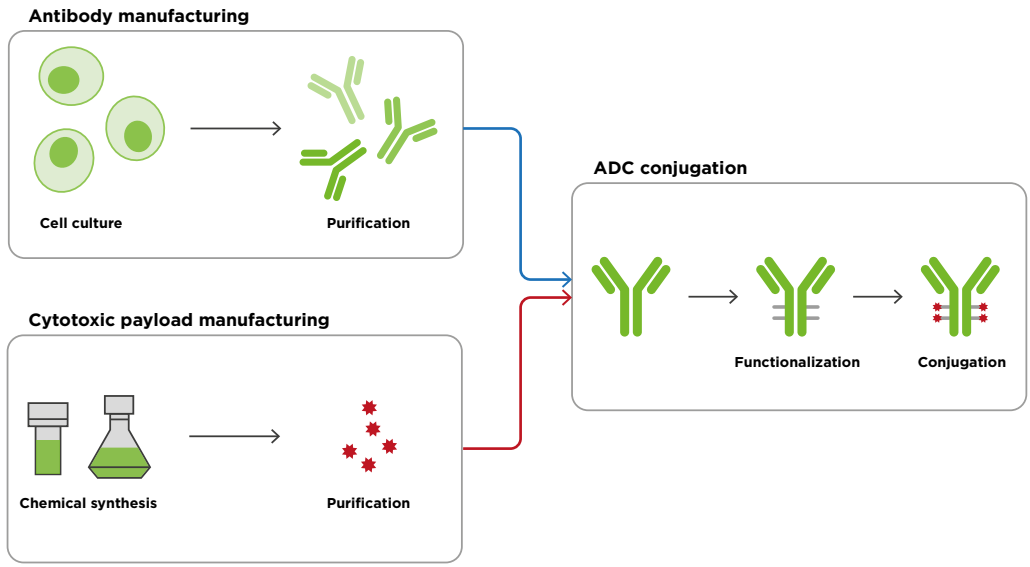

Figure 2: A simplified overview of the ADC manufacturing process

ADC trends

The use of different payloads and dual linker/dual payload strategies for ADC-based therapies will continue to drive ADC innovation. Despite the potential bottleneck of high costs, ADCs will continue to be attractive and beneficial to develop and manufacture.[1]

1. A toxic relationship

Handling ADC cytotoxicity with care

ADCs are highly effective against cancer cells and therefore offer great hope for the treatment of patients. However, the chemical structure of ADCs and their manufacturing process have their pitfalls, making it a challenge to produce and deliver a safe and effective product to the patient. Before an ADC is formulated to a patient-ready drug product, its toxicity can be a safety challenge for those involved in its development and manufacture. The cytotoxins used in ADC manufacturing – unconjugated or conjugated – pose a health threat to operators, which is why primary and secondary containment strategies are aimed at preventing exposure to the cytotoxins through inhalation or skin contact.[2]

Limiting bag breakage

Primary packaging - typically a 2D single-use bag or a bottle - can be prone to breakage if not properly protected during fluid management and cold chain operations. Using protective secondary packaging reduces the risk of bag breakage, which could result in product loss and exposure of production personnel to hazardous liquids.

The unique characteristics of ADCs mean that, to maximize the safety and efficiency of their manufacture, safe infrastructure and comprehensive facility design is necessary.

EU GMP Annex 1 is one of the current regulatory standards that promotes exactly this: aseptically closed systems and container closure integrity to reduce the risk of contamination and exposure to toxic liquids.

Operator safety through protective secondary packaging

Single Use Support's RoSS® shell is secondary packaging for 2D single-use bags that enhances manufacturers' safety, efficiency, and reliability in ADC handling. The robust shell supports single-use bags of all types and sizes while significantly reducing product loss from bag breakages. The robust and tamper-evident protection remains a closed system throughout the (cold chain) logistics process, exemplifying a secondary containment strategy that safeguards employees and the environment from exposure to cytotoxins during ADC manufacturing process steps, including temporary and long-term storage, transportation, freezing and thawing. Similarly, the Bottle RoSS provides a robust secondary packaging solution that protects tubing and the assemblies on bottle caps from damage during cold chain handling.

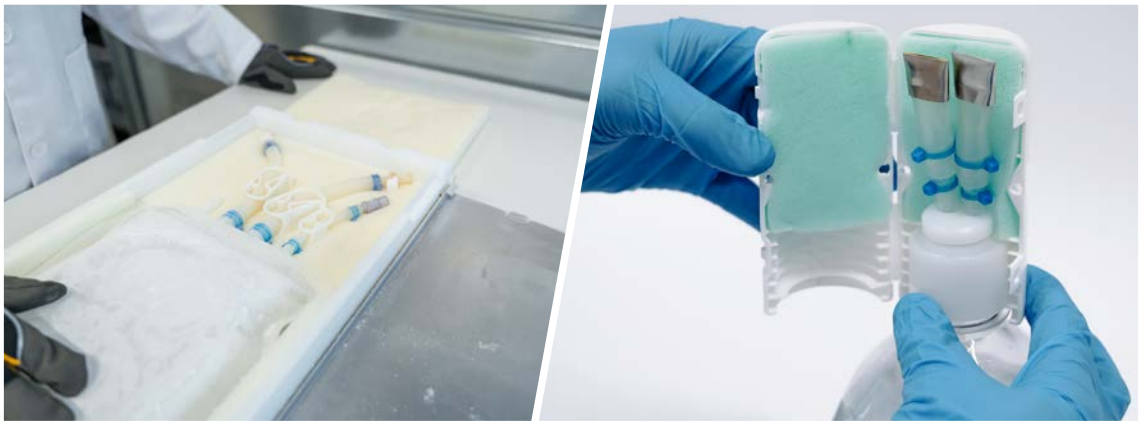

Figure 3: RoSS® shell (left) and Bottle RoSS (right) offer robust protection against ADC cytotoxicity

2. Every drop counts

Precision in fluid management

The accurate dispensing of ADCs into single-use bags or bottles is a challenge in ADC manufacturing. Therefore, there is a need for precision equipment, for the following reasons.

- **Precise dosing at all scales:** In clinical studies, development and upstream bioprocessing ADCs are handled in small volumes, requiring high-precision filling equipment. However, it is also important when handling larger volumes of cytotoxic drug substances to ensure that each bag or bottle contains the intended filling volume with no deviation, as overfilled bags are more likely to break during freezing.
- **Bag-to-bag variability:** It is important to have one type of primary packaging that is filled precisely. Maintaining this precision consistently throughout all packaging types is even better. Consistent counts of active pharmaceutical ingredient (API) per single-use bioprocess container can improve the operational reliability of the process.
- **Holdup volume:** Drug substance product losses can occur when fluids are left in single-use assemblies. Technologies that help maximize the recovery rate of such residual volumes improve bioprocessing yield.

Automation breaks new ground

Aseptic filling is a necessary process step when aliquoting raw materials, intermediates, or conjugated APIs into bioprocess containers for safe storage and shipping. Due to the sensitivity of the protein structures and the high cytotoxicity of the drug substance, the liquid transfer of ADCs is a high-risk process for manufacturing personnel. Seamless fluid paths in aseptically closed single-use systems, following EU GMP Annex 1, help establish a safer environment and mitigate the risk of contamination when aliquoting. Automated filling platforms reduce product loss by addressing three key causes of losses: inaccuracy, inefficiency, and residual volumes. Manual handling introduces inefficiencies due to limited scalability and slow, repetitive operations. In addition, residual product losses occur through holdup volumes in single-use tubing after the filling process step.

All-in-one: Homogenize, fill, filter, seal

RoSS.FILL is a modular platform designed for the filling and filtration of drug substances, capable of dispensing volumes from a few milliliters up to 250 liters per hour into single-use bags, bottles, and more. Meeting 21 CFR Part 11 and EU GMP Annex 1 quality standards, this single-use technology provides manufacturers with consistent process safety, precision, and maximum recovery rates for holdup volumes. Flow sensor or gravimetric technologies allow operators to aliquot with the greatest precision. Its advanced sealing valves automatically decouple the filled container from the assembly.

Figure 4: RoSS.FILL precisely fills high-value biologics into single-use bags and bottles with aseptic sealing valves.

3. Tough cookies?

How ADCs maintain stability after freeze-thaw cycles

Freezing and thawing means stress for ADCs as protein-based biologic drug substances. The greatest challenges when preserving the quality of an ADC during cold chain processes are as follows[3]:

- **Cryoconcentration:** Freezing leads to the concentration of solutes before and during the phase transition, which alters buffer chemistry and pH, creating destabilizing microenvironments for ADC components.
- **Denaturation:** Local pH changes and ice-interface stresses can partially unfold the antibody, compromising its native structure.
- **Aggregation:** Denatured or stressed ADC molecules can self-associate, reducing biological activity and increasing the risk of immunogenicity.
- **"Weakest link":** Temperature- and pH-sensitive linkers represent a vulnerable structural element; freeze-thaw stress can cause cleavage or altered conjugation, effectively separating ADC components.

Managing the stress of freezing & thawing

Proteins, including antibodies, must be handled with care to preserve their quality, identity, purity, and potency. It is important to avoid repeatedly freezing and thawing ADCs, as this makes them more susceptible to aggregation. To avoid jeopardizing their potency, they are often stored at 2–8°C, but for medium- to long-term storage, freezing at temperatures of less than -60°C is necessary. Using uncontrolled freezing technologies, such as static freezers, poses a higher risk for protein instabilities. It is a real challenge to maintain a product's critical quality attributes when there is insufficient control over the freezing process. It is effectively hoping for the best. However, it is possible to take control of the freezing and thawing process of biologics with recipe-driven freezing protocols that address the needs of each drug substance with regard to the optimal freezing rate and behavior during phase transition, regardless of the primary packaging or batch size used.

Controlled freezing & thawing to improve ADC stability

The most appropriate freezing technology depends on whether single-use bags or bottles are used to freeze intermediates or ADCs. The most suitable technologies are plate freezing and blast freezing. Recipe-based freezing protocols enable a controlled drop of temperature gradients to -80°C, and up to ambient temperature for thawing. Using controlled-rate freezers ensures a reproducible freezing process, which is typically around -1°C per minute. However, the freezing rate that results in optimal product stability is determined by the product specifications and the geometry and size of the container used. This approach to control the freezing according to what the drug substance needs minimizes the risk of cryoconcentration, unwanted pH shifts, and denaturation. The plate freeze-thaw platform RoSS.pFTU, designed by Single Use Support, is intended for the controlled freezing of single-use bags, whereas the blast freeze-thaw platform RoSS.BLST is intended for the controlled freezing of drug substances in bulky packaging, such as bottles. Both platforms adhere to the automation standards set out in 21 CFR Part 11, which help to maintain optimal product stability during cold chain processes.[3]

Figure 5: Single Use Support's plate freezer RoSS.pFTU (left) and blast freezer RoSS.BLST (right)

4. Road mAb for scaling

Transition from bench scale to commercial production

mAbs, cytotoxic payloads, and linkers are essential components in the development of ADCs. Considering scalability from the outset of ADC production planning is critical. This involves evaluating the potential for future growth and expansion during the early stages of process design. Such early consideration enables the implementation of adaptable systems that can seamlessly meet increasing demands as production progresses from research to large-scale manufacturing. Several factors must be taken into account, even when focusing solely on the scalability of mAb production:

- **Cell expansion:** To ensure efficient upstream bioprocessing and a smooth transition from bench to production, the growth of cell lines must be maintained during scaling to ensure yield and viability.
- **Process flexibility:** Not all mAb production processes are the same. They differ according to the mAb variant used, the scale of production, the primary packaging used, and the critical quality attributes applied. Modular equipment helps to balance the various parameters that influence the manufacturing process and thereby ensure process flexibility.
- **GMP readiness:** Automation helps make process steps more efficient at a variety of scales. It also facilitates standardization. Stringent quality control ensures that each mAb batch meets the highest quality standards, whether produced in laboratories or commercially.

Helping scale-up and scale-out

Modular systems enable scaling in every direction. This includes increasing the batch size and thus the size of the single-use bags and bottles used (scale-up), but also the scale to produce a larger number of bags and bottles with the same volume (scale-out). This depends on what you are producing and at which step of the manufacturing process it is applied, e.g., bioprocessing of a commercialized mAb or ADC intermediates in GMP production or cell line banking as part of seed train intensification.

Modularity forms the cornerstone of Single Use Support's solutions. Aligned with GMP relevant standards, Single Use Support offers scalable solutions across the full range of fluid and cold chain management as well as process automation protocols that are easily transferable across a variety of scales.

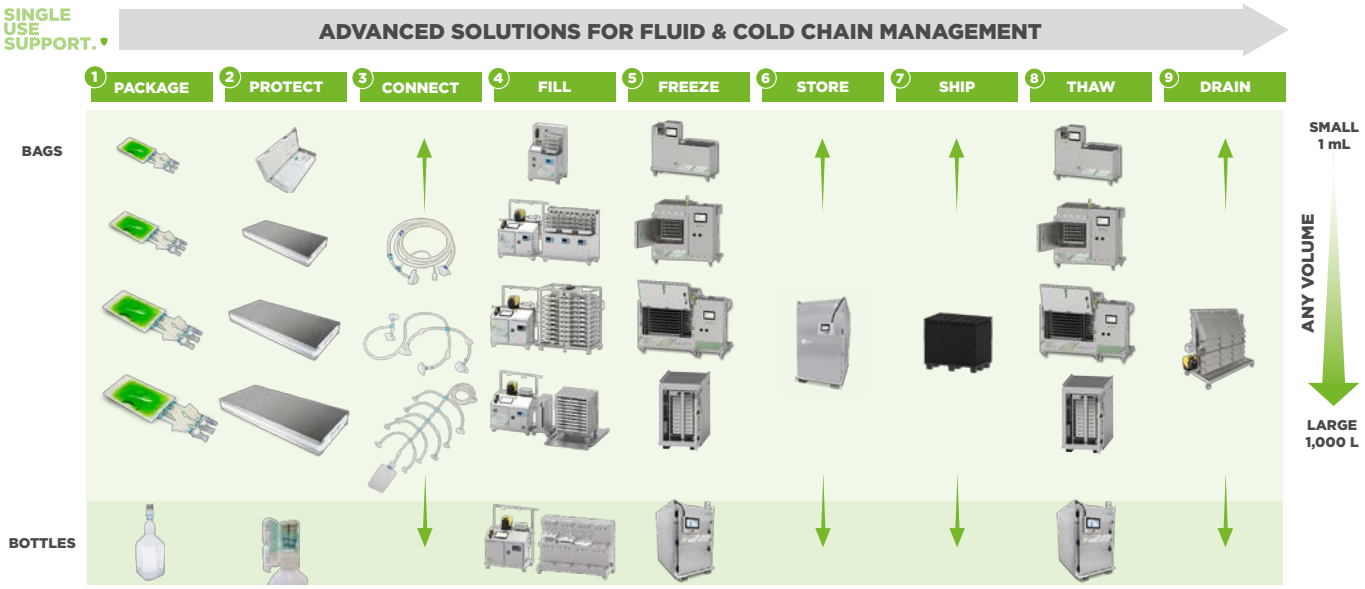

Figure 6: Single Use Support's process solutions for single-use bioprocess containers per volume range.

5. The price is right

Cost considerations for filling & freezing equipment

Not surprisingly, cost-, time-, and labor-intensive processes are major concerns for ADC manufacturers. However, reducing costs must not compromise the safety of a manufacturing facility. Ensuring safe fluid management and maintaining cold chain integrity are essential to protect both patient and personnel safety. New technologies and developments in the production process help streamline operations through fully automated aseptic filling, controlled-rate freezing and thawing methods, and closed, contamination-mitigating systems.

Integrated yet modular

For this reason, most contract development and manufacturing organizations (CDMOs) involved in ADC development or ADC processing, including antibody manufacturing, rely on single-use technologies. This makes modular and automated single-use technologies particularly attractive for production process developers, as they help save time and reduce costs across multiple process steps:

- **One system for all:** Moving away from vendor-specific proprietary systems and providing more universally compatible technologies simplifies the process flow by reducing the need for different platform systems. For example, the RoSS® Shell is a robust, vendor-agnostic secondary packaging solution for all 2D single-use bags currently on the market, helping to standardize cold chain operations.
- **Two-in-one solutions:** Less equipment and a smaller footprint further enhance cost efficiency by reducing the need for manual handling, staff training, cleaning, validation, and facility space. For example, Single Use Support's RoSS.FILL and RoSS.BLST are automated filling and controlled-rate freezing platforms that enable the reliable handling of single-use bags and bottles.
- **Fewer machines:** Efficient single-use technologies increase productivity with faster filling and controlled-rate freezing, as well as higher storage densities, which ultimately reduces the number of platform systems required.

Modular process solutions

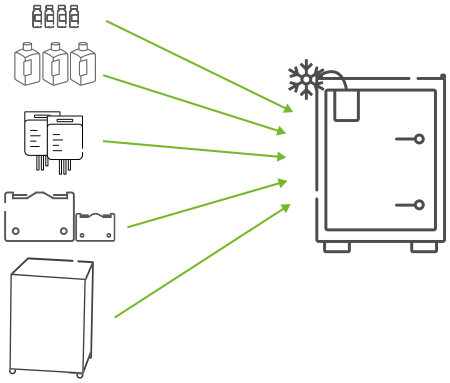

Figure 7: RoSS.BLST is suitable for freezing different container types, sizes, and formats.

Fewer, more efficient platforms

Figure 8: Equipment required to freeze 2,000 L within 48 hours

Lower costs with added value

Single Use Support's platform systems can be integrated into existing processes and ultimately be put together plug-and-play style to create an integrated end-to-end fluid and cold chain management solution. This means manufacturers are not limited in terms of size, scale, or bag manufacturer, as the RoSS® shell can accommodate them all.

The process flexibility provided by using Single Use Support's modular and process-independent solutions can result in significant capital expenditure (CAPEX) and operational expenditure (OPEX) savings. CAPEX savings increase even further for manufacturing processes that handle multiple primary packaging types, such as single-use bags, bottles, and more, across a variety of scales. Despite the need for consumables to protect primary packaging, single-use assemblies, and cold chain shipping containers, OPEX can still be reduced considerably. This is because of the reduced need for operators, operator training, cleaning, and validation. In addition, thanks to the efficiencies of automated single-use technologies, manufacturers benefit from greater space saving, faster production, and a lower overall risk of contamination or product loss due to improved container integrity as well as more accurate filling with a maximized holdup-volume recovery rate.[4]

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