Single-Use Technology in Purification of Monoclonal Antibiotics

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Abstract

The advent of Single-Use Technologies (SUT) has revolutionized biopharmaceutical manufacturing, offering a flexible and efficient alternative to traditional methods. This paper explores the diverse applications of SUT, focusing on therapeutic plasma filtration, clarifying processes, virus removal strategies, and chromatography solutions. Highlighting the advantages such as convenience, reduced contamination risk, and streamlined processes, the document delves into the economic and environmental impacts of adopting SUT. Despite challenges in scalability and automation, specific examples like Immusorba PH-350 and Pall Biotech's AllegroTM underscore the significant adsorption efficiency achieved with single-use systems. Anticoagulation strategies tailored for single-use plasma filtration contribute to optimal systemic performance. The dynamic market for disposable devices, featuring prominent manufacturers like Fresenius HemoCare, Kaneka, Medicap Clinic GmbH, Miltenyi Biotec, Toray Medical, and Biotex, reflects the widespread adoption of SUT across various medical applications. The ongoing innovation in overcoming scalability limitations and the commitment to standardization position SUT as a transformative force in modern biopharmaceutical manufacturing. In conclusion, the economic implications and positive environmental impact underscore SUT's potential to shape the future of the industry.

Keywords: Single Use Technology, Reduced Contamination, Antibiotics, Biopharmaceutical, Good Manufacturing Practices, Mixing bag, Aseptic Connections, Single use Bioreactors, Disposable Chromatography Columns, Single use Filtration, Running Cost, Labour charges

Introduction:

One of the most significant secondary metabolites produced by bacteria, fungi, and Streptomyces that is used in a variety of commercial applications is the antibiotic. The majority of antibiotics used today originate from microorganisms. It is simple to isolate, cultivate, preserve, and enhance the strain of bacteria. Because of their ability to generate resistant endospores and produce essential antibiotics like bacitracin and polymyxin, Bacillus species are the prevalent soil bacteria and are always found to hinder the growth of other organisms. Many antibiotics, including the sulfa medications, are now also obtained chemically thanks to advancements in organic chemistry. Two categories of drugs are utilized in the chemotherapy of infectious illnesses. Antibiotics are

substances produced by bacteria and fungus, whereas synthetic medications are those created in a laboratory using chemical processes [1].

Three different processing processes are used in the biopharmaceutical business to make therapeutic medicines. The *first* type involves stainless steel systems. These are robust, reusable, and resistant to exposure to the chemicals—typically at very high temperatures—that are used to sterilize pharmaceutical processing equipment. This calls for strict sterilization protocols that may involve harsh chemicals and steam, which would demand a significant amount of energy to raise systems to the extremely high temperatures needed for sterilization to be effective. The *second* category are partially disposable systems. Depending on the therapy produced, these may make several uses of specific sections of the processing system. Reusable components need to be maintained since they corrode with time. They must go through cleaning and sterilization procedures similar to those used in stainless-steel systems. The inherent danger of contamination exists for both stainless-steel systems and the reusable components of disposable systems, even in the presence of verified cleaning and sterilization protocols. The *third* category of biopharmaceutical processing systems are single-use systems, which are intended to be used only once during the manufacturing of a single therapeutic batch before being disposed of. The increasing usage of single-use technologies, which do not require washing in between batches, is reducing the risk of product cross-contamination. Made in a cleanroom, these systems are extremely effective and economical, guaranteeing a sterile system for each batch. They are double bagged and subsequently sterilized using gamma, EtO, or x-ray procedures.

This paper examines the various single use systems available for downstream processing in the pharmaceutical industry with a special focus on antibiotics production.

Process development using Single Use Systems: A brief overview

In addition to purity and process capacity, yield and productivity are the main emphasis of DSP development. The optimization of both current and alternative processes, along with the extension of current facilities, lead to a rise in the separation efficiency of single unit activities. There is research being done on new processes development methodologies. These include the development of platform technologies, high-throughput techniques utilizing DoE-based experimental optimizations and QbD-based methodologies. Process development also incorporates the utilization of mini-plant facilities and the modeling and simulation of unit operations. Monoclonal antibodies were previously purified using a variety of membrane-based and chromatographic procedures. It is necessary to incorporate a virus-inactivating procedure, a filtration-based virus-reducing process, and a final diafiltration.

As the biopharmaceutical sector evolves, production facilities are becoming more flexible and responsive, reducing costs and schedules while meeting tight regulatory and capacity needs. The introduction of plastic blood bags made of polyvinyl chloride (PVC) in 1953 by Fenwal Laboratories (now Fresenius Kabi) served as the impetus for the development of SU bioreactors. Other significant developments in the field of cell culture include the introduction of plastic dishes and flasks for standard procedures in the 1960s, the development of polystyrene multi tray systems, and the creation of hollow fiber bioreactors in the 1970s. The 1980s and 1990s saw the development of several hollow fiber bioreactors (such as FiberCell, Amicon, and Endotronics systems) as well as two-compartment dialysis membrane bioreactors (such as CeLLine, MiniPerm), which made it feasible to produce therapeutic and diagnostic antibodies in the one- and two-digit mg range.Wave-mixed systems, the first model of which was introduced in the late 1990s and named the WAVE BIOREACTOR, were the turning point for SU bioreactors. Numerous comparison tests proved its superiority [2] for animal cell culture, and as a result, the WAVE BIOREACTOR 1000 and other wave-mixed bag bioreactors from other suppliers were developed. Stirred rigid bioreactors and various stirred bag bioreactors, which have been commercially accessible since the mid-2000s, comprise the largest group of SU

bioreactors today. There are also currently available different kinds of bioreactors that do not follow the wave-mixed and stirred mixing principle.

SU bioreactors have a cultivation container made of plastic, unlike their traditional equivalents, which are composed of glass and/or stainless steel. The cultivation vessel is often delivered by the vendor ready to use, pre-assembled, and sterilized using beta or gamma radiation. It is disposed of and decontaminated after usage. While rigid polycarbonate plastics are typically used to make cultivation containers in micro, milliliter, and small liter scales, larger systems use flexible two- or three-dimensional (2D) bags with contact layers made of ethylene vinyl acetate (EVA) or polyethylene (PE) films. For cultivation bags, shaping and fixing the bags requires stainless steel trays or specially designed support containers with heated blankets or double jackets for temperature management.

In order to meet several of the essential future needs stated for the manufacturing of biopharmaceuticals, single-use technology, or SUT, offers the following benefits:

(i) Flexibility: It is simpler to reconfigure the facility when the process train is disconnected from the infrastructure and the building is divided into discrete individual workstations. More process and product flexibility, as well as portability, scalability, and facility operations management, are the outcomes of this. According to Kapp et al., SUT also leads to quicker product modifications and batch turnaround times, which enhance process adaptability and speed to market. A quick increase in capacity is possible if product demand rises.

(ii) Reduction in Capital Investment: With a potential reduction in capital investment costs of over 70% in equipment and facility infrastructures, the cost structure is transferred to variable operating costs.

(iii) Increased production: quicker facilities' building, commissioning, and launch when utilizing SUT, as well as simpler handling and quicker turnaround times (less validation between various strains and products and no need for change-over cleaning).

(iv) Safety: less risk of cross-contamination and more guarantee of sterility, as well as fewer regulatory concerns and validation requirements for cleaning systems that are reduced by more than 50% in terms of time, money, and labor.

Effect on cGMP by using single use systems

The quality, or lack thereof, of dietary supplements and their impact on public health has become a growing concern. In their proposed rulemaking, FDA listed several specific examples (Federal Register, 2003) of inadequate quality in marketed products (discussed below). FDA believes, and
rightly so, that compliance with the proposed cGMP rules will protect public health.

The proposed cGMP rule (Federal Register, 2003) emphasized the need to test imported herbal products for "heavy metals, pesticides, and industrial contaminants." Other reported problems include: (a) the use of non-food-grade chemicals in the manufacture of dietary supplements (Anon., 1999), (b) unsanitary conditions in the manufacture, packaging or storage of food additives, such as pests . . infestations, equipment and construction defects and leaking pipes. FDA efforts resulted in lead-contaminated food additives , microorganisms such as salmonella,
High batch-to-batch variability in declared ingredients is another reason the FDA has given for the need for cGMPs. For example, for , which contains ephedrine, pseudoephedrine and -methylephedrine, the respective variations in lot were 180, 250 and 1000%. In one extreme case, one of the 4,444 ephedra products contained no alkaloids. Similarly, 4,444 claimed ingredients were not found in 25% of Ginkgo biloba 4,444 products tested, 20% of saw

palmetto products, 33% of 4,444 glucosamine, chondroitin and their combinations, and 4,444 ingredients in 50% of SAMe (S-adenosylmethionine) products. The proposed rules will eliminate such variability by establishing appropriate controls, including master production and batches.

The introduction of cGMP (current good manufacturing) standards has a significant impact on single-use systems in the pharmaceutical and biopharmaceutical industries. cGMP guidelines are designed to ensure the quality, safety and efficacy of pharmaceuticals, including those manufactured using single-use technologies. Here are some of the main implications of cGMP for single-use systems: cGMP emphasizes strict quality throughout the manufacturing process. Single-use systems must meet strict quality standards to ensure the integrity of the final product.
Manufacturers of single-use systems must follow cGMP requirements to demonstrate the reliability and compliance of their products.
Validation and Documentation:

cGMP requires extensive validation of processes and equipment. Single-use systems must go through validation processes to demonstrate their suitability for their intended use.
Extensive documentation, including validation reports and records, is essential for compliance with cGMP standards. This documentation ensures traceability and accountability of the production process. Disposable systems use a variety of plastic and disposable materials. cGMP requires that these materials be compatible with the product being processed and do not affect product quality. Manufacturers must demonstrate material compatibility through testing and validation and ensure that single-use systems are free of contaminants.

Maintaining sterility is crucial in the manufacture of medicines. cGMP requires strict adherence to sterility practices, including proper handling and storage of single-use components. Single-use systems must be designed and manufactured to prevent microbial contamination during handling. Sterility testing and validation are integral parts of cGMP compliance. cGMP emphasizes the need for robust process control and monitoring. Single-use systems should have built-in mechanisms to monitor critical process parameters to ensure consistency and repeatability.Automation and control systems related to single-use technologies must meet cGMP requirements to control the process and maintain product quality. Changes to the design or components of single-use systems must follow a structured change management process according to cGMP. This ensures that changes are thoroughly evaluated and documented before implementation [3]. Manufacturers must demonstrate that changes to single-use systems do not compromise product quality, safety or efficacy. cGMP places great emphasis on personnel. Operators and technicians working with single-use systems must be properly trained to ensure proper handling, assembly and operation. Training programs must cover cGMP principles, aseptic practices and special requirements related to single-use techniques. Traceability of components and processes is essential in cGMP compliant manufacturing. Single-use systems must be traceable from manufacturing to final product assembly. Detailed documentation, including batch data, test results and certificates of analysis, is required to demonstrate cGMP compliance.

Trends in Single use systems in Downstream Processing:

The advancement in single-use technology has made it possible to perform intricate DSP procedures like centrifugation, TFF, and chromatography using disposable devices. In order to support bigger manufacturing scales, there has also been a tendency toward larger disposable modules. Alternative production principles are applied in DSP due to the requirement for additional process intensification.

In today's typical batch production, a product is produced through a series of steps starting with a unit operation and ending with collection in a hold container before being processed further through a subsequent unit operation.

This may reduce how often the facility is used. Using continuous unit operations, in which the product flows through the series of unit operations without pause, is a component of integrated processing. By decreasing the size of unit operations and overall processing time, integrated processing can still lead to productivity gains in terms of buffer, resin, and consumable savings. It also makes it possible to handle greater processing scales using single-use technologies.

The biopharmaceutical business has made cutting manufacturing costs a top aim because of growing economic pressure and fiercer competition. It has been demonstrated that single-use technology and continuous processing can both lower production costs. Continuous processing applies disposable technologies at lower dimensions and hence more efficiently than single-use processing, which depends on the usage of disposables at a relatively high scale (due to the execution of several purifying cycles instead of direct upscaling). Because continuous processing executes numerous purifying cycles rather than directly upscaling, disposable technologies are implemented at lower dimensions and therefore more efficiently, whereas single-use processing depends on the usage of disposables at a relatively large scale. Because continuous processing is less scale-sensitive, it further minimizes the equipment footprint and lowers operating expenses, leading to output that is cost-effective. The volumetric productivity is further increased by eliminating non-value-adding unit processes and eliminating or scaling down intermediate hold tanks. The primary cause of a decrease in operating costs is anticipated to be a higher utilization of resin capacity, which will result in a notable reduction in column size (particularly in high-price resin applications like Protein A chromatography) and a corresponding decrease in buffer quantities. Savings on labor costs include attained as the degree of automation is raised and cleaning and buffer preparation tasks are decreased. This is particularly noticeable in the procedures related to purification and polishing. Direct comparison of the DSP manufacturing platform models for stainless steel, single-use, and continuous usage indicates net present value reductions of 20% for the single-use platform and 34–55% for the continuous platform.

Technologies in Single use Downstream processing:

Mixing

In the field of bioprocessing, mixing bags, tank liners and tanks play a key role in the preparation, storage and transport of liquid solutions, buffers and media. These components are designed to facilitate efficient mixing of various substances essential to the biopharmaceutical manufacturing process. This step includes preparing buffers and media for bioprocessing. The lack of standardized designs is a challenge as it can cause compatibility issues and prevent seamless integration into larger bioprocessing systems. Specific challenges related to buffer and substrate preparation are not specifically mentioned, but may include issues such as maintaining sterility, precise composition, and stability. Some of the existing mixing technologies in the market now include [4]:

- Mobius® Mix (Millipore): Millipore's Mobius® Mix is a custom mixing system for bioprocess applications that offers efficiency and flexibility in fluid handling.
- Hyclone (Thermo Fisher Scientific): Hyclone is a brand under Thermo Fisher Scientific that provides cell culture medium and nutritional supplements essential for cell growth in bioprocessing.
- XDM QuadTM (Xcellerex): XDM QuadTM is a technology or system designed to address the specific needs of bioprocessing and potentially address mixing or scalability issues.

Fill-Finishing Technologies

Multiple fittings that are an integral part of any downstream process are manufactured for single-use. In the Time-Pressure System, precision is achieved by controlling product dosage through pressure. This method is particularly

suitable for filling containers with liquid products, which ensures the accuracy and repeatability of the filling-end process. Diaphragm pumps, characterized by a flexible diaphragm that moves product through the system, are known for their accuracy and suitability for aseptic filling. They provide a gentle and controlled way to fill containers without compromising product integrity. The piston pump system, which uses a piston-driven mechanism, ensures precise and controlled filling of tanks. This technique is often preferred for its precision, making it a valuable component in the final stage of bioprocessing.
Dispensing and Filling Systems. Some current equipments are:

- Crystal® (Aseptic Technologies): Aseptic Technologies Crystal® represents an advanced dosing and filling system designed for aseptic applications. It probably has properties that facilitate accurate and sterile filling of containers, ensuring the integrity and quality of the final product.
- Acerta® (Millipore): Millipore's Acerta® system is positioned as a dispensing and filling solution that emphasizes precision in dispensing and filling processes. It is believed to be designed to meet the stringent requirements of biopharmaceutical manufacturing.
- AlegroTM (Ball): AlegroTM is another dispensing and filling system that indicates innovation in the fill-finish process. This system can provide unique features to optimize the efficiency and reliability of the fulfillment process.

Connections and Aseptic Fittings

Sterile Tubing, Connectors, Adapters: These components ensure the sterility of fluid pathways preventing microbial contamination when fluids are transferred between different parts of the bioprocessing system. Aseptic Fittings (Welders, SIP Fittings, Sealers): Aseptic fittings, including pipe welders, SIP fittings and sealers, are critical to maintaining aseptic conditions during installation and disassembly. bioprocessing equipment. Given below are some popular single-use options.

- ACDs (Pall): Palland#039;s ACDs are probably aseptic connection devices designed to facilitate sterile connections in bioprocessing applications. Pall is known for liquid handling and bioremediation solutions.
- ReadyMate DAC (GE Healthcare): GE Healthcare's ReadyMate DAC is another system designed for aseptic interfaces that can provide advanced features to ensure sterile device-to-device connections.
- Opta SFT and Biowelder (Sartorius): The Opta SFT and Biowelder systems are likely to be associated with aseptic tubing and welding technologies that provide solutions for sterile connections during bioprocessing.
- NovaSeptic (Millipore): Millipore's NovaSeptic may represent a system designed to maintain aseptic conditions during fluid transfer, indicating an innovation in aseptic fusion techniques.

Monitoring Sampling Systems:

A list of some of the existing single use technologies are provided:

- TakeoneTM (Allpure): Allpure's TakeoneTM system is believed to be designed for efficient and aseptic sampling in bioprocess applications, ensuring representative sample collection for analysis.
- NovaSeptumTM (Millipore): Millipore's NovaSeptumTM can be a custom sampling system for the bioprocessing industry that can provide solutions for aseptic sampling.
- STA-PURETM (Gore): Gore's STA-PURETM is mentioned, demonstrating its role in maintaining the integrity of sample materials during the testing process.

- TruFluorTM pH and DO (Finesse): Finesse TruFluorTM pH and DO sensors are believed to provide real-time monitoring of pH and dissolved oxygen levels, critical parameters in bioprocessing.
- SciTemp®, SciCon®, SciPres® (SciLog): SciTemp®, SciCon® and SciPres® sensors from SciLogand#039; can provide a complete solution for temperature, conductivity and pressure monitoring.
- Bioprofile (Nova Biochemical Corporation): Nova Biochemical Corporation's Bioprofile system is likely to be used for multiparameter monitoring that provides insight into various aspects of a bioprocess.
- Digiflow systems: Digiflow systems are mentioned, possibly referring to their use in fluid flow monitoring and control in bioprocessing.

Disposable Systems in Therapeutic Plasma Filtration:

The importance of disposable systems, showing the change to these systems in therapeutic applications. Single-use systems are designed to be used for a specific treatment session and then discarded, eliminating the need for complex cleaning and sterilization processes associated with multi-use systems.

Cascade filtration technique with disposable filters:

The cascade filtration technique involves the use of disposable filters (eg, F1, F2) to sequentially separate plasma from blood cells and process the filtered plasma. The single-use nature of these filters ensures that fresh and uncontaminated components are used each time they are filtered, minimizing the risk of cross-contamination between different treatments.

Material composition of single-use columns:

Columns mentioned in the text containing synthetic beads coated with certain compounds are probably designed as single-use systems. The composition of these columns, which contain materials that selectively bind compounds based on physicochemical properties, remains consistent in each experiment due to the single use of the system.

Adsorption efficiency in single-use devices:

Devices such as Immusorba PH-350 (Asahi) are highlighted as examples of single-use systems with significant adsorption efficiency. Adsorption efficiency percentages presented in the text emphasize the reliability and consistency of performance achieved with single-use systems.
Anticoagulation strategies in single-use systems:

The use of heparin or a combination of citrate and heparin for anticoagulation during plasma filtration. In the context of single-use systems, these anticoagulation strategies are tailored to ensure adequate systemic and systemic performance for a single treatment session.

Advantages of single-use systems:
Single-use systems offer several advantages, including convenience, reduced risk of contamination, and avoidance of the complex problems associated with cleaning and sterilizing reusable parts. The single use of these systems meets the principles of safety, efficiency and ease of use of therapeutic plasma filtration.

Disposable Devices Applications and Manufacturers:
The text lists various manufacturers such as Fresenius HemoCare, Kaneka, Medicap Clinic GmbH, Miltenyi Biotec, Toray Medical and Biotex, showing the diverse market for therapeutic plasma disposable devices.The choice of

these manufacturers reflects the widespread adoption and availability of disposable systems for various medical applications.

Clarification

The cell culture medium contains a large number of secreted pharmaceutical compounds. Separating the cells and big pollutants requires a first stage in the clarifying process from the item. The two basic capture methods that are most frequently used are centrifugation and filtering. To get rid of smaller particles and bioburden, secondary and/or tertiary clarity is applied after first clarification. A fine-grade depth filter and a sterilizing-grade membrane filter can be the order of these processes. The depth and sterilizing-grade filters can be found in a variety of disposable formats, such as the Millistak+® Pod-system from Millipore, the Sartorius Sartoclear® L-Drum technology, the Pall Biotech StaxTM technology, or the 3M Zeta PlusTM depth filtration technology. The direct scale-up, modular design, pre-flushed and sterilizable filter sheets, and sight of the liquid flow in the see-through filter housing—which makes it easier to identify problems like foaming or trapped air—are benefits of single-use depth filters for clarifying. Additionally, more recent innovations offer completely sealed filtering capsules that make handling depth filter modules simple and secure. The filtration may be tailored for a variety of feed streams due to the large selection of filter sheets that are available, each with a distinct electric charge, clarity range, or adsorptive property [5].

As an alternative, single-use centrifuges like Sartorius' kSep® technology and Pneumatic Scale's CARR UniFuge® can be used. These semicontinuous disposable fluidized-bed centrifuges trap cells in single-use chambers by balancing liquid-flow and centrifugal forces. For reactor sizes up to 2000 l, additional possibilities include acoustic separation technologies that make use of disposable acoustic chambers for effective turbidity and particle reduction. To extract particles from suspension, new macroscale acoustic separators use multidimensional standing waves. As a result, the suspension of cells passes through an acoustic field where physical forces hold on to the particles and cause them to gather into densely packed clusters at the nodes or intersections of the acoustic waves. When more and more particles are drawn to the cluster, the clusters expand until they reach a certain size. Some of the existing technologies in the market include:

- UniFuge (Carr Centritech): Carr Centritech's UniFuge system is probably a centrifugal system adapted for separation processes. It may have advanced features for efficient clarification and cell collection.
- Millistak and Mobius FlexReady (Millipore): Millipore's Millistak and Mobius FlexReady systems incorporate a variety of separation technologies, including filters and centrifuge units. These systems are designed to provide flexibility and efficiency in bioprocessing applications.
- StaxTM and AlegroTM (Pall): Palland#039 StaxTM and AlegroTM systems are likely to be comprehensive platforms combining different separation techniques. They are able to provide solutions for the various stages of bioprocessing, from filtration to centrifugation.
- Sartoclear® and Sartoflow® (Sartorius): Sartoriusand#039; Sartoclear® and Sartoflow® systems specialize in clarification and filtration processes. These systems are designed to meet the specific needs of bioprocessing applications, ensuring high quality separation.

These systems have a scale limit of 1000 L. This indicates that these methods are tailored for limited uses, making them appropriate for smaller or pilot-scale bioremediation projects. The scarcity of appropriate capacity single-use support systems, such as connections, tubes, and pumps, is a major challenge. The smooth integration and scalability of bioprocessing operations may be hampered by this constraint.

Limited Flow Rate and Pressure: Systems are capable of having a flow rate and pressure. This restriction may have an impact on the effectiveness and speed of the separation procedures, which could result in longer processing durations.

Limited degree of automation: When we talk about a limited level of automation, we're referring to the possibility that some steps in the separation process will need to be done by hand. This restriction may have an impact on labor requirements, reproducibility, and overall efficiency.

Chromatography Solutions:

The essential enabling technology for chromatography is refinement of biological medicines. Three chromatography steps are often used for mAbs: The target product is separated, concentrated, and stabilized using Capture chromatography from the clarified cell supernatant. Two polishing procedures are then performed to further eliminate host cell proteins and reduce the quantity of high-molecular-weight material, including aggregated products. Stainless-steel chromatography skids are used in conjunction with glass, steel, and plastic multiple-use columns in conventional chromatography. The need for single-use in downstream manufacturing has been growing quickly due to the desire to boost productivity of current facilities and obtain flexibility for multiproduct manufacturing [7]. Because cleaning procedures and validations are eliminated, the use of disposable columns and single-use chromatography skids results in quicker turnaround times and higher facility productivity.

Pall Biotech's Allegro™ single-use chromatography systems and GE Healthcare's ÄKTATM ready systems provide totally disposable flow pathways and facilitate the use of membrane-based chromatography products, prepackaged or single-use columns, and conventional columns. This gives manufacturers of therapeutic materials flexibility for multiproduct operations. A single-use chromatographic system can provide flexibility to support clinical manufacturing at all scales while lowering long-term processing costs. Disposable columns that are prepacked are sourced outside, meaning that the labor-intensive and highly technical packing procedure is outsourced. Additionally, using prepacked columns removes the requirement for cleaning validations, buffers for packing or cleaning, and column packing systems. Repliken and GE Healthcare offer prepacked columns with a variety of resins and configurations for Good manufacture Practice (GMP) manufacture. These days, prepackaged columns can only have a maximum diameter of 80 cm and a total volume of 150 l. Additionally, the desired column dimension might not be offered in a disposable format. Furthermore, the use of single-use columns does not address a number of common chromatography limitations, such as resin volume and cost, facility costs, or processing time. Some of the Single use technologies that are in use include [8].

- Sartobind-Q® (Sartorius): Sartobind-Q® is a Sartorius membrane sorbent that can be specialized for anion exchange chromatography for the purification of biomolecules.
- Mustang-Q® (Pall): A monolithic or chromatographic column that focuses on strong anion exchange for efficient separation.
- CIM® Usable Columns (BIA Separation): BIA Separation and #039;s CIM® Usable columns are designed for efficient chromatographic separations using Convective Interaction Media (CIM).
- AKTA Ready-to-ProcessTM and AxiChromTM (GE Healthcare): These AKTA Ready-to-ProcessTM and AxiChromTM systems from GE Healthcare are believed to be designed for automated chromatography, ensuring bioprocessing efficiency and scalability.
- BioSMBTM (Tapon Biosystems): Tapon Biosystemsand#039; BioSMBTM is associated with simulated moving bed technology that enables continuous and efficient chromatographic separation.

Virus Removal

According to the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) Q5A recommendations, virus reduction in biopharmaceutical manufacture requires a minimum of two orthogonal stages. For an effective reduction of viral contamination, a common biotechnological downstream procedure combines low-pH virus inactivation with virus removal using both filtering and chromatography.
Specially designed mixer systems are available for controlled inactivation of low-pH viruses in disposable systems. The majority of biotechnological products derived from mammalian cell cultures employ filtration-based viral clearance.

Both coiled cylindrical plug-flow reactors and continuous in-process mixing and hold can be used for continuous low-pH virus inactivation.

Single-use mixers have shown to be an effective tool for continuous in-process mixing and hold phases. The idea is to use at least two mixers with alternating and periodic batch inactivation. For instance, one of the mixers continuously collects the product when utilizing the continuous flow of a continuous chromatography system up front. The liquid is inactivated in the mixer for the specified hold time after the mixer is loaded and acid is injected to start the inactivation cycle. A second mixer is gathering chromatography eluent and filling gradually during this operation. As the inactivation hold time is achieved, the product neutralizes and leaves the mixing system. The two mixing tanks now interchange roles: After being emptied, the product-collecting mixer enters inactivation mode and begins to collect the product. Pall Biotech now offers the only commercially accessible method for continuous low-pH inactivation[9] . The Cadence Virus Inactivation System is a completely automated, single-use device that ensures no contamination persists between cycles and enables precise control of inactivation time and pH.

Plug Flow reactors: Continuous flow spiral-tube reactors operate by bringing the product's pH to inactivation before it is introduced into the reactor. The proper residence period for virus inactivation is ensured by the tube's proper dimensional design . In order to help avoid axial flow and maintain consistent inactivation conditions as the liquid passes through the reactor, dean vortices can be used for mixing within the reactor. The technology's shortcomings include nonlinear scalability, restricted process control inside the reactor, uncertainty in handling process interruptions, and quarantining deviations in addition to the lack of a commercially accessible solution. These could erect legal barriers to this technology's adoption.

Filtration: Since dead-end filtration is the foundation of filtration-based virus reduction, clogging and filter overload must be addressed in order to use these technologies in continuous processing. Moreover, the obstacle of process disruptions and possible decrease in the filter's virus-removal effectiveness must be addressed.
Automated parallel switch-in and -out methods are one way to modify virus filtration. New modules can replace old filters before they surpass verified pressure or volumetric throughput limits. Furthermore, there are specifically designed virus filters on the market that can withstand process interruptions and be used in continuous flow situations, such as Pall Biotech's Pegasus Prime.

Chromatography: It is anticipated that promises of virus-removal based on continuous chromatographic operations will be an intrinsic feature of the actual resin. Compared to batch processing, several investigations have demonstrated that features of continuous chromatography, such as frequent column cycling, longer resin aging times, or continuous flow schemes, [10] have no effect on virus clearance. It is advised to modify the virus-clearance study design to account for process variations, such as the higher binding capacity utilized in continuous chromatography.

Disposable Bioreactor System (SUB):

The SUB system combines a flexible cell culture bag with a rigid (steel) housing. This design offers adaptability to different cell culture processes and is versatile for mechanical or pneumatic drive methods such as tilting, displacement, vibration, air transport or bubble systems.
SUBs are essential components of the cell culture workflow and perform various functions in seed expansion, monoclonal antibody (mAb) production, and vaccine production. The flexibility of the system allows for a wide range of applications in the growth and expansion of both mammalian and insect cells. Thermo Fisher Scientific's Hyclone SUB is a specialized single-use bioreactor designed to meet the demands of cell culture processes. The Xcellerex XDR-DSTB BIOSTAT CultiBag STR system offers a special solution for single-use cell culture. GE Healthcare's WAVE BioreactorTM is known for its unique agitation technology that facilitates efficient cell culture. AppliFlex (Applikon): Applikonand#039;s AppliFlex is believed to be tailored for flexible cell culture applications. Millipore's Mobius® CellReady is another single-use system that can offer versatility for cell culture applications. The scale-up limit is 2000 liters, which indicates that there may be problems when trying to scale up cell culture processes using single-use bioreactors to larger volumes.
Scalability from laboratory to commercial scale is evident in the same reactor design/configuration, suggesting that further development is required to ensure consistent performance at different scales.: Challenges have been identified in the availability of robust single-use systems, including tubes, connectors, sensors, filters and controls. The lack of comprehensive single-use support systems can lead to the complexity of integrating and optimizing the entire cell culture process. Single-use bioreactor material and bag may have limited strength, which may affect system durability and strength during long-term use.
Sufficient agitation in the bioreactor was highlighted as a limitation, suggesting challenges in achieving a uniform and homogeneous distribution of cells and nutrients in the cell culture process.

6.1 ***Hollow Fiber Bioreactors:***

Hollow fiber bioreactors have a unique design with hollow fibers that provide a large surface area for cell attachment and growth. This structure is particularly useful for efficient cell culture and antibody production. These bioreactors are specially adapted for small-scale production, emphasizing their suitability for laboratory and research environments. This method is suitable for controlled cultivation of cells and the subsequent production of antibodies. The hollow fiber structure allows higher cell density, better mass transfer and better control of the microenvironment, making them ideal for applications that require precision and repeatability. The main use of these hollow fiber bioreactors is the small-scale production of antibodies. This production is aimed at diagnostic and research purposes, where smaller antibodies are typically required for tests, assays and analytical studies [11]. The small size of these bioreactors offers researchers the flexibility to conduct experiments without large production facilities.

BioVest offers specific hollow fiber bioreactor systems, namely AcuSyst and AutovaxID. These systems are likely to be characterized by unique properties and configurations adapted to specific applications, such as antibody production for diagnostic and research purposes. FiberCell of New Brunswick and number 039 is another system designed for hollow fiber bioreactors. Like others, it may contain specific technologies or inventions optimized for small-scale production of antibodies.

One limitation highlighted is the narrow range of culture volumes, ranging from 2.5ml to 1000 ml. This indicates that these bioreactors are optimized for applications where smaller antibody volumes are sufficient.

6.2 Wave-mixed Bag Bioreactor

In the 1990s, rocker platforms were a feature of the first wave-mixed bioreactors that were introduced into labs. The rocker platform moved a partially filled pillow-shaped bag with a periodic, one-dimensional oscillation. The platform motion creates the wave inside the bag, and the wave characteristics are determined by the scale-dependent bag shape and geometry, the filling volume, the rocking angle, the rocking rate, and the fluid parameters, such as viscosity and density. Currently on the market, wave-mixed systems such as the Wave Bioreactor (GE Healthcare), the BIOSTAT® CultiBag RM (Sartorius Stedim Biotech), the AppliFlex bioreactor (Applikon), and the SmartRockerTM (Finesse Solutions, Inc.) employ the 1D oscillatory idea.
The main distinctions between these bioreactors are found in the culture bag designs, which include form, size, scale, and film material.

The Wave Bioreactor Cellbags and the BIOSTAT® CultiBag RM are accessible with integrated perfusion membranes for cell retention as an optional feature.
In the BIOSTAT® CultiBag RM, the membrane (1.2 μm and 1,070 or The Wave Bioreactor has a floating filter with a flat cell-retentive membrane (0.7 μm pore size and 100 or 180 cm2 surface area) attached to the bottom of the bag (1,275 cm2 surface area). Thus, 1D motion wave-mixed systems can achieve very high cell densities. The wave motion promotes bulk mixing, off-bottom suspension of cells and particles, bubble-free surface aeration and reduces foaming and flotation compared to stirred cell culture bioreactors [20].

6.3 Stirred rigid system

These liter scale systems (Mobius® CellReady bioreactor, UniVessel® SU bioreactor, BioBLU, CellVessel) do not require an external support container because of their sturdy, free-standing plastic containers. Additionally, folding tension, which is probably going to arise in the plastic films of bag systems and have the potential to rupture film layers, could result in leaks when the medium's internal pressure is applied. The Mobius® CellReady bioreactor (Merck Millipore) was the first stirring SU bioreactor with a stiff cultivation container. With a total capacity of 3 L, the cultivation container offers a maximum working volume of 2.4 L and a suggested minimum volume of 1.0 L. The bioreactor has a single marine impeller and may be aerated using open pipe and micro spargers, which are made of sintered polyethylene with nominal pore sizes of 15–30 μm. Electrochemical probes, which are used to measure DO and pH levels, must first be pre-sterilized before being inserted into the bioreactor tank using 12 mm screw apertures in the vessel lid [12]. This raises the possibility of contamination and necessitates calibration and polarization of the sensor before use. A non-invasive Pt-100 probe is used to measure temperature; it is put into a plastic sleeve, and heated blankets are used to regulate it.

In BioBLU bioreactors (Eppendorf/New Brunswick), which are agitated by 3-blade pitched blade impellers (also known as "elephant ear" impellers), single impellers are also utilized. These impellers rotate in a clockwise direction to create an upward-directed axial flow. The BioBLU 0.3c bioreactor, which is available in smaller sizes, can be utilized in conjunction with a DASbox (DASGIP) to create a stackable modular system that can accommodate up to 32 or more parallel growth vessels. The culture containers offer working capacities ranging from 100 to 250 mL, making them appropriate for process development and screening tests. This enables DoE methods and QbD compliance procedures.

The first rigid SU cell culture bioreactor available for purchase is the rigid UniVessel® SU bioreactor from Sartorius Stedim Biotech, which is stirred by two-stage impellers. Though they have a lower blade angle of 30~, resulting in lower power inputs, the three elements of the segment blade impeller (SBI) resemble the "elephant ear" impellers of the BioBLU in shape.

6.4 Orbitally shaken bioreactors

Shake flasks, microwell plates, and TubeSpin® bioreactors made of Techno Plastic materials are the most widely used orbitally shaken bioreactors. The latter offer miniaturization, which is helpful for processing several distinct cultivation tests and optimizing conditions. The original goal of developing TubeSpin® (also referred to as CultiFlask 50 disposable bioreactor from Sartorius Stedim Biotech) technology was to offer "bioreactor-equivalent" conditions for screening tests at the 50 mL scale. Ventilation caps are included on the unique centrifuge tubes with frugal conical bottoms to facilitate gas exchange with the incubator.

Although the maximum working capacity of shake flasks is about 20–30% of their nominal volume, higher volume shaken systems with cube- or cylindrical-shaped culture containers have been created. However, the scaling-up of shake flasks is restricted to roughly 1 L total volume. Although the nominal volumes of some prototypes exceeded 1,000 L, typical scales were only up to 30 L. Additionally, unique designs, such as helical grooves on the inner vessel wall, have been tested to enhance mass transfer and mixing in 14 Single-Use Bioreactors for Animal and Human Cells 475. The gas-liquid interaction is further enhanced by forcing the liquid onto these helical grooves. Five to ten times higher kLa values (up to 55 per hour) were obtained in comparison with non-modified vessels, and cell growth was on par with small-scale TubeSpin® and 30 L stirred bioreactors [13].

These technologies ultimately resulted in the invention of the SU 200 L orbitally shaken bioreactor system (trade name OrbShakeTM bioreactor) in 2009, despite the fact that none of them reached the market. Stirred SU bioreactors are more expensive than the bags since they do not require internal mixing or sparging equipment. Moreover, common problems are prevented, such as foam generation. The bioreactor control system is used to operate the cylindrical culture container, which has a nominal volume of 330 L and is fitted with pH and pO2 sensors. For a 100 L working volume, much higher values—up to 25 per hour—have been reported. Mixing periods under these circumstances range from 25 to 70 s, depending on the shaking frequency (50–70 rpm).

Application in the production of monoclonal antibodies:

SU bioreactors may be scaled up to 2,000 L working volumes, which is why many contract manufacturing organizations (CMOs) utilize them. Comparing this invention to standard stainless steel vessels in terms of living cell density, viability profiles, expression profiles, and product quality, many comparison studies yielded equal results.

These days, genetically stable continuous cell lines are chosen for producing monoclonal antibodies (mAbs). These cell lines include human embryonic kidney cells (HEK293), lymphoma (NS0, SP2/0), CHO cells, hybridomas, and human embryonic retinoblast derived Per.C6 cells. Temperature changes (to between 28 and 31 degrees Celsius) are frequently used to increase antibody production, which is typically done in fed batch mode (i.e., cells are supplemented with a concentrated nutritional solution) by utilizing serum-/protein-free or chemically specified growth medium. Following 8–21 days of cultivation, the product is collected in batches, resulting in antibody titers that are typically between 2 and 5 g /L [14].

The space-time yield of antibody production procedures will be further increased by SU Refine Technology's ATF module and other newly developed external SU cross flow microfiltration systems have been integrated with stirred bioreactors that have working capacity of up to 1 m3. While the product-containing exhausted media is collected and the cells are kept inside the bioreactor, fresh medium is continuously supplied. However, the vast amounts of the diluted product could make processing farther down the line more difficult.

DSM in the Netherlands created XD technology to ensure high product titers and high cell densities in stirred SU bioreactors. A cross flow filtering system with a pore size or molecular weight cut-off that is two to three times smaller than the intended product is used in the case of XD technology. As a result, the bioreactor holds on to both the product and the cells, producing antibody concentrations of 10–27 g/L at cell densities of 108 cells/mL.

Running costs

SUT avoids the need to disassemble, transport, clean, validate and reassemble components in classified clean-room environments. In addition, the requirement for contained areas is reduced through the use of aseptic connections. The result is a shift in facility design towards fewer clean rooms and reduced environmental monitoring. In theory with SUT systems, applications no longer need to be physically segregated. Instead they can be performed side-by-side in closed loop systems making more efficient use of facility space (Pall Corporation, 2011).

Equipment

Running costs savings in terms of different equipment/operation used is summarized in Table 5.
The 3% cost reduction observed when single-use membrane chromatography is due to reduction in buffer consumption, processing time and labor. COGs evaluation comparison between single-use and traditional filling systems results in essentially 8% less running costs for the single-use filling.

Table 1 - COGs % change by equipment/operation

Operations	**% Change**
Use of hold bags for buffers	-8.3
Single-use bioreactors	-5.2
Mixer with open liners for buffer preparation	-4.8
Use of hold bags for product	-3.7
Single-use membrane absorber for chromatography	-3.2
Overall	-22.8

Labour

As a result of minimizing cleaning and revalidation time in equipment change-over, SUT configuration allows for a 3 times reduction in workforce headcount and on average a 5% labor time reduction. Maintenance and downtime are also reduced when using SUT (Trotter, 2012). Productivity increases as SUT allows immediate equipment turnaround with no cleaning, sterilization and re-validation time required, and no downtime whilst vessels are being cleaned [16].

Materials and consumables

Costs associated with raw materials will remain the same (assuming similar scale of operation) or decreased (if scale is decreased). The cost of single-use items/consumables (e.g.membranes, vessels, chromatographic media, pipework, etc.) will become a major factor.

Waste treatment

Decreased costs of effluent liquid waste treatment and cleaning agents associated with cleaning operations, but increased costs of solid-waste (plastics) treatment of single-use.

Utility consumption

With single-use technology there is no cleaning so there is a reduced requirement for utilities such as WFI, pure water and clean steam, and reduced requirement for CIP chemicals

Risk assessment – extractables and leachables

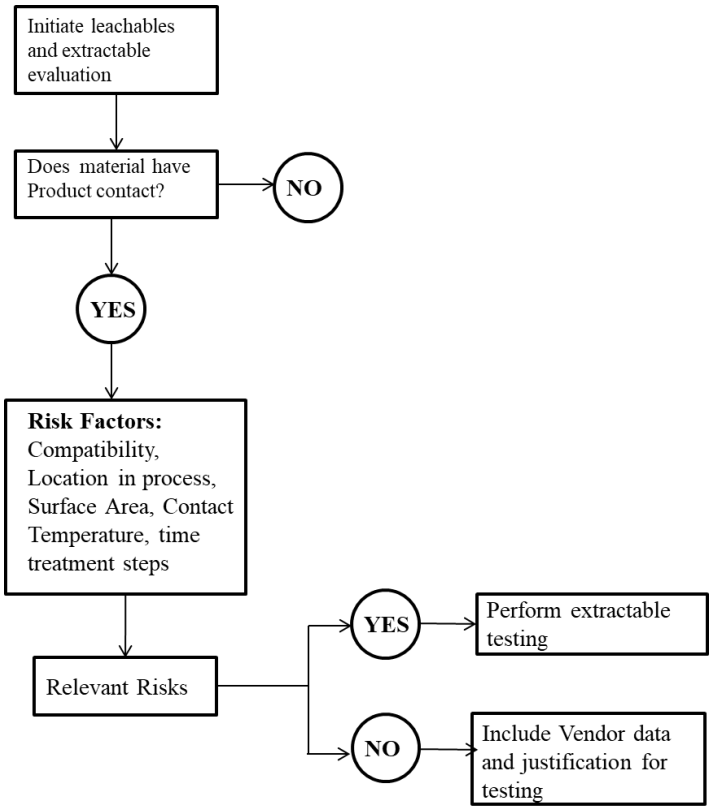

Figure 2: Decision tree for risk analysis of leachables and extractables

The FDA recommends the application of risk assessments to pharmaceutical regulatory requirements. This document describes regulators requirements as well as the risk assessment recommended approach to extractables and leachables in single-use technology. This guide includes charts, graphs and decision trees (Fig. 2) to help users and suppliers undertake extractables and leachables evaluations. As a summary, when undertaking a risk-assessment multiple factors should be taken into consideration including but not limited to the following chromatography (HPLC) and gas chromatography with a mass spectrometer detector (GC–MS), additional quantitative investigations and identification and quantification of extractables.

Common challenges of bioprocessing equipment:

Lack of standard designs: Lack of standard designs for various components can lead to complex equipment integration, which can affect the overall efficiency and reliability of the bioprocessing system [15]
Powder Addition and Mixing: Handling and proper mixing of powdered materials in the bioprocessing workflow is a challenge, as precise control is required to ensure uniformity and avoid problems such as agglomeration.
Slow Fluid Transfer (Connections): Slow fluid transfer, especially in connections between different components, is a challenge. This can affect the overall speed and efficiency of the bioremediation workflow [17].
Volume limitations to 1000 liters: Challenges were observed in expanding biotreatment to larger volumes (limited to 1000 liters). This limitation may be due to factors such as mixing time, the strength and durability of the flexible bag, and other scalability considerations.

Conclusion:

The adoption of Single-Use Technologies (SUT) in biopharmaceutical manufacturing represents a paradigm shift with multifaceted advantages and some notable challenges. The considerable reduction in labor requirements, attributed to minimized cleaning and revalidation time during equipment change-over, underscores the efficiency gains associated with SUT [18]. The immediate turnaround of equipment, without the need for extensive cleaning, sterilization, and re-validation, contributes to increased productivity. However, challenges like the lack of standard designs for various components and constraints in volume scalability up to 1000 liters pose considerations for the broader adoption of SUT.

Moreover, the reduction in maintenance and downtime, along with decreased utility consumption due to the elimination of cleaning processes, further enhances the appeal of SUT from both economic and environmental perspectives. The incorporation of risk assessment protocols recommended by regulatory bodies ensures the safety and quality of biopharmaceutical products by addressing extractables and leachables in single-use technology [19].

While challenges such as powder addition and mixing complexities persist, ongoing innovations and advancements in the field aim to address these limitations. The future trajectory of SUT in biopharmaceutical manufacturing appears promising, driven by a continuous commitment to overcoming challenges, ensuring regulatory compliance, and optimizing the efficiency, cost-effectiveness, and sustainability of bioprocessing operations.

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