Chapter 29

Single Use Technology and Equipment

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29.1 INTRODUCTION

29.1.1 History of Bioprocessing—1970–80s

In the mid-1970s, the advent of recombinant DNA technology spawned a radical new era in the treatment of disease and the role of biologics and their manufacture. Newly formed biotech companies, armed with powerful genetic engineering techniques, began to clone human forms of the legacy biologics at that time (insulin, Factor VIII, growth hormones) as well as potential 1st generation blockbusters heretofore unavailable, such as therapeutic enzymes, erythropoietin and the cytokines. Within a few years in the early 1980s, large scale manufacturing became an urgent new need. Out of necessity, biologics manufacturers turned to legacy manufacturing systems from the plasma purification, dairy, food and beer/winemaking and antibiotics industries, traditionally dominated by stainless steel manufacturing technology.

As the 1980s progressed, low cell line expression levels ($\ll$1000 mg/L) and ballooning blockbuster markets quickly drove manufacturing scales to 10,000 and to 50,000 L (for insulin, respectively), taking the form of large, single product "six-pack" facilities (6 × 10,000 L) with complex stainless steel biomanufacturing systems [1,2]. These required large clean utility facilities and miles of welded and boroscoped stainless steel piping (see Fig. 29.1). Facilities of this size and complexity demanded industrial grade mechanical, architectural and process engineering design and construction. Steam in place (SIP) and clean in place (CIP) systems are required to clean and sterilize their complex closed stainless steel structure. Many facilities require hundreds of validated SIP and CIP circuits with sophisticated automation systems to monitor and control all unit operations and support systems. Facility complexity drove up the capital cost and timescale to install validated GMP-ready manufacturing capability to the extent that the benefits of new single use technologies, despite their risks and disadvantages, began to draw attention [1–13].

29.1.2 Industry Drivers and Developing Trends—1990–2010

In the 1990s, further market growth and the advent of high-dose blockbuster monoclonal antibodies drove manufacturing scales to 20,000–25,000 L, further increasing the cost, complexity and timescale to install validated GMP-ready manufacturing capability [2]. In parallel, the desire to share the high cost risk of facilities spawned a growth spurt in the CMO industry. In these CMO facilities, the challenge was to change the single product facility to a multiproduct facility (for multiple clients). This forced the development of validatable SIP and CIP operations to reduce the risk of cross contamination from one product to another. Regulatory agencies required extensive validation and quality systems of these operations to reduce potential for cross contamination between different drug manufacturing campaigns.

Despite these challenges and predictions of capacity shortages, these large facilities were hugely successful in meeting the rapidly growing demand in western markets for 1st generation blockbuster biologics [2]. Many of these large stainless steel facilities and new facilities will likely continue to be needed to produce new blockbusters as well as biosimilars for the large United States and EU markets (see Chapters 2 and 3).

As the 1990s progressed and the industry became dominated by the 1st generation blockbuster drugs, large changes loomed for the next several decades. Patent expiration of the 1st generation drugs would be followed by "generic biologics" (now "biosimilars") and global competition, which would demand higher efficiency, lower costs and faster agility. In addition, 2nd generation drugs would be more potent with lower doses, cell line genomics would boost titers and yields and globalization would lead to smaller markets—all reducing the production demands and the average scale requirement on manufacturing facilities [2–4].

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FIG. 29.1 Example of a large scale stainless steel biomanufacturing facility at Rentschler Biotechnologie GmbH, Laupheim, Germany.

What was not anticipated by 2010 was the requirement by some nations for "in country, for country" manufacturing, forcing drug companies to build in those countries to supply that country [14–16]. This served to further shrink the scale of biomanufacturing facilities since many would serve smaller local markets. Another potential change that was difficult to predict was the rate of increase in productivity of biomanufacturing: large increases in mammalian cell expression levels and product expression levels as well as improvements in downstream purification yields that occurred in the 2005–15 timeframe [2,17,18]. These particular trends were further enabled by the domination of monoclonal antibodies whose manufacturing processes could be platformed and optimized for many different antibody drugs. Collectively these trends in productivity and towards smaller markets would combine to reduce manufacturing scale on average.

29.1.3 Perfect Storm: Industry Pressures, Changing Markets, and New Technologies

As the early 2000s developed, it became clear that the 2010–25 timeframe would witness a major transformation of the biotech industry that had been anticipated [17]. Biosimilar competition could trigger lower prices which despite of high or very high margins reported by the industry (see Chapter 55) would force a re-examination of the entire basis of drug production costs and supply chain. R/D success to market for new drugs would be more risky, challenging and expensive as more complex diseases were attacked, resulting in large pharma consolidations and mergers and acquisitions to share the risk. Cell line productivity and purification yield improvements coupled with more potent, lower dose and eventually personalized medicines would require facilities that were designed differently and potentially much smaller in scale [18]. Meanwhile however, the biomanufacturing industry had been focusing on scaling up blockbusters to meet market demand rather than investing in innovations in cost, quality and speed that could pose regulatory risk.

Finally, as industry pressures continued to mount, large and slow to build single product stainless steel manufacturing technology began to be re-examined from a financial risk and return basis due to the increased chance (from failure in the clinic and other trends) of becoming obsolete before completion or of being too slow to respond to industry changes [2,5,7]. Top driven economics and looming drug price pressure began to force biomanufacturers to examine all aspects of risk and cost and to look for new technologies that could transform cost and speed, without endangering product quality [3,4,6,13]. Manufacturing agility or flexibility for multi-product operations to maximize facility utilization and efficiency also came under scrutiny as an additional way to reduce costs [1,3,6,8,9]. The time was ripe for a new agile manufacturing technology that could meet the needs of a rapidly changing industry without triggering regulatory risk.

29.1.4 Cost, Quality, Speed, Flexibility—Agile and Flexible Single-Use Manufacturing

Early single use technologies that became available in the 1990s such as cartridge filters, t-flasks, cell factories, sample bags, media and buffer storage bags, etc. continued to increase in use mainly in support operations of stainless steel facilities. At first glance, single use systems afforded the ease and simplicity of speed of installation and turnaround combined with disposability, eliminating the need for costly and slow CIP and SIP sterilization systems [1–3,17]. In addition,

manufacturers could claim that single use systems avoided the chance of cross contamination between batches of the same product or between batches of different products, thereby increasing manufacturing quality assurance [3,4,8,9,13]. This latter advantage was of particular value as more manufacturers considered multi-product operations to increase facility utilization and to lower costs. To any trained biologics manufacturer, single use technologies had the potential to address cost and speed and could enhance manufacturing quality assurance as well.

29.1.5 Single Use Technologies Evolving From Support Systems to Production Systems

The transition of single use technology from "support systems" to "production systems" was pioneered by Wave Biotech [19] which launched the first bag bioreactor in the 1990s. This spawned a tipping point recognition of the simplicity and benefits of single use technologies. By the year 2010, the transformative potential of single use technologies became apparent as a possible new competitive "production" technology toolbox, and the industry drivers and pressures were converging to speed its assessment. In addition, while single use technology addressed some costs (reduced SIP and CIP capital and operating cost), increased manufacturing quality control (eliminated cross contamination thereby reducing risk of drug adulteration) and increased speed to install GMP manufacturing, it also improved flexibility and agility for adaptive manufacturing that could switch from one drug to the next [3–5,7,9]. This feature enabled manufacture of more diverse pipelines for in country, for country markets [14]. In addition, the parallel development of higher titers and downstream yields further reduced scales to the range where single use technologies are more practical. A single use manufacturing facility is shown in Fig. 29.2.

The concern that single use technology would not meet the capacity demands of the industry has been mitigated by the increase in cell culture titers over the last decade [2]. Table 29.1 shows the annual monoclonal antibody production capacity of a single use facility (in kilograms of purified bulk drug substance (BDS)/year) as a function of cell productivity (product titer in the bioreactor) and the scale and number of production bioreactors, assuming a 70% purification yield and 20 batches per bioreactor per year [20]. As shown in the table, up to 1000 kg/year can be produced at a cell culture titer of 6 g/L in a facility fitted with six 2000 L (working volume) single use bioreactor production lines. This broad range of capacity can satisfy the demands of many biologics (see Chapter 4).

29.1.6 Increasing Need for Flexibility, Agility and Economy—Increased Drug Diversity and Emerging Markets

Today, the biotech industry continues to grow rapidly, driven by new emerging markets, expanding indications and new drugs of greater diversity: hormones, fusion proteins, mAb fragments, multivalent mAbs, mAb drug conjugates, biosimilars, biobetters, cell and gene therapies, cell based and rDNA vaccines, therapeutic enzymes, etc. Fig. 29.3 shows the growth of the biologics markets, including traditional biologics, rDNA recombinant proteins (including mAbs), derivatives, virus vaccines, microbial products and tissue and stem cell therapies discussed extensively in Chapter 3).

FIG. 29.2 Example of a single use manufacturing facility at Rentschler Biotechnologie GmbH, Laupheim, Germany.

TABLE 29.1 Annual Production Capacity (Kilogram Purified Bulk Drug Substance Per Year) of a Single Use Manufacturing Facility as a Function of Cell Culture Titer and Number of Bioreactor Production Lines Assuming 20 Batches Per Year Per Bioreactor and 70% Purification Yield

		Cell Culture Titer						
		1 g/L	1.5 g/L	2 g/L	3 g/L	4 g/L	5 g/L	6 g/L
SU bioreactor trains	2×500 L	14	21	28	42	56	70	84
	2×1000 L	28	42	56	84	112	140	168
	2×2000 L	56	84	112	168	224	280	336
	4×2000 L	108	168	228	336	448	540	648
	6×2000 L	168	252	336	504	672	840	1008

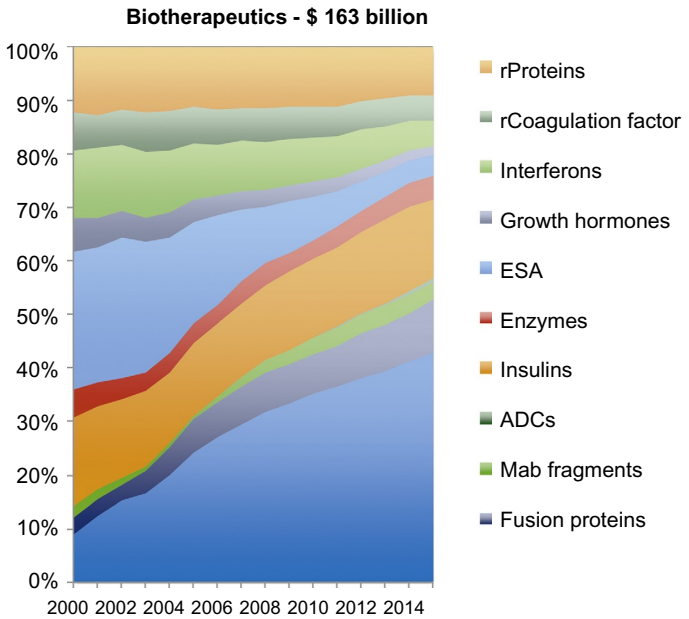

FIG. 29.3 Growth and diversity of the biologics industry by 2014 (see Chapter 3 for reference).

The scale range spans just several liters for cell and gene therapies to 20,000 L for large market biosimilars [2]. Increasing drug diversity also requires process diversity for their manufacture. Single use technology may afford greater flexibility to accommodate drug and process diversity compared to traditional manufacturing technology.

New emerging markets are shown in Fig. 29.4 and include China, India, Russia and Brazil. These territories and economies are less developed and may not be able to afford expensive biologics medicines. Hence these new markets present greater pressure on drug prices and drug manufacturing costs compared to western markets [14,15]. In addition, these territories may not have highly trained operators required to operate complex stainless steel biologics manufacturing facilities further forcing the need for less complex and easier to operate manufacturing technology.

29.1.7 Maturation From Development to GMP Clinical and Commercial Manufacturing

In the last decade, single-use technologies have rapidly increased in use because of industry pressures to reduce capital and operating cost and batch turnover time, and to increase speed of implementation and flexibility for multiproduct manufacturing. At the writing of this chapter, all types of biotechnology, biosimilar, CMO, cell therapy and vaccines companies have deployed some form of single use systems for either process development, pilot or GMP (good manufacturing practices) clinical manufacturing [21]. Regulatory acceptance of single use technology is evident from three companies that achieved commercial licensure for their single use facilities: Shire HGT in Lexington, Massachusetts, Serum Institute of India in Pune, India and SK Chemical in South Korea [22–24].

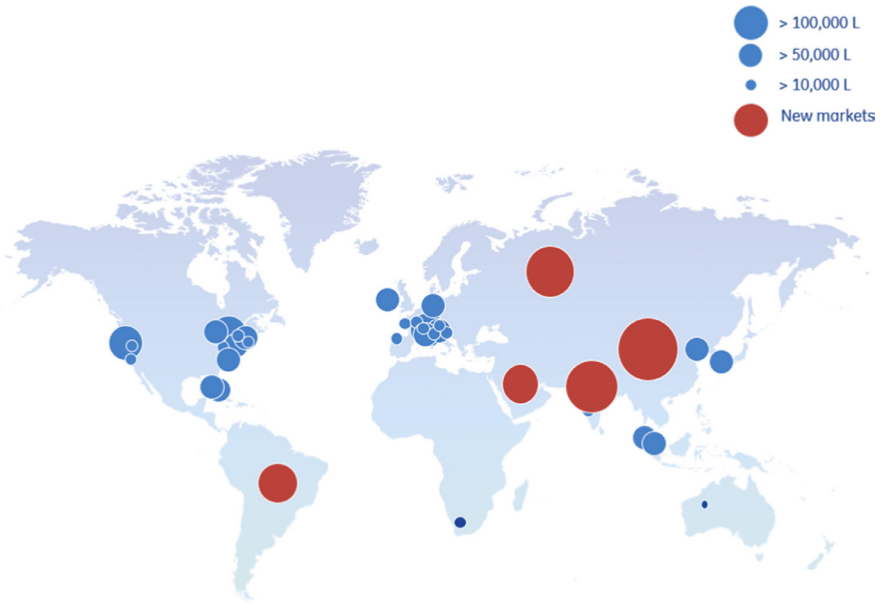

FIG. 29.4 Existing biologics markets are shown in blue, red indicates new merging markets. The relative size of the circles indicates relative market size and the bioreactor volume required to meet demand.

29.2 OVERVIEW OF SINGLE USE TECHNOLOGIES

29.2.1 Long History of Use of Plastics in the Medical Field and Stainless Steel Biomanufacturing Facilities

Plastic polymeric systems have been widely used in the medical field for decades and span a broad range of functionality and complexity as medical and surgical techniques have evolved. Infusion bags and blood bags are also made of single use films and components fused into simple and complete functional devices with fittings and tubing, irradiated for aseptic use. National blood supplies have been stored in these plastic blood bags for decades. At the other end of the spectrum open heart surgery using heart-lung bypass machines comprise significantly complex single use manifolds and connector systems. This long history of use, safety record, and familiarity of plastics in the medical field provided a rationale and precedent for their use in the field of biomanufacturing of medicines.

As the fermentation industry began to scale up the production of antibiotics in deep tank fermenters in the 1950s, and large automated dairy systems were developed for mechanized milk and cheese processing, a new generation of polymeric gaskets, o-rings, and flexible valve diaphragms were developed for these systems to provide sterilizable pipe/tubing joints and tank flanges. Just one standard stainless steel fermenter system contains hundreds of these o-rings, gaskets, and valve diaphragms. These very stable polymers were designed to withstand repeated cycles of high heat conditions of steam sterilization (121°C) and CIP operations. Any leachables produced by these polymeric components during these high heat cycles remain inside the closed system, coating the inner product contact surfaces and mixing with the drug or dairy product during operations. This practice continues today in large scale antibiotic and dairy processing operations.

Similarly, as the rDNA era took hold in the late 1970s, the biotech industry quickly adapted traditional stainless steel dairy and antibiotic fermenter designs to work for large scale mammalian and microbial processes. Today there are hundreds of thousands of liters of stainless steel mammalian and microbial bioreactor capacity in the industry and all of them rely on a system of polymeric seals. Steam sterilizable plastic filter cartridges were introduced into these facilities in the mid-1980s and are also subjected to in situ steam sterilization inside product contact stainless steel filter housings. Hence, the blockbuster generation of biologics has been produced with these polymer-enabled stainless steel systems.

29.2.2 Potential Toxicity and Effects of Leachables From Polymeric Materials on Cells and Product

Compared to stainless steel facilities, single use technologies present an increased potential for exposure of cell lines and product to leachables. Leachables from single use Wave bioreactor systems have been reported to have negative effects on

cell growth for some cell lines [10]. In this case, byproducts of an anti-oxidant formed upon gamma irradiation of the SU system had a negative effect on cell growth. Users of single use systems for cell culture should consider evaluating SU systems by purchasing small scale systems from each vendor and performing cell growth tests. In these experiments, attention should be given to the impact of length of exposure time, surface area to volume ratio, and exposure temperature. The next sections of this chapter delve into more detail on this subject.

For potential effects on the product by leachables, stability studies can be performed by incubating the product in the SU system in accelerated studies at elevated temperatures and compared against a control storage container such as glass or other inert material. Any changes in product aggregation, degradation or other quality attribute compared to the control could indicate adverse effects of the SU system on the product [11,12].

29.2.3 Best Practices for Qualification and Use of Single-Use Technologies

Best practices and harmonization of standards for single use technologies and their validation are a subject of great debate and discussions at the writing of this chapter [25]. A number of organizations including BioPhorum Operations Group (BPOG), Bioprocess Systems Alliance (BPSA), PDA, and others have been discussing best practices for the use and validation of SU technologies (Fig. 29.5, Refs. [26–30]). The goal of these working groups is to standardize and harmonize best practices for the qualification and use of single use systems, from vendor qualification and SU component extractables and leachables (E/L) testing all the way through process validation and supply chain security.

29.2.4 Regulatory Agency Guidelines for Validation of Extractables and Leachables From Single-Use Technologies

Regulatory agencies have been openly supportive [31] of single use technologies, potentially due to a long history of safety from wide use in the medical field (infusion bags are polyvinyl chloride films) and in blood bags used for the storage of the national blood supply. Additional quality and regulatory advantages of single use technology are elimination of cross contamination in bioprocessing and potentially lower costs which could improve patient access [31].

For upstream processing through cell harvest, the FDA has recommended that the SU components meet USP Class VI (USP 87 and 88) limits for extractables and leachables [22]. For downstream systems and container and closure systems, polymeric components may be validated following standard container closure guidelines to ensure product compatibility and stability [22].

Table 29.2 shows the relevant USP, ICH, FDA, and EU guidelines and other compendia for validation of polymeric and container/closure systems. Chapter 53 provides an extensive discussion of single use regulatory issues and security of supply.

In 2014 the PDA issued its Technical Report 66 “Application of Single Use Systems in Pharmaceutical Manufacturing” which was authored by many pharmaceutical and biopharmaceutical manufacturing experts as well as from the FDA [26]. This report provides a balanced guidance on all aspects of SU technology, systems, quality assurance, vendor supply chain management and business drivers. The reader is encouraged to access this report for specific references and to access these organizations to learn about the latest developments and consensus in this fast-changing arena.

	ASME BPE	ASTM	BPOG	BPSA	DECHEMA	ELSIE	PDA	PQRI	SUTAP	USP
Extractables	X	X	X	X	X	X	X	X	X	X
Leachables	X	X	X		X	X	X	X	X	X
Particulate	X	X		X	X		X		X	X
System integrity	X	X		X	X		X		X	X
Connectors	X			X	X					
Supply chain	X	X	X	X	X		X			
Design verification	X	X	X	X	X		X			
Biocompatability		X				X				X

For Further Information on this Table, Please Visit
www.bioprocessinstitute.com/single-use-news

FIG. 29.5 Working groups, associations and forums regarding validation and best practices for single use technologies. ASME BPE (American Society of Mechanical Engineers Bioprocessing Equipment), ASTM (American Society for Testing and Materials), BPOG (BioPhorum Operations Group), BPSA (BioProcess Systems Alliance), DECHEMA (Society for Chemical Engineering and Biotechnology), ELSIE (Extractables and Leachables Safety and Information Exchange), PDA (Parenteral Drug Association, PQRI (Product Quality research Institute), SUTAP (Single Use technology Assessment Program), USP (U.S. Pharmacopeia Convention).

TABLE 29.2 Overview of Compendia and Guidelines Applicable to Single Use Systems

International Standards	
• ICH Q1A: Stability testing of new drug products and substances: container closure system • ICH Q3A, Q3B: Impurities in new drug substances and products: threshold (%) dependency of the max dairy dose • ICH Q3C = E.P.5.4 = USP < 467 > (draft): Guidelines for residual solvents • ICH Q7A: Good manufacturing practice guidelines for active pharmaceutical ingredients • ISO 10993 part 13: Identification and quantitation of degradation products from polymeric medical devices • ISO 15747 (2003): Plastic containers for intravenous injection	
United States/North America	**Europe**
• 21 CFR Part 211.65: Equipment construction • Canadian Food and Drug Regulations, GMP, Part C, Division 2, Section C.02.005: Equipment • FDA CDER/CBER Guidance for industry: Container closure systems for packaging human drugs and biologics	• EU GMP, Medicinal products for human and veterinary use, Europena Commission, Volume 4, Chapter 3, Section 3.39 • EMEA/205/04 Guideline on plastic immediate packaging materials

29.2.5 Leachables in Manufacturing—Risk Assessment of Potential Product Exposure

The potential risk of exposure of a drug to extractables and leachables (E+L) from SU components can be estimated by employing the FDA's "risk based approach" assessment of risk [22]. Product exposure may be influenced by variables such as location of the step in the process (early upstream or late downstream), surface area/volume ratio, temperature, duration (time), chemical aggressiveness, and gamma irradiation of the SU component [25]. Table 29.3 shows an example of a SU risk assessment, with relative values assigned to each level of potential exposure risk. Values in each row are summed to the far-right column. Based on this assessment the highest risk steps (circled in red) would be targeted for further assessments such as stability studies, total organic carbon leaching, etc. Note that bioreactor steps or process hold points or storage steps pose the most exposure risk due to the long duration of exposure. In cases where product is not stored in bags, the exposure risk profile may shift to other steps in the process.

29.2.6 Leachables in the Upstream Process—Risk Assessment and Mitigation

Laboratory scale experiments should be conducted with small scale bioreactors fitted with SU bags to determine if there are any adverse effects on the growth and viability of the cell line [10–12,25]. Careful attention should be given to duration and temperature of the bioreactor run to simulate potential E+L exposures in future scale up runs. Similarly, storage of growth media in bags should receive similar attention, especially if the media storage bag is sourced from a different supplier than the bioreactor bag. Any inhibition of cell growth or reduction in viability (compared to control glass bioreactors or flasks) may be due to either E+L from the SU component or adsorption of critical nutrients to the SU film, or both. Toxicity of E+L can be proven by spiking healthy control flask cultures with growth media that was exposed to the SU component. Depletion of critical nutrients via adsorption can be proven by spiking additional nutrients to restore growth or precoating the bag with media components. Depletion of photosensitive nutrients that are critical for some cell lines (such as vitamin K) may also occur due to the transparent nature of the films. In this case, protecting the bags from light may be necessary. Pre-gassing of the media prior to inoculation may also remove volatile E+L components.

29.2.7 Leachables in the Downstream Process—Risk Assessment and Mitigation

Potential effects of E+L on the product in downstream steps may be observed in the form of changes to product quality [10–12,25]. It is critical therefore to establish stability indicating assays for the product to measure any impact on product quality. SU films and components are generally hydrophobic and may adsorb hydrophobic biomolecules. These may subsequently desorb and form aggregates upon renaturing in the solution. Other changes in product quality such as adduct formation or degradation may occur via metalloproteases that could be activated by the leachables [25].

Since single use components can potentially contain many different leachates, working with a toxicologist can help determine which leachates to focus upon. Some leachable assays may be masked by the product, therefore analyses for leachables should be performed with and without product present. It is important to consider that changes in product quality could occur at leachable levels that are below toxicity thresholds [10–12,25].

Mitigation methods include limiting duration of exposure, inclusion of non-ionic surfactants (such as tween 80) or other excipients to stabilize the biomolecule. Substitution of bags with glass or stainless containers for long term storage can avoid E+L exposure.

TABLE 29.3 Example Risk Assessment of Potential Product Exposure to Extractables and Leachables in a Typical Monoclonal Antibody Manufacturing Process

Risk assessment for extractables and leachables								
Step	Early or late in process	Bag film surface area/bag volume ratio	Time	Temp	Chemical aggressiveness	Gamma irradiated component	Total score	
Seed train	1	3	10	1	1	10	26	
Expansion bioreactor	2	2	3	1	1	10	19	
Production reactor	3	1	10	1	1	10	26	
Cell harvest	4	2	10	1	1	10	28	
clarified cell harvest process hold point	4	2	20	1	1	10	38	
Protein a column	5	5	1	1	1	1	14	
low pH hold (1 h) virus inactivation	6	5	1	1	5	1	19	
Post virus inactivation process hold point	6	5	20	1	1	10	43	
HIC column	7	5	3	1	5	1	22	
UF/DF	8	5	3	1	5	1	23	
IEX column	9	5	3	1	5	1	24	
UF/DF	11	5	3	1	5	1	26	
Bulk filltration/fill	12	5	3	1	5	10	36	
Bulk storage	13	10	10	1	1	10	45	

The circled rows indicate potential risk steps.

29.2.8 Mitigation of Overall Risk—Produce the Toxicological Batches in Small Scale Single Use Systems

Producing the toxicological material in smaller scale single use systems will expose the product to the maximum potential leachables due to the high surface area to volume ratio of smaller scale systems. Any toxicity of these leachables may potentially be revealed in animal toxicological studies. Care should be taken to produce the toxicological batches under conditions that will simulate larger scale clinical and commercial manufacturing runs that produce human destined material. For instance, attention should be given to simulate product exposure durations typical of processing times in larger tanks and bioreactors and hold points.

29.2.9 Commercial Licensure Viability of Single Use Technologies

The commercial viability of single use technologies is evident from the commercial licensure of single use manufacturing facilities. Regulatory agencies have proceeded to license three companies for commercial SU manufacturing: Shire HGT

for a therapeutic enzyme in Lexington MA, United States [23], Serum Institute of India for EPO biosimilar in Pune, India [24], and SK Chemical for seasonal Flu vaccine in Andong, South Korea [32].

29.2.10 Broad Impact on Operations, Flexibility, Agility, Process Economics, Product Quality and the Environment

In summary, despite the challenges with single use technologies, normal and to be expected during the initial phase of new technology introduction, they have become new tools for biologics manufacturing that address a broad number of limitations of legacy stainless steel facilities. Table 29.4 lists a summary of the limitations of traditional technologies that can be mitigated to some extent with SU technologies. SUT will not become a panacea for the industry (see Section 29.5 for gaps and limitations) but it constitutes an important addition to the biomanufacturing toolbox. Blockbuster drugs and very large scale producers of biosimilars will continue to require large stainless steel facilities to manufacturing multiple tons of drug for the treatment of these and other major diseases: cancer, inflammation, dementia, Parkinson's, and Alzheimer's, etc.[2].

TABLE 29.4 Summary of Comparison of Stainless Steel and Single Use Facilities and References

Issue	Stainless Steel Facility (SS)	Single Use Facility (SU)	Range of Impact Vs SS (%)	Reference and % Impact	Reference, Un Quantified Impact
Manufacturing area facility footprint	Larger	Smaller	5%–23% reduction	1(−23), 5(−5)	4, 9
CIP, SIP infrastructure, automation complexity	More	Less	50% reduction	1(−50)	3, 4, 5
Equipment capital cost	Higher	Lower	25%–75% reduction	1 (−25) 5(−30), 7(−40), 8(−50), 9(75%)	4
Consumable cost	Less	More	10%–50% increase	4(+10), 7(+50%)	
Operator labor	More	Less	17%–20% reduction	2(−20), 8(−17)	4
Operating cost	Higher	Lower	8%–67% reduction	3(−67), 4(−8), 6(−33), 7(−10), 8(−20), 9	
Speed to build	Slower	Faster	20%–50% reduction	1(−20), 2(−50), 6(−50), 7(−25)	4
Equipment validation burden and speed	Higher	Lower	20% reduction	3(−20),	1, 4
Extractables leachables validation burden	Less	More			3, 4, 11
Cost and speed of equipment modification	Expensive and slower	Less, faster		,	3, 4
Annual capacity increase due to faster turn around between batches	Slower	Faster	7%–20% increase	4(−7), 7(−20%)	4
Energy and/CO_2 footprint	Higher	Lower	20%–80% reduction	4(−20), 5(−80)	
Water use	Higher	Lower	45%–62% reduction	1(−45), 5(−62)	4
HVAC clean room requirement	Higher	Lower	33% reduction	5(−33)	

29.3 SINGLE USE MATERIAL OF CONSTRUCTION, COMPONENTRY, ASSEMBLY, STERILIZATION, INTEGRITY AND USE

29.3.1 Materials of Construction and Assembly

Single use components, bag films, connectors, fittings, filters and tubing are produced from high grade thermoplastic polycarbonate, polystyrene, polypropylene, polyethylene, PVC, ethyl acetate and other biocompatible polymers that can withstand gamma irradiation sterilization and still meet USP Class VI requirements for extractables and leachables [25]. In most SU fabrication operations, clean room facilities are employed for film casting or welding of films and components. Three dimensional bags are produced by heat or ultrasonic welding of flat panels of film. Injection molded rigid components such as fittings and rigid or semi rigid addition ports or agitator bases are usually of the same polymer type to be welded to the bag. For example, low density polyethylene (LDPE) is commonly used for bag films, and high density polyethylene (HDPE) is used for addition ports and/or agitator mounts that are welded to the LDPE film. Single use sensors are either assembled and welded into the bag during fabrication or re-used sensors are sterilized and inserted aseptically into the bag in the field.

29.3.2 Sterilization of Single-use Films and Components

Sterilization of the finished and packaged SU product assembly is typically performed by gamma irradiation at 25–40 kilogray dose. Validation of sterilization is first performed with dosimeters placed throughout the package which is rotated during the radiation process to ensure all irradiation shadows are exposed. Additional USP sterility validation of the bag or component is performed for aseptic applications and is becoming the standard in the industry. Autoclaving can also be used to sterilize some single use assemblies (not LDPE films) depending upon their thermal stability and size. The disadvantage to this approach is that the single use assembly must be vented to allow air to completely escape from inside the assembly and for steam to completely penetrate and saturate the inside surfaces. Any trapped air will compromise the bioburden reduction of the autoclaving process. Long coils of tubing are notorious for poor removal of air and inadequate penetration of steam.

29.3.3 Assurance of Single-Use Bag and Assembly Integrity

Weld strength and bag integrity is validated by subjecting a statistically representative number of welded samples of the film to tensile strength durability tests. Finished 3D bag integrity is performed by pressure testing a statistically representative number of sample bags, although pressure testing is prone to false positives due to stretching of the bag or temperature changes. Alternative methods to test bag integrity have been proposed (helium leak test) but these can produce false positives and negative results and have been found to be unreliable. In addition, these tests can increase the risk of bag damage just due to the handling.

After over 10 plus years of SU manufacturing across the industry, these problematic integrity testing methods are beginning to give way to an overall quality assurance system to ensure bag integrity:

1. Vendor requirements:
   a. Single-use (SU) film, components and 3D bags are manufactured per SOP and batch records
   b. Quality control checks the bag for defects and documentation for compliance
   c. The SU component is wrapped in an outer protective bag and placed in final shipping package
   d. Packaging and shipping (including shipping to and from the gamma irradiation facility) are validated to ensure that the packaging can withstand normal shipping duress
2. The User inspection procedures:
   a. Auditing the SU vendor facility on a routine basis to ensure that they are following agreed to procedures per a Vendor Quality Agreement
   b. Upon receipt inspecting integrity of the outer shipping packaging for damage
   c. Inspecting the outer protective bag before removal
   d. Inspecting the SU bag and components for any visible damage or breakage
3. The User installation and test verification procedures:
   a. Insert the bag into the support vessel per validated procedures per validated instructions (SOP)
   b. Inflate the bag to low pressure and hold, inspect for any large leaks or loss of pressure
   c. Proceed to fill the bag with media or buffer to a low level, inspecting for leaks
   d. Proceed to fill the bag to full volume and operation, inspecting the bag for leaks
   e. Proceed to start up the system mixing or bioreaction to ensure all functionality of the system is working

4. Post use filter integrity testing (for aseptic operations only or where necessary)
   a. After use, remove all sterile vent or liquid filters from the assembly
   b. Subject filters to post use integrity testing

Compared to the validated bag fabrication procedure and assembly controls at the manufacturer, training of the operator regarding handling the single use bag may be next the most critical aspect of operational success in single use manufacturing, beginning with unpacking the shipping container and extending all the way to insertion and filling of the bag in the system.

29.4 DESCRIPTION OF SU UNIT OPERATIONS AND GENERAL USER REQUIREMENT SPECIFICATIONS FOR A TYPICAL MONOCLONAL ANTIBODY SUSPENSION CELL PROCESS

Given the rapidly changing portfolio of single use systems from a variety of suppliers at the writing of this handbook, it is only practical to describe general user requirement specifications (URS) and performance selection criteria for SU systems. The descriptions and tables in this section provide hardware and performance URS and selection criteria for single use systems in the upstream portion of a typical monoclonal antibody process. Single use technology and its applications are extensively described in Chapter 5 (introduction), Chapter 9 (cell separation), Chapters 14, 15, 23 (filtration), Chapters 24, 25 (USP and DSP equipment), Chapter 34 (process intensification), Chapter 35 (SU process implementation), Chapter 37 (N-1 bioreactor perfusion), Chapter 43 (vaccines manufacturing), Chapter 44 (cell therapy), Chapter 45 (facility design), Chapter 53 (security of supply), Chapter 59 (list of consumable suppliers). Additional descriptions and applications of single use technology are described in Chapters 14, 23, 34, 35, 43, 44, and 53.

All product contact components of the single use systems described in the URS charts below must meet USP Class VI limits for extractables and leachables.

29.4.1 Upstream Mammalian Cell Operations

Cell Banking

Single use cell bank systems have been in use since the inception of industrial fermentation in the 1970s in the form of plastic polycarbonate or polystyrene slat tubes, vials, t-flasks and shake flasks. These are available from a variety of suppliers. These components are sterilized with gamma irradiation and are prepared with various coatings depending upon the cell type application: suspension or adherent mammalian cells, insect cells or suspension microbial cells. Table 29.5 provides general URS for single use cell banking operations.

TABLE 29.5 General URS and Performance Selection Criteria for Inoculum Prep Systems Up to 25 L Working Volume for Suspension Mammalian Cells

Step	Device	Performance, Cell density range	Monitoring and control
Cell banking	Cryo vial or small bag	Up to 10 E6 cells/mL	Stable in liquid or gas phase N_2
Initial seed expansion and larger seed train expansion	T-flask or Hyper flask, Roller bottle	Up to 10 E6 cells/mL	Temp controlled 5% CO_2 incubator
	Spinner flask or Wave Rocker bioreactor	Up to 10 E6 cells/mL	Temp, pH, DO, agitation control, wave rocker angle and speed control, $pCO_2 < 100$ mm Hg
	Stirred bioreactor 3–25 L working volume	Up to 10 E6 cells/mL, impeller tip speed < 25 meters/second, sparge gas velocity < 40 meters/sec, power/volume 10–50 watts/cubic meter	Temp, pH, DO and agitation control, $pCO_2 < 100$ mm Hg
	Perfused high density inoculum bioreactor 3–25 L, Wave rocker or stirred bioreactor	Up to 100 E6 cells/mL impeller tip speed < 25 meters/second, sparge gas velocity < 40 meters/sec, power/volume 100–200 watts/cubic meter	temp, pH, DO, perfusion medium feed rate and agitation control, $pCO_2 < 100$ mm Hg See section 29.4.1.5 for URS requirements

Initial Seed Inoculum Expansion

Once expanded past the initial 10 mL t-flask or shake flask stage, larger spinners, shake flasks or bag rocker SU bioreactors can be used to expand the culture from tens of milliliters to liters of culture volume. Microbial cultures grow 10–20 times faster than mammalian or insect cells and thus will consume dissolved oxygen and nutrients quickly even in small systems. This requires that they be transferred to stirred tank fermenters with adequate oxygen transfer at smaller scales compared to mammalian cell cultures. Table 29.5 provides general URS for single use seed train expansion systems.

Seed Train Expansion—Liters to Tens of Liters

Expansion of mammalian or insect cell culture continues further in larger rocking bag systems or spinners up to 20 L or stirred tank bioreactors from the 3 to 25 L scale. Rocking bag systems are available from a variety of vendors. Table 29.5 provides the URS for seed preparation systems.

Production Single-Use Bioreactor— Medium Cell Density

This section describes the general URS for medium cell density bioreactors with cell density of 1–30 M cells/mL.

Single use production mammalian cell bioreactors, typically from 50 L up to 3000 L working volume scale are available from a variety of vendors. Table 29.6 lists the general URS and performance selection criteria for these systems.

Production Bioreactor—High Cell Density

High cell densities up to 200 M cells/mL have been reported for optimized perfusion cultures [33,34]. Bioreactor URS selection criteria for these higher cell density applications include:

a. Oxygen kLa increase to 50–100 h^{-1}
b. Higher kLa for CO_2 stripping so as not to exceed 100 mmHg
c. pure O_2 sparging may be required depending upon the kLa of the system chosen
d. ability to operate at elevated gas flow rates up to 0.1 volume of gas per volume of bioreactor per minute (VVM) with the option of pure O_2
e. large exit air filtration cartridges that will not over pressurize the bag when operating at 0.1 VVM.
f. Exit air condenser to reduce moisture in exit gas especially at high gas flow rates of 0.1 VVM
g. Enhanced heat transfer for high metabolic and motor power heat loads
h. Additional impellers to provide uniform mixing throughout the bioreactor volume
i. Modified liquid in and out ports (well below liquid level line) for perfusion device such as ATF system
j. Fast response pressure control systems due the high gas flow rates

TABLE 29.6 General URS and Performance Selection Criteria for Single Use Mammalian Cell Bioreactors

System Size, Max/Min Working Volume, Liters	Vessel Aspect Ratio at Max Working Volume Height: Diameter, With or Without Jacketing	Aeration Sparger Design, Gas Flow Rate Range, CO_2 Stripping	Max Impeller Tip Speed, Minimum Power/Vol., Max Sparge Gas Exit Velocity	Minimum O_2 Mass Transfer Coefficient (kLa) With Just Air (h^{-1}) at Full Working Volume	Minimum Process Monitoring and Control
50/10 200/40 500/100 1000/200 2000/400 3000/600	1.5:1, jacketed bottom and side wall	Microporous 2–150 µm, drilled hole 0.5–3 mm, 0.01–0.1 VVM separate CO_2 stripping sparger with 0.5–3 mm drilled hole	25–40 m/s (for eddy scale >20µm), 30 watts/cubic meter, 40 m/s	10 h^{-1} at 37°C, with 6 g/L NaCl and 1 g/L pluronic F-68	0.01–0.1 VVM[a] air sparging rate, cascade dissolved O_2, temperature, agitation, sparge O_2 gas enrichment to 100%, pressure, feed control, weight, CO_2 stripping sparger below 100 mmHg, head sweep 0.01 VVM, foam control, exit gas condenser, exit gas filter auto changeover, data historian, data trending/tracking

[a]*VVM stands for volume of sparge gas per volume of bioreactor per minute.*

TABLE 29.7 Bioreactor Operation Modes and Perfusion Device URS for High Cell Density Single Use Perfusion Devices

Operation Mode	Requirement	Perfusion Device	Bioreactor Bleeding	Other Requirements/Comments
Concentrated fed-batch, cell recycle only	Perfusion device to concentrate just cells in bioreactor	SU centrifuge, or ATF system with microporous filter that retains only cells, or Hollow fiber external loop with microporous filter	Not reported as a requirement	Large bore connections on bioreactor for exit and return ports for perfusion device ATF systems are now available in single use format Filtration methods have the additional advantage of cell free permeate enabling direct loading onto a capture column ATF filters will foul over time depending upon cell density and permeate flow rate, requiring a backup ATF unit Single use centrifuge disposable insert assemblies wear out and must be replaced periodically, potentially requiring a backup system SU centrifuges that have rotating seals may not provide suitable aseptic operation for cell recycling back to the bioreactor
Concentrated fed-batch with cell and product recycle	Perfusion device to concentrate cells and product in bioreactor	ATF system or external hollow fiber system with ultrafilter that will retain cells and product		
Continuous perfusion with cell recycle only	Perfusion device to concentrate and maintain high cell density	SU centrifuge, or ATF system with microporous filter retains only cells, or Hollow fiber external loop with microporous filter	ATF and external loop HF filtration system requires daily bioreactor bleed to remove dead cells	
Continuous perfusion with cell and product recycle	Perfusion device to concentrate and maintain high cell density and product in bioreactor	ATF system or external loop hollow fiber system with ultrafilter that will retain cells and product		

ATF stands for alternating tangential filtration.

Single-Use Flow Path Bioreactor Perfusion Devices for High Cell Density Cultures

Table 29.7 lists different bioreactor operational modes and URS for perfusion devices for high cell density and high titer cultures using concentrated fed-batch or perfusion operation [33,34]. At the writing of this chapter, acoustic cell separators are at developmental stage and have not yet been made available for large scale use.

Single-Use Primary Recovery and Cell Separation Systems

There are several methods for primary recovery of the spent cell culture media and removal of the cell mass. Fed batch processes require that a separate SU device such as a centrifuge, depth filtration system or acoustic separator are employed to remove the cell mass from the product stream. In contrast, perfusion processes using ATF systems described in the previous section generate a cell free stream which can be loaded directly onto a capture column, avoiding any intermediate cell removal system.

High cell density processes resulting from perfusion operation can reach wet cell mass of 15%–20% wet solids, challenging most depth filtration or acoustic separation systems. In these cases, a SU centrifuge is placed upstream of the depth filtration train.

Table 29.8 lists the various cell removal systems and their respective URS. At the time of writing of this chapter, acoustic separators are limited to lab scale operations and are not widely used yet by the biomanufacturing industry and are therefore not described in the URS. However, there is the expectation that these systems will find wide applications in bioprocessing as they are scaled up.

A typical single use depth filtration system for a 2000 L bioreactor harvest at 5 g/L mAb product titer (total of 10,000 g crude mAb) using typical flat sheet depth filters is shown in Table 29.9 [35].

29.4.2 Downstream Purification Systems for mAb Processing

This section addresses the major unit operations for downstream that are considered truly single use. For example, chromatography skids, membrane purification cartridges, virus reduction filter cartridges and tangential flow filtration systems are discussed as single use operations. Bioburden reduction or sterilizing filtersand vial filling systems are not discussed either due to too wide a variety (to encompass in this chapter), not in wide use or not truly single use.

TABLE 29.8 General URS and Performance Selection Criteria for Single Use Cell Separation Systems

Method	Device	Filter area URS:L/Sq. Meter Filter Area[a] or g-Force	Flow Rate Range	Other Requirements/Comments
Depth filtration	Cartridge, pod or flat sheet membrane	For 10–30 M cells/mL: Stage 1: 50 L/m^2 h, stage 2: 50 L/m^2 h For 30–50 M cells/mL: Stage 1: 20–30 L/m^2 h, stage 2: 50 L/m^2 h	Total flow rate is dependent upon total membrane surface area installed	Depth filtration systems require an extra 25% bioreactor volume to rinse out and recover product left in the depth filter system For very high cell densities, a SU centrifuge can be used to pretreat the stream to reduce the cell mass load on the depth filter Depth filters filtrate is filtered again using 0.2 µm cartridge filter
Tangential flow filtration (TFF)	Hollow fiber microporous filter Hollow fiber ultrafilter Alternating filter (ATF)	For 10–30 M cells/mL: 20–30 L/m^2h For 30–50 M cells/mL: 10–20 L/m^2 h	Total flow rate is dependent upon total membrane surface area installed and cell density	TFF systems require an extra buffer 10% volume wash to rinse out and recover product left in the system
Centrifugation	Tubular bowl Spinning tube Fluidized bed	Up to 4000×g Up to 360×g Up to 2000×g	Up to 360 L/h for tubular bowl Up to 120 L/min for spinning tube Up to 700 L/h for fluidized bed	Centrifuge flow rates are dependent upon cell density. High cell density will lower the flow rate to achieve the same cell separation efficiency Centrifuge centrate will contain residual cells requiring filtration before chromatography

[a]*Note: depth filtration stage 1 porosity is 0.5–10 µm. Stage 2 is 0.1–0.5 µm. Stage 1 permeate is loaded directly onto stage 2 filters.*

TABLE 29.9 A Typical Single Use Depth Filtration System for a 2000 L Batch at 5 g/L mAb Titer Containing a Total of 10,000 g of mAb

Depth Filtration	Parameter	Specification	Units	Description
Step 1	Stage 1 filters	20 m^2 area	0.5–10 µm porosity Flow rate 50 LMH	Stage 1 filtrate flows directly onto stage 2 filters
Step 2	Stage 2 filters	20 m^2 area	01.–0.5 µm porosity Flow rate 50 LMH	Stage 2 filtrate flows directly onto 0.2 µm depth filter
Step 3	Filter cartridge	1×30″	0.2 µm porosity, 2.6 m^2 area	
Step 4	Filter flush	400 L, 55 L/min flow rate		
	Recovery yield	95%	9500 g	
	Total product volume	2400 L (including flush)		
	Final titer in harvest volume	~4.0 g/L		

Prepacked columns, although not truly single use, offer the convenience of ready to use systems that relieve the operator of packing the resin into the column system, which requires significant expertise, high grade environment and expertly trained personnel.

Single Use Flow Path Chromatography Systems

Single use flow path chromatography systems are available from several vendors. The general URS and performance selection criteria for these systems is listed in Table 29.10.

TABLE 29.10 General URS and Performance Selection Criteria for Single Use Flow Path Chromatography Skid Systems

System	Flow Rate (L/min)	Monitoring Capabilities	Controlling Capabilities	# Ports, Inlet, Outlet	Gradient Capability	Other Attributes
Single use flow path chromatography skid systems	0.05–9	UV 280, pH, conductivity, flow rate, pressure, air bubbles	Flow rate	6 inlets, 6 outlets	Yes	Filtration capabilities (replacement of columns with filters)

TABLE 29.11 A Typical Single Use Flow Path Chromatography Operation to Process a 2400 L Batch of mAb Containing 9500 g of mAb

Protein A Capture	Parameter	Specification	Units
	Column	45 × 20	Diameter and height in centimeters
	Resin	Protein A affinity resin	
	Resin volume	32	Liters
	Binding capacity	60	g/L
	Total column capacity	1920	Grams
	# Column cycles	5	
	Column flow rate @ 300 cm/h	8	L/min
	Product recovery	95%	
	Total mass recovered	9025	Grams
	Total product volume elution	240	Liters
	Expected product titer in elution [pool]	~37.6	Grams

An example of a typical single-use flow path chromatography system operation is shown in Table 29.11. In this case 1920 g are processed in each cycle over the 32 L column, requiring a total of 5 cycles to process the entire batch [35]. Larger columns will reduce the number of cycles but require purchasing more resin.

Single-Use Membrane Purification Systems

Single use membrane purification systems continue to advance in selectivity and capacity with reduced holdup volumes and up to 4 bar operating pressure with 3–5 µm pores sizes. URS criteria for these systems are listed in Table 29.12.

As of the writing of this chapter, resin based chromatography operated in batch mode is too expensive to be considered single use. However, continuous chromatography has the potential to exhaust resin columns via multiple cycling to the point that the resin is consumed to a significant part of its maximum lifetime and therefore can be discarded as single use [36]. Continuous chromatography in the form of periodic counter current chromatography (PCC) and simulated moving bed chromatography (SMB) significantly reduces the size of the columns by maximizing resin utilization to the extent that the columns are operated for up to hundreds of cycles within one batch. Please see Chapters 14, 15 and 23 for extensive discussions of filtration.

Single-Use Virus Reduction Systems

There are a variety of single use virus inactivation solutions used in biologics manufacturing. A thorough review of potential virus contamination of drugs, virus inactivation methods, and guidelines is recommended for the reader [37–40]. Virus inactivation or reduction can be performed in single use systems including automated single use mixers for low pH hold and detergent treatment, and cartridge filters with very low porosities [41,42]. Table 29.13 lists the URS and performance selection criteria for single use virus inactivation or reduction systems. Please see Chapters 14, 15 and 23 for extensive discussions of filtration.

TABLE 29.12 General URS and Performance Criteria for Single Use Membrane Purification Systems

Type	Device and Volume Range	Operation Mode	Dynamic Binding Capacity	Impurity Load Capacity kg/L Membrane Volume	Max Flow Rate, Pressure, Porosity
2D Membrane purification	S Cartridge, cation exchange, 0.08–5 L	Flow through, shallow bed height	30 mg/mL	2 kg/L	30 Membrane vol/min, 4 bar, 3–5 µm
	Q Cartridge, anion exchange, 0.08–5 L		30 mg/mL		
	HIC Cartridge, phenyl HIC, 0.08–5 L		20 mg/mL		
	S Cartridge, cation exchange, 0.08–5 L	Bind/elute, longer bed height	30 mg/mL	2 kg/L	5 Membrane vol/min, 4 bar, 3–5 µm
	Q Cartridge, anion exchange, 0.08–5 L		30 mg/mL		
	HIC Cartridge, phenyl HIC, 0.08–5 L		20 mg/mL		
3D HydroGel membrane purification	Q Membrane, 0.0002–0.46 L	Flow through	N/A	10 kg/L	25 Membrane vol/min, 6 bar
	Q Membrane, 0.0002–0.46 L	Bind/elute	200 mg/mL BSA	N/A	25 Membrane vol/min, 6 bar
3D HydroGel membrane purification	Sulfonic acid/t-butyl (multimodal), 0.00087 L	Bind/elute	85–95 mg/mL IgG	N/A	10 Membrane vol/min, 6 bar

TABLE 29.13 General URS and Performance Selection Criteria for Single-Use Virus Clearance Systems

Type	Device	Log Reduction	Type of Virus	pH Range and Duration, Additional Equipment	Other Requirement/Comments
Low pH hold using HcL acid	Two Automated mixers in sequence	4.6–4.9	Enveloped	3.7 ± 0.1 for >30 min A second mixer is required to avoid the hanging drop problem	Continuous single use in-line mixers can be used with tubing that has sufficient length and residence time to provide for virus inactivation
Detergent treatment	Automated mixer	0.5%–1.0% triton X-100 or tween 80	Enveloped and retrovirus	Kinetics of inactivation are concentration dependent and must be validated A second mixer is required to avoid the hanging drop problem	Preferred method if the product cannot tolerate low pH treatment Lower temperature reduces effectiveness
Nano-filtration	Cartridge	4.0–4.96	Enveloped and nonenvelope, small parvovirus and large retrovirus	Post use integrity testing is required	Bleed through of parvovirus can occur during pressure excursions

Log_{10} reduction values (LRV) of 3–4 can be achieved for low pH and detergent treatments and can be conducted in single use mixers. For these methods, a second single use mixer is required to address the "hanging drop problem" which occurs when an untreated drop in the headspace of the first treatment mixer falls into the treated batch at the end of its hold period, thus re-contaminating the batch [37]. The solution is to add a second mixer into which the entire batch is transferred and held to incubate for the required duration. Alternative virus inactivation methods are described in Chapter 36.

Nanofiltration methods for virus clearance are available in convenient cartridge configurations from a variety of vendors. Comparable LRVs can be achieved with nanofiltration cartridges for both enveloped and nonenveloped viruses compared to low pH or detergent treatments [41].

An important user requirement for single use virus clearance systems is the ability to conduct representative small scale clearance validation studies with scaled down versions of the inactivation method. For instance, virus reduction has been

TABLE 29.14 General URS and Performance Selection Criteria for Single Use TFF Systems. TMP Stands for Transmembrane Pressure

Method	Device, Operation Mode	Retentate Flow Rate	Retentate Pressure	Permeate Flow Rate	Sensors, Monitoring and Control	Additional Comments/Key Options
Tangential flow filtration	Flat sheet, multiple pass	240–360 L/m^2 h	Up to 4 bar	30 L/m h	Monitored: pH, UV, conductivity, temperature, retentate delta pressure, TMP, permeate backpressure Controlled: retentate flow rate, ret. delta pressure, permeate backpressure, TMP, temperature	Low holdup volume and full drainability of the system Single pass systems may not offer as high a concentration factor as multiple pass systems Low foaming in the retentate vessel
	Flat sheet, single pass	100 L/m^2 h	Up to 6 bar	20 L/m^2 h		
	Hollow fiber, multiple pass	200–300 L/m^2 h	Up to 4 bar	20–30 L/m^2 h		

reported using disposable depth filtration [42], but some have raised questions about reproducibility in small scale model depth filtration systems [37]. Another important feature of single use filters is the ability to perform post use integrity testing [43].

Single-Use Tangential Flow Filtration Systems

Tangential flow filtration (TFF) in single use flow path format is available from a variety of vendors and is used for diafiltration, concentration, or formulation. There are two classes of TFF systems: recirculating and single pass and these are configured with flat sheet or hollow fiber membranes [44]. Table 29.14 lists the URS and performance selection criteria for single use tangential flow systems.

Single pass TFF systems do not require recirculation and thus reduce system complexity. However, since there is no need for a recirculating vessel for the retentate, buffer exchange is achieved by injecting a diafiltration buffer into the membrane cassette system flow path as the product moves through the membrane cassettes. This creates more of a step wise change in buffer concentration compared to recirculating TFF systems which produce a steady change of buffer concentration.

Recirculated TFF systems subject the product to multiple recirculations through the filter system and back to the retentate vessel. For shear sensitive products this exposure could cause foaming and shear effects. In addition, repeated recirculation can cause heating by the pumping action, so caution is advised unless a heat exchanger can be included in the system [44].

Sensors and controls include retentate flow rate and pressure, delta pressure (inlet retentate pressure minus outlet pressure), UV, transmembrane pressure, temperature, pH and conductivity. An additional option is to control the permeate flow rate to reduce membrane fouling.

Single Use Filling and Filtration of Bulk Drug Substance (BDS) or Active Pharmaceutical Ingredient (API)

Sterile fill/finish/filtration of bulk drug to produce the BDS or API using single use systems is commonly performed using sterilizing cartridge filters (0.1 µm). These custom systems are available from a variety of vendors and are assembled into a completely integrated and closed single use assembly [45]. The general user requirements for the assembly are:

a. starter bag containing the prefiltered bulk drug intermediate
b. connected via tubing to a peristaltic pump
c. followed by connection to a sterilizing filter (0.1 µm)
d. followed by a receiving bag to hold the filtered BDS/API which is shipped to the fill/finish site
e. The assembly includes various sample ports and satellite sample bags for post use QC sampling and post use filter integrity testing.

The prefiltered bulk is pumped through the sterilizing filter into the receiving bag. Several prefabricated assemblies are commercially available on the market at the writing of this chapter, however, many users assemble their own bulk filtration assemblies or outsource their assembly.

Single Use Bulk Drug Packaging, Storage and Shipping

SU bulk drug substance can be filled into preirradiated sterile bags connected to the integrated bulk filtration and filling assembly described above in Section 29.5. Stability of the drug to be stored should be carefully studied to ensure that no deleterious effects occur to the product from long term exposure to polymeric bags, especially if the product is not frozen. A common temperature range for bulk storage is −20 to −80°C. Generally, BDS/API is stored in bags up to volumes of 10 L.

Shipping of BDS in frozen or liquid bags can be performed using the "Bio Shell" shipping container whose construction is suitable for shipping bags containing product up to 10 L [46].

Single-Use BDS/API Freezing Systems

Bulk drug substance volumes above 20 L may require freezing in SU controlled rate freezers [47]. These freezers package and freeze the BDS in narrow pillow bags (1–2″ in depth) that are sandwiched between freezing plates.

The critical URS performance criteria are the freeze front velocity (FFV) or rate of cooling to the freezing point for the liquid to the last point to freeze time (LPTF), followed by subsequent cooling below the freezing point to the desired storage temperature range of −30 to 80°C [47].

Due to the variability in stability of proteins and the variation in excipient choice and formulations, there are no standard FFV or LFTF URS criteria. The FFT and LPTF performance of a single use system must be tested on a protein by protein, case by case basis.

29.4.3 General User Requirement Specifications for Other Single Use Systems

Single-Use Microbial Fermenters

High density bacterial fermentation in single use fermenters presents engineering challenges for mass and heat transfer [48,49]. Despite these challenges, single use fermenters are commercially available from several vendors. General URS and performance selection criteria are listed in Table 29.15.

Single Use Mixers

SU media and buffer preparation or product mixing systems include a variety of mixers that range from several liters to 2500 L scale. General URS and performance selection criteria for single use totes and mixers are listed in Table 29.16. Totes are simple non-agitated containers for storing solutions. Mixers come in 2 classes: simple mixers with just agitation and "smart mixers" for more complex operations such as pH adjustment and conductivity adjustment or both.

When mixing cell culture powdered media, especially hygroscopic powders tend to clump and float on the liquid surface. For this application, strong down pumping mixer action is required to draw the floating clumps under the liquid level. Salt addition to mixers for buffer preparation should be performed while agitation is turned on to avoid forming large mounds of salt on the bottom of the mixer on top of the film. These large mounds can become very warm or hot during slow dissolution and may soften or damage the film.

TABLE 29.15 General URS and Performance Selection Criteria for High Cell Density Single Use Microbial Fermenters

System Size, Max/Min Working Volume, Liters	Vessel Aspect Ratio at Max Working Volume Height: Diameter, With or Without Jacketing	Aeration Sparger Design, Gas Flow Rate Range, CO_2 Stripping	Oxygen Transfer Rate, Heat Transfer Rate, Power/Vol.	Minimum O_2 Mass Transfer Coefficient (kLa) With Just Air (h^{-1}) at Full Volume	Minimum Process Monitoring and Control
50/10 200/40 500/100	3.0:1, jacketed bottom and side wall	Drilled hole ring sparger 0.5 mm to 1/8″, 0.1–2.0 VVM	OTR = 400 mmoles/L h HTR = 220 BTU/h/L P/V = 0.016 Hp/L	100 hr-1	0.1–2.0 VVM air sparging rate, cascade dissolved O_2, temperature, agitation, sparge O_2 gas enrichment to 100%, pressure, feed control, weight, foam control, exit gas condenser, exit gas filter auto changeover, data historian, data trending/tracking

TABLE 29.16 General URS and Performance Selection Criteria for Single Use Totes and Mixers

Type	Operation	Mixing Performance	Process Control
Tote	Solution storage	None	None
Simple mixer: rocker, paddle, stirred tank	Mixing/heating/cooling/storage of solutions, with sampling, bottom and side jacketing, means to add powders	Mixing time of 1–2 min, no dead zones	Mixing speed variable, jacketed tank for cooling/heating
	Mixing/heating/cooling/storage of powdered media or buffers, with sampling, bottom and side jacketing, means to add powders	Vigorous surface mixing to dissolve clumped media, mixing time of 1–2 min, no dead zones	Mixing speed variable with down pumping, jacketed tank for cooling/heating
Smart mixer: rocker, paddle, stirred tank	Mixing/heating/cooling, pH, conductivity or temperature adjustments, with sampling, bottom and side jacketing, means to add powders	Vigorous mixing for rapid process control, mixing time of 1–2 min, no dead zones	Insertable (autoclavable) or single use pH, temp and conductivity sensors with pH, conductivity or temperature controllers Temperature ±0.5°C pH ±0.1 pH

Mixing time is an important performance criterion for mixers. Mixing efficiency can be measured by inserting pH or conductivity probes at various points throughout the height and width of the mixer to track pH or conductivity as they are adjusted. If homogeneity is not reached or takes more than several minutes, the mixer's efficiency should be questioned.

29.4.4 Single Use Facility Design

Single use facilities are discussed extensively in Chapter 45. These facilities are operated quite differently compared to traditional stainless steel facilities, which suggests the need for alternative designs for maximum efficiency [2,50–52].

Since the SU components and bags must be carried into the facility for use and carried out of the facility for disposal, the location, size and accessibility of the warehouse should be carefully considered to ensure that logistics are not slowed or inhibited. In addition, the number of hallways and airlocks that must be traversed can also slow down facility turnover time. For these reasons, open architecture facilities are being considered more frequently for single use operations as well as for potential significant capital and operating cost advantages along with reduced clean room requirements [2,48,50].

Clearly, open architecture facilities must address the issue of segregation of pre- and postviral inactivation operations that are normally segregated into separate suites in traditional multiple clean room biopharmaceutical facilities. Closed single use systems are reducing the risks of cross contamination if no breaches occur during processing, or if the risk of breach is considered acceptably low [52,53].

29.5 GAPS AND DISADVANTAGES OF SUT

Single use systems offer considerable advantages but also have substantial considerations and limitations [1–14,25,45,54]. Single use systems are suitable for almost all bioprocessing applications except for pressurized or higher temperature operations, or in the presence of certain organic solvents and higher concentrations (>50%) of alcohols [25]. Gaps and inefficiencies include immature supply chains, incompatible sterile connectors and lack of industry standards for extractables and leachables validation. The latter is an area of focus for several working groups who are collaborating to adopt industry standards, discussed extensively in Section 29.2.

29.6 CONCLUSIONS AND THE FUTURE OF SINGLE USE TECHNOLOGIES

Single use technologies are spreading throughout the industry, around the globe and into emerging markets [55]. They present several advantages despite their disadvantages [2,22,25,45,50,54]. They enable continuous manufacturing, which is well described in Chapters 28 and 35 [36]. Improvements and new applications are being reported frequently [44–48,50,53]. Harmonization and standardization should continue, but not to the point of limiting innovation.

Single use facilities will not be a panacea for the industry as stainless steel facilities will continue to be needed for very large (1000 kg/year) and very low cost biologics [50]. The advent of fully closed single use systems and open architecture facilities will pave the way for advances in efficiency, cost and quality improvements [51–53].

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