

This article describes the process, design, and fabrication considerations to take into account for large-scale chromatography control and dilution equipment.

## Large-Scale Chromatography Becoming State-of-the-Art

by Roy Greenwald and Bill Rochelle

### Introduction

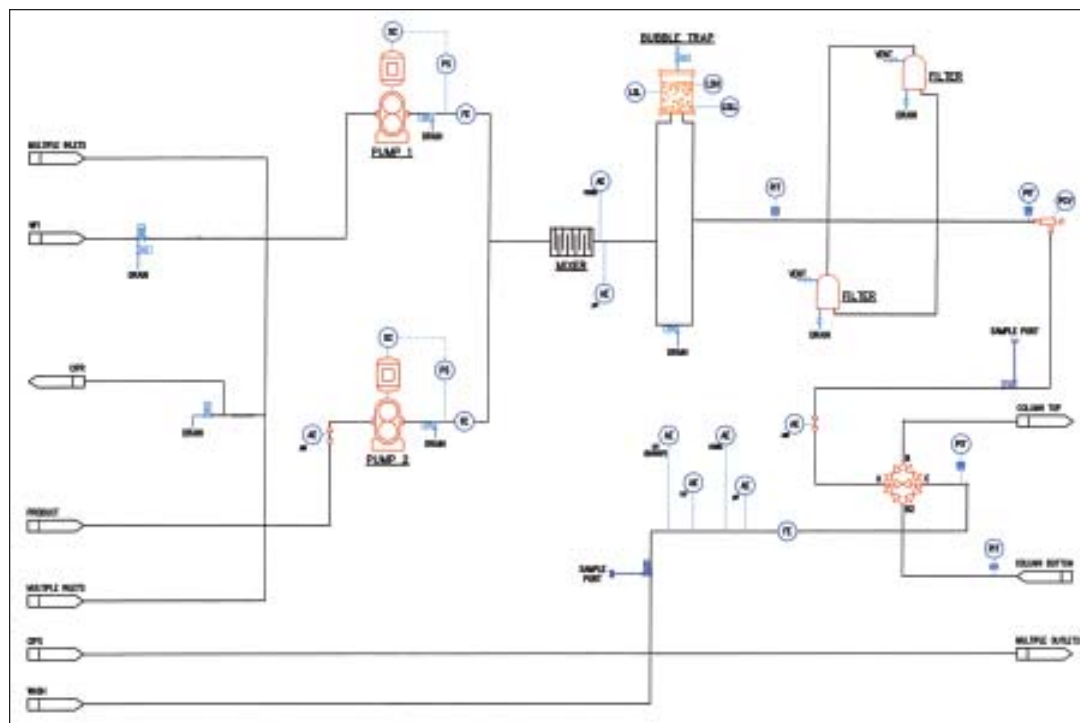
Over the past five years, the biopharmaceutical industry has seen a steady increase in the size of bioreactors. Whereas in the past it was typical for a large bioreactor to be in a range that might span 3,000 to 6,000 liters, it is not uncommon to now see bioreactors being installed that routinely exceed 10,000 liters and often reach 20,000 liters. Yet, in many installations, the purification process equipment has failed to keep pace or scale up at the same rate. Recently, DECCO Process Solutions (DPS) worked with a large New England biotechnology firm to confront the decision process and engineering challenges related to this scale-up in order to de-bottleneck the typical downstream processing suites. The result of this effort was the design, fabrication, testing,

and start-up of the largest biopharmaceutical chromatography skids built by anyone to date. This article describes many of the decision factors, unique design considerations, and challenges that were addressed and overcome in the process.

The manufacturing company recently constructed a new large scale production plant that is a cGMP facility for commercial scale protein and antibody production. Because the cost of biopharmaceutical facilities is often a function of its square footage, it seemed prudent to install as much reactor capacity as possible within the footprint. The new plant was to include three 20,000 liter stirred bioreactors with dedicated seed train reactors. The facility was to operate as a single harvest system.

The next key decision confronted by the

Figure 1. Control and dilution skid P&ID.



	TRAIN 1		TRAINS 2 - 4	
	SI	English	SI	English
Quantity	1	1	3	3
Number of Inlet Manifolds	2	2	2	2
Total Number of Feed Sources	16	16	16	16
Column Diameter Serviced	2 meters	6.6 feet	1.4 and 2 m	4.6 and 6.6 feet
Skid Inlet Line Size	63 cm	2.5 inches	63 cm	2.5 inches
Column Outlet Line Size	50 cm	2.0 inches	50 cm	2.0 inches
Design Pressure	7 bar	101.5 psig	7 bar	101.5 psig
Design Viscosity	1.3 cp	2.4 - 7.3 lb/hr-ft	1.3 cp	2.4 - 7.3 lb/hr-ft
Minimum Flow Rate	5.25 lpm	1.4 gpm	2.6 lpm	0.7 gpm
Maximum Flow Rate	262 lpm	69 gpm	129 lpm	34.2 gpm
Pump Power	11.2 kW	15 hp	7.5 kW	10 hp
Bubble Trap Size	40 liters	10.6 gallons	20 liters	5.3 gallons

Table A. Skid design and physical parameters.

design team was sizing of the purification equipment. In many facilities the purification suites represent the bottleneck in the production stream. The owner decided that would not be the case for their new project. In addition, there was to be a single equipment train dedicated to each specific purification suite. This decision represented a break from the traditional approach, wherein there are often multiple smaller units. As a result of these decisions, the owner worked with their engineering team to produce a performance specification for the largest chromatography control and dilution skids ever built. Table A contains some of the specific variables related to the chromatography skids.

## Chromatography Fundamentals

Chromatography has become one of the most frequently employed separation processes in the biopharmaceutical industry due to both the simplicity of its application and its ability to bring high resolution to the separation process. Fundamentally, the chromatography process most commonly selected is based on an ion exchange process that utilizes immobile ligands embedded in an insoluble matrix, packed into a chromatography column. Proteins, polypeptides, and other bio-molecules that are charged, or capable of retaining charge, are suspended in a solute. The solute is routed through the chromatography column under pressure, and based on the interactions of ionic charges may be retained or passed through the column. By changing the conditions within the column, the affinity of the matrix for various product or buffer molecules can be modified. A typical separation process may include several steps in order to produce the required separation. Some studies have indicated that the average number of steps in a biopharmaceutical purification process is four.<sup>1,2</sup>

As an example, a chromatography column will typically be prepared and packed with the proper matrix media. The "proper" media is based upon characterization studies using a combination of experience, biochemistry, and empirical

data. The media itself will have different characteristics, depending upon the product that the user is planning to separate. Normally these characteristics have been established in the laboratory well before production volumes have been anticipated with the purification method validated during FDA clinical trials. The goal in matrix selection, and indeed the operation of the process itself, is to maximize resolution of the separation. The resolution is a mathematically defined parameter that is proportional to three variables: 1. the selectivity of the ion exchange process, 2. the efficiency of the process, and 3. the capacity of the process.<sup>3</sup> Selectivity is influenced by several factors; some are determined by the matrix affinity for particular ions, while some are experimentally determined factors that can be manipulated by the process itself, such as ionic strength and pH. The second parameter, the efficiency of the separation process, is most influenced by diffusion across the matrix bed or channeling through the bed. Ideally, a chromatography column should exhibit characteristics as close as possible to plug flow. Therefore, factors such as bead size of the matrix, packing techniques, air bubbles, and other physical anomalies that can lead to channeling will have a major impact on efficiency. The final factor that influences resolution is the capacity of the process. Capacity is an indication of the ion exchange capability of the matrix which can be influenced by the surface geometry of the ion exchanger. For example, a porous matrix material may allow smaller molecules greater access to surface area than for larger molecules. In addition, the protein's ratio of charge to pH is an important parameter that can be manipulated by the experimental conditions, for instance, via buffer selection, and will influence capacity. Finally, the flow rate through the column has a great impact on capacity. The dynamic capacity of the ion exchanger will normally exhibit an inverse relationship to flow rate, decreasing as the flow rate is increased.

From the preceding paragraph, it can be seen that many of the factors that determine the resolution of an ion exchange

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chromatography separation are inherent in the selection of the matrix. This article will not discuss these areas. However, many of the factors previously noted are determined or influenced by the process and the hardware utilized for the separation process. Most of these must be considered in the selection of the equipment, its design, the control logic, and the operating procedures. These factors do form the basis of this article.

A final consideration that strongly influences the product recovery in a chromatography process is the number of stages, steps, or phases required to collect the product. At each stage, other than initial equilibration, a percentage of the product is lost. As an example, in the most simple of separations, a column might be brought to a set of initial conditions, or a starting state, by pushing a buffer solution through the column at a specified pH and ionic concentration. This buffer will establish an initial set of charged molecules on the surface of the matrix. During the second step, the solute loaded with the product is adsorbed onto the matrix surface, displacing the charged molecules that were loaded via the buffer. The third stage, called elution, changes the conditions within the column so that the product is no longer preferentially bound to the matrix. This is the product collection step. A further step or two may be required to flush the column of undesirable ionic products that remain within the column, followed by a re-establishment of initial conditions. This is the most simple of separations. In most instances, the separation may require additional steps or may involve other complexities, such as gradients, wherein concentrations of buffers vary over time to collect various fractions of samples. This latter technique is often employed in the laboratory to establish optimal separation parameters. The problem inherent in multi-step separations is that even with recoveries of 95% per step, simple math will show that with two, three, or four steps, total recovery would drop to 90, 86, or 81% respectively. Therefore, the abilities of the equipment and control system to minimize losses at each step are critical to effective chromatography system design.

Currently, the supply of the columns themselves, along with their media, is served by a small number of companies. These companies have extensive laboratory and experience bases that allow them to work with biopharmaceutical companies to properly tailor their purification processes. The balancing of recovery, resolution, processing time, and cost is their area of expertise. However, the biopharmaceutical industry also has begun to realize that for Large-Scale systems, the Control and Dilution (C&D) skids should be designed and built by specialty fabricators. This second group of companies has the ability to work closely with the client to design the control of the skid for the specific purpose for which

it is intended, and often with a lower cost structure. By necessity, for Large-Scale Chromatography, these are almost always “one of a kind” skids.

## Process Design Considerations

One of the design parameters for this project was the ability to purify 20,000 liters of bioreactor output within a set timeframe. In order to process the necessary volume of product, extremely large quantities of dilute buffer were going to be required. There were two options on how to meet this requirement. The first, and obvious one, was to invest in the necessary tank storage needed to inventory the dilute buffers. This would have represented an excessive use of real estate, as well as a large capital investment. The alternative approach was to use concentrated buffer and dilute it on demand on the chromatography C&D skid. This latter approach was the preferred option for the owner’s project team. The impact of this decision was multi-faceted. In-line dilution created the following demands:

1. a need for multiple pumps on each skid
2. a need for high turn-down ratios on each pump
3. a need for efficient, but low-impact, mixing on each skid
4. a control system that could quickly and accurately adjust and measure buffer properties

Once in-line dilution is selected as the operational approach for a facility, the C&D skids actually must combine the unit operation of mixing with chromatography control. A failure to recognize this fact may lead to irreconcilable problems in equipment selection and control due to improperly matched pumps, inadequate turndowns, or inability to exercise ad-



Figure 2. Chromatography control and dilution skid – back.

equate control. Another impact of the upsizing of production facilities is that the collection of fractions is usually not practical. This is again due to the size requirements of the hold vessels. Although clinical and characterization studies make extensive use of gradients, Large-Scale Chromatography tends to be for market supply quantities and often finds less application for gradients. This obviously assumes that the characterization studies have been completed and elution phases adequately defined. Typically, flow rates and ratios will be programmed from the two pumps with separate buffer feeds, and blended in-line to create the required gradients. It is also typically necessary to include a backpressure control loop ahead of the column in order to control low flow rates at the low end of the dilution turndown. A typical Large-Scale P&ID is shown in *Figure 1*. This figure indicates hardware requirements, but has omitted the proprietary or confidential control logic.

In accordance with *GAMP® 4* protocols, a User Requirement Specification (URS) was produced to define the owner's needs. This 30-page document included designations of the skid's control boundaries in addition to its physical boundaries. In addition to these baseline parameters, several objectives were outlined. Among these were:

1. ability to continuously perform GMP operations
2. ability to display all monitored equipment variables on-screen, in real-time
3. ability to report faults via a video display and to archive a real-time event log
4. ability to communicate bi-directionally with the plant-wide control system (Delta V)
5. ability to manage both clean-in-place and steam-in-place operations for the skid as defined within the given boundaries



Figure 3. Chromatography control and dilution skid – front.

6. ability to produce linear flow rates through the columns of 75 to 500 cm/hr (29.5 to 196.9 in/hr)
7. adherence to ANSI/ISA S88.01 Batch Control Model

In addition, a further requirement was added as the project evolved, which was the ability to archive and recall “Golden Batch” data for optimized separation runs. This data would be available for use as the baseline of future purification runs of the same product.

Another advantage that most Large-Scale systems present is the ability to work with the owner in a way that integrates the skid into the Plant-Wide Control System (PWCS). In fact, one of the greatest advantages of large-scale systems is that the incremental efforts and cost of PWCS integration is almost always justified. This is another factor that owners cite when making the decision to work with specialty fabricators to meet their needs. Although the smaller chromatography units are often provided by the column suppliers with off-the-shelf expertise in standard programming features, they normally are stand-alone units. The larger systems create an opportunity to interact with hold vessels, CIP, SIP, and other equipment that is not only useful, but provides safeguards as well. As an example of a safeguard that PWCS integration might provide, one might consider a skid flowmeter failure. In such an instance, alternate data collection points can be utilized in real-time (in this case, potentially using buffer tank weigh cells as an input) to calculate loadings to the chromatography columns.

However, the single most important item to recognize regarding the process and automation design of a large scale system is the need to build in flexibility. This is best done by assuring that the data acquisition will be provided by the instrumentation at the required level of accuracy. This must be addressed at the Functional Requirement Specification (FRS) stage. The control system will then be able to produce the necessary routines to allow for the desired level of automation. As an example, it is not unusual that a UV pre-peak might be excluded from collection. This may require incorporating a subroutine to determine the second instance of when  $d(\text{UV})/dt$  equals zero to begin collection. Signal data collection, averaging, or manipulation is easily obtained if the initial data is available and accurate; the key is to properly design and instrument the skid initially.

## Equipment and Layout Design Considerations

There are many equipment design considerations in the proper design of a Large-Scale Chromatography C&D skid. Although the following list is not all-inclusive, it does contain some of the more critical parameters. The design must address:

- hold-up volume of the skid
- blending instrumentation philosophy and selection
- pump selection, both type and size
- control and elimination of air entrainment
- blending and mixing equipment selection

- cleanability and drainability

Hold-up volume has always been a focal point for chromatography C&D skid owners and designers. This is because every liter lost not only represents concentrated product, but it scales with each separation step. From the previous example, for a 95% efficient, four-step separation (excluding equilibration), if there is a 2% volume loss with each step it will yield a final volume of only 92% of that which one would collect with no losses – that is, 8% additional product loss. Although this is unlikely within the equipment itself, it is possible with a poor equipment design in combination with a poor layout. This is rare in today's production-scale, fully engineered facilities, but may be of more concern in smaller laboratory applications. However, efficient equipment design and full drainability are still essential.

However, hold-up volume actually assumes a decreasing significance in larger chromatography control and dilution skids than in smaller ones. Although this may be counter-intuitive, the reasoning is simple. The ratio of the volume of the skids does not scale linearly with the volume of the bioreactors; it scales more closely with the flow rate through the skids. This flow rate is more related to the dynamics of the entire bioreactor processing cycle with its attendant ramp-up, ramp-down, and non-steady state transients. The goal remains to empty the product tanks quickly, but in a multi-reactor plant there may be adequate down-time between cycles such that the flow rate is set by these other parameters.

Nonetheless, there are several examples of where the flow rate does have a direct impact on hold-up volume. The most obvious of these is the bubbletrap that is typically used to remove entrained gases. A bubble trap is normally sized to provide between 10 and 20 seconds of hold-up volume at maximum flow. Therefore, the bubble trap scales linearly with maximum flow rate (again, not the bioreactor volume). However, because the maximum design flow rate in a larger skid is often not encountered as frequently as it might be on smaller skids, the C&D skid designer has the option of sizing the bubble trap on the lower end of its expected range. These are project-specific decisions.

Another of the large hold-up volumes on the C&D skid appears within the filter housings. These should receive special attention, as the trade-off is often between pressure-drop and hold-up volume. By decreasing filter area and housing size, the hold-up volume is decreased, but other operating factors may become part of the trade-off.

Despite the two equipment items that represent a major portion of hold-up volume, there still remains a significant portion within the tubing and instrumentation. It is noted that for most C&D skids and pump sizes, a standard design velocity is used. Therefore, as the flow rate goes up, only the cross-sectional area of the tubing increases, and it increases with the square of the tubing diameter. Since the velocity is unchanged and the "residence time" in the skid is thus unchanged, one might assume that the volume of the skid would increase linearly with the flow rate. This is close, but not strictly the case for reasons noted previously. In addition,



Figure 4. Chromatography column with C&D skid in the suite.

designers must pay careful attention to fitting dimensions. For instance, by comparing information given in Tables DT-4 and DT-7 of ANSI/ASME BPE-2002,<sup>4</sup> one can conclude that the volume contained in Automatic Tangent Weld (ATW) fittings increases in step increments. Therefore, the volume of a 2" diameter fitting will not be exactly four times that of a 1" fitting, as one might expect. Some C&D skid designers have elected to actually trim the ATW fittings in order to minimize this impact, as this non-linear relationship to the square of tubing diameter can cause larger skids with excessive fittings to trend adversely.

The primary conclusion to be drawn from these facts is that the best technique to minimize hold-up volume losses is to size the throughput accurately and bring additional focus on the volume of the filter housings and bubble trap. However, the percentage of product loss in a large skid, as a function of bioreactor volume, will still usually be significantly less than for a smaller purification train.

For the subject skids, the decision was made to supply all inlets via two separate headers. Header number one had provisions for six buffer feeds and one Water-For-Injection (WFI) feed. Header number two provided for one product inlet, two concentrated buffers, two concentrated NaOH inlets, and three outlets. The valve arrangements on such an assembly can become extremely complicated. If not designed carefully, they also can be a source of much of the non-recoverable hold-up volume as well. Another major factor that is unique to the larger skids is the physical size and weight of the valves themselves. As the valves become larger, the ability to both fit and support the valves becomes more challenging. A careful balance is needed to minimize hold-up volumes while being able to support the valves and yet provide access for potential diaphragm change-outs. For this project, a valve design that used multiple stacked actuators offered a particular advantage in this regard.

Because the facility where the skids would be installed required multi-product capability, the design dictated process flow turn-downs well-beyond normal ranges. As can be seen in Table A, the flow rate ratios were in the range of 50:1.

This required a combination of an in-line reducer system in tandem with variable frequency drives. In addition, the pumps themselves had to be carefully selected to assure that they had the ability to deliver the required flows within the pump RPM range produced by the selection of the VFD, motors, and reducers. As noted previously, this high turn-down requirement also dictated the use of a back-pressure control loop.

Once a combination of pump drives and variable frequency drives had been selected, the panel arrangement and operational methodology came into play. For this project, it was decided that a combination of auxiliary panels, local instrument panels, and local control panels would provide the greatest flexibility from an operator's perspective, while still providing appropriate separations with respect to power and signal applications.

The Auxiliary Panels (APs) were dedicated to housing solenoids, Festo blocks, and buss equipment. They were modeled off of a plant-wide standard that had been selected for the entire project. The C&D skid vendor then had the responsibility for design and fabrication of the panels and for their mounting on the skid. The local instrument panels were dedicated exclusively to the housing of analytical instruments and their local displays. These instrument displays had a requirement to display all information in real time. Finally, the control panels were mounted remotely from the skids themselves and housed in NEMA 4 panels. For this particular project, there was a desire to also utilize plant standards for the specifications and to drive the graphic user interfaces. This is another area in which the ability to customize a large skid for the owner creates benefits. Due to use of a Delta V platform, the C&D vendor wrote and provided the code, which was then loaded and run from the PWCS.

Even after the pumps and control systems have been properly specified and designed, it is necessary to bring a plant-wide view to the skids. Relative locations of the chromatography skids within a facility can have a direct impact on their functionality. In most instances, the purification suites will be located at an elevation below the buffer or product tanks. This can be problematic. As an example, there have been instances where, due to the combination of buffer tank sizes and relative head pressures above the skids, operators have experienced pump flow rates of up to 1/3 of the pump capacity with the pumps off! Proper piping design to allow throttling can resolve this issue.

In addition, the chromatography C&D skid vendor must receive direction on the inlet and outlet elevations for the skids prior to design. Having to lift product out of a trap in a suite, due to a low-level skid discharge point, can be a very expensive fix. However, with proper design and air blow-downs, it should be possible to keep losses between suites to well below 1%.

One more factor comes into play due to discharge elevation requirements imposed on the skid. This is the pump support system. Often the final skid product discharge height drives the pumps to a higher elevation, requiring them to be mounted mid-level on the skid. This can be seen in the photographs

designated as Figures 2 and 3, where the pump height was set by the discharge elevation of the lowest outlet on the header, which in turn was a function of piping locations within the suite. When the pumps are elevated, one must be careful to introduce both enough structural strength in the skid itself for shipping, and enough mass to damp out vibration. Noise also may appear to be elevated from the operator's perspective, as the pumps approach ear-height. These problems are real, but manageable.

## Fabrication and Testing

Because multiple skids were being constructed, DPS was able to use a phased approach for fabrication. This had several advantages. First among these was the ability to perform in-process inspections. These inspections allowed for changes on more than one occasion that created better value in the end product. Once an initial design was proven out, the other three units could be built in sequence. Because these were custom units, specific fabrication teams were selected to perform the same functions on each unit. This assured that delivery timelines were met, as there was no additional learning curve on each unit. Factory Acceptance Testing (FAT) was performed on the first unit 16 weeks after the project was begun by DPS with one skid completed every subsequent two weeks.

The FAT included complete dry and wet testing of the first unit, along with some loop tuning. All of the control modules were proven out, as were all of the instrument loops. Through judicious selection and standardization of the control modules and equipment modules early in the project, FAT was greatly reduced. This was done in a way that still assured compliance with GAMP. After FAT of the first skid, subsequent units were tested both wet and dry. However, the wet tests were limited to demonstrating pump curve compliance and functionality of all instruments that required flooded or flowing conditions. Final loop tuning on the three later units was able to be performed at the project site, based on the actual final conditions. Because this was an eventual commissioning requirement in any case, the time savings were significant. Figure 4 shows the 2-meter column with the C&D skid at its final location.

As is true in most projects, this one had its one major headache. This upset was in one of the simplest and most unexpected areas. Upon completion of all testing, when the drainability of the skids was being tested, the first skid failed. It was discovered that a machining error on the internal of the valves, visible only upon disassembly, had created an improper slope for horizontal configurations. This defect was impossible to detect during a typical incoming receipt inspection. The repair was fairly substantial, but the valve vendor accepted full responsibility to the extent of performing repairs during a normal factory shutdown period. The impact to the owner was minimal as repairs were able to be scheduled around the sequencing of the phased site testing of the skids. This turned out to be another welcome, albeit unexpected, benefit of the phased delivery of the skids.

## Conclusion

Large-scale equipment has become state-of-the-art from the bioreactors through the purification suites in today's biopharmaceutical production plants. Many facilities have not yet focused on the bottlenecks in their plants to the point where they have been willing to up-scale their filtration and chromatography processes. Yet, those facilities that have increased their purification equipment throughput have found they are an easily incorporated element of the process. In addition, the larger units allow for more customization and better integration into the overall plant philosophy of control. For this project, careful preparation of the User Requirement Specifications, coupled with a specialty skid fabricator, created a fully integrated set of large-scale chromatography skids. With attention to several of the key process design parameters, as well as critical equipment selection and design factors, the largest biopharmaceutical chromatography skids built to date have been successfully put into operation.

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