FROM PROCESS DEVELOPMENT TO MANUFACTURING:

Lab-intensive Courses in Downstream Bioprocessing

Gary L. Gilleskie and Baley A. Reeves
North Carolina State University, Campus Box 7928 • Raleigh, North Carolina 27695-7928

Almost 90% of chemical engineering graduates take jobs in industry, with the remainder entering government, education, or self-employment.[1] This fact underscores the need to provide students with experiences similar to what they are likely to encounter in industry in order to prepare them for the challenges ahead. And while students may find this type of experience through internships or cooperative education programs, providing experiential learning opportunities in an academic setting offers a unique opportunity to connect hands-on experience with fundamental principles—something that neither an internship nor participation in a co-op program may be able to provide. There are, of course, challenges to providing this kind of experience to students. Efforts to revise course content and curricula can be time consuming. Further, relatively few course instructors may have industry experience and therefore may not be prepared to incorporate topics that sufficiently benefit a student about to enter industry. This shortcoming in undergraduate and graduate teaching has been acknowledged for many years.[2-4]

In this article, we describe two courses taught at North Carolina (NC) State University's Biomanufacturing Training and Education Center (BTEC) that seek to benefit students by bridging the gap between the traditional academic lab experience and an actual work environment in industry. These courses provide hands-on training and education in downstream bioprocessing principles and practices to chemical engineering students, and to students from other disciplines. BTEC opened in 2007 with a mission to develop skilled professionals for the biomanufacturing industry. Located on NC State's Centennial Campus and operating within the university's College of Engineering, BTEC has 82,500 gross square feet of space that includes 63,000 square feet of labs and 9,000 square feet of classrooms. The labs range from bench scale to simulated cGMP (current Good Manufacturing Practice) pilot scale and are equipped with a multitude of process and analytical equipment required for biopharmaceutical manufacturing. BTEC offers a number of academic courses at the undergraduate, graduate, and post-baccalaureate level, most of which provide significant hands-on experiences in an industry-like setting. These courses support our own minor and master's programs and also support other degree programs throughout the university.

Gary Gilleskie *is currently the head of Operations and a teaching associate professor at the Golden LEAF Biomanufacturing Training and Education Center (BTEC) at North Carolina State University. After receiving his Ph.D. in chemical engineering from the University of Minnesota, he spent 15 years in the chemical and biopharmaceutical industries, focusing on the area of separations. While in industry, he managed downstream process development and cGMP manufacturing groups at Diosynth Biotechnologies, a contract manufacturer of biopharmaceuticals. Since coming to BTEC six years ago, he has been actively involved in the development and delivery of courses in downstream bioprocess development and cGMP biomanufacturing for both university students and industry professionals.*

Baley Reeves *is a senior scientist at the Golden LEAF Biomanufacturing Training and Education Center (BTEC) at North Carolina State University. She holds a Ph.D. in chemical engineering from Princeton University, where she focused her dissertation on developing novel methods for protein purification. At BTEC, she develops and teaches courses in downstream bioprocessing and contributes to process development projects in conjunction with industry partners.*

The two courses described in this article were designed with significant input from biopharmaceutical industry professionals and are titled "Introduction to Downstream Process Development" and "cGMP Downstream Operations." While by no means a new field, downstream bioprocessing is an area in which companies are keen to hire chemical engineers because their background in fluid dynamics, heat transfer, and mass transfer is well suited to the unit operations involved. The courses are closely linked and provide students the opportunity to design, transfer, and execute a downstream manufacturing process. The two courses are taken by undergraduate and graduate students from a wide range of departments at the university. Chemical engineering undergraduates, mostly seniors, make up one-third to one-half of our students, a majority of whom are fulfilling course requirements for the Department of Chemical and Biomolecular Engineering's Concentration in Biomanufacturing Sciences.

The chemical engineering students taking these courses come to us with a solid foundation in biochemistry. As part of their coursework for the Biomanufacturing Sciences Concentration, they take an introductory biochemistry course prior to taking any of BTEC's downstream courses. In addition, they are required to take an introductory downstream course at BTEC that serves as a prerequisite to "Introduction to Downstream Process Development." This eight-week prerequisite provides an overview of chemical and physical properties of biomolecules, including size, charge, and hydrophobicity, and links these properties to the downstream processing steps that are covered in the courses that are discussed here.

A number of examples of lab activities and courses related to downstream bioprocessing have been described previously in engineering education literature. Two of these deal specifically with the use of chromatography for protein separations,[5, 6] while two others cover multiple, integrated bioprocessing unit operations, including chromatography.[7, 8] Looking at these examples as a whole, they include features that build both technical and non-technical skills that will prepare students for what they are about to face in industry, including:

- *Hands-on lab experiences with equipment commonly used in industry;*
- *The use of actual process streams that include real impurities;*
- *Content related to process design;*
- *Opportunities to develop effective communication skills, including report writing and oral presentations;*
- *Experience in working with multidisciplinary teams that reflect the workforce in the biopharmaceutical industry.*

The two BTEC courses described in this paper include all of these features, but additionally include the following features that we believe distinguish them from previous approaches:

- *The courses are designed to follow a path from process design to process transfer and then to manufacturing.*
- *In addition to major unit operations, a number of ancillary operations are included that are part of any biopharmaceutical process, including solution preparation, filter integrity testing, and equipment cleaning/sanitization.*
- *Regulatory requirements—and there are a large number that apply to biopharmaceutical manufacture—are substantially incorporated into lectures and labs for the courses, because these requirements cannot be separated from the technical aspects of downstream processing.*

OVERALL COURSE DESIGN

The two courses described in this paper each run for half a semester—eight weeks—and include both lecture and laboratory components. Students spend one hour and 50 minutes a week in lecture and five hours per week in lab. We limit the number of students in a lab section to 12 to ensure hands-on lab experiences. Lectures present fundamental concepts needed to execute lab exercises and draw appropriate conclusions. Lecture topics are typically followed up by related lab topics in the same week.

As its title suggests, "Introduction to Downstream Process Development" introduces students to the basic concepts in downstream process design. By the end of the course, we expect students to be able to describe principles that underlie major unit operations; "sketch" a process to recover and purify a protein, given appropriate information; design and execute bench scale studies to determine appropriate process parameter ranges; perform basic scale-up calculations; and be familiar with downstream processing terminology.

The second course—"cGMP Downstream Operations"—is focused on the transfer and execution of the process designed in "Introduction to Downstream Process Development" to BTEC's simulated pilot-scale cGMP labs (see Figure 1). By

Figure 1. *An AKTAprocess™ system (GE Healthcare), located in BTEC's large-scale process area, used in the "cGMP Downstream Operations" course.*

the end of this course, we expect students to scale up unit operations based on lab data obtained in the first course; describe components on downstream equipment, including their purpose; execute the set-up, operation, and cleaning/sanitization of downstream equipment; explain how and why biopharmaceutical production is regulated; write standard operating procedures (SOPs) and batch records required for processing; analyze downstream process failures to determine root cause and recommend corrective and preventive actions (CAPAs); and be familiar with cGMP processing terminology.

Lab exercises for each course are based on the production of a model biopharmaceutical—green fluorescent protein (GFP). The *E. coli* cell line uses strain BL21(DE3) with plasmid pET17- b::*gfpuv* that encodes a green fluorescent protein variant known as GFPuv (referred to as GFP in the remainder of this article). GFP was chosen as a model biopharmaceutical because it glows when exposed to a UV light—making it an excellent teaching tool—but also because the downstream process for production of GFP is representative of other biopharmaceutical processes in which protein production is intracellular.

The downstream process for GFP production is shown in Figure 2. Cells are recovered from the fermentor by disc-stack centrifugation. To recover the intracellular GFP, cells are lysed by homogenization, and the resulting lysate is clarified by centrifugation and filtration. Multiple chromatography steps—anion exchange for capture (*i.e.*, the first chromatography step) and hydrophobic interaction for intermediate purification/polishing (*i.e.*, the second step)—are used to remove soluble impurities from the GFP. Finally, the GFP solution is formulated using ultrafiltration and then bulk filled. This process flow diagram is used as a course map for students and frequently referred to so that students keep the integrated process in mind to understand how decisions at one step affect other steps.

Major lecture and lab topics for each course are summarized in Table 1 (page 82). In "Introduction to Process Development," lectures are focused on the fundamental principles and theories related to four major unit operations in downstream processing—centrifugation, homogenization, chromatography, and ultrafiltration—all of which are included in the GFP process. Lectures prepare students to understand experimental design for each lab, correctly interpret results, and perform scale up. Labs are designed to determine relationships between process inputs—that is, process parameters and material attributes—and process outputs. This methodology is central to the principles laid out in ICH (International Conference on Harmonisation) guidance document Q11, Development and Manufacture of Drug Substances,[9] which describes approaches to designing a commercial manufacturing process capable of *consistently* producing drug substance of the intended quality. With these relationships established, process parameter ranges required for transfer to manufacturing scale can be specified and materials selected.

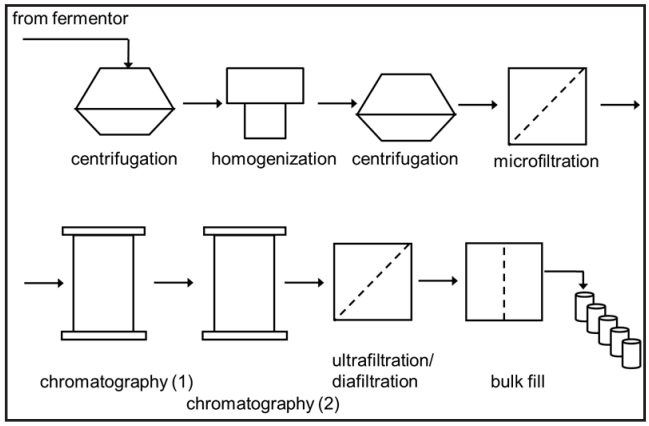


Figure 2. *The BTEC downstream process for production of green fluorescent protein using* E. coli.

It is worth noting that process development in the biopharmaceutical industry requires a mix of theoretical and empirical approaches[10]; consequently, this course involves both. For example, our coverage of centrifugation includes basic concepts such as sedimentation theory and sigma analysis and empirical (*i.e.*, lab) work in which students execute studies to determine a flow rate that results in an acceptable recovery of cells from fermentation broth using a bench-top Westfalia CTC1 Whisperfuge disc-stack centrifuge. Because flow rate is a scale-dependent parameter, students use sigma analysis to scale the lab results to production, which requires the following relationship: $Q_1/\Sigma_1 = Q_2/\Sigma_2$.[10, 11] Q is the volumetric flow rate, Σ is the sizing factor derived from sigma theory, and subscripts 1 and 2 refer to the different scales (*i.e.*, bench vs. production).

Labs for "cGMP Downstream Processing" cover the same major unit operations as the first course. However, the focus is no longer on process design, but on the transfer to BTEC's simulated cGMP large-scale processing area and execution of the process in a cGMP environment. Lectures focus on facility design, equipment design, and cGMP topics. The cGMP topics include the use of procedures and rules for proper documentation; how to address process deviations and develop appropriate corrective/preventive actions based on correct identification of root cause; and guidelines for process validation and equipment qualification. In addition, lab activities adhere to many of the basic cGMP principles required by 21 CFR Parts 210 and 211,[12] including wearing appropriate apparel (gowning), the use of written procedures such as SOPs and batch records, and the calculation of step yields.

CHROMATOGRAPHY

Chromatography is critical to a downstream process because of its role in ensuring a pure and safe biopharmaceutical product. Because of this and the complexity in designing chromatography steps, we spend more time on chromatography than any other topic.

In the "Introduction to Downstream Process Development" course, we devote three lectures and three lab sessions to chromatography. Table 2 provides a summary of chromatography lecture and lab topics.

In the first lecture, we cover basic concepts of chromatographic retention and velocity and provide an overview of product- and process-related impurities in biopharmaceutical processes. A brief introduction to the equipment and column packing is also provided.

AKTAexplorer™ chromatography systems (GE Healthcare

TABLE 1
Summary of Labs for Major Unit Operations in the "Introduction to Downstream Processing" and "cGMP Downstream Operations" Courses

Unit Operation	Introduction to Downstream Processing		cGMP Downstream Operations	
	Key Theoretical Concepts/Principles Covered in Lecture[1]	Key Lab Objectives and Equipment Used[2]	Key Concepts/ Principles Covered in Lecture	Key Lab Objectives and Equipment Used
Centrifugation	• Sedimentation principles • Sigma analysis for disc-stack centrifuges	• Determine the maximum feed flow rate that meets a predetermined % recovery of cells. • Use sigma theory to scale up to BTEC production. • Equipment: Westfalia CTC 1 Whisperfuge	• Disc-stack centrifuge components (pumps, valves, sensors) • cGMP documentation and procedures	• Harvest cells from a 300-L GFP fermentation using parameters determined from previous course. • Execute with cGMP documentation - batch record, standard operating procedure (SOP), etc. • Equipment: Alfa Laval LAPX 404 disc-stack centrifuge
Homogenization	• First-order kinetic expression relating the extent of lysis to pressure and passes in a high pressure homogenizer	• Specify homogenizer pressure and the number of feed passes required to maximize extent of disruption. • Equipment: Niro Soavi NS1001	• Homogenizer components (pumps, valves, sensors • Deviations, corrective and preventive actions (CAPA) • CIP (clean-in-place) cycle design, process equipment design for cleanability • Cleaning validation	• Homogenize cell paste harvested from a 300-L fermentation using process parameter values determined from previous course. • CIP a portable vessel used for homogenization. • Equipment: Niro Soavi NS3006H, Hartel Automated CIP System
Chromatography	Described in the section that follows		Described in the section that follows	
Ultrafiltration	• Modes of operation: concentration, diafiltration, fed batch • Relationship between permeate flux and key process parameters—transmembrane pressure (TMP), feed flow rate, and protein concentration • Guidelines for membrane selection and development studies • Scale-up	• Determine how permeate flux varies with TMP, feed flow rate, and GFP feed concentration. • Select TMP and feed rate values that maximize permeate flux. • Determine diavolumes required for "complete" buffer exchange. • Scale-up to BTEC production. • Equipment: SciLog PureTec TFF system with EMD Millipore Pellicon XL UF Module Biomax 10 kDa	• UF system components	• Using appropriately scaled parameters from previous course, write process instructions for execution of UF step. • Concentrate 30 L of purified GFP using 10 kDa molecular weight cutoff cassettes. • Aseptically bulk fill the final concentrate. • Equipment: Millipore ETU TFF system with EMD Millipore Pellicon 2 UF Module Biomax 10 kDa, ASI Aseptic Single-Use Filling System Model 4MP6000

1) Lectures also include an overview of equipment and typical operating procedures; 2) Product intermediate generated in one lab is used as starting material for the subsequent lab.

Life Sciences), controlled by UNICORN software, are used in the labs and were chosen because they are commonly used in industry for chromatography method development. The first lab exercise introduces students to the UNICORN software by teaching programming basics, analyzing data from inline sensors (*e.g.*, UV and conductivity), and interpreting UNICORN process documentation. Students also pack a 1.5-cm diameter Pall LRC chromatography column to a bed height of 25 cm with Q Sepharose FF, an anion exchange resin, to be used in subsequent labs. The column is flow packed, and students execute a tracer test to determine column asymmetry and height equivalent of a theoretical plate (HETP), two quantitative measures indicating that the flow distribution in the column is acceptable. To execute the tracer test, a pulse of 2M NaCl is injected onto a column that has been equilibrated with a 0.5M NaCl solution. Conductivity monitored at the column outlet produces a peak that is used to calculate column asymmetry and HETP.[13] Columns must meet acceptable asymmetry and HETP ranges before students move forward with subsequent labs.

In the following week, lecture is focused on different types of chromatography—ion exchange (IEC), affinity, hydrophobic interaction (HIC), reversed phase, and size exclusion—and the resin chemistry that makes this possible. We give special attention to the basic principles underlying ion exchange and hydrophobic interaction chromatography, because these are the resins that will be used in lab. We also cover band broadening and rate theory of chromatography using the Van Deemter equation. Our focus then turns to design principles as we cover elution modes—step and linear gradient—and the concept of dynamic binding capacity.

In the second week of chromatography lab, students execute a breakthrough study for the purpose of determining the dynamic binding capacity (DBC) of GFP in clarified lysate on the Q Sepharose FF resin. We define the DBC as the mass of GFP that is adsorbed per unit volume of resin at 10% breakthrough. Students load their Pall LRC column, packed in the previous lab, with clarified lysate until saturation is achieved as determined by UV absorbance. The flow rate is set to 10 mL/min (a linear velocity of 340 cm/hr) based on recommendation from the vendor. During the run, flowthrough samples are collected, and the GFP concentration in each is measured by direct fluorescence.

TABLE 2
Chromatography Lecture and Lab Topics in "Introduction to Downstream Process Development"

Week	Lecture Topics	Labs
1	• Basic retention and velocity concepts • Process- and product-related impurities • Bench-scale chromatography systems and their key components (including detailed coverage of UV and conductivity measurement) • Introduction to column packing and evaluation by measuring height equivalent of theoretical plate (HETP) and asymmetry	**Lab 1:** Introduction to AKTAexplorer™ systems **Lab 2:** Column packing and evaluation for bench-scale studies
2	• Stationary phase properties (bead size, pore size, chemistries) • Overview of chromatography techniques by separation mechanism (ion exchange (IEC), affinity, hydrophobic interaction (HIC), reversed phase, size exclusion) • IEC and HIC principles and procedures • Band broadening and rate theory (Van Deemter) • Step vs. linear elution, bind-and-elute vs. flowthrough chromatography • Equilibrium and dynamic binding capacity, linear and Langmuir equilibrium isotherms	**Lab 3:** Dynamic binding capacity of GFP lysate on anion exchange resin (Q Sepharose FF) **Lab 4:** Anion exchange (Q Sepharose FF) for GFP capture and elution step design
3	• Development study objectives—binding capacity, selectivity, and product recovery • Guidelines for resin selection using the capture → intermediate purification → polishing model • Guidelines for buffer/solution selection for IEC and HIC • Scale up	**Lab 5:** HIC step design for intermediate purification of Q Sepharose FF eluate

The DBC value is required so that students can determine the volume of clarified lysate that can be loaded onto the column, a key process parameter for chromatography. Overloading a chromatography column operating in bind-and-elute mode will result in product loss, and we suggest loading approximately 50% of the DBC to the column, which results in the following relationship: $C_{load} \times V_{load} = 0.5 \times DBC \times V_{bed}$, where C_{load} is the concentration of protein in the column load, V_{load} is the load volume, and V_{bed} is the volume of the packed bed.

Once an acceptable load volume has been determined, students execute a development run. Chromatography offers a large number of process parameters that can be studied. We choose to focus on parameters related to the design of the elution step. Specifically, students determine whether a step elution or linear gradient elution provides better separation with respect to two key process outputs—product purity and recovery.

To carry out this study, each three-member lab group is assigned a different elution step design. One group executes a step elution in which NaCl concentration in the 50mM Tris, pH 8.0 elution buffer is increased from 0M to 0.2M immediately upon the start of the elution step and held at 0.2M for the duration of the step. Another group executes a linear gradient in which NaCl in 50mM Tris is increased from 0.1M to 0.2M over four column volumes. A third group executes the same gradient over 10 column volumes. Data from each run are shared with all lab participants.

During week 3, lecture topics include a review of key development study objectives, including: maximizing purity and product recovery; guidelines for resin and buffer selection; and chromatography scale up. Scale-up guidelines are based on keeping residence time constant by maintaining a constant bed height and linear velocity, so that the column is scaled

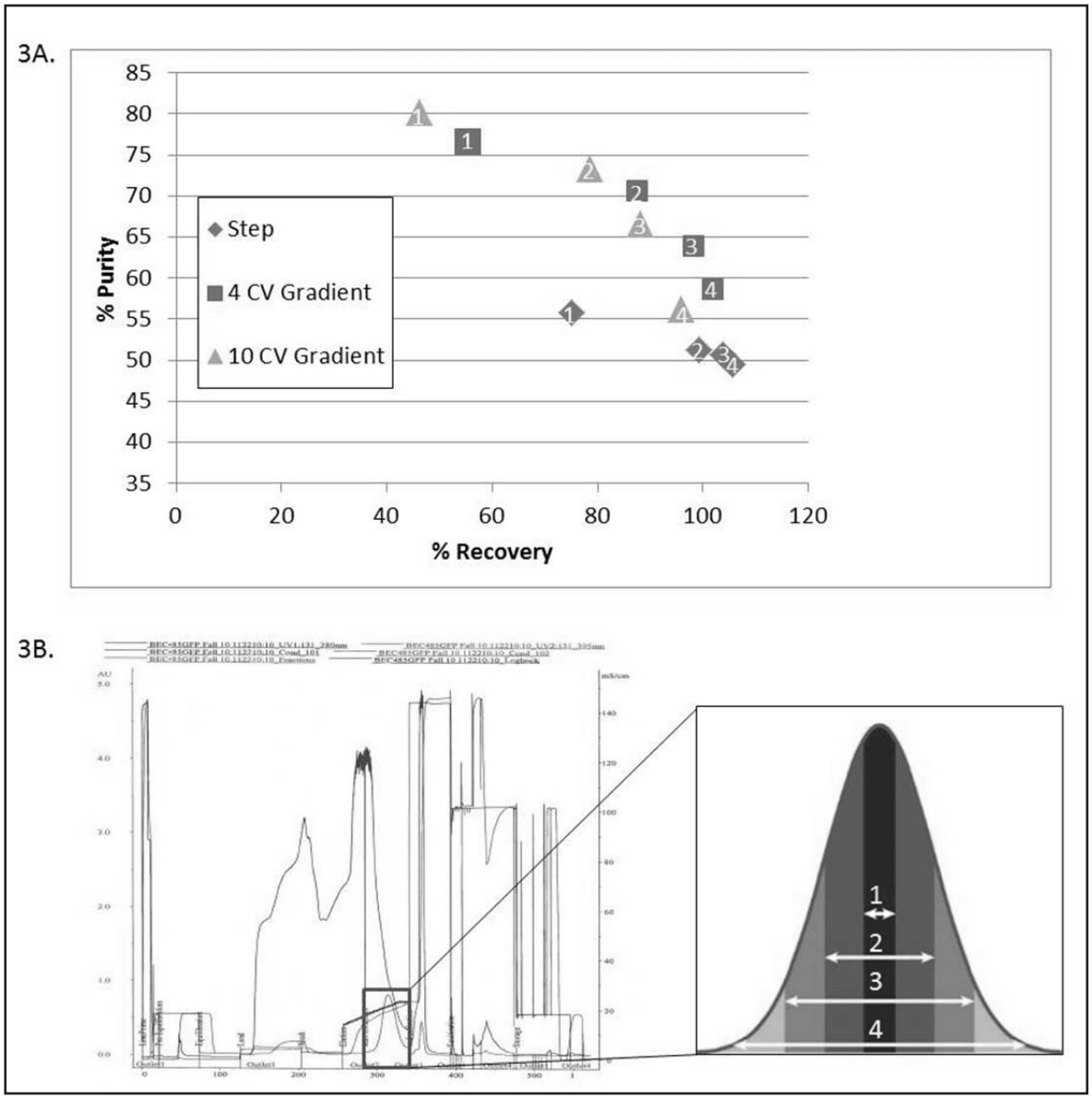


Figure 3A. *Typical student results comparing the percent purity vs. percent recovery for different fraction pooling scenarios (see 3B).*
Figure 3B. *A typical chromatogram for the GFP capture step and elution peak (inset). The elution peak (inset) is fractionated, and GFP concentration and total protein concentration determined for each fraction. From these values, students determine the percent purity and percent GFP recovery for different pooling scenarios, as plotted in 3A.*

by diameter. In the lab students evaluate data generated from week 2. To do this, fractions collected from each of the product peaks for each elution method are pooled computationally to produce a plot of % product purity vs. % product recovery as shown in Figure 3. Recovery is calculated as the mass of GFP collected during elution divided by the mass of GFP loaded to the column. Purity is determined simply as the ratio of the concentration of GFP in the pooled fractions to the concentration of total protein in the pool. GFP concentration is measured by a direct fluorescence assay, while total protein is measured either using the Pierce 660nm Protein Assay or the Pierce BCA Protein Assay. In addition, students perform mass balance calculations to determine the percentage of GFP loaded to the column that can be accounted for in the chromatography run. For many students, this is their first time doing mass balance calculations using real process data. Some are surprised when, as a result of assay variability or process variability, more or less than 100% of the GFP loaded can be accounted for.

As seen in Figure 3, there is an inverse relationship between purity and recovery; that is, the larger the number of fractions pooled, the greater the recovery but the lower the purity of the GFP "product." Because in an actual biopharmaceutical process, this capture chromatography step is typically followed by two additional chromatography steps, we emphasize the importance of recovery over purity for capture. To help students choose which fractions from their elution peak to pool and use for subsequent process steps, we set a minimum recovery requirement of 80%. They must choose the process parameter—in this case, the use of a step elution or one of the linear gradients—that meets the product recovery requirement. Students then pool the appropriate fractions that will give them the highest purity while still meeting the minimum recovery requirement. It should also be noted that the choice of fractions to pool sets the product collection criteria for capture chromatography at large scale. Specifically, the UV absorbance (at 280 nm) ranges that correspond to the fractions chosen become the UV "gates"—that is, the start and stop product collection criteria—for the elution peak at large scale, a key design parameter that must be transferred to manufacturing.

The resulting product pool is then subject to an intermediate chromatography step for further purification. This portion of the course is less protocol-driven, and freedom is given to the students to design their own step. As with the capture step, resin options are discussed with students, including the advantages and disadvantages of each. Once they have selected their resin—either cation exchange or HIC—we discuss the buffers required to execute the chromatography step. The students use a HiTrap column (GE Healthcare), which is much smaller than the Pall LRC column used for developing the capture step, packed with their resin of choice. The students program a UNICORN method to execute the step they have designed. They are given complete creative control in specifying the solutions and volumes required for each step of the chromatography run, the type of elution method used (step vs. linear gradient), and how to monitor their process. Students make these decisions based on their results from and analysis methods used in previous lab exercises. Upon completion of these chromatography activities, students summarize their work, including all design-related decisions, in a short lab report.

At this point in the "Introduction to Downstream Process Development" course, chromatography lab activities are complete, and students move on to the ultrafiltration lab described previously in Table 1.

In the follow-up course,"cGMP Downstream Operations," the in-depth study of applied chromatography is continued. Table 3 provides details of the topics covered. With a designed anion exchange capture step in hand, students execute a series of activities that are required for the transfer and execution of a chromatography step in a cGMP production environment. Students begin by performing an operational qualification (OQ) of the AKTAprocess™ (GE Healthcare) system that they will use in their actual production run. They then pack a 30-cm BPG column (GE Healthcare) for execution of the capture chromatography step for the GFP process. They develop a procedure by scaling up their results from the bench-scale studies performed in the previous course. Finally, they execute an actual run. These activities are described in greater detail below.

TABLE 3
Chromatography Lecture and Lab Topics in "cGMP Downstream Operations"

Week	Lecture Topics	Labs
1	• Installation and operational qualification (IQ and OQ) of equipment • Process validation requirements • Flow through a packed bed (Darcy's law and Blake-Kozeny equation) • Pressure-flow curves • Chromatography packing methods at large scale	**Lab 1:** Operational qualification of an AKTAprocess™ system **Lab 2:** Flow pack of a 30-cm BPG column
2	• Control charts and process capability analysis	**Lab 3:** GFP capture by anion exchange chromatography at production scale

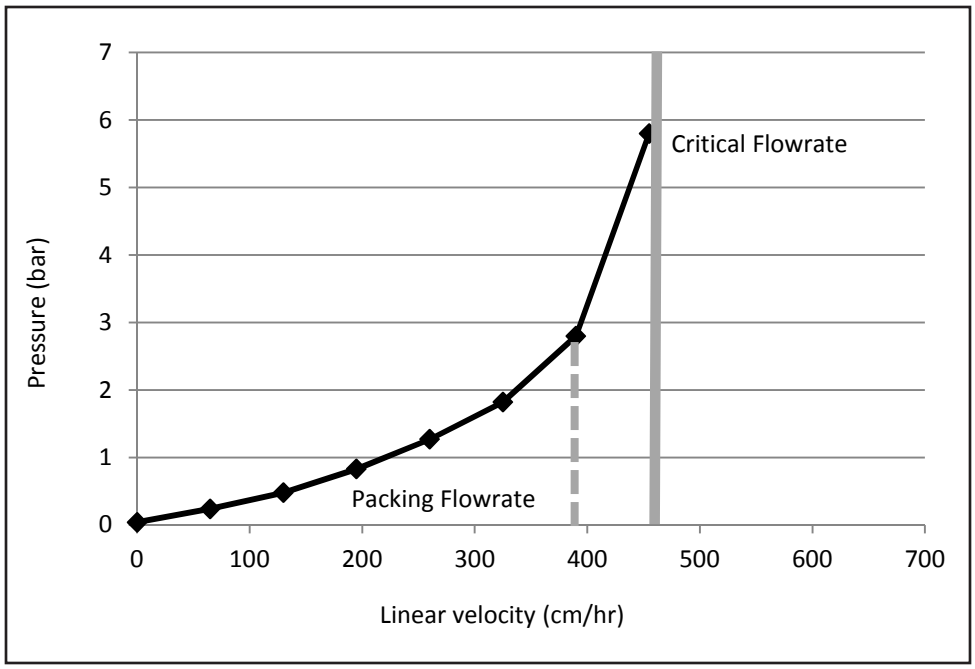


Figure 4. *Pressure-flow curve to determine packing flow rate. The critical flow rate, defined as the flow rate at which the slope becomes infinite, was measured as approximately 450 cm/hr; higher linear velocities could not be achieved due to pressure limitations of the column. The packing flow rate is calculated as 85% of the critical flow rate.*[16]

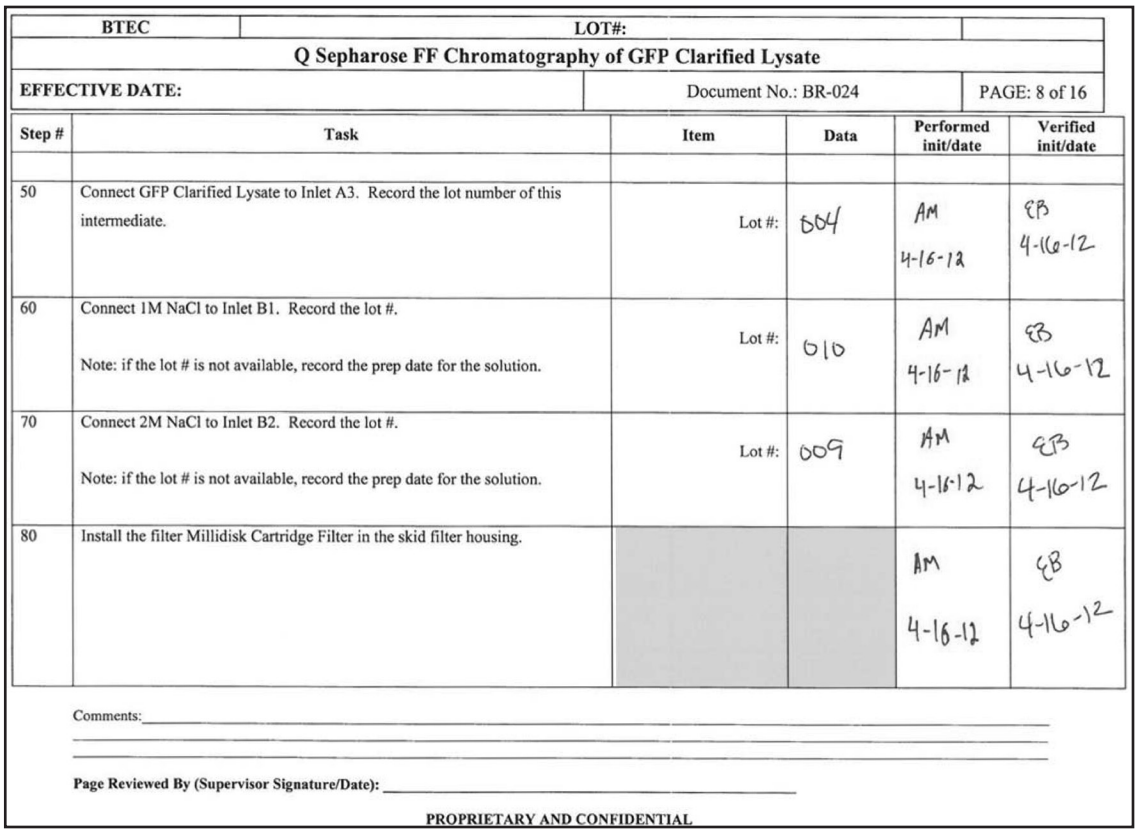
Equipment OQ is a key part of validating a biopharmaceutical process. It specifically refers to a documented activity that verifies that utility and equipment systems operate in accordance with the process requirements through all anticipated operating ranges. OQ must be executed prior to the "conformance" batches that are required to show processing consistency[14] and are central to a process validation program.

Students execute the OQ with a protocol that mimics the format and testing that would be used in an industrial setting. Tests include confirmation that valves perform properly when given a signal from the UNICORN controller; that valves are in the proper configuration in response to various controller commands; that column air sensors correctly detect the presence of liquid or air in the process lines; that system pumps actuate properly and deliver a flow rate that matches a set point; that conductivity and pH elements read accurately by checking their calibration with standard solutions; etc. Not only does this exercise teach students—in a hands-on way—what "qualifying equipment" means, but it also serves as an effective tool for teaching details of the piece of equipment they are working with.

Following the OQ exercise, students pack a 30-cm diameter BPG chromatography column in preparation for the anion exchange capture step that is part of the production-scale GFP process. Prior to packing, students generate a pressure-flow curve,[15] using the same resin and column to be used in the production process. The curve, which is generated by varying the flow rate of packing buffer, monitoring the resulting pressure values, and plotting pressure vs. flow rate, is used to define the target flow for column packing.[16] An example is shown in Figure 4. Upon completion of packing, the HETP and asymmetry are measured for the 30-cm column.

Once the column is packed, students go through a paper exercise of transferring the capture chromatography step developed in the process development course to the large-scale lab, using the 30-cm column. These calculations are done following the scale-up guidelines described previously for the "Introduction to Downstream Process Development" course. These guidelines are used to determine the volume of clarified lysate that can be processed by the 30-cm BPG column and the corresponding operational volumetric flow rate. Once the pertinent scaled-up parameters have been calculated, the process is executed in our simulated cGMP facility using appropriate cGMP written procedures, including both a batch record and an SOP. A sample page from the batch record used to execute the capture step is shown in Figure 5.

After the chromatography labs are completed, students are presented with several related assignments. One of those involves data trending. Per the FDA process validation guidelines,[14] it is expected that companies provide continual assurance that the process remains in a state of control—the "validated" state—during commercial manufacture. A key part of this demonstration is that data—both process- and product-related—be trended and analyzed to demonstrate that the process is under control. Students are presented with step yield data from a number of capture chromatography runs for the GFP process, and they perform a process capability assessment[17] to determine whether the process as designed is capable of meeting a pre-determined step yield range. Students numerically assess process capability using a capability index, C_p, defined as $C_p = (USL - LSL)/6s$. USL and LSL are the upper and lower specification limits, respectively, and s is the standard deviation of the data set. C_p values greater than one suggest that a process is capable of meeting the specification for a given parameter; C_p values less than one suggest the opposite. It is worth noting that process capability analysis is not unique to cGMP processing, but is used throughout many industries as a means to assess process variation, and is a key Lean Six Sigma tool.



BTEC	LOT#:	
Q Sepharose FF Chromatography of GFP Clarified Lysate		
EFFECTIVE DATE:	Document No.: BR-024	PAGE: 8 of 16

Step #	Task	Item	Data	Performed init/date	Verified init/date
50	Connect GFP Clarified Lysate to Inlet A3. Record the lot number of this intermediate.	Lot #:	004	AM 4-16-12	EB 4-16-12
60	Connect 1M NaCl to Inlet B1. Record the lot #. Note: if the lot # is not available, record the prep date for the solution.	Lot #:	010	AM 4-16-12	EB 4-16-12
70	Connect 2M NaCl to Inlet B2. Record the lot #. Note: if the lot # is not available, record the prep date for the solution.	Lot #:	009	AM 4-16-12	EB 4-16-12
80	Install the filter Millidisk Cartridge Filter in the skid filter housing.			AM 4-16-12	EB 4-16-12

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Figure 5. *One page from the batch record used to execute the capture chromatography step of the GFP process.*

In another assignment, students are presented with a deviation scenario in which step yield for a capture chromatography step falls out of range. The cGMP regulations require that the deviation be addressed. In a lecture earlier in the course, we teach our students about typical components of a deviation report. Central to addressing a deviation is root cause determination and developing appropriate corrective and preventive actions (CAPAs). We teach root cause analysis through use of fishbone diagrams. For the assignment, there are numerous possibilities for the root cause; students must identify the root cause, develop appropriate CAPAs, and complete all other components needed to address the deviation. Students perform this exercise in small groups of two or three students, and upon completion, they make an oral presentation to the class on their findings.

ASSESSMENT OF LEARNING AND PROGRAM EVALUATION

To assess student learning, a number of different tools are used throughout each eight-week course:

- *Periodic quizzes. These are focused on lecture content, which includes the principles underlying unit operations and regulatory requirements. They also test whether students are keeping up with the biopharmaceutical lexicon used throughout each course. The regularity of the quizzes ensures that students stay on top of material, a necessity for these lab-intensive courses.*
- *Lab reports and presentations. In the "Introduction" course, lab reports are used to ensure that students are making correct design decisions from the data they have obtained. They also help students to develop clear and concise writing skills. In the "cGMP" course, presentations are used for the deviation exercise to help build oral communication skills.*
- *Homework assignments. Homework is calculation intensive and gives students practice in applying the principles and theories taught in the courses.*
- *Final exam. The final exam is comprehensive, covering all lecture and lab material.*

BTEC obtains feedback from students through a university-administered end-of-course survey. Among the many statements that students are asked to rate, two of the most important are 1) "this course improved my knowledge of the subject" and 2) "overall, this course was excellent." Results from all surveys administered since the "Introduction to Downstream Process Development" course was first offered in 2007-2008 show that 95% of all students agree or strongly agree with statement 1 and 93% agree or strongly agree with statement 2. For the "cGMP Downstream Operations" course, 99% of all students agree or strongly agree with statement 1 and 96% of all students agree or strongly agree with statement 2.

In addition, we recently sought feedback regarding course effectiveness from former students who are currently working in industry. These students were asked to respond to a series of 12 questions related to their current position and the courses covered in this paper. We received feedback from nearly 40 former students. When asked whether the downstream courses

TABLE 4
Responses to Survey Questions Asked of Former Students Who Have Taken "Introduction to Downstream Process Development" and "cGMP Downstream Operations"
Note: 5 indicates strongly agree and 1 indicates strongly disagree.
Only former students actively working in a position involving downstream processing responded to this set of questions.

Question	Mean	5	4	3	2	1
BTEC's downstream processing courses covered material (*e.g.*, centrifugation, homogenization, chromatography, ultrafiltration, design, scale up, cGMP, quality systems, etc.) that you find relevant to your current job.	4.83	15	3	0	0	0
BTEC's downstream processing courses provided skills (*e.g.*, operating and cleaning downstream equipment, packing a chromatography column, addressing deviations, etc.) needed to carry out the responsibilities of your current job.	4.71	12	4	1	0	0
BTEC's downstream processing courses have given you a professional advantage, relative to co-workers who did not take these courses.	4.47	11	4	1	1	0
If you were a supervisor, would you send your employees to a BTEC course in downstream processing?	4.89	18	0	1	0	0

prepared them for their current position, 87% responded "yes." The 13% who responded "no" did so because they are not working in the area of downstream processing. In addition, 95% of all respondents felt that they started their job with more fundamental knowledge and better job skills than if they had not taken these courses.

Among those working in an area related to downstream processing, we asked a series of more detailed questions related to the relevance of material taught in "Introduction to Downstream Process Development" and "cGMP Downstream Operations." A summary of those questions and responses is given in Table 4.

Responses indicate that most former students thought that course content and actual skills acquired as part of the BTEC courses were useful to their current position and gave them a "professional advantage" over co-workers who did not take these courses.

Some of the comments we have received from former students and supervisors of our former students are shown below:

> *"Very informative. Loved the fact it was hands-on. By far the most informative and helpful course I took in my Undergrad."* (from survey)

> *"My hiring managers cared more about my BTEC minor than my degrees in chemical engineering and biochemistry."* (from survey)

> *"I have found that simple things like how to go about using a fishbone diagram for investigations... people at my job find amazing that I learned in school and they do not have to show me how to do."* (from survey)

> *"I had never heard of anyone coming out of school that knows how to use a disk-stack centrifuge or knows how to calculate discharge intervals."* (Process Development Engineer from Genentech)

CONCLUSIONS

BTEC has developed and delivered courses that cover topics associated with the development of downstream bioprocess and cGMP manufacture of biopharmaceuticals. These courses were designed with a number of features that enable them to provide an industry-like experience, including: the use of real process streams with real impurities; content that is focused on process design, process transfer, and execution at manufacturing scale; group work in multidisciplinary teams that mirror those in industry; and incorporation of regulatory requirements that apply to biopharmaceutical production. At the same time, they cover the basic principles and theories underlying downstream bioprocessing. Based on a survey of former students currently working in industry, student satisfaction is high, and the courses seem to be effective at preparing students for careers in biomanufacturing. Thus our primary objective of developing skilled professionals for the biomanufacturing industry is being met. Perhaps the best evidence of the program's success is the continual growth that we have seen over the five years that the courses have been offered. When the courses started in the 2007-2008 academic year, enrollment in each course totaled about five students; that number has steadily increased to 20-30 students in each course per semester. For the courses to continue to provide an industry-like experience, it is important that they constantly evolve, to keep up with industry practices and regulatory changes. To this end, we actively seek input from our industry collaborators.

We believe that some of the concepts used in these two courses can be integrated into other chemical engineering courses, even without the equipment and laboratory resources available at BTEC. For example, unit operation labs can focus on establishing the relationship between process inputs and

process outputs that are relevant to a manufacturing environment. And while many students may not take jobs in the pharmaceutical/biopharmaceutical industry, many different industries are implementing quality management systems (*e.g.*, ISO 9001) and operational excellence programs (*e.g.*, Lean Six Sigma) with many features in common with cGMP. Consequently, there is value in introducing basic cGMP concepts such as the use of detailed procedures, addressing deviations through proper root cause analysis, developing CAPAs, and validating a process, etc., into lab courses regardless of the type of equipment available.

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