This paper presents a case study in the use of a CAPE tool for the development of a protein synthesis and purification process. The paper has three main objectives. First, it discusses the use of the software in representing the process and in determining the overall material balance and flows. It indicates how this information is used in meeting environmental and safety regulations and to assist with process troubleshooting. It also describes the unit operations typically employed in bulk protein manufacturing, how these are modeled with the software and learning points from the model development process. Second, the paper briefly discusses the context and environment within which the software was deployed at the company. Business drivers, project structure and linkages to other corporate efforts are all described. Third, the paper discusses the evolution of the tool and the direction of its technical development, including expanded CAPE functionality and utilization within the pharmaceutical industry.

1. BUSINESS DRIVERS FOR CAPE IN THE PHARMACEUTICAL INDUSTRY

The pharmaceutical industry faces several significant challenges today. Increased pressure to lengthen the protection accorded by patents has resulted in the need for rapid and effective process development. Improved development reduces costs and leads to processes with higher economic efficiency. In the current climate of increasing globalization, economic efficiency will dictate whether a manufacturer will be able to remain competitive, an issue of special relevance to the pharmaceutical industry, which faces a long government approval process for its products.

The use of CAPE tools can reduce the duration of the development cycle as well as support the development of processes that are more efficient, environmentally friendlier, safer and more cost effective by allowing more alternatives and options to be evaluated [1]. They can support recipe development, material selection, equipment sizing and design, scale-up and process optimization. The use of these tools can lower the barriers between development and manufacturing, thus shortening the facility delivery process. They can also support engineering workflows and technology transfer among laboratory chemists, pilot-plant engineers, process engineers and others who play a role in process development. Batch Plus™ [2] is one such tool currently available.
2. PROCESS DESCRIPTION

In the protein business many of the products utilize batch manufacturing in multi-step processes. Fermentation is typically used to make the protein while chromatography, tangential flow filtration (TFF) and drying are often used to isolate and purify it.

2.1. Protein Expression

A recombinant-derived cell typically expresses the protein. The cells are fermented and then harvested. The unwanted cell parts are then separated from the desired protein. The protein is then purified and chemically altered using chromatography, tangential flow filtration and chemical reactions. A recombinant-altered cell has DNA added to its plasmid to give it instructions to do something it normally does not. In the pharmaceutical industry, the cells are given instructions on how to express the required protein chain. In some cases, the cell cannot make the exact product required; therefore a protein that is close to what is needed is produced. This “similar” protein is then altered and purified to give the final product, using multiple unit operations. When the protein is relatively small, e.g., a peptide or polypeptide, the use of solvents does not irreparably damage the structure of the protein and reverse-phase chromatography can be used to provide an extremely pure product.

2.2. Protein Purification Chemistry and Equipment

Chromatography, enzymatic reactions, tangential flow filtration and drying are typical unit operations used in biotech processing.

Chromatography is a separation technique based on the protein’s affinity to adsorb to and desorb from a solid surface (resin). There are several types of chromatography such as ion exchange, reversed phase and size exclusion. A typical batch process would call for an equilibrating solution to be run across the column to prepare the resin for the protein solution. The protein solution would then be charged onto the column. This might be followed by a wash to remove any material not bound to the resin or weakly bound to the resin. Next, the column is eluted with either a gradient or isocratically, the mixture of proteins then desorb based on their relative affinity for the resin and elution fluid. When the product protein leaves the column it is then segregated from the rest of the material for forward processing. At the end of the elution one or more fluids are run across the column to remove any remaining material; this is referred to as regeneration.

Tangential flow filtration is a separation technique used to do size separation. In order to be separated, the difference in size of the molecules needs to be an order of magnitude or greater. A membrane is used to separate molecules of different sizes. Based on the size of the membrane, TFF can be referred to as reverse osmosis, ultrafiltration, microfiltration or viral filtration. The basic principles are the same for all membrane sizes. The fluid is circulated across a membrane with higher pressure on the feed side than the permeate side. The molecules that are small enough to go through the membrane are the permeate and those retained by the membrane are the retentate. Typically, a large quantity of low concentration material is circulated across the membranes until a portion of the carrier fluid and smaller
unwanted proteins pass through the membrane leaving the retentate more concentrated. Sometimes it is desirable to change the carrier fluid, and therefore, the retentate would be diafiltered. The new buffer would be added to the retentate as permeate is leaving, thus keeping the concentration of the protein constant.

Drying can take several forms including spray, vacuum and lyophilization. The technique used most often is lyophilization since most proteins cannot be crystallized. In lyophilization, the protein solution is frozen, then a vacuum is pulled and the product is slowly warmed. During the warming period, the protein solute is removed by sublimation. At the end of the cycle all volatile material has been removed, leaving a stable powder.

3. CHALLENGES FOR PROCESS ENGINEERS AT LILLY

The challenge for the process engineer is to quickly translate the development process to a scaled manufacturing process. At the manufacturing scale, many factors that are transparent during the development can become rate limiting, at best, and detrimental to the process, at worst. The rate of heat transfer for a reaction, the size of a column, the quantities of raw materials required could all be issues when the batch size is changed significantly. A macro-level look at the equipment and utilities required is usually done with a spreadsheet. The spreadsheet would list the manufacturing steps (TFF, chromatography, reactions, etc.), expected yields, step sizes (mass in), expected capacity, column dimensions, ultrafiltration membrane area, vessel working volumes, raw material requirements and production costs.

Once a draft of the manufacturing process is determined, issues and feedback are typically given to development to have new ideas incorporated into the production scheme. Once most of the design criteria are agreed to, it is then possible to start creating a model of the process. The model usually takes several iterations. Each process may be modeled separately, then combined with the other steps and finally linked to raw materials and utilities. One of the larger challenges is to determine the environmental effects of the process. In most cases a mass balance must be determined which details VOC emissions, fugitive and point source, along with solid and liquid wastes generated.

3.1. Description of CAPE Efforts at Lilly

At Lilly, spreadsheets have long been the tool of choice for almost any kind of computational engineering problem involved with process analysis. Only in the past four to six years have commercial off-the-shelf tools been considered for wide deployment to support process engineering efforts. Seeing a growing CAPE market, Lilly decided in 1996 to license a suite of engineering software tools with both batch and continuous process modeling capabilities. In 1997, after completing a selection process, Lilly chose AspenTech as its primary vendor of choice for several reasons including the availability of many CAPE applications in its Aspen Engineering Suite.

After the selection and procurement were completed, the software deployment phase began late in 1998. The company’s central engineering organization managed the initial deployment
as expertise with the software developed and continues to develop today. The current deployment focuses specifically on the Batch Plus tool for performing material and energy balances, emission calculations and graphical and text-based recipe descriptions.

Currently, the effort to utilize Batch Plus as a standard tool is being driven by the evolution of the technology (bug-fixes and functional enhancements), developing the infrastructure, and learning how to apply it with a critical mass of users [3]. The Batch Plus infrastructure includes several central support functions such as: on-site training, internal user group meetings, troubleshooting support, bug tracking and model-development support that also encompasses contract modeling.

Although the deployment was initially targeted to the company’s small molecule, i.e., “synthetic” late-stage development and manufacturing, environmental obligations later drove the deployment to include the large molecule, i.e. “protein” side of late-stage development and manufacturing. Future applications at Lilly may include fill-finish manufacturing and technical knowledge transfer and scale-up in mid and late stage development. The case study presented in this paper provides one example of the benefits and current limitations of utilizing the Batch Plus technology in a protein purification process.

4. PROJECT SCOPE AND DESCRIPTION

The scope of this project was to determine a mass balance for solids and liquids entering and leaving the process. The types of generated waste in the process are aqueous, non-aqueous and urea. The aqueous waste can be sent to the local sewage treatment plant for further processing while the non-aqueous waste is sent to a liquid incinerator and the urea waste is sold to a vendor. Due to the nature of the product being manufactured, any amount of product in the urea waste would exclude it from sale to our vendor. The characterization of the waste stream quantities and components was required to ensure we remained within our operating permits. Due to time constraints, this needed to be done quickly and in a manner that allowed updates to be done with minimal effort.


Several methods for determining the mass balance were considered: hand calculations, spreadsheet and available software. Using hand calculations was quickly eliminated as a viable method due to the number of calculations required, the need to re-work entire sections if quantities of buffers or concentrations were changed and the difficulty of ensuring accuracy and widespread review.

The advantage of using a spreadsheet is that it is easily tailored to the specific process being modeled and can be set-up to the engineer’s particular style and needs. Once completed, it is fairly easy to update changes in quantities and concentrations in the spreadsheet. The disadvantages are that the spreadsheet is typically difficult for others to use, it is time consuming to set-up, the calculations need to be checked and it can be difficult to add or change sub-steps.
The three software options considered were SuperPro Designer™, Batches™ and Batch Plus™. Batch Plus was chosen primarily because it is an Eli Lilly supported tool and is becoming the corporate standard. Process engineers and consulting engineers from Lilly’s Engineering Technology Center have been working with AspenTech to improve the product (specifically the biotech capability) and so this was an excellent opportunity to review recent upgrades and give input on future improvements. As a result, it is expected that application of the model by future engineers will be fairly straightforward. Also, if needed at a later date, the air emission calculations could be programmed into the model without much additional effort. One potential benefit not yet capitalized on is the link to our historian, Aspen Process Explorer.

4.2. Model Development

The model development was segmented into smaller activities: information procurement, recipe detail, backbone assembly, workarounds, model refinement, error checking and updates. The information gathered for use included flow documents, batch records, buffer recipes and equipment diagrams. Once the information was gathered, agreement was reached around the level of detail that would be required and how to construct the model (hierarchy, standards, etc). In this situation, since timelines were a significant constraint, focus was placed on the mass balance and some of the details required to get accurate emission calculations and cycle time estimations were left out of the model.

The backbone of the model was then assembled and reviewed by an engineer experienced with the software but not the process. During the assembly it became apparent that some operations could not be directly modeled. For example, the use of a pre-column that goes directly to another column could not be done since a column output could not become another column’s input. To remedy this situation a tank was added to the model so the pre-column sent its output to the tank, which was then the input for the next column.

Model refinement and error checking was performed by an engineer versed in the process but with little experience (other than a two-day training course) in using Batch Plus. The combination of experts in both modeling and processing made for efficient time use. Error checking was done using the spreadsheet mentioned above, making sure the inputs went somewhere and using the Batch Plus Run History report. This report helped to determine that more was going into some vessels than they would hold or when a vessel feeding a column didn't have enough material in it to meet its charge requirements.

4.3. Model Outputs

Since the output reports were available as Excel spreadsheets, this made further data manipulations or use by others more transferable. In this case, the basic material balance needed to be captured, and specifically, any waste quantities. This was done using macros to adjust the stream table. One of the drawbacks of the stream report was that if 100 g of 15% by weight NaOH was used, for example, the stream table showed it as 15 g NAOH and 85 g water. If it is desired to total how much 15% NaOH would be required to process a lot or
campaign, then the user would need to take the NaOH total and back-calculate the amount since the report captured quantities by pure components only and not by mixture.

4.4. Learning Points
The abilities of Batch Plus to model a process are extensive. Therefore, deliberate decisions as to what is needed and how the model will be used are required. It worked better on this project to limit the initial scope with the potential of building onto the core model later as needs evolved. This allowed for a small success, with the building blocks for larger, more complicated models firmly planted.

The use of the having a modeling expert design and program the backbone of the model with a process expert later refining it made the modeling go smoothly and the task reasonable for both parties. The model can now be easily updated for process improvements.

The software proved that it could provide a valuable output. The mass balance was reported in a manner that was understandable, supportable and maintainable. A few “work-arounds” were required for chromatography and tangential flow filtration but these were fairly straightforward. As feedback from this case and others is integrated with future releases, the utility of the program will increase for biotech process modeling. This model used relatively little of the software’s potential functionality but it demonstrated that expanded use in the future would likely be valuable. The next evolution of this model will likely be for cycle-time analysis and emission calculations.

5. EVOLUTION OF SOFTWARE

Batch Plus is an advanced simulation and data management tool for the modeling of complex, recipe-based batch processes such as those found in the pharmaceutical, agricultural chemical, biotech and specialty chemical industries. The tool supports a wide spectrum of engineering workflows over the full lifecycle of a process. The evolution and technical development of Batch Plus is expected to continue along several dimensions that expand its ability to support these workflows and address significant business problems. This section describes some of these enhancements.

Batch Plus contains a comprehensive library of unit operations and models that supports the incremental development of a recipe at different scales and permits the construction of a single composite model of the entire process. These models range from shortcut models that perform mass and energy balances to rigorous models that allow predictions of process performance. The enhancement and extension of the operations library is one path along which the evolution of Batch Plus is expected to continue. Each successive version of the software has included new operations and models or enhancements to existing ones. The protein purification process model described in this paper made extensive use of the chromatography models in Batch Plus. The updates and enhancements to the unit operation models that rendered them appropriate for use in this project were made in direct response to Lilly’s requests. As a result of Lilly’s recent biotech modeling efforts, additional
functionality has been requested including non-linear elution gradients, regeneration with material originating from a vessel, factor-based calculation for concentration operations, hierarchical material and energy balance reporting and the ability to utilize the “custom” operation with biotech equipment.

A related path along which Batch Plus is expected to develop is the continued expansion of its modeling coverage for secondary, packaging and solids processes. In the pharmaceutical industry, a distinct manufacturing process is usually responsible for producing and packaging a drug in its final finished form. This secondary process, as it is often referred to, has as its starting point the active ingredient or bulk chemical produced by the primary process. Several new features were implemented in a recent version of Batch Plus that allows it to support engineering projects involving secondary processes [4]. These include new unit operations such as mill, granulate, freeze-dry, screen, tablet and fill, the ability to represent particle size distributions for solid materials and the ability to account for the consumption of packaging materials, e.g., bottles, cartons and vials, in the process.

In the coming years, the pharmaceutical, biotech and other batch process industries are expected to face several new challenges in complying with the regulatory requirements promulgated by environmental bodies such as the US EPA and state agencies. The new rules that are being formulated will introduce several new calculation methods that have to be implemented at an early stage of process development. The Pharma MACT rules are an example of such a new regulation. The US EPA 1978 CTG equations, the US EPA 1994 ACT equations and the recent US EPA MACT equations are currently implemented in Batch Plus [5]. These emission models allow users to estimate the vapor emissions from the batch operations in a process. These calculations may be carried out for either one batch or multiple batches, thus allowing the user to determine how emissions will build up over an entire production campaign. The incorporation in Batch Plus of additional emission models and methods for the characterization of other waste streams is another path along which the software is expected to develop.

Information is a key resource in today’s commercial environment and organizations that have the ability to manage their information effectively will have a competitive advantage over those that do not. Effective large-scale information management can yield tremendous business benefits by preventing loss of information, hand-off delays and duplication of effort. The development of an enterprise database architecture for Batch Plus is another dimension along which the software is evolving. This architecture will allow the tool to act as a central repository of process data as well as permit multiple users to work concurrently on the same project by sharing process information and data.
6. SUMMARY

This paper described the application of Batch Plus in a biotech process development project. The use of the software in representing the process and in determining the overall material balance was discussed. The unit operations typically employed in bulk protein manufacturing, how these were modeled with the software and learning points from the model development process were described. The paper also discussed the context and environment within which the software was deployed. Business drivers, project structure and linkages to other corporate efforts were all described.

Several conclusions were drawn regarding the application of Batch Plus in this project. Key among these was that it was worth using the tool because of the numerous benefits derived. The overall potential of the software was recognized as well as the fact that it was also evolving. The paper concluded by discussing some of the dimensions along which the tool was evolving and the direction of its technical development.
REFERENCES


