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Mathematical Programming for the Design and Analysis of a Biologics Facility—Part 1
Donald L. Miller, Derrick Schertz, Christopher Stevens, Joseph F. Pekny
An algorithmic approach can fine tune facility design to increase plant capacity, avoid operational difficulties, and predict the required level of critical support services.

CLEANING VALIDATION
Statistical Equivalence Testing for Assessing Bench-Scale Cleanability
Cynthia Chen, Nitin Rathore, Wenchang Ji, Abe Germansderfer
How to apply the two-one-sided t-test (TOST) method to assess the comparability of two groups of cleanability data generated from a bench-scale study.

DOWNSTREAM PROCESSING
The Purification of Plasmid DNA for Clinical Trials Using Membrane Chromatography
Miladys Limonta, Gabriel Márquez, Martha Pupo, Odalys Ruiz
A case-study that describes the production of a therapeutically suitable pDKE2 plasmid by combining size exclusion and membrane chromatography.

ON THE WEB

Podcast: Quality by Design
The second in BioPharm’s 2010 monthly podcast series. This month, join Laura Bush, Editor in Chief of BioPharm International and John Towns, Senior Director of Global CMC Regulatory Affairs at Eli Lilly, as they discuss the current state of QbD. biopharminternational.com/BioPharmNow

Webcast: Extractables Testing
On March 23, Jerry Martin of BPSA chairs a panel on conducting extractables & leachables testing for single-use bioprocessing equipment. Watch live or on demand. biopharminternational.com/webcasts

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QbD Gains Momentum

Colleagues from other departments in my company sometimes ask what topics are hot in the field I cover. My answer always seems to start with, “Many of the same things that have been hot for the past four or five years . . . ” As those who work in this area know, the science and regulation of drug manufacturing don’t change very fast.

The evolution of Quality by Design is no exception. The concept was born in 2002 with the US FDA’s twenty-first century initiative. As we now enter the second decade of the new century, QbD seems to be coming out of early childhood and entering that difficult transition into adolescence.

We could look at QbD’s plodding growth and conclude that it is never going to make it to graduation. I prefer to see the last eight years, however, as a necessary development process. We may think that our kids should grow up faster than they do (who hasn’t wished that a teenager would act more mature?). But as we all know, wishing won’t make that happen.

The same holds true for a concept as big as this one. Yet, we have seen important progress. Industry now seems fairly comfortable with certain core aspects of QbD, particularly in terms of what it means to carry out product and process development in a QbD framework—what studies to carry out, how to determine critical quality attributes (starting from the patient’s needs), how to take advantage of design of experiments (DOE), even how to develop a design space.

This understanding has resulted from intensive work—within company walls, and through cross-company collaboration and industry–regulator dialogue—and has laid the foundations for the next phase in QbD’s development. In that next stage, several big challenges remain to be sorted out, including:

1. **How to file.** How do you clearly demonstrate your product and process understanding? How much information should you provide?

2. **Lifecycle management.** How will process validation, change control, deviations, and inspections be managed in a QbD environment? How will regulatory commitments be defined? How much flexibility will companies have to make postapproval changes without regulatory filings? (See my January editorial).

We are now at an exciting juncture for QbD. The QbD Working Group of the PhRMA Biologics and Biotechnology Leadership Committee has published its white paper, the CMC Biotech Working Group has released its 278-page A-MAb case study, and EFPIA is progressing on its mock CTD S2 and P2 filings. These groups have approached the goal of advancing QbD from different angles, and thus are contributing to the overall effort in complementary ways. Meanwhile, of course, two major regulatory efforts also are underway—ICH Q11 on applying QbD to biologics and the FDA QbD pilot for biologics.

In the coming year or two, through public workshops and meetings, the industry and regulators will begin, collectively, to digest all the recent efforts and start to figure out how to take the next steps forward, particularly on the tricky pending issues mentioned above. And I am confident that from all this effort, will we see important progress toward getting QbD out of school and into the real world.

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Vaccine Report

Phase 1–2 Completed for Universal Influenza Vaccine

BiondVax Pharmaceuticals Ltd, an Israeli biopharmaceutical company, has completed Phase 1–2 clinical trials of its Multimeric-001 universal flu vaccine. According to the test results, the Multimeric-001 universal flu vaccine, produced in E. coli, activates both the antibody and cellular arms of the human immune system. “This makes the BiondVax universal vaccine different from other universal vaccines, which only stimulate a humoral response,” said Tamar Ben-Yadidia, PhD, director of research and development at BiondVax. “It also contributes to the universality of the vaccine.”

BiondVax’s Multimeric-001 universal flu vaccine also stimulated both humoral (antibody) and cellular responses against both influenza type A (including H1N1) and influenza type B. Other universal vaccines that are currently under development provide protection against influenza type A only.

The Phase 1–2 trial was a randomized, single-blind, placebo-controlled, escalating double-dose safety study in which a total of 60 participants, males and females aged 18–49, received two intramuscular injections containing the Multimeric-001 vaccine, either with or without the Montanide ISA 51 VG adjuvant, and at two different dose levels. The clinical trial results showed that BiondVax’s Multimeric-001 universal flu vaccine is safe to use at all doses tested, both with and without adjuvant.

This trial is the first of two Phase 1–2 clinical trials of the Multimeric-001 universal flu vaccine being conducted by BiondVax. The second trial has already commenced with 60 participants aged 55–75, and is expected to be completed in the first quarter of 2010.

BiondVax is one of several companies developing a universal vaccine. VaxImmune, Dynavax, and Acambis (now part of Sanofi Aventis), all have universal vaccines in various stages of development.

—Haydia Haniff

Genzyme Outsources Fill–Finish Operations to Hospira

Genzyme (Cambridge, MA) will outsource fill-and-finish operations for its drugs Cerezyme, Fabrazyme, Myozyme, and Thyrogen to Illinois-based Hospira, Inc. The move follows a series of manufacturing problems at Genzyme’s Allston Landing facility in Boston, MA.

The Allston Landing plant was temporarily shut down last summer for decontamination following the detection of bioreactor contamination with Vesivirus. That shutdown led to shortages of Cerezyme and Fabryzyme.

Then in November, the FDA warned healthcare professionals about the potential for foreign particle contamination of Genzyme products Cerezyme, Fabrazyme, Myozyme, Thyrogen, Lumizyme, and Aldurazyme.

Since last fall, Genzyme has moved some of its fill-and-finish operations to its plant in Waterford, Ireland. The rest will be outsourced to Hospira. The transfer to Hospira is expected to take six to eight months, over which period Genzyme will seek regulatory approval.

Per Genzyme’s December 28 filing with the United States Securities and Exchange Commission, the initial term of the agreement will expire on December 31, 2015, followed by a two-year extension.

—Chitra Sethi

FDA Approves MAb for Rheumatoid Arthritis

The US Food and Drug Administration has approved Genentech’s Actemra (tocilizumab) to treat adults with moderate to severe rheumatoid arthritis who have not adequately responded to or cannot tolerate other approved therapies for rheumatoid arthritis.

Actemra works by blocking the action of interleukin-6, an immune system protein that is overabundant in people with rheumatoid arthritis. The FDA is requiring the sponsor to conduct a postmarketing clinical trial to further evaluate the long-term safety of Actemra. In addition, a risk evaluation and mitigation strategy (REMS) will require the drug sponsor to implement a communication plan for physicians informing them how to appropriately monitor their patients for liver or gastrointestinal side effects. The REMS will include a medication guide to ensure that patients are informed of the benefits and risks of Actemra.

Actemra’s recommended use is limited to patients who have failed other approved therapies because of serious safety concerns that were noted in clinical studies. These safety concerns include elevated liver enzymes, elevated low-density lipoprotein (LDL) or bad cholesterol, hypertension, and gastrointestinal perforations.

Genentech, Inc., is a subsidiary of the Roche Group.
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Last year was a difficult one in the biotech-manufacturing arena. The H1N1 vaccine shortage in October and November brought home the perils of influenza vaccine development and production, especially when working with new viral strains each year. A leading biotech manufacturer suffered major losses caused by serious problems on the production and compliance front. Despite several decades of experience in monitoring bioreactors and refining cell culture operations, the production of safe, pure, and potent biologics remains a tricky business.

DEBACLE AT GENZYME

The troubles at Genzyme began two years ago when it sought to scale up production of its new treatment for Pompe disease, Myozyme (agalglucosidase), at its flagship Allston Landing facility in Massachusetts. With six large bioreactors, that plant produces Genzyme’s lead product, Gaucher disease treatment Cerezyme (imiglucerase), along with Fabrazyme (agalglucosidase) for Fabry disease. Unfortunately, initial commercial batches of Myozyme made at Allston Landing differed from those made at Genzyme’s smaller Framingham facility, requiring the company to conduct additional clinical trials and submit a new license application for the product.

In addition, an inspection of the Allston Landing plant by the US Food and Drug Administration in the fall of 2008, found deviations from good manufacturing practices (GMPs), including inadequate procedures for preventing contamination, equipment maintenance, maintaining in-process controls, and for computer systems validation. Genzyme thought it had addressed all these deficiencies by February 2009, but an FDA warning letter requested additional actions and information, as did another FDA communiqué in May.

As Genzyme struggled to address the FDA’s concerns, in June 2009 it discovered viral contamination of a bioreactor used to produce Cerezyme in-process material. The company had to close the Allston Landing facility altogether for several weeks for decontamination. Almost all of the starting material used to make Cerezyme was scrapped, and several thousand patients were left with limited access to therapy for these very serious conditions.

As the cleaning process concluded, Genzyme then discovered problems with its fill–finish process at Allston; evidence of metal particulates, fibers, and other minute particles in certain batches of Cerezyme and Fabrazyme prompted a shutdown of those operations. Genzyme transferred those activities to facilities in Waterford, Ireland, and Geel, Belgium, along with all large-scale bulk production for Myozyme. Genzyme also contracted with Hospira to perform fill–finish work for its major drugs to support what it hoped would be expanded production in the coming year. The company is anxious to ramp up output because the FDA has moved to alleviate the Cerezyme shortage by fast-track approval of alternative therapies made by UK-based Shire and by the Israeli firm Protalix Biotherapeutics, which has linked up with Pfizer to commercialize its Gaucher treatment.

By the end of 2009, Genzyme was beginning to ship new lots of Cerezyme and Fabrazyme. But by then, revenues had slumped, along with the company’s standing with investors. New competition was on the horizon, and the company had to implement a two-year corrective action plan to address the FDA’s concerns about manufacturing processes and controls. Prominent stockholders raised questions about the leadership of Genzyme CEO Henri Termeer, who moved to deflect these
challenges by bringing in new senior executives to oversee manufacturing, operations, and regulatory affairs. It remains to be seen if these responses are too little, too late.

**EGGS VERSUS CELLS**

Despite the perennial risk of contaminated cell cultures, there’s a big push underway to switch influenza vaccine production from eggs to cellular methods. The campaign accelerated after vaccine makers failed to deliver some 100 million doses of the new H1N1 influenza last summer as expected; industry and government officials lost considerable credibility when only 40 million doses were available in September, and some 20 million Americans already were hit by the new flu. Health and Human Services (HHS) Secretary Kathleen Sebelius said she relied on industry predictions that turned out to be wildly overoptimistic, although manufacturers claimed they kept health officials informed of their technical problems.

Manufacturers have used fertile chicken eggs to grow flu vaccine since the 1930s, because the influenza virus grows well in eggs, an important feature for fast production. Such methods have been operational in 2011. But with the H1N1 flu, production took longer than expected because the yield in each egg was much lower than with seasonal flu viral strains. The resulting delays have prompted government officials and manufacturers to accelerate the shift to cell-culture flu vaccine production. Such methods have been used for decades to produce vaccines for mumps, measles, polio, rubella, and other diseases, explained Jesse Goodman, FDA chief scientist, at a December 2009 seminar on advances in influenza vaccine technologies at the National Institutes of Health (NIH). But that approach has not been adopted for influenza because it can take a long time to get a good yield out of cell cultures.

The change will not transform influenza flu vaccine production, said Goodman, but could lead to more reliable products and faster scale-up. Anthony Fauci, director of NIH’s National Institute of Allergy and Infectious Diseases, agreed that shifting to cell culture doesn’t cut off that much production time, but could establish a more flexible surge capacity. Making the transition from egg- to cell-based production “will take some time,” Fauci said, “but eventually we will get there.”

Support for this transition comes from HHS’ Biomedical Advanced Research & Development Authority (BARDA), which funds efforts to expand manufacturing capacity for influenza vaccine as part of its mission to expand research and production of public health countermeasures. BARDA has awarded grants and contracts to construct new facilities and retrofit existing plants with an eye to creating more robust, flexible, and scalable manufacturing systems, explained BARDA Director Robin Robinson at the NIH seminar. A big winner is Novartis, which received $487 million (of $1.3 billion in grants to manufacturers) to construct a cell-based pandemic and seasonal flu vaccine manufacturing facility; the new plant opened a few months ago and is scheduled to be operational in 2011.

Potency has been another issue anticipated problems emerge.”

“Shifting to cell culture doesn’t cut off much production time, but could establish a more flexible surge capacity.”

though clinical trials indicated that the new vaccine was effective with a single dose in adults, manufacturers found some lots inadequate: Astra-Zeneca’s MedImmune had to recall nearly 5 million doses of its nasal spray vaccine in December, and Sanofi Aventis pulled 800,000 doses in prefilled syringes for young children. But by then, the pandemic was ebbing, and manufacturers faced a new problem: more vaccine than anyone wanted. Last month, the French government moved to sell off millions of doses to countries in the Middle East and Latin America, and to cancel sizeable orders from Sanofi, Glaxo, Novartis, and Baxter.

The real “endgame answer” to pandemic and seasonal flu vaccine production problems, according to Fauci, is to develop a universal influenza vaccine that could be administered in childhood and last a lifetime, as with most vaccines against infectious disease. Such a discovery could reduce the need to produce 120–150 million doses of a new influenza vaccine each year in the US, at a cost of $2.8–4 billion. “And it is doable,” Fauci said, noting that researchers already are identifying possible vaccine targets that don’t change with every different influenza virus.

Even though the FDA received some of the blame for the pandemic flu vaccine manufacturing woes last fall, Commissioner Margaret Hamburg prefers to regard the response from the FDA and from the industry as “quite remarkable in terms of how much has been mobilized in response to a previously unrecognized strain of flu virus.” Some of the disappointment may stem from over-optimism in predicting how much vaccine would be available when. “Sadly, we know that it’s the nature of manufacturing vaccines to have some unexpected delays, and it’s the nature of science to have unanticipated problems emerge.”

**February 2010 www.biopharminternational.com BioPharm International 15**
I wrote this month’s column while on my way back from this year’s JPMorgan Health Care Conference, the premiere industry showcase for healthcare companies—especially bio/pharmaceutical companies—seeking to pitch their stories to investors. Held annually during the second week of January, the conference is a great place to gauge the mood and prospects of the industry for the coming year.

A significant number of public contract research and manufacturing companies (CROs and CMOs) told their stories, including Covance, Charles River Laboratories, Icon, Cambrex, Patheon, and Wuxi Pharmatech. As might be expected by anyone who followed the pharmaceutical services business in the past year, the CROs and CMOs told two widely-divergent stories. The companies focused on early development (preclinical through Phase 1) and manufacturing talked mostly about how they cut costs to preserve profitability in the face of the precipitous fall in development spending while assuring everyone that they are still poised to take advantage of the resurgence that has to come. On the other hand, the CROs offering Phase 2–4 clinical research services were able to talk about the remarkably good year they had despite the general industry downturn.

A DIFFICULT OUTLOOK
What conference attendees really wanted to know from the CRO and CMO executives was when they expect the industry’s fortunes to improve. All the executives could really deliver was a sense of cautious hopefulness based on positive conversations with customers, a slight uptick in requests for proposals, and a sense that things couldn’t get much worse. The past year really shook the confidence of many CEOs, who had gotten used to annual double-digit increases in sales and profits.

That was really exemplified by Covance Chairman and CEO Joe Herring, who said the company had experienced too many “false positives”—contract wins for projects that were subsequently delayed or cancelled—in its preclinical business during 2009, to allow him to feel overly optimistic about 2010. Herring was still able to project overall growth for Covance, thanks to the spectacular performance of its central laboratory business.

The difficult outlook was further underscored by the announcement from Charles River Laboratories that it would close its Shrewsbury, MA, preclinical toxicology laboratory facility in 2010, as a cost saving measure. Charles River expects to improve profitability by $20 million annually by closing the 400,000 sq. ft. Shrewsbury operation and consolidating work at its other facilities. Preclinical toxicology facilities are high fixed-cost operations, much like manufacturing, and are unprofitable without high utilization.

Jim Miller is president of PharmSource Information Services, Inc., Springfield, VA, 703.383.4903, jim.miller@pharmsource.com.
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The closure reflects both the general downturn in early development activity and the serious overcapacity problem in the preclinical toxicology sector, following an explosion of facility building and expansion in recent years. Shutting down the facility suggests that Charles River executives don’t expect a rebound in the business sufficient to absorb all of the available capacity for at least several more years. That’s not good news for any company supporting clinical development, including manufacturers of clinical trial materials.

MAJOR EVENTS OF 2009
The CEOs’ perspectives at the JP Morgan Conference were offered against a backdrop of a series of major and minor industry developments at the end of 2009 and the beginning of 2010.

In addition to the Charles River Laboratories’ facility closure, there was the announcement by Lonza in early January that it would close three of its manufacturing and distribution sites in the US and Europe, including its pharmaceutical chemical manufacturing site in Conshohocken, PA. The closures are actually part of the restructuring plan Lonza executives announced in October 2009, following a rash of manufacturing cancellations and delays. That announcement highlighted the changing inventory management practices at the major bio/pharmaceutical companies.

December 2009 saw the sale of two well-known biomanufacturing companies, as Merck announced its plan to buy Avexia, and Cobra Biologics was acquired by Recipharm. Merck’s acquisition of Avexia has the most impact because Avexia was a well-established player in microbial fermentation for manufacturing of therapeutic proteins, with capacity at the 5,000-L and 1,000-L scale, and because it appears likely that under Merck, Avexia will no longer pursue new contract manufacturing business. According to financial information available on Avexia’s website, the business has never really been profitable and its private equity owners were probably relieved to be rid of the negative cash flow.

Profitability also has been an issue for publicly held Cobra, which offers process development and very small-scale manufacturing services for both microbial and mammalian fermentation. Recipharm executives clearly believe they can change that situation. The company has been an aggressive buyer of manufacturing facilities in Europe in the last several years and has been aggressively building its large molecule capabilities in the last two years. In 2008, it bought a liquid injectables manufacturing facility in France from AstraZeneca, and the manufacturing assets of Switzerland-based Innotech, which offers formulation and lyophilization cycle development as well as clinical scale fill-and-finish services. In 2009, it purchased a majority interest in a biomanufacturing facility in Sweden from AstraZeneca.

IT COULD GET NASTY
The general market outlook and financial performance challenges faced by Avexia and Cobra suggest that contract service providers need to be somewhat wary of the near-term prospects for the biologics market. Yet, most contract services companies are excited about the opportunity presented by the growing number of biologics in the pipeline, especially fill-and-finish service providers. Notable announcements of new entrants into the market last year included Vetter, which is building a clinical-scale operation near Chicago.

Overall, 2009 was a rough year for clinical-scale injectables manufacturers. A number of new facilities and suites came on line during the year but they didn’t get much use. Some injectables companies were forced to give away fills and at least one new entrant was reportedly on the verge of closure less than a year after it opened.

There is widespread agreement throughout the bio/pharmaceutical industry that outsourcing will continue to grow long term, especially as major bio/pharmaceutical companies adapt their business models to the more hostile market environment. The near-term prospects are not so clear, however, and industry participants are forced to walk the line between maintaining current profitability while positioning themselves for future opportunity.
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At the start of this new year, we would like to thank our readers for actively following our column and for your positive feedback through the BioPharm International web site. Feedback is important to us and we encourage you to continue sending it.

Over the last year, several end-users have asked for status updates of disposables for a wide variety of final filling applications. The availability of technology in this area has grown rapidly in the last two years. Here we discuss a recent example of a successful end-to-end deployment of single-use technology in aseptic filling. This case study was first presented by Nigel Bell, sterile product lead at GlaxoSmithKline (GSK, Barnard Castle, UK) at IBC’s Biopharmaceutical Manufacturing & Development Summit in San Francisco, CA, in December. For this article, Miriam Monge (MM) talked to Nigel Bell (NB) of GSK, along with Ernie Jenness (EJ), a development engineer at Millipore (Billerica, MA), one of the main vendors who actively collaborated with GSK throughout this project.

MM: Nigel, in what context did you start evaluating the use of disposable technologies?

NB: GSK operates two aseptic vial and two syringe facilities, both using fixed and mobile stainless steel systems. These filling systems are not flexible enough to respond to the needs of a fast growing, varied product portfolio. The evaluation of disposables for final filling had already started at GSK, but the acceleration was precipitated by the need to rapidly fill a large quantity of vials for the flu pandemic because we did not have sufficient capacity with the existing setup.

MM: I understand that one factor that motivated you to switch to disposables was that cold fill products challenged the integrity of hard-piped steam-sterilized stainless steel systems.

NB: The thing that puts a lot of strain on the joints in the stainless steel systems is the polytetrafluoroethylene (PTFE) gaskets. These plastics do not have a cold flow memory and so do not react well when being heated to 121 °C for sterilization purposes, then to 5 °C for filling. There have been some problems with leakage over the years. But even though cold fill products provided the greatest risk from the integrity point of view, there were many other reasons for the need for change. Stainless steel is fine when you are working with one dedicated product, but as we move to filling multiple biopharmaceutical products, the cleaning and sterilization issues rapidly become unwieldy.

MM: You talk about the cost and capacity loss through qualification and routine steam-in-place (SIP) as one of the key challenges when working with traditional systems.

NB: When you carry out this analysis, you rapidly realize that the opportunities to improve efficiency, increase productivity, and reduce cost...
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are huge. The time required for cleaning and sterilizing stainless steel; the space and money required for dedicated areas for clean and dirty vessel storage; the downtime required for cleaning, steaming, and product changeover; the massive energy consumption required to generate thousands of liters of water for injection (WFI) to clean and steam equipment, and the cost to make the WFI, are all highly time consuming and offer the potential for efficiency improvement.

Another key concern for us with the traditional systems was the complexity of operation and the associated level of grade A intrusions. With the existing arrangement using restricted access barriers (RABs), the doors between grades A and B would be open taking out and replacing equipment, and so on.

**MM:** What was the timeline for resolving these issues and implementing disposables?

**NB:** The project started in June 2009 and we needed to start filling in September 2009.

**MM:** How was the project managed? What type of team did you put in place?

**NB:** The new product introduction department set up the URS, working in collaboration with the quality and engineering departments. Nigel Wood, our engineering project manager, played an important part, particularly looking at the integration of the Bosch system, as did our quality assurance (QA) department which took a pragmatic approach to making sound, risk-based decisions. We also worked in close collaboration with the Millipore Mobius experts.

We carried out a full critical review of all aspects of the filling systems and identified many areas of over complication. We challenged each of these aspects with local QA, then Millipore Mobius expert advisors, and then with the MHRA regulators. We took the approach that every item, regardless of its excellence or complexity, has a failure rate. Thus any reduction in items will have a beneficial effect, and many component parts were removed or at least reduced in complexity.

Other principles were that the product passage from point 1 to 2 should be as simple as possible with as few manipulations as possible (to reduce training and error rates) and wherever practical, as few grade A manipulations as possible. No aseptic connections (including needle assembly) means no SIP to point-of-fill, and minimal grade A intrusions. Our other main motto is, “where there are connections, use the best available.”

**EJ:** Our approach on such a project is to work hand-in-hand with the end-user, a collaborative approach aimed at finding the best overall solution.

**DISPOSABLES SELECTION AND IMPLEMENTATION**

**MM:** How did you evaluate the operational aspects of the technology and the cost of the options? How did you go about risk mitigation?

**NB:** If it hadn’t been for the pandemic, the selection and implementation of disposables would no doubt have taken twice as long. In this situation, convincing the sponsors was somewhat quicker than in a normal situation. QA pragmatism through risk assessment also helped immensely.

One of the key technologies we selected was the Millipore Mobius magnetic drive mixer for bulk drug formulation. We had already done previous testing with this system and found it to work efficiently. The Millipore STS connector was used to secure the sterile connection of the irradiated filter in the grade C environment. In both cases, we went through a series of trials and training, using 10 assemblies for each connection for each process step. It is important that the operators are trained to make the connection, because if you don’t make the final click properly it can leak. We chose 50-L bags from Sartorius Stedim Biotech for the bulk drug hold. We selected the Sartorius Stedim bags because we preferred the integrity testing methodology that they have developed. We also carried out a full risk assessment using failure mode effect analysis (FMEA). Other key technologies included the Getinge La Calhene aseptic trans-
fer system, which was premounted with the disposable beta transfer port to allow aseptic transfer of the seven pre-assembled manifold bags mounted with the Bosch needles into the RABs.

The Millipore team worked to rapidly qualify the assembly of the Bosch needles on the manifold—not a simple task when ensuring the safety of the disposable assembly prepacked with stainless steel needles. The peristaltic pump filling systems from Groninger and Bosch were selected for their high level of accuracy and simplicity. We also work with ready-to-use presterilized stoppers from Helvoet & West Pharma, again eliminating washing requirements.

EJ: Cost was not the primary concern for pandemic flu. Time to get a solution and flow path security and sterility were the primary drivers. For other applications, GSK had a cost target, but was flexible with this as they learned of the operational efficiencies they would gain in using a single-use solution.

MM: Were procurement supply chain issues a consideration?

NB: The pandemic has clearly stretched the global supply of producers and manufacturers. Within our organization, there are strong supply chain management groups with regular reviews and audits of suppliers. We have particularly rigorous controls in place and also exchange a great deal with colleagues in the vaccines division who have many years experience with disposable selection and implementation.

EJ: Our client voiced concern about single sourcing from Millipore. Millipore has met with several clients to review our strategy around this critical area and is taking action such as developing specific bag holder systems that facilitate the deployment of bags from alternate vendors.

MM: What did you see as the biggest challenges with single-use systems?

NB: The fact that we are contracting out the sterility assurance, cleanliness, particulates, and endotoxin control to a third party requires careful auditing and supply agreements. We are of course concerned about the overall system integrity and the perceived weakness of polymeric materials. We were concerned about the reliability of sterile connections in terms of mold variation. Concerning the Bosch needles, we are concerned about ongoing control of the physical, chemical, and micro quality.

MM: One common concern when evaluating disposable technologies for final filling is the question of pre- and post-integrity sterile filter and disposable system testing. How was this handled?

EJ: Pre-use system and filter integrity testing is performed by Millipore. Package validation testing by ISTA 2A drop and vibration and post ISTA package integrity testing demonstrates that the system is integral pre-use.

NB: We then perform pre- and post-filter testing where appropriate.

MM: What do you see as the biggest limitations or risks when working with disposables in the context of final filling operations?

NB: Solvent-containing products are not ideal for disposables because of the potential for evaporative molecular loss through tubing by permeation. We had two preserved multi-dose products that couldn’t be filled in a single-use setup.

Having said all of this, I think the best proof of the fact that these systems are a sound solution is that we have now filled 85 million doses and we have had no integrity issues and no sterility failures.

MM: In terms of the benefits, have you been able to measure efficiency and productivity improvements and related cost savings since implementing disposables?

NB: With the disposable setup, the amount of grade A intrusions for the wetted path assembly and strip down has been taken from 2 h to 0 using the Getinge La Calhene grade C to grade A door. This door has a disposable beta door that is premounted with the disposable assembly, and is pre-gamma sterilized and prepared by the vendor with all the relevant documentation. Also, with this arrangement there is no need for the operators to touch the needles, avoid-

**Figure 2.** Bosch single-use filling needles. GSK’s use of Bosch needles began early in the development of the Bosch systems. The Bosch manufacturing process has been improved to control all aspects of quality control, including chemical and microbial levels.
“Since implementing disposable technology, our site energy usage has dropped, significantly reducing our carbon footprint.”

ing the need for SIP post-assembly. The system is considerably simplified. The regulators have commented that it could reduce aseptic risk.

The overall time savings are significant. The system preparation time is minimal, the system build time is 30 minutes versus 2 hours for the stainless steel system, sterilization before filling is 0 versus 3.5 hours, operator training time is 2 days versus 2 weeks, SIP qualification is 0 and no six-monthly requalification, the aseptic connections are 0 versus approximately 50 (which were SIP sterilized), and of course there is no cleaning validation. To sum it up, we previously required 36 hours for a campaign fill and we are now down to 12 hours!

In addition, we had a stretch target to reduce energy. Since implementing disposable technologies for final filling, our site energy usage has dropped, significantly reducing our carbon footprint. In addition, we have significantly reduced the carbon footprint and increased capacity by 40%. Validation and routine SIP are key contributing factors, but of course there are many other factors, as outlined earlier. We haven’t yet carried out a full cost analysis, but again we expect the overall impact to be significant in terms of less water usage and less chemical usage. And of course the increased capacity means that the number of days gained in manufacturing capacity is huge.

MM: What are the key take home messages you have gained from this project?

NB: Disposables offer a platform-enabling simplification of many activities. The end-to-end solution will provide the biggest benefits, including reducing assembly time, limiting aseptic intrusion, and enabling modular use of setups, allowing for minimum hold and maximum flexibility. Consider all connections as highly critical, assume leakage will occur, and build in security. Lastly, control suppliers through quality and supply chain agreements.

All of our forecasts indicate that there is going to be an explosion in the need for vaccines. Previously we were constrained by capacity and now, thanks to disposable platforms, we can rapidly gear up to meet demand.

CONCLUSION

The track record and experience gained in the use of disposables in a good manufacturing practices (GMP) environment for active pharmaceutical ingredients (API) manufacture has led to an upsurge in interest in recent years in the use of disposable technologies in the fill–finish arena. Recent developments in disposable filling sets for small- and large-scale liquid filling lines, together with disposable rapid transfer port technologies has meant that, for the first time, a disposable fluid path from the formulation vessel to the vial or syringe can be established, thus realizing the full potential of the technology.

The case study confirms that the benefits seen in bulk API manufacture also are realized in the fill–finish facility, specifically with regard to reduced costs, reduced energy usage, and reduced labor.\textsuperscript{1,2} Based on the outcomes of this case study, the future for disposables use in the final filling arena has significant potential to simplify process operations. This would reduce process risk, significantly reduce carbon footprint, and save considerable time in process operations, notably through reducing the time required for assembly and eliminating the requirement for disassembly of equipment, eliminating time required for cleaning and steaming operations, thus increasing available capacity (in this case by 40%).

Although a full cost analysis was not carried out, it is clear that the cost savings are significant, notably in terms of the number of extra days of manufacturing that become available, along with significantly reduced WFI requirements.

Of course, disposable technology requires further testing and fine-tuning before being suitable for implementation on a routine basis. In particular, the issue of supply chain security—validating a second source—must be addressed. With regard to integrity testing of disposable systems, an industry consensus on what are acceptable and sensible detection limits would be helpful. This is where the industry association disposable user groups within the ISPE, PDA, BPSA, and ASME must work together to provide one unique recommendation.

ACKNOWLEDGEMENTS

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Mathematical Programming for the Design and Analysis of a Biologics Facility

Donald L. Miller, Derrick Schertz, Christopher Stevens, Joseph F. Pekny

ABSTRACT

In this paper, we describe the use of mathematical programming methods for automated schedule generation. Our method creates production schedules that encompass all necessary process constraints and span a sufficiently large time scale to produce statistically meaningful results. We illustrate the approach using a new industrial biologics facility. In Part 1 of the article, we describe the process and results of the study. In Part 2, we will summarize the formulation, compare our approach to discrete event simulation, and discuss the algorithmic methods used to produce high quality production schedules.

A large-scale biologics facility represents an enormous capital investment. When the cost of the underlying research effort required to discover and gain approval for a drug is considered, this investment can be considerably higher. The ability to predict, analyze, and improve the performance of such a large capital investment represents a huge business opportunity. At a minimum, predicting the expected plant capacity with some level of confidence is necessary to ensure the quality of the design. Far more valuable is the ability to actually study the details of everyday plant operation while still in the design phase. If potential operational problems or bottlenecks can be identified at this point, the design can be improved to mitigate or even eliminate anticipated problems. This level of detailed analysis also can determine whether the anticipated need for a newly approved drug can be met by time sharing production at an existing facility. Thus, the economic payback for developing a high fidelity model capable of performing detailed analysis of process operations can be many times the cost of the development effort.

Even when the chemistry of a process is reliably known, it is not possible to effectively analyze the performance of a large-scale biologics facility unless detailed operational schedules can be produced. Understanding the dynamic behavior of the process requires fine resolution of the timeline, although, as shown in the results in this article, some of the phenomena of interest only emerges over long time scales. This is because of the batch nature of the processes, intermittent material storage in process vessels, biological variability, and the need to perform certain periodic maintenance operations on critical pieces of equipment. Thus, unless detailed schedules can be produced—schedules that satisfy all of the con-
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straints associated with the process—even such basic properties as plant capacity cannot be accurately predicted.

In this paper, we describe the results of using mathematical programming methods for automated schedule generation to address the above goals. Our method creates production schedules that encompass all necessary process constraints and that span a sufficiently long time scale to produce statistically meaningful results. Furthermore, the algorithm used to solve the mathematical programming formulation uses a Monte Carlo selection of mutually exclusive terms that represents a sampling of stochastic parameters. This provides a means of addressing process uncertainty on realistic problem sizes. We illustrate the approach using a new industrial biologics facility. The data have been altered to protect proprietary technology without changing the intellectual significance of the results. In Part 1, we describe the process and results of the study. In Part 2, we will summarize the formulation, compare our approach to discrete event simulation, and discuss the algorithmic methods used to produce high quality production schedules.

THE ENGINEERING PROBLEM AND BUSINESS OPPORTUNITY

Almost as soon as computers first appeared, engineers recognized their potential as tools for use in the simulation and analysis of processes. As early as the 1950s, chemical companies developed in-house sequential modular simulation systems. By the 1970s, use of commercial sequential modular simulators was widespread. These systems were well suited to predict and analyze the behavior of continuous single-product facilities that operated at or near steady-state conditions over long periods of time. Indeed, many would find it unthinkable to carry out process development, and the design and startup of a large-scale continuous chemical plant, without an accurate computer simulation. Yet, nearly a decade into the 21st century, we routinely design and construct large-scale biologics facilities based on bench-scale and pilot plant data, and using primitive tools such as spreadsheets, which can provide only limited insight into dynamic processes. Why do we see such a marked disparity in the level of sophistication applied to modeling these two very different types of processes?

The answer is that biologics facilities are beset with a myriad of complicating factors that require dealing with dynamics to get sufficiently accurate results.

Manufacturing processes for biologics typically consist of a series of batch reactors followed by purification and concentration steps that use chromatography columns and ultrafiltration skids to isolate the protein of interest and concentrate it to manageable volumes. The batch nature of these reactions renders steady-state simulators unusable for detailed dynamic analysis. Although a steady-state simulator might approximate multiple batches with a single continuous rate, this approximation breaks down when the series of batches must be interrupted. Such interruptions occur routinely in biologics processes, e.g., for clean-in-place (CIP) operations or periodic repacking of chromatography columns. In addition, many processes require enforcing batch integrity and individual batch tracking. This means that when a vessel is used to store an intermediate material, all material from that batch must be emptied before any material from a subsequent batch may be added to the vessel. Typically, the vessel also must be cleaned before the introduction of new material. In the presence of such constraints, no modeling technology will be accurate unless it explicitly tracks the location of inventory from individual batches.

The biologic nature of these processes also induces stochastic variability in batch yields (titers) and cycle times. Successive batches of the same reaction produce different yields of the desired products, even when executed under identical conditions. Furthermore, the cycle time often is correlated to titers so that high titer batches may take more time. Batches with different titers will produce effluent with different concentrations of the protein of interest and thus different loadings on the chromatography columns. To describe and predict the true capacity of the facility, it is necessary to model both the expected uncertainty in titers and the effect that it has on the required frequency of column repacking. Bioreactor titers also affect the batch-processing duration in the columns. Other complicating factors may arise, such as failed batches. These variables alter the true capacity of the process and many of them interact in a nonlinear fashion with the avail-
ability of parallel equipment available at the various process stages. As a result, without high fidelity approaches that can produce detailed feasible schedules for the process over a sufficiently long time period to reflect stochastic behavior, and that satisfy all necessary constraints, it is not possible to predict how a process design will in fact behave in the real world with an accuracy commensurate with the capital investment.

One approach to this challenge has been to model such processes using discrete event simulation. This method is more appropriate than steady state simulation. However, discrete event simulation requires a significant investment of time to develop the logic necessary to accommodate complex processes. Discrete event simulators work by advancing the time variable in a monotonically increasing fashion with an attendant design of supporting data structures (e.g., event stacks). Therefore, if we consider events happening at time $t$, it is no longer possible to initiate events that can occur at an earlier time. This complicates the logic to such an extent that high fidelity simulations of large-scale biologics facilities present a significant technical challenge. In highly constrained applications, such as the one studied here, temporally restricted decision-making often encounters local infeasibilities. Discrete event simulation approaches address these infeasibilities with heuristics such as restarting the simulation sufficiently far in the past in an attempt to avoid problematic behavior. Practically, these simulation heuristics degrade as the models consider increasing levels of process detail. This happens because (1) the number of events becomes large and the sequential processing of events results in prohibitive algorithmic complexity, (2) the extension and maintenance of simulation heuristics becomes arduous and their behavior unpredictable as model complexity grows, and (3) complexity tends to grow in successive studies as the model evolves to address more detailed interactions and answer increasingly sophisticated questions of interest. These limitations, combined with the combinatorial burden of searching the enormous
number of timelines needed for design and schedule optimization, argue for an alternate approach supporting implicit search and designed to scale well with increasing detail and model evolution.

A mathematical programming approach transcends both steady state and discrete event simulators. This approach allows the solver to range freely over the entire timeline of interest and insert or remove scheduling events and activities whenever it is useful to do so. The approach can not only mimic the left-to-right behavior of simulation to capture causal reasoning, but also can range over the timeline to enhance algorithm speed and facilitate global reasoning about constraints. In this article, we show how to handle stochastic process variability, disposal of batches, periodic repacking of chromatography columns, and other process constraints necessary to accurately predict the real world performance of a large-scale biologics facility during the design stage.

THE RESOURCE TASK NETWORK AND MATHEMATICAL PROGRAMMING APPROACH TO BIOLOGICS FACILITY MODELING

A resource task network (RTN) description is used to provide a structured description of process details. We developed a high fidelity model of an industrial biologics facility using the VirtECS version 7.1 software system, which consists of a core mathematical programming solver designed around a uniform discretization model (UDM) and a customized outer layer that is specifically tailored to address biologics process behavior.

Model Description of the Biologics Process

The process and model descriptions in this paper reflect the actual industrial process for which this work was performed. Diagrams, figures, and results are taken from a sanitized model that is similar in nature and complexity. A diagram of the process is shown in Figure 1. In the inoculum stage, a working cell bank vial is thawed and expanded in a series of flasks and Wave bioreactors. The resulting material is then fed through a series of three bioreactors of increasing capacity and then sent to a large production bioreactor. There are three complete parallel trains for scaling up the vial thaw and five parallel production bioreactors. The cell culture growth in the production bioreactor requires 9 to 11 days. When cell growth is completed in the bioreactor train, the lot is

Figure 1. Biologics process schematic
harvested using centrifugation and filtration. Purification follows through a series of chromatographic columns and filters.

The model we developed considers each step including preparation time, processing time, and the time required to clean the equipment following processing. An important consideration in the model is the handling and storage of intermediate materials. Unlimited intermediate storage can be available for certain types of materials, e.g., frozen cell cultures that are sufficiently compact that storage space is never a problem. But for most materials in this process, storage is limited and must be modeled explicitly if the resulting model is to have real world validity and for confident engineering decisions to be made. This limited storage availability may be classified into two types: dedicated and process. Dedicated storage describes the case where dedicated tanks are available to hold an intermediate material. Both the capacity and identity of these tanks must be modeled explicitly because lots cannot be mixed. Process vessel storage takes place when the material produced by a given process step can be stored only in the vessel where it was produced. When process vessel storage is used, no activity can occur in that processing vessel until all of the stored material has been removed and fed to the downstream stage. Thus, even the preparation or cleanout activities must be delayed until the vessel is finished being used as a storage tank for the previous batch. In this process, there are many nontrivial material transfers that tie up both the feeding and receiving tanks, e.g., charging cycles to a column from an eluate tank. These were modeled in detail because they can force delays in processing the subsequent lot. The resulting model consists of 22 mainline manufacturing steps, 151 pieces of equipment, 479 materials, 353 activities (tasks), and 186 resources (e.g., operators, etc). For a representative instance, the final solution to mathematical programming formulation contained approximately 87,500 nonzero continuous variables, approximately 19,250 nonzero binary variables, and approximately 300,000 active nontrivial constraints. All of the instances reported below had comparable final solution statistics.

The manufacturing process begins with the thaw of a working cell bank that is fed to the flasks in the inoculum stage. Through subsequent scale-up processing, a new lot becomes available approximately every 3.5 days. By using parallel scale-up equipment, lots can be produced at a variety of rates. From an operational perspective, it is desirable to schedule bioreactor starts at regular intervals (cadence). This cadence is one of the main operational parameters investigated during the design study. Faster cadence produces more batches, but increases the likelihood of batch failure because the chance of holding a batch longer than the permitted hold time increases. By exploring cadences ranging from one lot every two days to one lot every four, we studied the effect of cadence on realizable plant capacity. Because of the biological nature of the process, the production bioreactors experience variability in run length, volume, and titers. As described above, the processing time varies from 9 to 11 days. The run length of the production bioreactor also affects the volume and titers of the resulting product. The volume and titer variables in turn affect the batch size and processing time of the columns. We modeled this behavior by specifying a series of time-volume-titer tuples that represent possible outcomes from bioreactor harvests—the Monte Carlo versions of the stochastic tasks described above. Each tuple has a specific probability of occurrence. We modeled product demands so as to require a single bioreactor lot for each demand. In this way, we are able to select among the literal time-volume-titer tuples for each lot/demand with a random number generator used by the solution algorithm. Then, during the scheduling of this particular lot, the algorithm only authorizes steps whose processing times and batch sizes correspond to the tuple that was randomly assigned to this demand. In addition to variability in bioreactor performance, we modeled a 1% random failure rate on bioreactor batches to account for the anticipated likelihood of both primary and backup sterility failure. The purification system begins with a series of four chromatography columns. The columns are not large enough to consume an entire bioreactor lot in one pass, so each lot is fed into the columns in a series of cycles. Here the behavior of the process is affected by the stochastic variability inherent in the bioreactors. The columns require periodic repacking after a given number of cycles. During repacking, the column is out of service for two days. This requirement introduces irregularities into the schedules, making
it very difficult to analyze plant capacity using simplified methods like spreadsheets. Because lots with higher volume and titer load the column more heavily, we weigh the cycles of these lots more heavily, meaning that they will cause the column repacks to occur with a greater frequency. This accurately represents the true variability expected in actual plant operation.

Most of the equipment in this process requires a CIP procedure to be performed after each batch. This is an example of implementing process-specific constraints in the custom logic layer. The solution algorithm schedules CIP tasks as soon as possible after each piece of equipment completes a batch and assumes that equipment can be held in a clean state until its next use. This assumption was validated by inspection of early Gantt charts and expectation of clean-hold times. Because CIP requires a CIP skid, and a limited number of skids are available, manufacturing can be delayed because of this activity. This allows us to determine the minimum number of skids required if the plant is to avoid losing capacity because of CIP, and to assess the capacity reduction expected if fewer skids are provided.

**STORING BIOREACTOR PRODUCT AND DROPPED LOTS**

The storage of bioreactor product is a major factor in analyzing process behavior. Bioreactor product may be stored in the bioreactor itself while waiting for the centrifuge to become available. This happens when batches from two parallel bioreactors finish processing at nearly the same time. Although no hard limit was enforced in the core solver for the length of this storage, we did track bioreactor storage time for all lots. This can be used to determine the distribution of the hold time durations that will be encountered. This distribution can be used to determine the hold time that should be validated with the regulatory agency to facilitate manufacturing. Following the centrifuge and filter, the resulting material is collected in the harvest tank where it remains until it is fed to the purification stage. The storage duration in the harvest tank is subject to a strict time limit, approximately a day for this representative process. However, bioreactor batches that exceed this limit must be discarded, and are referred to dropped lots. To explore stochastic behavior and still obey causality, this harvest tank limit is not enforced by the core solver lest the solver introduce idle time before the start of a production bioreactor to noncausally avoid a dropped lot because of contention for the downstream purification stage. When deciding to start a production bioreactor, an operator cannot know that a particular batch will face a busy purification stage because of a confluence of future factors, such that delaying it will avoid a dropped lot. To ensure an accurate analysis, the customized solution algorithm does not act on future information either.

The dropped lot effect is a significant factor in determining expected plant capacity. There are a number of circumstances that can lead to a dropped lot. First, anytime the bioreactors produce an 11-day batch followed by a 9-day batch, the lots arrive at the purification stage in rapid succession, forcing storage of the second lot. Depending on the presence of other delaying factors, such as waiting for a chromatography column to be repacked, the storage duration for this lot may be too long, forcing a dropped lot. In addition, the presence of high harvest titers can cause processing delays. Batches with high concentrations of product take longer to process on the columns and can lead to unacceptable delays in processing subsequent batches. It is not easy to avoid dropping lots of high harvest batches because by the time the harvest titer of a lot is known, the subsequent lot may already have started. The processing of individual batches also may be delayed because of column repacks, repack failures or, in general, any unexpected events that reduce the capacity of the columns.

**USING DECOMPOSITION TO TREAT AUXILIARY ACTIVITIES SUPPORTING MAINLINE MANUFACTURING**

In addition to the mainline process, there are a number of auxiliary activities involved in plant operation. These consist of support functions like buffer and media preparation and quality control (QC) testing. We used a classic problem decomposition method to model the support activities (for an LP analogy see the Dantzig and Thapa paper). Limitations on labor and support activities were not allowed to affect main line capacity. The level of required support services was also considered, but it was assumed that these constraints would not be rate limiting. This
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assumption is justified because it suffices for the model to predict the required level of resource availability required at every point in the timeline for any particular process schedule. These data are then used to determine the level of support services that would be required to ensure that they would impose no limit on real production. This method was used in modeling such support services as buffer and media preparation, QC testing, and labor. Following the initial evaluation of plant capacity, a separate set of studies was performed in which constraints were added to the model, allowing the impact of limited support and staffing to be quantified. The model explicitly accounts for the fact that support activities can be rate limiting because the outcome of these activities (e.g., QC results) may influence downstream processing decisions. This model decomposition was carried out as follows: Materials were classified as either primary or secondary. Primary materials (those materials used in the main manufacturing model) were scheduled using the solver, ensuring all constraints were met. Secondary materials (manufacturing support areas) were included in the formulation equations, but their constraints were tracked rather than enforced during the main solution process. In practice, the subproblems are broken into independent models for buffer and media prep, and QC.

There are two primary benefits to this approach. First, the decomposition allows us to solve multiple related smaller problems instead of a single large one. This computational benefit is especially important as the number of support areas and their complexity grows. Second, the solution to the primary problem describes the behavior of the plant in the absence of support area restrictions. This aligned well with the industrial philosophy of first designing the manufacturing process and then sizing the support areas to fit the needs of manufacturing. Whether because of unanticipated factors or facility growth, support activities can become the de facto bottleneck of manufacturing operations. Initially, the model was solved with several constrained support areas. Later, it was resolved with fully unconstrained support areas. A comparison of results provided guidance into which support areas had a critical impact on manufacturing capacity.

RESULTS

In this section, we describe the model used to study and optimize the expected behavior of this process. Figure 2 [available at www.biopharminternational.com/stevens] illustrates a portion of a schedule for a typical timeline. The first goal was to determine the production capacity for the process. The major independent variable for plant operation was the cadence with which new cell culture lots were started. Simplistically, faster cadence should lead to higher production rates. Indeed, in the absence of process variability, batch failure, column repack, and other unexpected events, this would be true; if every activity in the plant went according to plan every time, we could calculate the maximum cadence that would allow successive batches to begin as soon as possible, subject to not violating any of the process constraints. Unfortunately, this is not a valid picture of the real world and certainly does not adequately describe a real biologics process. For example, the need to periodically repack the columns produces a problem in which the determination of maximum cadence cannot be determined without a realistic model that accounts for all process constraints. When process variability is considered, the situation becomes more interesting and complex. Proper analysis requires Monte Carlo methods involving the generation of hundreds or even thousands of timelines. Each timeline represents one possible outcome for a scheduling horizon. In this study, we generated timelines that spanned a full year of operation.

Using our approach, we were able to automatically generate schedules for a full year of operation in about 10 seconds on a desktop computer. These schedules honor all constraints in the UDM formulation and all process-specific constraints described above. Each timeline produced a different randomly generated set of time-volume-titer data and thus each timeline produced different results. Thus, when a timeline is generated with a given cadence, we can examine the results to calculate the plant capacity in terms of lots per year. By generating hundreds of timelines for a given cadence, we begin to get an accurate picture of how the plant would behave if the process were driven at that rate. In principle, the plant could be operated so that one batch completely exited the process before the next were started, which would
also minimize dropped lots. In the limit of extremely low cadence, the batches move through the plant independently and the only dropped lots correspond to the random 1% batch failure rate. However, this makes very poor use of the capital investment. As the cadence is increased, production increases as the batches follow one another more closely. The higher the cadence, the greater the probability of interaction or coupling between successive batches. An example of this coupling would be when batch \( k \) must wait at some point in the process because material from batch \( k-1 \) continues to occupy a storage vessel. Another example would be a batch waiting for column repack necessitated by preceding batches, as shown in Figure 3 [available at www.biopharminternational.com/stevens]. Material must be held in production BioReactor-5 because downstream processing is held up while Column2 is repacked. Because we drive the system at a fixed cadence, the production rate is essentially fixed, assuming all of the batches finish successfully. For this reason, interbatch coupling that produces a delay here and there does not reduce capacity. However, as cadence is increased, the process reaches a point at which interbatch coupling begins to cause dropped lots. In Figure 4 [available at www.biopharminternational.com/stevens], the repack of Column2 disrupts the normal flow of lots through the purification train and ColTank1 becomes a local bottleneck. Three lots later, the harvest material expires while waiting for Column1/ColTank1 and must be discarded. Here, because a 9-day bioreactor lot followed a 11-day lot, two lots must be harvested in rapid succession. However, ColTank1 is still catching up from the recent repack and thus the second Harvest lot cannot be sent to Column1 in

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**Figure 5.** Annual production (A) and dropped lots (B) as a function of cadence. Although the plant capacity with a cadence of 2.75 is higher than with a cadence of 3.0, the performance of the plant at 3.0 is considered more desirable.

**Figure 6.** Peak and average labor requirements. A: labor pool levels (high); B: labor pool levels (low).
time. This illustrates how process variability can give rise to rich behaviors not anticipated by a deterministic model.

We studied the process with cadences ranging from one lot every 2.75 days to one lot every 4 days. Even at the slowest cadence studied, dropped lots can occur. This is because of the coupling of batches caused by variable bioreactor process times (9–11 days), and the occasional column repack failure. As the cadence becomes more aggressive, the process becomes less tolerant of delays in the purification system, thus even successful column repacks begin to play a role. This effect is enhanced because processing higher titer batches requires more time on the columns and increases the frequency with which repacks must be run. Figure 5 illustrates annual production capacity as a function of cadence. Figure 5 also shows the number of lots in which bioreactor product was held in the reactor for more than 1 or 1.5 days. Although the plant capacity with a cadence of 2.75 is higher than with a cadence of 3.0 (Figure 5A), the performance of the plant at 3.0 is considered more desirable. At 2.75, the plant experiences a dropped lot rate of 2% and another 6% of the lots have been stored for more than 24 h in the bioreactor. Given the labor and material costs associated with creating a new batch, and the disruption to normal work flow caused by handling dropped lots, operation at this faster cadence is simply not economical.
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The model also was used to investigate the effect of labor availability on plant performance. We defined high load and low load scenarios for staffing levels. For the high load case, we used a seed cadence of three days. For the low load case we used a cadence of four days accompanied by more conservative harvest projections and more aggressive batching of QC samples. These cases represent the extremes of expected operating conditions and serve to bracket the expected support area load. Labor levels represent the number of persons needed to staff production for 24/7 operations.

The profiles were smoothed somewhat before recording peak levels in an effort to account for the fact that many jobs can be done slightly earlier or later than dictated by the schedule. Figure 6 shows the peak and average labor requirement for the four labor pools for the high and low load scenarios. The disparity between peak and average levels indicates that a significant benefit can be gained by cross-training.

We also investigated the effect of staffing levels on QC turnaround time. Additional resource studies were designed to quantify the turnaround time by measuring how fast the laboratory processed different assays. The laboratory cycle time is measured from when the sample is taken to when the test (or set of tests) is complete. This includes any time the sample spends waiting to be processed plus the actual duration of the test, including any reprocessing required for failed tests. Completion of the release testing is key in particular because it is the final step of the process at the site. Four staffing scenarios were tested, with Case A being well-staffed and Case D being very lean.

Figure 7 illustrates the behavior of QC turnaround time for these four cases. As the results indicate, the turnaround time initially decreases rapidly with increasing labor. With Case B, however, we have reached a point of diminishing returns, because the further increase in labor supply for Case A yields little improvement. Figure 8 shows data for the 10 longest turnarounds over any particular timeline, contrasted with the average turnaround time. As these data indicate, we see a marked improvement as labor availability is increased. In Case D, the minimum labor case, the outlying turnaround times can be significantly more than twice the average. In the other three cases, we see much better performance on average, with Cases A and B indicating significantly faster turnaround times only on about 20% of the timelines. Still, these data indicate that substantial variability in turnaround time may be expected on rare occasions.

**CONCLUSIONS**

We have described the results of a mathematical programming-based approach to modeling a large-scale biologics facility for design and analysis of the process. The method permits a Monte Carlo type treatment of stochastic parameters, even for very large problem sizes. As the results have shown, including many aspects of daily plant operation in the analysis allows the design to be fine tuned to increase capacity, anticipate and avoid operational difficulties, and provide insight into the required level of many critical support services, such as CIP and labor. The results for many different cadences show that because of process variability, notably in titers and cycle time, the optimum cadence must balance throughput with lots dropped because of excessive hold time. Furthermore, this mathematical programming approach permitted the analysis of other biologics products by industrial users, without any change to the customized algorithm. ♦

For Figures 2, 3, and 4, please visit www.biopharminternational.com/stevens or contact Associate Editor Haydia Haniff at hhaniff@advanstar.com.

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Statistical Equivalence Testing for Assessing Bench-Scale Cleanability

Cylia Chen, Nitin Rathore, Wenchang Ji, Abe Germansderfer

ABSTRACT
Regulatory authorities expect biopharmaceutical manufacturing facilities to demonstrate that they have an effective and consistent cleaning process in place. For a multiproduct facility, bench-scale characterization offers a useful and cost-effective means to support cleaning validation by comparing the cleanability of a new product to a validated one. Because of the challenges posed by experimental variability in such evaluations, such relative cleanability assessments should be based on a sound statistical analysis. This article describes the application of a two-one-sided t-test (TOST) method to assess the comparability of two groups of cleanability data generated from a bench-scale study.

A n effective cleaning process is critical to ensure that product quality attributes are not compromised by contamination or carryover through product contact equipment surfaces shared among different lots. Regulatory agencies, therefore, require all biopharmaceutical manufacturing facilities to establish effective and robust cleaning validation programs.\(^1\) Multiproduct facilities can use a worst case–based cleaning validation approach in which cleaning cycles are demonstrated to be capable of cleaning the most difficult-to-clean product; no further large-scale verification is needed for other products.\(^2\)\(^-\)\(^4\)

Such an approach, however, requires that the cleanability of all new products be compared to the validated worst case. Bench-scale cleaning studies provide a useful tool to evaluate the relative cleanability of new products and determine the need for revalidation.\(^4\)\(^-\)\(^7\)

In one of our earlier studies, a scale-down model was developed to study the effect of key operating parameters on the performance of the cleaning process.\(^7\) That bench-scale model uses stainless steel coupons spotted with product samples (Figure 1), followed by cleaning under simulated thermal and chemical conditions that are representative of large-scale cleaning cycles. Product removal from the coupon surface is visually monitored and the time required to clean the spot is recorded as the cleaning time.

In this study, we apply the bench-scale model to evaluate the relative cleanability of different protein products. Because of the variability observed in the cleaning times, data...

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Cleaning Validation

points were collected in replicates and the statistical error was estimated. After multiple cleaning time data points were generated for each product, a robust statistical method was needed to adequately assess the comparability of these cleaning time distributions. The two-one-sided t-test (TOST) is a commonly used statistical tool for comparability purposes, especially for method transfers between two laboratories, when the goal is to demonstrate equivalency between the receiving and transferring laboratory. This method is well accepted by the FDA and is widely used in the industry.

This study applies TOST to compare the cleanability of protein drug products.

A STATISTICAL METHOD

When comparing two or more groups of data, the more common approach is to determine if the difference in group means (\(\mu_a - \mu_b\)) is sufficiently large to be declared statistically significant. The test statement or the null hypothesis is that the groups are not different. The effect of declaring the difference statistically significant indicates that the null hypothesis is rejected; the groups represent two or more different distributions of values and are in fact not equal. In practice, given sufficient sample size, even differences that are too small to be meaningful may be declared statistically significant.

The opposite cannot be declared, however, when no statistically significant difference is observed. One can only reject the null hypothesis or show that the groups are different using the common t-test. This is inconvenient when the goal is to show comparability between two or more groups.

An approach widely used in clinical trial statistics and which is gaining popularity in pharmaceutical and biotech settings, the TOST is a method for declaring the comparability of equivalence that is built around comparing two or more group means and their respective mean difference confidence intervals against predetermined equivalence limits. If the difference between the confidence intervals is within a predefined equivalence limit, then the true difference will be within the limit as well, thus making it possible to claim equivalency between two data sets. The key goal for the cleanability assessment is to compare the cleanability of the two products by an equivalency test.

Experimental data generated during a cleaning characterization study using the bench-scale model showed that some inherent variability exists because of the nature of the cleaning process. In addition, analyst and experimental error contribute to further variability. To adequately establish the predefined equivalence limit, each component contributing to variability should be considered. If the equivalence limit is set too wide, the resolution of the method may be reduced because it would be more difficult to distinguish between two products. If the equivalence limit is set too narrow, the results may not be accurate in assessing whether two products are truly equivalent. For the scale-down cleaning model, an evaluation of the different components of experimental variability showed that two times the upper 95% confidence limit of the standard deviation estimate of a controlled data set is adequate to differentiate between the cleanability of two products. Variability in the controlled dataset is one of many potential equivalence limit justifications. Often, when specification or acceptance

**Figure 2.** Datasets are statistically equivalent when the 90% confidence intervals (shown as horizontal bars) of the difference in the group means (\(\mu_a - \mu_b\)) are within the predefined equivalency limit (\(\pm \theta\)). The green bar is considered equivalent whereas blue and red bars are deemed not equivalent.
criteria are available, maximum differences that ensure the capability of meeting these criteria maybe used as equivalence limits.

**SETTING A NULL HYPOTHESIS AND EQUIVALENCY LIMITS**

The null hypothesis (also referred to as the equivalence hypothesis) states that the means of the cleaning times of two products are different by an amount $\theta$ or larger:

$$H_0 = \mu_A - \mu_B > \theta$$

in which $\theta$ is the equivalence limit and $\mu_A$ and $\mu_B$ are the means of the two groups. To test for equivalence, the 90% confidence intervals for the difference between two groups are constructed. The null hypothesis that the groups differ by at least $\theta$ is rejected if the limits of the interval fall outside the $\pm \theta$ bounds. Conversely, comparability is demonstrated when the bounds of the 90% confidence interval of the mean difference fall entirely within the $\pm \theta$ bounds, as shown in Figure 2.

Note that the confidence interval width increases with smaller sample sizes of collected data and with less variability within each data group. The specifics behind the sample size calculation are outside the scope of this article. Larger sample size, however, would naturally result in a narrower confidence interval of the mean difference and hence would make declaring comparability easier. Likewise, although equivalency does not explicitly compare an individual group’s variability, wider variance would result in wider confidence intervals, making it more difficult to declare comparability.

This equivalence limit was computed as two times the upper 95% confidence limit of the standard deviation estimate of the controlled dataset. For the case of cleaning experiments, equivalence limit was equal to 2 x $[1.6 \times 1.4] = 4.48$, in which 1.6 was the standard deviation of a controlled data set (product A) and 1.4 was the multiplier for the 95% confidence limit of a standard deviation estimate, based on a sample size of 18.11 Using the upper confidence limit of the standard deviation estimate accounts for the uncertainty of such estimates based on a given sample size.

Therefore, the acceptance criterion for equivalency was that the upper and lower confidence limit of the difference between the two means should be within $\pm 4.48$. The following two case studies show the application of this statistical approach to comparing the cleanability of different protein drug products.

**Case Study 1:**
**Products A and B are Not Equivalent**

Two protein products were cleaned using the bench-scale method. A total of 18 data points (for cleaning time) were recorded for each product. Commercially available statistical software (JMP) was used to perform the TOST analysis.12 The one-way analysis “Fit Y by X” function was used with a set alpha level (probability of type 1 error) of 0.1, which represents the 90% confidence interval discussed earlier. Figure 3 shows the distribution of cleaning times for the two products. The box and whis-
Cleaning Validation

Figure 4. Cleaning time distributions for product A and Y along with box and whisker plots. The two products are deemed equivalent to each other in terms of cleanability.

Table 2. Upper and lower confidence limits of the difference between two groups as determined using the two-one-sided t-test

<table>
<thead>
<tr>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Difference between two group means</td>
<td>0.8056</td>
</tr>
<tr>
<td>Standard error of the difference between two group means</td>
<td>0.4431</td>
</tr>
<tr>
<td>Upper confidence limit difference</td>
<td>1.5547</td>
</tr>
<tr>
<td>Lower confidence limit difference</td>
<td>0.0564</td>
</tr>
<tr>
<td>Degrees of freedom</td>
<td>33.98923</td>
</tr>
<tr>
<td>Confidence</td>
<td>0.9</td>
</tr>
</tbody>
</table>

in which \( s_A \) is the standard deviation of group A, \( n_A \) is the sample size of group A, and \( s_B \) and \( n_B \) represents the corresponding values for product B. This value provides an estimate of the variability of the difference between the two data sets. The degrees of freedom are adjusted based on the variability of each data set, which is determined by the statistical software (JMP) using the Satterthwaite approximation.\(^1\) The 90% confidence interval for the difference between two means is reflected by the upper confidence limit difference of 70.36 and the lower confidence limit difference of 62.91 of the two group means. Because the equivalence limit is \( \pm 4.48 \), and the upper and lower confidence limit of the difference between two means fall outside the set equivalence limit, it is concluded that product A and product B are not equivalent. Based on the average cleaning time and confidence interval, product B is considered more difficult to clean than product A.

In this case study, the products failed to meet cleanability equivalency mainly because of the large difference (66.64 min) in the mean cleaning times, as shown by the blue bar in Figure 2. It is also possible to fail the equivalency test when the two group means are similar but product B has a high degree of variability, resulting in broad confidence intervals as the one shown by the red bar in Figure 2. In such a scenario, the variability in product B should be further evaluated and the outcome of the cleanability ranking (B<A or B>A) can be made based on an appropriate risk assessment and business considerations.

Case Study 2: Product A and Y are Equivalent

The TOST analysis, as described in the previous case study, was repeated for two other products. Figure 4 shows the distribution of cleaning times for these two products: A and Y.

Table 2 shows the output of the TOST analysis using JMP. The difference between two group means represents the point estimate of the true difference between the two means. This can be calculated by subtracting the sample mean for data set A from the sample mean for B. The standard error (SE) of the difference between two group means can be calculated by applying the following equation:

\[
SE = \sqrt{\left(\frac{s_A^2}{n_A}\right) + \left(\frac{s_B^2}{n_B}\right)}
\]
ADDITIONAL CONSIDERATIONS

To ensure consistency and adherence, a procedure should be established and analysts should be trained to perform such experiments. Because this method provides relative product cleanability, it is important that each experiment be conducted in a consistent manner. When performing cleaning evaluations to compare new products to the validated worst case, an additional check can be incorporated to ensure that each evaluation is conducted in a consistent manner. This is achieved by comparing the data for a control molecule (e.g., a worst-case product) to the established data set or the “gold standard” generated for the control during the characterization study. The same statistical method, the TOST, can be used to fulfill this requirement. For example, an analyst may need to perform an experiment to determine the cleanability of new product N relative to the validated product W. The cleanability of validated product W has been pre-established by prior characterization work. To ensure that the analyst performed the experiment adequately, a comparability test using the TOST can be used to compare the equivalency between data generated by an analyst for product W to the established data set. The equivalency between the two data sets would demonstrate that the experiment was indeed adequate and reliable.

SUMMARY

The two-one-sided t-test (TOST) is a statistical method well accepted by the FDA and industry for evaluating the comparability between two groups of data. In the case of a scale-down cleaning evaluation, this statistical approach has been applied to determine the relative cleanability of two products. The TOST compares two group means and their confidence intervals by comparing them to a predefined equivalence limit. The predefined equivalence limit should be established by evaluating the variability involved with such experimental evaluations. To incorporate an additional check for analyst consistency, TOST can be applied to ensure that the data obtained from different analysts for a particular product (control molecule) are equivalent.

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REFERENCES


The Purification of Plasmid DNA for Clinical Trials Using Membrane Chromatography

Miladys Limonta, Gabriel Márquez, Martha Pupo, Odalys Ruíz

ABSTRACT
Membrane chromatography offers a good solution to the challenge of developing an efficient chromatographic procedure for plasmid DNA purification. The large convective pores of anion exchange membranes allow plasmid DNA to access all the anionic binding sites of the membrane at high flow rates. Here we demonstrate that the pIDKE2 plasmid can be purified from a recombinant Escherichia coli lysate using a Sartobind D membrane combined with size exclusion chromatography to render material with 95% purity and an average yield of 50%. This process yields therapeutically suitable plasmid DNA that meets all regulatory requirements.

Gene therapy and DNA immunization are important and promising possibilities to successfully develop preventive and therapeutic strategies for various diseases. Thousands of people have already received plasmid DNA (pDNA) without serious adverse effects. In the last decade, an increasing number of clinical trials for gene therapies and vaccines based on pDNA have reached the final phases. However, the amount of highly purified pDNA that will be required for these products, should they ultimately reach the market, has been largely underestimated. Commercial-scale processes for plasmid production must be able to manufacture grams or even several kilograms of purified pDNA per batch while meeting the quality standards required by the health authorities.

The chromatographic supports used in purifying such plasmids play a major role these processes, the development of which can be challenging. In particular, RNA removal is a challenge because its chemical composition and structure are so similar to those of pDNA, and because it is so abundant in crude plasmid preparations. Also, the large pDNA molecules adsorb only on the outer surface of the particulate supports, and consequently their capacity is very low—usually on the order of only hundreds of micrograms of plasmid per milliliter of chromatographic support.

Here we describe a procedure for purifying the pIDKE2 plasmid, which encodes the hepatitis C virus (HCV) core, E1, and E2 structural proteins, using a Sartobind D membrane (Sartorius-Stedim Biotech, Goettingen, Germany) at high flow rates. The quality of the pDNA produced using this membrane-based purification process was demonstrated using the analytical methods recommended in relevant...
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- **Extractables & Leachables Testing for Single-Use Bioprocessing Equipment**  
  March 23, 2010  
  As the biopharmaceutical industry’s adoption of single-use bioprocessing equipment continues to increase, end-users continue to have questions about how to conduct extractables and leachables testing in a way that ensures patient safety, meets regulatory expectations, and is appropriately scaled to the true levels of risk. This session will explain the sources of extractables and leachables from plastics and elastomers, present the updated Bio-Process Systems Alliance (BPSA) guide to conducting extractables and leachables testing, and outline risk-based approaches to assessing the impact of extractables and leachables on product quality and structuring an E&L program to address those risks.

- **Quality by Design in Biotech: Keys to Implementation**  
  June 8, 2010  
  Applying the concepts of Quality by Design to the manufacture of biotech products involves some nuances and complexities. This web seminar will provide guidance for implementing QbD with biopharmaceuticals, including recommendations from the QbD Working Group of the PhRMA Biologics and Biotechnology Leadership Committee and industry case studies of applying QbD to various steps of process development for different unit operations.

- **Addressing the Challenges of Downstream Processing, Today and Tomorrow**  
  September 21, 2010  
  As upstream titers continue to improve, companies need solutions to ensure that cell culture gains are not lost in a downstream bottleneck. This web seminar will share data on new strategies and disruptive technologies that can streamline current processes and facilitate productivity, as well as improvements in traditional unit operations.

- **Characterizing Protein Products: Critical Questions and New Technologies**  
  November 11, 2010  
  Protein characterization has always been critical for setting product specifications, determining process controls and release tests, and for establishing comparability after scale-up or site changes. Today, the analysis of protein structure has become even more important, given its key role in the debate over what constitutes “similarity” for biosimilar molecules. This seminar will analyze accepted and emerging methods for protein characterization, including the strengths and weaknesses of the methods and their use in regulatory filings.

**Visit [www.biopharminternational.com/webcasts](http://www.biopharminternational.com/webcasts) today to register for the 2010 webcasts!**
US FDA guidance. The procedure delivers the pIDKE2 plasmid with an average yield of 50% and with 95% purity. The biological activity of the purified plasmid was confirmed in vivo: vaccinated mice developed a positive antibody response against all HCV structural antigens—86.6% and 60% for the core and E2 proteins, respectively.

**MATERIALS AND METHODS**

**Materials**

All the reagents used to make the buffers were purchased from Merck (Darmstadt, Germany) and Sigma (St. Louis, MO). G25 coarse chromatography medium, Sepharose CL-4B, and Sephacryl S-1000 were purchased from Amersham BioSciences (GE Healthcare, Piscataway, NJ).

**Recombinant proteins**

Recombinant Co.120 and E1.340 were obtained from E. coli. Co.120 comprises the first 120 amino acids (aa) of the HCV nucleocapsid protein, whereas E1.340 encompasses aa 192–340 of the viral protein. E2.680 comprises aa 384–680 of the HCV polyprotein and is obtained from recombinant *Pichia pastoris*.

**Production of pIDKE2**

*E. coli* DH10B cells harboring pIDKE2, a plasmid for DNA immunization expressing the first 650 aa of the HCV polyprotein from the 1b-Cuban isolate genotype, were grown overnight, at 37 °C, in 100 mL shake flasks containing 50 µg kanamycin/mL, at 250 rpm. The engineered *E. coli* was cultured in a 5-L fermenter (Marubushi, Tokyo, Japan) using a complex media containing 50 g/L of yeast extract in fed-batch mode.

**Bacterial lysis and plasmid recovery**

The bacterial cell paste (typically 200 g) was re-suspended in 2.4 L of a buffer containing 61 mM glucose, 10 mM Tris–HCL, and 50 mM EDTA, at pH 8. Bacteria were lysed using a previously described procedure. The clarified lysate was concentrated five times on a tangential flow filtration (TFF) system (nominal molecular weight cutoff 100,000 kDa, 0.1 m²). All experiments were conducted keeping a constant transmembrane pressure (TMP) of 0.8 bar at 4 °C, using Sartocon Slice equipment (Sartorius-Stedim Biotech). To remove RNA, the concentrated clarified lysate was loaded on Sepharose CL 4B in a BPG 113 column equilibrated with 20 mM Tris–HCL, 3 mM EDTA, and 1.5 M (NH₄)₂SO₄, at pH 7.2.

Plasmid capture and elution using the Sartobind membrane was carried out after buffer exchange on a G25 matrix using 25 mM Tris, 10 mM EDTA, and 0.5 M NaCl, at pH 8 and a rate of 50 mL/min, to reach a conductivity of 45 ms/cm. The pDNA fraction was pumped at 150 mL/min to the membrane holder 91-D-01K-15-03. After loading the feeding solution, the membrane was rinsed with a pH 8 buffer containing 25 mM Tris, 10 mM EDTA, and 0.5 M NaCl, at pH 8 and a rate of 50 mL/min, to reach a conductivity of 45 ms/cm. The pDNA fraction was pumped at 150 mL/min to the membrane holder 91-D-01K-15-03. After loading the feeding solution, the membrane was rinsed with a pH 8 buffer containing 25 mM Tris, 10 mM EDTA, and 0.5 M NaCl, at pH 8 and a rate of 50 mL/min, to reach a conductivity of 45 ms/cm. The pDNA fraction was pumped at 150 mL/min to the membrane holder 91-D-01K-15-03.
The Parenteral Drug Association Presents

2010 PDA Annual Meeting
Manufacturing Excellence

March 15-19, 2010
Gaylord Palms Resort and Convention Center
Orlando, Florida

For 64 years, the Parenteral Drug Association (PDA), an industry non-profit, has been joining industry regulators, educators and policy-makers at the PDA Annual Meeting to discuss the latest scientific and technological innovations. We invite you to join our 500 industry leaders in this year's dialogue.

The theme and focus of the 2010 meeting is Manufacturing Excellence and productivity in the regulated healthcare product industry. This paramount issue not only affects your company’s bottom line, but the optimization of benefits and values. As an industry we must realize the need for better productivity, identify the obstacles and use our scientific and technical knowledge as a means to achieve Manufacturing Excellence.

The complete 3-day program includes more than 70 presentations given by some of the most knowledgeable professionals in our industry. To help you navigate the comprehensive program agenda, we have segmented the concurrent sessions into subject matters, including:

- Development Science
- Manufacturing Process Science
- Manufacturing Science
- Media Fills
- Microbiology
- Process Analytical Technology
- Process Development
- Process Validation
- Quality by Design
- Quality Science
- Rapid Micro Methods
- Risk Management

In addition to the extensive program agenda, PDA provides attendees with several formal and informal networking opportunities. These strategically placed gatherings allow you to interact with your speakers and peers in an idea exchanging environment to help you generate solutions for your specific challenges.

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by TFF until reaching a concentration of 2 mg/mL as determined by the absorbance at 260, using a VivaFlow 200 system (nominal molecular weight cutoff 100,000 kDa, 200 cm²). The final pDNA was filtered (0.22 µm) before further analyses were performed.

Analytical Methods
Flow-through and eluted fractions were precipitated by adding one volume of 2-propanol for 15 min on ice and then centrifuged at 14,000g for 10 min at 4 °C. The pellet was washed with 70% ethanol and centrifuged again at 4,000g for 10 min. The pellet was air-dried for 5–10 min and dissolved in 50 µl 10 mM Tris–HCl at pH 8. The pDNA purified as reported was used as a positive control.

Identity was evaluated by restriction endonuclease digestion using a panel of enzymes followed by analysis on agarose gels. Binding capacity and pDNA purity were determined by measuring absorbance at 260 (A260) and 280 (A280) and expressed as the ratio A260/A280 in a low-salt buffer. The pDNA purified as reported was used as a positive control. Identity was evaluated by restriction endonuclease digestion using a panel of enzymes followed by analysis on agarose gels.

Mice immunization and biological activity
Fifteen female Balb/c mice (6 to 8 weeks old), purchased from CENPALAB (Havana, Cuba),
were immunized intramuscularly three times every second week with 50 µg pIDKE2 + 5 µg Co.120, obtained using a method previously reported, or following the method described in the present paper. The presence of antibodies against recombinant HCV structural antigens were determined by ELISA, as previously described. The induction of antibody titers of above 1:50 against HCV structural antigens in >25% of the mice on day 42 was considered a positive response.

**RESULTS AND DISCUSSION**

**Lysis and plasmid recovery**

The most common method for isolating pDNA is based on alkaline treatment and detergent-mediated solubilization of the bacterial cell membranes. In the second stage, the pH of the solution is adjusted to a value close to 5.5 by adding potassium acetate. The change in the physicochemical conditions of the solution causes the renaturation and flocculation of the chDNA as well as the precipitation of protein–SDS complexes and cell wall debris. The insoluble material can then be separated from the liquor containing the pDNA by filtration or centrifugation; once separated, the liquor containing pDNA is subjected to downstream processing to recover and purify the product. In our process, the insoluble material is separated from the liquor containing the pIDKE2 plasmid by centrifugation. Loading the crude lysate containing large amounts of impurities such as chDNA, RNA, proteins, and endotoxins directly to a chromatography matrix is not recommended; primary purification is essential. The clarified lysate containing the plasmid is concentrated five times using a TFF system; however, this step is not sufficient to remove all the RNA (Figure 1, lane 2). RNase is commonly added to degrade the RNA but this procedure is not recommended by regulatory agencies; consequently the RNA content in the clarified lysate is very high—about 25 times the amount of the pDNA in weight. In the present process, 100% of the remaining RNA is removed from the concentrated cell lysate during the first chromatographic step on Sepharose CL-4B,
with 91% of the pDNA recovered. The analysis on the agarose gel shows that pDNA eluted in the void volume can be efficiently separated from RNA (Figure 1, lane 4).

**pIDKE2 purification on a Sartobind D membrane**

Large biomolecules such as pDNA bind only to the surface of traditional chromatography beads; hence, the capacity for pDNA is much lower than it is for small biomolecules which are able to access the full volume of the beads.\(^4\)

Figure 2 shows the chromatographic profile for the separation of pIDKE2 under the selected conditions. During loading, no significant amount of pDNA was detected in the flow-through at the high flow rate of 150 mL/min. We calculated the average dynamic binding capacity for 10 batches at 3.3 ± 0.8 mg pDNA per mL. This means that the capture of pDNA from *E. coli* lysate is more efficient and rapid using the Sartobind D membrane, which has a dynamic binding capacity between 4.1 and 2.5 mg pDNA per mL support using a flow rate of 4.3 column volumes per minute. This is a more desirable result than what can be achieved by conventional anion-exchange resins, which bind about 1 mg of pDNA per mL of resin at flow rates that typically are lower than 0.5 column volume per minute.\(^6\) Seventy percent of the pDNA was eluted with 90% purity as determined by agarose gel, and no RNA is found (Figure 1 lane 6, Table 1). In addition, the assays for proteins and endotoxins indicate that there was a reduction of these contaminants.

The loading, washing, elution, and membrane regeneration procedures took place in 1.5 h. Thus, this approach significantly reduced the separation time and increased the throughput and productivity for pDNA recovery. The Sartobind D membrane was repeatedly used for 10 batches. After each run, the membrane was regenerated with 0.5 M sodium hydroxide, because of its high stability in alkaline solutions. During the process, no change in backpressure was observed, demonstrating the stability and reproducibility of the membrane.\(^7\)

The final purification step was similar to that reported previously:\(^4\) pDNA fractions...
Biologics Forum & Networking Reception:
The Rapid Evolution of BioManufacturing and the New Supplier Reality

Wednesday, March 17, 2010
Program: 1:30pm-4:15pm
Reception: 4:15pm-5:30pm

Biologics are gaining a growing share of the commercially approved therapeutics and therefore pharmaceutical companies are increasing their R&D and/or manufacturing capacity investments in this area. However, the complex manufacturing processes and the requirement for even more complex analytical characterization (relative to small molecules), requires flawless execution across the entire value chain, from sourcing to manufacturing and distribution, to ensure consistent product quality.

Leading manufacturers will discuss how to design and implement a successful supplier management program to ensure effective biologic manufacturing. They will provide their insight on how economic forces, the changing regulatory landscape and advances in cell-line productivity and disposable technology are driving a rapid evolution of biologics manufacturing and what suppliers need to do to adapt to this new reality.

Suppliers will discuss their current strategies to deliver increased flexibility, improved process economics and reduced time-to-market to support this burgeoning segment of the market. A question and answer session will follow the presentation.

Why should you attend this forum?
• This program will be very informative for established biologics manufacturers as well as companies considering entry into biologics development or manufacturing.
• This program will bring out the subtleties and nuances of managing the complex supply chain of raw materials used in biologics manufacturing.
• Participants will hear the key trends in biomanufacturing and learn how their organizations – be it a biologic manufacturer, a contract manufacturer or a supplier of materials and components – need to adapt to remain competitive.

The networking reception following the event will allow participants to interact and develop relationships with a variety of industry leaders.

Speakers: Richard Crowley, Sr. Vice President, Biopharmaceutical Operations, ImClone Systems, a wholly owned subsidiary of Eli Lilly & Company, Nigel Darby, Vice President, BioTechnologies, GE Healthcare Life Sciences, Dr. Michael Hawaldt, VP Biotechnical Manufacturing, Boehringer-Ingelheim, Jim Skrine, Executive Director and Site Head of Quality, Amgen, Inc. and Karen King, President, DSM, Biologics Division. Moderated by Marc Lampron, Sr. Director - Procurement, Genentech

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Select Member button and in the comments/code section please enter DWbio-10 so your special pricing can be accepted.

An e-ticket to the event will be sent to you.
were pooled, concentrated to 2 mg/mL by tangential flow filtration, and finally filtered (0.22 μm). The purification steps render a final high purity pIDKE2 plasmid with an average yield of 50%. The yield is influenced also by the homogeneity of pDNA in the cells. A summary of the analytical specifications according to FDA criteria is shown in Table 2. The purified plasmid pIDKE2 had 95% purity.

Plasmid identity was confirmed by restriction enzyme digestion and its activity confirmed by an in vivo assay. The purified pIDKE2 plasmid induced a positive response against HCV core and enveloped proteins, with 87% seroconversion against Co.120 and 60% against E2.680 proteins, respectively.

**CONCLUSIONS**

Our process produces plasmid DNA (pDNA) with 95% purity, and the process fulfills all regulatory requirements and renders pharmaceutical-grade pDNA. The content of genomic DNA is lower than 5 ng per dose, RNA is not detectable by agarose gel electrophoresis; endotoxin content is 0.77 EU per kg body weight, and the protein content is 4.1 μg per dose, which is lower than the limit established.

In conclusion, this process, which combines size exclusion and membrane chromatography, met the criteria of purity, robustness, and reproducibility required for manufacturing pharmaceutical-grade pDNA for human clinical trials.

**REFERENCES**


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**Table 2: Plasmid recovery, yield, protein, and endotoxin reduction for the Sartobind D capture step.**

<table>
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<tr>
<th>Fraction</th>
<th>Total volume (L)</th>
<th>pIDKE2 (mg/mL)</th>
<th>Endotoxin (EU/mg of pIDKE2)</th>
<th>Protein (mg/mL)</th>
</tr>
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<tbody>
<tr>
<td>pDNA fraction loading</td>
<td>2.4</td>
<td>0.086</td>
<td>9.3 x 104</td>
<td>5.62</td>
</tr>
<tr>
<td>pDNA elution</td>
<td>0.5</td>
<td>0.29</td>
<td>2.7 x 102</td>
<td>1.21</td>
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</table>
FLUID PATH ASSEMBLIES
Meissner Filtration Products has just released literature featuring its One-Touch single-use fluid path assemblies. The assemblies are designed for secure fluid transfer in critical biopharmaceutical processing applications. The literature illustrates a variety of common fluid path assembly process configurations that use Meissner’s library of prequalified components. Additionally, fluid path assemblies are shown with attached capsule filters for applications requiring filtration in volumes from 10 mL to over 10,000 L. The assemblies can be customized to end-user requirements and include combinations of connectivity, tubing, and various other components to achieve the functionality necessary for process demands.

POLYPROPYLENE TUBING
Prolite polypropylene tubing from NewAge Industries is made from an NSF-61 (National Sanitation Foundation) compound. Prolite’s properties include excellent chemical and corrosion resistance, very low moisture absorption, and high dielectric strength. It meets UL94 HB burn rating, is lightweight, thermoformable, is weldable, and has low surface friction. These combined attributes make Prolite a viable and reasonably priced alternative to fluoropolymer tubing in many applications. Prolite is stocked in nine sizes (1/4” through 1-1/4” OD) for same-day shipment. Custom options consist of non-stock sizes, colors, heat-formed shapes, coils, and cut-to-length pieces. Although Prolite may be welded, it is typically joined by means of a push-to-connect fitting such as Newlco. NewAge Industries, Inc., 215.526.2300, www.newageindustries.com/polypropylene_tubing.asp.

2010 LIFE SCIENCE EQUIPMENT CATALOG
New Brunswick Scientific’s 2010 catalog is now available online or can be ordered in hard copy format. New products include the 5- and 14-L CelliGen BLU single-use stirred-tank bioreactor, the versatile BioFlo 610 sterilizable-in-place mobile pilot plant fermenter, and the new benchmark for SCADA software user-friendliness: NBS’s next-generation PC-based BioCommand. The catalog also features over 40 biological fermenters, advanced CO2 incubators, and ULT freezers, as well as several new bioprocessing systems. Color-coded product sections and at-a-glance model comparison tables make this catalog easy to use.

SMALL CAPACITY INCUBATORS
Thermo Fisher Scientific’s smaller capacity Thermo Scientific Midi 40 CO2 incubator delivers the reliable culturing performance of a full-sized incubator in a convenient, space-saving footprint. The Midi 40 is ideal for researchers who require separate, undisturbed incubation conditions caused by the contamination risk associated with shared incubators or lack of available space. The incubation parameters of the Midi 40 are managed using the incubator’s IntrLogik II user interface, which features a vacuum fluorescent display and membrane touchpad. The unit also incorporates a non-corrosive stainless steel chamber, with 1.4 cubic feet (40 L) of culture space, and a heated humidity pan to generate relative humidity values of up to 95%. Thermo Fisher Scientific, 781.622.1000, www.thermo.com/incubators.

CELL BANK MANUFACTURING
Lancaster Laboratories offers capabilities to manufacture, store, and characterize a wide variety of mammalian cell banks, including master, working, and research banks with expertise in cold-chain management. Our cGMP-compliant manufacturing facility includes multiple ISO 7 clean rooms and ISO 5 critical areas designed to meet current aseptic processing guidelines. Fully validated manufacturing procedures include process simulation studies to ensure the integrity of the manufacturing process.
Lancaster Laboratories, 717.656.2300, lancasterlabspharm.com.

SINGLE-USE 3L BIOREACTOR
The Mobius CellReady 3L Bioreactor from Millipore Corporation demonstrates all the benefits typically associated with single-use processing, while incorporating standard design features familiar to customers already using benchtop, stirred-tank bioreactors. Designed to replace traditional glass benchtop bioreactors, the bioreactor has a standard stirred-tank format for use in development and optimization of cell culture processes. This vessel design features a universal design, ensuring compatibility with most benchtop bioreactor control systems. By eliminating time-consuming steps associated with cleaning, assembly, and sterilization, the CellReady 3L Bioreactor significantly reduces the turnaround time typically associated with glass bioreactors. The CellReady 3L Bioreactor was co-developed under agreement with Applikon Biotechnology B.V.

BioPharm International  www.biopharminternational.com  February 2010  55
**Integrated Single-Use Solutions**

FlexAct from Sartorius Stedim Biotech offers new possibilities for designing flexible production processes by using out-of-the-box disposable unit operations. The FlexAct BP system is based on a multifunctional central operating module that accommodates preconfigured single-use assemblies and a pump and a control unit. FlexAct BP addresses production capacity needs from 50 to 1,000 L for buffer preparation. The integration of monitoring and control features for pH, temperature, pump speed, and fluid level control reflects a new milestone for implementing process-relevant analytical tools.


**Online Guides for Protein Purification**

GE Healthcare has launched a set of online guides for protein purification. The site includes interactive selection guides and a variety of educational materials. There are four selection guides in the series: purification of histidine-tagged proteins, purification of GST-tagged proteins, antibody purification, and prepacked columns selection guide. The site also offers information on all types of protein separation technique, in-depth handbooks, and advice on developing successful protein purification protocols. GE Healthcare, www.gelifesciences.com /protein-purification.

**Aseptic Sampling Systems**

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Location: San Diego, CA
www.pda.org

28–March 5: Pittcon 2010
Location: Orlando, FL
www.pittcon.org

MARCH

15–18: International GMP Conference
Location: Athens, GA
www.internationalgmp.com

15–19: PDA Annual Meeting
Location: Orlando, FL
www.pdaannualmeeting.org

20: ACS Division of Biochemical Technology One Day Short Courses for Professional Development
Location: San Francisco, CA
http://acsbiotn.alwhs.com/BIOT/

23–25: Aseptic Processing
Location: Princeton, NJ
www.agalloco.com/aseptic_processing_course.php

APRIL

12–15: 2010 PDA Pharmaceutical Cold Chain Management Conference
Location: Bethesda, MD
www.pda.org/coldchain2010

13–14: CHI Protein Scale Up
Location: San Diego, CA
www.healthtech.com/Conferences_Overview.aspx?id=94573

20–22: INTERPHEX
Location: New York, NY
www.interphex.com

26–28: CMC Strategy Forum Europe
Location: Vienna, Austria
www.cass.org/cde.cfm?event=265809

MAY

3–6: BIO International Convention
Location: Chicago, IL
www.convention.bio.org

16–19: AAPS National Biotechnology Conference
Location: San Francisco, CA
www.aapspharmaceutica.com

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QUALITY BY DESIGN: WHERE WE STAND NOW
What aspects of Quality by Design now have consensus within the industry and regulatory bodies, and what remains to be sorted out? Join Laura Bush, Editor in Chief of BioPharm International and John Towns, Senior Director of Global CMC Regulatory Affairs at Eli Lilly, as they discuss the current state of QbD.

BIOPHARMACEUTICAL JOB MARKET: 2010 & BEYOND
Which job functions will be the most sought-after five years from now? Listen as Eric Langer, President and Managing Partner at BioPlan Associates, and Chitra Sethi, Managing Editor of BioPharm International, compare the findings of BioPlan’s recent biomanufacturing job market research and BioPharm’s new salary survey.

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