A publication of
The Williamsburg BioProcessing Foundation

Winter 2006

BioProcessing JOURNAL

The Most Trusted Source of BioProcess Technology®

Vol. 5 No. 4

www.bioprocessingjournal.com

Current Therapeutic Antibody Production and Process Optimization

By FENG LI, JOE X. ZHOU, XIAOMING YANG, TIM TRESSEL, and BRIAN LEE

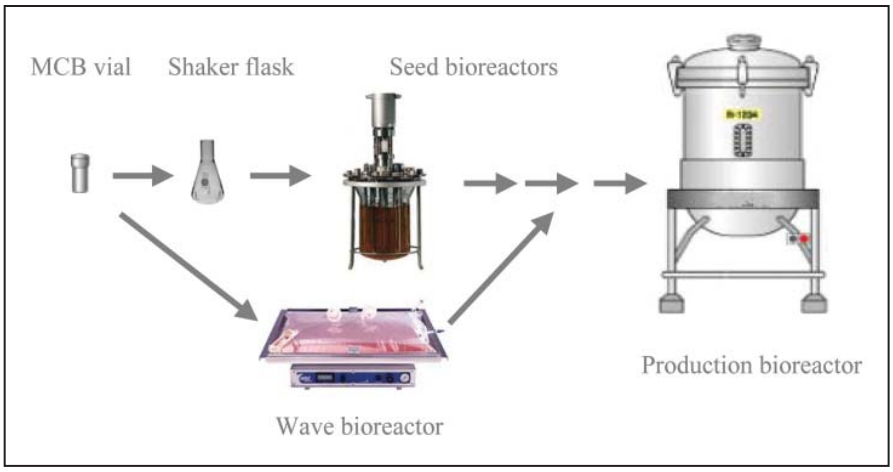


Figure 1. A typical cell culture process comprises vial thaw, seed expansion, and production stages.

In today's biopharmaceutical pipeline, monoclonal antibodies are a predominant modality for a broad range of clinical indications, including inflammatory disorders, oncology, and infectious diseases.[1] More than two dozen antibody-based products are commercially available. In 2004, six of the 12 new biopharmaceutics that gained approval in the United States and Europe were antibody-based products.[2]

Most antibody therapies require high doses over a long period of time, which requires large amounts of purified product per patient. Therefore, manufacturing capacity to meet the demands of antibody production is a real challenge. It is desirable to have highly productive and consistent manufacturing processes. In addition, speed to market is critical to deliver health benefits to patients quickly and to achieve business success. For early-stage clinical studies (Phase I and II), an initial standardized platform process is usually applied to satisfy the material demand and quality requirements within a short period of time, despite the relatively low productivity and robustness.[3,4] Once the product candidate is proven safe for patients in an early-phase clinical study, the process is further optimized in order to maximize product yield and process robustness, and reduce the cost of goods for commercial manufacturing.

Two major areas of commercial antibody process development are described in this review article: upstream cell culture and downstream purification processes. Upstream process development includes cell line development, media optimization, and cell culture process optimization. Downstream process development comprises cell harvest, antibody capture, viral inactivation, and polishing steps. This article provides an updated review and discussion of the technologies used in recent therapeutic antibody production processes.

Cell Culture

Recombinant mammalian cell culture is the most popular expression system to produce monoclonal antibodies. Chinese hamster ovary (CHO) and murine myeloma (NS0) are the most widely used cell lines.[5] A typical cell culture process starts with thawing a frozen vial of a working cell bank (WCB), followed by expanding the cell population through a series of seed trains in different culture vessels. The culture is then transferred to a production bioreactor where the cells continue to grow and the expressed product accumulates in the culture broth. In order to achieve a high product titer, high cell mass and cell viability need to be maintained in the production bioreactor. Two of the most popular process modes are fed-batch (feeding concentrated nutrient solutions to a batch production bioreactor) and perfusion (maintaining and recycling the cells to a bioreactor while continuously replacing spent media with a fresh supply). The fed-batch process is often used due to its scalability, ease of operation, and high volumetric productivity. An illustration of the unit operations of a typical upstream process is shown in Figure 1.

Although stainless steel bioreactors are still the major choice for large-scale

Feng Li, Ph.D.* *(fengl@amgen.com) is a scientist, and Brian Lee, Ph.D., is a principal scientist in the process engineering department;* ***Joe X. Zhou, Ph.D.**** *(joez@amgen.com) and Tim Tressel, Ph.D., are principal scientists in the purification process development department; and Xiaoming Yang, Ph.D., is a principal scientist in the cell science and technology department; Amgen, Thousand Oaks, CA. *These two authors contributed equally to this article.*

production, disposable bioreactor systems have become available. The Wave Bioreactor® system, which uses a plastic disposable bag, is commonly used during seed culture expansion. Disposable bioreactor systems can benefit the manufacturing process by eliminating the clean-in-place (CIP) and steam-in-place (SIP) operations and by reducing the expensive capital investment for stainless steel bioreactors. The Wave Bioreactor system at the 500-liter scale has achieved high cell densities with CHO cells which shows its potential application as a final production vessel, especially for rapid material supply during early stage clinical studies.[6]

Cell culture process optimization is an integrated activity involving cell line selection, medium development, and optimization of bioreactor conditions. High titers up to approximately 5 g/L and cell densities of more than 20 million cells/ml have recently been reported in fed-batch cultures.[7,8] The specific productivity of over 20 pg/cell/day can be routinely achieved for production cell lines.[9] Enhancement of specific productivity per cell is accomplished not only by selecting highly productive cell lines, but also by optimizing medium compositions and bioreactor operating conditions.

Clone Selection

Cell line development and master cell bank (MCB) generation comprise one of the most critical steps of process development. After transfection with plasmids bearing the antibody light- and heavy-chain genes, cells are screened for highly productive cell lines through growth recovery, single-cell cloning, serum-free and suspension adaptation, amplification, and final clone selection. Screening and selecting a highly productive and stable clone from the transfectant population in a limited time frame is a major challenge. Most commonly, the transfected cells are diluted and cultivated in 96-well plates with a basal growth medium and screened to identify those with fast growth. The productivity among the fastest growing cell lines is then analyzed for product titer. The candidates with the best productivity and growth characteristics are adapted to suspension culture using serum-free medium in shake flasks.

At this stage, in order to predict the performance of each clone candidate in a large-scale production bioreactor, an enriched medium similar to the final production medium formulation, with a similar feeding regime, can be employed in shake flasks. Once promising candidates are identified, the cell lines can be amplified to increase the copy number of the antibody gene by using higher concentrations of inhibitors of selective markers. For example, increased concentrations of methotrexate (MTX), a dihydrofolate reductase (DHFR) inhibitor, are added to culture medium where CHO cells are growing. Under these conditions, only the cells containing a high copy number of the DHFR gene can continue to grow. During amplification of the selective marker gene, the genes of interest expressing the antibody may be co-amplified, which consequently results in a higher expression level and enhanced productivity of the antibody. A similar approach can be taken with NS0 cells containing glutamine synthetase (GS) by using its inhibitor, methioninesulfoximine (MSX). The amplified cell lines need to be evaluated for genetic stability in the absence of selective pressure because the production stage usually does not employ selective pressure.

High-throughput screening and selection technologies have been developed to shorten the time required for clone selection. Screening for cells with high expression and secretion levels can be accomplished using a fluorescently tagged antibody against the product expressed either on the cell surface or secreted in a micro gel bead, followed by fluorescence-activated cell sorting (FACS).[10,11] Another approach involves miniaturized bioreactors or shake flasks that can simulate standard production bioreactor conditions, including nutrient feeding. This technology usually employs high-speed automation to streamline liquid handling and analytical capability for early evaluation of growth and productivity profiles for candidate clones. Once the production cell line is identified, MCBs and WCBs are generated under GMP conditions before initiating process development for commercial production.

Medium and Feeding Strategy Development

In general, medium development for a fed-batch process involves batch medium and feed concentrate development, as well as feeding strategy optimization. Optimization of cell culture processes is often regarded as cell line-dependent and is, therefore, based on the metabolism and nutrient consumption of a specific cell line. Several approaches can be used systematically for medium optimization, such as single-component titration, spent medium analysis, and medium blending.[12] Due to regulatory concern regarding serum and other animal-derived components, the addition of animal-component-free hydrolysates to chemically defined media is a common approach to increase cell density, culture viability, and productivity. Hydrolysates are protein digests composed of amino acids, small peptides, carbohydrates, vitamins, and minerals, which provide nutrient supplements to the media. In addition, hydrolysate peptides can also act as growth factors and stimulate production. Non-animal-derived hydrolysates from soy, wheat, and yeast are commonly used in cell culture media and feeds. However, due to their complex composition and lot-to-lot variations, hydrolysates can be a significant source of medium variability.

The most common approach to developing a feed medium uses concentrated basal media without salts (to avoid high osmolality). Certain key feeding components (*e.g.*, phosphate) have also been identified.[13] During medium preparation, pH and/or temperature may need to be adjusted to completely dissolve some low-solubility components.[14] To optimize a feeding strategy, consideration should be given to nutrient consumption, by-product accumulation, and the balance between growth and production.

Previous studies indicated that byproducts such as lactate and ammonia could be minimized by maintaining low glucose and glutamine concentrations through frequent feeding.[15] However,

This Bioreactor is So Advanced, You'll Think We're "Out of Control"

Introducing the Supercharged CelliGen® 310

This compact bioreactor / fermentor is so powerful, it's virtually impossible to outgrow its control capabilities:

- Control 1 to 4 vessels & regulate over 120 parameters, simultaneously!
- Integrate up to five mass flow controllers for sparge and gas overlay — up to 10 sensors, scales, analyzers or other external devices, per vessel, for total process optimization.
- Large 15" touchscreen controller is exceptionally easy to navigate.
- cGMP-compliant with validation packages available.

For downloadable brochure see
www.nbsc.com/wbf.htm or email us at bioinfo@nbsc.com

New Brunswick Scientific

Innovative equipment for culture growth, detection & storage

frequent feeding is less desirable for large-scale production in manufacturing, due to its complexity. Stepwise bolus additions of the feed solution to the production bioreactor is widely used in industry, due to its simplicity and scalability. In general, medium development is labor-intensive and time consuming. Approaches that combine high-throughput screening platforms with statistical design of experiment (DOE) are commonly applied to shorten the development time.

Bioreactor Monitoring and Control

Process improvement can be achieved through optimization of bioreactor physicochemical environments. Optimization of culture conditions needs to balance cell growth with antibody production. A preferred cell growth condition may not be the optimal condition for productivity and vice versa.

For mammalian cell culture, pH is normally controlled within the range of 6.5–7.8. High pH (≥7.0) is preferred for initial cell growth, whereas low pH can facilitate antibody production.[16] However, high pH is usually associated with increased anaerobic cell metabolism that converts more glucose into lactate. When lactate accumulation exceeds the medium's buffer capacity, the pH of the medium decreases. Because most bioprocesses are pH controlled at a certain level, lower pH of the culture medium induces base addition to the medium, which increases osmolality. This can cause delayed cell growth and accelerated cell death. Thus, the initial pH control condition needs to be optimized at an early stage of the cell culture process in order to maximize initial cell growth. Once fast cell growth is achieved, the pH set point can be shifted to a lower level to facilitate antibody production. Figure 2 shows typical pH and lactate profiles in a fed-batch process. As lactate was consumed in the later stage of the process, pH was allowed to increase.

Manipulating the cell cycle in CHO cell culture through a temperature shift has been reported to extend culture longevity. A temperature shift from 37° C to 30° C after 48 hours post-inoculation can retain cells in the G1 phase longer and therefore delay the onset of apoptosis.[17] Dissolved oxygen (DO) is usually considered a less critical parameter which can be controlled in a wide range (between 20–100%) of air saturation. Cell growth may appear unaffected under a certain DO level; however, DO can have significant impact on product quality. For example, it has been reported that a reduction in DO caused decreased glycosylation of antibody N-glycan chains.[18]

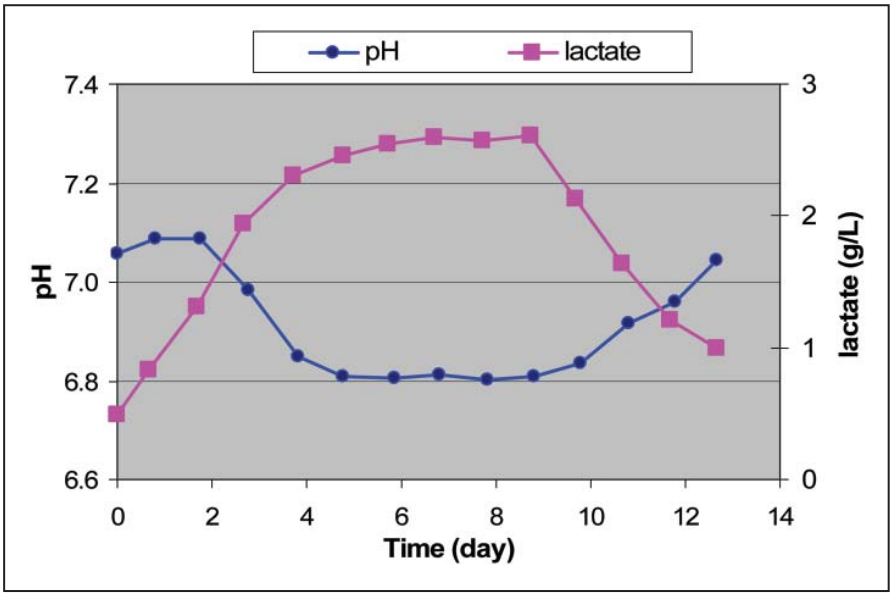


Figure 2. Typical pH and lactate profiles in a fed-batch process.

Although a bioreactor's physical environmental parameters, such as pH, DO, and temperature, can be monitored on-line and controlled, monitoring of chemical and biological parameters is limited due to the lack of reliable on-line sensors. The measurement of cell density, metabolites, and protein concentrations usually occurs through daily bioreactor sampling and subsequent analysis by off-line instrumentation assays such as the Cedex cell counter (innovatis AG, Bielefeld, Germany), the Nova BioProfile, (Nova Biomedical, Waltham, MA), YSI analyzer (YSI Inc., Yellow Springs, OH), and high-pressure liquid chromatography (HPLC). Within the last decade, many new on-line monitoring and control techniques for bioreactors have been developed. Some novel biosensors are commercially available (*e.g.*, biomass sensors and YSI's dissolved CO_2 probe). These can measure on-line oxygen uptake rates to optimize nutrient feeding strategy and at-line HPLC is used to analyze cell culture broth amino acids and glucose.[19,20] Near-infrared (NIR) spectroscopy is a nondestructive technique that can monitor multiple components simultaneously from a single probe. With recent advances in NIR instrumentation and data analysis algorithms, its applications have been demonstrated in the measurement of glucose, glutamine, lactate, ammonia, and even recombinant protein concentrations in cell culture broth.[21,22] With FDA's regulatory framework on Process Analytical Technology (PAT), these new technologies for in-process, on-line, and at-line monitoring are being heavily investigated as tools to identify critical process variables and initiate the appropriate control actions to maintain process and product consistency.

Cell Culture's Impact On Product Quality

Monoclonal antibodies are complicated glycoproteins subject to glycosylation heterogeneity and other modifications such as N-terminal pyroglutamate and C-terminal lysine variants, methionine oxidation, asparagine deamidation,

disulfide bond scrambling, aggregation, and fragmentation. Multiple process factors have the potential to change these product quality attributes, potentially resulting in clinical implications including efficacy and safety. While optimizing processes to achieve high yield, it is critical to monitor product quality changes at every stage of development.

Glycosylation variation, which can impact *in vivo* IgG functions and product stability, is one of the most sensitive quality-related attributes that is dependent on cell lines and cell culture conditions. Most mouse-derived cell lines are known to add Gal-α1,3-Gal onto antibody heavy chain N-glycan. High Gal-α1,3-Gal content, which is not found in human antibodies, raises immunogenicity concerns. It has been reported that this glycoform is varied in different NS0 clones. A high-throughput screening approach was used to select low Gal-α1,3-Gal clones in early process development stages.[23]

The effects of cell culture media and conditions on antibody glycosylation have been extensively studied.[24,25] Factors such as medium serum, glucose, ammonia, DO, dissolved CO_2, and culture osmolality have been reported to cause glycosylation changes in different cell lines. These factors can likely affect the activity of monosaccharide transferases and/or sugar transport to the Golgi apparatus, which is the major glycosylation site in mammalian cells.

Although the integrity of a protein's backbone is usually unchanged across different cell lines and culture conditions, some modifications can occur during cell culture processes. The IgG heavy-chain C-terminal is conserved as lysine, which is normally cut off through post-transcriptional modification. The presence or absence of C-lysine can result in product charge variants. Carboxypeptidase B specifically cleaves C-terminal lysine residues. The activity of such metal enzymes can be affected by some medium trace element concentrations.

Downstream Processes and Optimization

As enhancement of the final product titer and cell density of a cell culture process continues, the subsequent purification stage can become a bottleneck that impedes achieving a cost-effective and robust manufacturing process. A cell culture process with high product titer generates a heavy burden on the downstream process not only due to increased non-product impurities, but also because of a high amount of undesirable product-related isomers. Major challenges to developing an efficient downstream process for monoclonal antibody manufacturing with highly productive cell culture materials include the following three areas:

1. **Process capacity:** The equipment and manufacturing facility need to have an adequate capacity to handle the high-titer cell culture broth. This includes resin-binding capacity, process time, number of process cycles, overall facility size, schedule and facility flexibility, buffer and water consumption, and compliance with cGMP regulations.

2. **Removal of product-related impurities:** Heterogeneity is common in antibodies expressed by recombinant mammalian cell culture processes. These impurities include dimers, aggregates, and various isoforms resulted from amidation, deamidation, oxidation, and shuffling of disulfide bonds. Removal of these species cannot be achieved by protein A affinity chromatography alone, and requires additional polishing steps.

3. **Removal of process impurities:** Process impurities include host cell protein (HCP), nucleic acids (DNA, RNA), leached protein A, and potential viral contamination, all of which need to be cleared according to FDA guidelines. Currently accepted levels of impurities for therapeutic antibodies reported in the literature are: < 5 ppm for HCP, < 10 ng/dose for rDNA, < 0.5% for dimers or aggregates, and < 5 ppm for

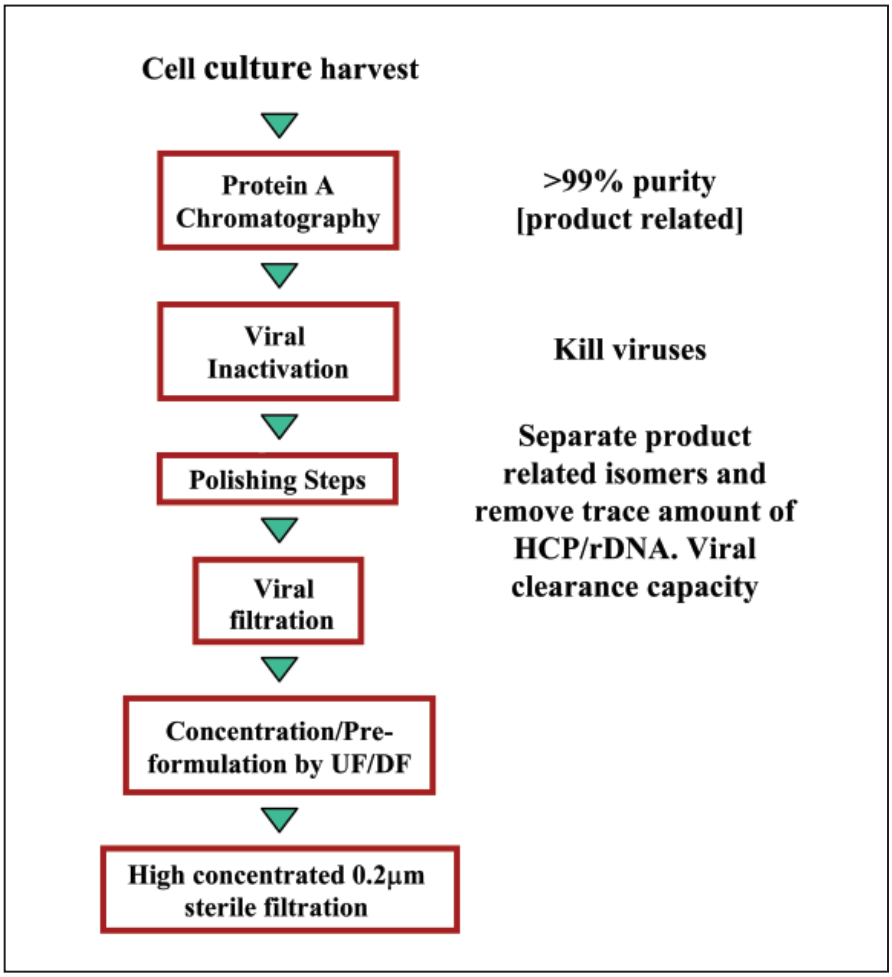


Figure 3. A typical antibody downstream process.

leached protein A.[26]

A typical antibody downstream process flow is shown in Figure 3. The most common industrial purification process for an antibody uses protein A affinity chromatography as a capture step followed by two or three additional chromatography polishing steps. The bulk product is then filtered through a 20 nm filter for viral removal, concentrated to a certain target concentration in pre-formulation buffers using an ultrafiltration/diafiltration device, and finally, sterile filtered through a 0.2 µm membrane. The entire purification process for GMP manufacturing must be validated by demonstrating a 12 to 20 log reduction of viral load using at least four different model viruses following FDA guidelines.

Centrifugation and Depth Filtration

The harvest process separates the antibody product released in the culture broth (conditioned medium) from the cells. A typical harvest diagram is illustrated in Figure 4. Continuous centrifugation is commonly used to harvest large-scale cell culture broth ranging from 500 L to 20,000 L, resulting in a cell-free supernatant. Generation of a supernatant with low cell turbidity depends on the choice between a full and partial shot, as well as the total number of shots and buffer volume per shot during the continuous centrifugation process. The supernatant is then further clarified through depth filtration followed by a 0.2 µm filtration. For optimal depth filtration, selection of filter type, flux, and total membrane surface area need to be considered. The depth filtration step with a well-selected filter can not only reduce the cell turbidity, but can also remove up to 50% of DNA impurities and 15% of host cell protein at neutral pH. The filtrate is then loaded on a purification column.

Protein A Affinity Chromatography

Protein A chromatography is the most efficient purification step for antibodies—it can purify the product to more than 98% purity and remove most process impurities, including proteases.

Two major types of protein A resins are commercially available: agarose-based resin from GE Healthcare and glass bead-based resin from Millipore. Both resins are robust enough to handle a high flux and have acceptable chemical resistance, including resistance to high concentrations of urea, GuHCl, and reducing agents. Dynamic binding capacity is in the range of 20 to 50 g per liter of resin, and both resins can be reused up to 200 times. Because of different physical properties, each type of resin has its advantages and disadvantages. For example, agarose-based resin has very low non-specific binding. However, high operational backpressure is observed when the resin is used in a column with an inner diameter (i.d.) of greater than 45 cm and a bed height of greater than 20 cm. In order to maintain an operating backpressure below 30 psi, a low linear flow rate of less than 200 cm/hr and a bed height lower than 20 cm need to be employed. In contrast, glass-bead resin can be used with a high flow rate in a high bed height column with an i.d. of 45 to 160 cm without significant backpressure. However, the glass-bead type resin is sensitive to caustic solutions and has higher non-specific binding with process contaminants compared to the agarose-based resin. Additional washes with a solvent-containing buffer are required in order to reduce the HCP level in the protein A pool. A low loading temperature around 15° C (±3° C) is recommended to minimize potential proteolytic degradation of the product and the protein A resin.[27]

Operating conditions of the protein A affinity chromatography step need to be optimized in three areas: resin type and sample loading residence time for optimal binding capacity; composition, pH, and volume of wash solution to minimize the HCP and rDNA levels in the pool; and composition and pH of elution solution to minimize turbidity, conductivity, dimer, and aggregate levels, as well as volume of the final pool which is important for the subsequent step.

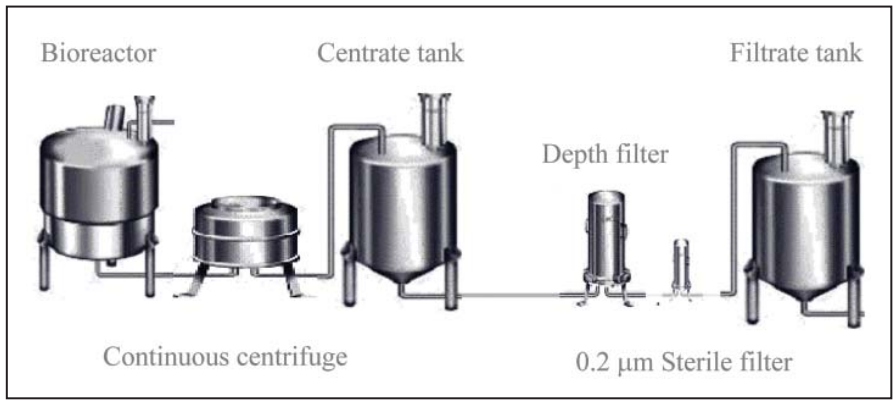


Figure 4. A typical harvest diagram for an antibody process.

Low pH Viral Inactivation

A viral inactivation step at low pH usually follows the protein A affinity chromatography step. Two model viruses, minute virus of mice (MVM) and murine leukemia virus (MuLV), are commonly used for process optimization. The protein A column pool is titrated with 10% acetic acid or 1 M citric acid to a pH between 3.3 and 3.8 and incubated for 45 to 60 minutes at room temperature.[28] The choice of pH level largely depends on the stability profile of the antibody product and buffer components. Use of low pH for viral inactivation is effective for MuLV, whereas MVM viral particles are not efficiently killed at low pH.

After viral inactivation, the pH of the protein A pool is titrated up with 3 M Tris buffer prior to the next step. Turbidity of the solution increases as the pH of the pool rises. The degree of turbidity of the solution after pH adjustment varies depending on process conditions for each product. In order to remove the turbidity, viral inactivation is followed by depth filtration or 0.2 µm filtration. An optimized depth filtration step can greatly reduce turbidity, as well as HCP and rDNA levels in the post-viral inactivation pool solution. In addition, depth filtra-

tion can efficiently remove MVM and MuLV viruses with log reduction values (LRV) of four and two, respectively.[28,29] Optimization of the depth filtration step must consider the filter type, surface area, and the operation flux rate.

Polishing

Two to three polishing steps are usually required to remove impurities. Typical polishing objectives are to remove:

- high molecular weight aggregates
- trace amounts of host cell proteins
- isomers of the product
- residual rDNA
- leached protein A
- viral contaminants

Cation-exchange chromatography (CEX), hydrophobic-interaction chromatography (HIC), and anion-exchange chromatography (AEX), are commonly used for polishing (Table 1).

CEX Chromatography

CEX chromatography used for bind-and-elution has proven to be a powerful tool for removing product-related impurities left behind by protein A affinity chromatography. CEX resin screening criteria include less HCP binding, high product dynamic binding capacity at a relatively high conductivity, and high resolution to remove target protein variants. An ideal resin will leave about 70–80% of HCP and most of the DNA/RNA and endotoxin in the flow-through fraction. Deamidated or acidic material can be separated in the front peak while the amidated or basic material and dimers/aggregates can be isolated in the tailing part.

Elution can be performed stepwise or as a linear gradient. Linear gradient elution provides better purity, process control, process monitoring, reproducibility, and PAT conformance.[30] In contrast, stepwise elution has proven to be mechanically simpler and results in higher product pool concentration.[31]

Recently, a linear pH gradient elution mode in a narrow pH range has been developed. Compared to salt gradient elution, pH gradient elution can provide similar product purity, higher yield, a smaller pool volume (up to 50% of the volume produced by salt gradient), and the lower pool conductivity required for the next step. pH gradient CEX chromatography has been demonstrated at large scale with a recovery rate of over 95%.[32] Figure 5 illustrates the separation power of Fractogel COO- (EMD Chemicals, Inc., Gibbstown, NJ) using a pH gradient from 5.0 to 6.0. Fractogel COO- is a weak CEX resin with high antibody-binding capacity (>50 g/L).

HIC and CHT Chromatography

HIC is an efficient mode to remove dimers and aggregates in bind-and-elution fashion. However, this mode has relatively low yield and separation resolution for other product-related isomers, and has to contend with high salt concentration in the elution pools. Therefore, bind-and-elution through HIC is becoming less popular in antibody production. Instead, HIC chromatography in a flow-through mode is gaining interest as a way to remove a large percentage of aggregates. A slow flow rate is necessary because, like other protein binding during HIC chromatography, aggregate binding on HIC resin is residence time-dependent.

CHT chromatography also can be a robust step to remove dimers and aggregates.[33] Several technical issues regarding CHT need to be addressed, however, including lot-to-lot viability, extractables, and the half-life of the resin.

AEX Chromatography

Several chromatography modes have proven very useful to remove trace amounts of impurities (*e.g.*, DNA and endotoxin) and viruses. Among these, AEX is perhaps the most powerful. In most cases, AEX chromatography is carried out using flow-through (FT) fashion, in which impurities bind to the resin and the product of interest flows through. However, the use of conventional packed-bed chromatography with FT-AEX requires columns with a very large diameter to permit high volumetric flow rates necessary to avoid a process bottleneck at the polishing step.[26]

Table 1. Applications of chromatography as polishing steps.

Impurity	Mode of Chromatography
High MW. Aggregate/Isomers/DNA	HIC, CEX, CHT (Ceramic Hydroxyapatite)
Host cell protein impurities/DNA	AEX, HIC, CEX
Leached Protein A	CHT, HIC, CEX
Viral clearance	AEX, CEX, HIC, CHT

Table 2. Comparison of performance and operation between column.

Items	Q Sepharose Fast Flow	Q Membrane
Flux/Flow rate	100–150 cm/hr	450–600 cm/hr
Capacity	50–70 g/L	>3000 g/M^2 or >10.9 kg/L
Buffer used	100%	5%
Operation Time	8–9 hrs	2–2.5 hrs
Cleaning Validation	Yes	Single use
Viral Clearance	Good	Good

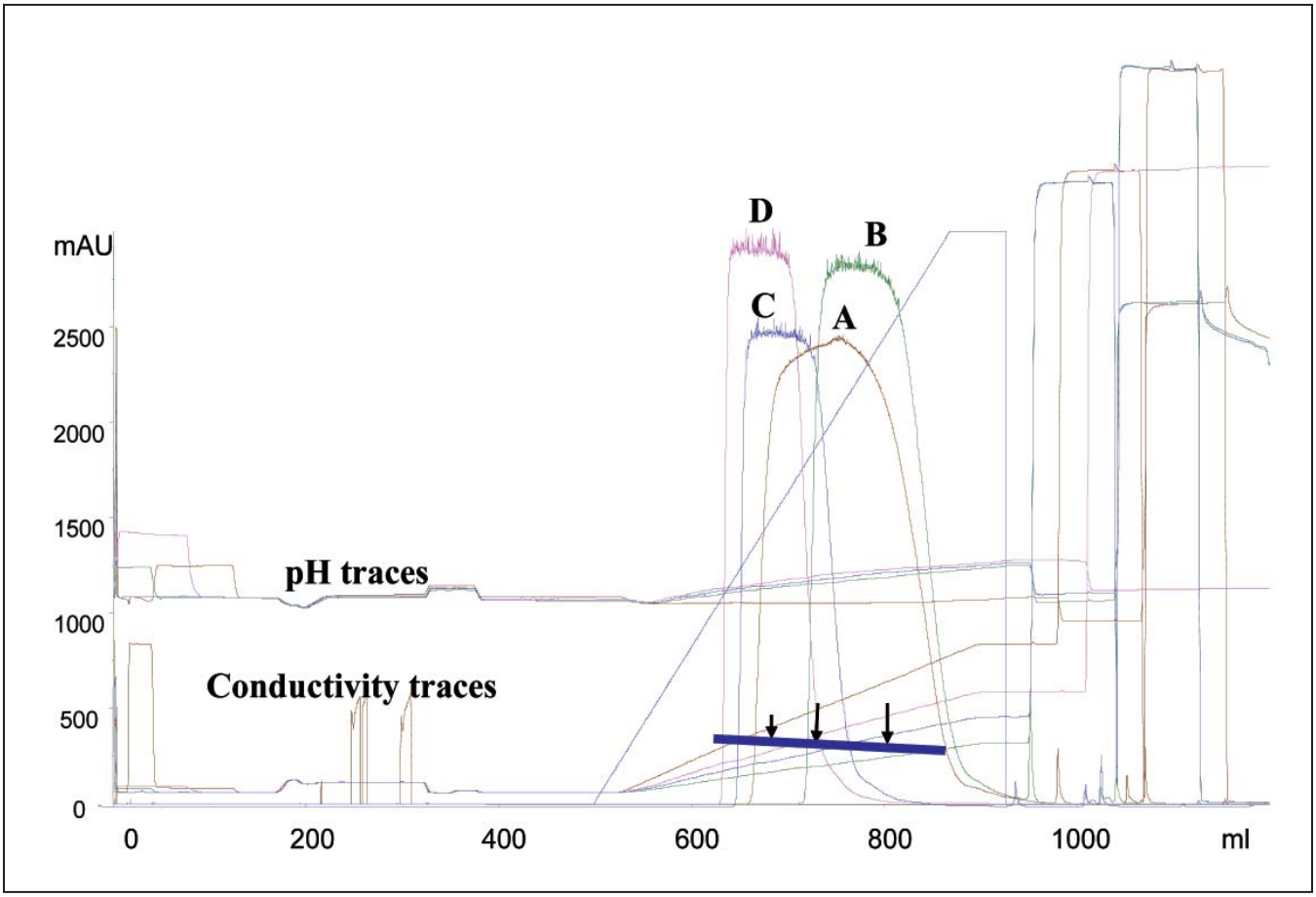


Figure 5. Comparison of antibody separation in CEX with pH-gradient and salt-gradient. A: Salt-gradient; B, C, D: pH-gradient with different buffer B concentrations. Binding Capacity: 40 g/L, Linear Velocity: 300 cm/hr. Condition C was chosen due to low pool conductivity and small pool volume. Arrows indicate pool conductivities by conditions B, C, and D, respectively.

In order to minimize the effect on the column operation by inadequate header design, a specific bed height is required for proper flow distribution.[34] This leads to a large column volume which is needed for fast flow but is not optimized for binding capacity. This disadvantage with AEX columns has led to the development of membrane chromatography or membrane adsorbers. Current membrane chromatography offers a convenient alternative to resin chromatography in the purification of antibodies.[35]

Q membrane chromatography devices have been in development for almost 15 years and are a very useful approach in viral vaccine production and DNA purification for gene therapeutics. The devices have also been used for endotoxin removal in both laboratory and large-scale processes. Current Q membrane technology offers major advantages over packed-bed resin chromatography in antibody purification, including fast operation, no requirement for cleaning or storage validation, significantly low buffer consumption, easy and quick qualification, good scalability, and antibody recovery higher than 98%.[35,36]

It was demonstrated that, with a newly developed representative scale-down model, a process capacity of >3,000 g/m^2 (total membrane surface) or >10.7 kg/L (membrane volume), a MVM viral log reduction of >6.0, and a MuLV viral log reduction of ~5.0 could be achieved.[37,38] Q membranes can significantly save operating time and buffer consumption. Table 2 compares the performance between Q Sepharose Fast Flow (GE Healthcare) column chromatography and the Sartobind Q membrane (Sartorius AG, Goettinger, Germany) device.

Conclusion

Recent developments and improvements on cell culture processes and separation technologies make it possible to lower the manufacturing cost of goods for antibody manufacturing, and significantly reduce the overall development time from clone selection to clinical manufacturing. High product titers greater than 2 g/L by the end of cell culture, and purification recovery yields greater than 75% are readily achievable. While productivity and process efficiency continues to be improved, it is important to monitor product quality attributes to ensure the comparability with any process changes during commercial manufacturing. Recent publication of the Q5E comparability guideline for biotechnological and biological products by FDA provides framework to consider for development and manufacturing processes.[39]

MILLIPORE

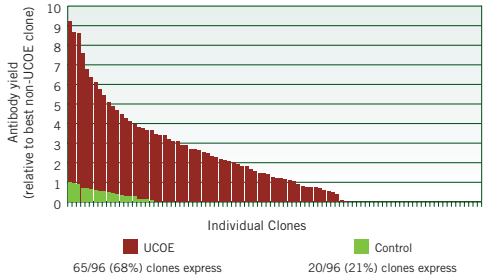
UCOE™ Expression Technology

The Key to Protein Expression

UCOE™ (Ubiquitous Chromatin Opening Element) expression technology can unlock the door to protein expression in mammalian cells, yielding strong results within your existing process without the need for amplification. The majority of clones produced with UCOE™ have over 50% substantially higher expression than the best non-UCOE clones. These clones have been shown to be stable over 130 generations and high-yielding cell lines can be derived in less than 60 days.

Speed, Stability, Success with UCOE™ expression technology. Visit www.celliancecorp.com/ucoe or call 1.678.728.2246 or toll-free 1.800.431.4505.

Substantially More Expression Using UCOE™



Vectors encoding heavy and light chains (separate plasmids) were co-transfected into CHO-S cells followed by drug selection and screening of 96 randomly selected clones.

Celliance
now part of Millipore

ACKNOWLEDGEMENT

The authors sincerely acknowledge Tony Hong for proofreading.

REFERENCES

1. Chadd HE, Chamow SM. Therapeutic antibody expression technology. *Curr Opin Biotechnol* 2001;12: 188–194.

2. Walsh G. Biopharmaceuticals: Approvals and approval trends in 2004. *BioPharm International* 2005; 18(5):58–65.

3. Hubbard B, Shukla A. Platform approaches to monoclonal antibody purification. *Paper presented at the IBC Antibody Production and Downstream Processing meeting*: 2005 March; San Diego, CA.

4. Tressel T. Development of a generic platform and use of statistically designed experiments to enable rapid development of several antibodies and increase throughput for first in human antibodies. *Paper presented at the IBC Antibody Production and Downstream Processing meeting:* 2004 February; San Diego, CA.

5. Chu L, Robinson DK. Industrial choices for protein production by large-scale cell culture. *Curr Opin Biotechnol* 2001;12:180–187.

6. Pierce LN, Shabram PW. Scalability of a disposable bioreactor from 25 L – 500 L run in perfusion mode with a CHO-based cell line: A tech review. *BioProcessing J* 2004;4:51–56.

7. Gay R, Birch J. Approaches to improving the performance of mammalian cell cultures for protein production. *Paper presented at the BioLOGIC conference*: 2004 October; Boston, MA.

8. Li F, Pendleton R, Lee B. Scale-down model development and process characterization for a fed-batch cell culture process. *Paper presented at the ACS BIOT conference*: 2005; San Diego, CA.

9. Burky J. Improving NS0 fed-batch culture productivity. *Paper presented at the IBC Cell Culture and Upstream Processing conference*: 2003; San Diego, CA.

10. Akselband Y, Trnovsky J, Trnovsky PM. Transfected cell line enrichment using the gel microdrop (GMD) secretion assay. *BioProcessing J* 2003;2:83–88.

11. Holmes P, Al-Rubeai M. Improved cell line development by a high throughput affinity capture surface display technique to select for high secretors. *J Immunol Meth* 1999;230(1-2):141–147.

12. Fletcher T. Designing culture media for recombinant protein production: A rational approach. *BioProcess International* 2005;3:30–36.

13. deZengotita VM, *et al*. Phosphate feeding improves high-cell-concentration for monoclonal antibody production. *Biotechnol Bioeng* 2000;69:566–576.

14. Robinson DK, *et al.* Optimization of a fed-batch process for production of a recombinant antibody. *Annals NY Acad Sci* 1994;745:285–296.

15. Xie L, Wang DIC. High cell density and high monoclonal antibody production through medium design and rational control in a bioreactor. *Biotechnol Bioeng* 1996;51:725–729.

16. Sauer PW, *et al*. A high-yielding, generic fed-batch cell culture process for production of recombinant antibodies. *Biotechnol Bioeng* 2000;67:585–597.

17. Moore A, *et al*. Effects of temperature shift on cell cycle, apoptosis, and nucleotide pools in CHO cell batch culture. *Cytotechnol* 1997;23:47–54.

18. Kunkel JP, *et al*. Dissolved oxygen concentration in serum-free continuous culture affects N-linked glycosylation of a monoclonal antibody. *J Biotechnol* 1998;62:55–71.

19. Zhou W, *et al*. Fed-batch culture of recombinant NS0 myeloma cells with high monoclonal antibody production. *Biotechnol Bioeng* 1997;55:783–792.

20. Larson TM. Chemometric evaluation of online HPLC in mammalian cell cultures: analysis of amino acids and glucose. *Biotechnol Bioeng* 2002;77:553–563.

21. Yeung KS, *et al*. Near-infrared spectroscopy for bioprocess monitoring and control. *Biotechnol Bioeng* 1999;63:684–693.

22. Harthun S, Matischak K, Friedl P. Determination of recombinant protein in animal cell culture supernatant by near-infrared spectroscopy. *Anal Biochem* 1997;251:73–78.

23. Zhu GJ. A high-throughput system to analyze N-glycans of NSO cell produced antibodies. *Paper presented at the IBC Cell culture and upstream processing conference*: 2003; San Diego, CA.

24. Jenkins N, Parekh RB, James DC. Getting the glycosylation right: Implications for the biotechnology industry. *Nature Biotechnol* 1996;14:975–981.

25. Jefferis R. Glycosylation of recombinant antibody therapeutics. *Biotechnol Prog* 2005; 21:11–16.

26. Fahrner RL. Industrial Purification of Pharmaceutical Antibodies: Development, Operation, and Validation of Chromatography Process. *Biotechnol Gen Eng Rev* 2001;18:301–327.

27. Laverdiere, A. Reduction of ligand leaching during protein A affinity chromatography by controlling proteolytic degradation of protein A. *Paper presented at the 229th ACS national meeting*: 2005 March 13–17; San Diego, CA.

28. Zhou J, *et al.* Development of A Cost-Effective, Robust Process for Recombinant Monoclonal Antibody Production. *Paper presented at the Williamsburg BioProcessing 2nd Annual Europe Antibodies & Recombinant Proteins meeting*: 2005 May 22–24; Amsterdam, The Netherlands.

29. Tipton B, *et al*. Retrovirus and parvovirus clearance from an affinity column product using adsorptive depth filtration. *BioPharm* 2002;Sept:43–50.

30. Blank GS. The future of process development for MAb manufacturing. *Paper presented at IBC's 14th International Antibody Production & Downstream Processing*: 2005 March 9–11; San Diego, CA.

31. Gagnon P. The quest for an industrial purification method for generic IgG. *Paper presented at the Williamsburg BioProcessing Foundation's 2nd Annual Europe Antibodies & Recombinant Proteins meeting*: 2005 May 22–24; Amsterdam, The Netherlands.

32. Zhou J, *et al*. pH-gradient cation exchange chromatography for process-scale antibody purification. *Paper presented at the 229th ACS national meeting*: 2005 13–17; San Diego, CA.

33. Franklin S. Removal of aggregate from an IgG 4 product using CHT ceramic hydroxyapatite. *BioProcess International* 2003;January:50–51.

34. Yuan QS, *et al*. Flow distribution in chromatographic columns. *J Chromatog A* 1999;831:149–165.

35. Gottschalk U, Fishcher-Fruehholz S. A cutting edge process technology at the threshold. *BioProcess International* 2004;May:56–65.

36. Knudsen H, *et al*. Membrane ion-exchange chromatography for process-scale antibody purification. *J Chromatog A* 2001;907:145–154.

37. Tressel T, Zhou J. Process development of an anion exchange membrane. *Paper presented at the North American Membrane Society Conference*: 2005 June 4–6.

38. Dermawan S, *et al*. Process development of a flow-through anion exchange membrane chromatography in protein purification. *Paper presented at the 229th ACS national meeting*: 2005 March 13–17; San Diego, CA.

39. Food and Drug Administration [FDA]. FDA guidance for industry: Q5E comparability of biotechnological/biological products subject to changes in their manufacturing process. <www.fda.gov/cder/guidance/index.htm> June, 2005.