Novel Tools Streamline Purification Process

Technologies Are Being Developed and Applied to Overcome Downstream Logjams

K. John Morrow Jr., Ph.D.

Participants at a recent downstream processing meeting sponsored by **Sartorius Stedim Biotech** (www.sartorius-stedim.com) considered a host of new technologies to speed and streamline protein purification, including membrane chromatography, nanofiltration, ultraviolet irradiation for viral inactivation, and ultracentrifugation concentration technology.

"Downstream has an ongoing processing bottleneck, which continues to affect biomanufacturers, especially those retrofitting existing facilities to accommodate modern fermentation processes," explained Uwe Gottschalk, Ph.D., vp for purification technologies at Sartorius Stedim Biotech.

There is a major push, at present, to deal with increasing titers during purification. As the antibody titer increases over 1 gram per liter the cost benefit levels out, so going over 5 grams per liter yields little economic benefit in per gram outlay. As the yield in grams per liter increases, the same equipment is employed, whereas buffers and other material requirements expand along with antibody yield. This means that processing equipment must be able to handle the larger amounts, otherwise a significant bottleneck will develop.

Sartorius-Stedim Biotech offers a range of modular purification tools that can be used together so that all the process steps, including clarification, crossflow, membrane chromatography, and viral clearance, can proceed efficiently.

Column chromatography has become the central enabling technology of bioprocessing, according to Dr. Gottschalk, who cited the many improvements that have been made in chromatography media over the years, ranging from diffusive to perfusive particles to monoliths and stacked membranes. He specifically discussed the use of UV inactivation, depth filtration, nanofiltration, and membrane chromatography for the final steps of antibody purification.

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In looking toward the future, Dr. Gottschalk believes that crystallization and precipitation will eventually supersede chromatography for the penultimate stages of purification. The crystallization-precipitation approach would represent a major financial advantage, since it would allow the elimination of protein A or other expensive affinity reagents. "By optimizing the various steps in the purification process we should realize an overall 10-fold productivity increase and a significant reduction in costs," he stated.

Protein Precipitation

Alahari Arunakumari, Ph.D., senior director for process development at **Medarex** (www.medarex.com), also spoke on the need to move beyond affinity ligands in protein (especially antibody) purification. Her team is working on the optimization of fully human monoclonal antibody purification processes. She cited protein production levels as high as 13.8 g/L in CHO cell culture, establishing the need for maximum efficiency at the downstream processing end.

Improvements in the company's processing protocols include precipitation of contaminants or impurities followed by ion-exchange chromatography and a change from resin to Q membrane chromatography, resulting in significant increases in loading capacity—up to a 20 g/mL increase in load protein concentration for viral clearance.

"Further improvements in our system are being put in place," Dr. Arunakumari continued. "These include increasing the binding capacity on the ion-exchange column so as to minimize the required number of cycles and increasing the load conductivity on Q membrane chromatography so that no dilution is needed. Moreover we have reduced the dilution factor to accommodate the tank sizes and raised the formulation concentrations."

The Polishing Step

Membrane technology is an area where major improvements in technology are anticipated in the next few years. Amelie Raveneau, purification technologies application specialist, and Suma Ray, Ph.D., virus clearance technologies product manager, both at Sartorius Stedem Biotech, discussed current chromatographic technologies in the polishing step of antibody purification.

At this stage, the goal is to reduce host-cell protein impurities, DNA, and leached protein A to acceptable levels. Flow-through membrane chromatography appears to be the superior method because of its ease of handling, high flow-through rate, and single-use character.

Virus removal is a critical component of the polishing procedure, and here **Bayer Technology Services'** (BTS; www.bayertechnology.com) UVivatec device can be integrated into the viral-clearance step. The instrument, which is jointly manufactured and marketed by BTS and Sartorius Stedim Biotech, is a helical flow, single-use reactor

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with hydraulic design for uniform treatment.

It employs UVC irradiation, a shortwave, germicidal wavelength that acts by causing dimerization and nicks in the DNA. The 254 nm wavelength is effective at inactivating viruses without damaging the proteins of interest. The disposable modules are robust and scalable, up to 1,000 L/hour. According to the companies, these properties make it useful in a GMP environment for polishing media, plasma and blood products, vaccines, and therapeutic proteins.

The UVivatec component inactivates either nonenveloped or enveloped virus, and due to its viral-clearance mechanism can be used in addition to virus adsorption and removal through the use of Sartobind Q and Virosart CPV membranes.

Eckhard Flechsig, Ph.D., head of virus validation at **Biotest** (www.biotest.com), described his company's work optimizing the process of viral removal. Despite the risk of transmission of blood-borne diseases, there are still numerous biological products that are obtained from human blood. Therefore, it is incumbent upon producers to reduce the risk of viral transmission to an absolute minimum.

While donor selection and PCR screening for selected viruses will eliminate a large portion of the potential contamination, a viral inaction and removal step is de rigueur. These may include filtration and chromatography, but UV inactivation has proven to be one of the most robust and cost effective.

Scientists at Rentschler Biotechnologie have developed a novel approach to aggregate removal using phenyl membrane adsorption technology.

Dr. Flechsig concurred with Drs. Raveneau and Ray that UVC inactivation at a wavelength of 254 nm brings about the requisite damage to nucleic acids without causing aggregation, alteration in S-H bonds, or other adverse effects on proteins. The maximum wavelength for energy absorption by proteins is a narrow window in the higher range of 280 nm. The UVivatec system is a continuous flow system, which operates by pumping the biological fluid through a hose spiraling around the irradiation source. In model experiments it was possible to define the dose required for a greater than 4-log reduction in viability, which was required for each individual virus.

Viral Preps for Validation Studies

Dana Cipriano, director of project management at **WuXi AppTec** (www.pharmatechs.com), described her company's extensive experience with viral purification—more than 1,600 studies on a wide range of recombinant proteins and plasma products have been conducted. For quality control studies, it is essential that viral stocks be well-characterized and uniformly free of contaminating proteins and nucleic acids.

The purification procedure employed by scientists at WuXi AppTec consists of viral harvest, clarification of crude preparations, followed by ultracentrifugation. New methods, known as Ultra 2 preparations, incorporate column or membrane chromatography. Nonenveloped viruses such as parvoviruses and reoviruses appear to lend themselves to this approach. In the case of enveloped viruses (such as bovine viral diarrhea virus) better purification was achieved with the addition of a sucrose gradient concentration step.

Cipriano reported that virus removal was efficiently achieved using nanofiltration for all viral types that were investigated while improving flux rates for customers.

Getting It Together on Aggregates

"Aggregate formation may occur due to the natural properties of protein monomers, but it can be a major impediment for generating good yields in recombinant proteins," Sybille Ebert, Ph.D., manager of protein chemistry development at **Rentschler Biotechnologie** (www.rentschler.de), explained.

While some multimer formation is expected, especially in protein preparations that are heated or concentrated at high densities, the consequences can be quite serious, including a decrease in activity and stability and an increase in toxicity and immunogenicity. In the clinical evaluation of protein therapeutics, this can result in hypersensitivity and even anaphylaxis in severe cases.

According to Dr. Ebert, downstream processing employs a variety of treatments that may cause aggregation. These include pH changes, mechanical stress, and high protein concentrations that arise during the elution phase. To evaluate these conditions and minimize aggregate formation, it is necessary to carefully monitor the aggregate levels. This can be done using a variety of detection methods, especially size-exclusion HPLC.

Dr. Ebert and her colleagues used Sartobind phenyl membrane adsorbers in a 96-well format to develop an effective approach to aggregate removal. The Sartobind membranes are made from regenerated, stabilized cellulose, with ligands attached covalently and then rolled up in the form of a cylindrical chromatographic bed. The group carried out lab-scale experiments using 3 mL nanocapsules to define the optimal conditions for aggregate removal, flow through, and binding to the membrane.

"We found that the optimal removal of the aggregates was accomplished on a 3 mL nanocapsule," Dr. Ebert stated. "On this basis we can conclude that the phenyl membrane adsorption technology is quite suitable for the efficient removal of aggregates in downstream processing."

Moving the Downstream Upward

Downstream processing technology has advanced rapidly in recent years largely in response to demands from the vaccine industry. As emphasized by Rodney Carbis, Ph.D., head of vaccine development at the International Vaccine Institute in Korea, there is no generic technology for vaccine manufacture. Because pathogenic viruses vary considerably, there will continue to be demand for a whole range of approaches, a reality that will test the inventiveness of downstream developers in the years to come. GEN

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