

# BIOPROCESSING

## Manufacturing of Biopharmaceutical Proteins

### Tutorial: Process Design and Implementation of Viral Clearance

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One of the challenges in downstream processing is the design of robust process steps to efficiently isolate instable products, such as recombinant proteins, from complex fermentation broths to the required pharmaceutical degree of purity. Downstream operations usually represent 50–80% of the total processing cost, therefore, optimization of downstream processing technologies is considered the central element in appropriate process design.

The purification process includes chromatographic and filtration technologies. The combination and design of the single steps is of paramount importance for economic process development. In the overall process design the engineer has to consider different aspects such as elimination of contaminants, process scalability, automation, capacity of the production line, and regulatory compliance. Every single step of the process has to be validated and optimized in terms of yield, purity, and stability.

Protein purification is still the bottleneck of process development, and it is the improvement in chromatographic matrices that accounts for modern downstream processing.

At **Rentschler Biotechnologie** (Laupheim, Germany), process design additionally comprises the implementation of new technologies such as expanded bed adsorption (EBA) and membrane adsorbers. These technologies increase yield, cut overall processing time and costs, and shorten time-to-market.

**State-of-the-art chromatographic matrices.** The requirements for novel matrices are demanding. Novel high throughput and high-capacity chromatographic matrices must meet GMP-quality requirements and be suitable for upscaling. The supplier must be able to provide the batches with tight specifications in order to assure a supply with consistent quality over a long period of time.

Furthermore, the matrices have to be stable over a broad pH range, permit column regeneration and cleaning, and should support increasing cycle numbers for economic considerations. In the case of nonadherence, the contract manufacturer cannot guarantee the manufacturing of a product over several years.

#### Expanded Bed Adsorption

With purification technologies, the challenge is to recover the target protein in its active form and in high yields from the hundreds or thousands of other proteins found in the host organism, as well as from the many components of the medium in which the cells were grown. Manufacturers have traditionally tackled this problem with a multi-step combination of techniques, commonly involving centrifugation and filtration.

Once clarified, the target protein can be concentrated via ultrafiltration/diafiltration and then purified further, usually by fixed-bed chromatography. More recently, however, expanded bed adsorption has emerged

as a single-step alternative that achieves cell separation, clarification, concentration, and initial purification in one operation. EBA increases yield, cuts overall processing time, decreases labor and running costs, and reduces capital expenditure compared with conventional steps.

**Membrane adsorbers.** Membrane adsorbers are chromatographic membranes carrying functional groups for the binding of biomolecules. They are not filters, although the structure looks similar. Separation is achieved by reversibly binding the protein to the ligand.

Methods and buffers known from conventional gel chromatography can be directly applied. Compared to a conventional chromatographic resin, the major kinetic effect of the macroporous membrane adsorber is the convective flow—molecules are transported to the binding sites by pump pressure—and rapid film diffusion. The diffusion limitation in chromatographic beads

development.

Then, the single purification steps are carefully looked at for their capability to deplete contaminants, the load capacity of the columns, buffer requirements, yields, process timelines, and scalability, to name just a few. The necessity to implement ultra-, dia- and nanofiltration steps and the effectiveness of DNA depletion and virus removal steps is discussed and optimized, if required.

A successful process design also includes the selection of suitable buffers to minimize the number and quantity of buffers used. Furthermore, the regeneration of the column matrices has to be established and optimized. The degradation of the product has to be examined regarding process conditions and storage times to define hold steps.

Each step of the purification process needs to be optimized in terms of individual and overall yield, and the product quality of the intermediates in the hold steps and of the bulkware has to be controlled extensive-

ly. The scale of the individual steps of the purification process has to be adjusted according to the intended product quantity.

#### Validated Down Scale

Once the robustness has been successfully checked, the process is repeated three to five times with defined parameters, starting from cell culture up to bulkware production. The aim is to demonstrate reproducibility of the single steps concerning quality and characteristics of intermediates and bulk drug substance.

After successful establishment of the lab-scale process, the process is gradually upscaled by the factor of 10 or more to the pilot scale and further to the production scale. All parameters of the single steps are thoroughly checked and after successful upscaling, the process is downscaled again for validation to comply with all technical parameters of full scale. At this point, when the downscale process is validated, virus clearance steps are performed.

#### Virus Clearance

An essential component of process validation studies for products derived from eukaryotic cells is to assess the capacity of the purification process to remove/inactivate potential viral contaminants. Viruses that contaminate biological processes are divided into three categories.

Endogenous viruses exist as a part of the cell line used to express the protein product (e.g., murine retrovirus). Non-endogenous viruses are viruses from external sources present in the Master Cell Bank (e.g., Epstein Barr virus). Adventitious viruses are introduced into the product during the manufacturing process either through the addition of contaminated raw materials or through extraneous contamination (e.g., parvovirus).

To date, biotechnology products derived from cell lines have not been implicated in the transmission of viruses. But some manufacturing processes are, by their nature, susceptible to virus contamination from extrinsic sources. Therefore, to assure product safety, validated virus clearance steps have to be a part of the manufacturing process.

#### Virus Panel

Virus clearance steps must be validated before Phase I products may be administered to humans. It is common for biotech manufacturers to use rodent cell lines to validate virus clearance steps for the removal/inactivation of murine leukemia virus, which serves as a specific model virus for the retrovirus-like particles produced by murine cell expression systems, and to insert and validate a parvovirus clearance step into the manufacturing process for Phase I products.

Virus inactivation can be achieved by heat, pH, detergents, irradiation, and solvents; virus removal can be accomplished by filtration and chromatography.

In a virus validation study, done before production of Phase III material, the manufacturer will choose a panel of two to four additional viruses for testing. The choice of the viruses depends on the cell line and the origin of the media and includes both rele-



Downstream processing (DSP) small-scale development

One of six state-of-the-art DSP GMP-production suites at Rentschler Biotechnologie



is generated mainly by poor diffusion due to a small pore size in the nanometer range.

Adsorbers allow large flow rate ranges and show high binding capacity for large proteins. However, the use of innovative products, such as membrane adsorbers, can also be a risk rather than a blessing for process development. Therefore, Rentschler Biotechnologie is co-developing membrane adsorbers with **Sartorius**, among others.

#### Development Depends on Project Aims

A thorough process design requires both a deep comprehension of the chemical and biological characteristics of the product and detailed information about the cell culture conditions and the harvest (including, media components, cell vitality, enzyme activity, product stability, titer). The final bulk (bulk drug substance) needs to be defined as to product identity, quality, product concentration, and formulation prior to

ly. The scale of the individual steps of the purification process has to be adjusted according to the intended product quantity.

#### Check of Process Robustness

Cells are tiny bioreactors that produce, more or less efficiently, recombinant protein depending on known and unknown factors. The fermentation harvest will contain variable amounts of protein, although the process is well established at this stage. This has certain implications on the purification steps.

In a robust process, the column matrices are able to bind various amounts of protein (10–20% more or less of the expected outcome). The column flow rates and the load volume can be varied without losses in duration of the column run and purity of separation.

Tests need to be performed to evaluate the lifetime range of the column and of the product to be purified to define the condi-

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vant/specific model viruses and nonspecific model viruses. The virus panel is selected with the goal of inactivating and/or eliminating as many types of viruses as possible during the manufacturing process without compromising activity and quality of the product.

A relevant virus is either the identified virus, or of the same species as the virus that is known, or likely to contaminate the materials in the production process. A specific model virus is closely related to the known or suspected virus (same genus or family), having similar physical and chemical properties. Finally, a nonspecific model virus is used to characterize the capacity to remove and/or inactivate virus in general, i.e., to characterize the robustness of the purification process.

Obviously, viruses of the relevant category are the first choice. If the use of a relevant virus is not possible, the manufacturer chooses the best specific model virus to serve as a model for the relevant virus. To satisfy the "general virus clearance" objective, the study sponsor will evaluate clearance of a group of nonspecific model viruses. These viruses are combined with the relevant and specific model viruses to create a virus panel that represents viruses of different genomes (DNA and RNA), sizes, and surface properties (enveloped and non-enveloped).

#### Spiking Studies

In spiking studies, those steps within a manufacturing process with the greatest potential for effective removal and/or inactivation of virus are challenged with a high titer of virus. High virus titers allow larger virus reduction factors and allow a more rigorous challenge of the clearance capacity of a process step.

For this reason, regulatory guidance states that the amount of virus added to the starting material for the production step being studied should be as high as possible. However, so as not to alter the product composition unacceptably, the spike volume has to be kept low, typically less than 10%.

In spiking studies, the model

viruses are directly added to appropriate steps of the validated downscale process. These studies allow the manufacturer to evaluate the overall virus reduction capability of the manufacturing process and determine whether the process is capable of providing an appropriate level of virus clearance to assure final drug product safety.

#### Reduction Factor

It is necessary to know how much virus is likely to be in the unprocessed bulk, since the process must be designed with excess

capacity to remove viruses. Typically, virus in unprocessed bulk is quantified by transmission electron microscopy of the concentrated bulk or by infectivity assays.

The reduction factor is defined as the  $\log_{10}$  of the ratio between the total virus load before clearance and the total virus load measured in the product after clearance. The reduction factor requirement generally is established specifically for viruses that are known to contaminate the process.

Retrovirus-like particles produced by murine protein expres-

sion cell systems are the most common example. With a process-specific reduction factor target in mind, the manufacturer will add virus clearance steps to the process and run validation studies to document the virus clearance effectiveness of several unit operations.

On the basis of the reduction factor and an assessment of robustness, a unit operation may be classified as effective, ineffective, or moderately effective. Effective steps provide a reduction factor of at least four and are unaffected by small perturbations in process vari-

ables. Ineffective steps provide a reduction factor of one or less, and moderately effective steps fall between these two extremes.

Process design is still a challenge in downstream development and analytics since the process must be suitable to manufacture biologically active protein in a regulatory compliant manner with a high safety profile according to pharmaceutical drug safety. **GEN**

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