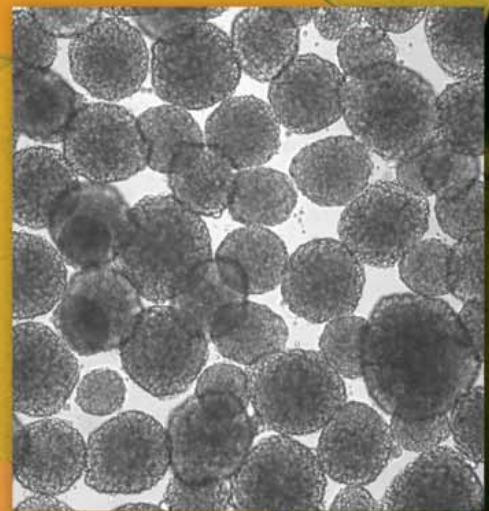
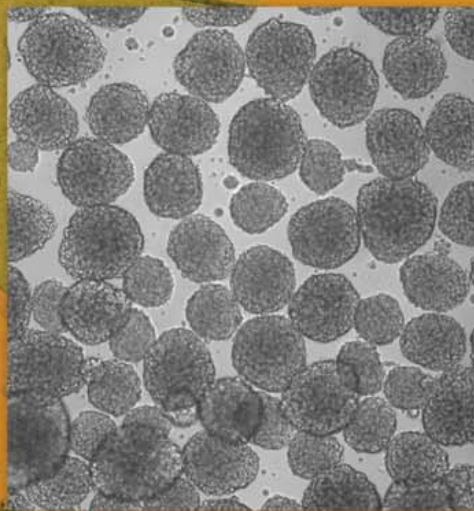
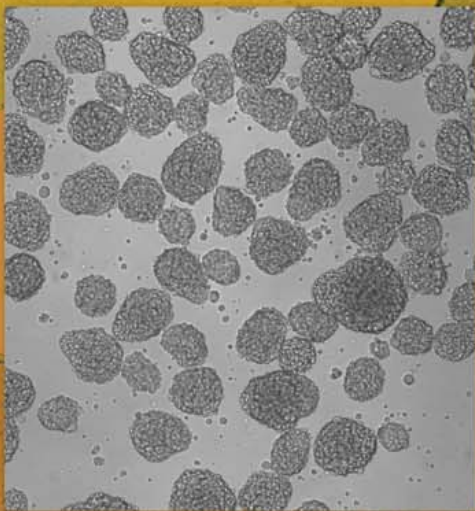
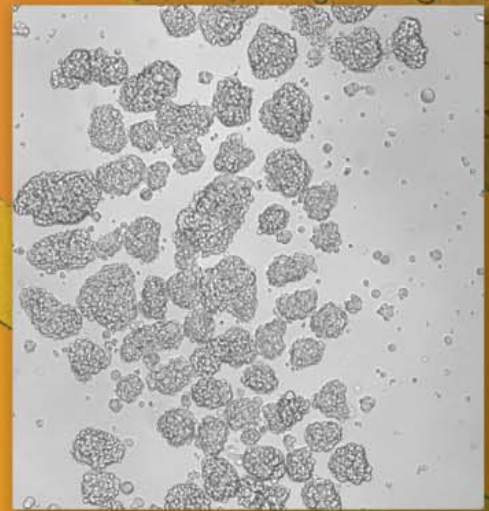
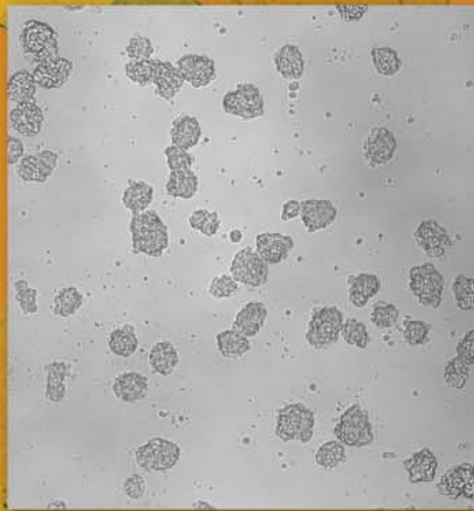
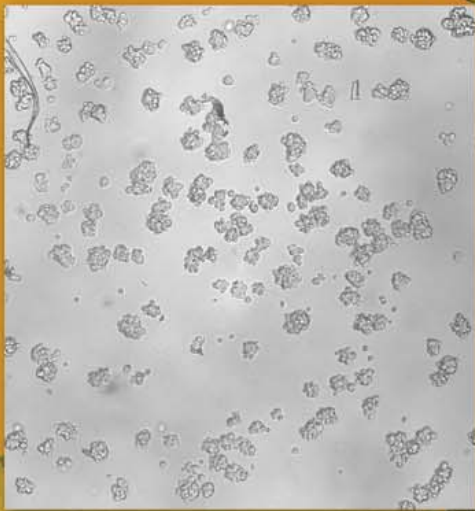


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# Process Economy of Disposable Chromatography and Nanofiltration In Antibody Manufacturing

By KLAUS TARRACH

**M**onoclonal antibodies and recombinant proteins have increased in importance and gained success as therapeutic agents in treating various diseases.

Biomanufacturing of such a biopharmaceutical product by cell culture follows a main route. Upstream processing is strictly biology-driven, while on the other hand, purification is engineering driven.<sup>1</sup>

Fermentation is setting the pace. To some extent, that pace is a result of recent advances in cell culture, greatly increasing the densities of cells along with cell-related contaminants.

CHO cells are the main workhorse in cell culture processes, and the impor-

tance of human cell lines is increasing. However, they all feature the same challenges to downstream processes (DSP), addressing both technical feasibility and economics. Today's technologies in the initial recovery process of the DSP require the principal capability to handle cell densities of  $10^7$ /ml and beyond, with product titers of up to 5 mg/ml and cell viabilities of less than 50%.

Within such recovery steps, the main target is to remove cells and debris with increased focus on contaminants such as "CHOP" (host cell DNA and viruses). DNA contaminant levels in direct bioreactor offloads have increased up to  $10^6$  ppm and pose a major challenge to the entire chromatography strategy applied to a biopharmaceutical.<sup>2</sup>

The challenge remains to align upstream and downstream processing and integrate them, in order to manage the challenges of purifying the

biopharmaceutical while effectively meeting the highest requirements for consistency and reproducibility.<sup>1</sup>

The biomanufacturing of products such as monoclonal antibodies (MAbs) are characterized by high production costs which are mainly derived from the fact that capital investments into a cGMP biotech facility are very high and can exceed \$1 billion.<sup>3</sup> However, the industry is realizing that biopharmaceutical drugs are subject to the same economic principles as products in other industries. The cost pressure is ongoing and the industry has to come up with smart concepts to meet expectations; not only shareholder expectations, but also governmental expectations for more affordable healthcare solutions.

Benchmarking with other industries experienced in handling larger-scale volumes of high value product will definitely be beneficial to biopharmaceutical



Figure 1. Disposable, single-use Sartobind® Q membrane chromatography setup.

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processes. Biomanufacturing processes are driven by fixed costs, and there is a need to bring the capital expenditures down with disposable manufacturing concepts.

The impacts are manifold. Besides significantly reducing capital costs for DSP, key aspects are: production flexibility, risk reduction, and ease and speed of operation. The ideal solution is a simple, robust, and controllable technology which can be made disposable; thereby providing a viable way to overcome these challenges.

### Why this Focus on Downstream Processing with Respect to Cost Scenarios?

Because the overall manufacturing costs are shared between upstream (30–40%) and downstream processing (60–70%), and it becomes clear that the larger leverage of cost-saving lies in the hands of downstream processing. DSP has to deal with the challenges of accommodating the output of this fast-paced biosynthesis development. The good news is that there are solutions available from the industry and its partners to meet these expectations.

Inquiries to the industry reveal clear messages that membrane technologies are expected to provide answers to downstream processing improvements. Also, cost-effective disposable technologies will inevitably help to curb the increasing problems of capacity constraints.<sup>4</sup> The concept of disposability is not just focused on DSP, but the entire process, as virtually everything can be made disposable. The challenging question is: “Can biomanufacturing a blockbuster MAb at its most important steps be made disposable to reduce capital investments?”

### There are Positive and Promising Answers

Because downstream processing is so costly, new disposable technologies are providing answers: integrated cell removal and clarification platforms, sterile grade filtration, cross-flow applications, and chromatography steps for purifying and polishing the target protein.

A typical large-scale purification

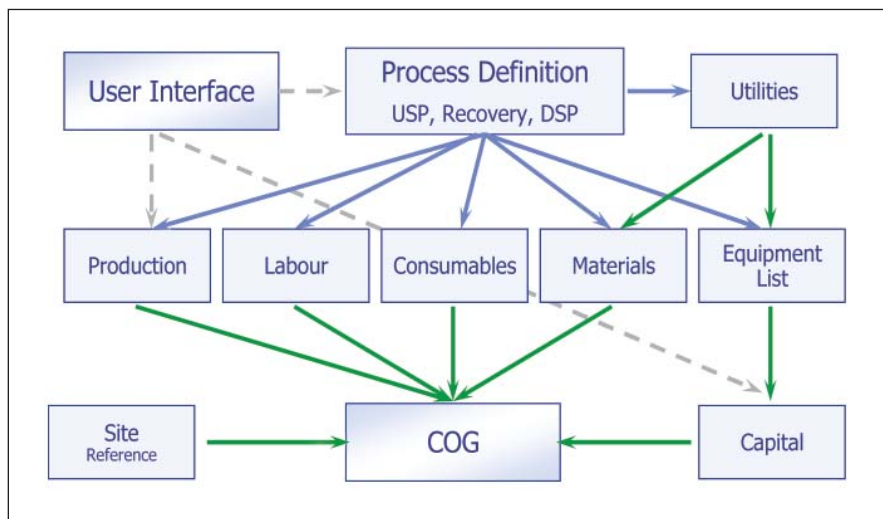


Figure 2. Structure of the cost-of-goods (COG) model.<sup>8</sup>

process is usually set around protein A as the capture step, followed by purification and polishing steps.<sup>5</sup> The polishing steps remove contaminants such as aggregates, protein fragments, host cell protein, and DNA. There is an increased focus on viruses, both retroviruses as well as small, non-enveloped viruses (mainly minute virus of mice [MVM]).

Increasingly, engineers are eager and willing to reduce the number of chromatography steps during purification and polishing ideally to two: a) protein A capture; and b) e.g., a single, disposable anion exchange step.

Non-protein A platforms are also being developed with high binding resins, for example: cation exchange (CEX) and hydrophobic interaction. Chromatography platforms incorporating protein A or non-protein A resins can also reduce costs substantially, provided the number of chromatography steps can be reduced.<sup>6,7</sup>

Among those chromatography techniques available, flow-through anion exchange (AEX) is the most powerful tool used for polishing. However, conventional packed-bed AEX chromatography systems require large diameter to meet flow requirements.<sup>5</sup> Therefore, packed-bed columns are significantly oversized in relation to the overall contaminant load, as the required volumetric flow can only be achieved by larger diameter columns. One of the main goals in polishing is speed, and disposable membrane-based technolo-

gies—being significantly faster—reveal their main benefit to such applications.

To support the implementation of membrane-based chromatography systems, cost calculation models have been developed which enable the end-user to determine the best fit for their specific polishing application.<sup>8</sup> Basically, the analysis considers the operational aspects of maintenance, validation, capital investment, labor, buffer and water costs, etc., to obtain a detailed comparison between the two technologies. The exact calculation principles and detailed analysis structure has been recently published. The main structure of such a model is outlined in Figure 2.

All assumptions revolve around a flow-through step using AEX chromatography for polishing. Calculations were done comparing post-protein A steps (2 kg of MAbs per liter) and post-CEX steps (10 kg of MAbs per liter) with the outlined loadings of antibody per liter of chromatography media. When comparing both technologies, it becomes clear that packed-bed chromatography systems show a very high capital charge; the major cost contributor. Packed-bed systems can be reused up to 100 times and beyond, and therefore distribute material costs of resins over each chromatography cycle.

In the case of the membrane-based system, consumable costs for the disposable device contributes significantly to the overall polishing expense. One of the biggest cost-saving factors for the mem-

brane-based technology is reflected in the amount of technical equipment needed to run such an operation. The outlined cost model indicates savings of more than 50% due to lower capital investments, design, and installation costs.<sup>8</sup>

Going disposable in chromatography removes column packing as such by being a “plug-and-play” technology. Labor costs can be significantly reduced as well, mainly because buffer consumption and staffing needs are significantly less. With respect to the overall disposable material used, membrane systems are used only once and are renewed for every batch being processed. However, the increased expense for membrane-based disposables is more than compensated by the reductions in capital expenditures, buffer consumption, and labor.<sup>9</sup>

It has been shown that disposable purification technologies will change the fixed capital costs for chromatography hardware into variable costs with single-use membrane technology. Those expenditures become relevant as cash-out only when the plant is in operation and the polishing step is up and running. For the data shown in the case study, a comparison revealed that operating costs of packed bed and membrane-based chromatography systems break even when membranes are loaded to about 2.0 kg of MAbs per liter of chromatography media.<sup>8</sup> One of the main influences in the overall cost scenario is the water for injection (WFI)—used for flushing and regeneration—which varies significantly from one plant to another. In the US, the expense of WFI is said to be as high as \$6.50 per liter. However, for our scenario, a best-case assumption of \$0.27 per liter has been used.

### ***The Disposable Option Doesn't End Here***

Viral clearance is one aspect of manufacturing that should be considered as well. With the new European draft guidelines for virus safety in place for investigational biologics, orthogonal strategies for virus clearance should be employed early on. This requires implementation strategies for virus inactivation and removal for Phase I and II study materials, and for both enveloped and small,

non-enveloped viruses.<sup>10</sup> Again, disposability will simplify material requirements, reduce labor, increase flexibility, and eliminate cross-contamination—a cornerstone in each virus safety concept.

Especially during early stage development where it is not known whether the drug candidate will make it through clinical trials or not, capital expenditures can be reduced by employing disposable technology platforms. With respect to virus clearance technologies being implemented at that stage, approaches to virus removal must assure robust and effective removal, regardless of their position within the purification scheme.

Disposable 20 nm nanofiltration technologies for the removal of adventitious viruses have made their way into the downstream processing of biopharmaceuticals. A state-of-the-art technique, 20 nm nanofiltration is used at the very end of the production process where the purity of the MAb or recombinant protein is at its highest, and filter breakdown due to contaminants is at its lowest.<sup>11-13</sup> Disposability at that stage—even for large volumes of >1,000 liter—is definitely an option.

Today's disposable technologies for 20 nm nanofiltration offer the capability to handle ultra large-scale batches (10,000 liters and beyond) while maintaining speed of operation and financial balance.<sup>14</sup> Figure 3 shows an ultra large-scale nanofiltration rig capable of handling more than 5,000 liters of product subject to nanofiltration.

### **Summary**

The principle decision to run with the disposable option has made its way into modern biomanufacture. Increasingly, disposable technologies are being considered for polishing applications within chromatography or nanofiltration. These new concepts feature integrated and robust disposable technology platforms to purify biotech products at large scale. Models have been established to help evaluate the economic justifications of sourcing disposable technologies.

The results are positive, as capital costs are reduced, a significantly higher flexibility in manufacturing is gained, ease and speed of operation is increased, and cross-contamination risks are eliminated.

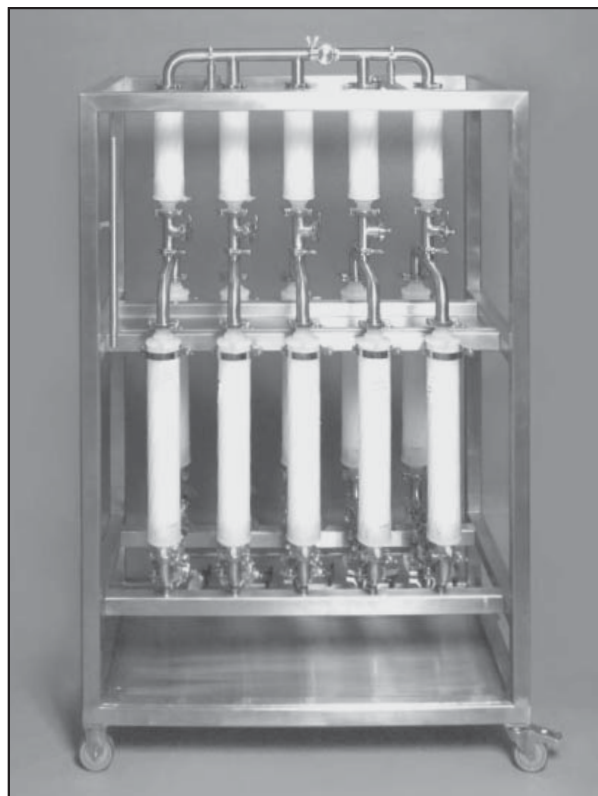


Figure 3. Large-scale setup for disposable Virosart® CPV nanofiltration.

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