High-Throughput Monoclonal Antibody Purification

A review of new technologies and products introduced in recent years that not only target key processes in Mab purification, but also offer scalable solutions to enable efficient and seamless transition from laboratory bench through to large-scale manufacturing.

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Therapeutic monoclonal antibodies (Mabs) represent the pharmaceutical ‘success story’ of the past decade. Mab-based pharmaceuticals command a worldwide market of more than $15 billion, and that figure will grow to exceed $30 billion by 2010. The proven safety and efficacy of Mabs as a drug class are driving faster developmental and regulatory timelines, and simplified routes from discovery to the clinic and from the clinic to the market. The dramatic growth in demand for Mabs manufactured under GMP conditions – combined with advances in upstream processes that have greatly improved the efficiency of mammalian cell culture – have focused attention on the need for advanced purification tools and platforms to enhance the speed, robustness and scalability of the downstream processes required for Mab manufacturing. In recent years, new technologies and products have been introduced that not only target key processes in Mab purification (including product harvest, capture and polishing), but also offer scalable solutions that allow for an efficient and seamless transition from the laboratory bench through to large-scale manufacturing.

Improved productivity – achieved through greater process efficiency, and measured in terms of both time and cost – is the driving force behind Mab process technology development. Ease of operation, reproducibility, quality control and process validation have long been key factors in the design of Mab manufacturing protocols. In recent years, though, these factors have filtered their way up the discovery pipeline, and now play a significant role in the selection of tools and technology for process development and optimisation at laboratory scale, and are being carried through from research to pilot-scale production of material for clinical studies, and on to large-scale manufacturing of an approved product.

In an ideal scenario, researcher teams would develop and optimise processes at the bench using equipment, methods and software systems they could then hand over to the production group. These instruments and techniques would be directly scalable, would be capable of processing from hundreds of millilitre to thousands of litre culture volumes, and would yield highly pure monoclonal antibodies.

MAXIMISING BINDING CAPACITY FOR ANTIBODY CAPTURE

As Mab drug candidates move into the clinic, large-scale production begins using established techniques of mammalian cell culture. Improved cell lines and high-yielding expression systems – with expression levels of up to 3-5g/L – are generating concentrated feedstocks that demand more effective purification tools. In the first step of the purification process – harvesting of the Mabs – the introduction of membrane-based techniques offers an efficient and gentle alternative to centrifugation-based methods. Hollow fibre membranes separate the Mabs from physical impurities in the feedstock – including cells and cell debris – rapidly and efficiently with...
minimal disruption of the integrity of the discarded cells to prevent the release of destructive proteases. At GE Healthcare, we have developed a family of hollow fibre membranes ranging in capacity from research-scale to full-scale production, allowing users to apply the same technology for harvesting Mabs at the bench and, ultimately, in the manufacturing plant.

Following product harvest is the critical capture and polish steps that represent the heart of the downstream Mab purification process. These procedures isolate and purify the Mabs from other proteins and impurities in the feedstream – including DNA, virus and host-cell proteins. Innovations in this area have focused primarily on improving the speed, capacity and robustness of the capture and polish steps, while at the same time reducing process costs. Protein A-based chromatography has evolved to become the industry-standard for Mab capture, due to its exquisite binding specificity and ability to generate up to 95% pure Mab in a single step with high yield.

New product developments in recombinant protein A-based chromatography media are helping to meet the growing demand for high dynamic binding capacity and improved media stability. Media with a dynamic binding capacity of approximately 40mg/ml (for example, GE’s MabSelect Xtra™) translates to a 30% higher binding capacity than conventional protein A media; this is especially useful for Mab capture from feedstock with expression levels higher than 1mg/ml.

For existing processes, the ability to capture antibody rapidly and efficiently from high expression feedstreams reduces the number of cycles required per batch. This lowers raw material costs for buffers and cleaning agents, as well as simplifying quality control testing. In the design of new processes, it enables a 30% reduction in unit operations and a smaller column footprint.

For processes in which the cost of cleaning is a key issue or for which the effectiveness of the cleaning regimen is cause for concern, a high-capacity Protein A-based antibody capture combined with rigorous CIP using 0.1-0.5 M sodium hydroxide can be used (GE’s MabSelect SuRe™). The media contains an alkali stabilised Protein A-derived ligand that improves stability in alkaline cleaning conditions, allowing for the use of less costly cleaning agents. Use of the media can reduce the cost of cleaning by more than 90%. The media remains relatively stable for at least 200 cycles of CIP with 0.1 M sodium hydroxide, and retains approximately 90% of its initial dynamic binding capacity.

ONE-STEP ANTIBODY CAPTURE

As an alternative to the traditional two-step procedure for isolating Mabs from culture broth, it is possible to combine the harvest and capture steps into one process in which antibody is recovered directly from unclarified cell-culture feed in a single-pass operation. Although not without its limitations, this approach can further reduce process times and increase product yield. Expanded bed adsorption chromatography (EBAC) offers advantages over techniques that rely on a combination of filtration and fixed bed chromatography. For example, EBAC does not introduce the shear forces associated with filtration – which can disrupt the cells during the harvest step, exposing the antibody to proteolytic enzymes. This reduces yield and generates degradative by-products that must be removed in subsequent downstream purification steps.

In EBAC, the bed is expanded by a controlled upward liquid flow that causes the adsorbent beads to remain suspended in equilibrium. Unclarified feed is applied to the expanded bed through an upward flow, and proteins are captured on the adsorbent while cells, cell debris and...
particulate matter pass through. Reversal of the flow allows the beads to settle and the proteins to be desorbed in an elution buffer.

In a comparative study of EBAC, STREAMLINE™ Protein A matrix was tested against a combination of tangential flow filtration/microfiltration, ultra/diafiltration and fixed-bed chromatography for large-scale capture of an FC-fusion protein from a Chinese hamster ovary (CHO) cell culture; EBAC resulted in a 92% product yield, compared with 78% for the multi-step approach. EBAC also reduced total process times from 16 to 10 hours. Electrophoretic evaluation of the output derived using each method revealed an extra band for the product eluted from the fixed-bed chromatography column, consistent with degraded Fc-protein resulting from disruption of the CHO cells during filtration.

PERFECTING THE POLISH STEPS

Product purification following antibody capture typically requires two polishing steps – one of which most often is flow-through Q anion exchange chromatography to remove process impurities. The second polishing step may vary depending on the characteristics of the particular Mab, the culture method and personal preference. The most common techniques used tend to be SP cation exchange or a hydrophobic interaction chromatography (HIC) step.

The quality and robustness of each individual purification step determines the needs and challenges facing the next step in the process and, ultimately, impacts the robustness of the process as a whole. Purification needs to be sufficiently robust overall to be able to handle the occasional batch that may have a higher than usual level of background impurities, without the need to redesign downstream processing or discard an entire batch. Having to redesign the purification protocol could prove particularly catastrophic for a product that has already received regulatory approval, and losing a ‘dirty’ batch would prove to be quite costly at large scale.

To address these issues, GE Healthcare has developed a novel anion exchange medium for packed bed chromatography (Capto Q™), designed to maximise throughput and productivity of Mab purification. The media is capable of high dynamic binding capacity (>100 mg bovine serum albumin/ml medium) at high flow, with a typical flow velocity of 700 cm/hour in a 1 m diameter column with a 20 cm bed height. Low back pressure (<3 bar) contributes to high-volume throughput and reduced process-times.

The media is based on a rigid agarose matrix that provides underlying mechanical strength and a pore structure compatible with fast mass transfer of antibody. The high rigidity allows for a larger window of operation at large scale than Sepharose 6 Fast Flow, particularly at bed heights of 25-30 cm and higher – and even for high viscosity feedstreams. Flow velocity is 235 cm/hour, compared to 80 m/hour for Sepharose 6 Fast Flow for feedstock with a viscosity of 2 cP at a bed height of 30 cm. The media contains a functional group comprised of a quaternary amine linked to the matrix via a dextran surface extender, yielding a total ionic capacity of 0.16-0.22 mmol Cl-/ml.

SCALE-UP EFFICIENCY IS KEY TO PRODUCTIVITY

Ideally, the research group that has laboured over the development and optimisation of a process for producing an attractive therapeutic Mab candidate in the laboratory will hand over to the production team not only an exciting new drug prospect, but also a ‘tried-and-true’, cost-efficient and reproducible strategy for making the antibody that is directly scalable to pilot-scale production. The ability to adapt the same tools, technology, hardware and software used to develop the process facilitates this seamless transition. Integrated scalability has become a chief goal in the development of process technology, including filtration and chromatography systems, membranes and media, and the control software that oversees the various processes.
At GE Healthcare, scalability is a driving force in the design of a broad range of Mab production and purification technologies that span the early stages of process development through to industrial-scale manufacturing. As an example, Capto Q is available in prepacked HiTrap™ columns for use in the laboratory for rapid screening of selectivity and process conditions; the same media can then be used in Tricorn™ or XK columns for process optimisation and method development, and in pilot- and production-scale columns. Scale-up is achieved by keeping the residence time (bed height ÷ flow velocity) constant while increasing the bed height. Similarly, principles and protocols identified using bench-scale systems (such as ÄktaXpress™ or Äktacrossflow™) translate to larger scale operations required for product manufacturing.

With the same goals in mind, MabSelect Xtra and SuRe were designed for antibody purification at laboratory- to production-scale. Both products are available in quantities as small as 1ml in prepacked Hitrap™ columns, and up to 100s of litres as bulk media. Using the new ÄktaXpress Mab software modification, researchers working at lab scale can perform pre-programmed multi-step Mab purification (eight to 48 steps with integrated CIP) in one day. Scale-up protocols take into account factors that affect column packing and clean-in-place procedures, aiming to maximize dynamic binding capacity, flow velocity, purity and yield as volumes increase.

The process control technology linking these applications and ranges of scale is embedded in the control software (Unicorn™), which is used to design, monitor, modify, analyse, and drive the performance of the full spectrum of process steps. Maintaining a common software interface from development through production facilitates scale-up by enabling accurate simulation studies and prediction of conditions at larger scale, promoting familiarity with the software at all levels of an organisation, and ensuring compatible data stores for analysis, reporting and validation functions.

**CONCLUSION**

The stunning success of monoclonal antibody drugs in the clinic and on the market – combined with advances in the engineering of expression systems and process design – is driving innovation in downstream purification technologies. High antibody expression levels – predicted to nearly double over the next three to five years have led to concentrated feedstreams that necessitate robust separation strategies for isolating high-purity Mabs. Furthermore, productivity and cost-cutting goals are the driving force behind advances aimed at reducing process times, minimising media and buffer consumption, and improving overall process efficiency.

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