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PROCESS



INCREASED PRODUCTIVITY FOR DOWNSTREAM PROCESSING OF BIOTHERAPEUTICS

Polymer-based chromatographic resins featuring high mechanical and chemical stability and high protein binding capacities can help to increase the throughput and robustness of biopharmaceutical manufacturing processes.

Advances in genetic engineering and cell culture technology have raised upstream productivity in the production of recombinant proteins. Increased titres of up to 10 g/l stress the need for highly efficient and robust downstream processes (DSP). Most industrial bioprocess development groups apply generic DSP platforms that are designed to purify various candidates of the same class of proteins applying the same process design⁽¹⁾. Besides various filtration and virus inactivation steps, they usually involve two or three chromatographic unit operations with orthogonal separation modes. The use of high capacity chromatographic resins can help to overcome the DSP bottleneck by increasing process throughput and robustness. High sample amounts can be loaded and higher velocities applied without changing existing columns and other hardware.

Finding the best conditions for each chromatographic unit operation is of critical importance to establish a robust and efficient biopharmaceutical manufacturing process at large scale. Today the Design of Experiments (DoE) methodology and high-throughput screening (HTS) tools support efficient resin screening and method development. Applying these technologies also contributes to a better process understanding by increasing 'prior knowledge'. This is mandatory when following the Quality by Design (QbD) approach to establish robust and validated platforms.

PURIFICATION OF MONOCLONAL ANTIBODIES

Monoclonal antibodies (mAbs) represent the fastest growing segment of the biopharmaceutical industry. A variety of preparative modes of chromatography have been employed for the large-scale purification of mAbs. Most schemes involve the use of protein A affinity chromatography in the capture step, exploiting the specific interactions that take place between the Fc regions of the monoclonals and immobilised protein A, a cell wall component of *Staphylococcus aureus*. Today, recombinant protein A ligands are used for protein A affinity resin production. Protein A affinity chromatography is well established and highly specific to mAbs. More than 95 per cent purity can be achieved

in a protein A capture step. In a typical three-step mAb process, protein A chromatography is usually combined with other modes of chromatography – such as cation exchange, anion exchange or hydrophobic interaction (HIC) – to achieve pharmaceutically acceptable purity levels.

Other strategies are focusing either on reducing the number of chromatographic unit operations to a two-step process, or on developing non-protein A platforms. Eliminating a protein A step would overcome the known drawbacks of protein A chromatography such as high resin costs, protein A leaching and the formation of aggregates due to the acidic conditions needed for elution. Recently Lain et al evaluated the use of cation exchange chromatography as a mAb capture step to replace the protein A step⁽²⁾. They developed a high capacity capture step for mAbs based on TOYOPEARL GigaCap S-650M.

In 2009, the CMC Biotech Working Group published the A-MAb Case Study, summarising the joint efforts of several large biopharm manufacturers in exemplifying a QbD approach to monoclonal antibody product development⁽³⁾. A-Mab – a humanised IgG₁ monoclonal antibody – was used to follow typical groups or sequences of activities that occur in the development of a monoclonal antibody including upstream and downstream processing.

CHROMATOGRAPHIC PROCESS RESINS

A broad range of commercial chromatographic resins can be applied in large-scale mAb purification. Base particles range from soft agarose based matrices to porous glass and more rigid polymer and silica-based matrices. Common processes often combine resins of various suppliers in order to achieve maximum performance for each single step. Besides binding capacity, purity, robustness towards sanitisation and cleaning-in-place, the mechanical properties of the resin also have to be taken into account when selecting the chromatographic resin for a specific step. The rigidity of the resin influences both the ease of column packing and the bed stability in large-scale industrial columns at high velocities.

TOYOPEARL® resins are based on a rigid, cross-linked polymethacrylate particle. The high mechanical stability pays off in excellent pressure flow characteristics and straightforward column packing. Now, that the first Toyopearl based protein A process resin – TOYOPEARL AF-rProtein A-650F – is being introduced, a complete mAb

purification platform based on TOYOPEARL can be established.

EVALUATION OF PURIFICATION STEPS

A generic mAb process assumes that a pre-defined purification template works for all monoclonals. However, physicochemical differences among mAbs require a flexible platform approach. The overall scheme of the downstream process and the limits of operating conditions can be pre-defined, but individual conditions have to be adapted to the specific target mAb. HTS and DoE are powerful tools when defining the design space of downstream processes and considerably speed up parameter scouting.

The results of the CMC study can be used to set up a mAb purification platform based on polymeric high capacity resins. HTS techniques and the DoE methodology were applied to evaluate chromatographic steps for mAb purification. Various resins and method parameters for the protein A affinity step and subsequent ion exchange steps were evaluated. Robotic platforms for parallel microscale chromatography substantially facilitate the screening of chromatographic resins and support a fast optimisation of method parameters⁽⁴⁾; they process small screening columns in 96-well plate format in a few hours. Today, high-throughput systems complement or even replace the tedious and sample consuming column-based screening in many industrial process development groups.

PROTEIN A AFFINITY

TOYOPEARL AF-rProtein A-650F is an attractive candidate to improve the efficiency of a protein A based mAb capture step because it retains its high dynamic IgG binding capacity at a broad range of velocities and also when loaded with high titre feedstocks (Figure 1).

HCP REMOVAL

	Bed volume (µl)	Protein load (mg/ml gel)	pH	Flow rate (cm/h)	HCP (ppm)
Toyopearl AF-rProtein A	200	5	3.9	100	19.2
Competitive rProtein A resin	200	5	3.9	100	59.7
Toyopearl AF-rProtein A	200	5	3.9	250	9.8
Competitive rProtein A resin	200	5	3.9	250	30.5
Toyopearl AF-rProtein A	200	25	3.9	250	47.3
Competitive rProtein A resin	200	25	3.9	250	629.6

Table 1

Effect of residence time on the CHO host cell protein (HCP) removal for two alkali-stable rProtein A resins: Toyopearl AF-rProtein A-650F and a competitive agarose-based resin. HCP content detected by ELISA (Cygnus Technologies). Data kindly provided by U. Breuninger, University of Applied Science Esslingen.

DYNAMIC BINDING CAPACITY

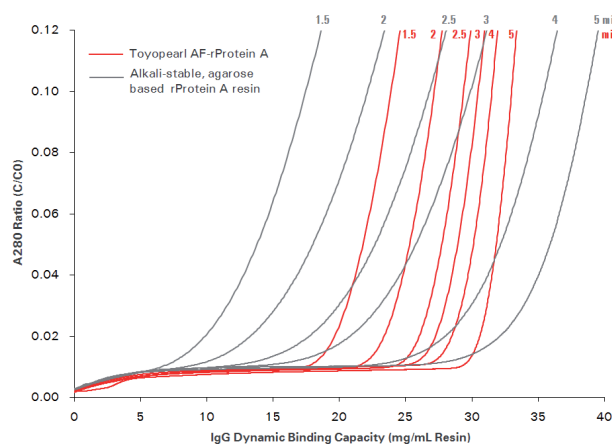


Figure 1

Breakthrough curves for hlgG loading (polyclonal, 10 mg/ml); Typical DBC at 10% breakthrough: 33 mg/mL @ 60 cm/hr (5 min residence time) - 24 mg/mL @ 200 cm/hr (1.5 min residence time) Column: 5 mm ID x 5 cm L; Mobile phase: 20 mM sodium phosphate buffer pH 7.2 containing 150 mM NaCl Sample conc.: 10 mg/mL; Residence time: 1.5, 2.0, 2.5, 3.0, 4.0, 5.0 min

Its recombinant protein A derived ligand is attached to the rigid base matrix by multiple stable bonds resulting in low ligand leaching and high stability towards alkaline clean-in-place (CIP) conditions. Different protein A affinity resins, including the Toyopearl AF-rProtein A-650F, packed in MediaScout® MiniColumns (Atoll GmbH, Weingarten, Germany) were tested by varying the binding buffer pH, the sample load and the residence time.

In order to simulate a real feedstock sample, a pure monoclonal antibody (IgG1) was spiked into a Chinese hamster ovary (CHO) cell lysate. Purity and recovery of the antibody in the eluate were monitored. The purity of the antibody fraction was tested by measuring the amount of CHO host cell proteins (HCP), and the amount of leached protein A by immunoassays. Table 1 shows the HCP removal when varying sample load and/or flow rate (residence time) at fixed bed volume and pH.

Fractions purified by the Toyopearl AF-rProtein A-650F showed a lower amount of remaining host cell proteins under all conditions tested when compared to those purified by a widely used competitive rProtein A resin providing a similar IgG binding capacity.

ION EXCHANGE CHROMATOGRAPHY

The reduction of CHO proteins (CHOP) was also evaluated for a cation exchange step by varying the parameters protein load and pH, as well as ionic strength of binding and/or elution buffer in a DoE approach.

At low pH of the binding buffer, the ionic strength of the elution buffer (shown as conductivity) has no significant influence on the amount of residual HCP eluting from the cation exchange resin (Toyopearl GigaCap CM-650M, see Figure 2). With increasing pH of the binding buffer up to neutral pH values, an increased influence of the ionic strength on the HCP removal can be observed. Similar high throughput screening experiments were performed for anion exchange and hydrophobic interaction chromatography (data not shown). By applying the ideal conditions for each unit operation, various downstream process platform strategies can be simulated easily at lab scale.

INCREASING DSP THROUGHPUT

Future challenges of mAb purification will be provided by the further increase in cell culture titres. Chromatographic operations thus become limited in terms of the throughput they can provide. The ability to run a chromatographic purification step at a broad range of velocities and sample loads expands the design space provided by a validated purification platform. The use of resins providing a high mechanical stability and maintaining high protein binding capacities at high velocities supports these requests.

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EVALUATION OF CATION EXCHANGE STEP

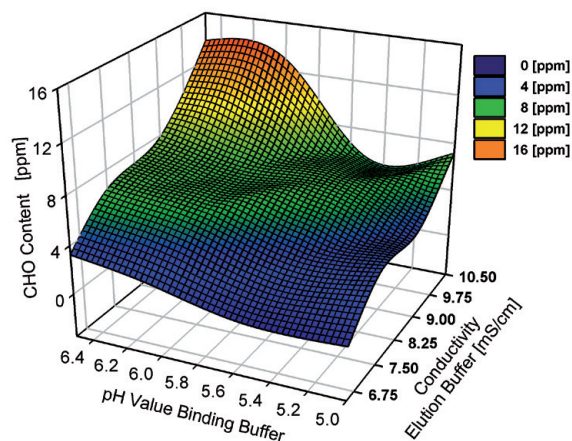


Figure 2

Influence of binding buffer pH and elution buffer ionic strength on the CHO protein content of the mAb fraction purified by cation exchange chromatography on Toyopearl GigaCap CM-650M. Data kindly provided

PRESSURE/FLOW CHARACTERISTICS

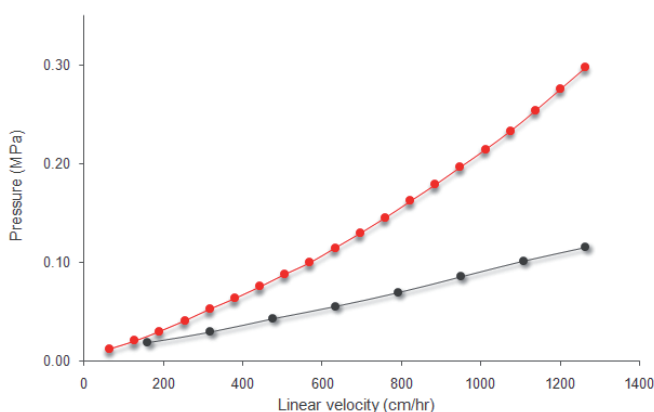


Figure 3

Pressure flow characteristics of polymethacrylate-based process resins. Rigid, polymeric process resins can be used at high flow rates. Toyopearl GigaCap S-650M cation exchange resin with a mean particle size of 75 μm (grey), as well as Toyopearl AF-rProtein A-650F (red) with a mean particle size of 45 μm can be used at linear velocities of more than 1,000 cm/hr.

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