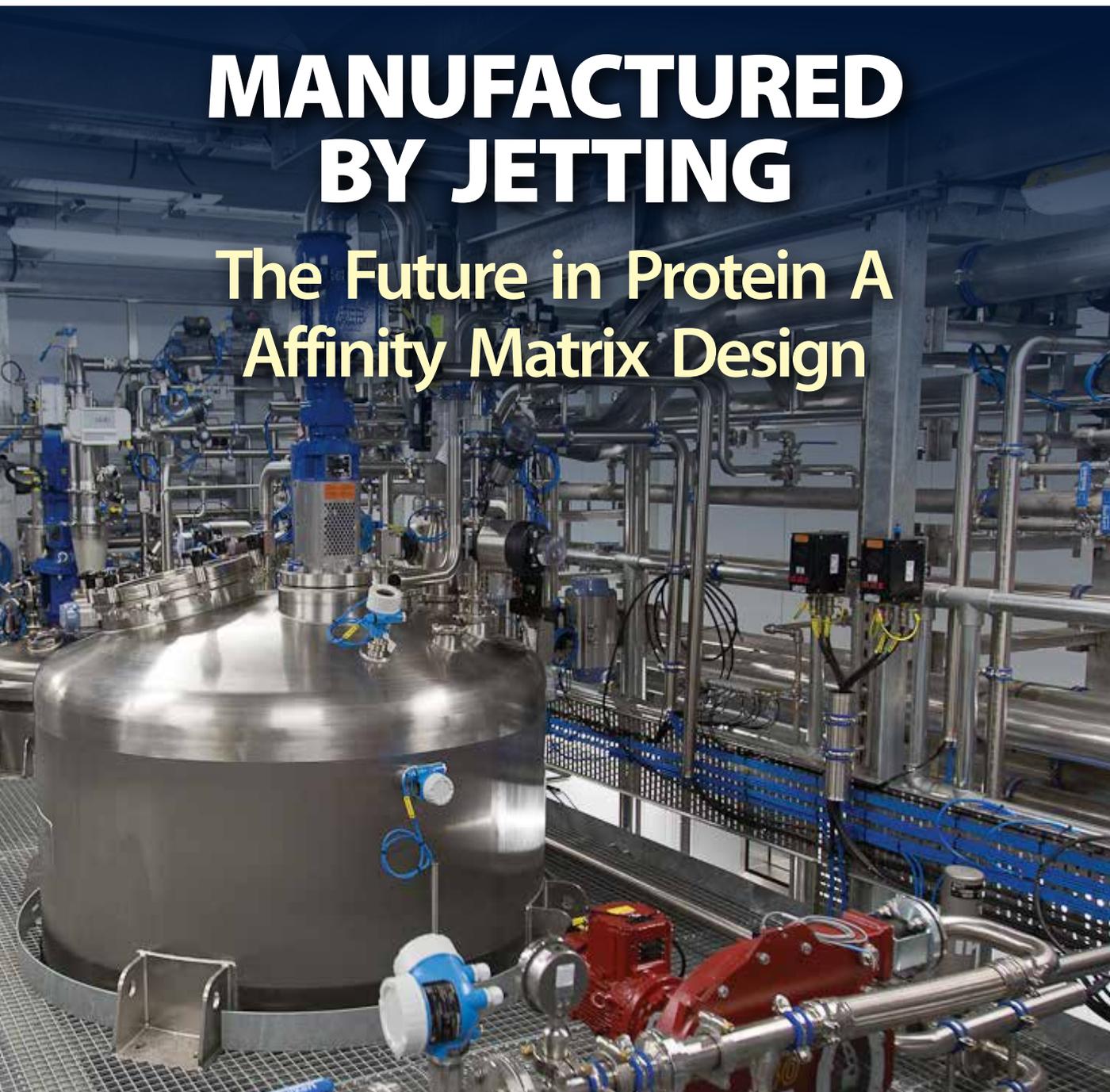


# MANUFACTURED BY JETTING

## The Future in Protein A Affinity Matrix Design



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# Manufactured by Jetting

## The Future in Protein A Affinity Matrix Design

Hans J. Johansson, Patrick Gilbert, Mark Hicks, and Oleg Shinkazh

**P**rotein A affinity chromatography continues to be the preferred method for commercial purification of antibodies because of its high selectivity and robust resin performance over repeated purification cycles. Reports estimate that US\$125 billion of yearly sales will be generated from monoclonal antibody (MAb) products by 2020 (1). Most of those will be purified by large-scale protein A affinity chromatography. With the continued growth and commercial importance of MAb production, availability of high-quality resin material and options for secondary sourcing are growing concerns. As current commercial patents for therapeutic antibodies expire and biosimilars enter the market, the cost of manufacturing will be of increasing interest.

The workhorses of today's commercial MAb purification processes still are porous resins based on styrenic, acrylic, or agarose chemistry and produced by the same batch emulsification methodology that has been used since the mid-1900s. That technology produces resins that have a wide particle-size distribution and require extensive screening to achieve the column performance demanded in modern processes. By contrast, a scalable continuous emulsification technology termed *jetting* can be used to produce beads with a narrow particle-size distribution, without sieving, and resulting in almost quantitative yield. Here, we present performance data of a new protein A resin, Praesto® Jetted A50, manufactured by jetting technology. Interestingly, the particle-size distribution has been shown to have a significant effect on resistance to fouling (2, 3).

### WHAT MATTERS?

It is not uncommon to overlook some important aspects of protein A affinity chromatography. For practical reasons, process development work is performed typically using laboratory-size columns (2–100 mL). Because of wall effects in small columns and sometimes a lack of representative feed material, phenomena such as deterioration of

pressure/flow performance, fouling, and large-scale packing are difficult to predict and could cause severe problems when scaling up. The most important factors to consider during evaluation of protein A resins are summarized below. Some factors will be impossible to evaluate at scale during design of a clinical process but still should be considered before selecting a resin.

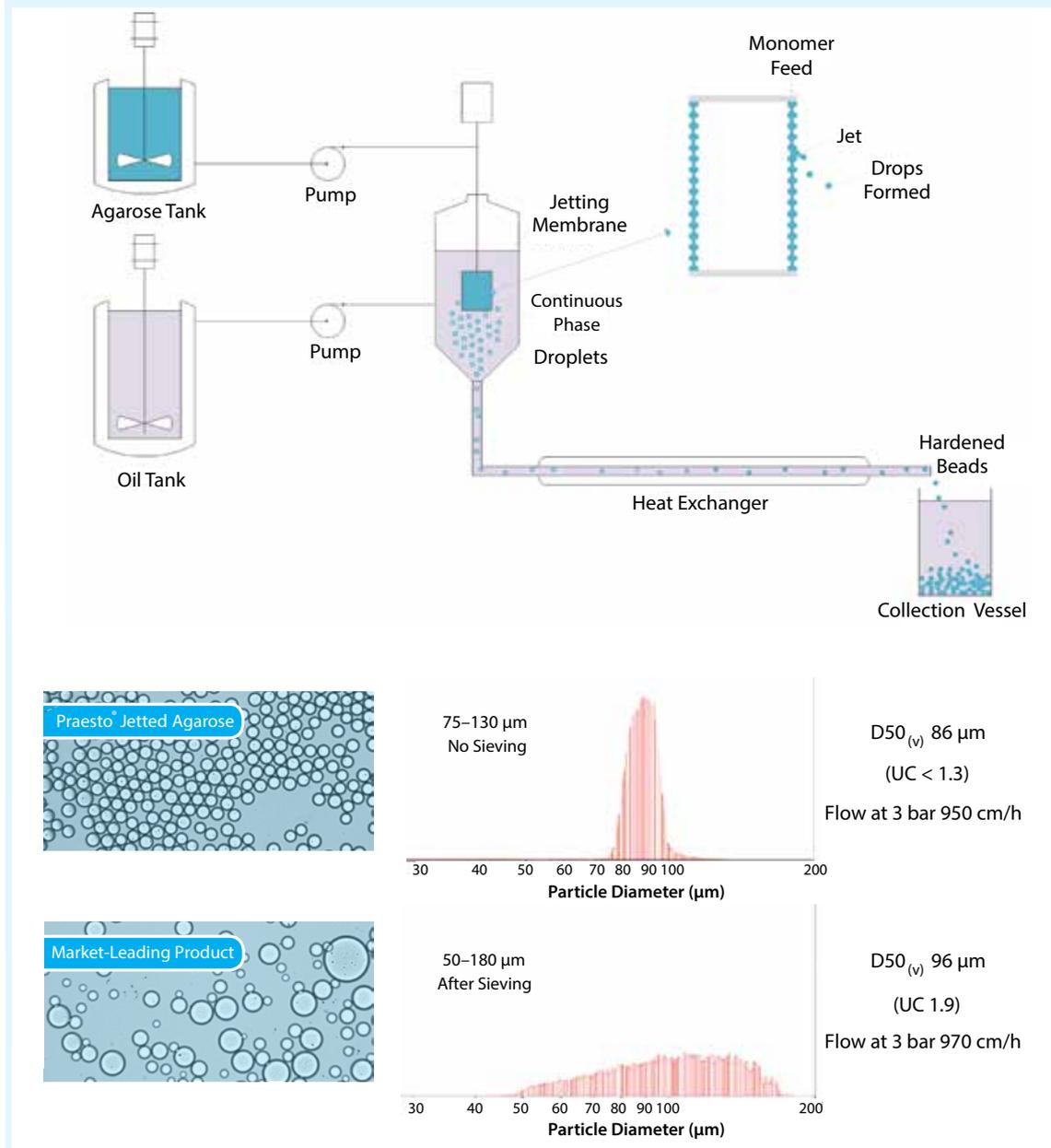
**Dynamic binding capacity (DBC)** typically is the starting point of every protein A resin evaluation. One important factor to consider at this stage is the anticipated column format and pressure/flow-rate restrictions at larger scales. DBC has a significant impact on both productivity and buffer consumption. A small bead will result in a relatively higher DBC, especially at short residence times. But small beads also will generate higher back pressures and will be more sensitive to fouling.

**Purification Performance:** The most critical contaminant in many processes is host-cell proteins (HCP). Copurification of HCP depends on both binding to a target MAb and the design and material used in a resin base matrix. In general, highly hydrophilic materials such as agarose show the best performance with respect to unspecific binding (4). The type of protein A used could affect the required elution pH needed to obtain quantitative recovery and the resulting product pool volume.

**Cleaning in place (CIP):** Development of effective cleaning protocols after purification is instrumental, both to eliminate carry-over and to maximize resin lifetime. Effective cleaning and sanitization will help prevent microbial growth and inactivate potential endotoxins. Because of their high costs, protein A resins are reused over many cycles in commercial manufacturing.

**Fouling** of chromatography resins could be the result of many factors. It is feed dependent, and proteins, lipids, lipoproteins and anti-foam agents all can cause fouling. However, in the case of purification of MAbs from Chinese hamster ovary (CHO) cell cultures, the most common cause of

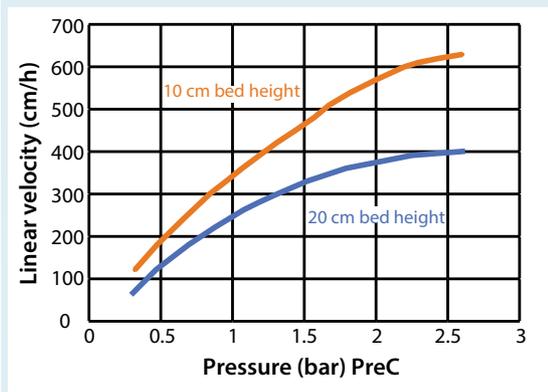
**Figure 1:** A jetting process produces beads continuously and uniformly. Using one membrane can produce resin with average particle size of 25–100  $\mu\text{m}$ .



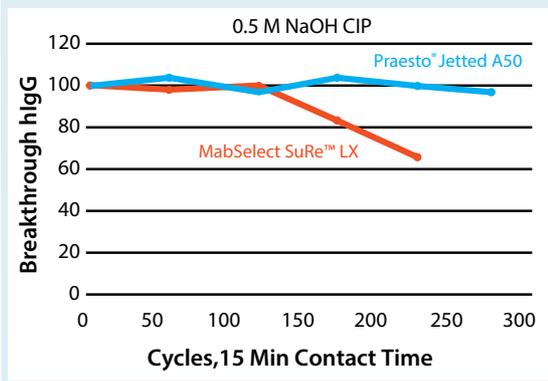
fouling is protein fouling by a target protein or target protein variants. The most effective agent for removing precipitated proteins is sodium hydroxide. With modern, alkaline-stable, protein A ligands, 0.1 – 0.5 M sodium hydroxide is the standard cleaning agent. For difficult feed streams, sodium hydroxide might be insufficient, and more sophisticated clean-in-place (CIP) protocols must be developed (5). Severe fouling might require further optimization of harvesting and clarification methods.

**Process Economics:** Clearly, the price of a resin significantly affects raw material costs. In many cases, protein A resins are the most expensive raw material in MAb manufacturing. To accurately estimate the contribution of protein A resin cost for a given process, take into account DBC, anticipated numbers of resin reuse, and buffer cost and consumption. Many companies are pursuing different types of continuous chromatography as a technology to decrease resin cost and buffer volumes. But such technologies are not yet

**Figure 2:** Praesto® Jetted A50 was packed into an axial compression column (AxiChrom 200, GE Healthcare) at two different bed heights, 10 and 20 cm respectively. Plate number, asymmetry and pressure vs flow were determined.



**Figure 3:** Columns were statically stored in 0.5 M NaOH for 15 hours at 20 °C between each DBC measurement.



**Functional Lifetime:** At commercial stage, the number of cycles in which a resin can be used is an important factor that significantly affects overall process economy. With “easy” cell culture supernatants, more than 200 cycles often can be achieved. However, every cell culture supernatant and MAb are unique, and true resin lifetime cannot be predicted from simple alkaline stability data. For example, Gilead presented cycling data (5) for the newest resin from GE Healthcare, MabSelect Prisma, with and without sample load using 0.3 M NaOH for CIP (15 minutes contact time). The difference in DBC after 150 cycles was 17% when comparing with and without sample load.

**Packing and Unpacking:** In commercial manufacturing, resins are expected to last for more than 50 cycles and in some cases, at least 200 cycles. So resin aspects such as ease of packing and unpacking are important. For some processes, standard operating procedures require repacking every 25 cycles. Long-term storage stability,

resistance to shear forces (to determine whether a resin slurry can be pumped), and generation of fines (which could clog column filters and frits) are important considerations.

#### **Security of Supply and Risk Management:**

Commercial processes could be operated for more than 20 years. It is important to evaluate the financial stability of a vendor and ensure procedures are established for disaster recovery and product discontinuation. Production capacity and lead time are also key considerations when selecting a vendor to ensure a consistent, stable supply of adequate volumes of resin.

#### **WHAT IS JETTING?**

Jetting is a continuous emulsification technology for manufacturing chromatography beads. Unlike batch emulsification, jetting produces beads without the need for additional sieving (Figure 1).

#### **Benefits of a Narrow Particle-Size Distribution:**

Absence of both fine and coarse particles provides a number of benefits or potential advantages over the use of other technologies:

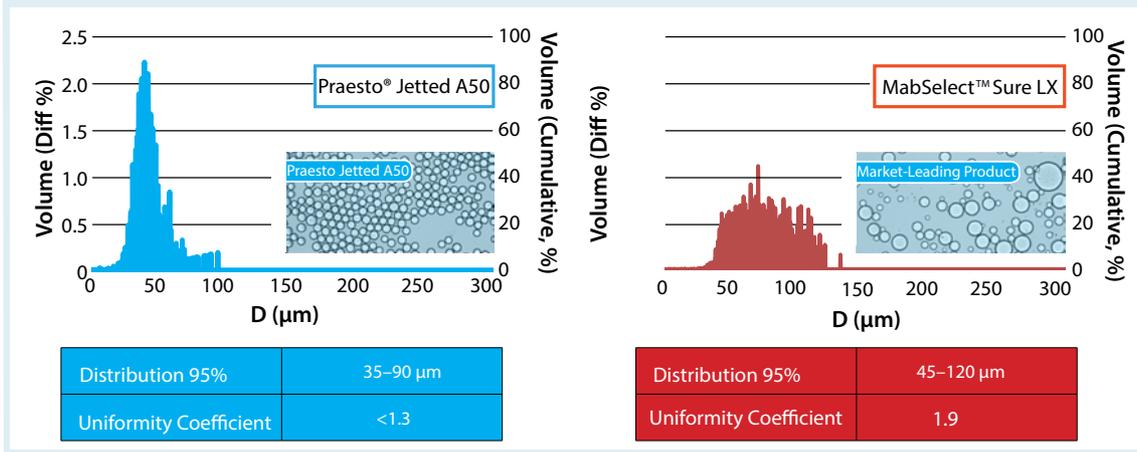
- Improved flow properties
- Decreased eddy diffusion (improved resolution and decreased buffer consumption)
- Reproducible column packing
- Decreased resin fouling
- Improved virus clearance
- Potential use of larger mesh-size column filters (less risk of clogging).

#### **COMBINING JETTING WITH A NEW ALKALINE-STABLE PROTEIN A**

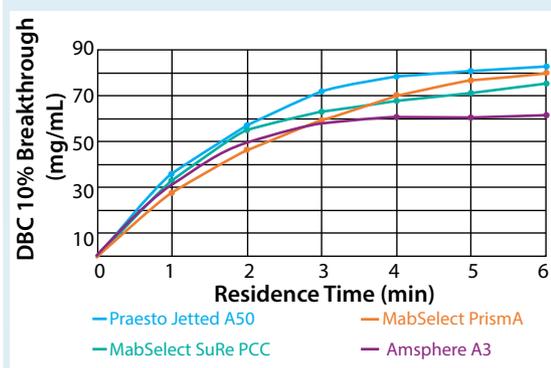
One size does not fit all, but based on our experience, particle sizes of 45–90 μm are the most useful beads for direct capture of MAbs from cell culture supernatant. Purolite decided to first launch a 50-μm bead to support the need of resins suitable for continuous chromatography, which typically involves high DBC at 1–2 minute residence times and 5–10 cm bed heights. Based on the jetting technology and high-flow agarose chemistry, the new resin still has sufficient pressure-flow properties to enable traditional large-scale column chromatography (Figure 2).

To support effective, alkaline-based, CIP (Figure 3) and sanitization protocols, a new ligand from Repligen was used. The new recombinant protein A ligand — NGL-Impact™ A — was developed by Navigo by screening of a large library of protein A constructs for alkaline stability.

**Figure 4:** Particle-size distribution of the novel protein A resin based on jetting technology vs. traditional batch emulsified agarose resin; results determined by static imaging analysis.



**Figure 5:** Comparing four different protein A resins packed to 10-cm bed height with respect to dynamic binding capacity (DBC, 10% breakthrough)



### CHARACTERISTICS OF THE NEW PRAESTO JETTETED A50 PROTEIN A RESIN

Praesto Jettted A50 resin is the first product designed by combining the new technology for continuous manufacturing of agarose beads with a novel alkaline-stable protein A ligand. Process economy calculations based on characteristics of the new resin show the potential reduction of resin cost by >75% in clinical manufacturing. Figures 4 and 5 show narrower particle-size distribution and dynamic binding capacities of the Praesto Jettted A50 resin compared with three different resins.

### PRODUCTIVITY AND PROCESS-ECONOMY SIMULATIONS

Another important factor to consider when selecting a resin is process economy, which is driven by resin price and resin lifetime, facility constraints, hardware requirements, and buffer consumption. Column dimensions vary widely

**Table 1:** Cost comparison of four agarose-based protein A resins (RT = residence time, DBC = dynamic binding capacity)

	Praesto Jettted A50	MabSelect PrismaA	Praesto AP	MabSelect SuRe LX
RT (min)	2	2	6	6
DBC (g/L)	57	46	50	53
Loading capacity 80% (g/L)	45	37	40	42
Number of cycles	14	17	5	5
Column dimensions (ID x BH cm)	45 x 10	45 x 10	60 x 20	60 x 20
Column volume (L)	16	16	57	57
Buffer volume (L)	3,785	4,596	4,807	4,807
Total processing (h)	7.9	9.6	8.5	8.5
Resin cost (per L)	10,720	17,000	10,720	16,313
Resin cost (\$)	170,494	270,373	606,202	922,478
g MAb/L resin/h	79.2	65.3	20.8	20.8
USD/g MAb	17.05	27.04	60.62	92.25

from the standard 15–25-cm bed height typically used in traditional batch chromatography to 5–10-cm bed height columns that are used often in different continuous strategies. We determined DBC using a purified biosimilar of Avastin, bevacizumab. Table 1 shows four agarose-based protein A resins based on a common case, as follows:

- Capture of a MAb from cell culture supernatant at 2000-L scale
- Feed titer of 5.0 g/L

**Table 2:** Feedback from customer testing of different monoclonal antibodies (RT = residence time, NT = not determined)

Customer	1 min RT	2 min RT	3 min RT	4 min RT	5 min RT
1	50	NT	NT	85	NT
2	NT	60	NT	76	NT
3	NT	NT	NT	75	NT
4	NT	NT	70	NT	85
5	NT	76	NT	NT	NT
6	70 (Humira IgG1)				

- Capture of 10 kg MAb.
- Load to 80% of the initially determined DBC at 10% breakthrough
- Limit process time (purification only) to 10 hours
- Residence time of two or six minutes
- Bed heights of 10 and 20 cm and diameters of 45 and 60 cm
- Buffer volume/purification cycle estimated to 17 CV
- Elution pool volume of 3 CV/cycle.

### PROCESS MODELING

Our calculations provide an estimate of the effect of resin price and performance (Table 1). Factors related to risk, time, and cost of implementation, and fulfilment of regulatory requirement also must be considered. However, it is clear that use of new, high-capacity resins can lower cost of goods (CoGs) significantly. Even for conventional batch chromatography, applied savings of >75 % can be achieved.

### SELECTED CUSTOMER RESULTS

The Praesto Jetted A50 resin was introduced to the market in April 2018. Table 2 summarizes feedback from initial trials by several different pharmaceutical companies.

### THE FUTURE: JETTING TECHNOLOGY

The industry is rapidly advancing MAb technology. Fc-fusion proteins, bispecific antibodies, IgG fragments, and drug-conjugated MAbs now make up most biologics in clinical pipelines. We believe that with the evolution of new classes of IgG-related biologics and novel expression systems, the industry also will have a constant need for new types of chromatography resins.

Successful implementation of jetting technology for continuous emulsification of

agarose beads enables the design of a new platform of protein A resins. We now can jet beads with 25–200- $\mu$ m mean particle sizes.

Jetting is a robust and quantitative process that allows a fast design of new agarose beads, matching requirements from both new chromatography ligands and novel pharmaceutical molecules and process technologies. The box on the next page shows data on a 25- $\mu$ m bead with an open pore structure designed for countercurrent tangential chromatography.

### REFERENCES

- 1 Levine HL, Jones SD, Ecker DM. The Therapeutic Monoclonal Antibody Market. *mAbs* 7(1) 2015: 9–14.
- 2 Johansson H, et al. Does Particle-Size Distribution Matter? Recovery of Biological Products Conference, Ashville, NC, 7–12 October 2018.
- 3 Johansson HJ, Cardillo D, Gerwe B. Are All Protein A Resins the Same? *BioProcess Int.* 15(11) 2017: insert.
- 4 Gronberg A, et al. A Tool for Increasing the Lifetime of Chromatography Resins. *mAbs* 3(2) 2011: 102–202.
- 5 Dransart B, et al. Evaluation of a Next-Generation Protein A Chromatography Resin for the Purification of Monoclonal Antibodies. *BioProcess Int.* 16(1–2) 2018: insert. 

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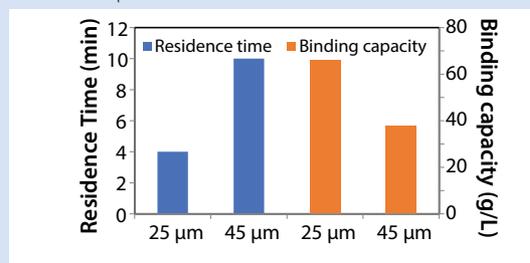
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Purolite and ChromaTan corporation have collaborated to produce a unique agarose resin specifically suited for the column-free continuous countercurrent tangential chromatography (CCTC) platform (1–2). CCTC has overcome many limitations of batch-column chromatography. Unlike column chromatography, in which resin particles are packed in a fixed-bed column, CCTC uses resin in the form of a slurry, which flows through static mixers and hollow-fiber membrane modules in a countercurrent staged configuration. The slurry is treated as a conveyor belt that is simultaneously “operated on” by the addition of specific buffers in different chromatographic steps through a staged configuration (Figure 1A). Unlike in column chromatography, the product elutes at steady-state without peaks or gradients (Figure 1B). The CCTC system has been used for capture and mixed-mode polishing as well as flowthrough operations showing ~5-15-fold increase in productivity when compared with batch-column systems. Figure 1c shows an example of protein A capture CCTC operation.

One benefit of using CCTC is that the pressure drop through the system essentially is independent of particle size, in sharp contrast to the  $1/d_p^2$  dependence seen with column chromatography. Thus, CCTC can be operated with much smaller particles than is possible with packed columns, providing a significant reduction in diffusional mass transfer resistance. A CCTC system also allows the use of highly porous/softer beads, which cannot be packed in columns because of high-pressure limitations but behave favorably in a column-free system. Softer, less-crosslinked particles provide more surface area for the protein A ligand and thus enable significantly higher dynamic binding capacity.

Figure 2 shows key characteristics of the new particle. Binding capacity of the 25- $\mu\text{m}$  Purolite Praesto resin was 66 g/L, which is ~50 % higher than the commercially available 45  $\mu\text{m}$  resin. The kinetics of the 25- $\mu\text{m}$  resin were significantly faster: Only four

**Figure 2:** Comparison of binding kinetics and capacity for small-particle (25- $\mu\text{m}$ ) Purolite resin and commercially available 45- $\mu\text{m}$  resin



minutes were needed to capture 95% of the MAb with the 25- $\mu\text{m}$  particle rather than 10 minutes with the larger particle size resin. The excellent performance of this new resin enabled a significant increase in the overall productivity of the CCTC system for protein A capture to 140 g/L resin/h for the 25- $\mu\text{m}$  particle. Buffer consumption also was reduced by about 50% because of the higher binding capacity.

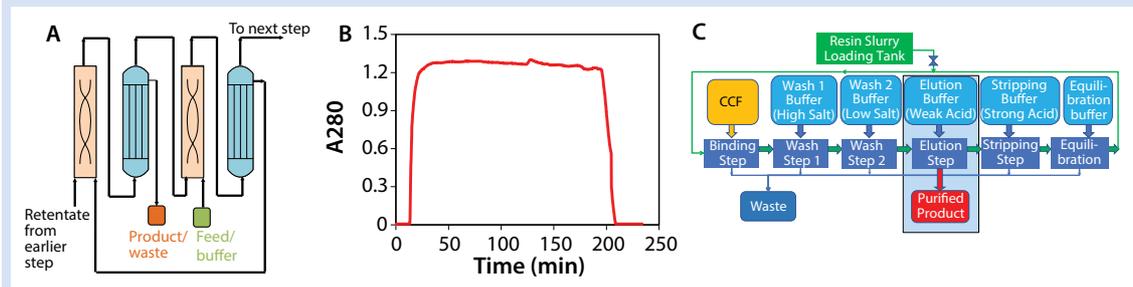
Overall, the productivity of the CCTC system with the new Purolite resin was ~12x higher than the packed column (12 g/L resin/h). This work showed tremendous promise and we are excited that further optimization of the resin could enable even further improvements of the CCTC system performance.

**References**

- 1 Shinkazh et al. Countercurrent Tangential Chromatography for Large-Scale Protein Purification. *Biotechnol. Bioeng.* 108(3) 2011: 582–591.
- 2 Dutta et al. Continuous Countercurrent Tangential Chromatography for Mixed Mode Post-Capture Operations in Monoclonal Antibody Purification *J. Chromatogr. A.* 1511 2017: 37–44.



**Figure 1:** (A) A single step in the CCTC system showing two stages, each consisting of a static mixer and hollow-fiber membrane module; (B) steady state UV profile; (C) CCTC system with continuous recycle of the protein A resin; the system consists of six steps (binding, wash 1, wash 2, elution, strip, and equilibration).





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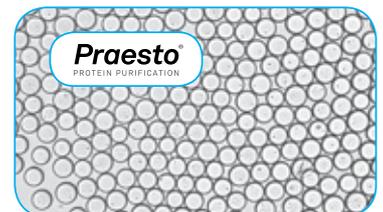


# The future of Protein A chromatography is here...

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- Exceptional alkaline stability at concentrations up to 1.0 M
- Novel 'Jetted' base bead offers 40% higher capacity than current market-leading Protein A resin



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