SEPTEMBER 2021

BioPharm INTERNATIONAL®

Reducing the Costs of Biopharmaceutical Separations and Purifications with Novel Resins

This custom ebook is sponsored by Purolite and presented in partnership with *BioPharm International*.

Purolite®

Welcome to this ebook focused on purification of monoclonal antibodies.

Four articles covering different aspects of Protein A affinity chromatography are included. Dr. Thomas Mueller Spath presents an in-depth description of the principles of CaptureSMB, a twin column continuous system, and how the implementation can reduce buffer volumes and resin cost with more than 50 %. Hans J. Johansson explains how purpose-designed agarose beads are manufactured by continuous emulsification. The performance of a novel Protein A resin, designed for purification of acid sensitive antibodies and antibody derived molecules, is also presented.

Included is your free pass to our on-demand version of the recent mAb purification workshop, which features talks from six mAb purification specialists.

Bringing Resin Experience to Today's Industrial Separations

Interview with Hans Johansson

Continuous vs. batch manufacturing makes more uniform particles, in a greener and more effective way.

For the purposes of chromatography, agarose is widely considered to be the best material available for protein purification resins. It is a useful material because it does not absorb biomolecules to any significant extent, has sufficient flow properties, and can tolerate extremes of pH and ionic strength. Most agarose-based resins on the market are produced using a batch emulsification technology developed more than 50 years ago. This produces a wide particle size distribution even after extensive sieving to remove the course and fine beads.

BioPharm International recently spoke with Hans Johansson, global applications director at Purolite Life Sciences, about the company's use of a patented manufacturing process called "jetting" to produce uniform agarose beads, as well as how his background in life sciences led him to Purolite to develop resins based on agarose.

BRINGING RESIN EXPERIENCE TO TODAY'S INDUSTRIAL SEPARATIONS

"Purolite's knowledge in large-scale manufacturing of resins includes a technology called jetting to make very uniform particles."

BIOPHARM: **Tell us about your professional background before joining Purolite.**
JOHANSSON: In the early 1980s, before life science and biopharma really existed, I joined a company called Pharmacia. In those days, it was really a pharma company, but they had a biotech division called Pharmacia Biotech. I came in when biopharmaceutical industry was just starting to be established as a separate industry and companies like Amgen and Genentech were recently founded. Pharmacia Biotech then became Amersham Pharmacia, which got acquired by GE and is now Cytiva. I worked there for roughly 30 years, involved mainly in R&D. We were developing chromatography resins that really form the basis for the industrial separations of today.

Seven years ago, I left GE and joined Purolite. At that time, Purolite was one of the larger resin companies and did not have much contact with the life science industry. I came from life sciences, and along with a small group of people, started a project to develop agarose-based resins for the life sciences market.

BIOPHARM: **What drew you to the life sciences industry and separation science?**
JOHANSSON: I came into the industry in a very interesting time, when the Nobel Prize had just been given for the principle of producing monoclonal antibodies (George Kohler and Cesar Milstein, 1984). People were talking about antibodies as a magic bullet that would cure everything. In those days, researchers were using mouse antibodies. And of course, there was a learning curve, like with all new technologies. Eventually in the late 1990s, antibodies started to take off and now they are a very substantial part of the pharma industry.

BIOPHARM: **What do you do now at Purolite, and how do you feel your work is advancing the biopharmaceutical industry as a whole?**
JOHANSSON: When I joined, they did not really have a life sciences business. Seven years ago, the industry was dominated by a few vendors. I think competition is the mother of innovation and we added competition into the market for the benefit of industry. Purolite, with a long history of manufacturing resins in a very cost-effective way, had all the technology to do that. I came in with the experience of working in the life science environment, which is a bit different from both scientific and regulatory aspects.

BIOPHARM: **Can you talk about a few projects that you've worked on at Purolite of which you're most proud?**
JOHANSSON: Purolite's knowledge in large-scale manufacturing of resins includes a technology called jetting to make very uniform particles. We adopted this technology to making agarose beads in

a continuous way rather than the more traditional batch way of producing resins via batch emulsification. With the exception of Purolite's, nearly all the industrial resins on the life science market today are made with a technology that was developed in the 1950s and 1960s.

Jetting has an impact as it is a green way of manufacturing. We don't use any solvents and the yield is almost quantitative. It benefits both the manufacturer and the end user. It's also a very flexible technology. Most commercial antibodies today are purified in basically the same way. You use large columns and a traditional batch strategy. However, the industry is now advancing toward more continuous and cost-efficient manufacturing.

This is particularly important in clinical manufacturing, where the impact of the resin cost is quite significant. By developing purpose-designed resins, we support the introduction of new cost-effective technologies for both clinical and commercial production.

BIOPHARM: **What are some of the most pressing challenges that the biopharmaceutical industry faces now?**
JOHANSSON: Supply issues are a problem for the industry. We hear from a lot of customers that lead times for resins are starting to get very long. When it comes to security of supply, we have expanded our plant in Wales, UK, and have started development of a second plant in the US that will be commissioned by the end of this year, using jetting technology to produce resins for the life science market. Purolite will be the only supplier able to produce bioprocess volumes of agarose resins with two independent manufacturing facilities two continents.

BIOPHARM: **What projects are you currently working at Purolite that you can discuss and/or what's in store for the future?**
JOHANSSON: We plan to expand the chromatography program, especially both on the ion exchange side and on specific affinity resin targeting new molecules for the next generations of pharma molecules ranging from mRNA to novel antibody formats.

BIOPHARM: **Do you have any comments about security of supply in this sector?**
JOHANSSON: The global pandemic caused supply challenges across the globe in many industries and markets, including the biopharmaceutical sector where the supply of process critical raw materials was especially affected, delaying clinical trials.The pandemic has taught the biopharmaceutical industry to consider the entire process from start to finish and highlight key process-dependent raw materials. Once completed, this exercise identifies in particular process critical singly sourced items.

In the case of the production of monoclonal antibodies, chromatography resins in particular Protein A resins are highlighted. Depending on the risk classification of the raw materials, process owners work to

implement strategies that mitigate the future supply risk. Today, it is common practice to understand your suppliers' capabilities to manufacture including their own supply chain and associated key high-risk items.

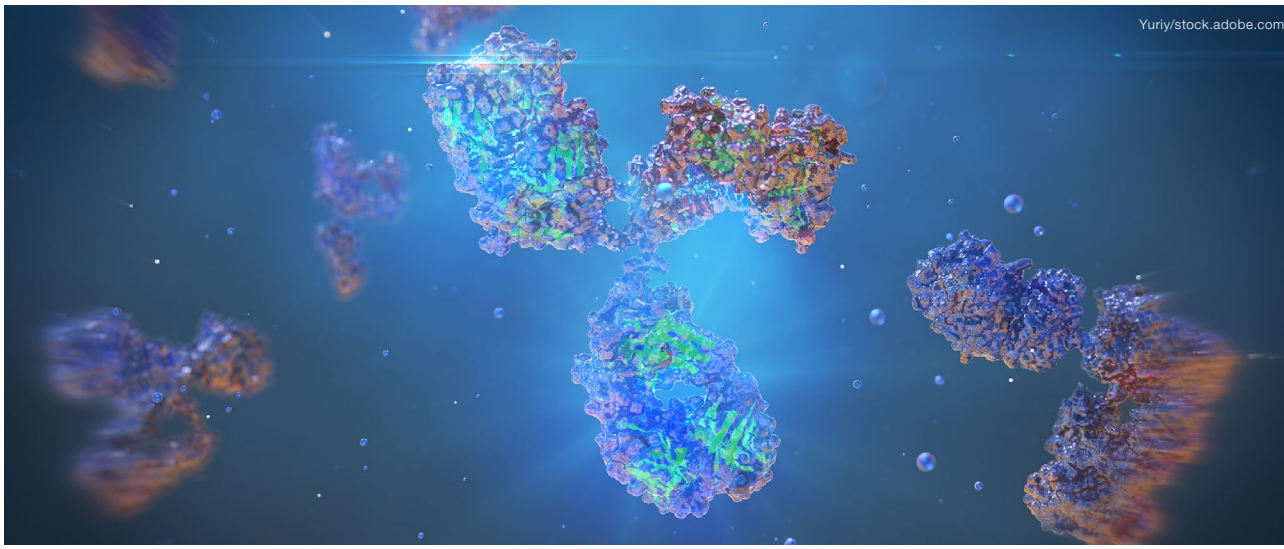
The ultimate goal of a responsible supplier whether that is raw materials, or the final drug product is to ensure the uninterrupted supply.

BIOPHARM: **What about supply and demand challenges?**

JOHANSSON: In supply/partnership discussions involving suppliers and biomanufacturers within the BioPhorum's Supply Partner Phorum around the surety of supply, it is clear that demand is growing along with the surety of supply pressure for the supply of Protein A to the market. As a result of these discussions, Purolite considers the current security of supply of protein A resins in the industry to need strengthening to ensure uninterrupted supply. Having listened to the needs of the market and key customers, Purolite has taken the strategic decision to improve security of supply and has invested heavily into our manufacturing capabilities that will provide faster supply of bioprocessing resins to the industry.

BIOPHARM: **How has this been achieved?**

JOHANSSON: This investment gives Purolite the capability to meet 100% of the global demand for bioprocess chromatography resins. The increased output will come from a second dedicated agarose manufacturing facility located in Pennsylvania, USA coming on-line at the end of 2021. For biopharmaceutical producers located in the USA, this new facility ensures they are not dependent upon imports from outside the country. This new high value state-of-the-art manufacturing facility, alongside the current facility located in the United Kingdom, makes Purolite the first bioprocess resin supplier to have manufacturing sites on two continents with the ability to flex as required output across two independent locations. This approach sees a new advent of Security of Supply, version 2.0.

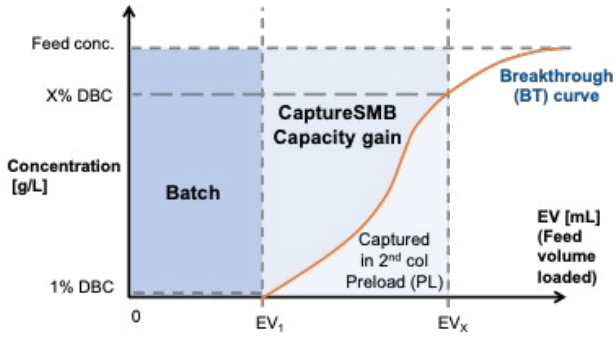


CaptureSMB for the Continuous Purification of mAbs with High Productivity and Load

Achieving high load and high throughput with CaptureSMB column format.

CaptureSMB IS A TWIN COLUMN PERIODIC COUNTERCURRENT (PCC) PROCESS FOR CONTINUOUS AFFINITY PURIFICATION OF THERAPEUTIC PROTEINS SUCH AS MONOCLONAL ANTIBODIES (MABS). CaptureSMB HAS BEEN IMPLEMENTED AT LAB AND GMP PRODUCTION SCALE WITH SIGNIFICANT BENEFITS OVER SINGLE-COLUMN BATCH CHROMATOGRAPHY. THE MAIN IMPROVEMENTS INCLUDE: AUTOMATED AND CONTINUOUS CAPTURE OF MABS, TYPICALLY 40-60% REDUCTION IN AFFINITY RESIN COSTS AND IN BUFFER REQUIREMENTS, A 2- TO 3-FOLD INCREASE IN PRODUCTIVITY COMPARED TO BATCH CHROMATOGRAPHY AND REDUCTION IN EQUIPMENT FOOTPRINT. THIS ARTICLE DESCRIBES THE DEVELOPMENT OF A LAB-SCALE MAB CAPTURE PURIFICATION STEP EMPLOYING THE CaptureSMB PROCESS USING COLUMNS OF ONLY 5 CM BED HEIGHT. DUE TO THE CaptureSMB PROCESS PRINCIPLE, HIGH LOAD AND HIGH THROUGHPUT COULD BE ACHIEVED SIMULTANEOUSLY WITH THIS COLUMN FORMAT, EXCEEDING 70 G/L/H.

FIGURE 1: Schematic illustration of the overloading principle of CaptureSMB. In twin column CaptureSMB the 1st column is loaded beyond 1% DBC (the maximum load that is achievable without yield loss with single column capture) and the flow through ("Preload" – PL) is captured by the 2nd column. The area above the breakthrough curve (at the Preload position) corresponds to the CaptureSMB capacity utilization gain vs. batch chromatography



Introduction

Increasing productivity (throughput) and column load at the affinity capture step is the main driver for implementing continuous processing. Both aims can be achieved using CaptureSMB. In batch chromatography, an affinity column is loaded up to the point of breakthrough of the product and then loading is stopped to prevent product loss. Usually, a safety margin is applied. As the shape of the breakthrough curve is sigmoidal, a significant portion of the expensive affinity resin is not utilized. With CaptureSMB, a second identical column is connected to the outlet of the first column, allowing it to continue loading beyond breakthrough of mAb from the first column, thereby almost fully saturating the first column. This overloading principle is explained in FIGURE 1 that schematically shows a breakthrough curve and the degree of loading that can be achieved with single column capture chromatography and with CaptureSMB. Due to the loading beyond breakthrough that can be carried out at low residence time, resin utilization is maximized, and productivity greatly increases compared to a single column batch method.

The process principle of CaptureSMB is shown in FIGURE 2. After interconnected loading and washing to transfer unbound material from the first into the second column, the fully loaded first column is disconnected from the second column, washed, eluted, cleaned and re-equilibrated for further use (FIGURE 2). In parallel, the second column is loaded with feed. The first cleaned column is now placed behind the second column to again allow the capture of the breakthrough product. This cyclic process is then repeated multiple times until the entire feed material has been processed. CaptureSMB uses a dual loading flow-rate strategy with different feed flow rates during interconnected and parallel loading phases to further enhance process performance and to match loading and recovery and regeneration protocol durations. CaptureSMB can be combined with UV-based dynamic process control (AutomAb), which automatically keeps the continuous capture process at an

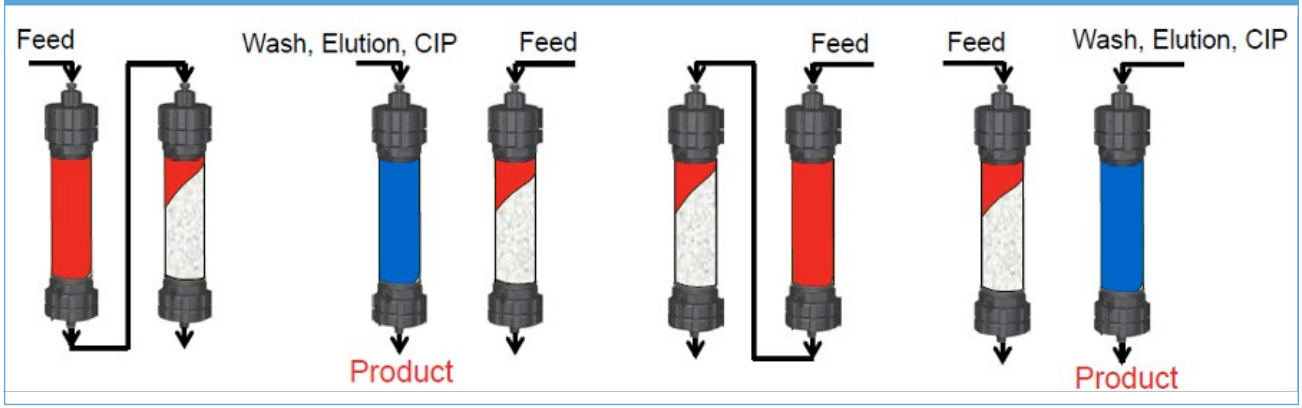
FIGURE 2: Schematic illustration of a single cycle of the twin-column CaptureSMB process. Step 1 & 3: interconnected phases, Step 2 & 4: batch phase.

Step 1 – In the first interconnected phase, Column 1 is placed upstream of Column 2. Loading of feed proceeds until Column 1 reaches maximum binding capacity while all breakthrough of antibody is captured on Column 2. A wash step is then used to flush any remaining unbound antibody from Column 1 to Column 2.

Step 2 – The process switches to a batch (parallel) phase, where Column 1 is washed, eluted, and cleaned. In parallel, Column 2 is directly loaded with feed until Column 1 is regenerated and ready for Step 3.

Step 3 – In the second interconnected phase, Column 2 is placed upstream of Column 1. Column 2 is loaded to maximum binding capacity while Column 1 captures the breakthrough of antibody in the downstream position. Once loaded, Column 2 is washed in series and Column 1 captures the unbound antibody.

Step 4 – In a second batch (parallel) phase, Column 2 is now washed, eluted, and cleaned. In parallel, Column 1 is directly loaded with feed until Column 2 is regenerated and ready for the next cycle.



optimum, preventing product yield losses due to decline of affinity resin capacity or variations in feed titer.

CaptureSMB operates on all Contichrom CUBE benchtop systems (FIGURE 6). ChromIQ, the operating software of the Contichrom systems, provides a wizard tool for designing and operating the CaptureSMB process. Any CaptureSMB process developed with the Contichrom CUBE system is directly scalable to the Contichrom TWIN GMP system (FIGURE 7). Several publications about the manufacturing-related aspect of CaptureSMB are available, including viral clearance (1, 2) and process characterization and validation (3).

TABLE 1: Batch method & materials

Buffer A – Equilibration / Wash	20 mM Na phos, 150 mM NaCl, pH 7
Buffer B – High Salt Wash	20 mM Na phos, 1 M NaCl, pH 7.0
Buffer C – Elution/Desorption	0.1 M Sodium Citrate, pH 3.0
Buffer D - CIP	0.1 M Sodium Hydroxide
Feed	clarified harvest 4.8 g/L mAb
Column dimensions (x2)	Volume: 1 mL (Diameter: 0.5 cm, Bed height: 5 cm)
Resin	Purolite Praesto Jetted A50

TABLE 2: Batch method parameters

Step	Column Volumes (CV)	Flow rate (cm/h)	Buffer
Equilibration	5	600	Buffer A
Load	5.6	300	Feed
Wash 1	3	600	Buffer A
Wash 2	5	600	Buffer B
Wash 3	5	600	Buffer A
Elute	4	600	Buffer C
CIP	5 (2 for 1 min CIP)	100 (600 for 1 min CIP)	Buffer D
Re-equilibration 1	3	600	Buffer B
Re-equilibration 2	5	600	Buffer A

CaptureSMB for mAb purification

Three steps, that are described in the following, are required for CaptureSMB process design and to execute a successful CaptureSMB run.

Step 1: Defining batch run parameters

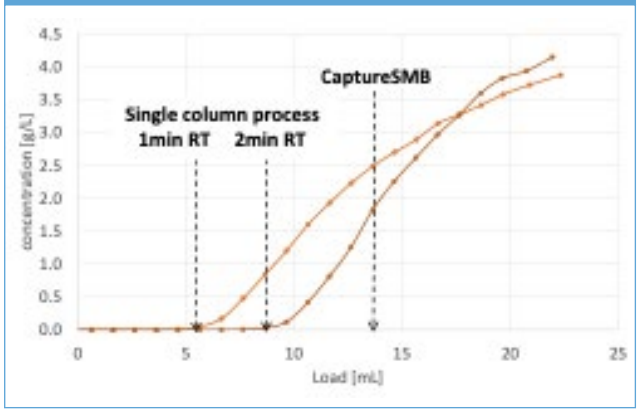
CaptureSMB uses many of the same process parameters as batch purification and most of the protocol optimization should be carried out in batch mode to save feed material. Improvements made to the washing, equilibration, elution, or cleaning-in-place (CIP) steps in batch mode translate directly to the CaptureSMB method. A generic purification protocol for mAbs as shown in TABLES 1 & 2 was carried out using the Contichrom CUBE system. The feed material was clarified mAb harvest with a titer of 4.8 g/L. Prepacked columns of 0.5 cm inner diameter and 5 cm bed height, packed with Purolite Praesto Jetted A50, were used.

Step 2: Generating a breakthrough curve

Before initiating a CaptureSMB method, a single experimental breakthrough curve is required to determine CaptureSMB operating parameters such as the load volume of feed material during the interconnected and batch process phases. The characteristics of the breakthrough curve depend upon a combination of factors including the Protein A resin, feed composition and loading flow rate.

Single column breakthrough curves were generated at flow rates of 300 cm/h (residence time RT = 1 min) and 150 cm/h

FIGURE 3: Single column breakthrough curves at 1 and 2 min residence times. The vertical arrows indicate the degree of loading of the single column processes and CaptureSMB as reported in Table 3.



(residence time RT = 2 min). In each case, the flow-through of the column was fractionated and the mAb concentrations in the fractions and the feed were determined by offline analytics (see FIGURE 3). For CaptureSMB design, only the 1 min RT curve was used. The 2 min RT curve was used to determine the optimal load for a single column reference run (see TABLE 3).

Step 3: Designing and running CaptureSMB

The CaptureSMB methods to be run on the Contichrom CUBE system can be easily generated using the CaptureSMB wizard, a software tool embedded in the ChromIQ operating software. For process design, the breakthrough curve data from a single column, column dimensions, feed concentration and the recovery and regeneration protocol are required. From these inputs, the CaptureSMB wizard

FIGURE 4: UV 280 nm signals from three cycles of the CaptureSMB run. The interconnected loading phases are indicated by the shaded areas.

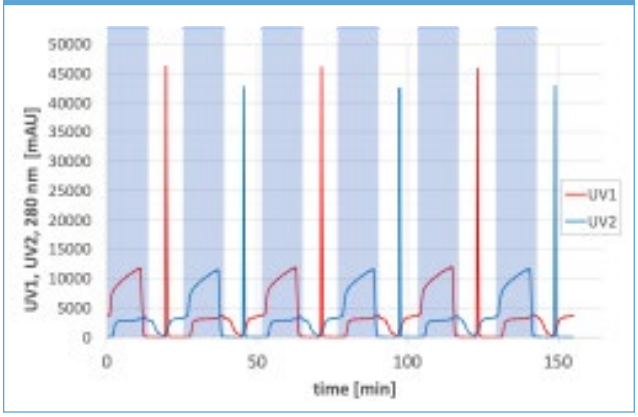
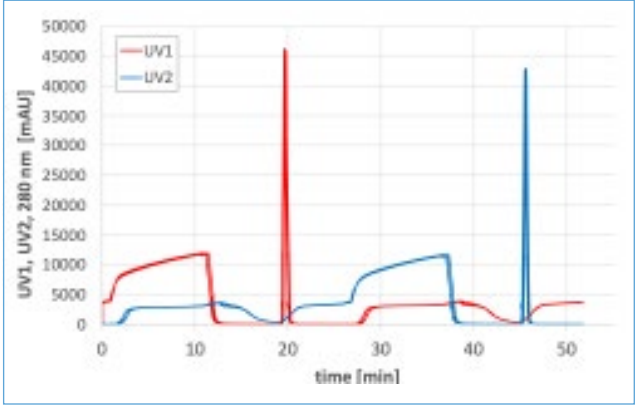


FIGURE 5: Overlay of the UV signals of three consecutive CaptureSMB cycles.



determines the CaptureSMB operating parameters, including the feed flow rates and the loading time. Thus, the load volume of CaptureSMB exceeds the load volume of single column chromatography (see FIGURE 3). Next, the UV-based dynamic process control AutomAb is enabled and the number of CaptureSMB cycles is entered by the user depending on the amount of starting material to be processed. Using AutomAb, fluctuations in feed quality, target protein concentration (as for example in perfusion cell culture) or gradual decrease in the capacity of the affinity matrix are automatically compensated for and process performance remains stable over time. AutomAb monitors and controls column saturation levels by automatic adjustments to the interconnected loading time based on UV detection. Finally, in the CaptureSMB wizard, the methods are generated, and the process is run on the Contichrom system. The process design of CaptureSMB was carried out using the 1 min RT breakthrough curve data.

CaptureSMB Results

The CaptureSMB process was run over six cycles. FIGURE 4 and FIGURE 5 show the 280 nm signal for Column 1 and Column 2 recorded during three cycles of the CaptureSMB run. FIGURE 4 gives an overall visualization of the CaptureSMB run whereas FIGURE 5 is an overlay for cycle comparison. Having a UV detector placed at the outlet of each column, the profiles for both columns result in an alternating pattern of loading and elution phases. The interconnected loading phases clearly show breakthrough of mAb from the upstream columns with only minimal rise of the UV signals of the downstream columns above the impurity baselines (i.e., minimal product loss from the outlet of the downstream column of the two interconnected columns). An overlay of the UV signals of three CaptureSMB cycles shows that there are negligible deviations in the UV signals from cycle to cycle. This indicates that the process has

TABLE 3: Data summary

	CaptureSMB	Batch (2 min)	Batch (1 min)
Feed volume processed/cycle (mL)	27.8	8.8	5.6
Feed concentration (g/L)	4.8	4.8	4.8
Product pool conc. (g/L)	15.8	10.1	6.4
Yield (%)	97.6%	95.3%	95.5%
Productivity/resin volume (g/L/h)	73.2	47.3	63.9
Buffer consumption (L/g)	0.43	0.77	1.25
Load (g/L)	68.0	42.2	26.9
Capacity utilization (%)	93%	58%	37%
Cycle time (min)	22.6	41.1	24.1

TABLE 4: Scale-up scenario 1 (fixed processing time)

	CaptureSMB	Batch (2 min)
Harvest volume (L)	2000	2000
Feed concentration (g/L)	4.8	4.8
Processing time (hrs)	24	24
Column i.D. (cm)	25	30
Bed height (cm)	5.6	12
Total resin vol. (L)	5.5	8.5
Buffer consumption (L)	4130	7390

reached a cyclic steady state (i.e., product concentration and quality is expected to be the same from cycle-to-cycle).

TABLE 3 shows a summary of the CaptureSMB steady state process performance data compared to single column reference processes with 2 min and 1 min residence times in the loading step and the same recovery and regeneration protocol. The data clearly show a trade-off between Load and Productivity for the single-column process.

The results demonstrate the superiority of CaptureSMB over the batch chromatography processes, including up to 50% improvement in productivity (compared to 2 min batch), or a 2.5-fold improvement in resin capacity utilization, a 2.9-fold reduction in buffer consumption and a 2.5-fold higher product concentration (compared to 1 min batch). Additionally, the results show that

TABLE 5: Scale-up scenario 2 (fixed column volume)

	CaptureSMB	Batch (2 min)
Harvest volume (L)	2000	2000
Feed concentration (g/L)	4.8	4.8
Processing time (hrs)	16	24
Column i.D. (cm)	30	30
Bed height (cm)	6	12
Total resin vol. (L)	8.5	8.5
Buffer consumption	4130	7390

CaptureSMB enables the use of short bed heights (5 cm), allowing to reach high productivity and high resin utilization at the same time. For batch chromatography, despite a high productivity value at small residence times (1 min), the resin utilization is extremely low (<40%), which makes short bed height columns very unattractive for capture applications in single column mode.

Scale-up scenarios

Scenario 1: Fixed processing time

The experimental data from small-scale runs was used to simulate large-scale manufacturing scenarios: Under the constraint of a processing time of 24 hours, in this case study, with a productivity of 73 g/L/h and a load of 68 g/L, CaptureSMB can process a harvest volume of 2000L (at 4.8 g/L titer) with just 5.5 Liters of Protein A column volume (2x 25 cm i.D) and a buffer demand of approximately 4130 Liters.

Conventional batch chromatography would require 8.5 Liters of Protein A column volume (30 cm i.D. column) and a buffer demand of almost 7400 Liters (see TABLE 4). Thus, in this scenario, CaptureSMB can save 3 Liters (35%) of Protein A resin per 2000L harvest, which is especially interesting in clinical trial manufacturing where the columns are used only for one harvest. Moreover, the smaller columns size in CaptureSMB makes it more attractive to use pre-packed columns.

Scenario 2: Fixed column volume

Under the constraint of a fixed resin volume of 8.5 Liters, with a productivity of 73 g/L/h and a load of 68 g/L, CaptureSMB can process a harvest volume of 2000L (at 4.8 g/L titer) with (2x 30 cm i.D and 6 cm bed height) in 16 hrs while conventional batch chromatography would require 24 hrs processing time (30 cm i.D. column and 12 cm bed height), as shown in TABLE 5. The buffer demand would be the same as described in scenario 1. Thus, in this scenario, CaptureSMB can save 50% of the processing time, reducing it from a 24 hours operation to a 16 hours operation, freeing up time for the next project. The scenario would also allow the change from a three-shift operation to a two-shift operation, while maintaining the same output per day.

Summary

Using the Contichrom CUBE system (FIGURE 6) with the CaptureSMB wizard, a batch capture method could be quickly converted into a continuous process. CaptureSMB greatly improves the

FIGURE 6: Contichrom CUBE benchtop chromatography system.

FIGURE 7: Contichrom TWIN process scale chromatography system.

economics of antibody purification. Due to the overloading principle, even with short columns of 5 cm bed height, a high column load exceeding 60 g/L could be achieved. The results showed:

- Productivity of 73 g/L/h (up to 50% improved)
- Resin capacity utilization of 93% (up from 58%)

"Due to the overloading principle, even with short columns of 5 cm bed height a high column load exceeding 60 g/L could be achieved."

- Buffer consumption of 0.43 L/g (down from 0.77 L/g)
- Product concentration of 15.8 g/L (up from 10.1 g/L)
- Simultaneous achievement of high productivity and load (capacity utilization)

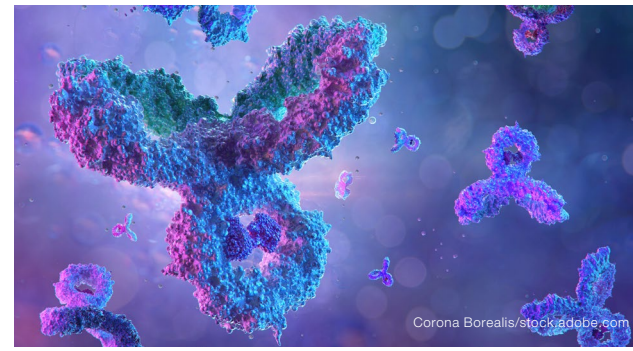
CaptureSMB is the least complex and most robust multi-column configuration for affinity capture. The simple twin column process configuration of CaptureSMB leads to reduced regulatory effort and operational risk compared to other multi-column configurations that use more columns. CaptureSMB is a scalable process. Several users in the biopharmaceutical industry are operating scale-up equipment, the Contichrom TWIN series, in a GMP environment (FIGURE 7).

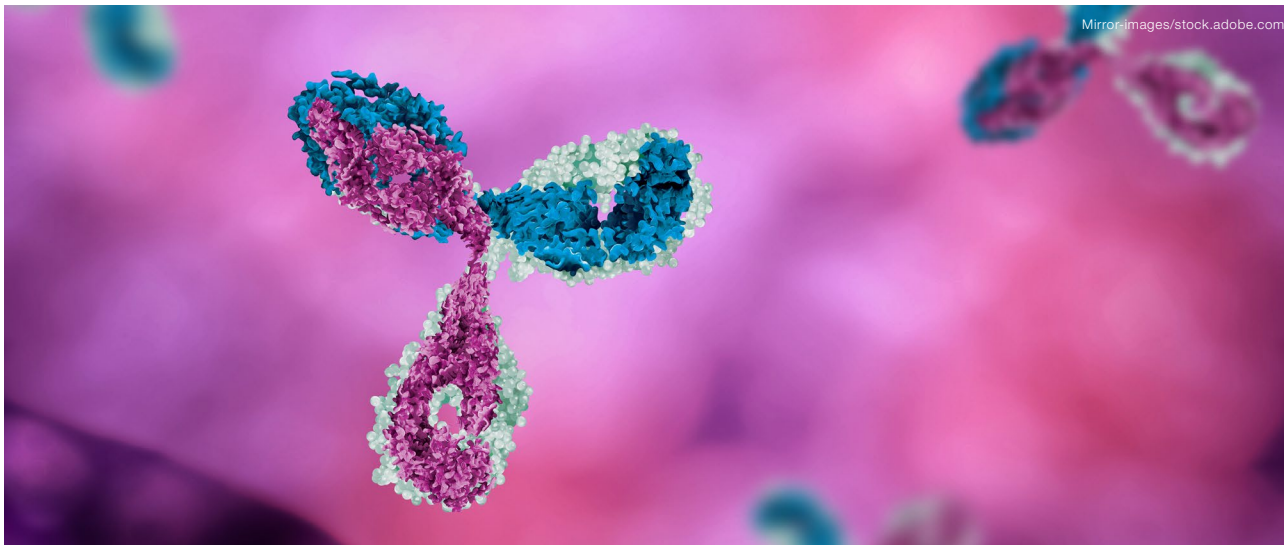
References

1. Angelo, J., Chollangi, S., Müller-Späth, T., Jusyte, S., Xu, X., Ghose, S. and Li, Z. (2019) Virus clearance validation across continuous capture chromatography. *Biotechnology and Bioengineering*, 116, 2275-2284.
2. Angelo, J.M., Potter, K., Müller-Späth, T., Xu, X., Li, Z.J. and Ghose, S. Continued insights into virus clearance validation across continuous capture

chromatography. *Biotechnology and Bioengineering*, n/a.

3. Baur, D., Angelo, J., Chollangi, S., Müller-Späth, T., Xu, X., Ghose, S., Li, Z.J. and Morbidelli, M. (2019) Model-assisted process characterization and validation for a continuous two-column protein A capture process. *Biotechnology and Bioengineering*, 116, 87-98.





Manufactured by Jetting

Hans J. Johansson, Patrick Gilbert, Mark Hicks, and Serguei Kosvintsev

The Future in Protein A Affinity Matrix Design

Protein 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, the 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. Interestingly, the particle-size distribution has been shown to have a significant effect on resistance to fouling (2, 3). Here, we present performance data on a new protein A resin, Praesto® Jetted A50, manufactured by jetting technology.

What Matters?

It is not uncommon to overlook some important aspects of protein A affinity chromatography. For practical reasons, process development work is typically performed 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 the 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 herein. 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). 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). The 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. This 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 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 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 available.

Functional lifetime. At the 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 Cytiva, 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).

MANUFACTURED BY JETTING

FIGURE 1: A jetting process produces beads continuously and uniformly. Using one membrane can produce resin with average particle size of 25–100 µm.

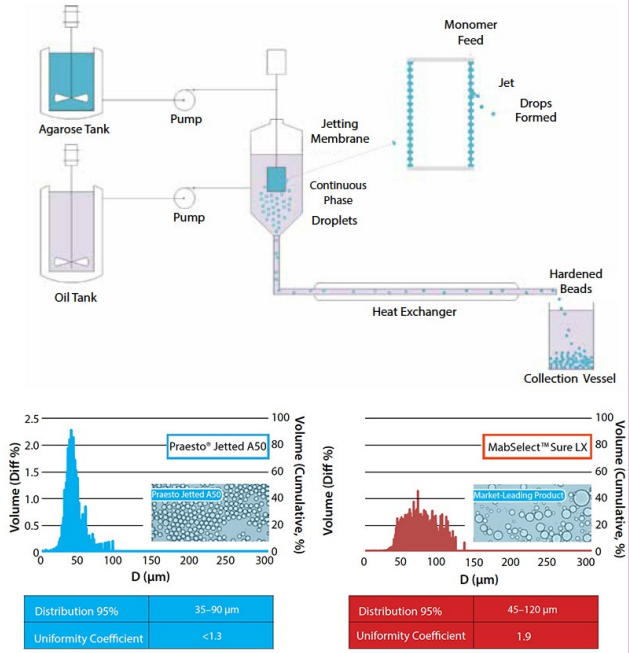
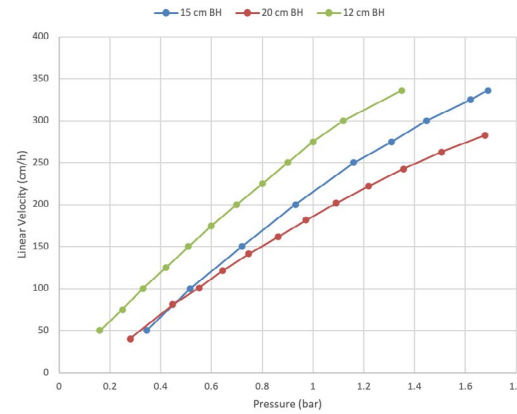


FIGURE 2: Praesto® Jetted A50 was packed into an axial compression column (AxiChrom 200, Cytiva) at two different bed heights, 10 and 20 cm, respectively. Plate number, assymetry and pressure vs flow were determined.



Bed height (cm)	Compression Factor	Maximum recommended flow (cm/h)
12	1.22	275
15	1.2	300
20	1.2	250

Maximum recommended flow at a pressure of 1.5 bar.

Benefits of a narrow particle-size distribution

The absence of both fine and course particles provides a number of benefits or potential advantages over the use of other technologies:

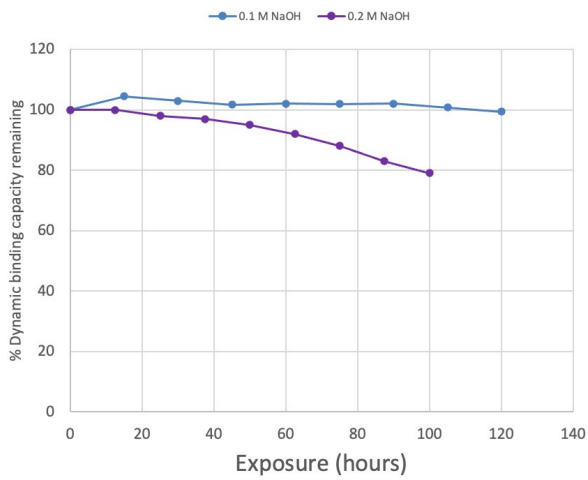
- Improved flow properties when compared to a batch emulsified product of equal d50v (µm)
- Decreased eddy diffusion (improved resolution and decreased buffer consumption)
- Reproducible column packing
- Decreased resin fouling
- Potential use of larger mesh-size column filters (less risk of clogging).

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

MANUFACTURED BY JETTING

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



Polyclonal hIgG dynamic binding capacity (10% breakthrough) was measured followed by exposure to 0.1 M and 0.2 M NaOH.

The dynamic binding capacity was retested at pre-determined time intervals.

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.

Characteristics of the New Praesto Jetted A50 Protein A Resin

Praesto Jetted 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 1 AND 4 show narrower particle-size distribution and dynamic binding capacities of the Praesto Jetted A50 resin compared with three different resins.

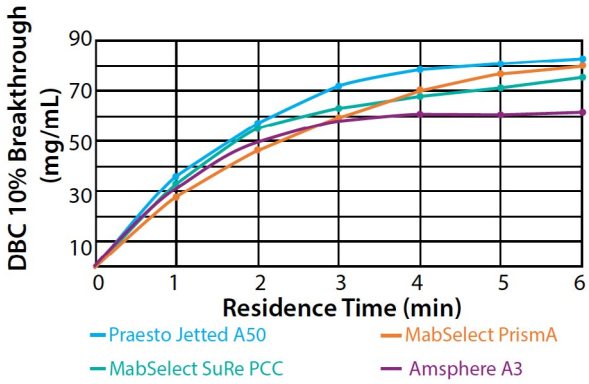
Process Modeling

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 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

MANUFACTURED BY JETTING

FIGURE 4: Comparing four different protein A resins packed to 10-cm bed height with respect to dynamic binding capacity (DBC, 10% breakthrough).



- Feed titer of 5.0 g/L
- 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.

Discussion

Our calculations provide an estimate of the

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

	Praesto Jetted A50	MabSelect PrismA	Praesto AP	MabSelect SuRe LX
Residence time (minutes)	2	2	6	6
DBC10% (g/L)	57	46	50	54
Loading capacity 80% DBC10% (g/L)	46	37	40	43
No. of cycles	12	14	4	4
Column ID (cm)	45	45	60	60
Bed height (cm)	10	10	20	20
Column Volume (L)	16	16	57	57
Buffer volume (L)	3244	3785	3845	3845
Process time (h)	11.0	12.1	10.3	10.3
Resin cost ($)/L	10720	17000	10720	16313
Resin cost ($) - Total	170494	270373	606202	922478
Productivity (g/L/h)	57.2	51.9	17.1	17.1
USD/g mAb	17.05	27.04	60.62	92.25

effect of resin price and performance (TABLE 1). Factors related to risk, time, and cost of implementation, and fulfillment of regulatory requirement also must be considered. It is clear that use of new, high-capacity resins can lower cost of goods (CoGs) significantly, however. Even for conventional batch chromatography, applied savings of >75 % can be achieved.

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-µm 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.

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.
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.

Hans J Johansson
Global Applications Director
Purolite Life Sciences

Patrick Gilbert
Research and Development Manager
Purolite Life Sciences

Mark Hicks
Lead Application Scientist
Purolite Life Sciences

Serguei Kosvintsev
R&D
Purolite Life Sciences

On Demand
Advanced mAb purification workshop

Purolite®

Duncan Low
(Claymore Biopharm LLC)
Managing change to approved commercial processes

Duncan Low is a consultant providing technical services in raw materials management and risk management to both manufacturers and suppliers in the biopharmaceutical industry. His broad experience includes his role as a Scientific Executive Director at Amgen (2003 – 2017), where he led the Raw Materials Global Network and Materials Science teams. Prior to joining Amgen he held VP positions at Millipore and Pharmacia Biotech.

Ronald Maurer
Bristol Myers Squibb)
Protein A resin evaluation and implementation into a continuous capture process

Ron is a Principal Scientist in Downstream Process Development at Bristol Myers Squibb in Devens, MA, where he has worked since 2014. His interests include continuous processing, Protein A capture resins, and biophysical characterization.

Adam Nelson
(Repligen Corporation)
Scalable Pre-Packed Column Performance 1mL - 100L

Adam Nelson has worked at Repligen for over 10 years in various roles contributing to the development, production, and growth of the OPUS Pre-Packed Chromatography product line. After 7 years in Operations, he transitioned to his current role on the Commercial team as the subject matter expert for OPUS.

Elena Gustchina
(Advanced Bioscience Laboratories, Inc)
Case study: Protein A resin screening for IgG1 and IgG2 Fc fusion protein capture

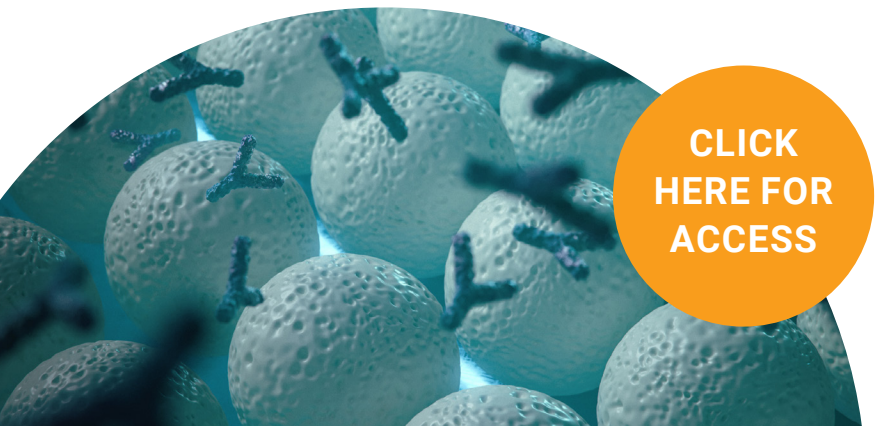
Dr. Gustchina joined Advanced Bioscience Laboratories, Inc., Innovation and Translation group to support downstream development components of the government contracts that I&T services. Previously, Dr. Gustchina worked at Lonza Pharma&Biotech on development, support and improvements of testing products and prior to that at Merck (MSD) and National Institutes of Health.

Carter Mitchell
(Kemp Proteins)
Exploring Modes of Mini & Midi-Scale Automated Purification using Protein A Affinity Capture.

Carter Mitchell is a protein chemist and structural biologist with 20 years of direct experience isolating and characterizing proteins from a variety of recombinant and natural sources. He joined Kemp Proteins in 2020 and is currently working towards the implementation of high-throughput automated methodologies for purification of proteins from various recombinant sources.

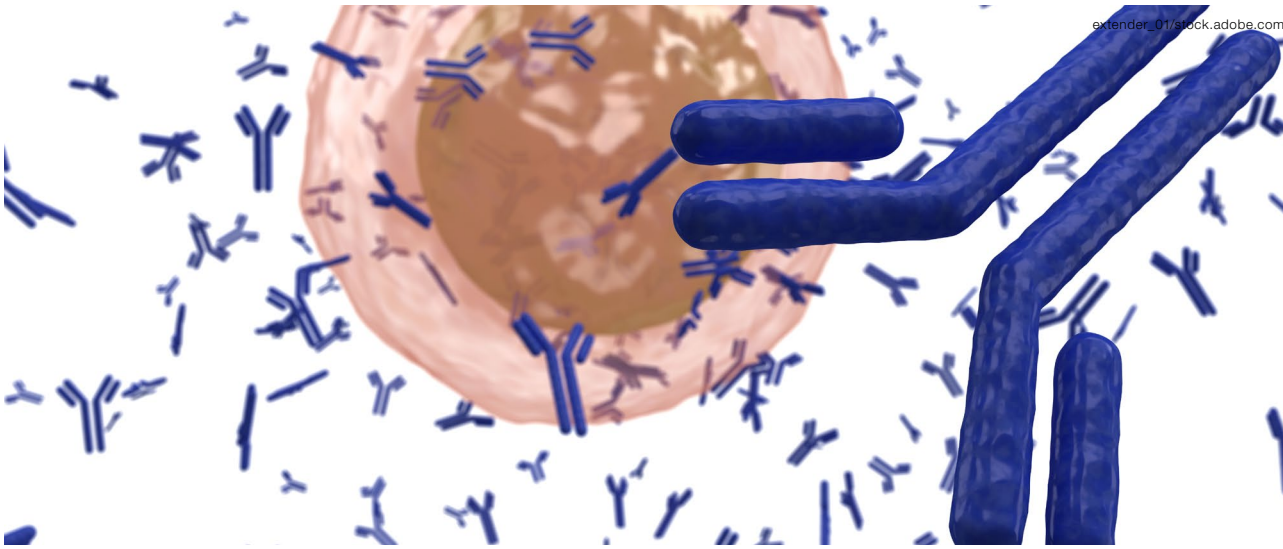
Thomas Müller-Späth
(YMC ChromaCon AG)
Maximizing Performance of Twin Column Capture Chromatography (CaptureSMB) by Resin Selection

Thomas Müller-Späth, Ph.D, holds the position CTO at YMC ChromaCon AG, Switzerland. Thomas is inventor of 10+ patents and has authored and co-authored 30+ scientific articles and book chapters on continuous chromatography for biopharmaceuticals and downstream processing. Thomas gained R&D experience at Bayer Healthcare in Berkeley, CA, and Beiersdorf in Hamburg Germany. He obtained his PhD in 2008 in the area of the multi-column-technology for the purification of proteins at the Swiss Federal Institute of Technology (ETH Zurich) in the group of Prof.



CLICK HERE FOR ACCESS

www.purolite.com



Praesto Jetted A50 HipH, a New Protein A Resin Enabling Mild Elution of Antibodies and Other Fc Containing Proteins

Protein A resin for complete elution of Fc binding proteins at a higher pH than existing Protein A resins.

Introduction

For large-scale purification of antibodies and Fc fusion proteins, Protein A affinity chromatography is the most used capture step in almost every established purification platform. Combining high purity and yield with concentration and rapid transfer into a stable intermediate makes Protein A affinity chromatography the choice. Today, all Protein A resins on the market requires acidic condition (pH 3-4) to achieve a quantitative elution into a reasonable pool volume (2-3 column volumes). This is rarely a problem with most antibodies. However, with the advancement of antibody engineering and drug development, new antibody types such as Fc-fusion proteins and other antibody derived drugs are emerging. This type of molecule is commonly more sensitive to acidic conditions and will in some cases aggregate when typical Protein A elution buffers are used (1). Several publications (2–4) address this by engineering Protein A binding domains for milder elution conditions. However,

"To facilitate rapid development of the new Protein A resin, an established base matrix, already used in commercial manufacturing was chosen."

to the best of our knowledge, there are currently no commercially available resins that fulfill the performance requirements of a modern Protein A resin while allowing elution at a significantly increased pH.

To address this issue, Repligen and Purolite have developed a new type of Protein A resin where complete elution of Fc binding proteins can be achieved at a significantly higher pH compared to existing Protein A resins on the market. In addition to milder elution, a new resin also needs to fulfill other requirements of a process resin described below.

Background: Protein A Affinity Chromatography for Large-Scale Manufacturing

Protein A affinity chromatography has gone through several development phases since the introduction of the first resin intended for large-scale chromatography by Pharmacia (now Cytiva) in 1988. The Protein A itself has been engineered for increased alkaline stability and high capacity and are manufactured without any contact with materials of mammalian origin. The base matrices have been further developed to handle requirements for large-scale batch manufacturing as well as different formats of continuous manufacturing. When looking at the future of Protein A chromatography, one could start from the following checklist of resin properties and their potential impact on downstream processes.

- **Dynamic Binding Capacity at different residence times:** Buffer consumption, productivity, resin volume needed, cost, product pool volume
- **Purification performance:** HCP, DNA, product stability, Protein A leakage
- **Packing and pressure/flow properties:** Scalability, window of operation, packing and un-packing
- **Alkaline stability:** Functional lifetime, cleaning and sanitization options
- **Unspecific binding:** Fouling, CIP protocols (conditions and frequency), functional lifetime, carry-over
- **Elution pH:** pool volume, product stability, HMW content, link to next chromatography step

Praesto Jetted A50 HipH

The base matrix

To facilitate rapid development of the new Protein A resin, an established base matrix, already used in commercial manufacturing was chosen. The base matrix is a 50 µm agarose bead manufactured by Purolite's proprietary jetting technology. Jetting is a continuous emulsification process that generates agarose beads with a narrow particle size distribution, Uc (uniformity coefficient) <1.3, without the need of additional screening (FIGURE 1). A narrow PSD is beneficial from both a manufacturer

PRAESTO JETTED A50 HipH, A NEW PROTEIN A RESIN ENABLING MILD ELUTION OF ANTIBODIES AND OTHER FC CONTAINING PROTEINS

FIGURE 1: Comparison of particle size distribution (PSD) by number. In red is a batch emulsified agarose resin after sieving. In blue is a jetted resin manufactured without sieving to narrow PSD.

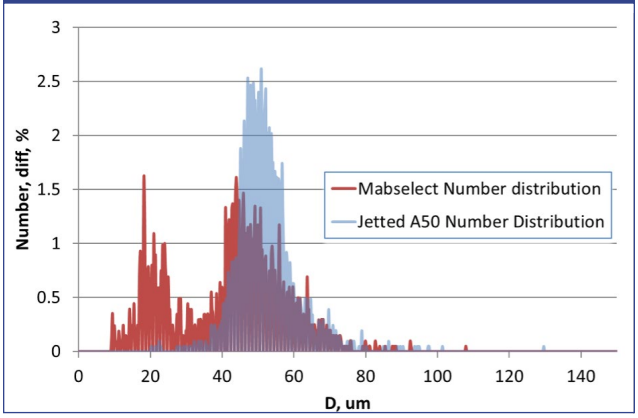
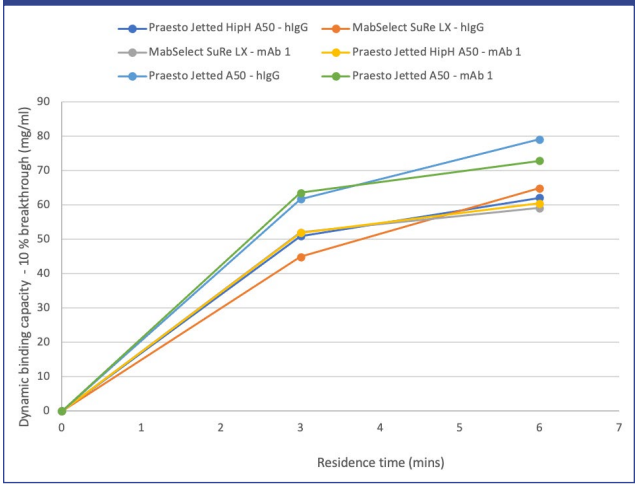


FIGURE 2: Breakthrough capacity for polyclonal immunoglobulin G (hIgG) and an IgG1 monoclonal antibody (mAb 1) of Praesto Jetted A50 HipH, Praesto Jetted A50 and MabSelect SuRe LX.



and user perspective. The continuous jetting process minimizes the consumption of raw material and energy while generating an almost quantitative yield. The traditional batch emulsification method uses solvent, significantly more energy, and requires extensive sieving to achieve a useful particle size distribution. Yield is typically 50-70 % with a Uc of 1.5-2.0 after extensive sieving. A more uniform PSD without fine particles "fines" facilitates reproducible column packing, generates lower back pressure, and decrease the risk of fouling when used in downs stream processing.

The product

The new resin fulfills all of the typically required properties for use in a mAb platform process while allowing quantitative elution at pH 5.0 for all tested antibodies including the VH3 containing subgroup.

Dynamic binding capacity (DBC)

DBC varies between different antibodies and Fc containing proteins. Praesto Jetted A50 HipH has a capacity for mAbs of subclass 1 within the same range as most modern Protein A resins on the market (FIGURE 2).

Host cell protein (HCP) clearance

HCP clearance varies with both target molecule, type of Protein A resin, and buffer conditions. Elution at a higher pH can have a positive impact on HCP clearance as shown in FIGURE 3.

Generation of aggregates during elution

While most antibodies could be eluted at pH 3.5 without generation of high molecular weight aggregates, it is relatively common to see less aggregation if higher pH is used

PRAESTO JETTED A50 HipH, A NEW PROTEIN A RESIN ENABLING MILD ELUTION OF ANTIBODIES AND OTHER FC CONTAINING PROTEINS

FIGURE 3: Log reduction of host cell protein levels associated with mAb X after protein capture using Praesto Jetted A50 HipH with elution buffer pH from 3.5 to 5.0.

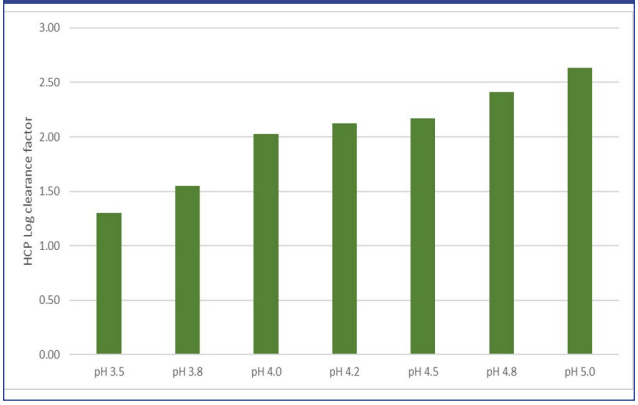
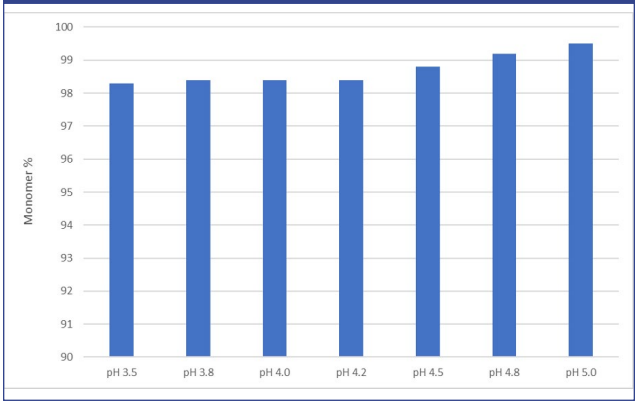
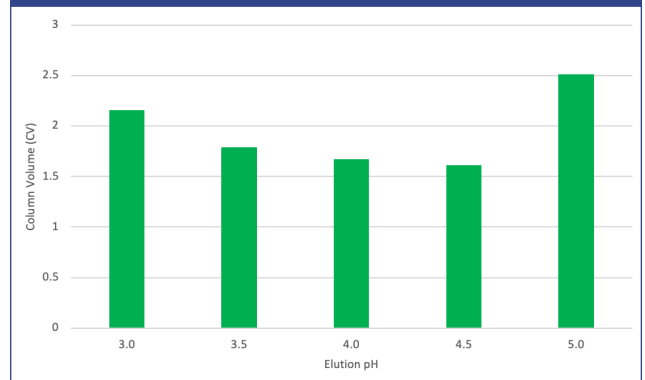


FIGURE 4: Monomer content of mAb 2 after protein capture using Praesto Jetted A50 HipH with elution buffer pH from 3.5 to 5.0.



for elution. One example is shown in FIGURE 4. If a low pH hold for virus inactivation is part of the downstream process, it can be beneficial to elute at milder condition to enable acid virus inactivation in a more controlled way.

FIGURE 5: Elution volumes (column volumes) at pH 3-5.



Yield and pool volumes

Ideally, product pool volume should be compatible with standard facilities. The pool volume depends on several factors such as attributes of the target molecule, sample load, buffer conditions, flow rate, type of base matrix, and Protein A ligand. In most cases, a pool volume of less than three column volumes is acceptable. Praesto Jetted A50 HipH allows elution with conventional elution buffers at pH 5.0 with high yield and acceptable product pool volumes (FIGURE 5).

Alkaline stability

The new ligand is also engineered for alkaline stability and allows the use of 0.1 M sodium hydroxide for over 100 hours with over 90 % of the initial DBC retained. FIGURE 6 shows the impact of sodium hydroxide as a function of exposure time and concentration.

Protein A leakage

Protein A leakage depends on the resin, operating conditions and properties of the

PRAESTO JETTED A50 HipH, A NEW PROTEIN A RESIN ENABLING MILD ELUTION OF ANTIBODIES AND OTHER FC CONTAINING PROTEINS

FIGURE 6: Sodium hydroxide stability of Praesto Jetted A50 HipH at different sodium hydroxide concentrations. DBC was tested initially and after every 20 hour using human polyclonal IgG.

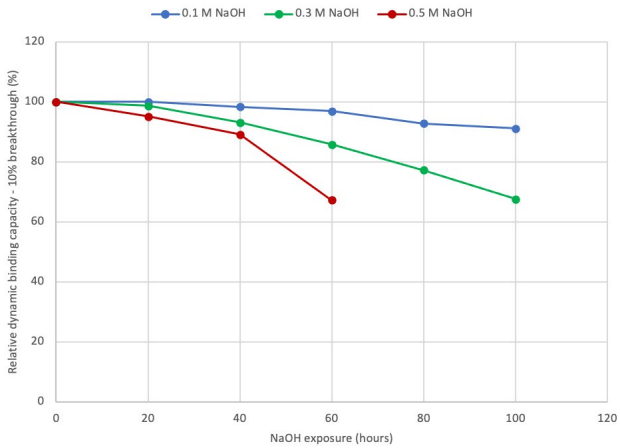


FIGURE 7: Protein A leakage in ppm (ng Protein A/mg IgG).

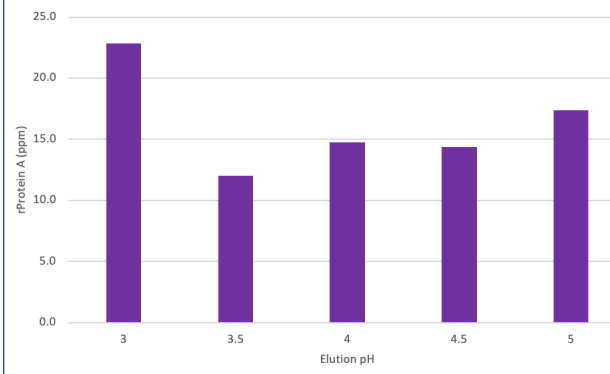
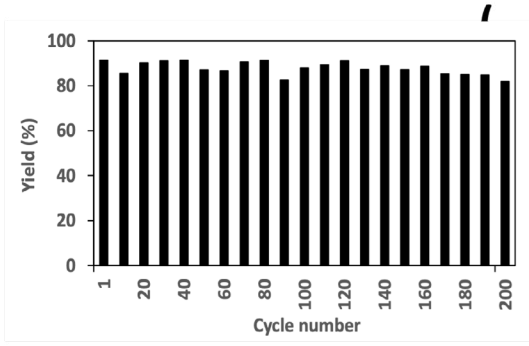
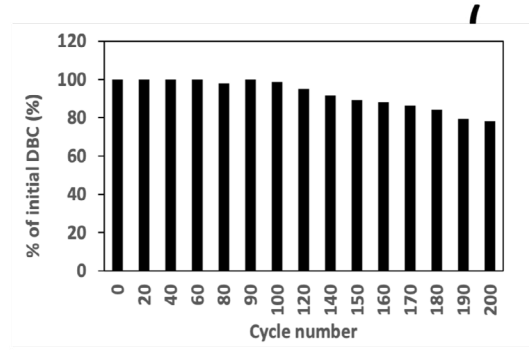
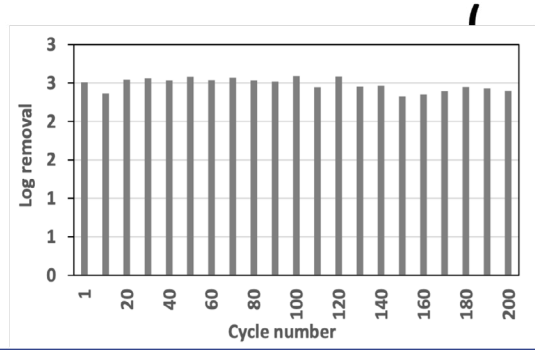
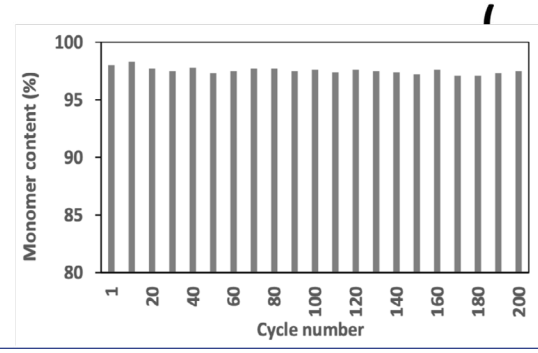


FIGURE 8: Re-use study over 200 cycles.









feedstock (protease content). For a typical mAb CHO culture supernatant, the leakage was 10-25 ppm (FIGURE 7).

Functional lifetime study over 200 cycles

To evaluate the performance over time, a study performed under typical conditions, using a CHO based cell culture supernatant, was conducted. Every purification cycle was followed by a cleaning with 0.1 M NaOH with a minimum contact time of 15 minutes except every 10th cycle when a 0.5 M NaOH solution was used. Product quality remained stable over the entire study. The initial dynamic binding capacity remained constant over the first 100 cycles followed by a gradual decrease to approximately 80% of the initial DBC after 200 cycles. Yield was robust until cycle 160 but dropped approximately 5% over the last 40 cycles.

Summary

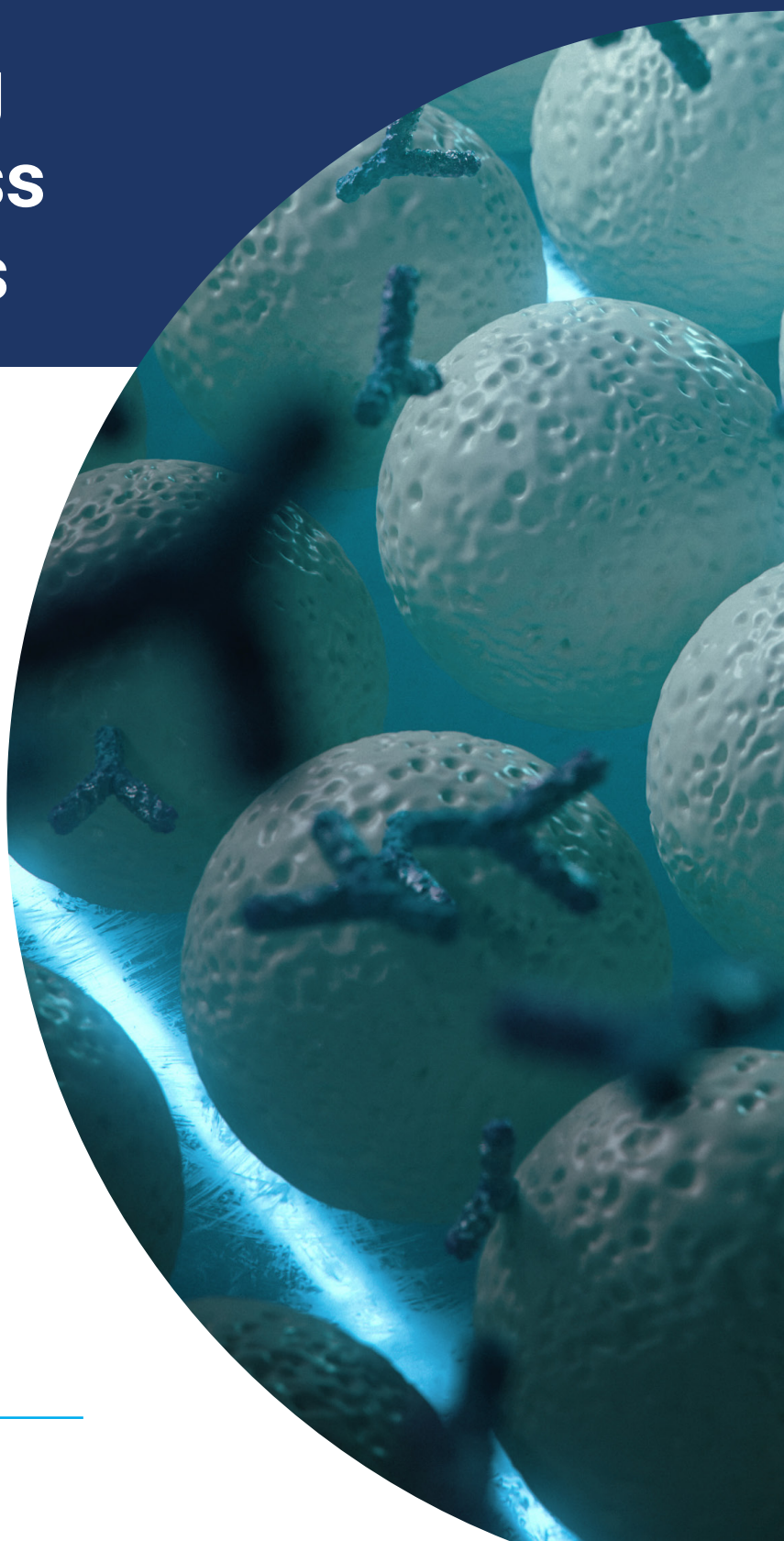
In this paper, we present a novel Protein A resin, Praesto Jetted A50 HipH. Based on a proven base matrix already used in commercial purification of therapeutic mAbs, the new resin meets all the typical requirements of a modern Protein A resin. In addition, the new ligand allows the elution of all types of human IgG as well as other Fc-binding protein to be eluted using mild elution conditions at pH values up to at least pH 5.0.

References

1. Shukla AA, Gupta P, Han X. Protein aggregation kinetics during Protein A chromatography. Case study for an Fc fusion protein. *J Chromatogr A* 2007;1171(1e2):22e8.
2. Pabst TM, Palmgren R, Forss A, et al. Engineering of novel Staphylococcal Protein A ligands to enable milder elution pH and high dynamic binding capacity. *J Chromatogr A* 2014;1362:180e5.
3. Watanabe H, Matsumaru H, Ooishi A, Honda S. Structure-based histidine substitution for optimizing pH sensitive Staphylococcus protein A. *J Chromatogr B Analyt Technol Biomed Life Sci* 2013;929:155e60

Purolite®

The only supplier with dual manufacturing facilities across two continents



Is the global pandemic causing supply challenges?

Ultra-fast, zero-risk Protein A and IEX resin supplier

Two continents, two dedicated agarose manufacturing facilities

www.purolite.com

Despite the global pandemic causing supply challenges across the global to many industries and markets, including the biopharmaceutical sector where the supply of process critical raw materials became particularly effected, delaying clinical trials. The pandemic has taught the biopharmaceutical industry to consider the entire process from start to finish and highlight key process dependent raw materials. This exercise once completed identified in particular process critical singly sourced items. In the case of the production of monoclonal antibodies, chromatography resins in particular Protein A resins are highlighted. Depending upon the risk classification of the raw materials, process owners work to implement strategies that mitigates the future supply risk. Today, it is common practice to understanding your suppliers' capabilities to manufacture including their own supply chain and associated key high-risk items.

The ultimate goal of a responsible supplier whether that is raw materials, or the final drug product is to ensure the uninterrupted supply.

Supply and demand challenges.
In supply/partnership discussions within the BioPhorum's Supply Partner Phorum around the surety of supply, discussions involving suppliers and biomanufacturers it is clear that demand is growing and with-it surety of supply pressure for the supply of Protein A to the market. As a result of these discussions, Purolite considers the current security of supply of protein A resins in the industry to need strengthening to ensure uninterrupted supply. Having listened to the needs of the market and key customers, Purolite has taken the strategic decision to improve security of supply and has invested heavily into our manufacturing capabilities that will provide faster supply of bioprocessing resins to the industry.

How has this been achieved?
This investment gives Purolite the capability to meet 100% of the global demand for bioprocess chromatography resins. The increased output will come from a second dedicated agarose manufacturing facility located in Pennsylvania, USA coming on-line end of 2021. For biopharmaceutical producers located in the USA, this new facility ensures they are not dependent upon imports from outside the country. This new high value state of the art manufacturing facility, alongside the current facility located in the United Kingdom, makes Purolite the first bioprocess resin supplier to have manufacturing sites on two continents with the ability to flex as required output across two independent locations. This approach sees a new advent of Security of Supply, version 2.0.

'The Pandemic has highlighted that for the biopharmaceutical manufacturing industry security of supply and supply chain resilience is best served when major suppliers are able to manufacturer the same products to the same quality standards in multiple regions of the world.'

Bob Brooks, Supply Partner Phorum Lead, BioPhorum