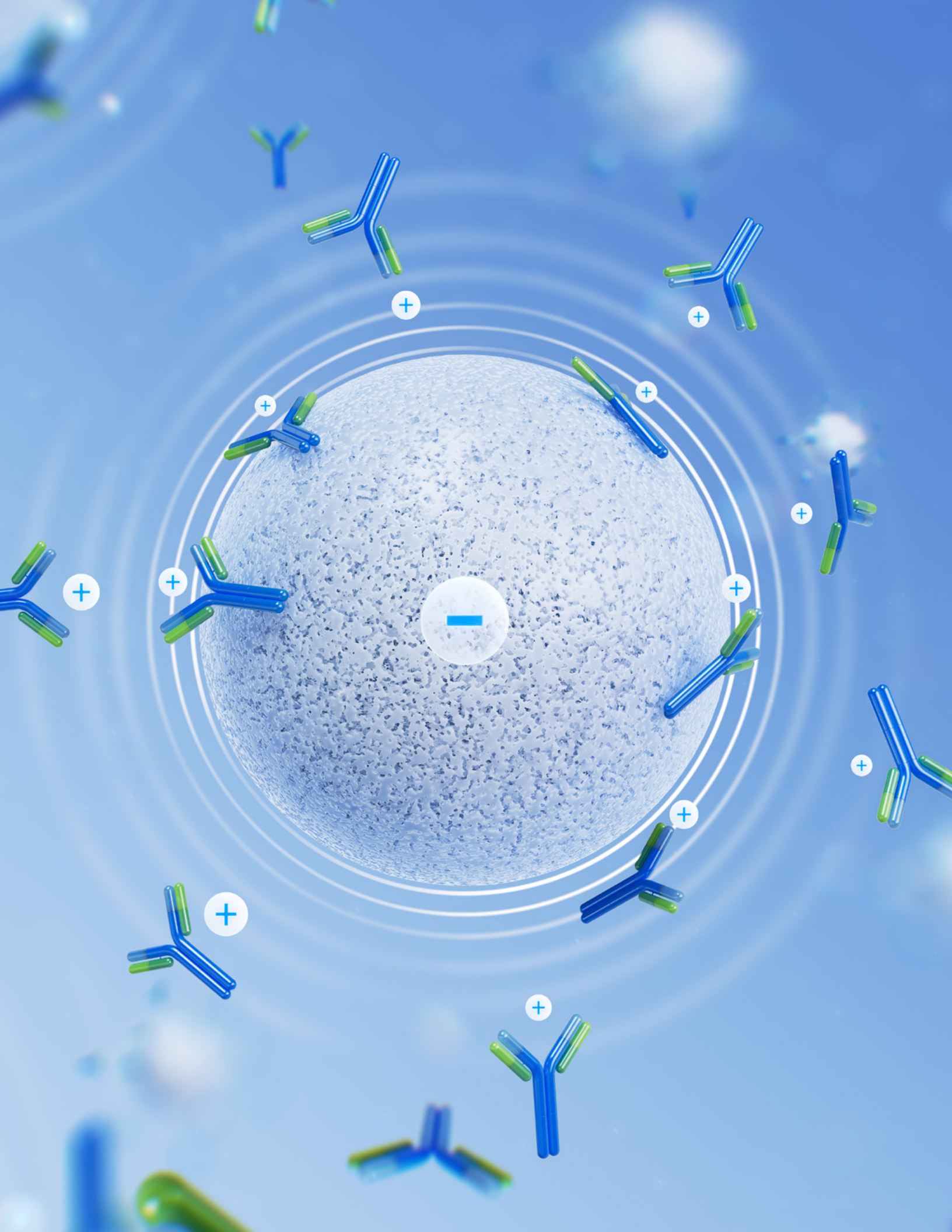
Praesto™ SP & Praesto Q Data File

Praesto SP and Praesto Q are agarose-based ion exchange chromatography resins designed for laboratory to process scale purification of recombinant proteins, monoclonal antibodies, and other biomolecules.

ECOLAB®



Introduction

Praesto SP and Praesto Q are available in 35, 45, 65, and 90 µm particle sizes, covering the use of ion exchange in high productivity capture steps as well as high resolution polishing applications.

Based on highly cross-linked agarose, they offer good flow and pressure properties, excellent chemical and physical stability, high capacity, and are readily scalable.

Purolite Resins — Designed with Patented Jetting Technology

All chromatography resins are manufactured using our patented Jetting technology. Unlike resins synthesized by older emulsification processes, which result in a wide range of particle sizes requiring extensive sieving, our novel Jetting process creates more uniform particle distribution size monodisperse beads for enhanced flow properties without using harsh chemicals, resulting in higher yields with less waste.

Key Benefits

Praesto SP and Praesto Q agarose-based ion exchangers provide the following benefits:

- Excellent dynamic binding capacities and good pressure/flow properties for high productivity operations and easy scale-up
- High resolution/selectivity for demanding separations with high yields
- 35, 45, 65, and 90 µm particle sizes match the goals of capture, removal, and polishing steps
- Excellent chemical and physical stability result in long functional life and reduce operating costs
- Manufactured by patented Jetting technology producing a high degree of uniformity
- Secure, validated supply and regulatory support

Agarose is widely considered the best material available for protein purification resins. It is highly hydrophilic (resulting in less non-specific binding) and alkaline stable. In biomolecule purifications, this translates to high yields and very long functional lifetime.

Purolite resins are manufactured with a porosity and pore structure ideal for high performance protein chromatography. Purolite ion exchange resins are strong anion (Q) and strong cation (SP) exchangers which maintain charge and subsequent capacity over a wide pH range.

FIGURE 1

Shows the ligands of Praesto SP (left) and Praesto Q (right). These are well established in large scale purification.

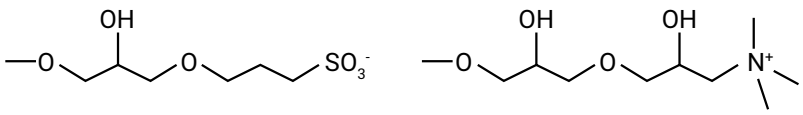


Table 1 shows the general characteristics of Purolite ion exchangers. Praesto SP and Praesto Q are compatible with all ranges of temperature, pH, chemical and physical conditions typically used in biopharmaceutical processes. The physical and chemical stability allows cleaning with sodium hydroxide, resulting in a very long, functional lifetime.

General Characteristics of Praesto SP/Q Media

TABLE 1

	Praesto SP				Praesto Q			
Matrix	Cross-linked agarose							
Functional Group	$CH_2CH_2CH_2SO_3^-$				$CH_2N^+(CH_3)_3$			
Ionic Capacity, mmol/mL resin	0.11–0.18 (H^+)/mL medium	0.11–0.18 (H^+)/mL medium	0.11–0.18 (H^+)/mL medium	0.11–0.18 (H^+)/mL medium	0.11–0.18 (Cl^-)/mL medium	0.09–0.18 (Cl^-)/mL medium	0.09–0.18 (Cl^-)/mL medium	0.09–0.18 (Cl^-)/mL medium
Average Particle Size (d_{50v}), µm	35	45	65	90	35	45	65	90
Flow Velocity+	Up to 120 cm/h (30 x 20 cm)	Up to 200 cm/h (20 x 20 cm)	Up to 350 cm/h (20 x 20 cm)	Up to 550 cm/h (20 x 20 cm)	Up to 120 cm/h (30 x 20 cm)	Up to 200 cm/h (20 x 20 cm)	Up to 350 cm/h (20 x 20 cm)	Up to 550 cm/h (20 x 20 cm)
Binding Capacity, mg/mL Resin at a 6-minute Residence Time	≥ 90 mg IgG*	≥ 80 mg IgG*	≥ 70 mg IgG*	≥ 50 mg IgG*	≥ 80 mg BSA**	≥ 70 mg BSA**	≥ 60 mg BSA**	≥ 50 mg BSA**
Operating pH Stability	Short Term	3–14			2–14			
	Long Term	4–12			2–12			
Working Temperature	4–30 °C				4–30 °C			
Chemical Stability	All commonly used aqueous buffers, 1 M NaOH, 8 M urea, 6 M guanidine, 30% isopropanol, 70% ethanol				All commonly used aqueous buffers, 1 M NaOH, 8 M urea, 6 M guanidine, 30% isopropanol, 70% ethanol			
Avoid	Oxidising agents, cationic detergents				Oxidising agents, anionic detergents			
Storage	20% ethanol, 0.2 M sodium acetate, 4–30 °C				20% ethanol at 4–30 °C			

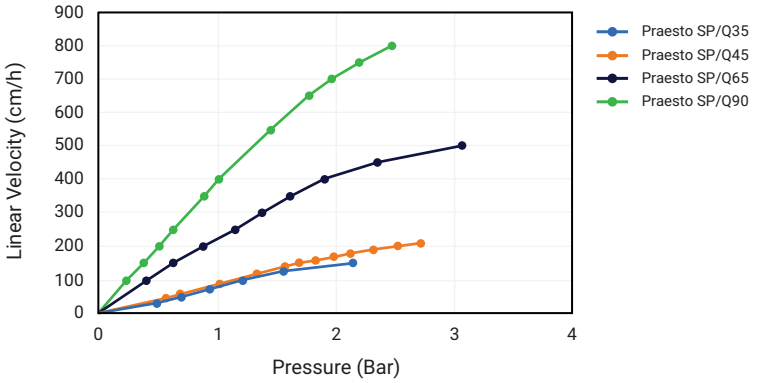
* Dynamic binding capacity — 10% breakthrough at 6 minutes residence time, 50 mM sodium acetate, pH 4.7 in a 1 x 3 cm column format.
** Dynamic binding capacity — 10% breakthrough at 6 minutes residence time, 50 mM Tris-base, pH 8 in a 1 x 3 cm column format.
+ Pressure flow determined using 0.1 M NaCl at 20 °C.

The uniformity of the Purolite chromatography resin range, allows the user flexibility in selecting high surface area, high capacity, and high-resolution products without severely diminishing expected flow properties.

Bead rigidity also means that process and column design are flexible and can be optimized for capacity utilization and economic performance. Bed height, column diameter and flow rate can be varied to accommodate existing equipment with the ability to reduce column size and optimize residence time for maximal capacity and the management of challenging feeds.

FIGURE 2

Packed bed pressure flow curves for Praesto SP/Q35 (30 cm ID), Praesto SP/Q45 (30 cm ID), Praesto SP/Q65 (30 cm ID), and Praesto SP/Q90 (30 cm ID) at a 20 cm bed height in 0.1 M NaCl at 20 °C.



High Dynamic Binding Capacity

Purolite resin base matrices have porosities optimized for medium-sized proteins, resulting in very high and robust binding capacities. With the continuing development of high titer cell lines, high capacity is especially important to facilitate processing of high concentration eluates from modern protein A resins such as Praesto Jetted A50.

FIGURE 3

Praesto SP capacities for polyclonal IgG at 5 g/L using a loading buffer of 50 mM sodium acetate, pH 4.7 in an Omnifit 1 x 3 cm column (CV 3.42 mL).

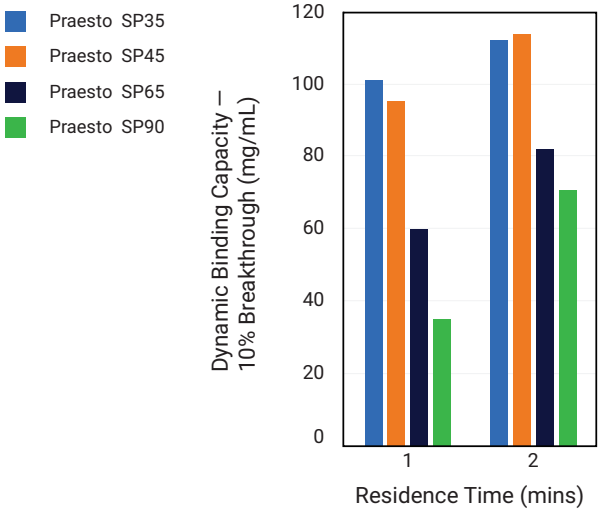
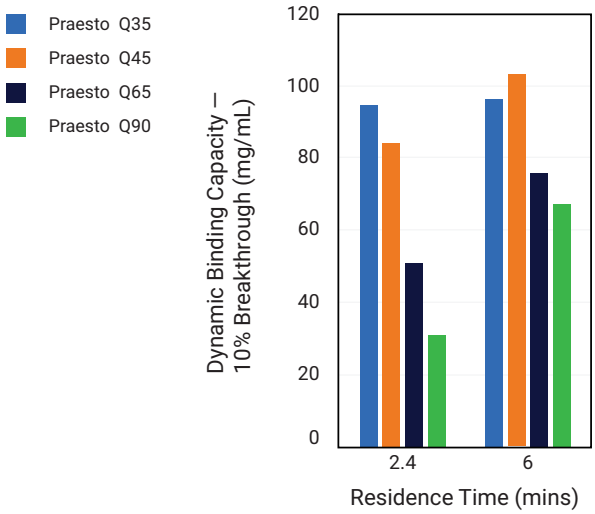


FIGURE 4

Praesto Q capacities for bovine serum albumin (BSA) at 5 g/L using a loading buffer of 50 mM Tris, pH 8.0 in an Omnifit 1 x 3 cm column (CV 3.42 mL).



Selectivity and Resolution

When comparing ion exchange resin, minor differences in selectivity still occur due to differences in base matrix, ligand density and the presence or absence of surface extenders. The Praesto SP and Q are strong cation and anion exchangers with no surface extenders.

With the four different particle sizes available, demands on resolution in various purification steps can be met and difficult separation challenges can be solved.

FIGURE 5

Resolution profiles for Praesto Q45, Praesto Q65, and Praesto Q90 upon application of a protein mixture containing 0.3 mg/mL apo-transferrin, 0.4 mg/mL α-lactalbumins, 0.6 mg/mL soybean trypsin inhibitor.

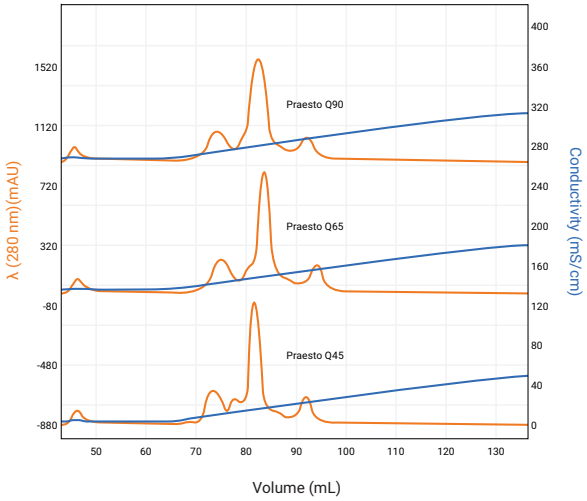
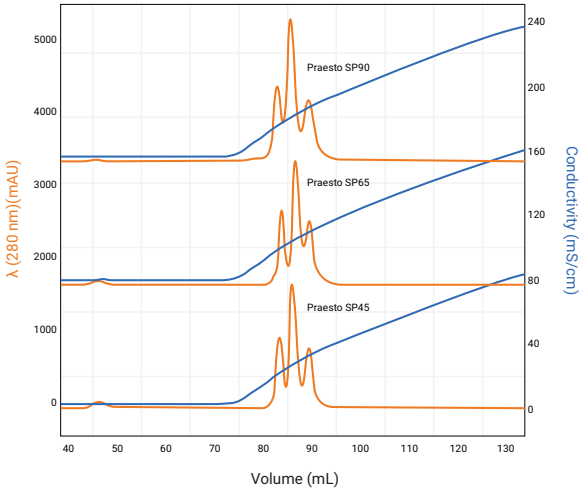


FIGURE 6

Resolution profiles for Praesto SP45, Praesto SP65, and Praesto SP90 upon application of a protein mixture containing 2.0 mg/mL Chymotrypsinogen, 1.6 mg/mL Lysozyme and 3.9 mg/mL Cytochrome C.



Application

Praesto SP45

Working together with Prof. Anurag Rathore at the Department of Chemical Engineering Indian Institute of Technology in Delhi, Praesto SP45 was evaluated for capacity and aggregate removal for three different monoclonal antibodies. DBCs at specified residence times for the 3 mAbs are shown in figure 7, fractionation of mAb A is shown in figure 8 whereby close to 90% mAb recovery is achieved at less than 1% aggregates.

FIGURE 7

mAb capacity evaluation on Praesto SP45, performed by the Department of Chemical Engineering, Indian Institute of Technology, Delhi (Prof. Anurag Rathore).

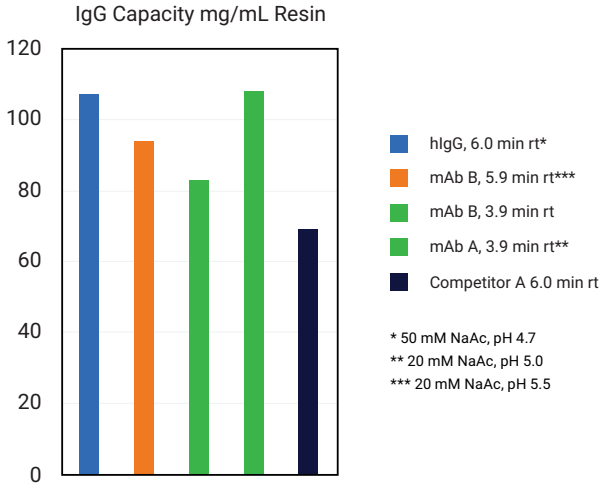
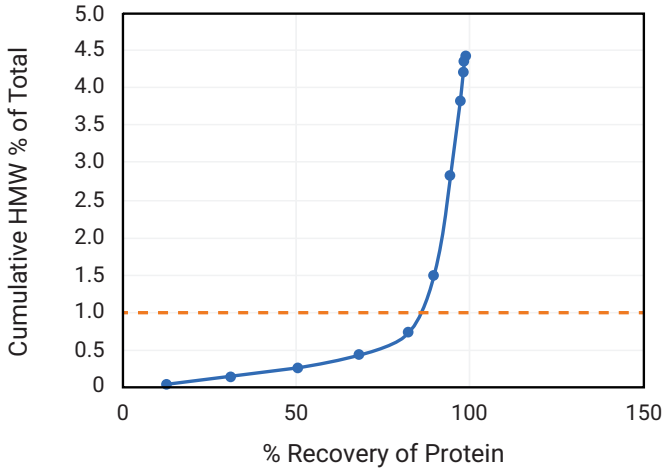


FIGURE 8

Percentage aggregates for mAb A against expected yield when using Praesto SP45.



Praesto Q

An IgG_1 mAb captured using protein A chromatography was applied to the standard Praesto Q anion exchangers in flow through mode using a buffer system of 50 mM Tris, pH 8.5. Recovery, protein A and host cell protein carry over were determined.

TABLE 2 **Percentage mAb recovered, residual Protein A and residual CHO HCP during flow through anion exchange mAb purification.**

Product	Percentage mAb Recovery (%)	Residual Protein A (ng PtA/mg mAb)	Residual CHO HCP (ng HCP/mg/mAb)
Protein A Eluate	98.0	60.5	970
Praesto® Q45	99.4	2.7	2.9
Praesto® Q65	100.1	1.9	1.7
Praesto® Q90	101.2	1.8	0

Excellent recovery, protein A clearance and host cell protein clearance were observed for Praesto Q45, Praesto Q65, and Praesto Q90.

Small Scale Formats for Method Development

Praesto ion exchange resins are available in prepacked formats for method development. Early screening and optimization experiments can be carried out in high throughput mode on 200 µL RoboColumns™. Chromatographic conditions such as pH and conductivity can be screened in parallel, saving both time and sample. Further optimization of operating conditions and verification of process robustness can be completed using 1 and 5 mL available prepacked columns. Packing instructions are available for most readily available empty laboratory columns.

Scale Up

Praesto chromatography resins are easily scalable, but it is important to develop a process at laboratory scale that is within expected process scale operational limits. Scaling a process is typically done by keeping the bed height and linear velocity constant while increasing the column diameter and volumetric flow rate. Because parameters such as DBC are frequently optimized on shorter bed heights than are used at final scale, it is important to keep the residence time constant. This ensures unchanged dynamic binding capacity for the target molecule.

Use and Storage

Praesto ion exchange media are shipped in a 20% ethanol slurry (0.2 M sodium acetate added to Praesto SP). To use, directly decant the ethanol and replace with your required buffer prior to column packing. Wash the packed column with at least 5 bed volumes of buffer before use. Regeneration protocols are application dependent. For soluble proteins, a high conductivity wash with 1 M NaCl is normally sufficient. Unused or used and cleaned Praesto media can be stored at 4–30 °C in 20% ethanol. Add 0.2 M sodium acetate to the Praesto SP storage buffer.

Cleaning in Place (CIP) and Sanitization

Specific cleaning in place (CIP) protocols should be designed and tested for each process depending on the target protein and the type of contaminants likely or known to be present. These may include lipids or denatured proteins.

After processing a harvest, prior to storage or after approximately every 5 cycles of operation, CIP is recommended to remove any aggregated or bound proteins, adhering particulate matter or lipids. If not removed, these can affect resin and column performance. For most common protein purifications, high conductivity regeneration followed by 1 M NaOH at a contact time of one hour is sufficient for both CIP and sanitization.

Innovative Solutions for Bioprocessing

In partnership with Repligen, Purolite™ develops and supplies innovative solutions for the bioprocessing industry, working with many of the top pharmaceutical companies to deliver the next-generation of healthcare. Our resins are used across the globe to deliver lifesaving medicines.

Global Support Network

No matter the location, our expert field application team members are positioned to help you solve your technical and downstream purification challenges, together. We provide the guidance necessary to develop robust, scalable, high productivity purification processes for mAbs and recombinant processes using Praesto™ Jetted chromatography resins. For wherever you are in your biomanufacturing journey, we are here to help.

Purolite Affinity Resin Toolbox

Purolite's diverse toolbox offers Protein A resins, Praesto Jetted A50 and Praesto AP+80, designed for high performance and increased sustainability, as well as novel resins, Praesto Jetted A50 HipH and Praesto 70 CH1, designed to enable cost-effective and reliable purification of bispecifics and Fc fusion proteins.

Purolite Ion Exchange Toolbox

Purolite's ion exchange toolbox consists of Praesto SP and Praesto Q resins in four particle sizes to ensure predictable selectivity across particle sizes, allowing for rapid performance screening.

Ecolab is a global developer, manufacturer, and supplier of Purolite™ Resins including ion exchange, catalyst adsorbent and advanced polymers that make the world cleaner and healthier.

www.puroliteresins.com

We're ready to solve your process challenges.

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