JSR Life Sciences

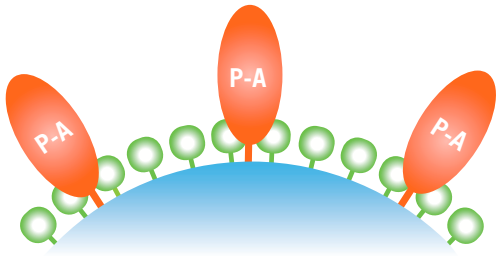
Comparison of purification performance of Amsphere A3 to other commercially available Protein A resins

Protein A affinity chromatography represents the key initial capture method in monoclonal antibody (mAb) purification. There are three major types of protein A "resin" based on a different matrix chemistries – glass, agarose and synthetic polymer. State of the art resins must offer good specificity, high mass transfer and binding capacity, low non-specific adsorption, low ligand leakage, suitable back pressure under high flow operation, and good chemical stability – particularly during alkaline sanitization. Until now, selecting a protein A resin for bioprocessing applications involved balancing among high specificity, high mass transfer and binding capacity, low non-specific adsorption and ligand leakage, incompressibility, resistance to alkaline condition for sanitization, chemical stability and cost effectiveness. Recent results using new Amsphere A3 versus Polymer H and Agarose show that the Amsphere A3 design minimizes compromise in performance criteria.

TABLE 1: HOST CELL PROTEIN LEVEL OF LOADING SAMPLES

LABEL	MOLECULE	LOAD HCP (ppm)
mAb1	Trastuzumab	350,000
mAb2	Adalimumab	220,000
mAb3	Bevacizumab	315,000
mAb4	Palivizumab	1,170,000
mAb5	Rituximab	560,000

Amsphere™ A3



Amsphere A3 is a new protein A resin designed with a surface modified base bead and alkali-resistant optimized ligand.

Protein A ligand

- High DBC via controlled conformation and orientation
- High alkaline stability from protein engineering

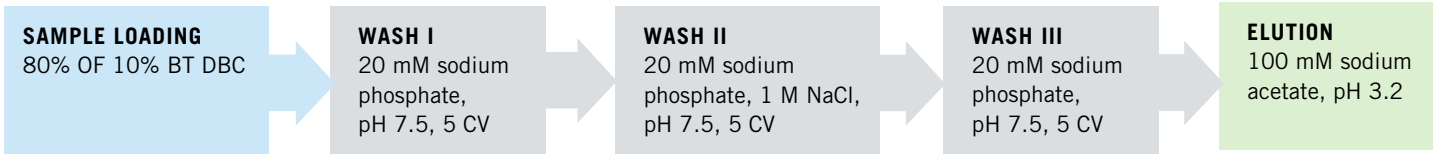
Surface modification

- Low HCP levels by surface hydrophilization

Base bead formulation

- High DBC at high flow rate
- Excellent pressure and flow properties via rigid crosslinking

FIGURE 1: TEST CONDITIONS OF CHROMATOGRAPHY PROCESS



Study Methods and Materials

Agarose and polymer (H) protein A affinity chromatography resins were tested in comparison with Amsphere A3. Five mAb samples (as in Table 1) expressed in CHO cells and clarified by centrifugation and 0.22 µm filtration were tested. Purified polyclonal human IgG was also used. Dynamic binding capa city (DBC) at 10% breakthrough versus residence time, alkali stability (0.5 M NaOH), host cell protein (HCP) clearance, pressure-flow properties, and protein A ligand leaching were evaluated according to common literature protocols. The details of the protein A column chromatography processes are given in Figure 1. Other experimental details are given in association with the results below.

Results

1. DYNAMIC BINDING CAPACITY

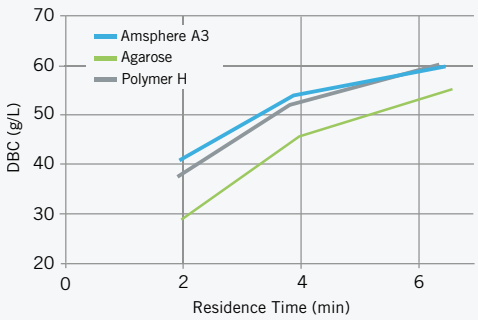


FIGURE 2: DYNAMIC BINDING CAPACITY VERSUS RESIDENCE TIME (RT)

Item	Details
Column	0.5 x 20 cm (4 mL)
Loading Buffer	20 mM sodium phosphate 150 mM NaCl (pH 7.5)
Detection	UV at 280 nm
Sample	Polyclonal human IgG ~5.3 mg/mL
DBC	10% BT

Conclusion: Amsphere A3 performed as well or better than the two other top performing resins studied.

2. mAbs DYNAMIC BINDING CAPACITIES AT 5 MINUTES RESIDENCE TIME

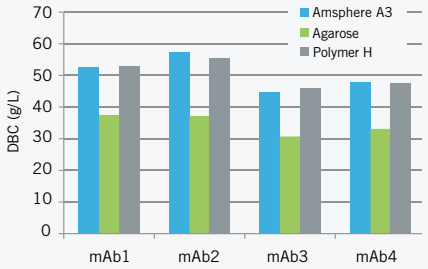


FIGURE 3: DYNAMIC BINDING CAPACITY FOR DIFFERENT mAbs

Item	Details
Column	0.5 x 5 cm (1 mL) Except DBC (mAb1): 0.5 x 20 cm (4 mL)
Residence Time	5 min (except for mAb1: 4 min)
Detection	HPLC
Sample	mAb in CHO cell culture fluid

Conclusion: Amsphere A3 performed as well or better than the two other top performing resins studied.

3. HCP CLEARANCE – TARGET POOL HCP LEVELS

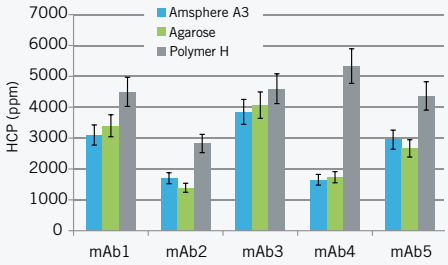


FIGURE 4: HOST CELL PROTEIN CLEARANCE (ppm IN TARGET POOL)

Item	Details
Column	0.5 x 5 cm (1 mL)
Residence Time	5 min
Detection	Cygnus F550 HCP ELISA kit

Host cell protein (HCP) values were measured using an enzyme-linked immunoassay and expressed relative to the recovered target as parts per million (ppm). Target feed HCP ppm values are given in Table 1.

Conclusion: Amsphere A3 performed as well or better than the two other top performing resins studied.

4. LEACHED PROTEIN A INTO TARGET POOL

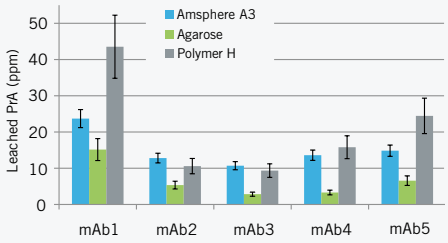


FIGURE 5: LEACHED PROTEIN A LIGAND (ppm) IN TARGET POOL

Item	Details
Column	0.5 x 5 cm (1 mL)
Residence Time	5 min
Detection	Cygnus F740 ELISA kit

Leached protein A ligand values were measured using an enzyme-linked immunoassay (Cygnus Kit) and expressed relative to recovered target as parts per million (ppm).

Conclusion: All three resins exhibited reasonably low levels of leached protein A ligand with the mAb targets.

5. ALKALINE STABILITY (% RETENTION OF DBC) TO 0.5 M NaOH

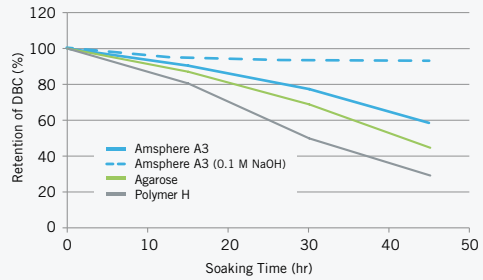


FIGURE 6: DBC RETENTION AFTER SOAKING IN 0.5 M NaOH

Item	Details
Column	0.5 x 20 cm (4 mL)
Loading Buffer	20 mM sodium phosphate 150 mM NaCl (pH 7.5)
Detection	UV at 280 nm
Sample	Polyclonal human IgG ~5.3 mg/mL
DBC	10% BT

This rigorous acceleration study is run to demonstrate resistance to high concentration of NaOH within an experimental time frame of 45 hr. This data can quickly highlight differences in NaOH resistance of various resins. Resins in packed columns were exposed to 0.5 M NaOH for various times and then tested for DBC at 10% breakthrough.

Conclusion: Amsphere A3 offered best-in-class performance. The resin maintained >90% of its starting DBC after 12 hr in 0.5 M NaOH and >80% after 25 hr of total contact time.

6. PRESSURE-FLOW PROPERTIES

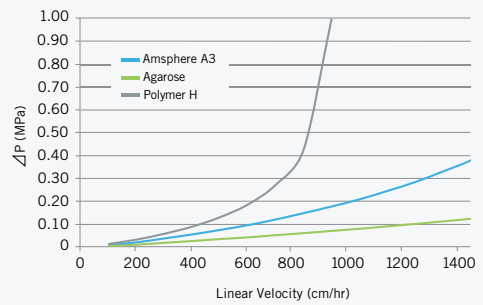


FIGURE 7: PRESSURE FLOW PROPERTIES

Item	Details
Column	1.6 x 10 cm (20 mL)
Mobile Phase	Water
System	AKTA avant 150

Conclusion: Suitably low back pressure at high linear velocities was observed for the agarose resin and Amsphere A3.

Overall Conclusion

Amsphere A3 has incorporated many resin design attributes that minimize compromise in key selection criteria such as DBC, HCP reduction, and caustic stability. The selection of ligand design, bead design and surface modification result in a superior product, fit for a wide range of operating conditions and scale.

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