JSR Life Sciences

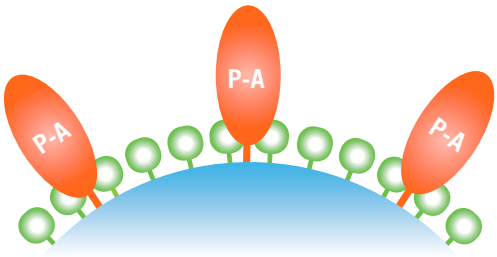
Lifetime study for JSR Amsphere A3 – protein A resin

Many factors are required to assess and implement a protein A resin into a mAb purification process. Dynamic binding capacity, purity, operating flow rate and caustic compatibility are a few among many evaluation parameters. Resin lifetime is a key parameter that needs to be assessed as it is directly related to both resin volume and process costs. The resin should maintain performance parameters over the intended number of process cycles. The following describes the effects of process cycling and caustic CIP on the purification performance of Amsphere A3 over 100 cycles.

Materials and Methods

- Biosimilar mAb, 1.1 g/l titer, pI 8.45, from CHO cell line
- Initial HCP level 350,000 ppm
- Clarified by centrifugation and 0.22 micro filtration
- HCP determination by Cygnus Kit (F550)
- DNA determination by Picogreen® assay (Invitrogen)
- Leached protein A determination by Cygnus Kit (F740)

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

TABLE 1: CIP CYCLE TEST CONDITIONS

CIP CYCLE TEST CONDITIONS	
Number of cycles	100 cycles
Column	0.5 x 5 cm (1 ml)
Sample	Biosimilar mAb (Trastuzumab)
Loading amount	>10% BT (Run 1, 11, 21…) 80% load of 10% BT DBC (Run 2, 12, 22…)
Loading frequency	Added to every 10 cycles
Residence time	5 min

FIGURE 1: TEST CONDITIONS OF CHROMATOGRAPHY PROCESS

SAMPLE LOADING
Over 10%BT DBC (Run 1,11,21,31,,,,)
80% of 10%BT DBC (Run 2,12,22,32,,,,)

WASH I
20 mM sodium phosphate pH 7.5, 2 CV

WASH II
20 mM sodium phosphate 1 M NaCl pH 7.5, 3 CV

WASH III
20 mM sodium phosphate pH 7.5, 3 CV

ELUTION
100 mM sodium acetate pH 3.3

STRIP
100 mM acetic acid pH 2.8

EQUILIBRATION
20 mM sodium phosphate pH 7.5

CIP
0.1 M NaOH: contact time 30 min
0.5 M NaOH: contact time 10 min every 10 cycles

Results

1. DYNAMIC BINDING CAPACITY

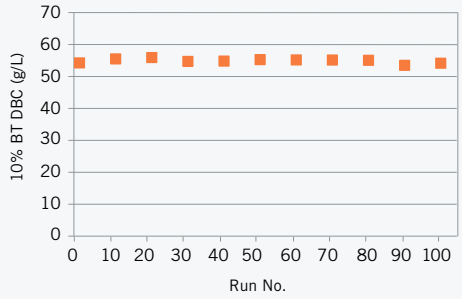


FIGURE 2: DBC IS MAINTAINED AT INITIAL LEVELS FOR 100 CYCLES

2. mAb RECOVERY

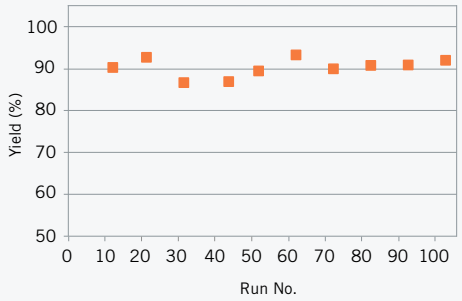


FIGURE 3: YIELD IS ABOVE 80% AND CONSTANT FOR 100 CYCLES

3. HCP REDUCTION

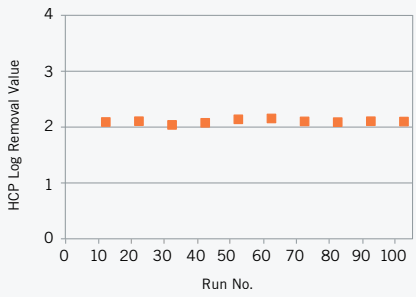


FIGURE 4: HCP REDUCTION IS APPROXIMATELY 2.0 LRV AND IS CONSTANT FOR 100 CYCLES

4. DNA REDUCTION

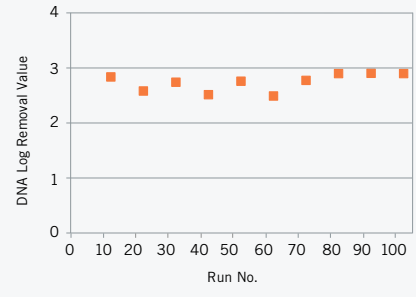


FIGURE 5: DNA LRV IS CONSISTENT AT APPROXIMATELY 2.9 THROUGHOUT THE 100 CYCLES

5. LEACHED PROTEIN A INTO TARGET POOL

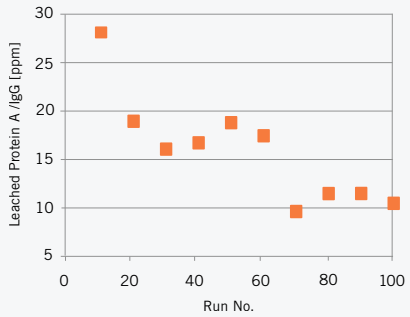


FIGURE 6: LEACHED PROTEIN A DROPS TO 15-20 ppm AFTER 20 CYCLES AND THEN REMAINS CONSTANT.

6. ELUTION POOL

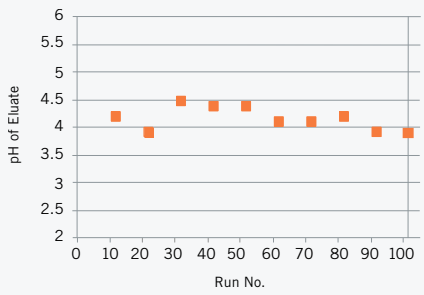
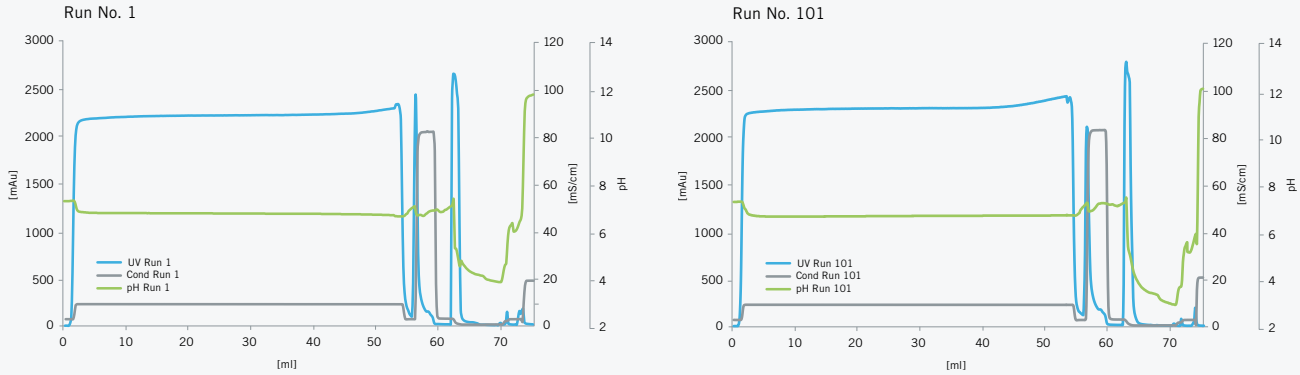


FIGURE 7: ELUTION POOL PH AS A FUNCTION OF RUN NUMBER IS STABLE AT APPROXIMATELY 4.0 – 4.5

7/8. CHROMATOGRAMS COMPARISON



FIGURES 8 AND 9: COMPARISON OF CHROMATOGRAMS BETWEEN RUN 1 AND RUN 101. NO SIGNIFICANT DIFFERENCE IS OBSERVED. THE ELUTION PROFILE, ELUTION VOLUMES AND PEAK SHAPE ARE NEARLY IDENTICAL FROM FIRST TO 101ST RUN.

9. ELUTION PROFILE

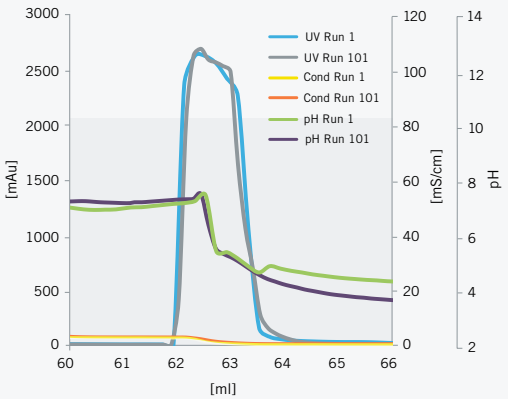


FIGURE 10: THE ELUTION PROFILE FOR THE FIRST RUN AND THE 101ST RUN ARE OVERLAID ABOVE. THE ELUTION PROFILE, ELUTION VOLUMES AND PEAK SHAPE ARE NEARLY IDENTICAL FROM FIRST TO LAST RUN

Summary

The CIP test results indicate stable performance over 100 operating cycles, including NaOH exposure of 0.1 M NaOH every cycle and 0.5 M NaOH every tenth cycle. The mAb recovery, HCP reduction, DNA reduction and elution pool pH are consistent across the 100 cycles. The chromatograms from the first to the 101st cycle also show nearly identical response both in terms of elution profile and column volume.

This data set indicates stable resin performance can be expected after multiple cycles.

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