JSR Life Sciences

Caustic Stability of Amsphere A3: Cycling Study with 0.5 M NaOH

Introduction

Sodium hydroxide (NaOH) is most commonly used for cleaning in place (CIP) of Protein A media and caustic stability is therefore an important asset. During its whole lifetime, performance of the resin needs to remain within set specifications. In view of cost benefits, resin lifetime in number of cycles should be as long as possible and will depend on operating conditions, e.g. the sodium hydroxide concentration and contact time. We have investigated the stability of Amsphere A3 when using 15 minutes of contact time with 0.5 M NaOH as CIP step.

Materials and methods

All experiments were done on a 1mL MediaScout® MiniChrom column (0.5 cm inner diameter; 5.0 cm bed height), pre-packed by Repligen. Lyophilized human polyclonal antibody from Equitech-bio was used for DBC measurements. Experiments were performed on the AKTA avant 25 from GE Healthcare.

Concentration of the polyclonal antibody feed solution was measured by UV280 absorbance with the Eppendorf Bio-Spectrometer. Feed concentrations were between 5.1 and 5.4 g/L.

The DBC value at 10% breakthrough was determined at the beginning of the study. Next, cycling with buffers was done and DBC was measured every 15th run. The protocol for the DBC runs and buffer runs is shown in below tables.

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-flow properties via rigid crosslinking

To confirm the resin performance after the study, a purification of Tocilizumab (column load: 40 g/L) was done on the column after 101 cycles with 0.5 M NaOH and on a new, unused column. Host cell protein (HCP) and DNA levels were measured in the feed and eluate. For HCP measurement, the CHO Host Cell Proteins Kit (Cygnus Technologies, cat no F550) was used. The Quant-iT™ Picogreen® dsDNA kit (P11496, Life Technologies) was used for DNA measurement.

STEP	SOLUTION - BUFFER	FLOW (cm/hr)	RES. TIME (min)	CV
Equilibration	20 mM sodium phosphate pH 7.5	150	2.0	5.0
Elution	150 mM sodium acetate pH 3.0	150	2.0	5.0
CIP	0.5 M NaOH (15 min contact time)	100	3.0	5.0
Equilibration	20 mM sodium phosphate pH 7.5	150	2.0	5.0

Table 1: Protocol used for the buffer cycling

STEP	SOLUTION - BUFFER	FLOW (cm/hr)	RES. TIME (min)	CV
Equilibration	20 mM sodium phosphate pH 7.5	150	2.0	5.0
Load	Human polyclonal IgG dissolved in equilibration buffer	75	4.0	Until >10% breakthrough
Wash 1	20 mM sodium phosphate pH 7.5	75	4.0	5.0
Wash 2	20 mM sodium phosphate; 0.5 M NaCl; pH 7.5	75	4.0	5.0
Wash 3	20 mM sodium phosphate pH 7.5			3.0
Elution	150 mM sodium acetate pH 3.0			5.0
CIP	0.5 M NaOH (15 min contact time)	100	3.0	5.0
Equilibration	20 mM sodium phosphate pH 7.5	150	2.0	5.0

Table 2: protocol used for determining the DBC of Amsphere A3

Results

DBC for the polyclonal IgG was 58 g/L at the start of the study. After 100 cycles, DBC was still 53 g/L.

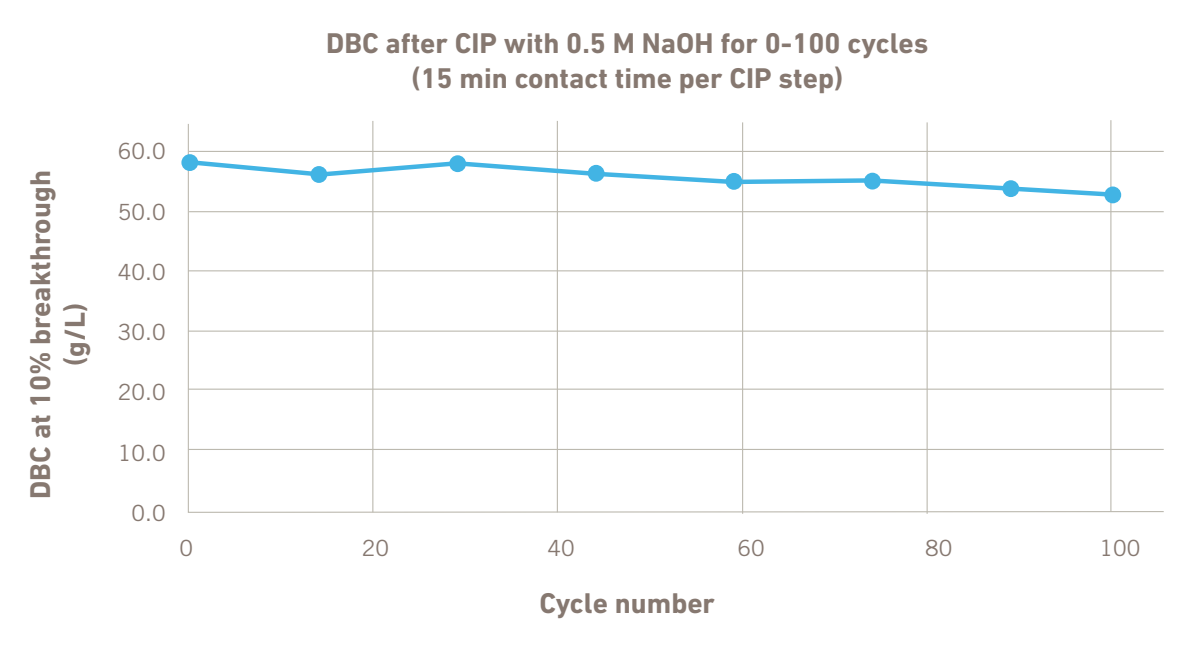

Fig. 1: DBC values at 10% breakthrough after a given number of cycles

Amsphere A3 shows a good maintenance of its dynamic binding capacity. After 100 cycles, the DBC was still 91% of the starting value.

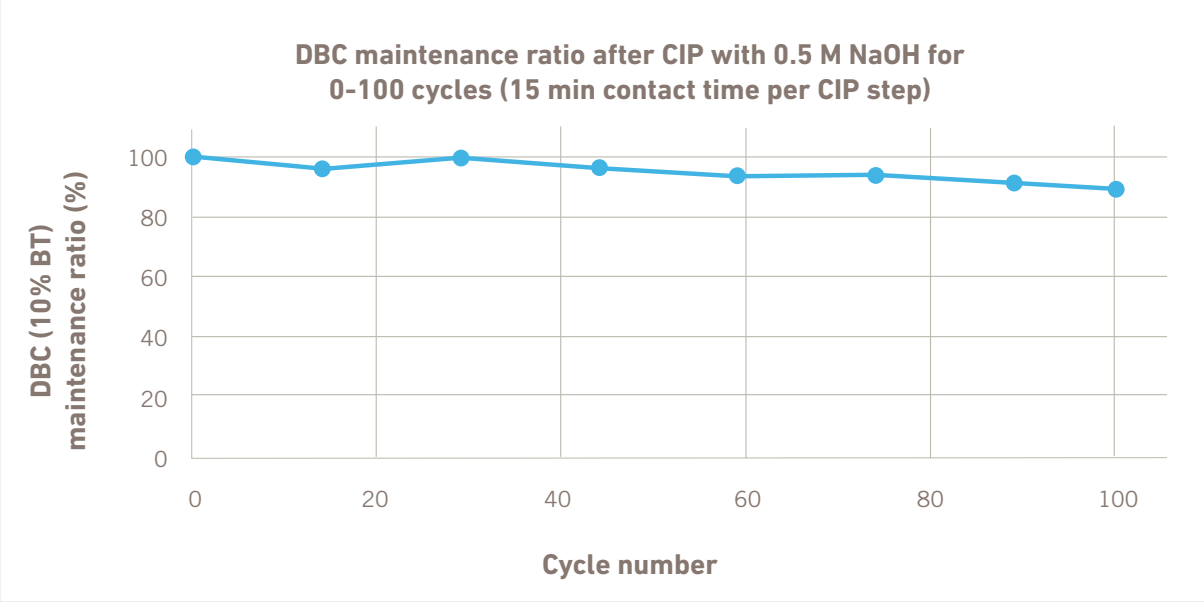

Fig. 2: Maintenance ratio of the DBC after a given number of cycles

Purification performance is maintained after the 100 cycles. HCP and DNA clearance can be considered the same after 100 cycles with 0.5 M NaOH.

	HCP CONCENTRATION (ppm)			DNA CONCENTRATION (ppm)		
	Feed	Elution pool	LRV	Feed	Elution pool	LRV
Run 1	735644	1269	2.8	9432	6	3.2
Run 102		1260	2.8		8	3.1

Table 3: Comparison of HCP and DNA clearance between a new column and after 101 cycles

Conclusions

Amsphere A3 has good caustic stability. DBC gradually decreases when using 0.5 M NaOH as CIP solution, but the data shown above demonstrates that the media can be used for 100 cycles with 15 minutes contact time without impact on purification performance. Columns are typically loaded at 80% of the DBC value at 10% breakthrough. There is no issue in recovery of IgG after 100 cycles.

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NORTH AMERICA
JSR Life Sciences
JSR Micro, Inc.
1280 North Mathilda Avenue
Sunnyvale, CA 94089
408-543-8800
bioprocess.us@jsrlifesciences.com

ASIA
JSR Corporation
1-9-2 Higashi-Shimbashi
Minatoku, Tokyo
105-8640 Japan
+81-3-6218-3557
bp@jls.jsr.co.jp

EUROPE
JSR Life Sciences
JSR Micro NV
Technologielaan 8
3001 Leuven
Belgium
+32-16-668-721
bioprocess.eu@jsrlifesciences.com