JSR Life Sciences

Amsphere A3 - protein A resin: an optimized media to minimize the impact of elution buffer selection

Protein A chromatography is widely used as the affinity capture step of both mAbs and Fc-fusion proteins because of its high degree of selectivity. Variations in elution behavior of the protein A capture step require more process development work and could have an impact on the polishing step in the downstream process. Thus, minimizing the variation in elution pH between different molecules for example, makes it easier to platform the capture process.

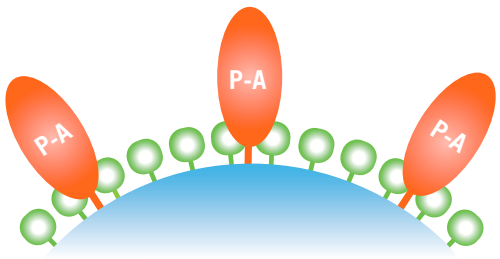
Amsphere A3 was designed so that differences in washing and elution buffers (type, conductivity and pH) have minor effects on the characteristics of the elution pool (pH, impurity levels, yield, etc). In this application note, the impact of the elution buffer counter-ion, pH and molarity were evaluated on the key purification performance parameters (HCP removal, yield, elution volume and pH of the elution pool) of Amsphere A3. In total, 5 different mAbs were used for the study.

TABLE 1: mAbs USED FOR THE STUDY

MOLECULE	LOAD HCP (ppm)
Trastuzumab	350,000
Adalimumab	220,000
Bevacizumab	315,000
Palivizumab	1,170,000
Rituximab	560,000

Table 1: Protocol used for the buffer cycling

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

Study Methods and Materials

Agarose was tested in comparison with Amsphere A3. Five mAb samples (Table 1), expressed in Chinese Hamster Ovary (CHO) cells and clarified by centrifugation, were tested. The impact of the antibody loading quantity on elution pH was investigated using Trastuzumab and 3 loadings (10, 20 and 30 mg) with the experimental conditions displayed in Figure 1. The on-line pH measured at the UV peak maximum during product elution (UV 300 nm) was reported. The impact of the antibody loading on the UV300/pH relation was investigated.

The conditions for the impact of various elution buffers (type, molarity, pH) on purification performance of Amsphere A3 are given in association with the results below.

Results

1. EVALUATING THE IMPACT OF ANTIBODY LOADING QUANTITY ON ELUTION pH

FIGURE 1: LOADING VERSUS ELUTION pH FOR AMSPHERE A3

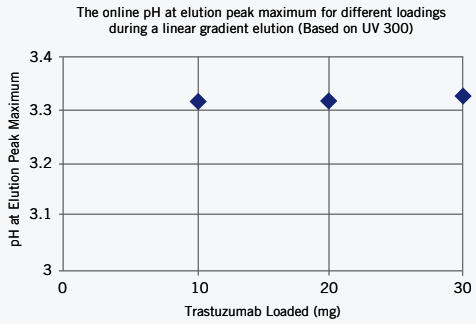


TABLE 2: EXPERIMENTAL CONDITIONS USED FOR TESTING ELUTION pH VARIABILITY

STEP	DETAILED EVALUATION
Column	Amsphere A3 (0.5 cm x 5 cm (1 mL))
mAb	Trastuzumab
Loading amount	10 mg, 20 mg, 30 mg
Residence time	1 minute
Washing steps	50 mM Sodium Citrate pH 6 (5CV)
Elution	Linear gradient 50 mM Sodium Citrate pH 6-2.5 (15CV)

Conclusion: There is negligible impact of the amount of antibody loading on the elution pH during antibody desorption.

2. EVALUATION OF THE VARIABILITY IN ELUTION pH

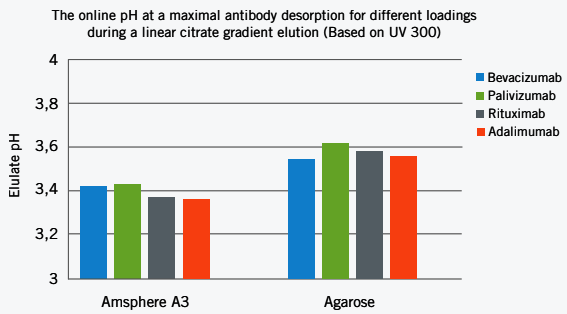
TABLE 3: STANDARD DEVIATIONS ON THE ELUTION pH

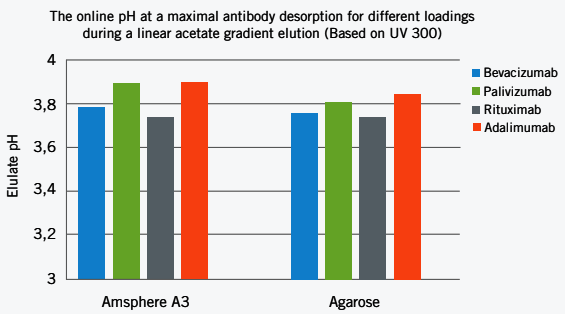
	CITRATE ELUTION	ACETATE ELUTION
Amsphere A3	pH 3.41 ± 1.00%	pH 3.85 ± 2.1%
Agarose	pH 3.59 ± 0.85%	pH 3.80 ± 1.2%

TABLE 4: EXPERIMENTAL CONDITIONS USED FOR TESTING ELUTION pH VARIABILITY

STEP	DETAILED EVALUATION
Column	Amsphere A3 (0.5 cm x 5 cm (1 mL)) Agarose (0.5 cm x 5 cm (1 mL))
mAb	Bevacizumab, Palivizumab, Rituximab, Adalimumab
Loading amount	10 mg
Residence time	1 minute
Washing steps	50 mM Sodium Citrate pH 6 (5CV)
Elution	Linear gradient 50 mM Sodium Acetate pH 5-3 (10CV) Linear gradient 50 mM Sodium Citrate pH 6-2.5 (15CV)

FIGURE 2: VARIABILITY IN ELUTION pH FOR 4 DIFFERENT mAbs FOR AMSPHERE A3 AND AGAROSE

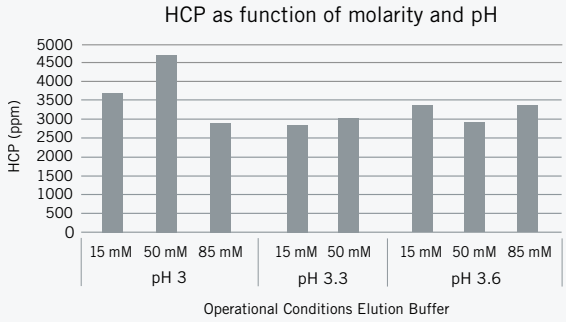


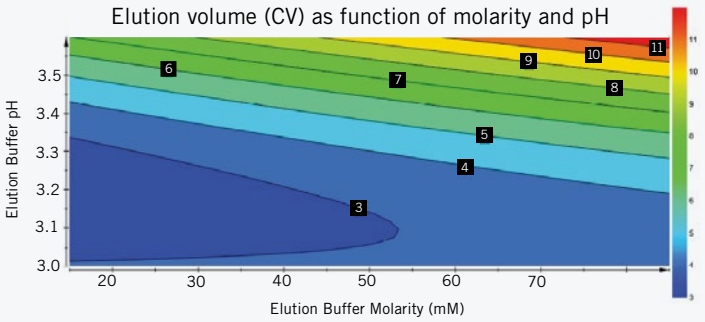


Conclusion: Amsphere A3 has comparable standard-deviation on elution pH than Agarose.

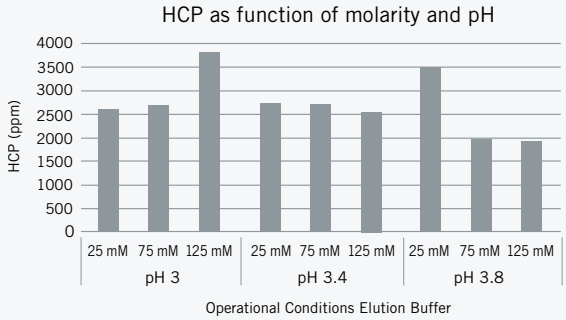
3. IMPACT OF VARIOUS ELUTION BUFFERS (TYPE, MOLARITY, pH) ON PURIFICATION PERFORMANCE OF AMSPHERE A3

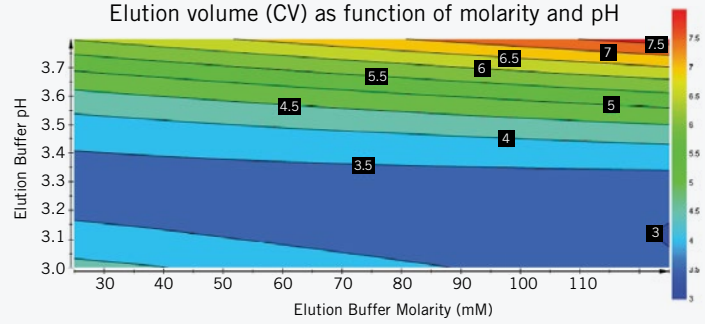
Citrate elution condition



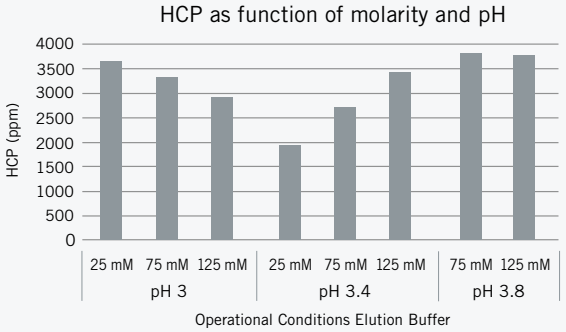


Acetate elution condition





Glycine elution condition



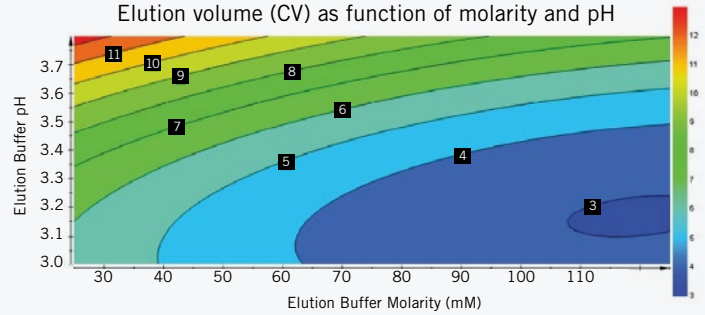


FIGURE 3: Different pH values and molarities were screened for glycine-HCl, sodium acetate and sodium citrate (up to 10 CV). All experiments were done with Trastuzumab at a loading of 80% of 10% DBC. The fractionation threshold was set above 50 mAU at 280 nm. HCP values were measured using an enzyme-linked immunoassay (Cygnus F550 HCP ELISA kit) and expressed relative to recovered target as parts per million (ppm). Target feed HCP ppm values are given in Table 1.

Conclusion: No impact was observed on HCP clearance and yield for all elution buffers.

Overall Conclusion

For Amsphere A3 the impact of elution buffer counter-ion, pH and molarity on key purification performance parameters was investigated. The HCP clearance was good for all mAbs tested. There was also no dependence of buffer counter-ion, pH and/or molarity on the HCP clearance.

Amsphere A3 is a platform-ready resin with outstanding performance. This allows use of preferred elution conditions while still obtaining good performance. Furthermore, there is almost no need to tailor or optimize buffers, allowing fast process development.

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