TOSOH

Application Note

How to...improve performance of the Protein A capturing step

DIFFERENT POST-LOAD WASH SOLUTIONS INCREASE HOST CELL PROTEIN REMOVAL OF TOYOPEARL AF-rPROTEIN A HC-650F

Protein A has become a well-established platform for capturing of monoclonal antibodies and Fc-fusion proteins. Various Protein A resins have been commercialized, one with a higher dynamic binding capacity than the other. Especially the latest generation of Protein A resins was developed to bind large quantities of mAbs and Fc fusion proteins. One drawback of these resins is that host cell protein (HCP) clearance can be - depending on the feedstream - comparatively lower than for standard Protein A affinity resins. When aiming for a 2-step platform process, no compromises can be made with regards to HCP removal of the capturing step. The remaining impurity burden would have to be covered by just one subsequent chromatography step. Hence, development of strategies or procedures to reduce the HCP content of the Protein A elution pool are key for the development of such purification processes. Post load washing steps during Protein A chromatography may improve HCP clearance of the capturing step. However, the employed wash buffers must not affect other product related quality criteria, such as aggregate levels and mAb activity. Besides, the impact of different wash steps on the parameters determining process economics, that is product recovery, dynamic binding capacity and resin lifetime, should be evaluated.

BUFFERS FOR THE PROTEIN A POST-LOAD WASH STEP

Buffer	Additive Name	Additive Concentrations (mM)
100 mM sodium phosphate, pH 7.4	sodium chloride	150, 500, 750, 1,000, 1,500, 2,000
100 mM sodium acetate, pH 5.5	sodium chloride	0, 150, 250, 500
100 mM sodium citrate, pH 5.5	n.a.	n.a.
100 mM sodium phosphate, pH 7.4	arginine	100, 250, 500
100 mM sodium phosphate, pH 7.4	guanidium hydrochloride	500, 750, 1,000, 2,000

Table 1

Sodium phosphate, sodium acetate and sodium citrate were substituted with different concentrations of miscellaneous additives

MATERIALS & METHODS

Different buffers, pH, and agents to reduce non-specific binding were evaluated with regards to their potential to increase HCP clearance. A parallel chromatography approach with 200 µl TOYOPEARL AF-rProtein A HC-650F RoboColumns was chosen. Columns were loaded with mAb at 3 min residence time. After laoding, columns were washed with 100 mM sodium phosphate buffer, pH 7.4. In a second wash step, columns were flushed with phosphate, citrate or acetate buffered solutions of sodium chloride, arginine or guanidinium hydrochloride. The applied pHs ranged from 5.5 to 7.4. The different wash solutions are listed in Table 1.

After washing, mAb was eluted with 100 mM acetate at pH 2.9. Columns were cleaned with 0.2 M sodium hydroxide and re-equilibrated using 100 mM sodium phosphate buffer, pH 7.4. Except for loading, all steps were conducted at 300 cm/h. A detailed protocol can be found in Table 2.

PROTEIN A CHROMATOGRAPHY

Step	CV	Composition
equil	10	100 mM sodium phosphate, pH 7.4 + 150 mM sodium chloride
load	26	2 mg/mL mAb, 1 x CHOP in equilibration buffer
wash 1	5	100 mM sodium phosphate, pH 7.4 + 150 mM sodium chloride
wash 2	5	variable
wash 3	5	100 mM sodium phosphate, pH 7.4 + 150 mM sodium chloride
elute	5	100 mM sodium phosphate, pH 7.4 + 150 mM sodium chloride
CIP	5	0.2 M sodium hydroxide
reequil	10	100 mM sodium phosphate, pH 7.4 + 150 mM sodium chloride

Table 2

Buffers for the wash 2 step were chosen from Table 1. Buffers and solutions for the other steps are provided.

The mAb was recovered at ~10 mg/mL in total pool volumes of 1 mL ± 50 µl, which is due to drop formation at the RoboColumn outlet. Aliquots were analyzed with a CHO HCP ELISA, a protein A leaching ELISA and size exclusion chromatography. ELISAs were conducted according to the manufacturers' descriptions.

HCP CLEARANCE

CHOP log reduction values have been calculated and are shown in Figure 1. The employed reference protocol using 20 mM sodium phosphate, pH 7.4 + 150 mM sodium chloride has a log reduction value of 1.85.

PROTEIN A LEACHING

Protein A leaching results are shown in Figure 2. The post-load washing has no significant impact on the detected Protein A concentration. All samples have a Protein A content smaller than 1 ng/mL. Provided the mAb concentration in the elute pool is 10 mg/mL, this corresponds to 0.1 ppm of leached Protein A in the elute pools.

AGGREGATE CONTENT

SE-UHPLC with TSKgel UP-SW3000 (4.6 mm ID x 30 cm L) was used for determination of aggregate levels. 100 mM sodium phosphate, pH 6.7 + 100 mM sodium sulfate was used as liquid phase. 5 µl of the Protein A elution pools were injected. UV absorbance was measured @280 nm. An exemplary chromatogram is shown in Figure 3. Aggregate contents of all samples are 0.85 % ± 0.05 %.

RECOVERY

Recoveries were calculated from the UV absorbance @280 nm. A graph with the corresponding data is shown in Figure 4. Roughly, mAb concentrations of the elute pools range from 9.6 mg/mL to 11.0 mg/mL. Recovery slightly decreases in case of 100 mM sodium acetate, pH 5.5 and phosphate buffer, pH 7.4, containing high concentrations of arginine and guanidinium hydrochloride. However, recoveries exceed 90 % in all cases. Hence, these wash solutions can still be considered useful.

CHOP LOG REDUCTION VALUES

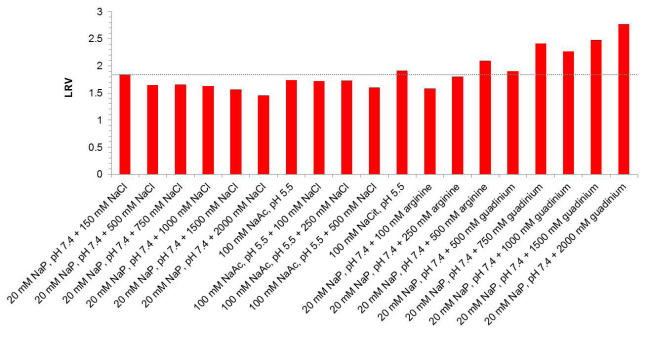

Figure 1

CHOP log reduction values of the parallel chromatography Protein A experiments using different buffers for wash 2. Comparatively higher log reduction values can be achieved using arginine and guanidinium hydrochloride for wash 2

PROTEIN A CONCENTRATION IN THE ELUTE POOL

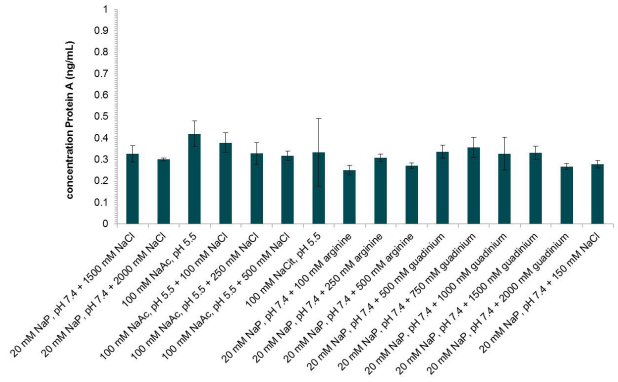

Figure 2

The determined Protein A contents are well below 1 ng/mL, which corresponds to roughly 0.1 ppm.

SE-UHPLC CHROMATOGRAM OF AN EXEMPLARY PROTEIN A ELUTION POOL

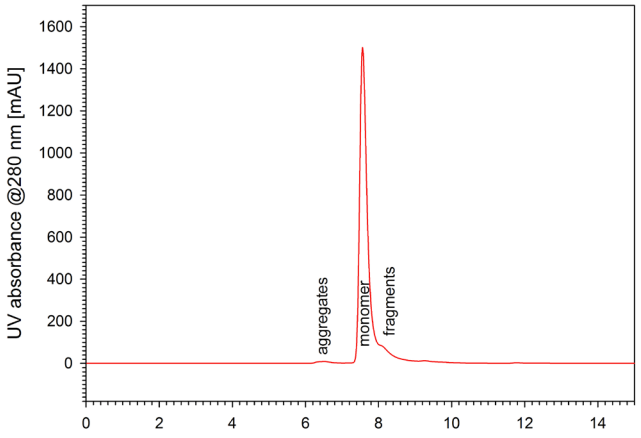

Figure 3

Aggregates elute at 6.5 min, the mAb monomer at 7.6 min. Fragments elute at 8.1 min

RECOVERY

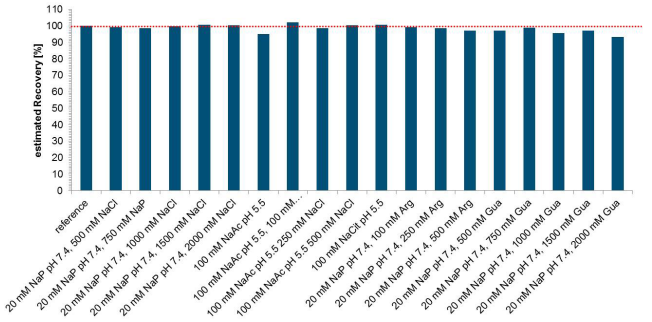

Figure 4

Recovery from Protein A after application of different wash buffers. Recovery is slightly affected by post-load washing with 100 mM sodium acetate, pH 5.5 and high contents of arginine or guanidinium hydrochloride. Recovery is greater than 90 % in all cases.

CONCLUSIONS

High capacity Protein A resins are highly beneficial with regards to process economics. Potential drawbacks with regards to CHOP clearance depend on a particular feed-stream and can be compensated by using post-load wash steps.

LITERATURE:

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