Maximizing Performance of AVIPure® Albumin Resin: A Case Study with rHSA from Pichia pastoris

Application Note

Introduction

Human albumin has a variety of applications, from clinical and medical devices to use as an excipient in drug and vaccine formulation. The protein can be purified from human serum or made recombinantly using different expression systems. One common system is genetically modified Pichia pastoris, also known as Komagataella phaffii. AVIPure® Albumin Affinity Resin can be used as a capture step in the purification of human albumin.

An HSA-expressing P. pastoris was developed in-house and loaded over AVIPure Albumin Affinity Resin at pH 4.5 to achieve highest capacity, as previous work has shown (See AVIPure Albumin Affinity Resin User Guide). HP-SEC and SDS-PAGE analysis showed that the recommended pH 3 elution was only eluting 59% of product. Repeating the experiment at a load pH of 6.5 resulted in 92% yield, bypassing the pH 3 elution issue but not utilizing the >70 g/Lres capacity of the resin.

To ensure optimum performance of the resin for customers, a method for improving yields when loading at pH 4.5 was investigated. It was found that a 1 M NaCl wash at neutral pH improved the pH 3 elution yield to 81%. Neutral pH elution buffers were also investigated as some HSA-fusion proteins do not tolerate a low pH elution. Two pH 8 elution buffers were developed based on sodium deoxycholate and sodium octanoate that achieve >90% yields for pH 4.5 loads.

The exact impurities observed for the P. pastoris fermentation used in this work may be different from other strains and fermentation conditions, however, the strategy demonstrated here for utilizing the resin capacity and maximizing yield is widely applicable.

The HSA titer for in-house P. pastoris fermentation was very low at 0.2 g/L, which required >100 CV to be loaded onto the column. However, the trends observed here may serve as a helpful guide for process development teams to optimize capacity and recovery of human albumin.

Optimizing Run Conditions of Low pH Load to Improve Yield of pH 3 Elutione

During development of AVIPure Albumin Affinity Resin it was discovered that lowering the load pH to 4.5 increased capacity to >70 g/Lres. Using the lower pH to maximize capacity, *P. pastoris* harvest was loaded at pH 4.5 following the procedure outlined in (Table 1). OPUS® MiniChrom® Pre-packed Columns (1 mL, 0.5 cm ID x 5 cm H) were used with a BioRad NGC FPLC. Resin was presanitized with 0.5 M NaOH for 15 minutes contact time prior to use. The harvest was pH adjusted with acetic acid and 0.22 µm filtered before use. HP-SEC titer of harvest was around 0.2 g/L. An acid strip with 0.1 M phosphoric acid prior to NaOH CIP helped prevent fouling of the resin. Both the strip and the CIP were allowed 15-minute contact time with the resin.

Table 1. Operating Conditions for 1 mL CV at 0.245 mL/min

Phase	Buffer	CV
Equilibration	150 mM NaCl, 50 mM Citrate, pH same as load	10
Load	*P. pastoris* harvest	120
Wash 1	1 M NaCl, 0.5 M MES, 50 mM Citrate, pH same as load	5
Wash 2	Equilibration Buffer	5
Elution	0.1 M Citrate, pH 3	8
Acid Strip	0.1 M Phosphoric Acid	4
CIP	0.1 – 0.5 M NaOH	4
Equilibration	Equilibration Buffer	10

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The chromatogram showed a tailing elution peak and large strip peaks suggesting that the pH 3 elution was not effective. HP-SEC analysis of the elution pool showed only a 59% yield. The run was repeated with equilibration, load, and wash buffer at pH 6.5 and showed an improved elution peak and smaller strip peaks (Figure 1). HP-SEC analysis of this elution pool showed a 92% yield.

Figure 1. Overlay of pH 4.5 and pH 6.5 Load

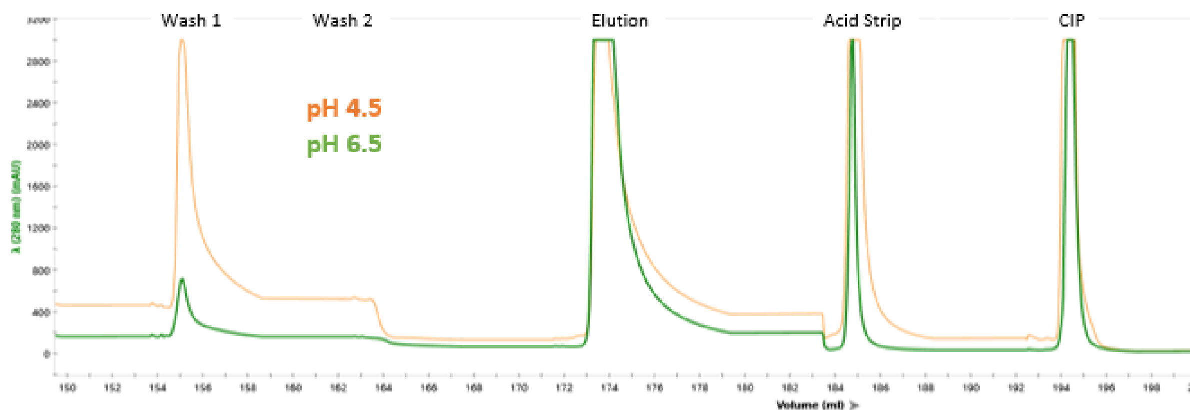
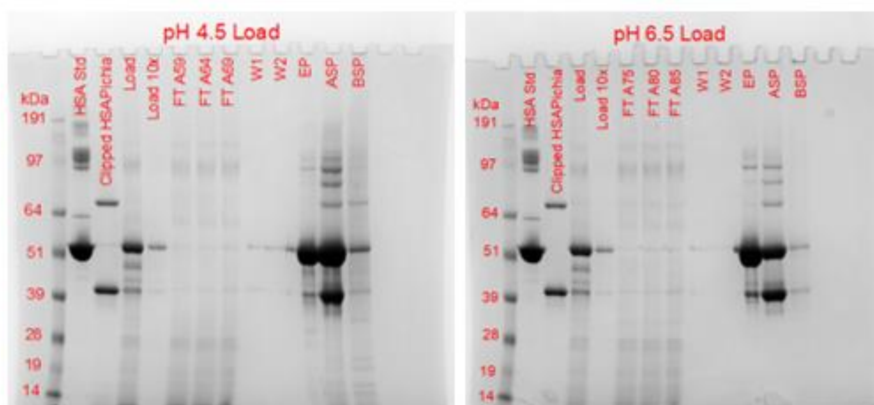


Figure 2. SDS-PAGE of pH 4.5 and pH 6.5 Load

- **FT: Flow Through**
- **EP: Elution Pool**
- **ASP: Acid Strip Pool**
- **BSP: CIP Pool**



SDS-PAGE analysis of the last three flow through fractions show no breakthrough for either pH load condition, meaning the load pH did not affect HSA binding. One noticeable difference on the gel was between the acid strip pools (Figure 2). The pH 4.5 load run had much more product retained on the column after elution than the pH 6.5 load run. The HP-SEC analysis showed 11% of the total HSA loaded in the acid strip pool for the pH 4.5 load run compared to only 3% for the pH 6.5 load run.

The SDS-PAGE in Figure 2 also shows that the elution, strip, and CIP pools from the load pH 4.5 run contain more low molecular weight (LMW) impurities, which correlates with the lower recovery. An extensive round of wash scouting was performed to try to remove the LMW impurities observed in the SDS-PAGE.

Wash Scouting Improves pH 3 Elution

The primary focus of the wash scout was to remove the species seen around 40 kDa on the gel as it is the main impurity observed by SDS-PAGE. Increasing the wash pH with a PBS wash did not improve the pH 3 elution as can be seen in the chromatogram (Figure 3).

Several different kosmotropes, chaotropes, pHs, and organics were tested in the wash buffer, but none were successful at removing the impurity. However, it was noticed that a neutral pH, high salt wash improved the pH 3 elution, despite not fully removing the

impurity. A confirmation run showed that the two separate washes outlined in Table 1 could be replaced by a single 1 M NaCl, pH 7.4 wash. The SDS-PAGE (Figure 4) showed a large amount of low molecular weight impurities being removed, less product in the acid strip, and HP-SEC of elution pools showed an 81% yield using a pH 3 elution. The major impurity running at 40 kDa binds strongly to the affinity resin and could not be washed off, suggesting that it may be related to an HSA cleavage product. Optimization of the elution condition was investigated to elute only the intact HSA, while leaving the potential cleavage product bound to the resin.

Figure 3. Overlay of Wash Scouting

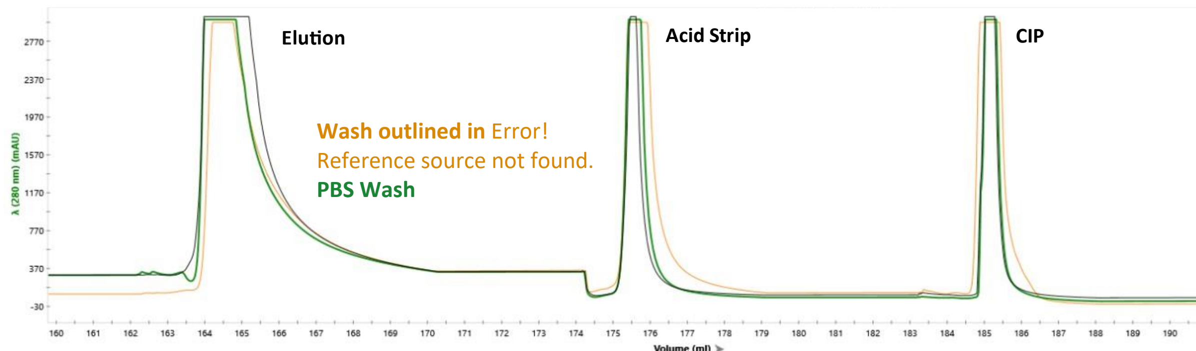
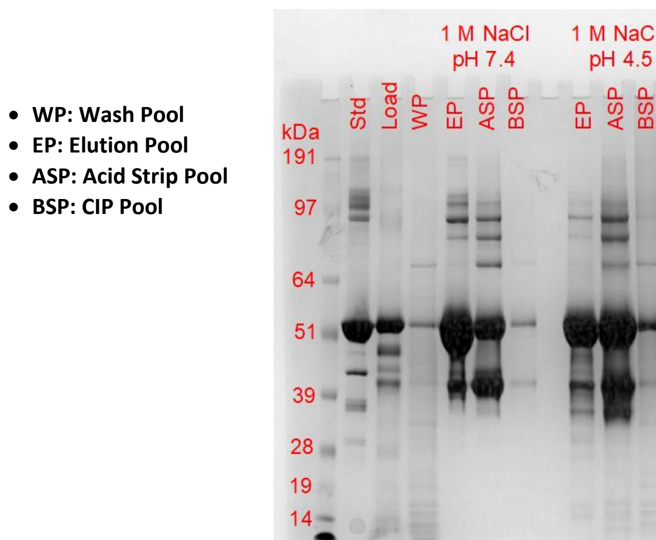


Figure 4. SDS-PAGE of pH 4.5 Wash Versus pH 7.4 Wash



Alternative Elution Buffers

One reason to investigate alternative elution options is to improve the yield with the *P. pastoris* harvest at pH 4.5, and another reason is that many HSA fusion proteins may not tolerate a low pH elution. During development of AVIPure Albumin Affinity Resin, it was found that 0.2 M sodium octanoate, 1 M NaCl, pH 8 worked well for a near neutral pH elution. That was tested on the pH 4.5 *P. pastoris* load with excellent results. The 25 mM sodium deoxycholate, pH 8 elution was also tested with excellent results (Figure 5). Optimization of buffer component concentrations using relevant harvest materials for each user is recommended to maximize yield and purity.

Both elution buffers require the pH to be raised prior to eluting as deoxycholate and octanoate have poor solubility at pH below 7.4. At first, both wash steps outlined in Table 1 were combined into one wash step using 1 M NaCl, pH 7.4, as was done when improving

the pH 3 elution. Further testing showed that a wash step using 150 mM NaCl, pH 7.4 also provided great elution and good HCP log reduction values (Table 2). It is recommended that the wash buffer contain at least a small amount of salt; based on product loading studies, 4x-diluted PBS in water, approximately 38 mM NaCl, was sufficient to facilitate binding to the resin. Using lower concentrations of NaCl in the load or wash buffer may result in a yield loss. The SDS-PAGE shows improved purity for both elution buffers.

Figure 5. Overlay of Deoxycholate and Octanoate Elutions

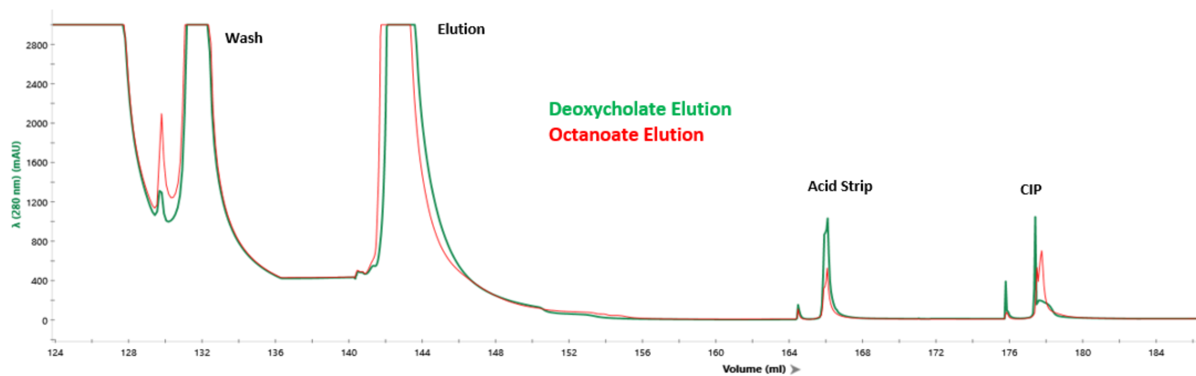


Figure 6. Non-Reduced SDS-PAGE of Deoxycholate and Octanoate Elutions

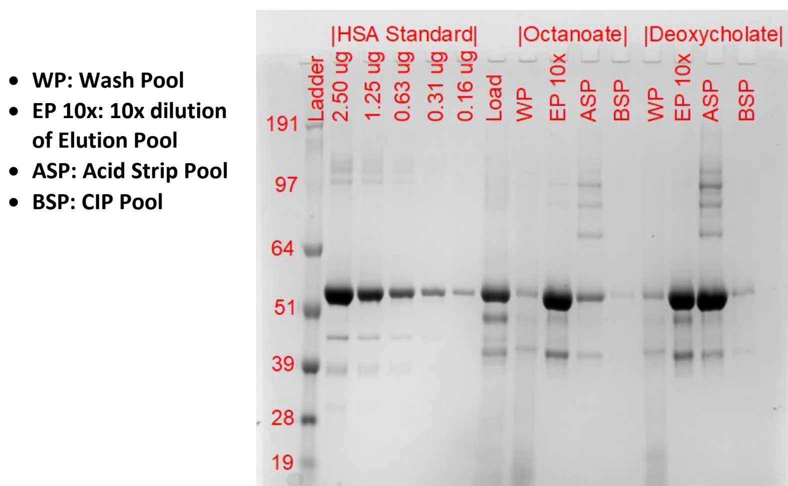


Table 2. HP-SEC and HCP LRV Values for Deoxycholate and Octanoate Elutions

Elution Buffer	% Yield	% HMW	LRV
Sodium Octanoate	≥ 95%	1%	2.49
Sodium Deoxycholate	≥ 95%	1%	2.16

Conclusion

AVIPure Albumin Affinity Resin has the highest capacity for HSA when loaded at lower pH. After loading *P. pastoris* at pH 4.5 onto the resin, a high salt, neutral pH wash is required to maximize product recovery with a pH 3 elution. Yields of >80% were observed using this single wash condition. Sodium octanoate and sodium deoxycholate serve as excellent, neutral pH alternatives to the pH 3 elution as they provided ≥95% yields with excellent product purity, i.e., 1% HMW species and greater than 2 log reduction of host cell proteins.

Ordering Information

Items listed here are available through your account manager, or customer service at the following emails:
US: customerserviceUS@repligen.com
EU: customerserviceEU@repligen.com
China: customerserviceCN@repligen.com

Description	AVIPure Albumin
Affinity resin, 10 mL	100HSA-10
Affinity resin, 25 mL	100HSA-25
Affinity resin, 100 mL	100HSA-100
Affinity resin, 500 mL	100HSA-500
Affinity resin, 1 L	100HSA-1L
Affinity resin, 5 L	100HSA-5L
600 µL RoboColumn® - Strip of eight columns, 0.5 x 3 cm	23052108R-30
1 mL Pre-packed MiniChrom Column, 0.5 x 5 cm	23052106
5 mL Pre-packed MiniChrom Column, 0.8 x 10 cm	23051004-100

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