Poster #334

Improving the elution step in capture chromatography: a mechanistic approach

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1. Introduction

Bulk recovery of adeno-associated viruses (AAV) in the capture step is often performed using affinity chromatography. Whilst this technique offers great capsid adsorption, the final recoveries can be lower than expected (< 80%). This disparity between loaded and recovered product suggests there are mechanisms not fully understood in affinity chromatography.

There is scarce understanding about the mechanistic governing phenomena during the elution phase in affinity chromatography for AAVs, thus affecting the optimal performance of this unit operation and hindering overall downstream productivity.

During the development of an AAV5-based product, the capture chromatography step exhibited lower than expected, and variable recoveries, of around 4% to 30% in the process. This situation affects the overall performance of the process with a final recovery of around 6% from capture to fill/finish.

The aim of this study was to improve the recovery in affinity chromatography step in the DSP operation and identify the main effects driving the elution behaviour.

Table 01. Mechanistic factors utilised to the execution of the first Design of Experiments (DoE) setup.

Factor	Range		
pH	2.0	2.7	3.4
Buffer system	Weak acid I (pK_a: +)	Weak acid II (pK_a: ++)	Strong acid (pK_a: +++)
Chaotropic salt	Average chaotropicity (±)	High chaotropicity (+)	Higher chaotropicity (+++)
Salt conc.	50 mM	250 mM	500 mM
Surfactant	0.00%	0.02%	0.20%
H_2O:Ethanol	100:0 (δ 48.0 $MPa^{1/2}$)	80:20 (δ 43.6 $MPa^{1/2}$)	70:30 (δ 41.5 $MPa^{1/2}$)

2. Mechanistic factors screening

To first gain understanding in the mechanisms governing the elution in the capture step, a Design of Experiments (DoE) study was performed to determine the main factors affecting the elution behaviour. The factors analysed with this DoE can be observed in Table 01.

The results of this DoE are highlighted in Figure 01. The model shows a good fit of the results obtained, with solvent use (Figure 01a), in the form of ethanol, as the most contributing factor of the Design, followed by the pH of the elution solution, and buffer system.

In Figure 01b can be seen that all the factors showing statistical significance for the model present good leverage goodness of fit, indicating that changes in them would influence the overall model greatly.

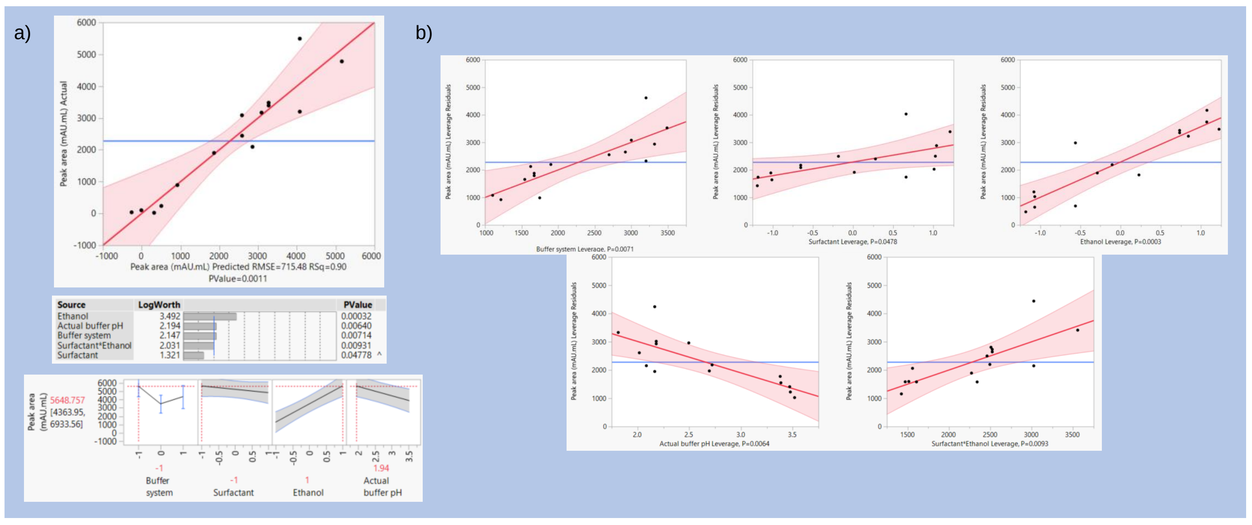

Figure 01. a) Model fit of the peak area of the chromatography runs performed in the DoE; b) Leverage residuals plots of the five main effects modulating the elution behaviour in capture chromatography.

3. Mass "imbalance"

When all the chromatography fractions from the mechanistic factors DoE were analysed, it was observed that the mass balance for capsids was closed in all the runs, with more than 95% of total VP quantified. However, less than half of the total genome copies were recovered in the elution on these runs, whereas on the rest of the chromatography phases these values remained constant (Figure 02).

It was theorised that if the genome copies were neither in the elution nor in any other process phase, there might be a disruptive mechanism releasing them outside the virions. To confirm this hypothesis, VG quantification by qPCR was repeated without the use of DNAse. It was found that repeating the qPCR without DNAse increased the genome copies in the elution, but not in the other phases, indicating that there is an unforeseen mechanism decreasing the yield in the capture process.

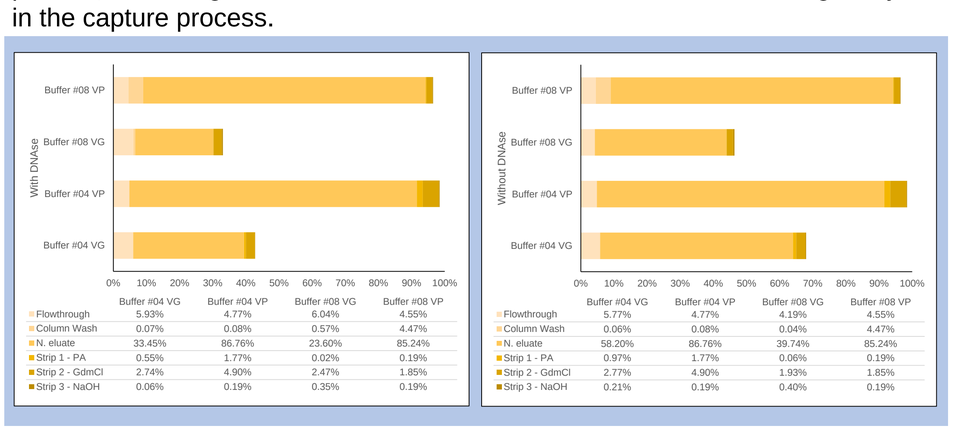

Figure 02. Mass balances of two conditions from the mechanistic factors DoE with and without DNAse, to evaluate the expulsion of the therapeutic genome from the capsid.

4. Genome escape

If the variables in the process are identically and independently normally distributed, we should expect a VG recovery like the VP recovery observed (e.g., $(\beta_0+\varepsilon_i)$ [VP%] ≡ $(\beta_0+\varepsilon_i)$ [VG%]). This trend was observed in all the other phases of the chromatography process, except in elution. Therefore, analytical error can be discarded as a possible explanation, and the discrepancies might be due to another factor affecting elution.

Thus, the genome escape mechanism has been suggested: expulsion of therapeutic DNA without capsid disassembly. When the virion is exposed to a given stress (i.e., low pH, chaotropic agents, salt-in effects), it needs to reach a state of lower entropy to avoid disassembly, thus ejecting the genome aids in decreasing the entropy of the virus system.

Genome escape is a time-independent variable, with an average VG loss of 45% across all timepoints. Escape happens regardless of the elution conditions. It was confirmed that escape is not a function of time, thus probably a "switch" mechanism might initiate it.

Figure 03b shows that there is a pH-dependent "switch" mechanism releasing VG from the capsids in at least two pH ranges.

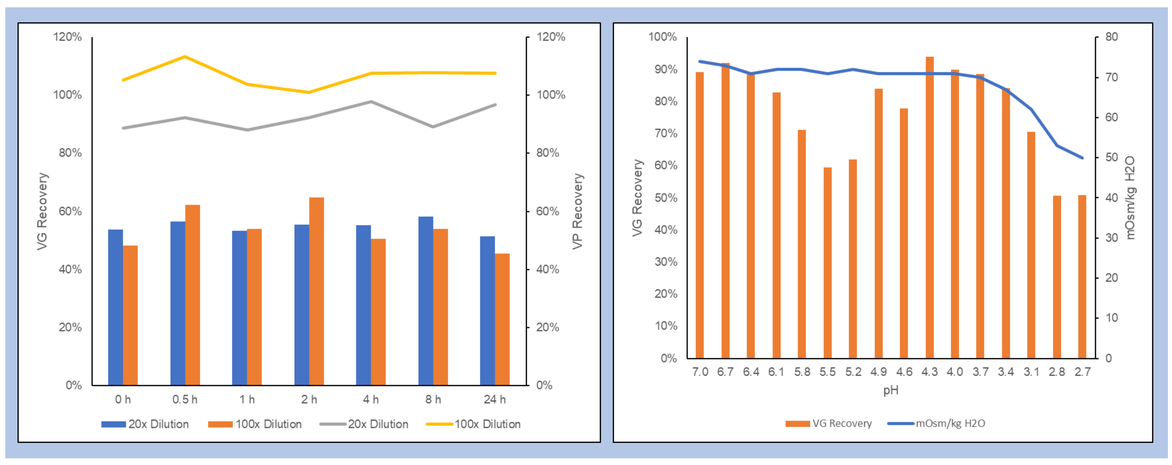

Figure 03. a) Time point study of AAV5-product at pH 2.7 to assess progressive escape of therapeutic genome from capsids; b) pH discrete gradient study of an AAV5-product to evaluate the hypothesis that genome escape is triggered by a switch mechanism.

5. Process conditions screening

With the learnings acquired from the first DoE and the description of the escape mechanism a second DoE was then executed, to characterise the process parameters affecting the elution of the AAV5 product (Table 02).

The findings from the previous DoE were confirmed with this second model, proving that solvent use, acid type, and pH were the main factors contributing to a yield increase (Figure 04a).

It was established that small changes in the pH led to a greater than two-fold increase in the recovery yield (Figure 04b). The results showed that small changes in pH of the elution buffer can increase genome copies recovery up to around 100% at pH 2.3 and lower. These small changes in pH were later confirmed with other therapeutic vectors in Figure 04c (AAV2-product "a" and AAV8-product).

Finally, the mechanistic knowledge acquired in this study was applied in the process development of two AAV2-products, named "b" and "c", (Figure 04d), showing that a pH drop of only a unit, the removal of stabilisers, and lower salt concentrations, increased the recovery 20% more compared to the previous iteration in both products.

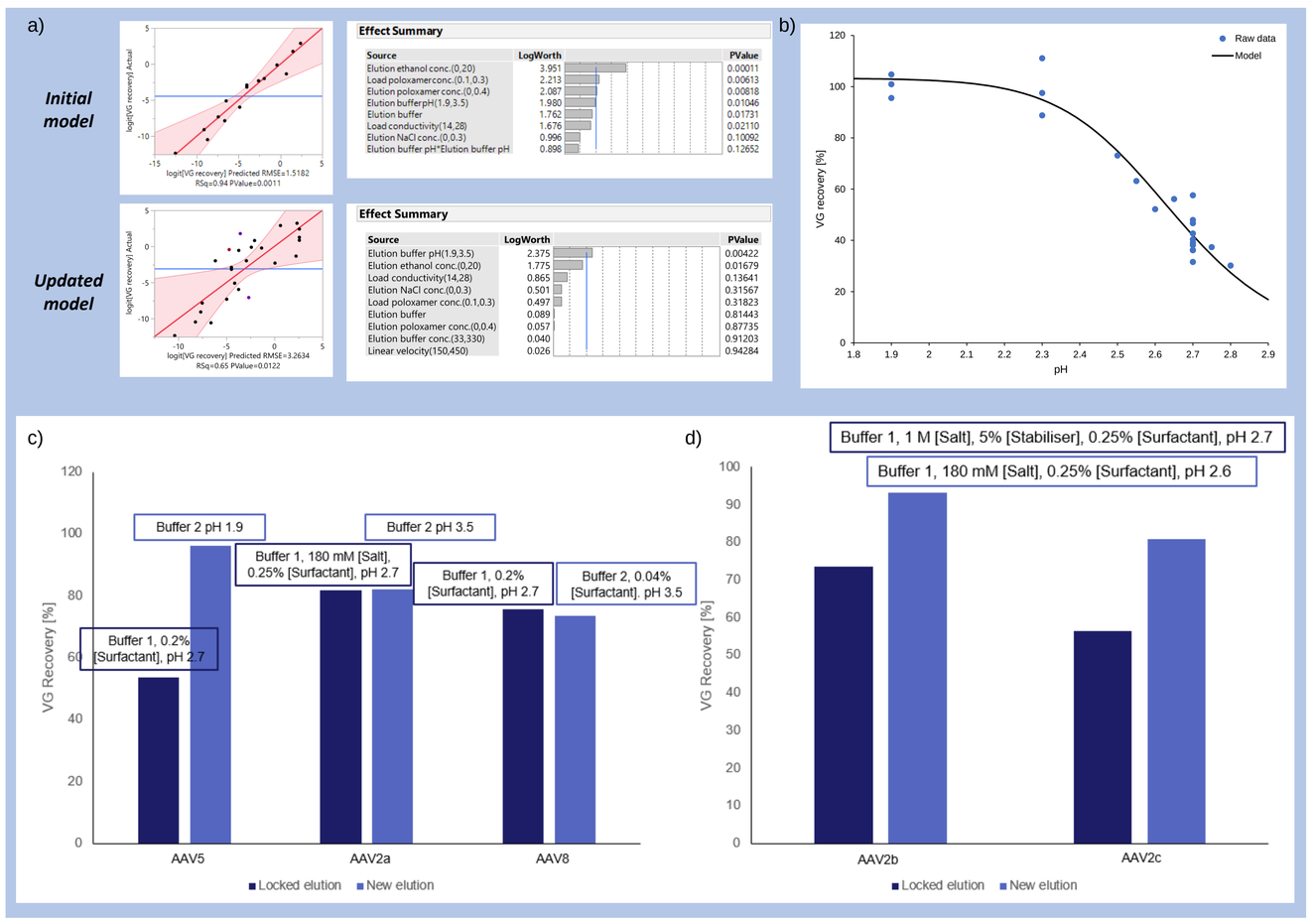

Figure 04. a) Initial DoE models of the VG recovery from the chromatography runs and updated model after an expansion design domain, and all the main factors contributing to the statistical significance; b) Small changes in pH can lead to increased recovery; c) Comparison between current locked conditions for three products, against new potential buffers exhibiting increased process, economic, and environmental performance; d) Comparison of current locked conditions and new small changes suggested to increase the yield of the capture step in two AAV2 products.

Table 02. Process conditions assessed in the second DoE performed

Factor	Range
Elution buffer pH	1.9, 2.3, 2.7, 3.1, 3.5
Linear velocity [cm/h]	150, 300, 450
Elution buffer	Buffer 1, Buffer 2
Elution buffer conc. [mM]	33, 181.5, 330
Load surfactant conc. [%]	0.2, 0.3, 0.4
Elution surfactant conc. [%]	0, 0.2, 0.4
Load conductivity [mS/cm]	14, 21, 28
Elution salt conc. [mM]	0.0, 150, 300
Elution ethanol conc. [%]	0, 10, 20

6. Conclusions

This work offers insights into the underlying mechanism of AAV elution from affinity chromatography, and how small changes in the process parameters can greatly increase the bulk yield of a therapeutic product. It also highlights a potentially novel ejection mechanism that can describe unexplained yield losses during the capture step.

Acknowledgements.

MSAT Downstream processing team for helping in the execution of this study, and MSAT Upstream processing team for producing the material used in the study.