Use of anion exchange chromatography in weak partitioning mode to provide high empty AAV capsid removal and product yields

Sian Davies and Jayan Senaratne

Meira GTx London

MEIRAGTx

Overview

- An anion exchange step to maximise full capsid enrichment for AAV-AQP1 (AAV2) was developed.
- The similar properties of the full and empty capsids led to a trade-off between product yield and full capsid ratio which made selecting optimal conditions difficult.
- Exploring the design space led to weak partitioning mode being established as a better alternative to bind-and-elute mode, and conditions that achieved the removal of **>50%** of the empty capsids were identified.
- As a result of the empty capsid removal, an eluate with a full capsid ratio of **>80%** (as confirmed by AUC) and a product yield **>50%** can be achieved.
- Understanding the design space led to the development of a robust and scalable process.

Key Results

Identifying Area of Interest

- Understanding how the parameters interact meant an optimal region could be identified, allowing the key process objectives to be achieved.

Alternative Mode of Operation

- Exploiting weak partitioning as an alternative to the typical bind-and-elute meant that almost **50%** of the empty capsids could be removed in the flow through alone. Coupled with a high NaCl wash, an eluate with **>80%** full capsids could be achieved.

1. Weak partitioning and where it is used

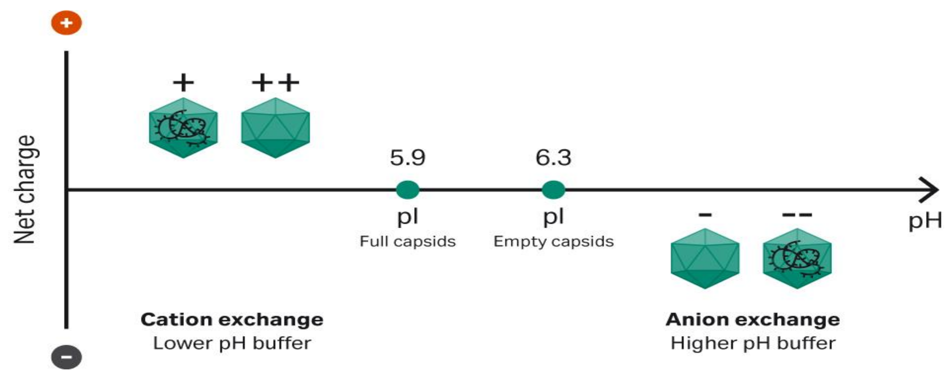

Figure 1. The charge of empty and full AAV capsids above and below the pI (1)

Weak partitioning describes the process of separating full capsids from empty capsids by creating an environment in which the binding of full capsids is more favourable.

- Anion exchange chromatography is a technique used to separate the full capsids from the empty capsids.
- This form of chromatography exploits the charge difference between capsids containing viral genome and those that are empty.
- Targeting pH values above the capsid pI means the VG containing full capsids carry a greater negative charge than the empty capsids.

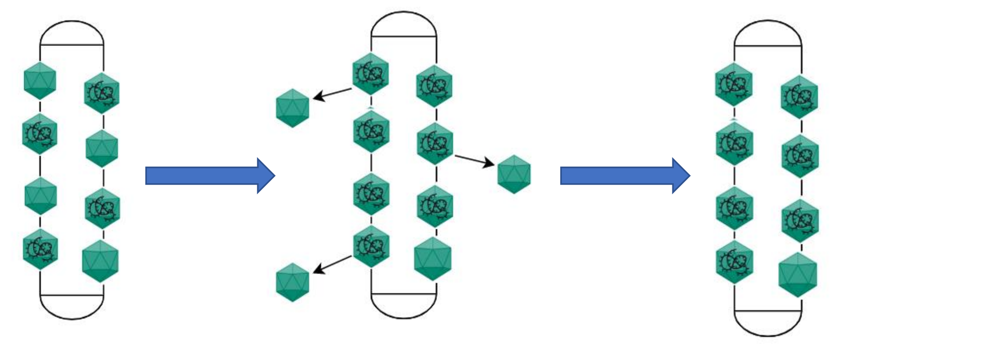

Figure 2: The weak partitioning process, where the empty capsids are replaced by full capsids due to the environment created

2. Initial design of experiments

Parameters and ranges for further optimization were identified from initial AEX platform-fit experiments.

Parameter	Range
Load challenge (VG/mL)	$5 \times 10^{13} – 1 \times 10^{14}$
Feed full capsid ratio (%)	<10
Eq./feed NaCl conc. (mM)	65 - 90
Wash 2 NaCl conc. (mM)	100 - 125
Elution NaCl conc. (mM)	200 - 300

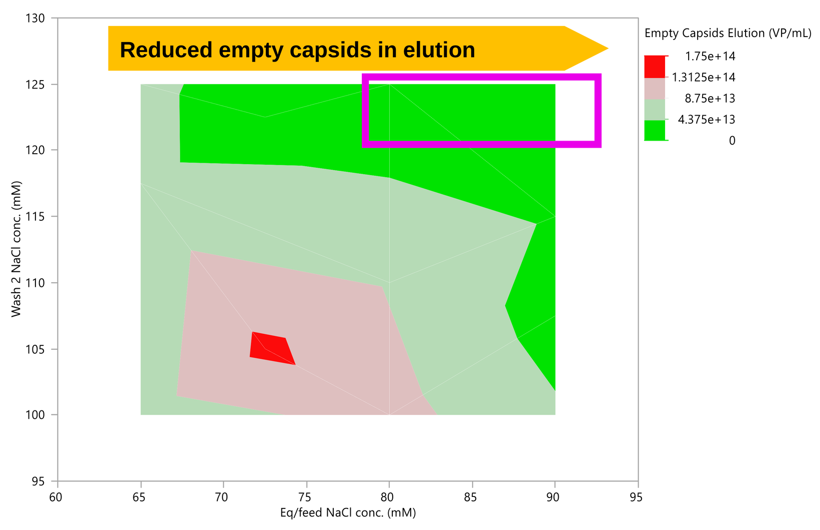

Figure 3: Relationship between wash 2 and eq. / feed NaCl concentrations (mM), and the empty capsids present in the elution (VP/mL)

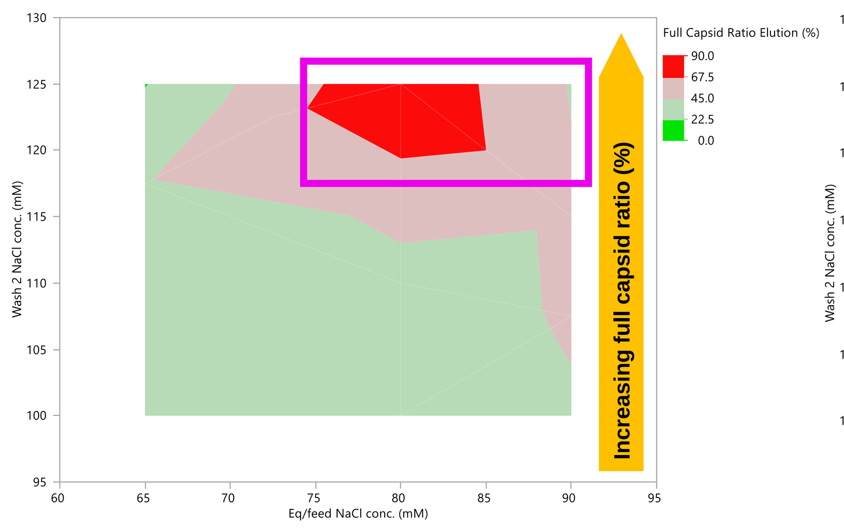

Figure 4: Relationship between wash 2 and eq. / feed NaCl concentrations (mM), and the full capsid ratio (%) in the elution

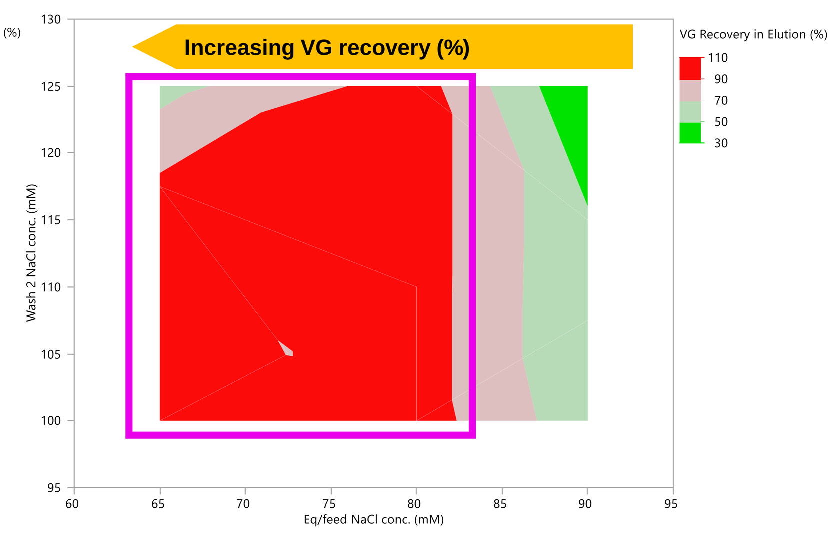

Figure 5: Relationship between wash 2 and eq. / feed NaCl concentrations (mM), and the VG recovery (%) in the elution

Results of DOE combined to highlight optimal region within the design space, in line with the desired outcome.

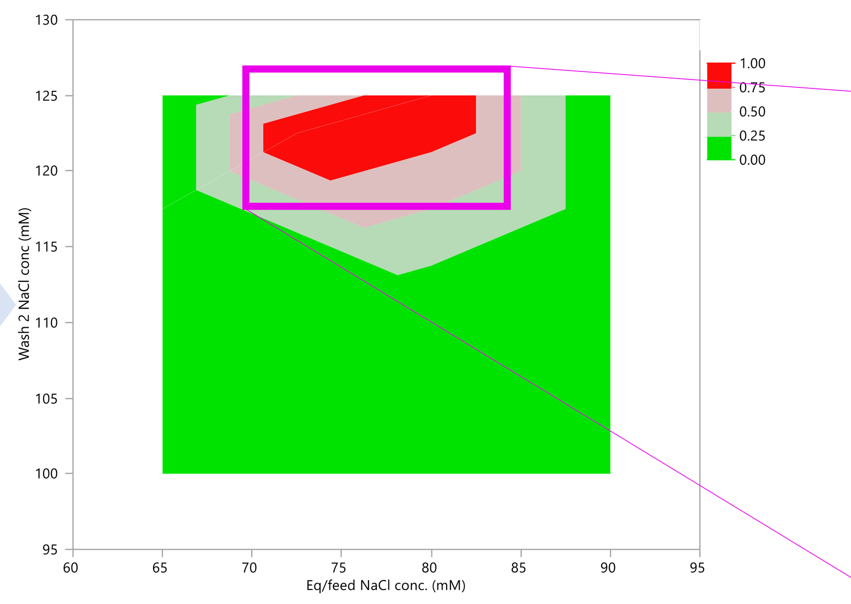

Figure 6: Optimal region based on the wash 2 and eq / feed NaCl concentrations (mM)

Parameter	Value
Empty capsid in flowthrough	Greater than 50%
Empty capsid removal in wash 2	Approx. 10%
VG recovery in elution	Approx. 50%
Full capsid ratio in the elution	Approx. 50%

3. Understanding how load ratio affects weak partitioning

The effects of weak partitioning are maximised by increasing the load ratio.

Parameter	Value
Load ratio (VG/mL)	1×10^{15}
Eq. / feed NaCl conc. (mM)	90

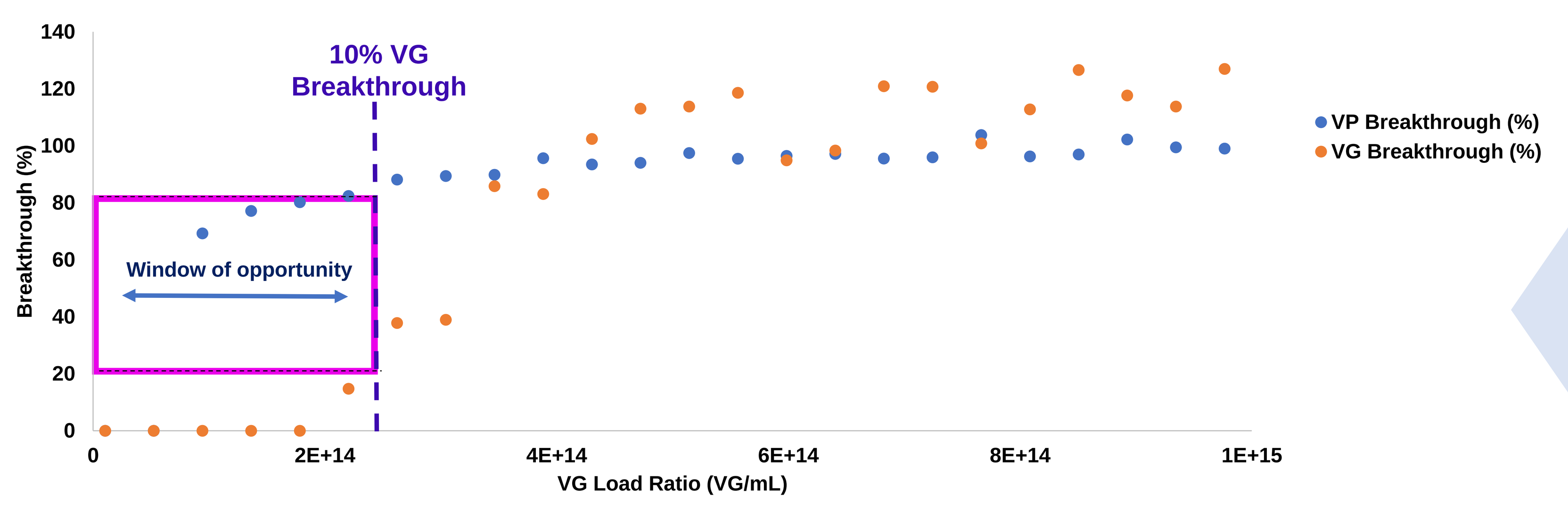

Figure 7: Relationship between load ratio, and full capsid (VG) and empty capsid (VP) breakthrough (%)

Exploiting the window of opportunity means up to 80% of empty capsids breaking through while only 10% of full capsids are lost in the flow through.

4. Collating findings

Parameter	Optimised Ranges
Load ratio (VG/mL)	1×10^{14}
Feed full capsid ratio (%)	31
Eq/feed NaCl conc. (mM)	90
Wash 2 NaCl conc. (mM)	125
Elution NaCl conc. (mM)	250

Figure 4 indicated that for the largest empty capsid removal, high NaCl concentration was required for both conditions.

Increasing the Wash NaCl causes VG losses of up to 30%, 50% VG recovery is still recovered in the elution phase.

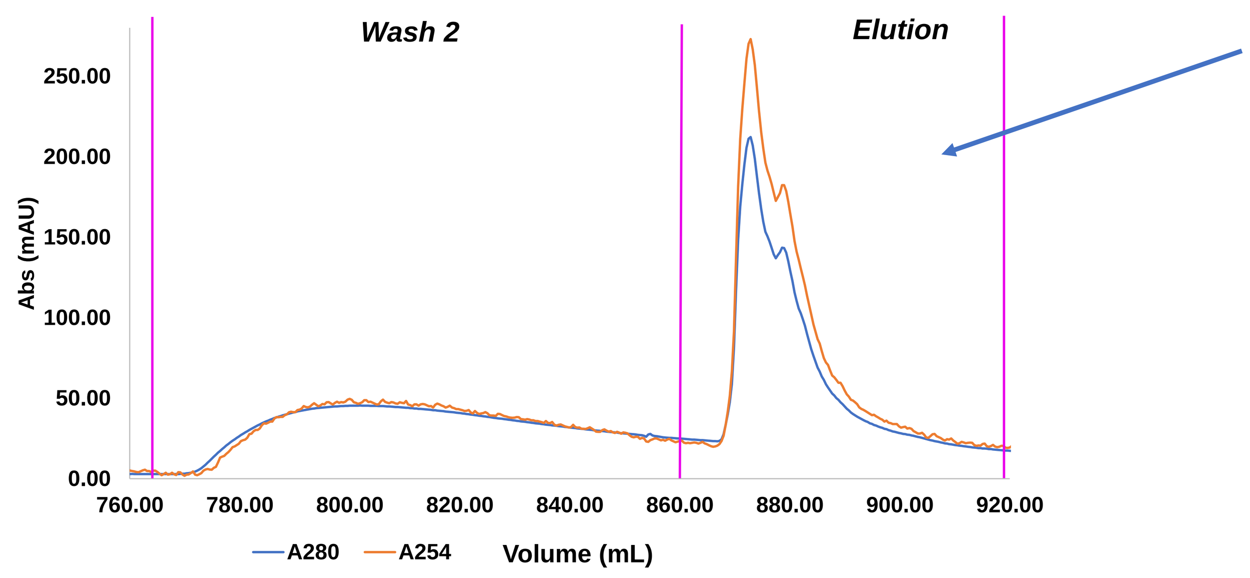

Figure 8: A chromatogram of an optimised AAV-AQP1 AEX step at 2 L scale

80% full capsid ratio highlighted by inversion

Results

Parameter	Value
Empty capsids in flowthrough (%)	>40%
Full capsids in flowthrough (%)	<1%
Full capsids in wash 2 (%)	33%
Empty capsids in wash 2 (%)	20%
VG recovery in elution (%)	53%
Full capsid ratio in elution (%)	81.5%

5. Confirmation by AUC

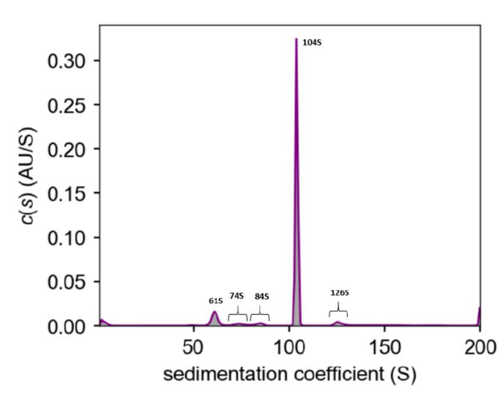

Figure 9: An AUC result confirming the elution peak in Figure 8 contains 81.5% full capsids

6. Proven scalability

The process is scalable from 2L to 80L for both the VG recovery and the full capsid ratio.

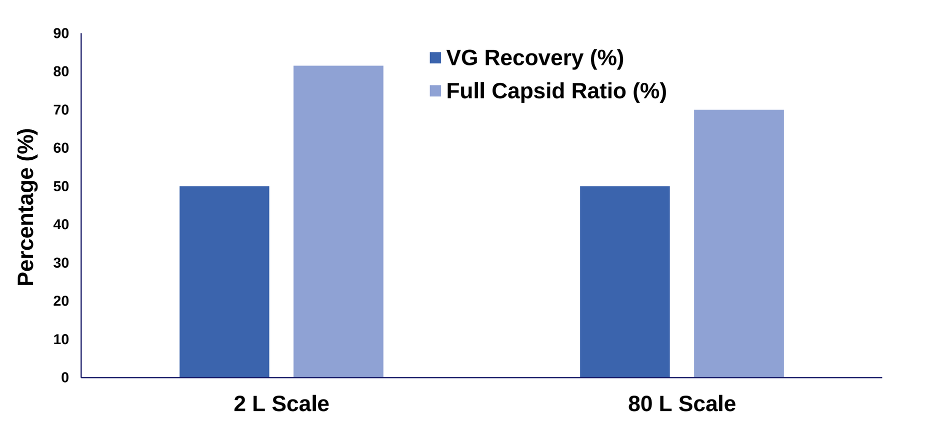

Figure 10: A comparison between the full capsid ratio and VG recovery of a 2 L and 80 L batch

Conclusion

Understanding how to exploit weak partitioning is vital to maximise the full capsid enrichment. When coupled with an understanding of the process 'sweet spot' a process with a full capsid ratio of greater than 80% is achievable for AAV-AQP1.

References

1. Cytiva. 2022. Enhanced AAV downstream processing - Cytiva. [online] Available at: https://www.cytivalifesciences.com/en/us/solutions/cell-therapy/knowledge-center/resources/enhanced-aav-downstream-processing.