Developing a Scalable Full Empty Separation Step for an AAV Capsid by Anion Exchange Chromatography in Step Gradient Elution Mode

R. Staffler, E. Tsiaousi, J. Weizenegger, J. Babic, B. Alkharrat, C. Weiss, M. Boscher, J. Wagner, S. Petrik, S. Ritter, C. Zach, M. Lott, T. Klötzler, A. Heinlein, M. Gora, A. Youssef, A. Schobert, M. Langhauser

Abstract

Packaging heterogeneity in recombinant adeno-associated virus (rAAV) manufacturing may lead to potential product-related impurities like 'empty' capsids, capsids containing partial vector genomes, and capsids containing different DNA impurities. It is technically very challenging to reduce or eliminate 'empty' capsids in down-stream processing, especially in a scalable manner. Here we describe a fully scalable method to efficiently enrich the percentage of full capsids by anion exchange chromatography.

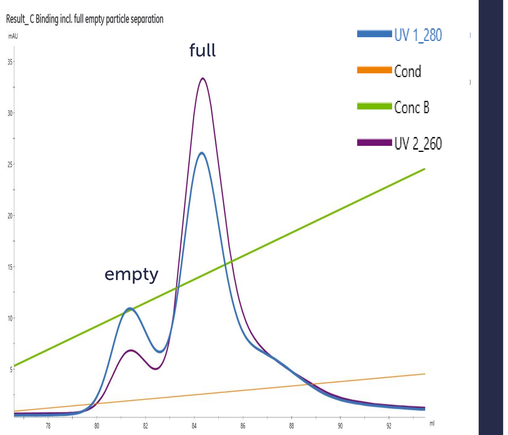
An anion exchange (AEX) chromatography step was established in isocratic elution mode and optimized by design of experiment (DoE) studies. After an initial screening of different resins, a strong anion exchanger was selected for further development in step gradient mode. DoE data were evaluated using chromatography UV and Stunner® (Unchained Labs) data. The DoE center point run results were confirmed by qPCR (recovery data) and mass photometry analysis (enrichment and percent full data).

Based on the DoE studies it was determined that the capsid load and salt concentration during the empty particle elution phase are the most critical process parameters to achieve a high percentage of full particles and a satisfactory yield. The scalability of the AEX step was demonstrated by polishing material from an entire 50-liter scale run applying the optimized step gradient conditions. The enrichment factor was dependent on the proportion of 'full' and 'empty' capsids in the load material. We obtained an up to 3-fold enrichment of full capsids compared to input material, or about 75% 'full' determined by mass photometry at a vector genome step recovery of ~70%.

The AEX chromatography process is designed to be optionally plugged into downstream processing depending on required product specifications. It is readily scalable and represents a tool that can be adapted for specific expression cassettes or capsids.

AEX screening

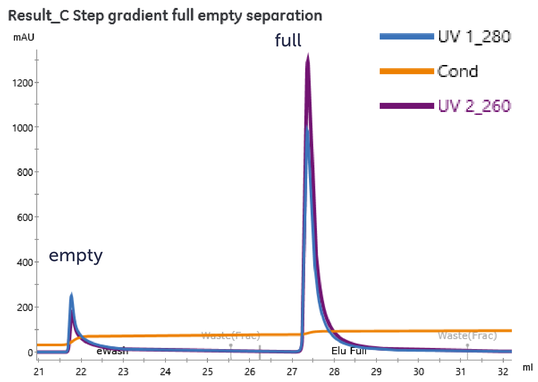
- Material purified by affinity chromatography was diluted 1:20 and applied to more than 20 different AEX resins/membranes/monoliths
- As elution a flat linear gradient with successive increasing salt concentration was used
- Different results were obtained:
  - A: No binding of capsids
  - B: Partial binding of capsids
  - C: Binding incl. visible full empty particle separation
  - D: Binding w/o or limited full empty particle separation
- One of the 7 exchangers that showed a C result was selected for further development work



Isocratic elution mode development and DoE study parameters

1. <u>Transform linear gradient into isocratic step elution mode for full empty separation:</u>
   - Two subsequent isocratic gradients with a constant salt concentration close to the conductivities of the elution peak maxima of the linear gradient runs were performed

2. <u>DoE study</u>
   - Unicorn® DoE tool
   - Optimization DoE with 11 runs
   - Input parameters of the DoE: c(NaCl) in mM of empty wash buffer & capsid load amount in E14 cp/CV
   - DoE Readout: vg-recovery and %-full (Stunner®)



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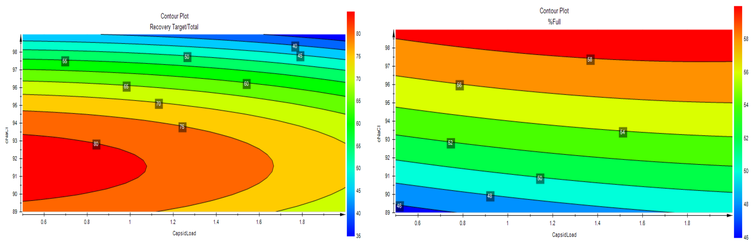
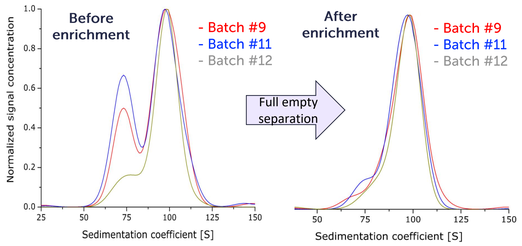
Conclusions DoE – Stunner® based evaluation



- No optimal range for maximum recovery and %-full at the same time → Final process conditions are a compromise between both output parameters
- Both input parameters impact the outcome in vg-recovery and %-full and should be controlled
- Vg-recovery of ~70% (Stunner®) and %-full of >52% (Stunner®), if salt concentration is 94 mM and loading is between 0.6 and 1.8E14 cp/CV

DoE Confirmation Runs & Analytics

AUC - Normalized sedimentation coefficient distributions*



*1st peak = empty, 2nd peak = partially and full particles

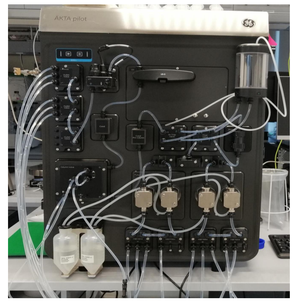
Clear qualitative difference (AUC) was observed by full empty separation

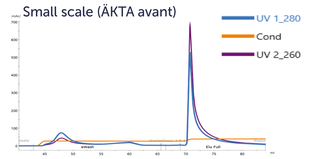
Batch	%-full Load	%-full Full eluate	Fold – enrichment	vg-yield
#6	28.0	57.1	2.03	73%
	37	74.5	2.01	74%*
#9	37.0	60.0	1.62	67%
	52	75	1.44	63%*
	45.89	61.97	1.35	
#10	12.0	37.2	3.10	72%
	16.4	*57.2*	*3.48*	90%*

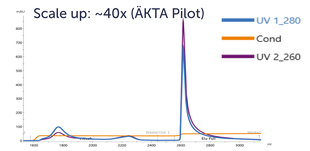
Stunner®/qPCR*/Refeyn mass photometry/cryoTEM

- The lower the %-full of the input material the higher the fold-enrichment (see table)
- Lower vg-yield obtained if the input material contains a higher proportion of full particles

Process Scale-up







Scale	%-full	Fold-Enrichment	Vg-recovery in %
Small scale	57.1±1.9	1.94	65±4 63±3*
~40x scale up	58.5±2.1	1.98	73±5
	70±14	2	69±8*

Stunner®/Refeyn mass photometry/qPCR*

Scalability was demonstrated until a scale of processing 50L of harvest sample

Ascend GCTx GmbH | Fraunhoferstraße 9b | 82152 Planegg | Germany

www.ascend-gctx.com
regina.staffler@ascend-gctx.com