On-the-Fly Optimization of Anion-Exchange Chromatography for High-Purity AAV Separation

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Introduction

Adeno-associated virus (AAV) vector purification remains a critical bottleneck in gene therapy manufacturing, particularly in achieving efficient separation of full and empty capsids. In this talk, we present the inAAVate™ platform for the development of anion-exchange chromatography (AEX)-based purification processes tailored to various AAV wild-type serotypes. By designing and evaluating multiple AEX resins and systematically tuning key process parameters, including gradient vs. isocratic elution, salt composition, column loading, residence time, and fractionation strategy, we demonstrate effective separation of full and empty capsids. Our approach yields up to 90% full capsid purity across most AAV serotypes, with some achieving complete removal of empty capsids (0% empty) as confirmed by analytical ultracentrifugation (AUC). Furthermore, we introduce a rapid optimization framework that enables rapid tuning of AEX conditions, shorten AEX development to days instead of weeks. This flexible, high-resolution platform supports efficient, scalable purification and sets a foundation for streamlined AAV manufacturing workflows.

Charge heterogeneity of AAV

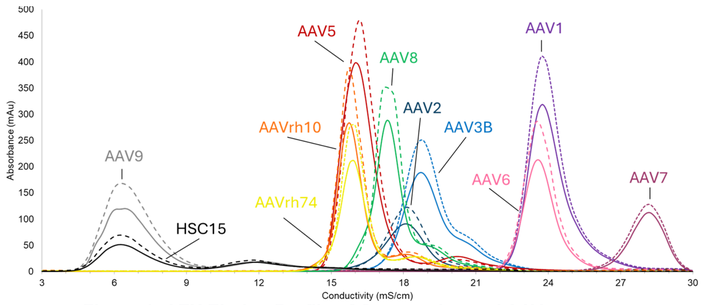

Figure 1: AEX Elution Profile of Commonly used AAV serotypes

- Charge profiles of AAV are vastly different
- Packaging efficiency varies from serotypes and the production systems used
- Having one process fit for all solution isn't practical to achieve consistent product purify profile

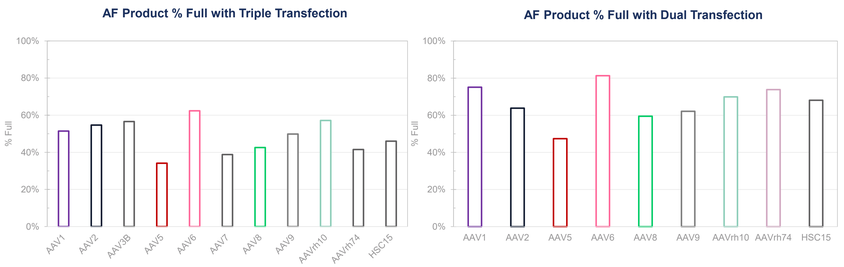

Figure 2: AAV Packaging Efficiency with Triple Vs. Dual Plasmid Transfection

Charge-based Separation is Serotype Dependent

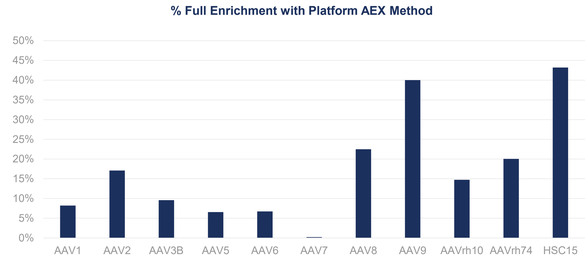

Figure 3: %Full Enrichment Using an Established AEX Separation method

- Charge difference between empty and full capsids varies from each serotype
- Certain serotypes demonstrated superior separation efficiency (e.g.AAV9) while others showed minimum enrichment through a standard AEX method (e.g.AAV7) ,

Building an AEX Optimization Toolbox

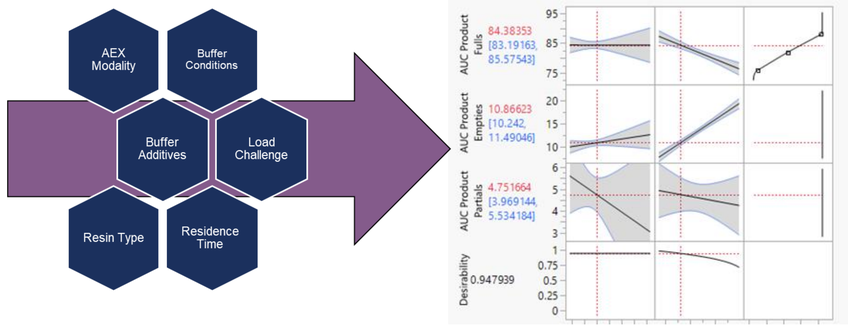

Figure 4: Identify Potential Levers to Improve AEX Separation

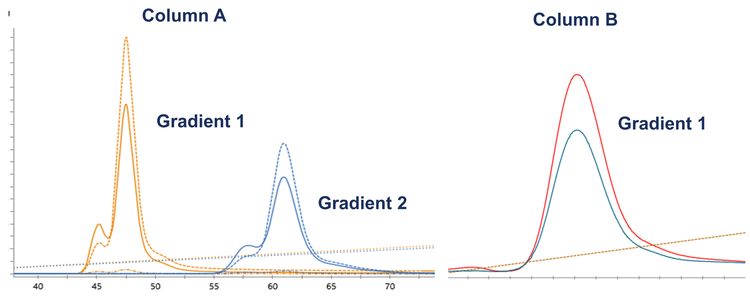

Figure 5: Comparison of buffer matrices and columns

- Both buffer matrices and column types affected resolution
- Other critical process parameters were evaluated for each serotype to understand the impact of separation efficiency

AEX Fine Tuning On-the-Fly

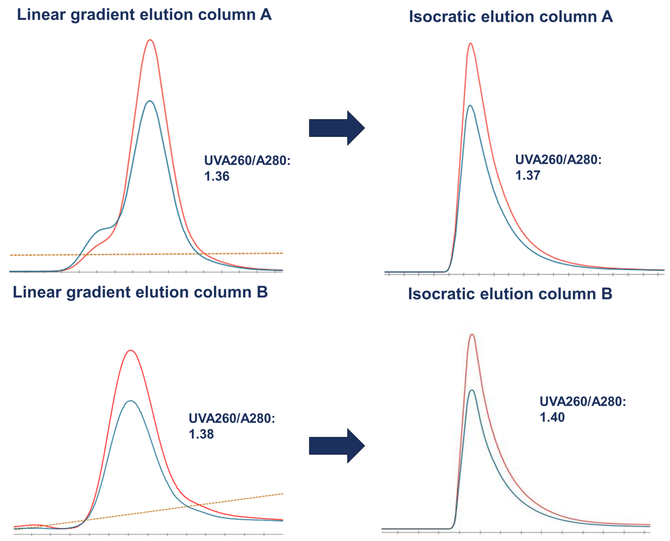

Figure 6: Finetuning isocratic elution using gradient elution profile

- Fine tuning critical AEX process parameters based on the physiochemical properties of AAV construct to achieve better separation

Conclusions

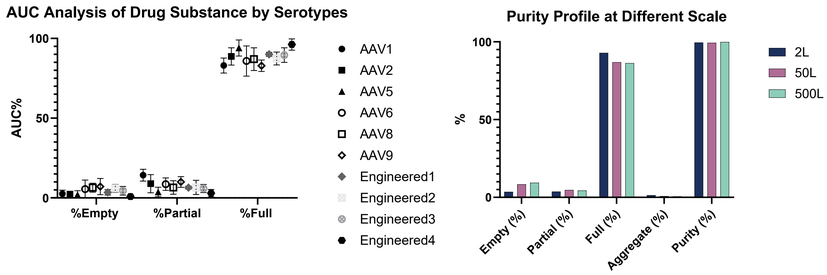

Figure 7: %Full purity summary of AAV serotypes and demonstration of scalability

- AAV charge profile is inherently heterogeneous as AAV serotype, GOI, production methods all affect the overall charge profile
- Therefore, charge-based separation using AEX chromatography requires process modification for every AAV construct
- The most efficient approach is to understand the correlations between different process elements, the physiochemical properties of capsid, and corresponding separation impact
- This knowledge enables rapid on-the-fly finetuning of AEX processes, achieving 80-90% purity for every construct

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