Separation of full and empty AAV capsids by AEX chromatography media with large pores

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Introduction

Adeno-associated virus (AAV) has emerged as a commonly used vector in gene therapy, with its application becoming increasingly widespread in recent years. However, challenges in the manufacturing process persist, notably the issue of empty particles that lack DNA in their capsids. Traditionally, CsCl density gradient ultracentrifugation has been the standard method for separating these empty particles. More recently, chromatographic methodologies has been chosen offering advantages such as scalability. In this study, we successfully purified AAV serotype 2 (AAV2) to a nearly 95% full particle ratio using anion exchange (AEX) chromatography employing monolith like particles (MLP) characterized by large pores.

Optimization of the base matrix

Our key technology for the purification of large molecules is the cellulose MLP, which features a large continuous pore structure. AEX chromatography resin based on MLP has demonstrated high capacity and superior resolution, depending on the pore size. The 50 µm diameter beads were selected to balance the resolution of the AAV empty and full capsids with their protein binding capacity.

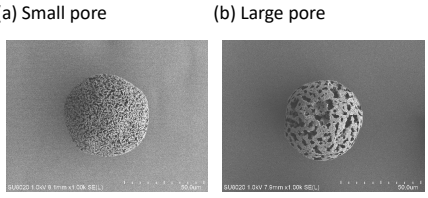

Figure 1. SEM images of MLP
Particle diameter is 50µm

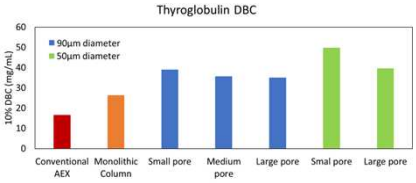

Figure 2. Comparison of thyroglobulin capacity
Protein has similar molecular size as AAV

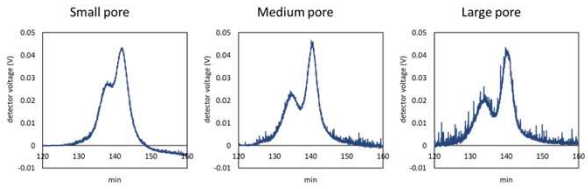

Figure 3. Impact of pore size on resolution of empty and full AAV2 capsids (90 µM bead size MLP)
Detection: multi angle light scattering (MALS)

Comparison with benchmarks

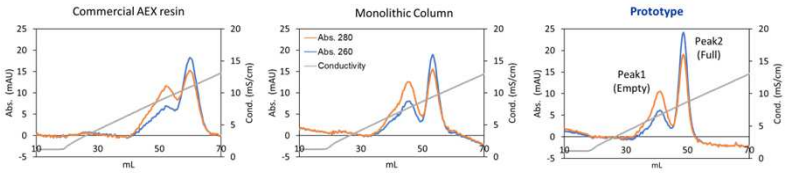

Figure 4. Comparison of chromatograms between MLP prototype and benchmarks

Column Vol	: 1.0 mL
Flow rate	: 1.0 mL/min (RT 1min)
Load	: AFF purified AAV2: 1.06 x 10^{12} vg
Equilibration buffer	: 50 mM Tris-HCl, pH 9.0 + 2 mM MgCl2
Elution buffer (B)	: 50 mM Tris-HCl + 150 mM NaCl + 2 mM MgCl2, pH9.0

Table 1. Comparison of quantitative results

		260/280		
	*Full (%)	Peak1	Peak2 (peak top)	Peak2 (Peak area)	Resolution
Before purified	15.9	-	-	-	-
Commercial AEX	-	0.60	1.20	-	-
Monolithic Column	42.8	0.64	1.22	1.19	0.83
Prototype	70.6	0.58	1.27	1.24	1.09

*Determined by Mass Photometry

✓ The prototype demonstrated superior separation of empty and full AAV2 particles compared to benchmarks.

Scale-up study (50L culture)

Following confirmation of efficient separation of empty and full AAV capsids with our MLP prototype, we conducted a purification study at a 50L culture scale. As shown in Figure 5, AAV2 production was initiated via triple transfection, followed by a pretreatment using a solubilizing and clarifying filter. The resulting crude solution was further purified using affinity (AFF) chromatography. Subsequently, our prototype was employed to eliminate empty particles. The purification's efficiency was assessed by qPCR for recovery and by AUC (analytical ultracentrifugation) to determine the ratio of full particles.

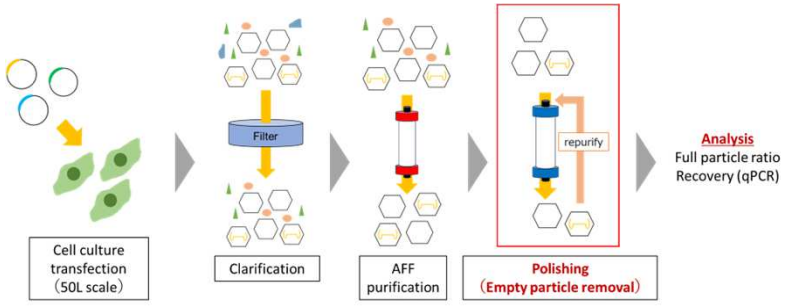

Figure5. Schematic workflow of the 50L scale-up manufacturing process of AAV2

Column	: HiScale 26 (ID 2.6 cm, 5.6 cm L) 30 mL
Flow Rate	: 7.5 mL/min (RT 4 min, 84.8 cm/h)
Load	: AAF purified AAV2 (2.08 x 10^{14} vg)
Equilibration buffer	: 50 mM Tris-HCl + 2 mM MgCl2, pH9.0
Elution buffer (B)	: 50 mM Tris-HCl + 90 mM NaCl + 2 mM MgCl2, pH9.0
Elution buffer (EL2)	: 50 mM Tris-HCl + 1.0 M NaCl + 2 mM MgCl2, pH9.0

※All kinds of buffer contain 0.1 % Poloxamer-188 (Pluronic F68)

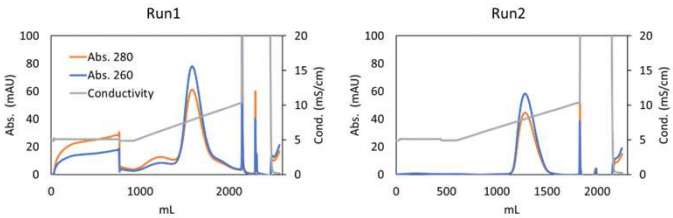

Figure 6. Chromatograms from the 50L scale-up process
The elution fraction collected from Run1 was then re-loaded onto the same column for Run2.

(a) qPCR recovery

	Recovery (%)
Run1	91.1*
Run2	97.4

*Correction value

(b) Full particle ratio (AUC)

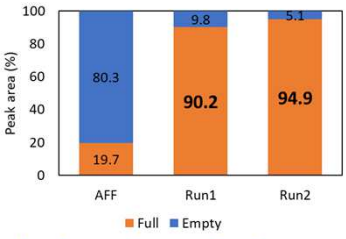

Figure 7. Analysis of fractions from scale-up purification

✓ We achieved a final full particle ratio of nearly 95% in the 50L scale-up process.

Conclusions

- The separation and adsorption capacity of AAV was optimized by varying the pore size of the MLP.
- MLP based AEX resin demonstrated high capacity and superior separation compared to benchmark.
- In the 50L scale-up culture, we could achieve nearly a 95% of full particle ratio and confirmed high recovery rates.

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