TOSOH

AEX polishing to separate full AAV from empty capsids

Your Challenge

- You need to remove empty capsids from your AAV preparation.
- Your preparative ultracentrifugation method is time-intensive and limited in scale.

Our Solution

SkillPak 1 TOYOPEARL GigaCap® Q-650S

- Handy column format for easy process development.

What was done?

- Optimized AEX process using non-toxic choline chloride as the eluting salt.

What was the result?

- Achieved full AAV content of 93%, comparable to preparative ultracentrifugation.

This rapid AEX chromatography polishing method is non-toxic, scalable, and adaptable to your serotype and purity requirements.

Your Benefit

You can rapidly remove empty capsids to a final full content of 90% or greater.

https://separations.us.tosohbioscience.com//process-development/id-8929/SkillPak_TOYOPEARL_GigaCap_Q-650S_1_mL_columns_qty__5_

Purification of adeno-associated virus (AAV)

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Application Note

Improved AEX Polishing of AAV Vectors Using Choline Chloride Gradients

Introduction

Recombinant adeno-associated virus (AAV) is used as a nucleic acid delivery vehicle in gene therapy. Recently, there has been an emphasis on maximizing full AAV content that has a complete genomic payload to improve AAV product potency and safety. Preparative ultracentrifugation is a commonly used non-chromatographic purification technique for the enrichment of full AAV capsids. However, it has scalability limitations, requires significant electrical power consumption, and is time intensive.

The differential charge between empty and full AAV, due to the encapsidated single-stranded DNA (ssDNA) genome, can be exploited as a means of separation. The negative charge of the ssDNA lowers the pI from 6.3 for an empty AAV particle to 5.9 for a full AAV virion. Therefore, full AAV has a higher affinity for AEX resin and requires a higher conductivity to elute the particle, thereby separating it. AEX has been used as a polishing step for full AAV previously, typically using sodium chloride; however, this usually results in significant peak overlap and loss of full capsids.

Choline chloride, a simple quaternary ammonium salt, is known to have significantly enhanced empty/full AAV separation ability in analytical AEX chromatography as compared to sodium chloride (Kurth et al. 2024). We show here that using choline chloride as the eluting salt on a SkillPak™ TOYOPEARL GigaCap® Q-650S preparative column enhances empty/full AAV separation to a degree comparable to that of preparative ultracentrifugation.

Experimental Conditions

The method described below has been optimized for AAV8. Because AAV capsid protein sequence identity between serotypes can be as low as 60%, a step elution method optimized for one serotype may not be optimal for others. For other serotypes, starting with a linear gradient from 0 mmol/L to 500 mmol/L choline-Cl is recommended, followed by process refinement with successive steps optimized for elution as necessary.

Preparative Anion Exchange Chromatography

Column:	SkillPak 1 TOYOPEARL GigaCap Q-650S, 7 mm ID × 2.5 cm		
Mobile phase:	A: 20 mmol/L bis-tris propane/HCl, pH 9.0, 0.001% poloxamer 188		
	B: 20 mmol/L bis-tris propane/HCl, pH 9.0, 0.5 mol/L choline chloride, 0.001% poloxamer 188		
Gradient:	Equilibrate:	5 CV	100% A
	Load:	typically ≥ 1 mL	
	Wash 0:	10 CV	0% B
	Wash 20:	20 CV	20% B
	Wash 25:	20 CV	25% B
	Elution (Full AAV):	5 CV	40% B
	Regeneration:	10 CV	100% B
Flow rate:	Equilibration:	1.9 mL/min	
	Load and Wash 0 steps:	0.2 mL/min	
	Wash 20, Wash 25:	1.9 mL/min	
	Elution step:	0.5 mL/min	
	Regeneration:	1 mL/min	
Detection:	UV @ 280 nm; UV @ 260 nm		
Temperature:	Ambient		
Sample:	Total of 10^{12} - 10^{14} affinity-purified AAV8 capsids diluted with mobile phase A + 2.5 mmol/L $MgCl_2$ (or alternative dilution buffer) to 1.0-1.5 mS/cm conductivity and pH ≈ 9.0, filtered through 0.2 µm PVDF or PES filter		

*Example of alternative dilution buffer: 20 mmol/L bis-tris propane base, 2.75 mmol/L $MgCl_2$, 0.001% poloxamer 188

SkillPak 1 TOYOPEARL GigaCap Q-650S is a prepacked column of 1 mL volume for preparative AEX chromatography on low-pressure liquid chromatography systems. The stationary phase consists of a hydroxylated methacrylic polymer HW-65 resin bead with a mean particle size of 35 µm and a mean pore size of 100 nm; the bead is functionalized with quaternary ammonium as a strong anion exchanger. The resin is packed in a polypropylene housing with standard 10-32 fittings.

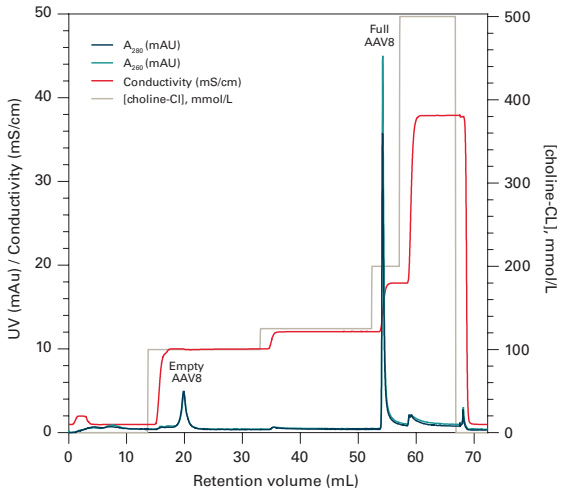
Analytical Anion Exchange Chromatography

Column:	TSKgel® Q-STAT (4.6 mm ID × 10 cm, 7 µm)
Mobile phase:	A: 20 mmol/L Tris-HCl, pH 9.0
	B: 20 mmol/L Tris-HCl, pH 9.0, with 1.0 mol/L choline-Cl
Gradient:	10 - 35 % B in 20 min, 100 % B for 5 min, 10 % B for 5 min
Flow rate:	1 mL/min
Detection:	UV @ 260 nm & UV @ 280 nm, Fluorescence Excitation 280 nm; Emission 350 nm

Results and Discussion

Affinity-purified AAV8 was loaded onto the SkillPak 1 TOYOPEARL GigaCap Q-650S column. *Figure 1* illustrates a typical chromatogram with two elution peaks to separate full AAV8 capsids from empty capsids. Monitoring absorbance at 260 nm and 280 nm enables identification of the predominantly full capsids peak. An A_{260}/A_{280} ratio of 1.3 indicates a predominantly full peak; an A_{260}/A_{280} ratio of 0.6 would suggest the peak is purely protein, with no encapsidated DNA, thus indicating empty capsids. A ratio between 0.6 and 1.3 would indicate partially filled capsids or a blend of empty and full particles. For AAV8, the full peak appears at 200 mmol/L choline-Cl. The column is usually regenerated with mobile phase B and re-equilibrated. For a more thorough cleaning-in-place (CIP) procedure, 0.5 mol/L NaOH may be applied with a 30-minute contact time, then neutralized.

Figure 1. AEX purification of full AAV8.



An elution peak at 56 mL, predicted to contain predominantly full AAV8 capsids (A_{260}/A_{280} peak area ratio = 1.27), was collected and analyzed by AEX chromatography on a TSKgel Q-STAT column[1]. The results are shown in *Figure 2*. Compared to a commercial preparation of full AAV8 purified via preparative ultracentrifugation, the choline chloride purification on TOYOPEARL GigaCap Q-650S was able to deplete empty AAV8 capsids to a fully comparable degree, resulting in a 93% full AAV8 sample *(Table 1)*.

Figure 2. Analytical AEX of affinity-purified (A), AEX-polished (B), and commercial full AAV (C).

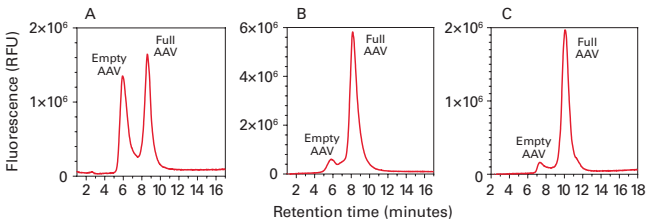


Table 1. Quantification of empty and full AAV peaks by analytical AEX column.

	Affinity-Purified AAV8	After AEX Polishing	Commercial
% Empty	48	7	7
% Full	52	93	93

Conclusions

This novel choline chloride-based AEX polishing method on TOYOPEARL GigaCap Q-650S provides a significantly enhanced solution for AAV full capsid enrichment. The step gradient can be adapted to meet diverse AAV serotype needs and material specifications. Choline chloride is an established excipient in the pharmaceutical industry with multiple applications and is included in the US FDA Generally Recognized as Safe (GRAS) list. The SkillPak column format makes process development and scale up rapid and straightforward. At the 1 mL scale, this process typically takes less than 2 hours starting from an affinity-purified AAV sample. This standardizable and scalable method reduces costs compared to preparative ultracentrifugation, which is labor-intensive, introduces operator variability, and has limited scalability.

Reference

1) Kurth, S., Li, T., Hausker, A., Evans, W. E., Dabre, R., Müller, E., Kervinen, J. Separation of Full and Empty Adeno-Associated Virus Capsids by Anion-Exchange Chromatography Using Choline-Type Salts. *Anal. Biochem.* **2024**, 686, 115421. DOI: 10.1016/j.ab.2023.115421

Featured Products

Part #	Description
0045274	SkillPak 1 TOYOPEARL GigaCap Q-650S (1 mL)
0045290	SkillPak 5 TOYOPEARL GigaCap Q-650S (5 mL)
0022881	TOYOPEARL GigaCap Q-650S Process Media (25 mL)
0021961	TSKgel Q-STAT, 7 µm, 4.6 mm ID x 10 cm Length