Effectiveness of Large-Pore Chromatography Media in AAV Empty/Full Separation: Cellufine MLP DexQ

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Abstract

JNC has developed a novel large-pore cellulose bead, “Cellufine MLP.” These beads are composed of highly crosslinked cellulose with large through-pore structures that enable intraparticle convective transport. As a result, even large targets such as viral particles can fully access the interior of the beads. Cellufine MLP DexQ is an anion-exchange medium whose surface is modified with a quaternary ammonium ligand. In this poster, we provide detailed insights into the development of MLP DexQ and explore its potential applications in adeno-associated virus (AAV) purification processes.

1 Introduction to Cellufine MLP DexQ

Bead structure

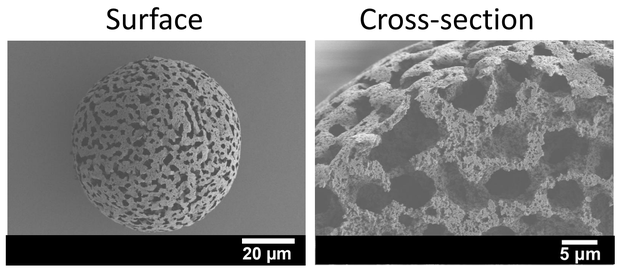

Fig. 1. SEM Morphology of Cellufine MLP

The through-pores of Cellufine MLP were estimated to have a mode pore radius of 1.5 µm using mercury porosimetry [1]. The average particle diameter of MLP DexQ is approximately 55 µm.

Flow property

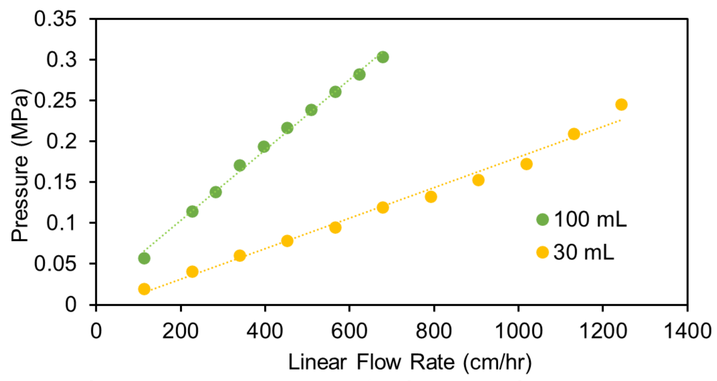

Fig. 2. Flow-pressure curve (subtracted system pressure)

Column: HiScale 26/20, 26/40 (ID 2.6 cm), Height: 6.4 cm (34.0 mL), 18.5 cm (98.2 mL), Mobile phase: Milli-Q water, Temperature: Ambient (20-25 °C)

A linear flow-pressure relationship was observed up to a 100 mL column volume so far.

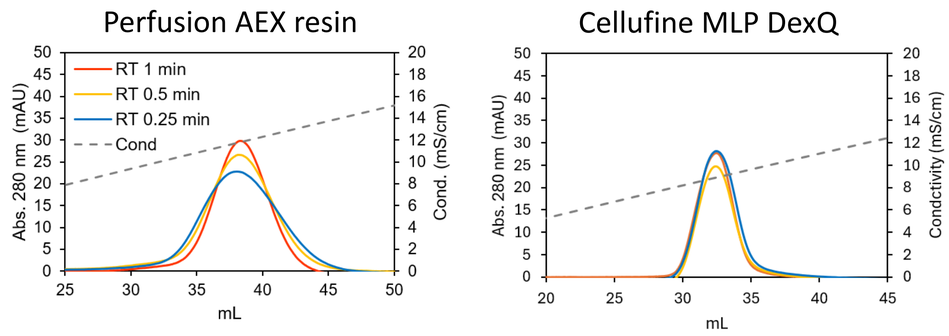

Fig. 3. Flow-rate dependence of purified AAV2 full particle elution peak shape

Unlike perfusion AEX resin, Cellufine MLP DexQ showed no flow-rate dependence in peak shape, indicating that mass transfer is governed by convection rather than diffusion, enabling use at high flow rates for large molecules such as AAV.

2 Comparison of AAV2 Empty/Full Particle Separation

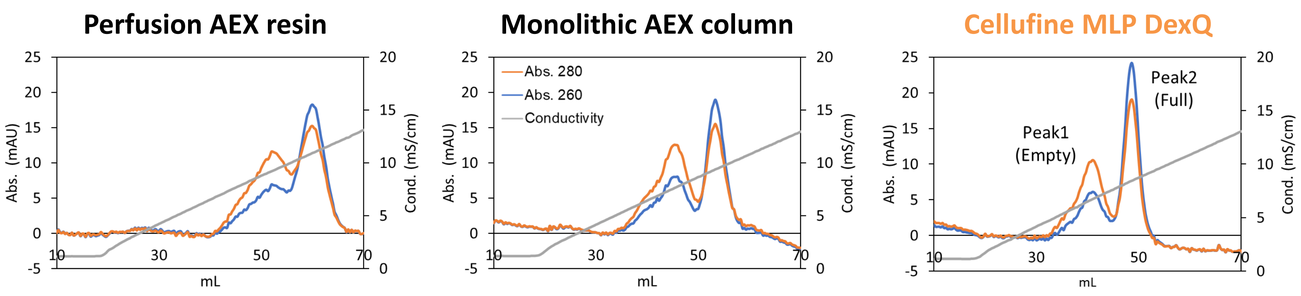

Fig. 3. Comparison of chromatograms from empty/full separation of AAV2

Column : Super Edge (ID 6.7mm x 30 mm) 1.06 mL
Flow rate : 1.0 mL/min (residence time: 1 min)
Load : Affinity purified AAV2 (1.06 x 10^{12} vg)
Equilibration buffer : 50 mM Tris-HCl, pH 9.0 + 2 mM $MgCl_2$
Elution buffer : 50 mM Tris-HCl + 150 mM NaCl + 2 mM $MgCl_2$, pH9.0

Table 1. Quantitative comparison of AAV2 empty/full separation on different media

		260nm/280nm UV absorbance ratio			
	Full*1 (%)	Peak1	Peak2 (peak top)	Peak2 (Peak area)	Resolution
Load	15.9	-	-	-	-
Perfusion AEX resin	-	0.60	1.20	-	-
Monolithic AEX column	42.8	0.64	1.22	1.19	0.83
Cellufine MLP DexQ	**70.6**	0.58	**1.27**	**1.24**	**1.09**

*1 Determined by Mass Photometry

Cellufine MLP DexQ demonstrated better separation of empty and full particles of AAV2 compared with the benchmarks.

3 Optimized and Scale-up Demonstration

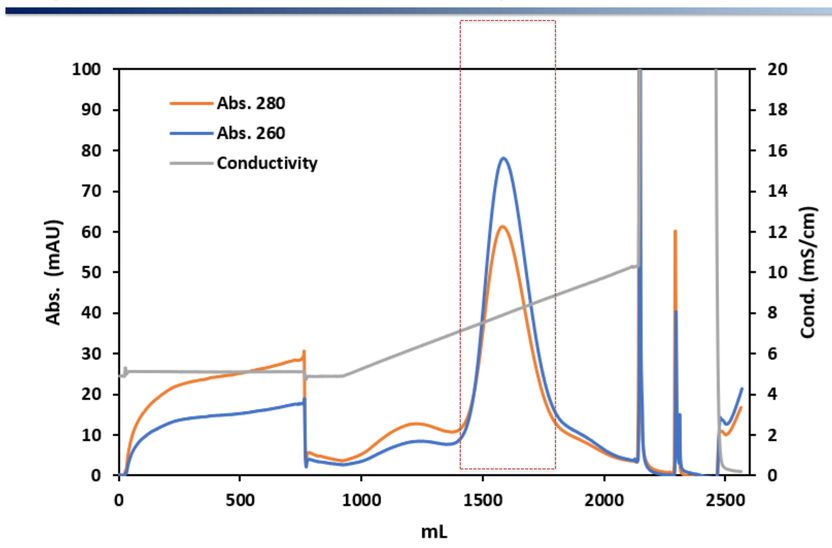

Fig. 4. Chromatogram of AAV2 purification in a 30 mL column process

Column : HiScale 26 (ID 2.6 cm x 5.6 cm) 30 mL
Flow Rate : 7.5 mL/min (residence time: 4 min; 84.8 cm/h)
Load : Affinity purified AAV2 (2.08 x 10^{14} vg)
Equilibration buffer : 50 mM Tris-HCl+ 2 mM $MgCl_2$, pH9.0
Elution buffer : 50 mM Tris-HCl + 90 mM NaCl+ 2 mM $MgCl_2$+ 0.1% poloxamer-188, pH9.0

Table 2. Fraction analysis from AAV2 scale-up purification

	Full particle ratio*2 (%)	Recovery rate*3 (%)
Load	19.7	-
Elution	**90.2**	**91.1**

*2 Determined by AUC (analytical ultracentrifugation)
*3 qPCR, correction value based on mass balance

We achieved a full-particle ratio of nearly 90% in the scale-up process using Cellufine MLP DexQ.

4 Conclusion & Key takeaways

✓ **Cellufine MLP is a novel large-pore, cellulose-based medium that is suitable for large biomolecules such as viral vectors.**
✓ **Cellufine MLP DexQ efficiently separates AAV2 empty and full capsids, achieving a full-particle ratio of > 90%.**

Reference
[1] K. Kadoi, E. Iwamoto, T. Nakama, Fabrication and characterization of a cellulose monolith-like particle for virus purification, *Biochem Eng J.* 192 (2023) 108849.

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