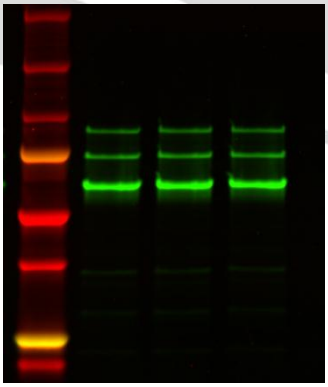
BIA separations

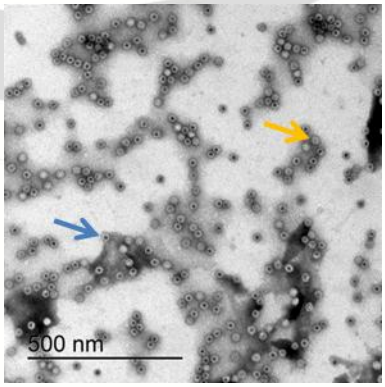
CIM Convective Interaction Media®

APPLICATION NOTE

A048 Chromatographic separation of full and empty AAV8 capsids

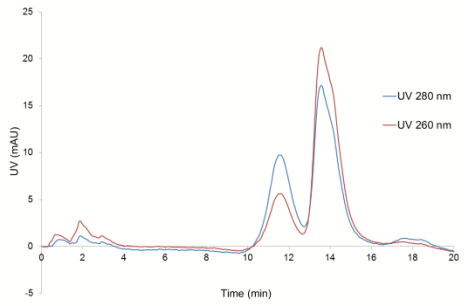
Adeno-associated virus (AAV) vectors of various serotypes are considered to have high potential for gene therapy applications. Currently, manufacturing of AAV vectors faces the challenge of co-production of incompletely formed particles lacking a recombinant viral genome. Empty capsids increase the dose of total AAV administered for efficient transduction and are thought to cause unwanted immunological reactions against the virus. Removal of empty capsids during manufacturing, as well as analysis of empty/full AAV particle content is therefore a critical requirement for any AAV production process. This Application Note demonstrates how CIMmultus QA monolithic columns can be used to remove empty AAV capsids from the product chromatographically in a single step.

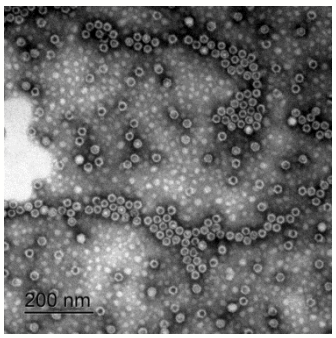




AAV8-containing cell lysate (HEK 293T; 3 freeze-thaw cycles) was benzonase-treated, filtered (0.45µm) and loaded onto AVB Sepharose (1 mL). AAV8 was eluted with 50mM Glycine pH 2.7 and neutralised by addition of Tris pH 8.8 (30 mM final concentration). AAV8 was then buffer exchanged into PBS supplemented with 2.5mM KCl and 1mM $MgCl_2$ until further treatment.

Figure 1: SDS-PAGE analysis of affinity-purified AAV8 shows the presence of VP1-3 (left). EM analysis reveals the presence of full (yellow arrow) and empty capsids (blue arrow).





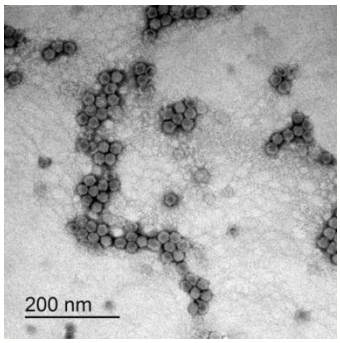


Figure 2: FPLC chromatogram and EM images of CIM® monolith ion exchange chromatographic separation of full empty and full AAV8 capsids using a linear gradient of NaCl. When 1.29E+12 GC of AAV8 (affinity-purified) were loaded onto CIMmultus QA (1 mL), 1.05E+12 GC were recovered in the second peak (80% recovery). EM images demonstrate enrichment of empty capsids in peak 1 (middle pannel) and of full capsids in peak 2 (right pannel). UV absorbance was monitored at 280 and 254 nm. Note the difference in 260/280nm ratios, suggesting a difference in DNA content. GC: genome copies

CONCLUSIONS:

A rapid method for separation of empty and full AAV8 particles by linear gradient elution on CIMmultus QA monoliths with 80% recovery is demonstrated.

MATERIALS AND METHODS

Anion-exchange capsid separation

Column:	CIMmultus® QA Monolithic column, bed volume 1 mL
Mobile phases:	Buffer A: 20 mM Bis-Tris propane (BTP), pH 9.0, Buffer B: 20 mM BTP, pH 9.0, 1 M NaCl
Flow rate:	3 mL/min
Gradient elution method:	Wash after load: 10 columns volumes (CV) buffer A Linear gradient: 0-200 mM NaCl, 60 CV High salt wash: 1M NaCl in 20 mM BTP, pH 9.0 for 10 CV
Sample:	All samples diluted in 1 mL buffer A
Sample loop:	1 mL
Detection:	UV detection, 280 nm and 254 nm

Electron Microscopy

Ion-exchange purified AAV8 capsids were examined under TEM using the negative staining method. Twenty microliters of fractions were applied on formvar-coated and carbon-stabilized copper grids (400 mesh) at room temperature for 5 min and stained with 1% uranyl-acetate (SPI Supplies, West Chester, PA, USA). The samples were observed using a Philips CM 100 transmission electron microscope operating at 80 kV, and images were acquired with an ORIUS SC200 CCD camera using Digital Micrograph Software (Gatan Inc., Pleasanton, CA, USA)

CIM monoliths for preparative-scale capsid separation

312.5113	CIMmultus™ QA-1 Advanced Composite Column (Quarternary amine)	1 mL
412.5113	CIMmultus™ QA-8 Advanced Composite Column (Quarternary amine)	8 mL
612.5113	CIMmultus™ QA-80 Advanced Composite Column (Quarternary amine)	80 mL
911.5113	CIMmultus™ QA-80 Advanced Composite Column cGMP compliant (Quarternary amine)	80 mL / cGMP
812.5113	CIMmultus™ QA-800 Advanced Composite Column (Quarternary amine	800 mL
921.5113	CIMmultus™ QA-800 Advanced Composite Column cGMP compliant (Quarternary amine)	800 mL / cGMP
1012.5113	CIMmultus™ QA-8000 Advanced Composite Column (Quarternary amine)	8 L
931.5113	CIMmultus™ QA-8000 Advanced Composite Column cGMP compliant (Quarternary amine)	8 L / cGMP

CIMac monoliths for QC analysis of empty/full capsid ratio

110.5113-1.3	CIMac™ QA-0.1 Analytical Column	0.1 mL

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