Development of a Full Capsid Enrichment Step in the Purification Process of an AAV8 Product by the CIMmultus® PrimaT Monolith

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Background and Objectives

Gene therapy treatments are promising for treating rare genetic diseases, particularly at Genethon, with a focus on muscular dystrophies and metabolic disorders. However, during the production of AAV vectors for these therapies, a certain amount of empty capsids is also generated, and their clinical impact remains unclear. CIMmultus® PrimaT monolithic column was evaluated for its ability to enrich the product in full capsids. Key parameters such as viral loading, residence time, and loading/elution conductivity were tested on an AAV8 product. Optimal conditions for full capsid enrichment and viral genome recovery were identified and successfully scaled up to 200L.

1. Introduction - CIMmultus® PrimaT monolithic column technology

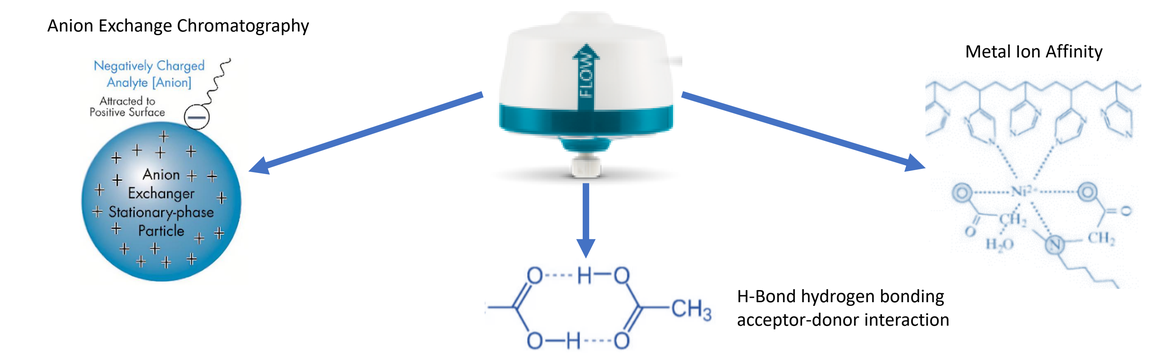

Fig. 1: CIMmultus® PrimaT technology overview.

CIMmultus® PrimaT is a mixed-mode monolithic column based on anion exchange, hydrogen bonding interactions, and metal ion affinity. It uses the physical differences between full and empty capsids to separate the two populations.

2. Method

The AAV8 polishing step was developed using a 1 mL PrimaT column on an AKTA pure 150 system. The product, previously clarified and purified by an affinity chromatography, was diluted X-fold before achieving low conductivity to ensure optimal capsid binding. Elution was performed using a conductivity gradient with a divalent salt. The resulting profile is as follows:

A first peak is obtained, containing primarily full capsids, followed by a second peak with mainly empty capsids and other contaminants. To optimize this separation, various elution volumes, residence times, dilutions, and loadings were tested.

The viral genome yield is measured by qPCR and HPLC, while the ratio of full capsids is determined using HPLC (SEC-MALS) and AUC.

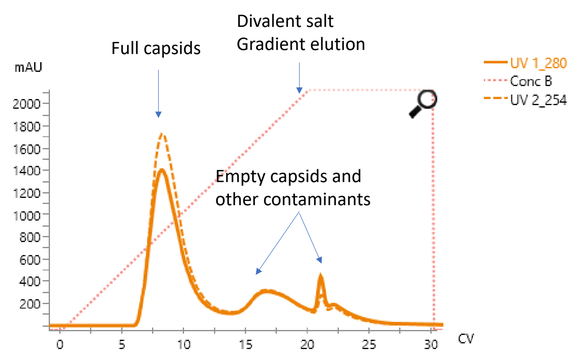

Fig. 2: Chromatogram profile of the PrimaT monolith

3.1 Results - Impact of viral loading

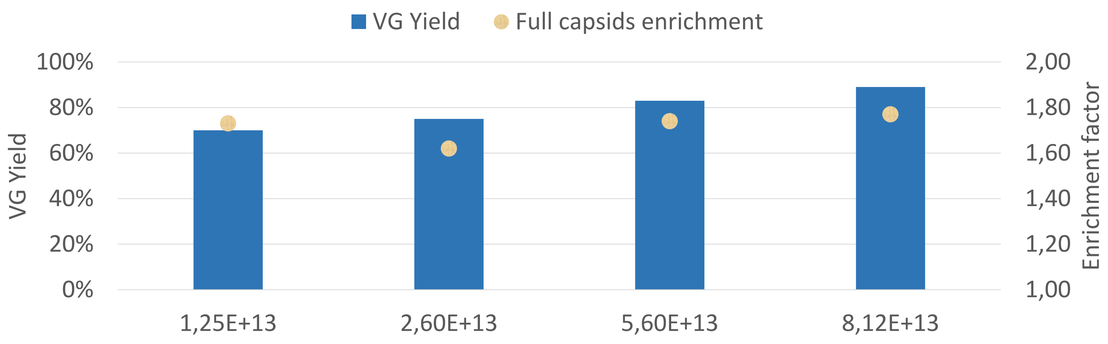

Fig. 3: Viral loading (VG/mL of monolith) evaluated on a 1 mL PrimaT

All viral loadings resulted in similar viral genome (VG) yields, ranging from 70% to 89%, and full capsid enrichment, ranging from 1.62 to 1.77. The selected loading corresponds to a 400L AAV8 production on an 80mL PrimaT monolith.

3.2 Impact of elution volume

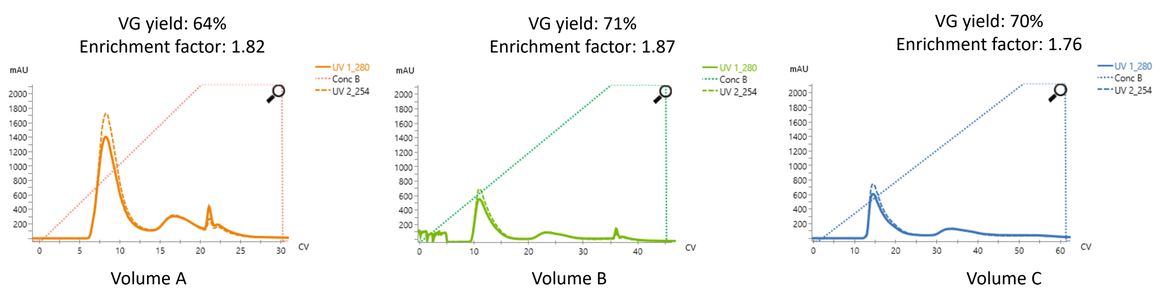

Fig. 4: Chromatography profiles obtained on a 1 mL PrimaT with different elution volumes

Increasing the elution volume further separates the different populations; however, there is no improvement in either the viral genome yield or the enrichment factor.

3.3 Impact of residence time and product dilution

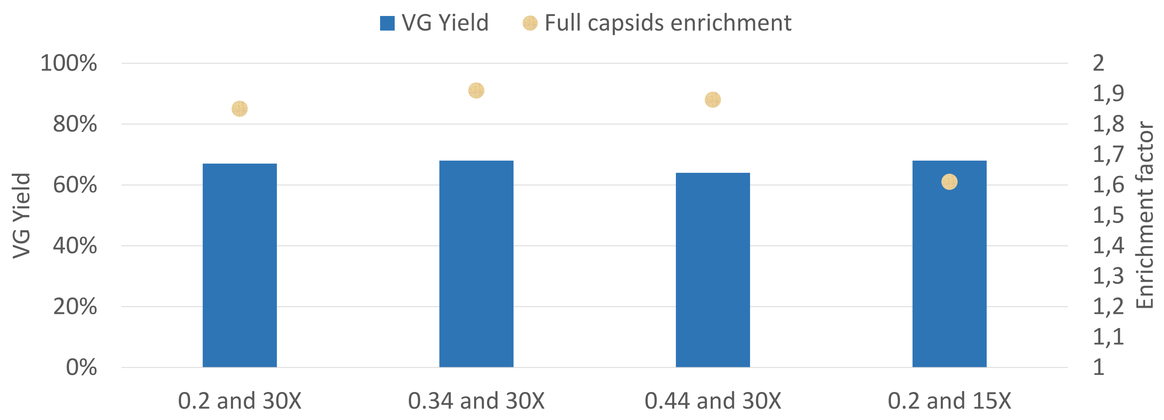

Fig.5: Residence time (min) and dilution impact on a 1mL PrimaT

There is no impact of residence time on the values tested. However, dilution appears to slightly affect the full capsid enrichment. Nevertheless, a 1.6 enrichment factor is still considered as correct.

3.4 Inter and intra batch repeatability

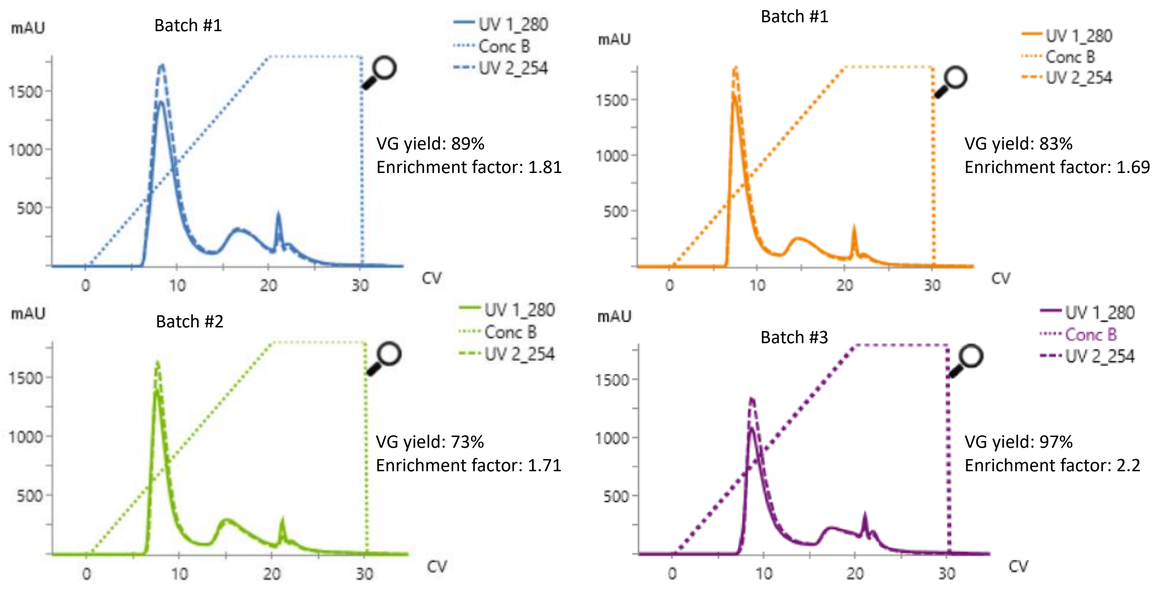

Fig.6: Chromatography profiles obtained on PrimaT 1 mL for the inter and intra batch repeatability

On the 1 mL PrimaT, similar chromatography profiles were obtained across four tests. The viral genome (VG) yield is approximately 80%, with a full capsid enrichment of around 1.8.

3.5 Scale up: 50L and 200L

Conditions developed at a scale of 1 mL were tested on 8 mL and 40 mL monolith scales.

Prima T volume	VG yield(1)	Full capsides enrichment(2)
1mL	80%	1.8
8mL	81%	1.7
40mL	110%	1.8

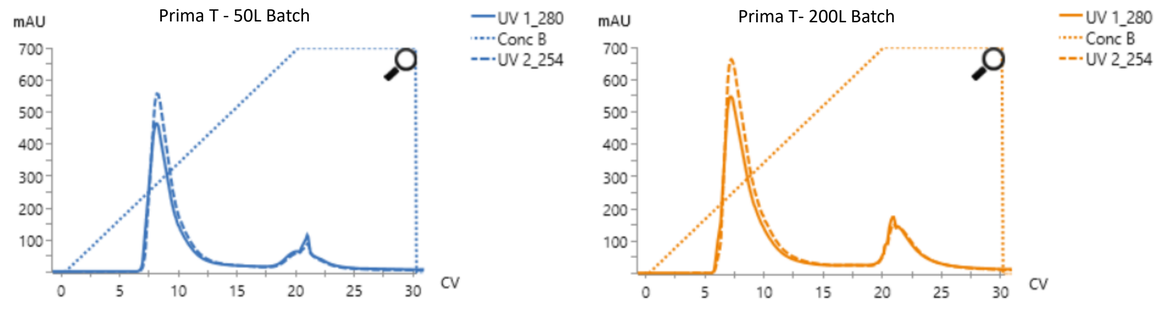

Fig.7: Scale-up results on PrimaT and chromatogram profiles

Conditions developed at a small scale are scalable to 50L and 200L production scales, achieving similar viral genome (VG) yield and full capsid enrichment.

Conclusion

Based on the data generated at multiple scales, conditions were established on the CIMmultus® PrimaT monolith for the industrial purification of a AAV8 product. The process achieves a full capsid enrichment of approximately 1.8 and a viral genome (VG) yield of 80%. Additionally, the PrimaT column effectively removes impurities, including some residual DNA and mainly host cell proteins, thereby improving the overall quality of the final product. These findings underscore the potential of the PrimaT monolith for large-scale AAV production in gene therapy applications.