Optimizing AAV Capture for Multiple Serotypes on BioRMB™ - A Novel Continuous Purification Platform

ChromaTan
Continuous Column-Free Chromatography

The future of **single-use, column-free purification** is here

Dalton Kinnard[1], Sreeja Edara[1], Dwayne Kenney[1], Alex Schaffer[1], Thiago Millen[1], and Oleg Shinkazh[1]*
[1]ChromaTan Inc., 727 Norristown Road Bldg. 3, Suite #103, Ambler, PA 19002

Abstract

BioRMB™ is a column-free and steady-state purification platform that is specifically tailored for sensitive modalities such as gene therapies, vaccines, mRNA, and complex antibodies, which combines the principles of Real Moving Bed (RMB) and columnless chromatography, allowing different operations to be performed simultaneously on the resin slurry as it is pumped in a continuous loop like a conveyor belt.

Previously, we have demonstrated integrated mAb capture and polishing using BioRMB™. In this work we will present capture data for two different serotypes of AAV, and the process development results for AAV9 capture.

Introduction

The cost of manufacturing life-saving gene therapies has become increasingly unsustainable. Consequently, the need for higher efficiency and productivity in AAV purification processes has been a major driver of R&D efforts in the biotechnology industry for the better part of two decades. While some progress has been made, the goal of developing a platform that delivers markedly improved productivity metrics, without comprising product quality, robustness and GMP suitability has been elusive.

The BioRMB™ platform combines the principles of Real Moving Bed (RMB) and columnless chromatography, allowing different operations to be performed simultaneously on the resin slurry as it is pumped in a continuous loop like a conveyor belt. BioRMB™ overcomes many limitations of batch columns as well as circumventing the drawbacks of multi-column systems, all while providing 5-10x greater productivity with a fully disposable, single-use flow path. Here, we demonstrate a novel AAV capture process that rivals column-based methods. Here, we demonstrate an optimized process for AAV capture of multiple serotypes with BioRMB™.

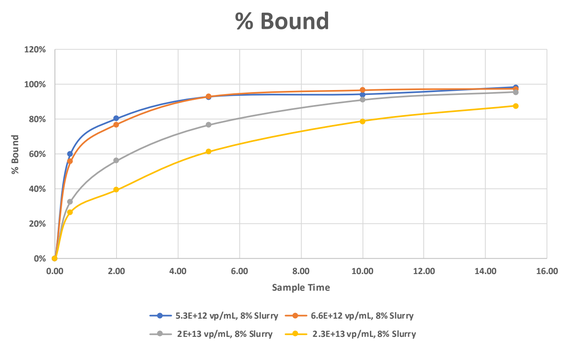
Methodology

The primary goal of this study was to develop and execute a pilot-scale BioRMB™ process for multiple AAV serotypes, focusing on optimizing AAV capture. This involved development and process optimization for binding, washing, and elution conditions, ensuring high recovery and productivity. A series of benchtop experiments were conducted to evaluate optimal binding and elution conditions before scaling up. The results were successfully transferred to the pilot scale, leading to successful Kascade™ BioRMB™ runs.

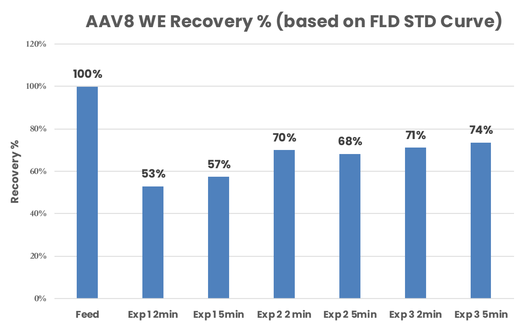
Results - AAV8 Capture

Process development (PD) experiments were performed on AAV8 crude lysate to evaluate the optimal binding and elution conditions before. First, binding studies were performed using off-the-shelf POROS™ AAVX resin (50 µm diameter) with a reported binding capacity of 1.0E+14 capsids/mL resin. The AAV8 feed concentration was determined to be 3.3E+11 vg/mL (by ddPCR), and target loadings ranged from 5.3E+12 to 3.3E+13 vp/mL. Samples were collected at 0.5, 2, 5, 10, and 15-minute timepoints. The optimal binding buffer was determined to be 20mM Tris, 150mM NaCl, 10mM Acetic Acid, and 0.01% Poloxamer P-188 at pH 8.4. Binding efficiency was evaluated using SEC-HPLC and dPCR, with the lowest loading condition (5.3E+12 vp/mL) achieving 98% binding by SEC-HPLC **(A)** and 99% by dPCR.

A)



B)



Wash and elution experiments were performed with a target loading ranging between 6.6E+12 vp/mL and 2.0E+13 vp/mL. Wash buffer conditions were varied to maximize elution recovery and impurity removal while minimizing product loss. The optimal wash buffers were identified as 20mM Tris, 150mM NaCl, 10mM Acetic Acid, 0.01% Poloxamer P-188, pH 7.5 for Wash I and 20mM Tris, 150mM NaCl, 10mM Acetic Acid, 0.01% Poloxamer P-188, pH 6.5 for Wash II. Three elution buffers were tested, containing 50mM, 170mM, and 400mM Acetic Acid, corresponding to pH values of 3.3, 2.9, and 2.65, respectively. At a resin loading of 7.50E+12 vp/mL, SEC-HPLC with fluorescence detection (FLD) showed that elution at pH 3.3 had the lowest recovery (57%), whereas pH 2.65 resulted in a 74% recovery after a 5-minute residence time **(B)**. Maximum AAV8 recovery was observed at pH values between 2.9 and 2.65.

A Kascade™ BioRMB™ run was designed in DoE format using optimized parameters. Process conditions **(C)** included optimized target loading, binding residence time, and wash and elution buffers, with elution pH values tested between 3.1 and 2.9. An increase in recovery and productivity was observed by dPCR and BLI as lower elution pH values were introduced.

The Kascade™ BioRMB™ run maintained a steady-state process **(F)**, with dPCR analysis indicating an average of 85% elution recovery for the pH 2.9 during steady-state operation, **(D)** and an average productivity of 7.0E+15 vg/L/h with the same elution condition **(E)**. Empty/full capsid ratio, analyzed using both SEC-MALS (Table 1) and Mass Photometry (MP), showed between 27% and ~29% full capsids (consistent with the feed) which demonstrates that capsid content was not altered by the BioRMB™ process, and thus product integrity was maintained.

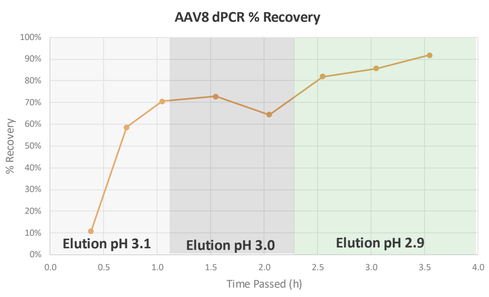
C)

Process AAVX Capture (AAV8 Run 02)	
Duration	4.1 Hours
Resin Slurry Ratio	11.5%
Retentate Flow	4 mL/min
Feed Volume	1.87 L
AAV titer	1.08 E+12 cp/mL 3.01 E+11 vg/mL
Cycles	7.13
Resin Volume	19.1 mL
Offline Sampling	Ramp up/ ramp down: 20 min Steady State: 30 min

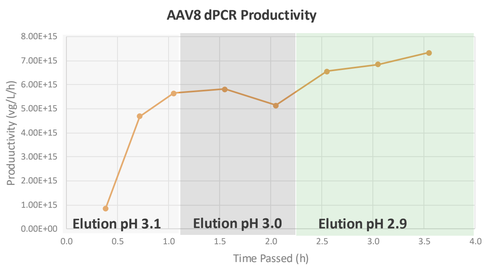
Flow Path	
Stages	21
Binding Time	15 min
Average Cycle Time	34.1 min

Process Outcomes	
Resin Loading (cp/ml)	2.95E+13 cp/mL
Average in-line Recovery Steady-state (%)	Elution Pool 1 - 65% Elution Pool 2 - 73% Elution Pool 3 - 85%
SS Productivity (vg/L/hr)	6.2E+15
Total Buffer Gamma	13.5
Buffer Usage (L/vg)	7.2E-10
Steady-state Product Concentration (vg/mL)	2.17E+11

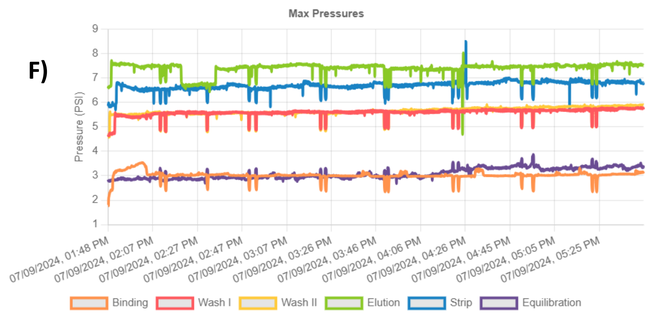
D)



E)



F)



Sample ID:	Number of replicates	Average % Full capsids
Feed (Crude Lysate)	2	27.1
Elution Pool 01 (pH 3.1)	3	27.3
Elution Pool 02 (pH 3.0)	3	28.9
Elution Pool 03 (pH 2.9)	3	28.4

Table 1: *Full-Total Capsid Ratio Analyzed by SEC-MALS*

AAV Capture Recovery and HCP Removal

An unspecified serotype of AAV was captured using AAVX affinity resin on Kascade™ BioRMB™. The bioreactor was harvested, and the feed was concentrated 10x for a total of 3.9 Liters prior to loading at two target loading conditions. Subsequent titer analyses by qPCR showed that when considering only the low loading condition, an average of 95% AAV recovery was achieved during steady-state. Overall AAV recovery of 89% **(G)** across both loading conditions with minimal loss **(H)** was observed. Furthermore, HEK293 HCP ELISA analyses revealed that Host Cell Proteins were reduced below detectable levels in eluted samples **(I).**

G)

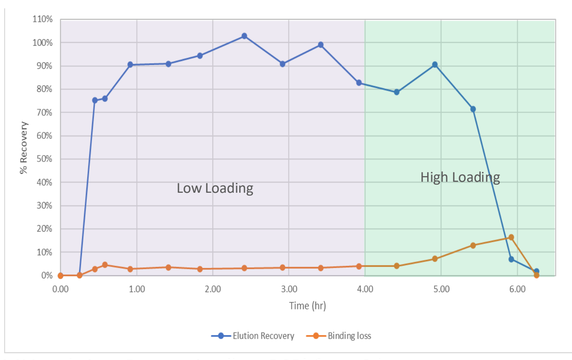
	Volume (mL)	qPCR titer (vg/mL)	Total capsids in (mass balance)
Feed (post-filter)	3891	**2.74E+12**	**1.06E+16**
Elution total	3961	2.39E+12	**9.47E+15**
Recovery			**89%**

I)

Elution/strip samples	LLOQ *	<8 ng/mL
Binding/wash samples	ULOQ**	>200 ng/mL

**Lower limit of quantification **Upper limit of quantification*

H)

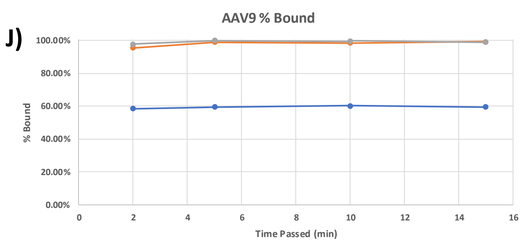


AAV real-time Recoveries (by qPCR) for a 6-hour run across two loading conditions

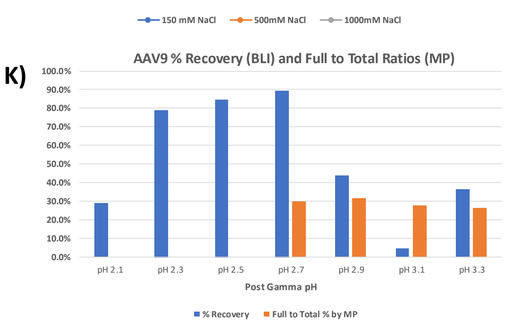
AAV9 Capture (Scale down Model)

Binding Kinetics and Wash-Elution studies were performed as previously described and evaluated by BLI. Binding was assessed with a target loading of 7E12 vp/mL in a variety of NaCl concentrations. 500mM and 1000mM salt resulted in 99% binding after 15 minutes **(J)**. Following this, Wash/Elution studies were run across different elution buffer conditions and with a target loading of 5.0E12 vp/mL. At a resin loading of 7.5E12 vp/mL, BLI indicated that 25mMm acetic acid (pH 2.7) yielded an 89% recovery after 2 minutes of residence time, and mass photometry confirmed full capsid % remained constant (27%) **(K)**. This process will be transferred from bench scale to the Kacakde™ BiRMB™.

J)



K)



Conclusions and Future Work

The Kascade™ BioRMB™ delivers recoveries of AAVs that meets or exceeds those reported by column-based processes and provides a promising platform for delivering life-saving therapies in a highly productive and efficient process without compromising product quality. Work is already underway on the development of a fully integrated AAV capture-polishing process which can be easily tech-transferred to GMP manufacturing as well as establishing a method for Real-Time analysis of whole capsid AAV titer as well as Empty/full % to monitor and optimize in-process recoveries.

***Special Thanks to*:**
Collin Britten, PhD
Waters-Wyatt Technologies

NIH National Institutes of Health
Turning Discovery Into Health
NIMBL
BLUE KNIGHT™
Anticipate. Activate. Amplify.

Contact:
Oleg Shinkazh, Founder & CEO
oleg.shinkazh@chromatan.com