Viral Removal and Process Performance via BioRMB™-A Column-Free Platform for Purification of Biologics, Gene Therapies, and mRNA.

Stop by **booth #210** for a tour of the BioRMB™ Kascade™!

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Abstract

The 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. In this work we will present viral clearance study data, as well as the general principles of operation for the BioRMB™ and showcase mRNA capture as well as AAV capture and impurity removal. The platform shows >3 log reduction of MockV® particle on a ProA resin and correlates well with process modeling. Additionally, BioRMB™ demonstrates >80% reduction in resin volume vs. batch columns, higher recoveries and lower HCP.

Introduction

The growing prevalence of complex diseases such as cancer and autoimmune disorders has led to an increase in demand for biopharmaceuticals[1]. Thus, the effective clearance of viral contamination from injectable biologics is necessary for mitigating the risks associated with viral contamination and is essential for regulatory compliance[2]. BioRMB™ (Formerly, CCTC) enables a purification process in steady-state continuous elution that results not only in high yield, high productivity purification, but also demonstrates effective clearance of viral contaminants.

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 that enhanced viral clearance capability is an added benefit of BioRMB™ technology.

Results

mAb harvest was spiked with mock viral Particle (MVP, Cygnus Technologies) at a concentration of 1.0E^7 VP/mL. Following commercial mAb capture with Protein A resin at 10% slurry concentration, performed in a 3-hour run on the Kascade™ BioRMB™ with a 21-stage system **(*Table 1*)**. To examine the impact of increasing wash buffer ratio to resin slurry flow rate (gamma) on overall viral log reduction value (LRV), a DoE was performed to vary Wash I gamma (γ) between γ 2.0-3.0. Subsequent permeate waste stream retains were subjected to RNA extraction using Cygnus' MockV® RVLP Kit and RT-qPCR analysis was performed with sRNA as a reference standard **(*Figure 1*)**.

Methodology – Viral clearance with BioRMB™

A ~3.5 log reduction in MVP was demonstrated for a continuous Pro A capture operation. It was further observed that increasing wash buffer gamma resulted in an additional 0.5 LRV in the eluted samples, leading to a subsequent increase in overall MVP log reduction of up to 3.9 LRV **(*Figures 2 and 3*)**. Finally, when compared with our modeled LRV for the BioRMB™ system with 4 or 5 stages, actual LRV for elution was aligned **(*Tables 2 and 3*)**.

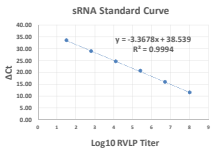


Figure 1: sRNA Standard curve generated on RT-qPCR using the MockV® RVLP Kit from Cygnus Technologies.

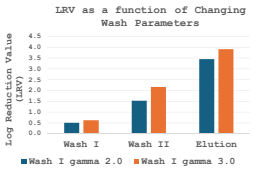


Figure 2: LRV as a function of changing wash gamma from 2.0 to 3.0.

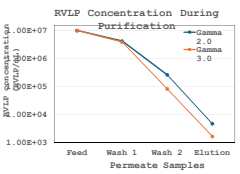


Figure 3: Recombinant Virus-like Particle (RVLP) concentration determined by RT-qPCR, as a function of stages and gamma.

BioRMB Step	# of Stages	Gamma	Gamma
Binding	1	2.7	2.7
Wash 1	4	2	3
Wash 2	4	2	3
Elution	5	2	2
Strip	3	2	2
Equilibration	4	2	2

Table 1: BioRMB™ diagram, 21-stage system.

	Wash II (LRV, modeled for 4-stage wash)	Elution (LRV, modeled for 5-stage elution)
γ 2.0	1.91	3.8
γ 3.0	2.41	4.6

Table 2: Modeled log reduction values for 4-stage wash and 5-stage elution steps.

	Wash II (actual LRV)	Elution (actual LRV)
γ 2.0	1.53	3.45
γ 3.0	2.16	3.91 (below limit)

Table 3: Actual log reduction values for 4-stage wash and 5-stage elution steps.

Conclusion

BioRMB™ platform demonstrates effective clearance of RVLPs from spiked IgG feed stock by showing >3 LRV for a continuous ProA mAb capture process. Furthermore, an increase in wash buffer gamma correlated with higher virus removal, correlating well with modeling. Study design can be used for viral removal testing for cGMP adoption.

Acknowledgments

- David Cetlin, *Cygnus Technologies*
- Prof. Zoltan Kis, *University of Sheffield, UK*
- Justin Parke, *ChromaTan*

[1]Proceedings of the 2023 Viral Clearance Symposium, Session 2: Viral Clearance Strategy and Case Studies
Frank Kohne, Astrid Schwantes. PDA Journal of Pharmaceutical Science and Technology Mar 2024, 78 (2) 147-156; **DOI:** 10.5731/pdajpst.2024.002242

[2]Rasouli-Nejad Mousavi SM, Hosseini SM, Ansari S. Evaluating the viral clearance ability of continuous monoclonal antibody purification steps, in order to inactivate and/or remove four model viruses. Iran J Microbiol. 2023 Oct;15(5):711-722. doi: 10.18502/ijm.v15i5.13877

More applications: mRNA Capture Recovery

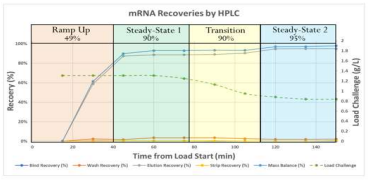
To study the possibility of achieving high-yield purification of mRNA on a continuous chromatography operation, IVT mRNA diluted to 0.26 g/L (based on IEX-HPLC) was purified on a 20-stage system (8-stage elution) on Kascade™ BioRMB™ during a 2.5-hour run using 50 µm POROS Oligo-dT 25 affinity resin (Thermo Scientific)(A). Two target loadings (1.25 and 0.75 g/L) were evaluated, and a mass balance (C) based on IEX-HPLC and NanoDrop indicated that steady-state recovery with a range of 95-97% (B) and an average productivity of 1.44 g/L/h (D) were achieved. These findings suggest that the process developed herein overcomes the current limitations and delivers higher yield than both batch column (90%) and multi-column (92%) continuous chromatography.

A)

BioRMB™ Step:	# of Stages	Gamma
Binding	2	1.25 / 0.75
Wash	3	2.5
Elution	8	3.0
Strip	3	2.5
Equilibration	4	2.5

BioRMB™ diagram, 20-stage system.

B)



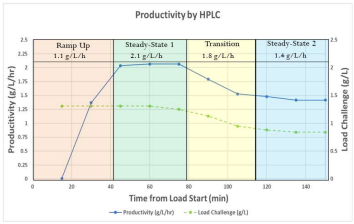
IVT mRNA recoveries for a 2.2-hour run with two loading conditions.

C)

	EP 01	EP 02	Total
Total Mass Balance (mg)	73.8	42.7	116.5
Elution Recovery (%)	92%	97%	94%
Bind Mass Losses (%)	0.2%	0.2%	0.2%
Wash Mass Losses (%)	3.4%	2.4%	3.0%
Total Mass Balance	95%	100%	97%
Productivity (g/L/h)	1.65	1.20	1.45

Mass Balance Summary.

D)



IVT mRNA productivity for a 2.2-hour run with two loading conditions.

AAV Capture Recovery and HCP Removal

An unspecified serotype of AAV was captured using AAVX affinity resin (Thermo Scientific) 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% (E) across both loading conditions with minimal loss (F) was observed. Furthermore, HEK293 HCP ELISA analyses revealed that Host Cell Proteins were reduced below detectable levels in eluted samples (G).

E)

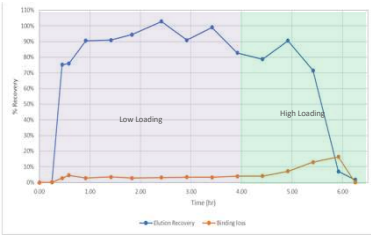
	Volume (mL)	qPCR titer (vg/mL)	Total capsids in (mass balance)
Feed (post-filter)	3891	2.74E+12	1.06E+16
Elution total	3961	2.39E+12	9.47E+15
Recovery			89%

G)

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

*Lower limit of quantification **Upper limit of quantification

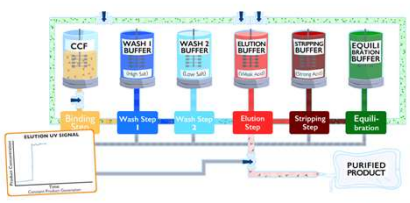
F)



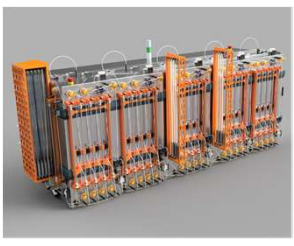
AAV real-time Recoveries (by qPCR) for a 6-hour run across two loading conditions

About ChromaTan and BioRMB™

ChromaTan strives to revolutionize bioprocessing productivity by introducing the first-ever, columnless, single-use, steady-state continuous elution chromatography platform, offering increased recovery and productivity, enhanced purity, flexibility and scalability, while dramatically reducing resin consumption and downtime for the cost-effective production of life-saving therapies.



BioRMB™ principles.



BioRMB™Kascade, PD unit

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

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