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High Throughput Protocol for Linear Scale-up of mRNA Capture Processes onto the BioRMB™ Kascade™ System

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Abstract

The BioRMB™ Kascade™ 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 a high throughput protocol for linear scale-up onto the BioRMB™ System. Using a recent mRNA study as proof of concept, we demonstrate the protocol for high-throughput screening of bind and wash conditions in 96-well filter plates, followed by confirmation optimal conditions in static-mixers using syringes. Applying ChromaTan's recipe maker and buffer model, an eGFP mRNA was purified on BioRMB™ resulting in 97% mRNA recovery, within 2% of the predicted model.

Introduction

ChromaTan's BioRMB™ Kascade™ system offers distinct improvements over traditional packed columns and simulated moving bed chromatography by being truly steady state, reducing resin usage, and improving recovery. One consideration for the BioRMB™ Kascade™ system is the minimum mass of material required for operation. To collect reliable and robust data, the system should be allowed to operate at steady-state for at least 3 cycles or approximately 1-2 hours. Process efficiency of the BioRMB™ Kascade™ system requires 90 mg for this operation time, which can lead to a high cost of development.

To minimize barrier to entry for the BioRMB™ Kascade™ for mRNA processes, a benchtop-scale development protocol has been created which allows users to quickly optimize binding and buffer conditions using less than 30 mg of material. In tandem with ChromaTan's recipe maker, buffer conditions can be designed to achieve a recovery within 2% of the modeled recovery value and a productivity within 10% of the modeled productivity value.

Scale 1: High Throughput Filter Plate Studies

High Throughput Process Development (HTPD) is becoming commonplace within the biologics and gene therapy space to quickly screen a wide array of conditions for binding, washing and elution. Filter plates are a great way to perform these experiments and allow for small-scale DOEs to be performed.

mRNA High Throughput Bind Study													
Feed Conc (g/L)	Load Challenge (g/L)	Experimental Wells											
0.2	0.75												
	1.00												
	1.25												
	1.50												
0.4	1.25												
	1.50												
	1.75												
	2.00												
Binding Time (Min)		15	10	5	3	2	1	15	10	5	3	2	1
Slurry Concentration (%)		15%						20%					

Binding: Resin is loaded into the wells of the plate as a slurry and feed solution can be pipetted into the wells to allow for binding. A Benchmark Scientific MultiTherm Touch Vortex Mixer is used at 850 RPM to mix the binding solution. After the desired binding time, the supernatant can be removed by centrifugation at 2,500x g for 2 minutes to quantify the unbound material.

mRNA High Throughput Wash Study (Conductivity in mS/cm)												
A	50.0			45.0			40.0			35.0		
B	30.0			25.0			20.0			15.0		
C	10.0			8.0			6.0			4.0		
D	3.0			2.0			1.0			1.0		
E	25.0			22.5			20.0			17.5		
F	15.0			12.5			10.0			7.5		
G	5.0			4.0			3.0			2.0		
H	1.5			1.0			0.5			0.5		
	1	2	3	4	5	6	7	8	9	10	11	12

Washing: Product is bound to resin at optimal conditions in bulk form. The supernatant is removed by vacuum filtration through a 0.22μm bottle top filter. The bound resin is then washed once with binding buffer to remove unbound product. The bound resin is then resuspended into the original slurry volume with binding buffer and aliquoted to the filter plate. Washes can be performed with varying buffers in a similar manner to Binding to measure desorbed contaminants or lost product.

Scale 2: Static Mixing Confirmation

BioRMB™ utilizes resin as a slurry, rather than a packed bed, which allows for direct translation of static binding studies rather than dynamic binding studies. Slurry and feed are mixed in a syringe wherein supernatant can be removed from the slurry by way of a syringe filter and analyzed. This is a more accurate scale-up from filter plates for confirmation of system parameters.

Load Challenge Confirmation				
Experiment	**Feed Concentration**	**Slurry Concentration**	**Load Challenge**	**Binding Time**
1	0.2 g/L	20%	0.75 g/L[resin]	10 min
2	0.2 g/L	20%	1.00 g/L[resin]	10 min
3	0.2 g/L	20%	1.25 g/L[resin]	10 min

Two-Stage Binding Assessment				
Experiment	**Feed Concentration**	**Slurry Concentration**	**Load Challenge**	**Binding Time**
Residence 1	0.03 g/L	20%	0.188 g/L[resin]	15 min
Residence 2	0.20 g/L	20%	1.250 g/L[resin]	15 min
Afterbinder	0.50 g/L	40%	1.250 g/L[resin]	15 min

Wash Buffer Confirmation				
Experiment	**Feed Concentration**	**Slurry Concentration**	**Load Challenge**	**Wash Conductivity**
1	0.2 g/L	20%	1.25 g/L[resin]	20 mS/cm
2	0.2 g/L	20%	1.25 g/L[resin]	25 mS/cm
3	0.2 g/L	20%	1.25 g/L[resin]	30 mS/cm
4	0.2 g/L	20%	1.25 g/L[resin]	0 mS/cm

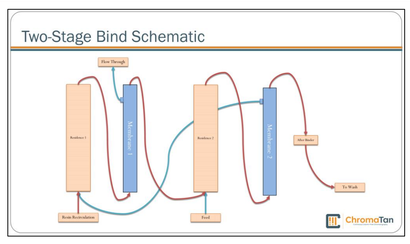
Scale 3: BioRMB™ Kascade Purification

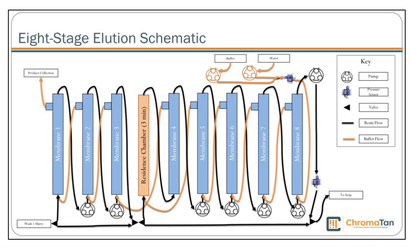
BioRMB™ operates at a true steady state throughout runs. Within each step, a steady state gradient is created through the principals of counter-current washout. This gradient can be mathematically modeled to determine the ratio of each buffer in a specific membrane then calculate the pH and conductivity utilizing ChromaTan's buffer model. Changes in staging and gamma will impact the gradient formation and buffer ratio. These changes can be applied advantageously to further optimized target pH and conductivity values at discrete points in the system beyond typical buffer development.

Acknowledgments

- Prof. Zoltan Kis, *University of Sheffield, UK*

Advanced Flow Path Design for mRNA

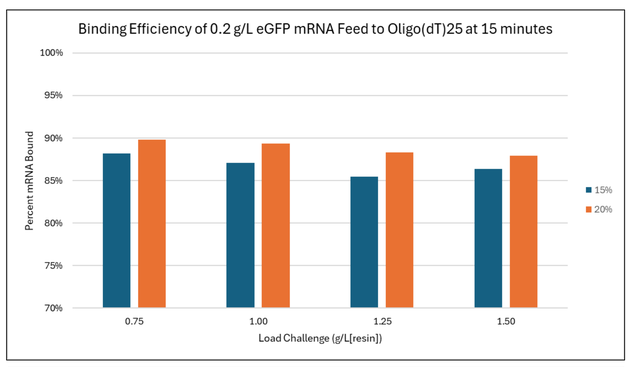


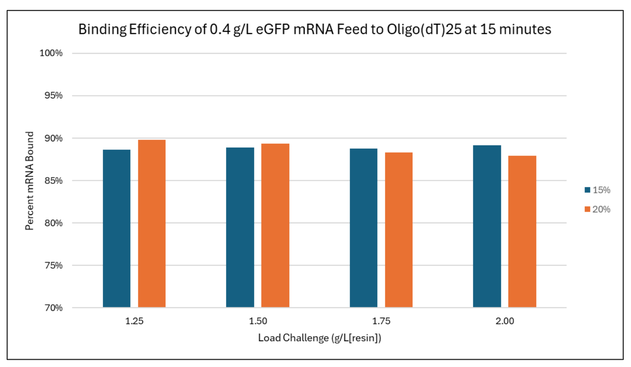


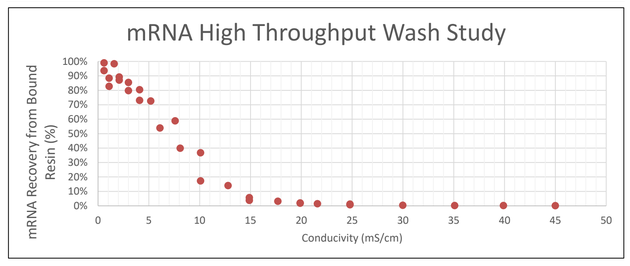
With novel Two-Stage binding on the BioRMB™, recovery of high value products can be significantly improved. Two-stage binding utilizes the theory of CCTC by recycling unbound product from membrane 2 into residence chamber 1 where dilute feed contacts fresh resin. Unbound product in the retentate passes through an after binder after being concentrated back to 20% slurry allowing dilute material to be in-contact with high concentration resin to promote further binding. This can decrease binding losses of mRNA on Oligo(dT)-25 from 8% to 3% or less.

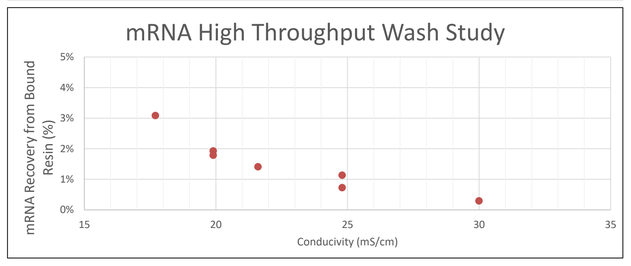
Eight-Stage elution on BioRMB™ Kascade™ allows for material eluted off in the transition from wash conditions into elution conditions to be captured. This allows the conductivity within the residence chamber to be below 1.0 mS/cm for complete elution while still retaining 5 membranes for 99.9% recovery of eluted product.

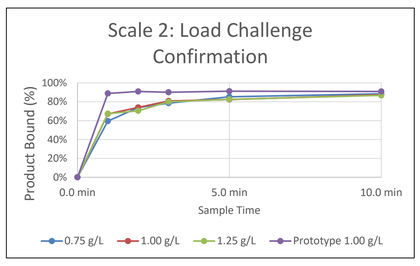
Development Results and Optimal Conditions

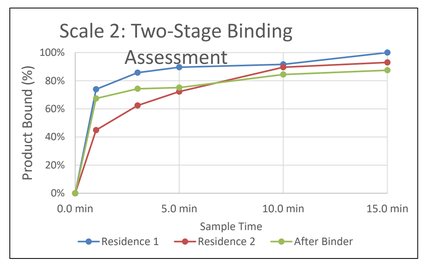


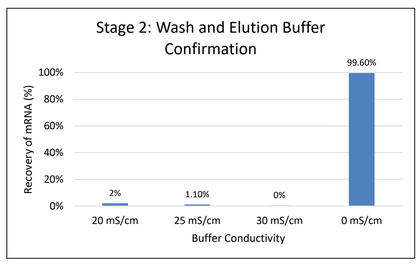










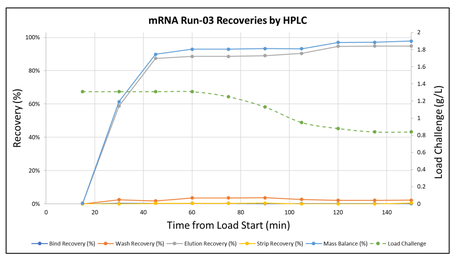


BioRMB™ Kascade Results and Linear Scale-up

With high-throughput screening plates, we were able to determine the binding efficiency of two feed conditions, two slurry conditions, and 6 load challenges. For a feed concentration of 0.2 g/L, a 20% slurry condition was significantly better. A lower load challenge improved recovery for all conditions tested, but 1.25 g/L was selected because of the balance between recovery and productivity. Models predicted a binding recovery of 95.2% for this condition. Static mixing confirmation experiments modeled a binding recovery of 94.7% recovery for the same conditions while the BioRMB™ Kascade™ scale up run had a binding recovery of 96.8% showing that binding experiments can accurately be modeled with high-throughput plates.

High-throughput plates were also used to model product losses in wash and recovery in elution. The data from the screening plate indicated a binding loss of 2% at 20mS/cm, 1% at 25 mS/cm, and 0.5% for 30mS/cm. Values were similar for the static mixing confirmation at 2.0%, 1.1%, and 0.0%, respectively. The screening plate and static mixing confirmation both predicted >99% recovery while the BioRMB™ Kascade™ scale up run had an elution recovery of 97%.

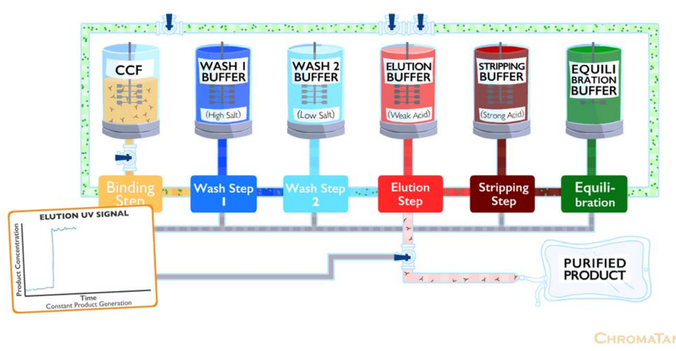
Condition	Feed Concentration	Slurry Concentration	Load Challenge	Residence 1 Time	Residence 2 Time	AfterBinder Time	Wash Conductivity
1	0.2 g/L	20%	1.25 g/L	10 minutes	3 minutes	5 minutes	>25 (26.4 mS/cm)
2	0.2 g/L	20%	0.75 g/L	10 minutes	3 minutes	5 minutes	>25 (26.4 mS/cm)



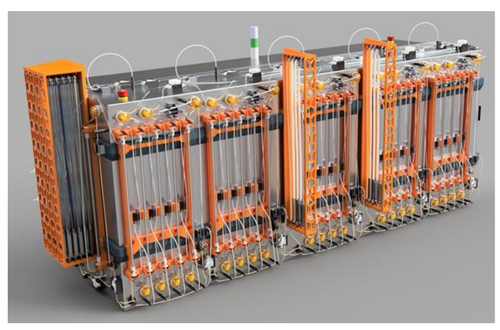
Scale	Process Step	Relative Product Recovery	Total Elution Product Recovery	Predicted Productivity
1	Binding	2.0%	95%	1.71 g/L/hr
	Wash	2.9%		
	Elution	100.0%		
2	Binding	0.5%	93%	1.66 g/L/hr
	Wash	4.8%		
	Elution	98.1%		
3	Binding	0.20%	92%	1.68 g/L/hr
	Wash	3.40%		
	Elution	97%		

About ChromaTan and BioRMB™

ChromaTan strives to revolutionize resin-based bioprocessing 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

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