Application Note

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Fast and Semi-automatic Optimization of Capture Step for rAAV Purification Using CIM® SO3 96-Well Monolithic Plate

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Abstract

Adeno-associated viruses (rAAVs) are commonly used vehicles for gene therapy applications, leading to increased demand for scalable purification tools. However, since rAAVs are a relatively new and highly variable modality, established platform processes do not yet exist. This can create long process development timelines.

Here, we show that CIM® SO3 monolithic 96-well plates can be used to screen cation exchange capture chromatography parameters and that results are scalable to preparative scale on the CIMmultus® line, which is suitable for industrial-scale applications.

Introduction

A robust capture step is a key parameter to increase yield during rAAV production. During monolithic chromatography capture, the rAAV is selectively bound to a strong cation exchanger (SO3) in acidic pH conditions.[1,2] In this step, the majority of host cell contaminants are removed, and rAAV particles are strongly bound to the matrix. The productivity of the step is strongly influenced by the dynamic binding capacity (DBC), which depends on the sample preparation and the availability of the binding sites at the chromatographic matrix.

As the demand for rAAV quantity and quality in clinical manufacturing continues to rise, effective solutions for industrial-scale purification of large volumes of upstream material are required. rAAV capture is the workhorse of the chromatography workflow and presents a valuable optimization opportunity through which the full potential of SO3 monolithic columns can be realized.

To reduce the development time using multi-factor screening and increase comparability during downstream process development, CIM® SO3 monolithic 96-well plates were introduced to the Sartorius BIA Separations portfolio. Here, we show that results on this small scale can be applied to CIMmultus® columns, which is scalable to large volumes.

Methods and Results

Fine-Tuning Mobile Phase Composition Using CIM® SO3 96-Well Plate Screening

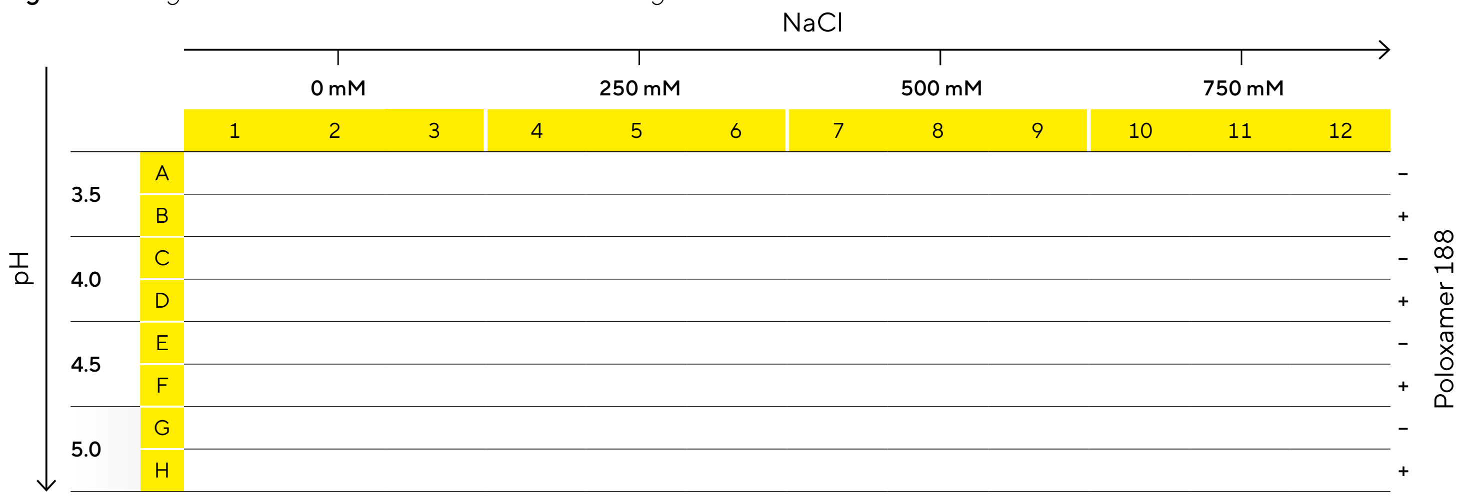
CIM® SO3 monolithic 2 µm 96-well plates were used to optimize mobile phase screening for rAAV downstream process development. Using a vacuum pump system, each well was prefilled with 0.05 mL of 2 µm SO3 monolith to ensure uniform flow and high flow rates across the plate.

To optimize mobile phase A, we screened buffers of different pH and NaCl concentrations. Each buffer combination was also tested in the presence of Poloxamer 188 to determine whether this additive influences the binding of the product to the SO3 monolith. Each combination was tested in triplicate (Figure 1). The elution buffer (mobile phase B) contained 2 M NaCl and the same pH and Poloxamer 188 concentration as mobile phase A for each condition.

Before loading on the SO3 96-well monolithic plate, clarified rAAV9 lysate obtained from Sf9 cells was processed by tangential flow filtration (TFF) coupled with nuclease treatment — salt-tolerant nuclease, as previously described.[3] The TFF retentate was acidified to the selected pH (pH 3.5, 4.0, 4.5, or 5.0) and filtered using Minisart® PES 0.45 µm filters.

After removing the storage solution and washing with diH_2O, the CIM® SO3 96-well plate was conditioned with mobile phase A for 10 CV (CV; column volume, 0.05 mL), mobile phase B for 10 CV, and mobile phase A once more for 15 CV. The wells were loaded with acidified filtered TFF retentate (1 mL), and the flowthrough fraction was collected into a collecting plate. Next, the column was washed with 10 CV of tested mobile phase A, and the wash fraction was collected into the collecting plate. Elution was performed using 4 CV of mobile phase B and also collected. CIP was performed with 10 CV of CIP solution (1 M NaOH 2 M NaCl). After this, the plate was regenerated and stored per the manufacturer's instructions.

Figure 1: *Design of SO3 Monolithic 96-Well Plate Testing*



Automating Analytics for CIM® SO3 96-Well Elution Plate Samples

One of the main advantages of using 96-well monolithic plates is the standard 96-well design compatible with all appropriate 96-well analytical methods. This saves a significant amount of time, is user-friendly, and reduces the number of steps needed to gain information about the viral titer and impurities for each of the tested conditions during process development.

The wash and elution fractions for mobile phases A and B were analyzed using a fluorescence plate reader with Tryptophan native fluorescence settings (ex. 280 nm, em. 348 nm). This allowed us to follow where concentrated AAV particles eluted under each condition (Figure 2). The results show that higher pH is negatively correlated with increased NaCl concentration in the wash buffer (mobile phase A). For pH 3.5, all tested NaCl conditions up to 750 mM showed binding of AAV to the SO3 monolith (high fluorescence detection values in elution fractions), whereas binding at higher pH resulted in tolerating lower NaCl conditions.

Figure 2: *Fluorescence Readings (A) Wash (Mobile Phase A) and (B) Elution (Mobile Phase B) Fractions*

A Wash

pH	NaCl →	0 mM			250 mM			500 mM			750 mM			Poloxamer 188
		1	2	3	4	5	6	7	8	9	10	11	12	
3.5	A	65233	65287	66259	65798	67156	68122	67159	68513	68884	67105	68370	66690	–
	B	64787	66494	66406	66662	67134	67265	66832	67821	67988	67914	66925	67034	+
4.0	C	66772	68385	68177	66125	68879	68415	69240	69276	70025	81885	81469	81405	–
	D	66560	68089	67259	67294	68827	67787	67254	68115	69321	81638	81007	80275	+
4.5	E	67265	67333	69174	69389	68808	69671	83254	83583	84566	85424	85168	84563	–
	F	65721	66717	67707	66986	67780	67879	81455	82679	82103	83709	83805	84200	+
5.0	G	66588	66672	67798	81413	81855	82225	88428	87930	88305	89215	90079	88251	–
	H	65627	66379	66821	79638	79784	80494	87216	87809	88554	88715	87967	87645	+

B Elution

pH	NaCl →	0 mM			250 mM			500 mM			750 mM			Poloxamer 188
		1	2	3	4	5	6	7	8	9	10	11	12	
3.5	A	57049	58073	58338	62732	63517	63877	64687	64699	63230	64552	65345	64528	–
	B	60374	60884	60969	60593	61033	60056	61057	62966	61622	62839	61278	61121	+
4.0	C	59292	59371	60820	62027	61732	60927	63985	62535	64211	52750	52993	53262	–
	D	64016	64642	64748	66076	64309	65874	65003	65700	64938	52560	52321	53178	+
4.5	E	61617	63219	63706	66022	62657	61910	50397	50305	40060	54142	54005	55507	–
	F	66733	68392	69320	68254	66904	67831	53875	53322	53203	52794	53693	53870	+
5.0	G	59611	62250	62281	53926	52547	51991	49957	51924	51158	54221	55022	55586	–
	H	71391	69522	69936	60368	59550	60353	54160	53145	54353	53883	53364	53815	+

To test the efficiency of rAAV9 binding and elution, capsid-specific ELISA was performed for the conditions that were close to maximum NaCl for each tested pH, as well as predicted negative binding conditions (low fluorescence signal in elution fraction). Viral titer (vp/mL) was the highest for mobile phase A, pH 3.5, and 500 mM NaCl in the presence of Poloxamer 188 (Figure 3A). To test the impurity profile, we performed SDS-PAGE analytics in the same elution fraction as ELISA was performed. Qualitatively, there is an observed trend towards increased impurities in the load (acidified TFF retentate) sample. More low-molecular-weight impurities were also observed in elution fractions from high pH samples, compared to lower pH elution (Figure B).

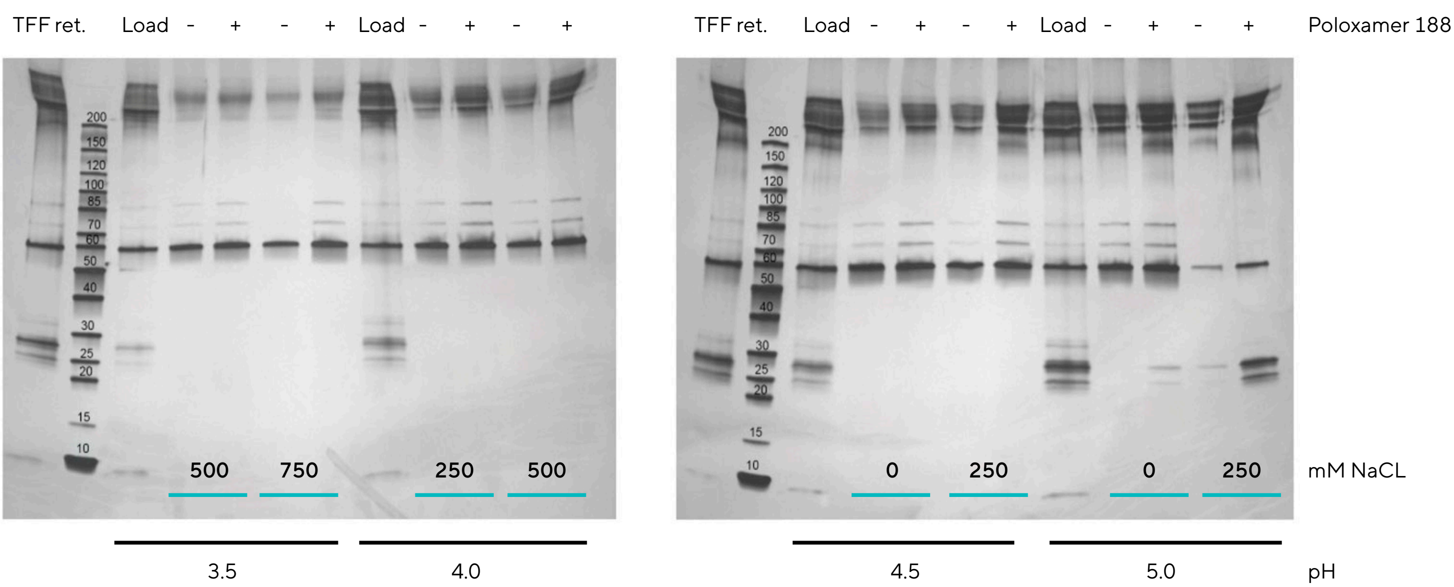
The combination of parameters (different pH, sodium chloride, and poloxamer concentration) that gave us the highest virus titer (vp/mL) value and lowest impurity content in elution fractions was selected as the most optimal mobile phase A (pH 3.5 and 500 mM NaCl in the presence of Poloxamer 188).

Figure 3: *(A) rAAV9 Viral Titers (vp/mL; (ELISA) and (B) Impurity Fractions (SDS-PAGE) in Elution Fractions*

A

pH		NaCl 0 mM (1 2 3)	NaCl 250 mM (4 5 6)	NaCl 500 mM (7 8 9)	NaCl 750 mM (10 11 12)	Poloxamer 188
3.5	A			1.49E+12	1.48E+12	–
	B			2.32E+12	2.10E+12	+
4.0	C		1.74E+12	1.81E+12	4.54E+10	–
	D		2.24E+12	1.83E+12	9.70E+10	+
4.5	E	2.04E+12	1.73E+12	4.77E+10		–
	F	2.11E+12	2.27E+12	9.73E+10		+
5.0	G	2.17E+12	3.14E+11			–
	H	2.11E+12	4.18E+11			+

B

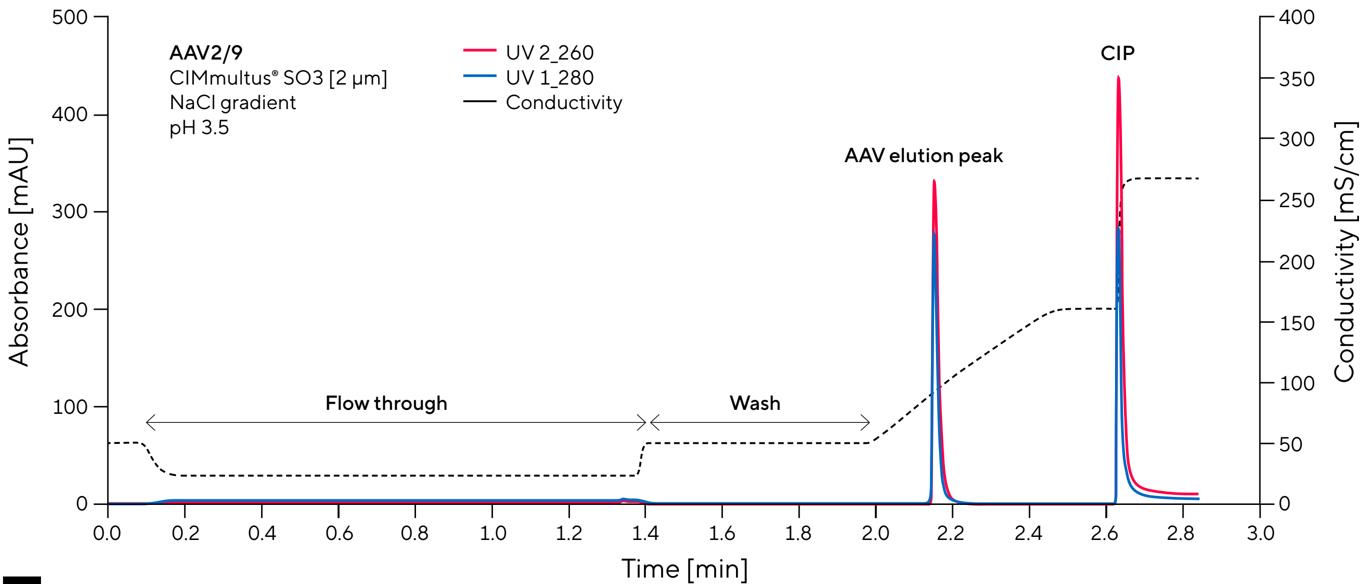


Scale-Up to CIMmultus® Line

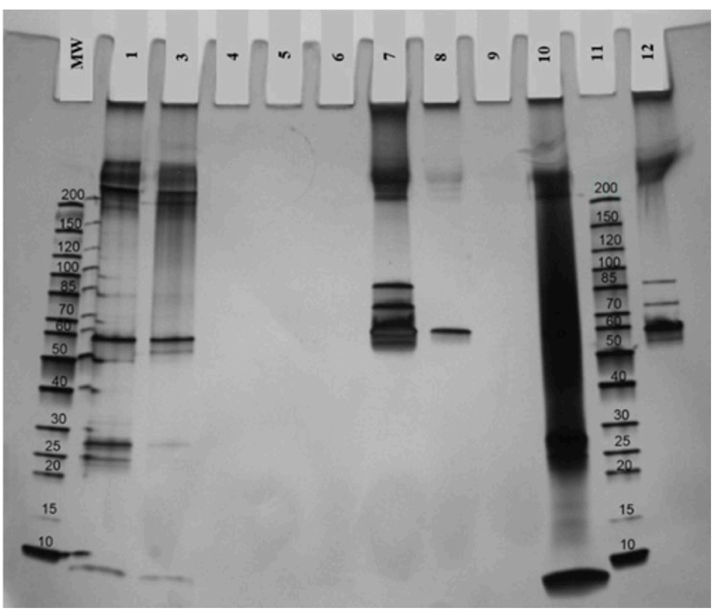
We then assessed the scalability of these results at preparative scale. We tested selected chromatographic conditions from the CIM® SO3 96-well monolithic plate on a 1 mL CIMmultus® SO3 column. We applied a linear NaCl gradient to observe the rAAV elution profile (Figure 4A). The results show that the capture step was robust under the selected conditions; high purity was observed in the elution AAV fraction (Figure 4B). With AAV9-capsid-specific ELISA, we detected 87.7% rAAV9 capsids in the E2 (main peak) fraction compared to the SO3 load material.

Figure 4: *(A) Preparative Chromatogram Using CIMmultus® SO3 1 mL Column (Conditions Selected From CIM® SO3 96-Well Plate Screening); (B) SDS-PAGE Analytics for Tested Fractions.*

A



B



Lanes

1: Size marker
2: TFF retentate 2
3: Load
4: Flowthrough
5: Wash
6: E1
7: E2
8: E3
9: E4
10: CIP
11: Size marker
12: E2 (diluted 5x)

Conclusion

CIM® 96-well plates are a useful tool for fast and automated screening of parameters during process development. A low sample amount is required for experiments, and several parameters can be studied in parallel to achieve high comparability. As such, CIM® SO3 96-well plates enable fine-tuning of an rAAV capture step, which can be transferred and adapted to the CIMmultus® SO3 product line.

References

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