Simplifying Progress

Improved Separation of AAV Capsids using AEX Chromatography

Timotej Žvanut[1*], Sergeja Lebar[1], Andreja Gramc Livk[1], Sebastijan Peljhan[1] and Aleš Štrancar[1]

[1] Sartorius BIA Separations, Slovenia
[*] Corresponding author: timotej.zvanut@biaseparations.com

Introduction

Strong anion exchange (AEX) chromatography is commonly used for separation of AAV capsids. The gold standard for target entities elution in ion exchange (IEX) chromatography is using an increasing salt gradient or an increasing or decreasing pH gradient, typically in a linear mode. Although AEX chromatography is a powerful tool in many manufacturing processes, it sometimes reaches its limits in purifying and separating complex samples comprising different AAV capsids or product-related impurities such as empty, partially-filled, overfilled AAV capsids, or aggregates (Figure 1).

Recently, Sartorius BIA Separations (Slovenia) has developed new high-resolution strategies to more selectively separate not only empty and full but also other product-related impurities (Figure 1). As part of well-known liquid chromatography, these strategies are, in contrast to some recently developed innovative techniques, robust and scalable to a preparative scale. Together with the new CIM QA HR column line, new AEX methods represent a powerful tool to meet increasingly stringent safety and efficacy criteria for the final AAV products.

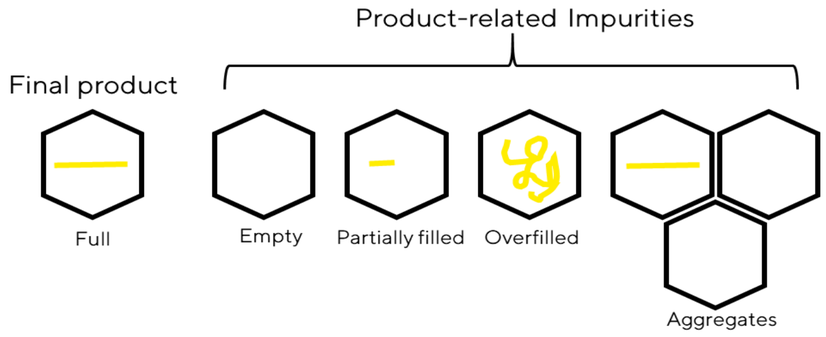

Figure 1: Schematic overview of final AAV (Full) product and common product-related impurities formed in a production process.

1. Experimental approach

The AEX approach used in the improved method utilizes a linear elution gradient similar to the conventional AEX method. Initial experiments involved applying a conventional AEX analytical method to separate full and empty AAV8 capsids.

- Buffer composition:

Buffer A : 20 mM BTP + 2 mM $MgCl_2$ + 1% sorbitol + 0.1% poloxamer 188, pH 9.0
Buffer B: 20 mM BTP + 2 mM $MgCl_2$ + 400 mM NaCl + 1% sorbitol + 0.1% poloxamer 188, pH 9.0

- Gradient composition: 10-minute gradient from 100% buffer A to 100% buffer B, followed by cleaning-in-place (CIP) to remove residual entities and to sanitize the column.

By adapting the elution mechanism of the conventional AEX method, an improved approach* was developed to enhance the separation of AAV8 empty/full capsids and other species of AAV8.

*patent application of the improved approach has been filed and is pending.

The study utilized a CIMmultus SO3 pre-purified AAV8 sample characterized by various orthogonal analytics (Table 1). The PATfix analytical system (Figure 2) was used in both the conventional and improved method approaches. The separation of AAV species was achieved using the CIMac QA HR monolithic analytical column (Figure 3). The HR designation signifies high reproducibility across column batches and scales. The CIM monolith QA HR ensures reproducible purity, enabling the enrichment of full AAV capsids from various AAV serotypes, chimeras or surface

Figure 2: PATfix analytical system.

Figure 3: CIMac QA HR 0.1 mL Analytical Column (Quaternary Amine) (2 µm channels)

2. Results – comparison of the two AEX methods

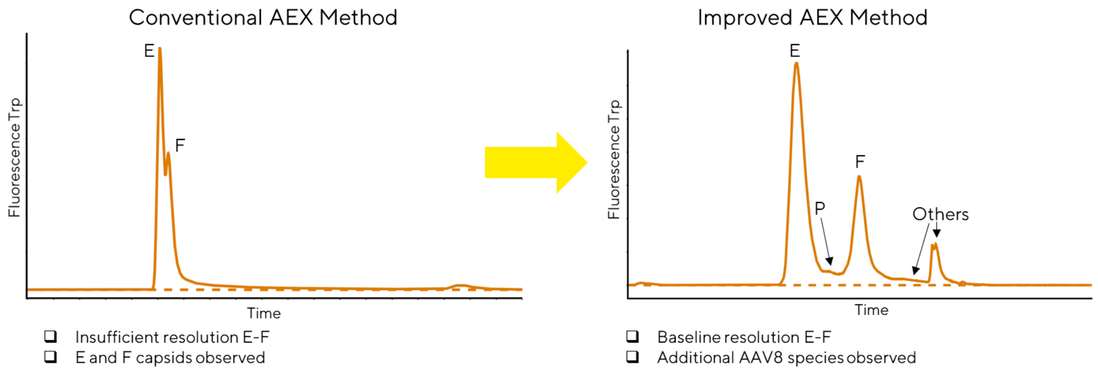

Figure 4: Comparison of pre-purified AAV8 sample analyzed by the conventional (left) and the improved (right) AEX analytical method. E=empty; P=possible partially filled; F=full and Others=other product related impurities (e.g. overfilled or aggregates).

Relatively poor empty and full resolution with considerable empty and full peak overlapping was obtained with the conventionally used AEX method compared to the improved one, as shown in Figure 4. With the new AEX method separation not only empty and full but also other subpopulations of AAV8 capsids was achieved.

3. Results – characterization of pre-purified AAV8 sample

Various orthogonal methods are used to comprehensively characterize AAV samples of interest and identify process characteristics crucial for process optimization (Table 1). Each of the utilized method has its own limitations. A shared constraint among all three orthogonal metods (Mass photometry - MP, Ultracentrifugation - UC and Transmission electron microscopy - TEM) is their lack of scalability, making them unsuitable for application on a larger scale. Additionally, e.g. MP represents a relatively innovative experimental technique, however it still requires better understanding and method validation to deliver reproducible results. With TEM analysis, subjective or biased evaluation of the sample might be obtained. As presented in Table 2, the percent of capsids obtained with TEM does not correlate with the other orthogonal methods.

Table 1: Pre-purified AAV8 sample characterized by conventional and improved AEX methods and some of the commonly used orthogonal methods. E=empty; P=possible partially filled; F=full and Others=other product related impurities. (e.g. overfilled or aggregates).

Pre-purified AAV8 Sample	Conventional AEX Method	Improved AEX Method	MP	UC	TEM
%E	70	60	65	70	>15
%P	/	<5	0	0	0
%F	30	25	35	25	80
%Others	/	>10	0	5	<5

4. Results – preparative run and orthogonal analytics

To characterize separated peaks obtained from the improved AEX method, the preparative run using CIMmultus QA HR 1 mL was performed to collect individual fractions (Figure 5) and to further analyse them by orthogonal analytics (Figure 6 and Table 2).

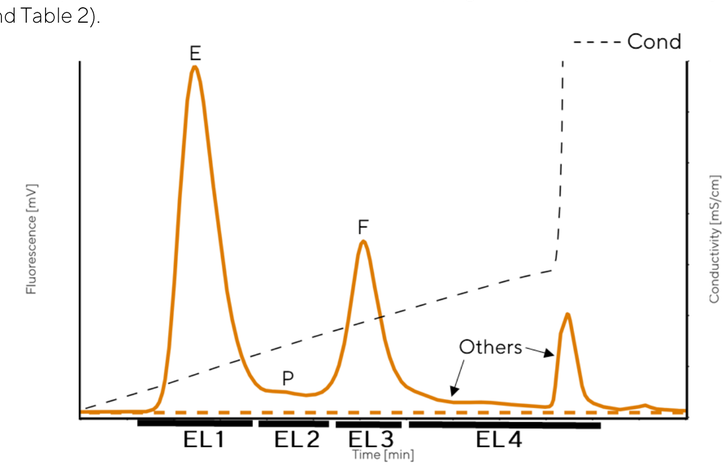

Figure 5: Preparative run of pre-purified AAV8 sample and its fractions collected, EL 1 – EL 4. EL=elution. E=empty; P=possible partially filled; F=full and Others=other product related impurities (e.g. overfilled or aggregates).

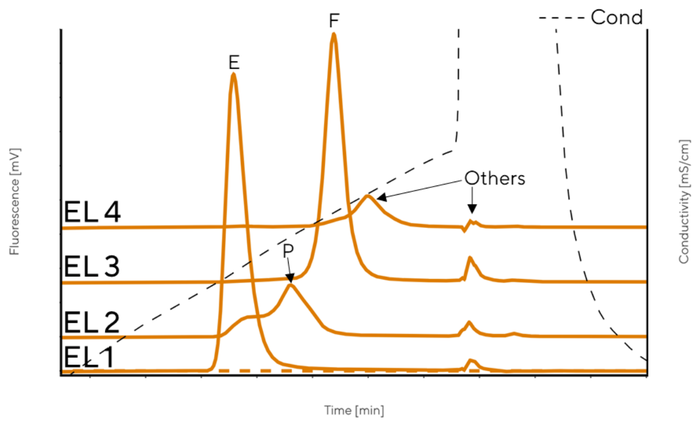

Figure 6: PATfix orthogonal analytics of fractions collected as shown in Figure 5 using fluorescent detector. EL=elution; E=empty; P=possible partially filled; F=full and Others=other product related impurities (e.g. overfilled or aggregates).

Table 2: MP compared to PATfix for fractions collected as shown in Figure 5. EL=elution. E=empty; P=possible partially filled; F=full and Others=other product related impurities (e.g. overfilled or aggregates).

MP				
	EL 1	EL 2	EL 3	EL 4
% E	100	90	10	30
% P	0	0	0	0
% F	0	10	90	70
% Others	0	0	0	0
PATfix AEX chromatography				
	EL 1	EL 2	EL 3	EL 4
% E	>95	25	0	0
% P	0	70	0	0
% F	0	0	95	15
% Others	<5	5	5	85

The orthogonal results of mass photometry and PATfix AEX chromatography show that elution 1 is predominantly empty, elution 3 is predominantly full, and elution 4 is a mixture of different AAV capsids. The main divergence between these two orthogonal methods is in elution 2, where possible partially filled AAV capsids were observed by AEX chromatography. To better specify all individual types of capsids present, additional orthogonal analytics are required.

6. Conclusion

The data presented demonstrates that:

- The improved AEX method provides, in addition to separation of empty and full AAV capsids, also separation of other impurities such as partially filled, overfilled capsids, and aggregates.
- The improved resolution enables higher purity of full AAV8 capsids.
- Scalability of chromatographic methods is a key advantage for AAV purification processes compared to other not scalable techniques.
- At least two orthogonal methods (e.g. PATfix-CIMac QA HR and Mass photometry) are required for characterization of individual separated fractions.

Acknowledgements

We thank Magda Tušek Žnidarič, Jana Krušič and Andrej Mihevc for assisting with the conventional orthogonal analytics. We also thank and appreciate the assistance of Ažbe Žnidaršič and our technician staff for buffer preparation.