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Empty and Full Capsids Separation Using Three Different Modalities

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1. Introduction

Removal of impurities generated in the production of adeno associated virus (AAV) is an important step as they may pose a serious health risk, as well as deteriorate the economics of the production process. The most critical subsets of these impurities include: host cell nucleic acids, host cell proteins, chromatin, capsid aggregates, capsid-DNA complexes and empty capsids.

This poster introduces two new columns for performing separation of empty (E) and full (F) capsids using multi-modal approach. A PATfix® HPLC system with three different detectors, i. e. absorbance, fluorescence, and light scattering in combination with three analytical columns traded as CIMac AAV Empty/Full, CIMac PrimaS® (AAV)-Beta, and CIMac PrimaT-Beta by Sartorius BIA Separations.

CIMac AAV column is strong anion exchanger, where separation of empty and full capsids is based on ionic interactions – charge differences between empty and full AAV capsids. Their elution is achieved using NaCl gradient. CIMac PrimaS® (AAV)-Beta column employs a new ion exchange-hydrogen bonding multimodal ligand that provides separation of empty and full capsids in pH gradient mode. This is directly opposite to QA exchangers where increasing pH causes capsids to bind more strongly. CIMac PrimaT-Beta is the latest multimodal column for empty and full separation. While it is still a weak anion exchanger-hydrogen bonding, it has also metal chelating properties. It is capable to separate different subpopulations of empty and full capsids using combination of $MgCl_2$ and NaCl elution gradient. The separation columns were used to determine and evaluate empty AAV capsids as one of the critical impurities in AAV samples. The analytical results using CIMac PrimaS® (AAV)-Beta and CIMac Prima T-Beta show that other fast and reliable orthogonal HPLC methods to the CIMac AAV full and empty column can also be used for the separation of empty and full capsids with monolithic columns.

2. Experimental Approach

For this experiment PATfix® HPLC system was used. It is powered by PATfix® software, a comprehensive, user-friendly toolbox for automated analysis of chromatographic data sets.

Detectors:
- Conductivity and pH
- Absorbance (260 nm, 280 nm), 50 mm UV cell
- Fluorescence (Ex/Em: 280/348 nm)
- Light scattering (angle: 90°)

Buffer composition (CIMac AAV Empty/Full, NaCl gradient):
- 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 $MgCl_2$ + 200 mM NaCl + 1% sorbitol + 0.1% poloxamer 188, pH 9.0

Buffer composition (CIMac PrimaS® (AAV)-Beta, pH gradient):
- Buffer A: 10 mM BTP + 10 mM TRIS + 2 mM $MgCl_2$ + 1% sorbitol + 0.1% poloxamer 188, pH 8.0
- Buffer B: 10 mm BTP + 10 mM TRIS + 2 mM $MgCl_2$ + 1% sorbitol + 0.1% poloxamer 188, pH 9.5*

*NaCl was added to achieve isoconductivity of Buffer A and Buffer B

Buffer composition (CIMac PrimaT-Beta, $MgCl_2$ and NaCl gradient):
- Buffer A: 25 mM HEPES, 1% saccharose, 0.1% poloxamer pH 7.0
- Buffer B: 50 mM Tris, 13.6 mM borate, 1% saccharose, 0.1 % poloxamer pH 9.0
- Buffer C: 50 mM Tris, 9.6 mM borate, 50 mM $MgCl_2$, 1% saccharose, 0.1% poloxamer pH 9.0
- Buffer D: 50 mM Tris, 12 mM borate, 2 M NaCl, 1% saccharose, 0.1% poloxamer pH 9.0

The results illustrated on the right were obtained with AAV 2/8 serotype sample (partly purified using CEX). The resolution between peaks is based on UV absorbance detector with the standard equation:

$$R = 2\frac{t_F - t_E}{w_F + w_E}$$

where t_F and t_E are retention times and w_F and w_E are peak widths at baseline of full and empty AAV capsids. Percentage of empty and full (%E and %F) was obtained considering tryptophan fluorescence.

PATfix® HPLC System

Figure 1: *PATfix® Is Powered by PATfix® Software, a Comprehensive, User-Friendly Toolbox for Automated Analysis of Chromatographic Data Sets.*

3. Results

CIMac AAV Empty/Full
NaCl gradient

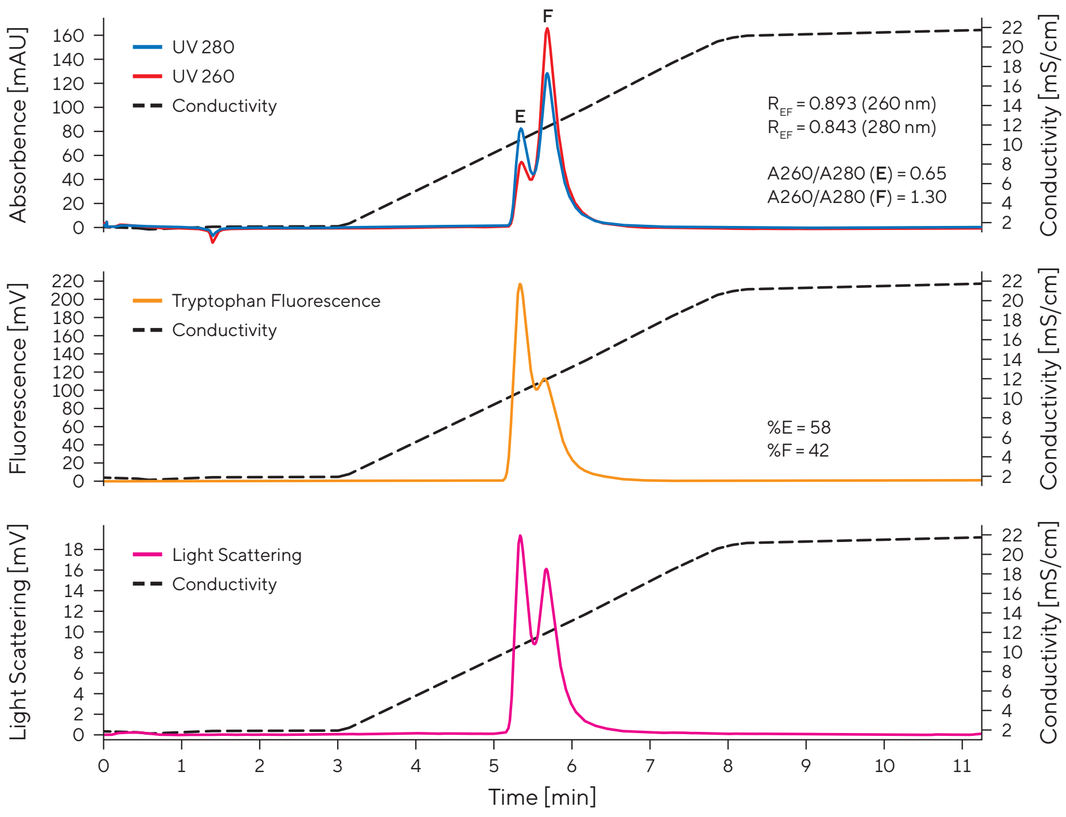

Figure 2: *Chromatogram of AAV 2/8 Serotype Sample Analysed by CIMac AAV Full/Empty Column. Gradient Composition: 5.0-Minute Gradient From 100% Buffer A to 50% Buffer B.*

CIMac PrimaS® (AAV)-Beta
pH gradient

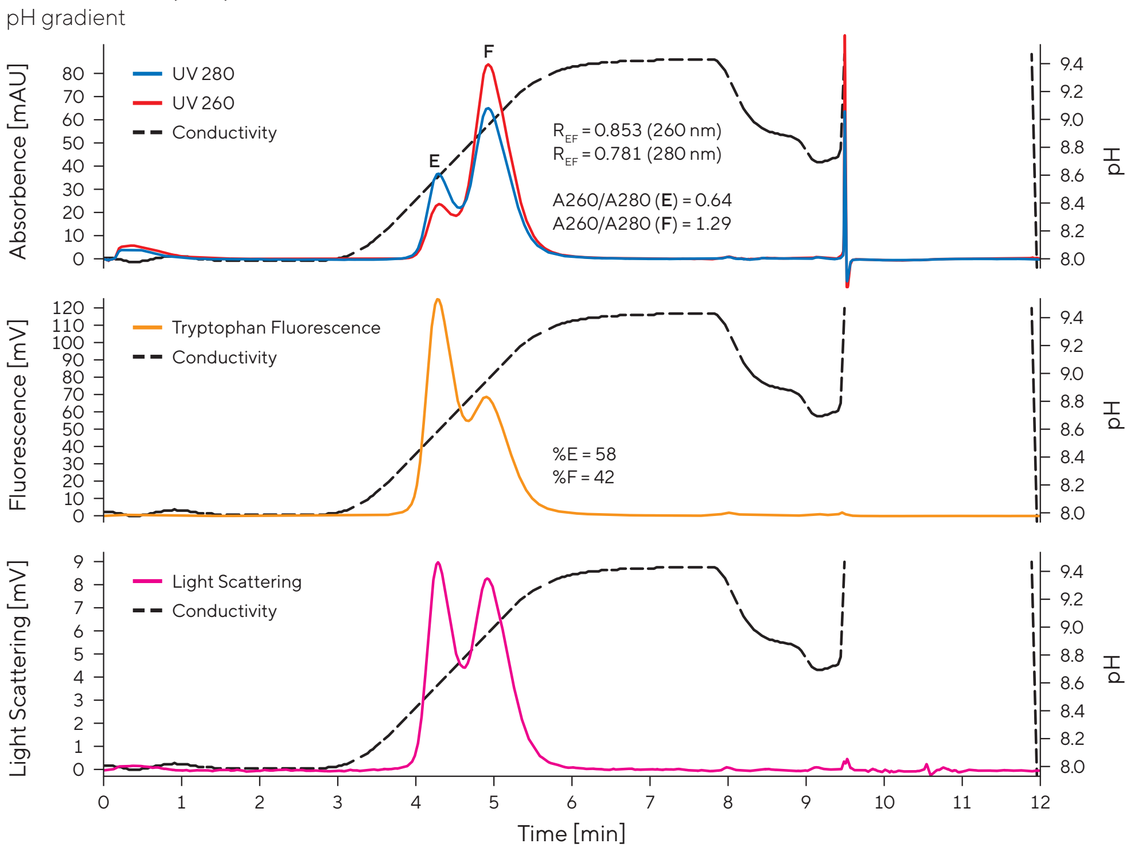

Figure 3: *Chromatogram of AAV 2/8 Serotype Sample Analysed by CIMac PrimaS® (AAV)-Beta Column. Gradient Composition: 2.5-Minute Gradient From 100% Buffer A to 100% Buffer B.*

CIMac PrimaT-Beta
$MgCl_2$ and NaCl gradient

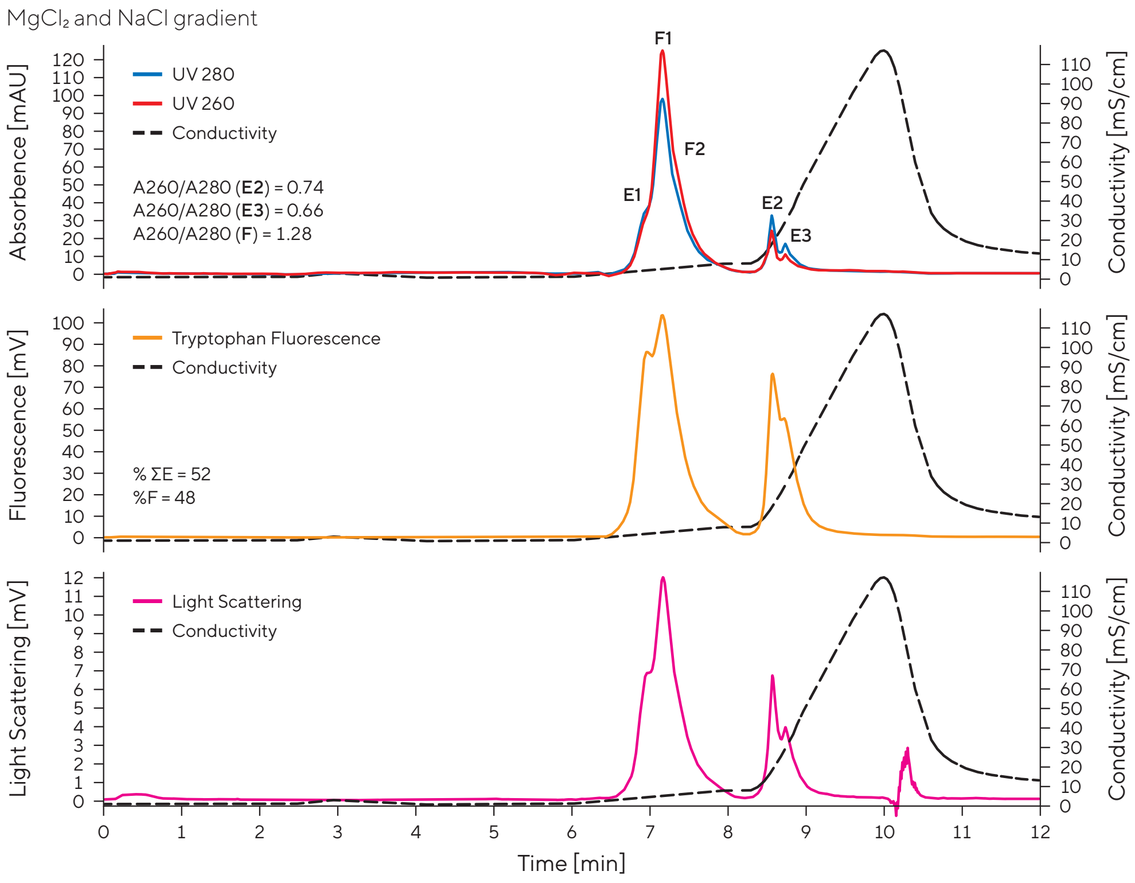

Figure 4: *Chromatogram of AAV 2/8 Serotype Sample Analysed by CIMac PrimaT-Beta Column. $MgCl_2$ Gradient is Marked With Dashed Black Curve. NaCl Salt Gradient (From ~8 to ~10 Min) Follows After $MgCl_2$ Gradient (From ~6 to ~8 Min). Gradient Composition: 2.5-Minute Gradient From 100% Buffer B to 100% Buffer C and 2.0-Minute Gradient From 100% Buffer B to 100% Buffer D.*

4. Conclusion

The data presented above demonstrates:
- Empty AAV capsids in AAV sample were characterized using three different empty/full separation columns.
- CIMac PrimaS® (AAV)-Beta and CIMac PrimaT-Beta are new multimodal orthogonal columns for empty/full separation.
- Each column has its own elution gradient of empty and full capsids (AAV: salt gradient; PrimaS® (AAV)-Beta: pH gradient and PrimaT-Beta: magnesium-salt gradient) and shows different profiles providing different information about empty and full separation.
- Three subpopulations of empty and two subpopulations of full AAV capsids were observed using CIMac PrimaT-Beta column.
- CIMac PrimaT-Beta: percent of empty capsids was obtained by summation of all three subpopulations of empty AAV capsids. Total percentage of empty AAV capsids are similar with the one obtained by CIMac AAV full/empty and CIMac PrimaS® (AAV)-Beta columns.