Simplifying Progress

Combining the Sensitivity of the PATfix® HPLC Platform With the Resolution of Ultracentrifugation for AAV Characterization

S. Peljhan, M. Štokelj, S. D. Prebil, B. Bakalar, P. Gagnon, A. Štrancar

BIA Separations d.o.o., A Sartorius Company, Mirce 21, 5270 Ajdovščina, Slovenia
Contact: monolith-purification@sartorius.com

Introduction

Density gradient ultracentrifugation (DGUC) is a well-established tool for Empty/Full AAV capsid separation, based on density differences between AVV sub-populations. However, DGUC practice is laborious and lacks any detection options, therefore fractions must be collected manually and analyzed later. Both shortcomings can be addressed by coupling post DGUC workflow to PATfix® analytical HPLC. BIA Separations PATfix® platform provides sufficient tools for liquid extraction and fractionation, as well as a comprehensive detector suite for precise fraction characterization. Baseline separation of capsid species was achieved in a density gradient of CsCl, producing a centrifugramthat reveals information traditional DGUC and anion exchange chromatography cannot.

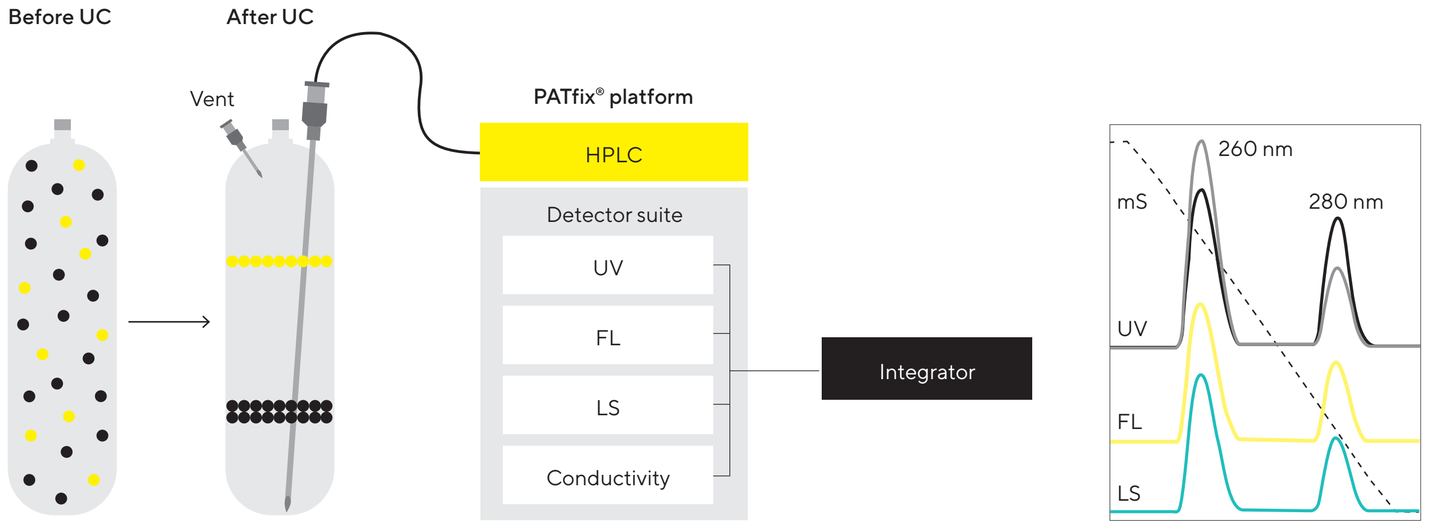
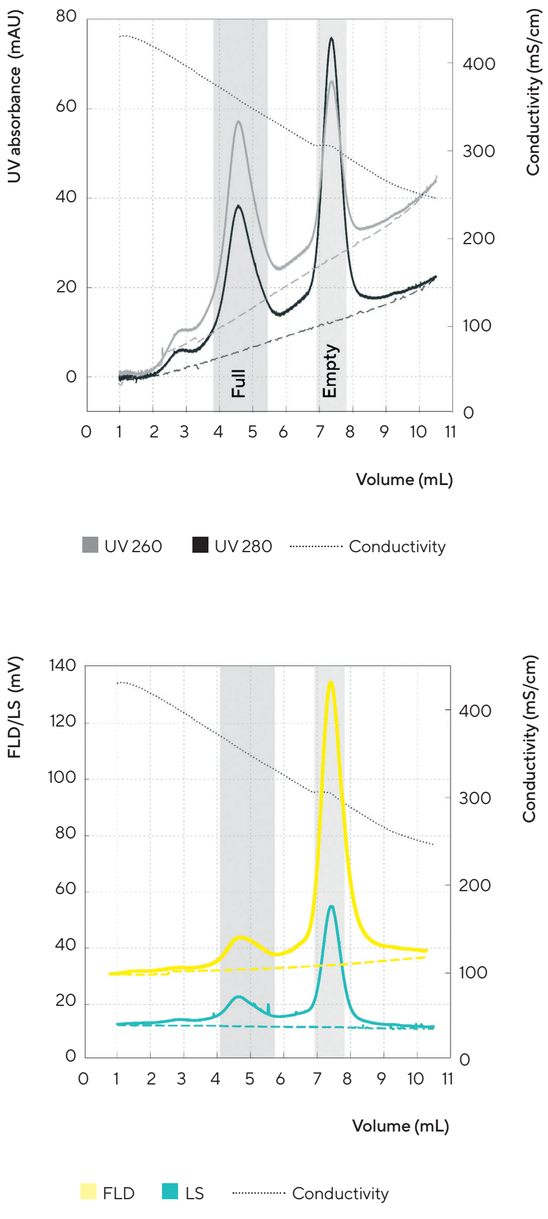


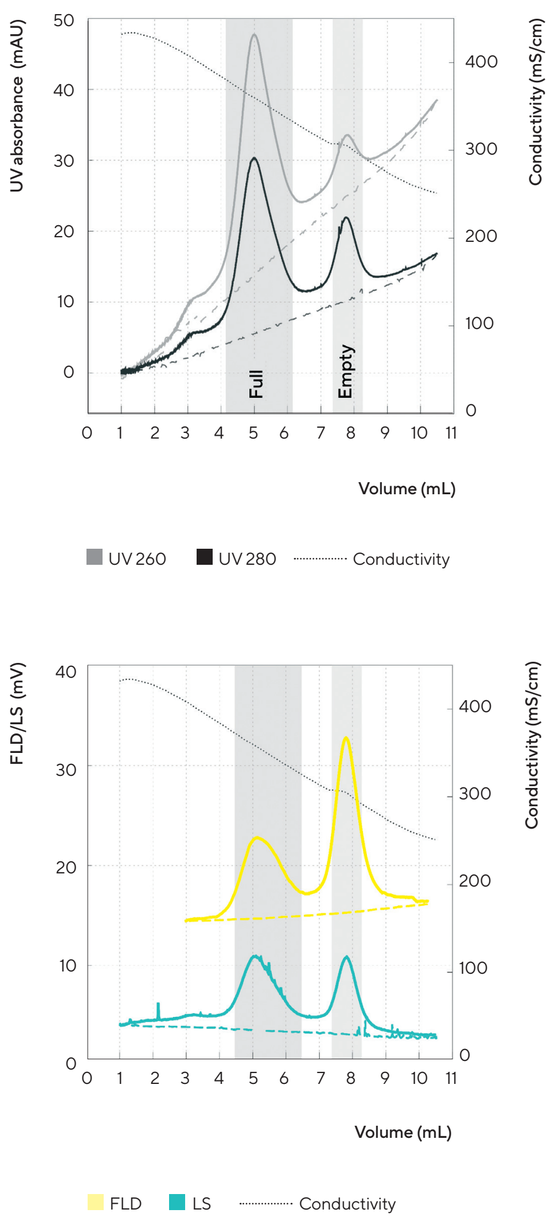
Figure 1: Schematic representation of PATfix® Ultracentrifugation

Results

After CEX: AAV Fraction



After AEX: Full Fraction



After AEX: Empty Fraction

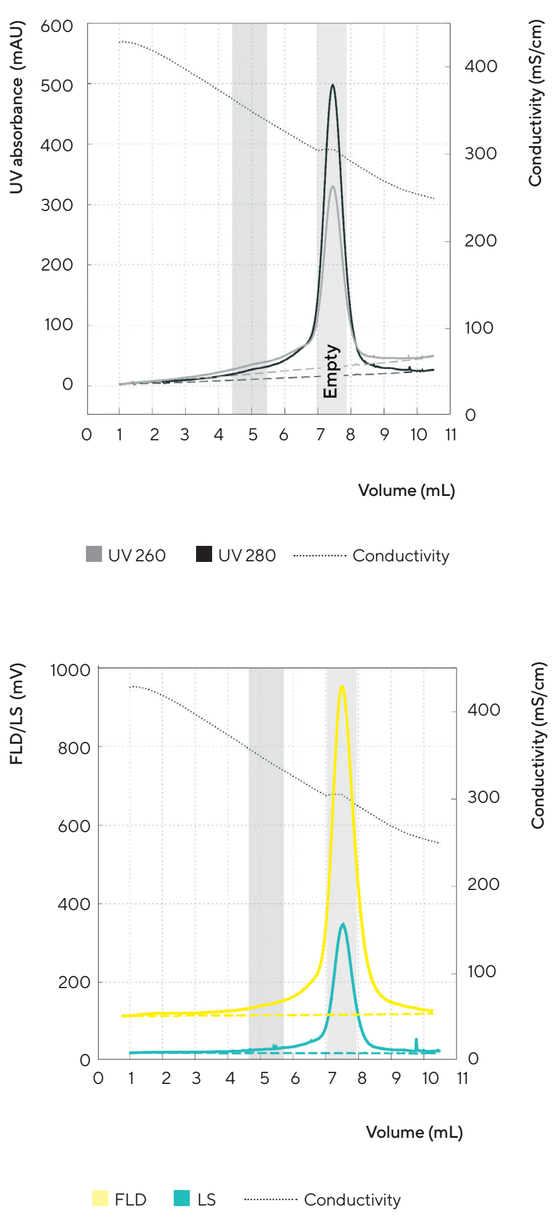


Figure 2: Centrifugrams after different chromo steps. 1.4+E11 vg of AAV8 capsids, obtained from Sf9/BEV cell lysate (provided by University of Nantes). Initial AAV purification was performed by Cation Exchange Chromatography (CEX) on a 1 mL CIMmultus® SO3 monolith (CAT#311.6157-2). The resulting centrifugram from the AAV fraction is shown on the left. Anion Exchange Chromatogrpay (AEX) fractionation was performed on 1 mL CIMmultus® QA monolith (CAT#311.5113-2). Centrifugrams resulting from fraction containing Full AAV capsids (centre) and fraction containing Empty AAV capsids (right) are shown. Tube contents were pumped from the bottom of the tube, from high to low CsCl concentration, directly through the monitor array of PATfix® analytical HPLC, equipped with UV detector (260 nm, black; 280 nm, grey), fluorescence detector (yellow), light scattering (teal) and conductivity monitor (dashed line).

PATfix® platform detector utilization and corresponding signal integration produces a multi-parameter DGUC "centrifugram" that corresponds in many respects to traditional chromatograms. The resulting centrifugrams of AAV capsid populations separated in CsCl, reveal a result that neither method can achieve separately. DGUC provides complete baseline separation between empty and full AAV capsids. PATfix® multi detector setup provides a comprehensive capability to detect AAV capsids within the CsCl gradient. A comparison of profiles originating from different detectors enables precise characterization of the corresponding capsid population. The peak at 4.5 mL with a UV260/280 ratio of 1.41 represents full AAV capsids, while the peak at 7.3 mL with a ratio of 0.61 represents empty capsids.

The ascending baseline of the UV profiles is caused by the change in refractive index of the CsCl gradient. This can compromise accuracy and sensitivity, but it does not interfere with peak identification. Intrinsic fluorescence is 20 – 50 times more sensitive than UV, its baseline is much less affected by refractive index changes, and it is not influenced by the encapsidated DNA. Intrinsic fluorescence sees only proteins, which makes it a more valid basis for judging relative size of empty and full capsid peaks. Light scattering fulfils the important role of confirming which peaks are populated by capsids, but is influenced by refractive index, so intrinsic fluorescence remains the best index for judging relative areas of empty and full capsids.

References

S. Peljhan, M. Štokelj, S.D. Prebil, P. Gagnon, A. Štrancar, Multiple-parameter profiling of density gradient fractionation for characterization of empty and full capsid distribution in AAV Preparations, Cell & Gene Therapy Insights, 2021; 7(2), 161–169