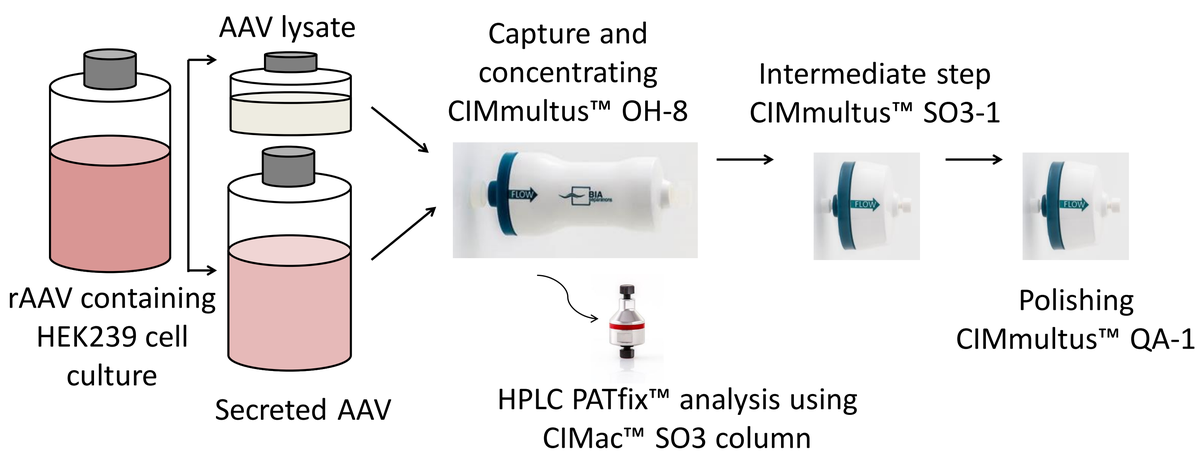
HEK293-derived Adeno Associated Virus (AAV) purification: comparison of small scale laboratory production towards industrial format using monoliths

M. Tajnik Sbaizero[1], L. Zentilin[2], M. Leskovec[1], B. Goričar[1], J. Merkelj Koren[1], P. Gagnon[1], A. Štrancar[1]

1 BIA Separations d.o.o.. Mirce 21. SI-5270 Ajdovščina, Slovenia. 2 International Centre for Genetic Engineering and Biotechnology (ICGEB), Padriciano 99, 34149 Trieste, Italy.

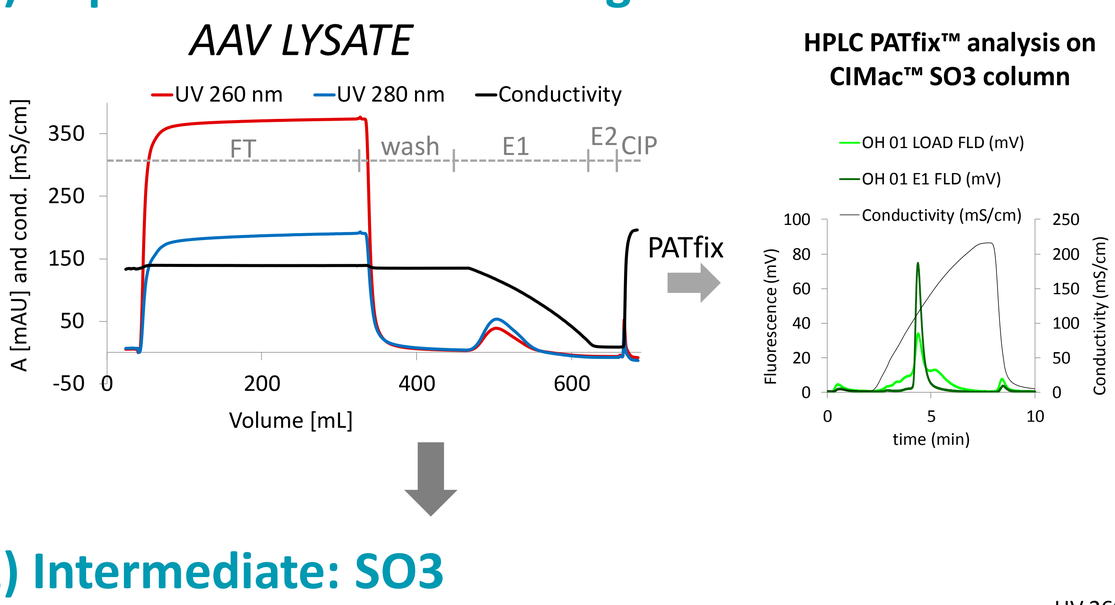
INTRODUCTION and AIMS of the study

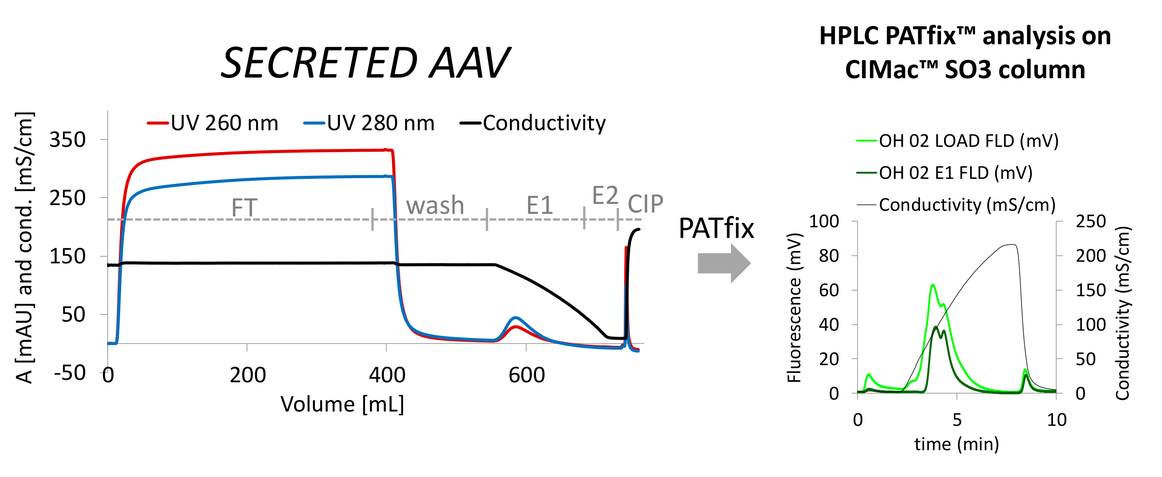
During recombinant adeno associated virus (rAAV) downstream processing, a large amount of host-cell and product related impurities needs to be removed from the product. Succesful process on laboratory scale, such as Cesium chloride purification, lacks scalability when the process is due to be transfered to larger industrial scale. The aim of the study was to develop robust, fast and effective rAAV virus purification platform, which can be used for several AAV serotypes with various inserts. Lysed harvest and supernatant of rAAV9 were first captured and concentrated on CIMmultus™ OH column, followed by intermediate step on CIMmultus™ SO3 column and further polishing on CIMmultus™ QA column. Derived purity of industrial scale monolith purification product was compared to laboratory scale purification.



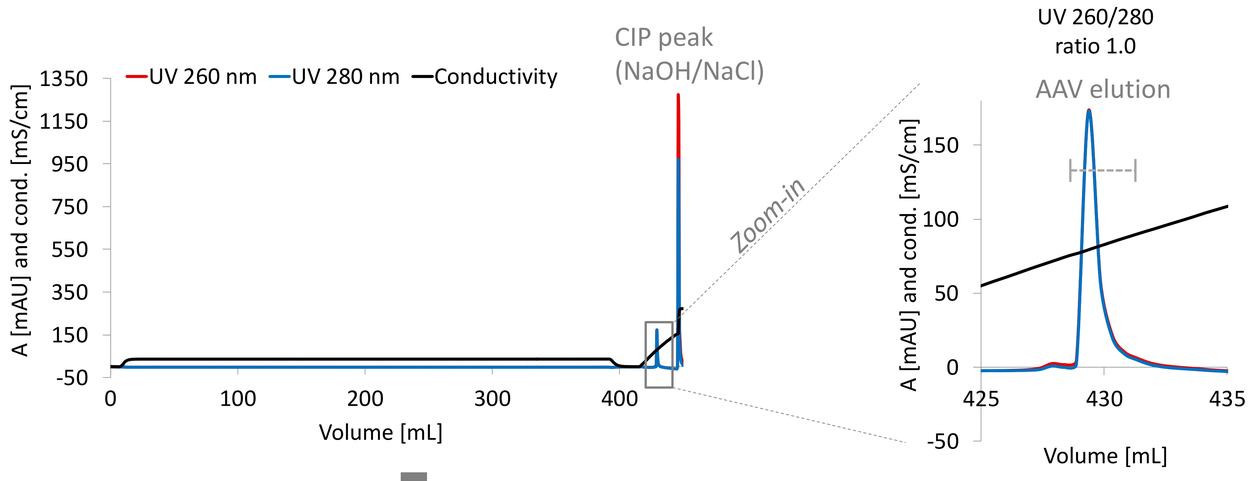
RESULTS

1) Capture and concentrating: OH

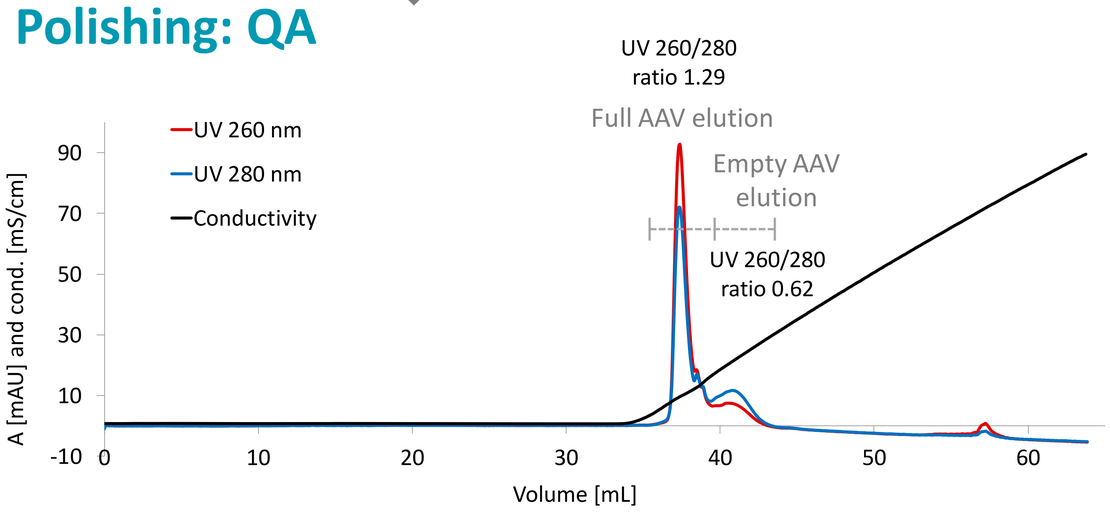




2) Intermediate: SO3



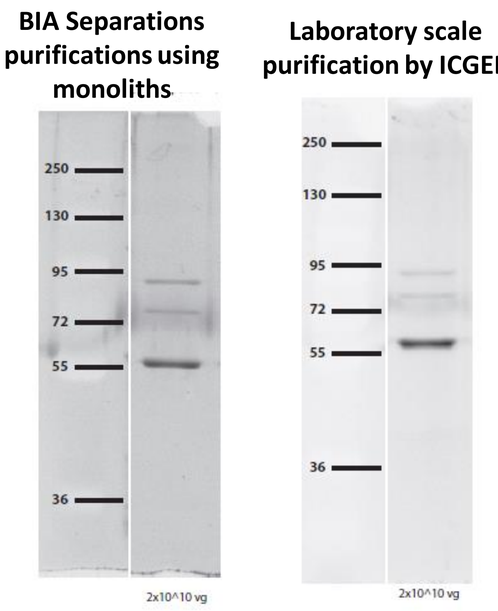
3) Polishing: QA



Conditions:

- OH CIMmultus™ 8 mL, 2µm pores, SO3 CIMmultus™ 1 mL, 2µm pores; QA CIMmultus™ 1 mL, 2µm pores
- For OH step: both samples diluted 1:1 with 3 M Na phosphate neutral pH, 3500 rpm 5 or 10 min, filter Minisart CA 1.2 µm, load 292 mL (lysed AAV) or 397 mL (secreted AAV); for SO3 OH lysed harvest E1 fraction diluted and acidified; for QA step SO3 AAV elution fraction, buffer exchanged
- Mobile phases: OH step - MFA: 1.5 M Na phosphate, neutral pH, MFB: 50 mM Na phosphate, neutral pH; SO3 step – acetate buffers, acidic; QA step: BTP alkaline buffers; for all steps linear salt gradient was used
- Analytical assays: total AAV method using HPLC PATfix™, SDS-PAGE, Real time PCR

Comparable purity profile of laboratory scale purification and purification using monolith technology



SDS-PAGE: SyproRuby staining.

CONCLUSIONS

- Capture step using CIMmultus™ OH column shows good virus recovery for both of rAAV9 materials tested, although elution profile using HPLC PATfix™ and SO3 column shows better peak resolution for the AAV lysate sample, which was used for intermediate and polishing step.
- Intermediate step furter eliminated sample impurities and AAV capsids were sucessfully separated during QA polishing step.
- Polishing step shows good separation of empty and full capsids.
- Derived product using BIA Separations' purification on monoliths shows similar purity than laboratory scale purification by ICGEB.