AN INDUSTRIAL PURIFICATION PLATFORM FOR AAV

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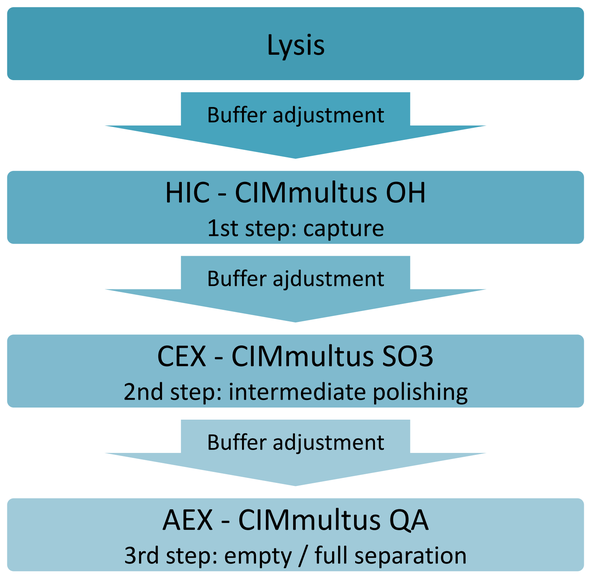
INTRODUCTION

This poster presents fully scalable non-affinity purification strategy that has been proven to be effective for all AAV serotype tested to date. Cell lysate is directly subjected to column purification after removal of cell debris without requiring a concentration step using tangential flow filtration. The process consists of three chromatographic steps.

Hydrophobic interaction chromatography on a CIMmultus OH monolith is used for initial virus capture and purification (Figure 1). Precipitating salts are used at 1.0–2.0 M to achieve virus binding. Most of the small molecule contaminants and proteins are eliminated in the flow-through (Figure 4 and Figure 5, OH FT). AAV co-elutes with a highly reduced population of contaminating proteins (Figure 4 and Figure 5, OH E). DNA-protein complexes are very strongly retained and require NaOH for removal.

Intermediate polishing is performed with a CIMmultus SO3 cation exchange monolith (Figure 2). The AAV fraction from the capture step (OH E fraction in Figure 1) is titrated to a pH value of 3.5—5.0 and diluted to binding conditions. Sugars and surfactants are added to suppress non-specific interactions with tubing and containers, and the product is eluted in a salt gradient.

Final polishing is conducted on a CIMmultus QA anion exchange monolith which separates empty capsids from full capsids (Figure 3). This is achieved in a salt gradient at alkaline pH. For more information please refer to BIA Application note A048 (www.biaseparations.com/applications).



RESULT

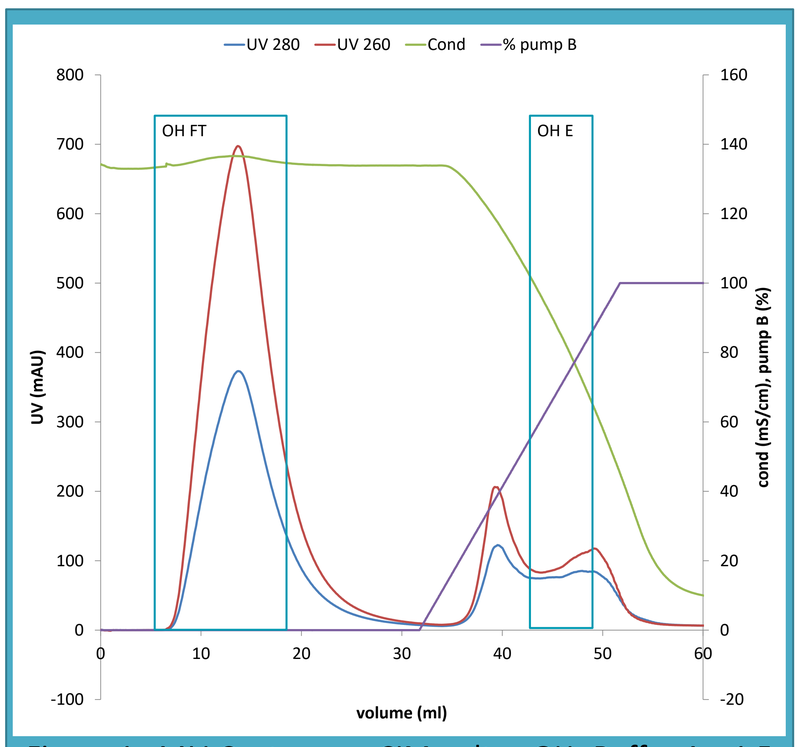


Figure 1: AAV Capture on CIMmultus OH. Buffer A : 1.5 M potassium phosphate, pH 7.0. Buffer B: 50 mM potassium phosphate, pH 7.0. Sample preparation: mix cell lysate supernatant with potassium phosphate to a final concentration of 1.5 M. Equilibrate buffer A. Load. Wash: buffer A. Elute: linear gradient to 100 % B in 20 column volumes (CV).

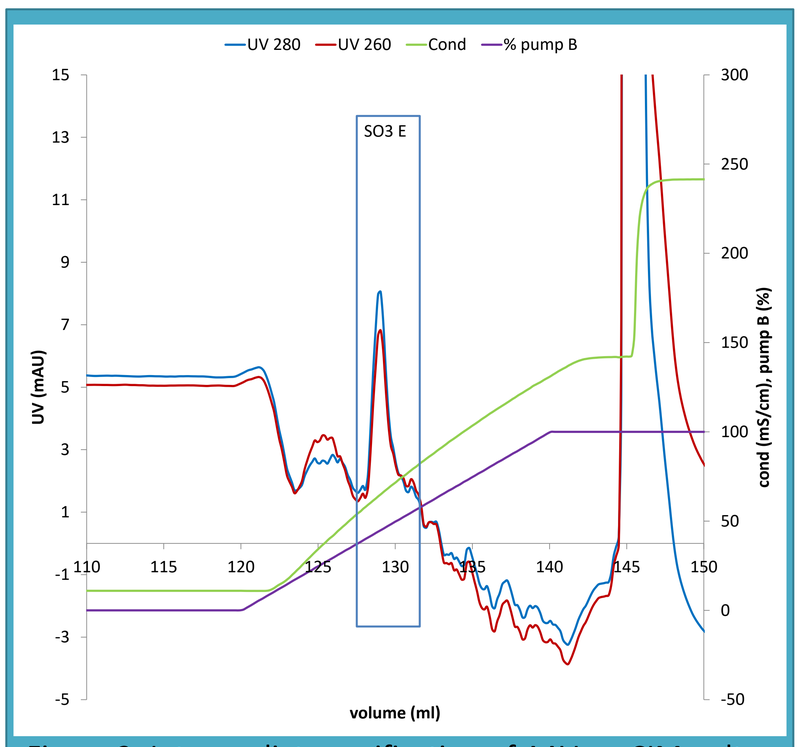


Figure 2: Intermediate purification of AAV on CIMmultus SO3. Buffer A: 50 mM acetate plus sugar and surfactant at acidic pH . Buffer B: 50 mM acetate, 2.0 M NaCl, plus sugar and surfactant at acidic pH. Equilibrate: buffer A. Load. Wash: buffer A. Elute: linear gradient to 100 % B in 20 column volumes (CV).

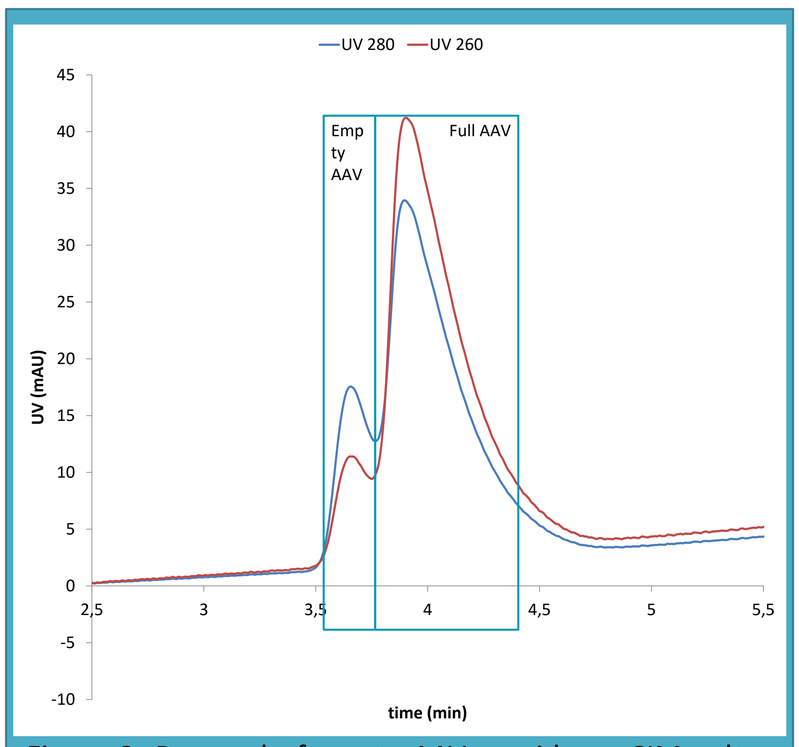


Figure 3: Removal of empty AAV capsids on CIMmultus QA. Buffer A : 20 mM bis-tris propane (BTP), 35 mM NaCl, pH 9.0. Buffer B: 20 mM BTP, 400 mM NaCl, pH 9.0. Equilibrate: buffer A/ Load. Wash: buffer A. Elute: linear gradient from 0 to 40 % B in 50 column volumes (CVs).

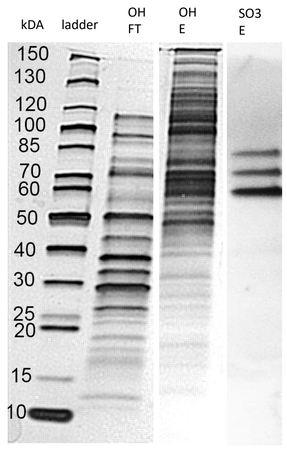
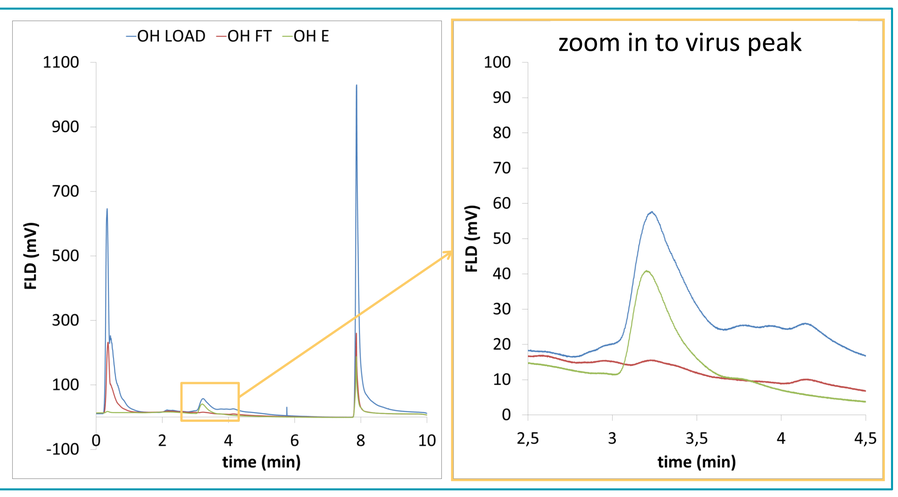


Figure 4. SDS PAGE showed good elimination of protein impurities in the flow-through of the capture step with no virus loss. Virus eluted in linear gradient fraction OH E, which was further on purified on SO3 column (SO3 E).

Figure 5. Analysis of OH fractions by fractionation on a 100 µL CIMac SO3 column, 5 µL injection. Analysis time: 10 minutes. Detection by fluorescence (280/348 nm). The sample was eluted with a salt gradient at acidic pH to match the requirements of the serotype.



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