ONE-STEP MONOLITH PURIFICATION OF ONCOLYTIC INFLUENZA VIRUS PRODUCED IN VERO CELLS

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INTRODUCTION and AIMS of the study

Chromatography is a useful purification method for large biomolecules and virus manufacturing and it is easily scalable to large production volumes. Convective Interaction Media (CIM) monolithic columns constitute of large flow-through channels and consequently have high surface accessibility of binding sites. Preferences of CIM monolithic columns are flow independent performance, resulting in fast separation, concentration, purification, impurities removal, and analytics of biopharmaceuticals.

The aim of the study was to develop Influenza virus purification platform, which can be used for several virus strains. The main objective was to develop a process with as little as possible of intermediate steps, especially omitting Tangential Flow Filtration (TFF) or other sample pre-treatments with high host-cell DNA and protein removal, as well as to achieve high binding capacity of the Influenza virus per mL of monolithic support.

Clarified benzonase treated Vero cell harvest

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Chromatography using CIMmultus™ SO3

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Formulation/sterile filtration

RESULTS

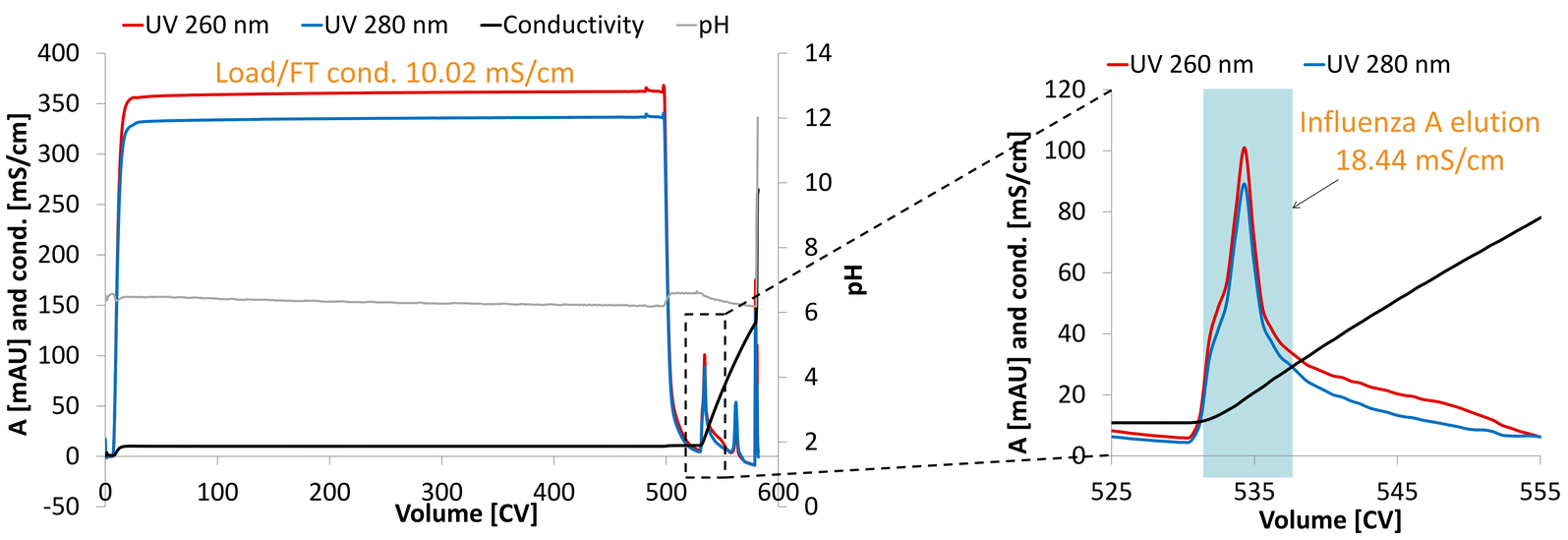

Figure 1: Chromatogram of Influenza A purification using CIM multus SO3 column and zoom-in to the main fraction. Virus elutes at 18.44 mS/cm under neutral pH conditions.

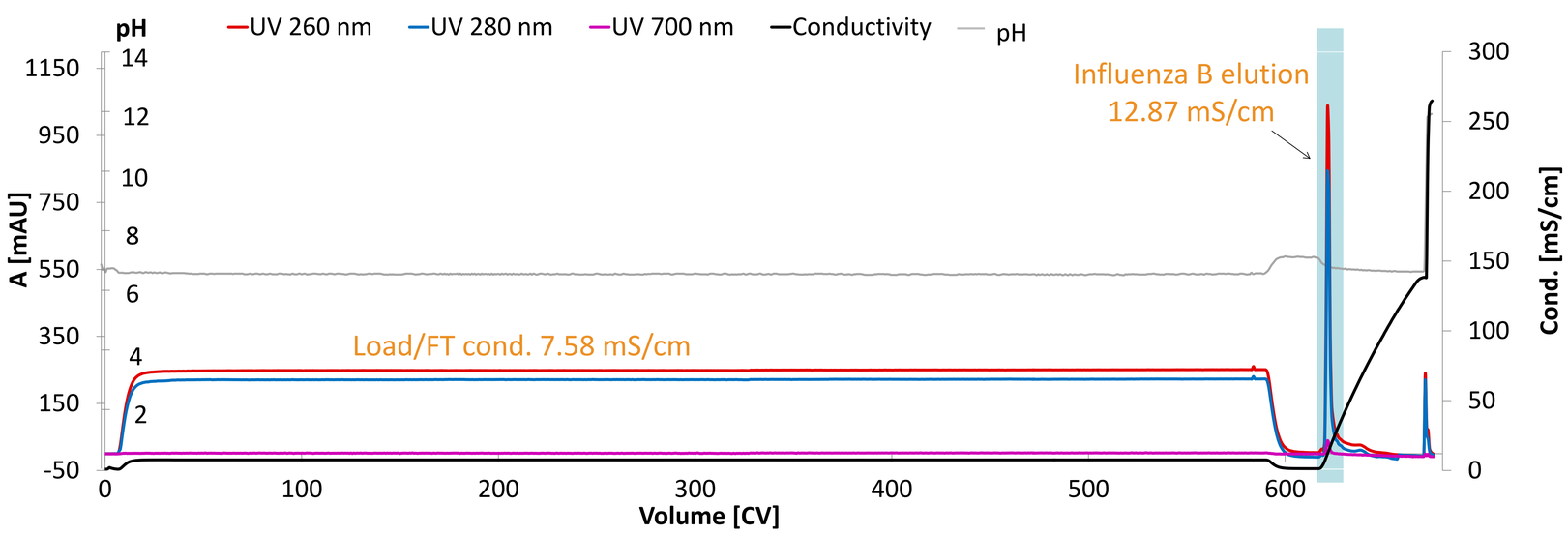

Figure 2: Influenza B purification chromatogram. Virus elutes at the beginning of the salt gradient at 12.87 mS/cm under neutral pH conditions.

Chromatography summary:

Dynamic binding capacity
> 3.16 E+10 FFU/ml SO3 resin

Protein reduction
> 95 %

Host cell DNA reduction
> 95 %

Infectious particle recovery
> 80 %

Column productivity (infectious particles/volume/time)*
~ 1.1 E+10 FFU/ml SO3 resin/ hour
- at flowrate 5 CV (mL) / min; FFU – focus forming unit

Conditions:

- Centrifuged cell culture harvest containing 1E+7–1E+9 influenza virus particles per mL was treated with benzonase, diluted 1.5-fold (influenza A; 2-fold for influenza B) with Hepes-sucrose buffer and applied without further treatment to a monolithic cation exchanger.
- Column: CIMmultus™ SO3-1 Advanced Composite Column (Sulfonyl) (Pores 2 um)
- Sample dilution / Column equilibration buffer A: 50 mM HEPES, 200 mM sucrose, pH 7.0
- Column equilibration buffer B: 50 mM HEPES, 200 mM sucrose, 2 M NaCl, pH 7.0
- Elution buffer A: 50 mM HEPES, 200 mM sucrose, 100 mM NaCl pH 7.5 (for Influenza A) and 50 mM HEPES, 200 mM sucrose, 50mM NaCl pH 7.5 (for Influenza B)
- Elution buffer B: 50 mM HEPES, 200 mM sucrose, 2 M NaCl pH 7.5
- Flowrate: 5 ml/min; Detectors: UV 260, 280, 700 nm; Conductivity, pH
- Analytical assays: hemagglutination (HA), Focus forming assay (FFA), Bradford, Picogreen, total Flu HPLC PATfix™

CONCLUSIONS

- VERO cell based production of Influenza viruses enables omitting Tangential flow filtration (TFF) prior to the chromatography step – reduces costs, operating time and improve overall process yields.

- Overall process yield are more than 80 % due to high binding capacity, determined as 3.16 E+10 FFU/ml per mL of CIMmultus™ SO3 advanced composite column resin

- Chromatography step using CIMmultus™ SO3 advanced composite column enables one-step process due to:
  - DNA and host protein contamination were reduced by 95%.
  - 50 times higher concentration of infective virus particles than the original cell culture.
  - Only dilution to achieve the target virus concentration is required to prepare the final formulation.