Inline-tandem purification of viruses from cell lysate by agarose-based chromatography

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Introduction

With the ever-increasing need for more viral-based material for vaccine production, there is an increased demand to develop time-efficient and optimized purification methods. Traditional purification of viruses and virus particles is typically a time-consuming and laborious multi-step endeavor with a high operator competence needed. In this study we investigate the use of an inline-tandem chromatography method to alleviate some of these issues and streamline the purification into a mostly automated single-flow procedure with high yields and specific infectivities (Fig. 1). The target virus in this project was the adeno-like phage-virus; PRD1.

Challenge: To replace a laborious and time-consuming precipitation and ultracentrifugation-based purification procedure with a rapid, easy to scale, single flow chromatography method for the adeno-like virus, PRD1.

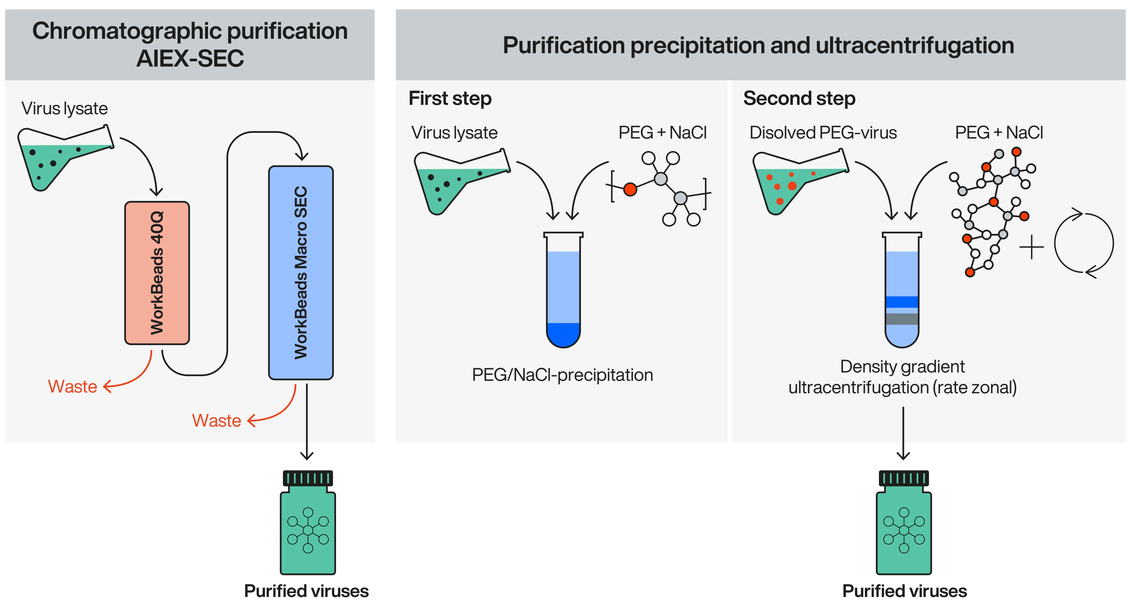

Figure 1. Setup for the inline-tandem purification of viruses by agarose-based chromatographic resins (A) and conventional two-step virus purification method by PEG-NaCl precipitation and ultracentrifugation in density gradients (B).

Method development and optimization

Initial development aimed to determine feasibility regarding the use of anion exchange chromatography (AIEX) and size exclusion chromatography (SEC) for PRD1 purification respectively was performed and confirmed (Fig 2.)

Resins:	WorkBeads™ 40Q	WorkBeads Macro SEC
Columns:	6.6 × 100 mm (3.4 mL)	10 × 300 mm (23.6 mL)
Samples:	40 mL virus lysate	5 mL pre-AIEX-purified sample
Flow rate:	0.7 mL/min (120 cm/h)	1 mL/min (50 cm/h)
Binding buffer:	20 mM K-PO_4, 1 mM Mg_2Cl, pH 7.2	20 mM K-PO_4, 1 mM Mg_2Cl, pH 7.2
Elution buffer	20 mM K-PO_4, 1 mM Mg_2Cl, 1 M NaCl, pH 7.2	
Linear gradient:	0–100% elution buffer in 30 CV	

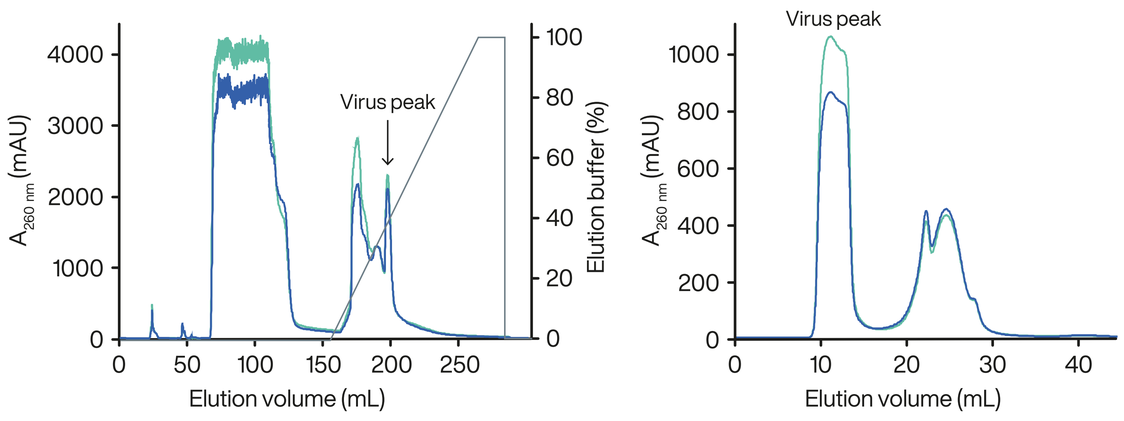

Figure 2. (A) Virus purification on WorkBeads 40Q (AIEX) using an elution linear gradient. UV 280 nm (blue), UV 260 nm (green) and elution buffer concentration (gray). (B) Purification of viruses pre-purified on WorkBeads 40Q using WorkBeads Macro SEC.

AIEX: PDR1 eluted in a single peak at high specific infectivity
SEC: PDR1 eluted in void – base line separation between impurities/virus

Finalized method and results

Based on these data the two methods where fused into a single flow where target virus was first accumulated on the AIEX column before a low salt concentration wash was applied to displace loosely bound impurities. This was followed by a step elution where the eluate was redirected onto the SEC column through a switch valve to separate the target from smaller residual impurities (Fig 3.). Results were compared with the traditional method and evaluated in Table 1.

Resins/columns:	1. WorkBeads 40Q, 6.6 × 100 mm
	2. WorkBeads Macro SEC, 10 × 300 mm
Sample:	40 mL virus lysate
Flow rate:	0.7 mL/min (120 cm/h)
Binding buffer:	20 mM K-PO_4, 1 mM Mg_2Cl, pH 7.2
Elution buffer:	20 mM K-PO_4, 1 mM Mg_2Cl, 1 M NaCl, pH 7.2
Step gradient:	1. 0–25% elution buffer in 10 CV
	2.25–100% elution buffer in 12 CV

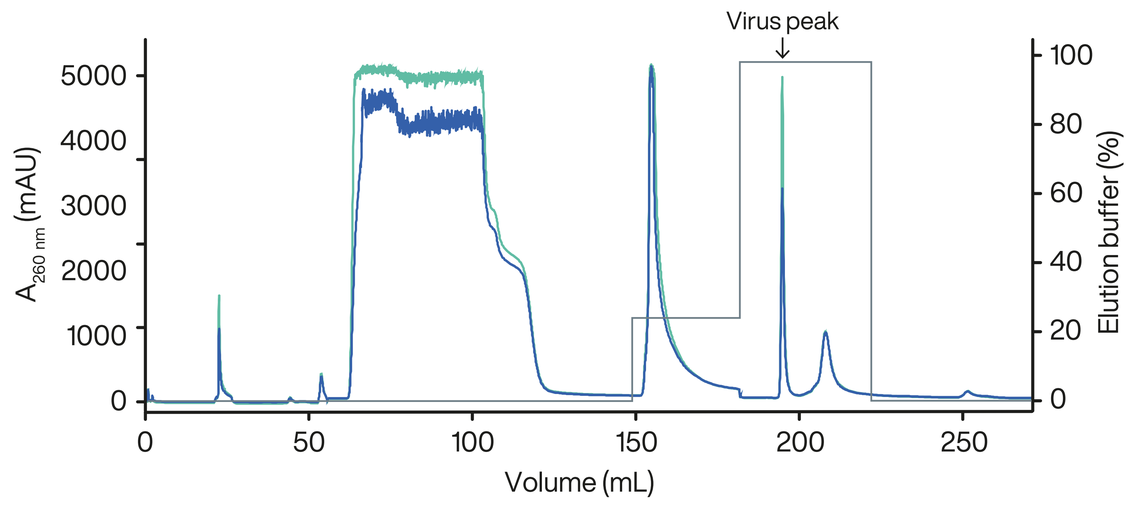

Figure 3. Tandem virus purification consisting of AIEX followed by SEC connected in series. UV 280 nm (blue), UV 260 nm (green) and elution buffer concentration (gray). Loading, wash and 25% elution step gradient are performed with SEC column bypassed. The flow was shifted onto WorkBeads Macro SEC at start of 100% elution gradient step.

Table 1. Recovery and infectivity data for PEG-precipitation-ultracentrifugation purification (old method) and tandem purifications (AIEX-SEC).

Method	Sample	Yield (pfu[1])	Specific infectivity (pfu/mg[2] of protein)	Purified particles (mg/L lysate)	Time (hours)
Old method	Lysate	100	3.4×10^{11}	-	-
	Pure virus[3]	13	6.6×10^{12}	3.5	> 24
AIEX-SEC	Lysate	100	4.4×10^{11}	–	–
	Pure virus[4]	53.5	5.8×10^{12}	12.8	6

[1] Determined by plaques assay analysis.
[2] Determined by Bradford analysis.
[3] PEG-NaCl precipitated, purified by rate zonal centrifugation in sucrose.
[4] Purified on WorkBeads 40Q and WorkBeads Macro SEC (no additional concentration step).

Conclusions

An inline-tandem chromatography setup was successfully developed to purify the adeno-like model virus PDR1. The method provided several advantages compared to a traditional precipitation/centrifugation type method as listed below:

- Fully automated setup
- Faster process
- Enables efficient scaling
- Very high yields (3.5x higher than traditional method) of infectious viruses
- 12.8 mg purified particles per liter of cell lysate (3.5 mg with traditional method)
- Robust and reproducible
- High purity of virus eluate

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