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WHITE PAPER

Virus purification using inline tandem-coupled WorkBeads 40Q and WorkBeads Macro SEC

Virus purifications

Viruses are very common and significant infectious agents that cannot be treated with any antibiotic-like substance, so vaccinations are essential to avoid infections. For production of many viral vaccines, an effective and successful purification of the virus is needed. This purification is however particularly challenging since the virus needs to be isolated from complex mixtures and purified (downstream processing) to extremely high levels. This frequently requires a lot of time-consuming optimization and often results in low yields. The same challenges also apply to virus vector preparation for gene therapy. Production and purification of a virus with high purity and activity levels is also needed for biochemical, virological, and structural work.

Preparative virus purifications are often based on various filtration, precipitation, or ultracentrifugation techniques. None of these techniques are automated and they require time-consuming manual handling. There is a clear need for more efficient approaches for virus downstream processing. Chromatography is an alternative that offers automated and affordable purifications. Here we present a virus purification setup that produces purified virus at high yield and with high activity.

Precipitation-Centrifugation *vs.* AIEX-SEC

A typical virus purification may consist of two steps, for example PEG-precipitation followed by ultracentrifugation. This will ultimately generate a pure product but at the expense of time (>24 h) and manual labor. PEG-precipitation alone is an efficient initial purification technique but is not sufficient. Replacing these time and labor intensive steps with two orthogonal chromatographic steps increases the efficiency of the virus purification. The purification was completed in only 360 minutes using an anion exchange (AIEX) step followed by a size exclusion (SEC) step connected in series, while still allowing the columns to be run individually. For an average of 4 biological replicates using AIEX-SEC, 12.8 particles per mg were purified per one liter of lysate (mg/mL), which is 3.5 times higher than that obtained using PEG-precipitation-ultracentrifugation. This means that a much higher yield was achieved using the chromatographic tandem purification while still reaching the same purity in terms of virus activity (1). The virus used in this study is a membrane-containing tailless icosahedral dsDNA prokaryotic virus with a size of approximately 65 nm.

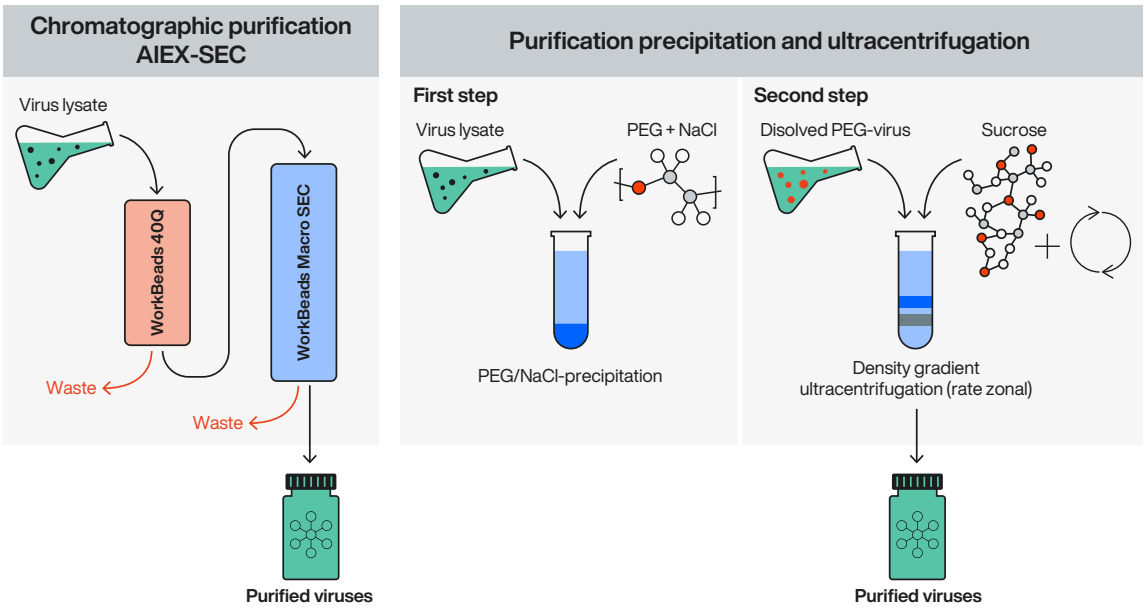


Figure 1. Chromatographic purification setup *vs.* purification by precipitation followed by ultracentrifugation. Schematic illustration of a tandem chromatographic setup consisting of AIEX and SEC connected in series (left). Schematic illustration of PEG-precipitation followed by ultracentrifugation for virus isolation (right).

Optimization of chromatographic steps

To be able to setup a chromatographic tandem method, the individual steps first had to be optimized, particularly the AIEX step since it is performed in binding-elution mode. WorkBeads™ 40Q was used in the first step followed by WorkBeads Macro SEC as the second step. WorkBeads Macro SEC is a resin with very high porosity. This resin has a cutoff of 30 000 kDa which is optimal for this purpose.

A physiological buffer system was used: 20 mM potassium phosphate, 1 mM magnesium chloride, pH 7.2, to maintain the virus in its active state. The loading capacity of the virus lysate was determined to be higher than 10 mL lysate per mL AIEX resin. The virus lysate was loaded onto the AIEX column to which the virus particles bind as well as other negatively-charged components, while the positively-charged components will flow through. The elution was performed in a long linear salt gradient to determine the salt concentration at which the virus eluted. Figure 1A shows that the virus eluted in a single peak at 0.4 M NaCl (40% of elution buffer) with no virus activity detected in other fractions (determined by plaque assays). The collected virus fraction was then applied to the WorkBeads Macro SEC column where it elutes in the first peak (Figure 1B).

Resin/column:	WorkBeads 40Q 6.6 x 100 mm (3.4 mL)
Sample:	40 mL virus lysate
Flow rate:	0.7 mL/min
Binding buffer:	20 mM potassium phosphate, pH 7.2
Elution buffer:	20 mM potassium phosphate, 1 mM $MgCl_2$, 1 M NaCl, pH 7.2
Linear gradient:	0–100% elution buffer in 30 column volumes (CV)

Resin/column:	WorkBeads Macro SEC 10 x 300 mm (23.6 mL)
Sample:	5 mL pre-purified sample (from AIEX run)
Flow rate:	1 mL/min
Running buffer:	20 mM potassium phosphate, 1 mM $MgCl_2$, pH 7.2

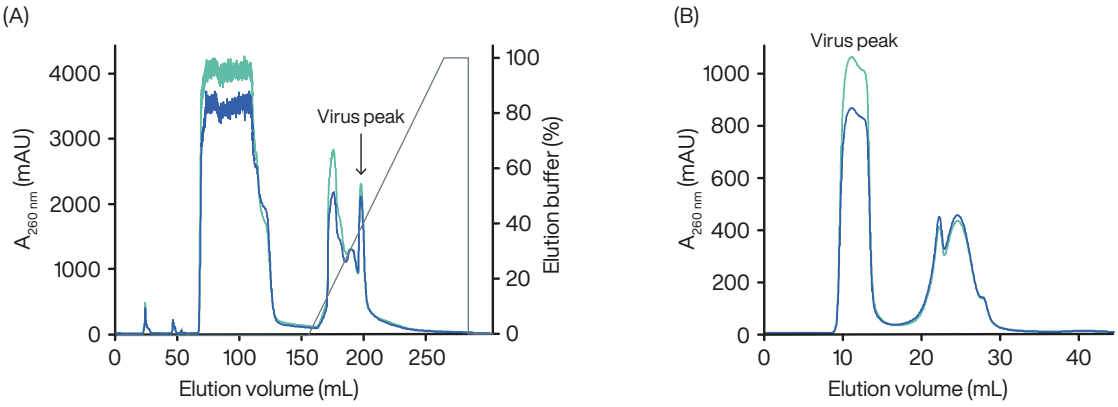


Figure 2. AIEX and SEC as stand-alone steps. (A) Virus purification on WorkBeads 40Q using an elution linear gradient. UV 280 nm (blue), UV 260 nm (green) and elution buffer concentration (grey). (B) Purification of viruses pre-purified on WorkBeads 40Q using WorkBeads Macro SEC.

Tandem chromatography

Four biological replicates were run on the optimized tandem setup to determine the robustness of the purification. We applied 40 mL of virus lysate onto a 6.6 × 100 mm column packed with WorkBeads 40Q. The virus titer in the lysate feed was 1.1×10^{11} pfu/mL with a specific infectivity of, on average, 3.4×10^{11} pfu/mg of protein. The wash step was followed by 25% elution buffer for 10 CV to elute loosely bound material. Elution of virus was then carried out using 12 CV of 100% elution buffer with the flow path shifted towards the WorkBeads Macro SEC via the column valve and fractions were collected for further analyses, such as plaque assays, Bradford analyses and SDS-PAGE. This automated method was performed in 360 minutes in total. Figure 3 shows the chromatogram for this optimized tandem setup.

Resins/columns:	1. WorkBeads 40Q, 6.6 x 100 mm (3.4 mL) 2. WorkBeads Macro SEC, 10 x 300 mm (23.6 mL)
Sample:	40 mL virus lysate
Flow rate:	0.9 mL/min
Running buffer:	20 mM potassium phosphate, 1 mM $MgCl_2$, pH 7.2
Elution buffer:	20 mM potassium phosphate, 1 mM $MgCl_2$, 1 M NaCl, pH 7.2
Step gradient:	1. 0–25% elution buffer in 10 CV 2. 25%–100% elution buffer in 12 CV

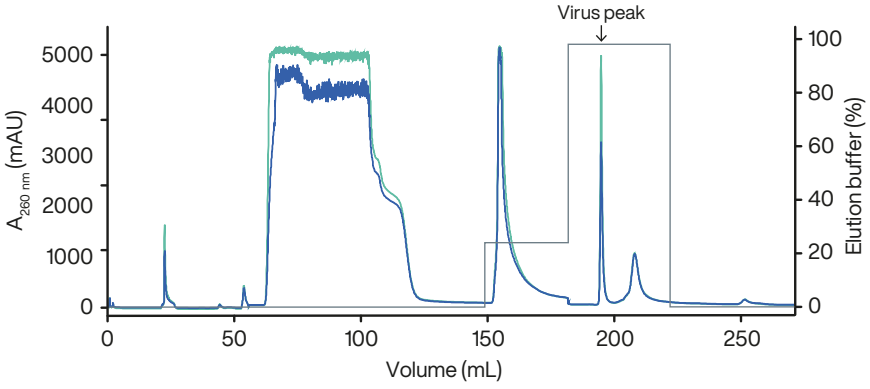
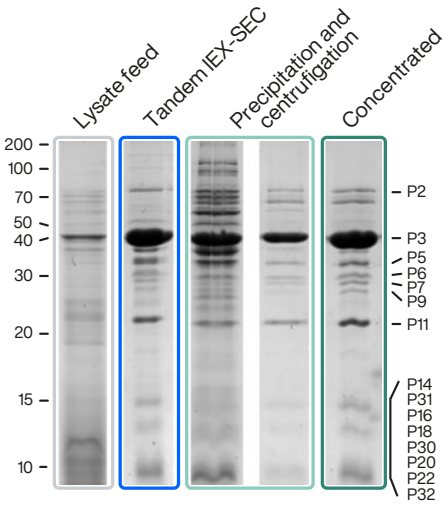


Figure 3. Tandem virus purification consisting of AIEX followed by SEC connected in series. UV 280 nm (blue), UV 260 nm (green) and elution buffer concentration (grey). Loading, wash and 25% elution step gradient is performed with SEC column bypassed. The flow was shifted onto WorkBeads Macro SEC at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

Table 1 below shows the average data collected from these four biological replicates using tandem chromatographic purification in comparison to the traditional purification using PEG-precipitation followed by ultracentrifugation. An enormous increase in performance is seen in yield/purified particles in mg per liter of lysate as well as in time saving, without compromising the specific activity of the virus. Using AIEX-SEC a 3.5 times higher yield was obtained compared to using PEG-precipitation-ultracentrifugation. The purity was evaluated using SDS-PAGE analyses with Coomassie staining, see Figure 4. This gain in performance is achieved without compromising purity. The four replicates gave very similar results, showing the robustness of the method.

One additional important factor is the ability to load lysate directly onto the column without any pre-treatment such as PEG-precipitation. The lysate contains large amounts of nucleic acids (DNA, RNA, chromatins) and has a high viscosity which might interfere with downstream steps.



Lanes 1 – 4:
Virus lysate feed (grey), virus sample post chromatographic tandem AIEX-SEC purification (blue), virus sample post precipitation and ultracentrifugation (light green), and finally virus sample post precipitation and ultracentrifugation concentrated by differential centrifugation (dark green). Molecular marker in kDa is on the left and positions of the PRD1 structural proteins are indicated on the right.

Figure 4. SDS-PAGE of virus samples.

Table 1. Recovery and infectivity data for precipitation-ultracentrifugation purification (traditional method) and new 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 (feed)	100	3.4*1011	–	–
	Pure virus[3] (final)	13	6.6*1012	3.5	>24
AIEX-SEC	Lysate (feed)	100	4.4*1011	–	–
	Pure virus[4] (final)	53.5	5.8*1012	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

Since many virus purifications have the disadvantage of being very time consuming and requiring a lot of hands-on time, there is a real need for purification setups such as the tandem chromatography described in this study. Based on four biological replicates and using AIEX-SEC, the yield of active virus particles was substantially increased in a fast and completely automated setup. Since the specific activity was still very high no disadvantage was apparent using AIEX-SEC. The final SEC step also removes all salt from the purified virus, so no additional desalting step was needed.

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

Reference

1. M. Andersson Schönn *et al., J Chromatogr B.*; 2022: **1192**: 1 March, 123140

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