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Virus Purification by Ion Exchange with Sartobind® Lab Q and S Membrane Adsorbers

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Abstract

Sartobind® Lab devices are available with various membrane adsorbers which can be utilized in purification of bioparticles and for impurity removal. The macroporous structure allows even large viruses to easily enter the membrane and to bind to the ligand-rich inner pore surface. This membrane adsorber technology offers a number of benefits compared to conventional chromatography, such as easy handling, high flow rates, minimal mass transfer effects, high capacities, low non-specific adsorption, reduced hardware investment, lower buffer consumption, and easy technology transfer to process-scale devices. This short review highlights publications related to the purification of viruses with Sartobind® Lab ion exchangers.

Find out more: www.sartorius.com/en/products/lab-filtration-purification/membrane-chromatography

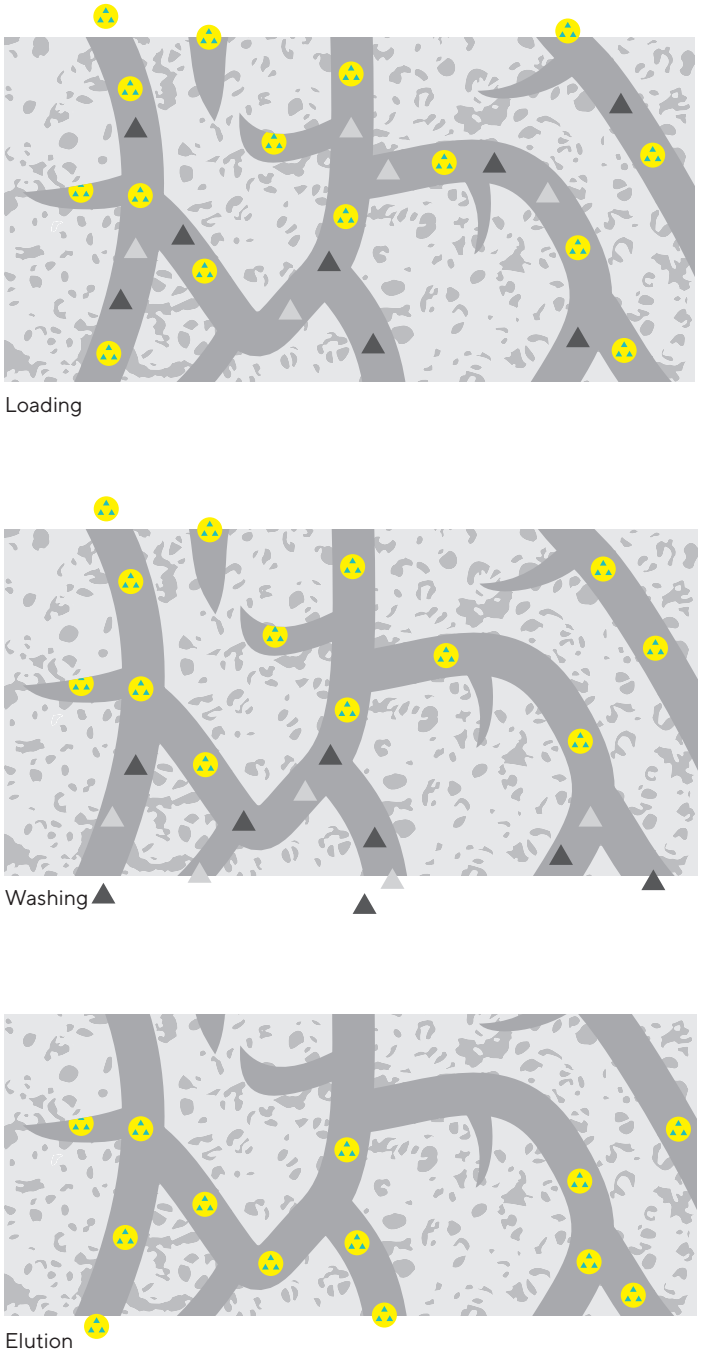
Introduction

An increasing focus on viruses, virus-like particles and viral vectors in research and development towards novel vaccines and disease treatments demand efficient purification techniques. Ion exchange chromatography has several advantages over sucrose or caesium chloride density gradient centrifugation, including increased sample capacities, higher virus yields, purity and infectivity, and significantly reduced processing times.

The Sartobind® ion exchange membrane adsorber technology developed by Sartorius offers further benefits over conventional ion exchange chromatography resins. The ready-to-use units can be operated at much higher flow rates, since the larger pores enable convective rather than diffusive movement of the target molecule through the matrix. This results in rapid target capture (adsorption) and elution (desorption), and therefore shorter cycle times. They also have lower buffer requirements thanks to the low bed volumes in comparison to resins. For research and development laboratories, this technology is available within the Vivapure® and Sartobind® Lab product ranges. By design, these provide the ultimate flexibility for sample handling, via centrifugation, syringe, peristaltic pump or FPLC system, using the equipment which is already available to the scientist.

The following examples from peer-reviewed literature illustrate the applicability of Sartobind® Lab devices to virus purification (Figure 1) in research and development laboratories.

Figure 1: *Schematic Representation of Virus Purification on Sartobind® Membrane Adsorbers*



Purification of Alphaherpesviruses With Sartobind® Lab S 100

The first ion exchange purification of herpesviruses by Sartobind® Lab devices was reported by Karger *et al.* in 1998 (Table 1).

Table 1: *Purification Conditions for PrV and BHV-1*

Sample	Infected cell culture supernatant
Device	Sartobind® Lab S 100
Purification Steps	1. Equilibration (20 mM MES, pH 6.2)
	2. Loading (supernatant diluted 1:2 in MES)
	3. Washing (25 mL MES)
	4. Elution (10 mL each of 200, 400, 600 and 1,000 mM KCl in MES)
	5. Particulate sedimentation (25,000 rpm, 1 h, 4 °C)
	6. Resuspension (100 µL of TBS (200 mM NaCl, 2.6 mM KCl, 10 mM Tris-HCl pH 7.5, 20 mM $MgCl_2$, 1.8 mM $CaCl_2$)
Flow Rate	10 mL/min

The results showed that most infectious pseudorabies virus Kaplan strain (PrV-Ka) and lacking glycoprotein gD (PrV-gD-Pass), and bovine herpesvirus 1 (BHV-1) was found after elution with 400 mM KCl in MES buffer (Table 2). More than 85% of the virus was eluted within a single fraction.

Table 2: *PrV and BHV-1 Yields After Cation Exchange Chromatography*

	PrV-Ka	PrV-gD-Pass	BHV-1
PFU Applied (100%)	$1.4 \times 10^{10} \pm 8 \times 10^{9}$	8.1×10^{6}	1.5×10^{9}
PFU Eluted in MES with 400 mM KCl	85.6 ±10.7%*	99%	93%

* Mean ±SD of 3 tests

Densonucleosis Virus Purification by Sartobind® Lab Q, D and S 75

In 2004, Specht *et al.* purified AeDNV particles with Sartobind® Lab 75 anion and cation exchange devices. Both exchangers could be used to adsorb the viral particles, depending on the feed pH (Table 3). The results showed no detectable size exclusion effects, indicating that membrane adsorbers are ideally suited to the capture of large viruses.

Table 3: *Sartobind® Lab Q, D and S Binding Capacities for Densonucleosis Virus*

Device	Q 75	D 75	S 75
pH	7.0	7.0	3.5
Virus Charge	Negative	Negative	Negative
Membrane Charge	Positive	Positive	Positive
Protein Capacity	60 mg BSA	45 mg BSA	60 mg lysozyme
Virus Capacity in Water, PFU	$>2.79 \times 10^{10}$	$>1.54 \times 10^{9}$	$>3.91 \times 10^{8}$
Virus Capacity in Medium, PFU	$>1.36 \times 10^{10}$	$>1.36 \times 10^{10}$	0

Summary

Sartobind® Lab devices offer an effective means for rapid and convenient purification of viruses. Thanks to their large pore size, viruses can enter the chromatography matrix unhindered. They are also flexible in operation, due to out-of-the-box compatibility with syringes, peristaltic pumps and FPLC systems. Alternatively, for parallel purification of low volume samples, the centrifugal Vivapure® devices are recommended.

For further applications of Sartobind® technology in process-scale virus purification and removal, readers are referred to the related Application Note titled "Virus Purification and Removal: Ion Exchange Chromatography with Sartobind® Membrane Adsorbers".

References

Karger, A., Bettin, B., Granzow, H. and Mettenleiter, T. C. (1998). Simple and rapid purification of alphaherpesviruses by chromatography on a cation exchange mambrane. J. Virol. Methods 70, 219-224.

Specht, R., Han, B., Wickramasinge, S. R., Carlson, J. O., Czermak, P., Wolf, A. and Reif O-W. (2004). Densonucleosis virus purification by ion exchange membranes. Biotechnol. Bioeng. 80(4), 465-473.

Note

Literature published up to c.2022 may reference the use of Sartobind® MA, which is a name previously used for the Sartobind® Lab membrane adsorbers. When these devices were renamed, there was no change made to fit, form or function. Therefore, results collected and methods established using Sartobind® MA devices remain valid also for Sartobind® Lab.

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