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# Bacteriophage PR772 Capture by Ion Exchange Chromatography With Sartobind® Lab Membrane Adsorbers

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## Abstract

To ensure the safety of biologics, downstream processing in biopharmaceutical manufacturing must contain at least three different methods for virus removal and inactivation. The robustness of the methods must also be demonstrated. There are different possibilities like filtration, and inactivation by UV, heat, or chemical treatment. Another method for virus removal is ion exchange chromatography. For example, negatively charged viruses can be bound to an anion exchanger. In this study, we demonstrate the efficient capture of the virus model bacteriophage PR772 by Sartobind® Lab membrane adsorbers.

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## Introduction

Since their hosts are bacteria, some key advantages of using bacteriophages (or phages) as virus models are their ease of handling and lack of human pathogenicity. They are also rapidly detected during analysis.

In this study, we chose the phage PR772, which belongs to the *Tectiviridae* family of icosahedral double-stranded DNA bacteriophages, as a model to assess the efficiency of virus removal. PR772 has been chosen by the Parenteral Drug Association virus filter task force to be the model bacteriophage to standardize nomenclature for larger-pore-size virus filters. This phage is stable, easy to handle, and high phage titers can be readily obtained. Unlike other members of the family *Tectiviridae*, production of PR772 does not involve the handling of pathogenic host bacteria. Furthermore, phage PR772 titers can be rapidly and easily enumerated by plaque-counting techniques<sup>2</sup>.

Here, we aimed to find the optimal binding conditions and estimate the binding capacity and flow rate of Sartobind<sup>®</sup> Lab membrane adsorbers (Figure 1) for the phage PR772.



Figure 1: Sartobind<sup>®</sup> Lab S 75 and Q 75 Membrane Adsorbers

## Materials and Methods

Each Sartobind<sup>®</sup> Lab device (Table 1) was equilibrated with 10 mL (5 MV) loading buffer (either 20 mM sodium acetate pH 5, 20 mM potassium phosphate pH 7, or 50 mM Tris-HCl pH 9). A stock phage PR772 suspension was prepared from *E. coli* K-12 culture. For loading onto each membrane adsorber, this suspension was diluted in the appropriate loading buffer to  $10^7 - 10^8$  plaque forming units per millilitre (PFU/mL) in a final volume of 100 mL and pre-filtered with a 0.1  $\mu$ m filter. Following loading, Sartobind<sup>®</sup> Lab devices were washed with approximately 15 mL (7.5 MV) loading buffer, followed by approximately 20 mL (10 MV) elution buffer (loading buffer containing 1 M NaCl). Membrane adsorbers were finally washed with approximately 15 mL (7.5 MV) loading buffer, regenerated with 1 M NaOH and re-equilibrated until the flow through pH matched that of the loading buffer used.

Table 1: Specifications of Sartobind<sup>®</sup> Lab Devices Used in This Study

Device	Ligand	Membrane Layers	Membrane Volume (MV)	Diameter	Bed Height
Sartobind <sup>®</sup> Lab S 75	S	15	2 mL	25 mm	4 mm
Sartobind <sup>®</sup> Lab Q 75	Q	15	2 mL	25 mm	4 mm

Phage titers were estimated in the initial sample, flow through (wash), and final eluate, by counting plaque forming units (PFU). These values were used to calculate log reduction values (LRVs).

To determine phage stability, suspensions were incubated for 3 h under agitation in buffers ranging from pH 1 – 13. Phage recoveries were determined and expressed as a percentage of the input material.

# Results and Discussions

## Screening for Optimal Phage Capture

Experiments were conducted to find the appropriate pH for optimal phage binding. One hundred milliliter aliquots of phage PR772 suspension with approximately  $10^8$  PFU/mL were loaded onto Sartobind® Lab S 75 cation exchangers at pH 5 or 7, or onto Sartobind® Q 75 anion exchangers at pH 5.

If the pI is higher than the buffer pH, the target molecules will bind to a cation exchanger. There was almost no binding to S 75 membrane adsorbers at both pH 5 and 7 (Table 2). Therefore, the phage was expected to have a pI lower than 5 and the most efficient capture required use of Q anion exchangers.

**Table 2:** Trials for Phage PR772 Capture by Sartobind® Lab S and Q 75

Device	pH	Load titer (PFU/mL)	Flow through titer (PFU/mL)	LRV
S 75	5	$1.60 \times 10^8$	$8.00 \times 10^7$	0.30
S 75	7	$0.87 \times 10^8$	$7.30 \times 10^7$	0.08
Q 75	9	$2.20 \times 10^8$	$3.10 \times 10^2$	5.85

Table 3 shows rates of phage recovery across a pH range of 1 – 13. The results indicate that phage PR772 was inactivated at pH 4 and therefore it could be deduced that its pI should be around this level. At pH 7 and 9, phage recoveries exceeded 100%. This could be due to phage colonies being detected, instead of individual phages.

The 0% recovery observed at pH 13 underlines our assumption that, following regeneration with 1 M NaOH (pH 14), all phages were inactivated so that no carry over was possible when using the same membrane adsorber device for further purifications.

**Table 3:** pH Stability of Phage PR772

pH	PR772 Recovery (%)
1	0
3	15.5
4	18.5
5	98.4
7	103.2
9	109.4
13	0

## Binding of PR772

Anion exchangers bind their target molecules at a pH which is higher than the pI. Consequently, these experiments were conducted at pH 7 and 9.

Sartobind® Lab membrane adsorbers were operated at flow rates up to 50 mL/min, with 100 mL phage suspension of approximately  $10^8$  PFU/mL loaded onto each device (Table 4). Sartobind® Lab devices showed a slightly reduced capacity at pH 7 but no PFU in the flow through at pH 9.

**Table 4:** Flow Rates and Binding Capacities for Phage PR772 With Sartobind® Lab Q 75

Device	Flow Rate	pH 7			pH 9		
		Load Titer (PFU/mL)	Flow Through Titer (PFU/mL)	LRV	Load Titer (PFU/mL)	Flow Through Titer (PFU/mL)	LRV*
Q 75	1 mL/min (0.5 MV/min)	$4.00 \times 10^8$	0	7.3*	$2.50 \times 10^8$	0	7.1
Q 75	10 mL/min (5 MV/min)	$1.30 \times 10^8$	$4.70 \times 10^1$	6.4	$1.20 \times 10^8$	0	6.8
Q 75	50 mL/min (25 MV/min)	$1.80 \times 10^8$	$1.20 \times 10^3$	5.2	$3.10 \times 10^8$	0	7.2

\* LRVs calculated using the plaque assay detection limit of  $2.0 \times 10^1$  PFU/mL.

## Conclusion

This study presents phages as useful models for virus removal trials under standard laboratory conditions, without the safety concerns associated with the use of mammalian or avian viruses.

For phage PR772, a pI of around 4 was estimated from the results of screening experiments with cation and anion exchange membranes. Further pH stability measurements showed that these phages were stable in the range of pH 5 to 9. These data were used as the basis for further characterization of capture efficiency and binding capacities.

Under optimized conditions, a 7-log reduction of the phage PR772 could be achieved, with no detectable PFU in the flow through (wash and elution) fractions. This capture efficiency was also possible at exceptional flow rates of 50 mL/min (25 MV/min), demonstrating that Sartobind® Lab membrane adsorbers can offer a rapid and effective means of virus removal.

## References

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## 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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