Capture of Bacteriophage PR772
Ion Exchange Chromatography with Sartobind® Membrane Adsorbers

Introduction
A downstream process at biopharmaceutical manufacturing must contain at least three different methods for virus removal and inactivation. The robustness of the methods has to be demonstrated. There are different possibilities like filtration, inactivation by UV, heat or chemical treatment. Another method for virus removal is ion exchange chromatography. E.g. negatively charged viruses can be bound to an anion exchanger.

In this study, bacteriophages (or phages) are used as virus material. Their hosts are bacteria. The advantages of the use of phage are easy handling, no human pathogenicity and fast result detection.

Aim of this study was:
– To find out binding conditions for phage PR772
– Estimation of binding capacity and flow rate of Membrane Adsorbers.

The phage PR772 belongs to the Tectiviridae family of icosahedral double-stranded DNA bacteriophages. PR772 has been chosen by the Parenteral Drug Association virus filter task force to be the model bacteriophage to standardize nomenclature for lager-pore-size virus filters. PR772 is stable, easy to handle, and high phage titers can be obtained readily. Unlike other members of the family Tectiviridae, production of PR772 does not involve the handling of pathogenic host bacteria. Phage PR772 titers can be rapidly and easily enumerated by plaque-counting techniques².

Materials
Phage PR772 (approx. 53 nm diameter, host: E.coli K-12)

Loading buffers
– pH 5: 20 mM sodium acetate
– pH 7: 20 mM potassium phosphate
– pH 9: 50 mM Tris/HCl

Table 1: Membrane chromatography Devices

<table>
<thead>
<tr>
<th>Device</th>
<th>Ligand</th>
<th>Layers</th>
<th>Volume [ml]</th>
<th>Diameter [mm]</th>
<th>Bed height [mm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sartobind® Q 75</td>
<td>Q</td>
<td>15</td>
<td>2</td>
<td>25</td>
<td>4</td>
</tr>
<tr>
<td>Sartobind® S 75</td>
<td>S</td>
<td>15</td>
<td>2</td>
<td>25</td>
<td>4</td>
</tr>
</tbody>
</table>

Method
1. Equilibration with 10 ml loading buffer
2. Load 100 ml phage suspension
   The suspension was diluted with loading buffer to $10^7$ - $10^8$ PFU/ml (PFU = Plaque forming units) and prefilled with 0.1 μm filter
3. Washing with approx. 15 ml loading buffer
4. Elution with approx. 20 ml elution buffer (each loading buffer + 1 M NaCl)
5. Second equilibration with approx. 15 ml loading buffer
6. Regeneration with 1 N NaOH
7. Neutralisation with loading buffer until pH shows the initial value of loading buffer

The titer of the phages was estimated in the initial sample, the flow through, fraction of washing and elution, and the log reduction value (LRV) was calculated. The phage recovery in % was determined.

The titer estimation was done by counting of plaque forming units (PFU). The plaques were formed by lysis of bacteria on the bacteria layer.

To find out the pH stability of the phage, the phage was shaken in buffer at certain pH for 3 h. Then the phage recovery in % was determined.

Results and Discussions
Screening for optimal binding pH
The experiments were conducted to find the appropriate pH for the phage binding.
Each 100 ml of phage suspension with $10^8$ PFU/ml was loaded on Sartobind® S 75 cation exchanger at pH 5 and 7 as well on Sartobind® Q 75 anion exchanger at pH 5.

Table 2: Trials with Sartobind® S 75 and Q 75

<table>
<thead>
<tr>
<th>Device</th>
<th>pH</th>
<th>Load titer PFU/ml</th>
<th>Flow through titer PFU/ml</th>
<th>LRV</th>
</tr>
</thead>
<tbody>
<tr>
<td>S 75</td>
<td>5</td>
<td>1.60E+08</td>
<td>8.00E+07</td>
<td>0.30</td>
</tr>
<tr>
<td>S 75</td>
<td>7</td>
<td>8.70E+07</td>
<td>7.30E+07</td>
<td>0.08</td>
</tr>
<tr>
<td>Q 75</td>
<td>5</td>
<td>2.20E+08</td>
<td>3.10E+02</td>
<td>5.85</td>
</tr>
</tbody>
</table>

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 at both pH 5 and 7 (Table 2). Therefore, the pI of the phage should be lower than 5.
Table 3 shows that at pH 4 the phages were inactivated.

It could be assumed from the results that the pI range of PR772 should be around 4. At pH 7 and 9 the recovery was even higher than 100%. The reason could be that no individual phage but the phage colonies were detected, which consist of different number of phages. The result at pH 13 underlines our assumption that through rinsing with 1 N NaOH (pH 14) all phages were inactivated and therefore no carry over was possible.

**Binding of PR772**

An anion exchanger binds the target molecules at higher pH than the pI, consequently the experiment was conducted at pH 7 and 9.

The Membrane Adsorber was operated at a flow rate up to 50 ml/min. 100 ml phage suspension of approx. 10^8 PFU/ml was loaded on the devices.

Table 4: Flow rate and binding capacity for Sartobind® Q

<table>
<thead>
<tr>
<th>pH 7</th>
<th>Flow rate [ml/min]</th>
<th>pH 9</th>
<th>Flow through [PFU/ml]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>0</td>
<td>*7.3</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>6.4</td>
<td>*6.8</td>
</tr>
<tr>
<td>50</td>
<td>1.20E+03</td>
<td>5.2</td>
<td>*7.2</td>
</tr>
</tbody>
</table>

* Calculation at the detection limit of 2.0E+01

Membrane Adsorber showed a slightly reduced capacity at pH 7 and no flow through at pH 9.

**Summary**

The pI range 4 of the phage PR772 was estimated by the screening tests with cation and anion exchange membranes. The pH stability experiments showed that phages were stable at pH 5 to 9. These data were used as base for further experiments.

At optimised conditions, 7 log reduction of the phage PR772 could be achieved with Membrane Adsorbers. It was shown that this reduction was also achieved at an exceptional flow rate of 25 membrane volumes (50 ml/min). It could be shown that phages may be an alternative to mammalian or avian viruses for trials under standard laboratory conditions without safety issues.