



## Concentration of Diphtheria vaccine in downstream processing



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Purification by  
Crossflow Filtration  
Hydrosart® 10kD

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**Abstract**

This trial was performed to investigate the downstream processing of Diphtheria vaccine and also to assess the suitability of the Sartorius Stedim Biotech Cross-Flow-Equipment for a diphtheria vaccine process.

**1. Background**

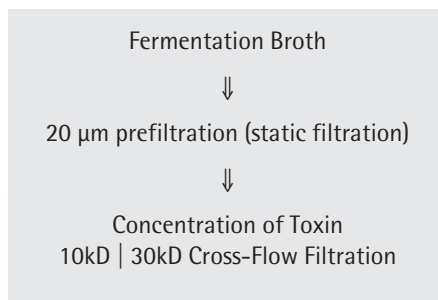
A batch of 100 liter diphtheria culture that was clarified by Sheet filter was prepared for the ultrafiltration procedure.

The batch was divided in two parts.

First batch of 54 liters were concentrated by 2 Sartocoon Cassettes, Hydrosart® 10kD; second batch of 46 liters were concentrated by 2 Sartocoon Cassettes, Hydrosart® 30kD.

**2. Description of Trial Procedure**

The following protocol was adopted for the trial:

**3. Materials and Methods****Crossflow system**

Sartocon 2+ ultrafiltration setup, the pump used to run the system was a peristaltic pump, double jacketed feed vessel cooled with cold WFI in order to ensure temperatures below 10°C. The temperature was carefully monitored during the whole experiment.

**Membrane**

Hydrosart® 10 kD,  
order no. 3021443906E--SG,  
membrane area 0.6 m<sup>2</sup>.

Hydrosart® 30 kD,  
order no. 3021445906E--SG,  
membrane area 0.6 m<sup>2</sup>.



**Part 1:**

**Concentration of Toxid by using a 10kD Hydrosart® Ultrafilter Membrane**

The cassette was first rinsed with 5 liters of DI-water, both retentate and permeate lines were directed down the drain. After rinsing the system was conditioned with 0.9% NaCl. This was done by rinsing the system with 5 liters of NaCl (retentate and permeate to feed tank), recirculation for 5 minutes. The purpose of this conditioning was to neutralize the charge of the membrane in order to minimize non specific binding of toxin to the membrane.

After the completion of conditioning the toxid (permeate of Sheet filter) was brought into the system. The inlet pressure at the beginning was 0.5 bar. Permeate flux was constant. To optimize flux rate, transmembrane pressure was increased by us during filtration. At the end we achieved a constant flux on a higher level of 30 l/hm<sup>2</sup>. The flux was independent from the time and concentration of toxid.

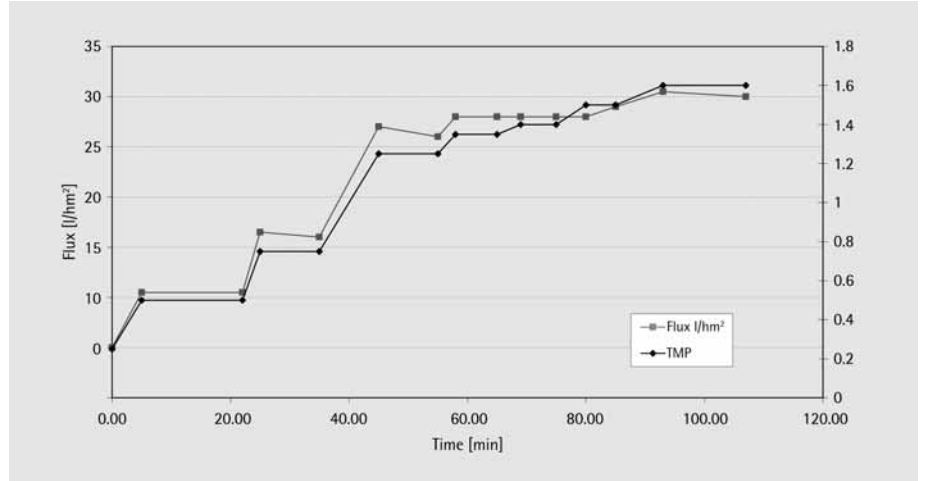
The concentrated toxid had a volume of approx 1 liter. This was flushed back in the vessel. For getting all toxid out of the system it was flushed with approx. 0.8 liter 0.9% NaCl. The total volume results to 1.8 liter.

**Recovery of toxid**

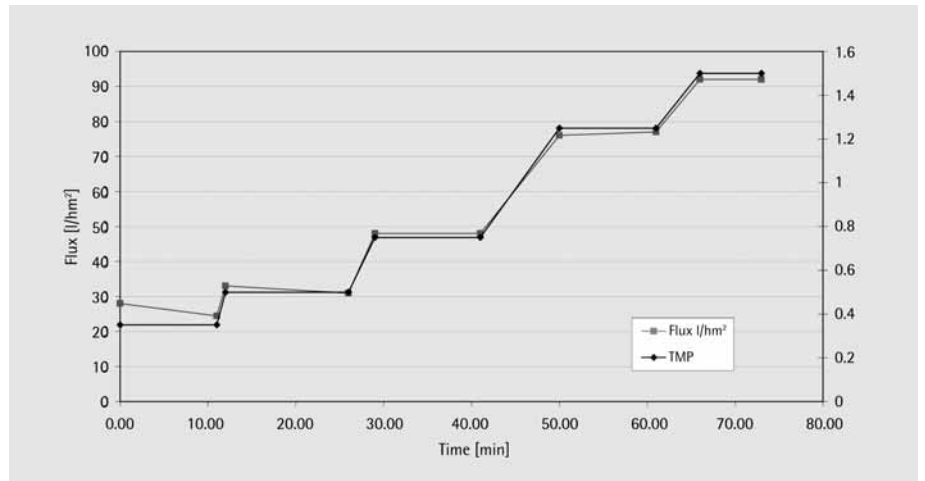
Permeate analysis during the entire concentration showed, that there is no passage of protein through the membrane.

Lateral-Flow (LF) assays (**LF assays** to detect specific nucleic acids) of this concentrate was measured. The total recovery from the diphtheria at the end of the concentration was about 85%.

**Diphtheria Concentration**



Testfiltration Concentration Diphtheria Toxid  
1 Cassette Hydrosart® 10 K, Filtration Area 1.2 m<sup>2</sup>, Filtration Volume about 50 l



Testfiltration Concentration Diphtheria Toxid  
1 Cassette Hydrosart® 30 K, Filtration Area 0.6 m<sup>2</sup>, Filtration Volume: about 50 l

## Part 2:

### Concentration of Toxid by using a 30kD Hydrosart® Ultrafilter Membrane:

The diphtheria protein has a molecular weight of about 70kD. For increasing the permeate flux Sartorius Stedim Biotech recommended to use a membrane with a cut off of 30kD. Protein loss caused by the higher cut off of the membrane was checked during the whole filtration and was not detected.

The preparation of the cassette was the same as in trial 1. The cassette was first rinsed with DI-water, both retentate and permeate lines were directed down the drain. After rinsing, the system was conditioned with 0.9% NaCl. This was done by rinsing the system with 5 liters of NaCl (retentate and permeate to feed tank), recirculation for 5 minutes. The purpose of this conditioning was to minimize non specific binding of toxin to the membrane.

After the completion of conditioning the toxid (permeate of Sheet filter) was brought into the system. The inlet pressure at the beginning was 0.5 bar. Permeate flux was constant. To optimize flux rate transmembrane pressure was increased by us during filtration. At the end we achieved a constant flux on a higher level of 90 l/hm<sup>2</sup>. The flux was independent from the time and concentration of toxid.

The concentrated toxid had a volume of approx 1 liter. This was flushed back in the vessel. For getting all toxid out of the system, it was flushed with approx. 0.8 liter 0.9% NaCl. The total volume results to 1.8 liter.

### Recovery of toxid

Measurements in the permeate during the entire concentration showed, that there is no passage of protein through the membrane.

Lateral-Flow (LF) assays (LF assays to detect specific nucleic acids) of this concentrate was measured. The total recovery from the diphtheria at the end of the concentration was about 90%.

### Cleaning efficiency

Product was flushed out by 0.9% NaCl (Clarification) or permeate (Concentration). The system was cleaned by recirculating 5 liter of 1m NaOH at room temperature for 30 min.

The cleaning was sufficient, decrease of water flux is in the recommended range. Increasing temperature up to 50°C is possible.

## 4. Summary

### Conclusion

The comparison of the 10kD and 30kD Hydrosart® membranes showed clearly that the performance of a 30kD Hydrosart® membrane is significant better for concentration of diphtheria toxid.

Within all process parameters the permeate flux rates are 3 times higher with 30kD Hydrosart® than using a 10kD Hydrosart® Cassette.

The flux increases proportional with TMP (transmembrane pressure). Perhaps higher flux rates could be achieved by using higher TMP as in the trials.

Even at the highest tested TMP and at the end of concentration there was no decrease of flux.

This investigation underline the superior performance of Hydrosart® ultrafilter membranes in:

- ... Excellent product recovery (approximately 100%).
- ... Superior flow rates (compared with Millipore trial).
- ... Excellent cleanability of the membrane.
- ... No fouling tendencies of the membrane.

### Note:

Flow rates (and therefore a reduction in the required membrane surface area) can be improved by optimizing the operating parameters prior investigation runs. Optimization procedures are performed under constant concentration and constant temperature conditions. Various crossflow rates combined with various TMP's supports the definition of optimum processing point.

**Recommendation**

Improvement of System design and cGMP processing supports an increase in yield performance. Customized equipment combined with important process optimization support this issue as well.

**Ultrafiltration|Concentration**

Membrane:	Hydrosart® 30kD
Average flux rate:	90 l/hm <sup>2</sup>
Processing volume:	200 l
End volume concentration:	2–4 l (dependent of system)
Estimated diafiltration volume:	2–4 l
Processing time:	2 hours

Based on the results of this comparison investigation, it is recommended to use two Hydrosart® (30kD) Ultrafilter Cassettes (1.2 m<sup>2</sup> membrane area) to perform 200 liter batches within approx. 2 working hours for concentration of diphtheria toxin.

During this investigation the concentration factor (CF) was limited to 50. If this CF is increased, the processing time might be extended. This can be overcome by increasing the filtration area.

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