



Concentration and Diafiltration of a Monoclonal Antibody



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Application
Note

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Purification by
Crossflow Filtration
Hydrosart® 30 kD

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Abstract

The monoclonal antibody is produced in genetically modified NS0 cells. The antibody is secreted into the growth media thus requiring separation from the cells prior to downstream purification.

The cell clarification (removal of cells) was performed using a 0.2 μm Hydrosart Crossflow step. **This application note describes the ultrafiltration procedure which incorporates volume reduction (concentration) and diafiltration (buffer exchange).**

The purpose of the trial was to demonstrate the suitability of the Sartoclon system and cassettes for concentration and diafiltration of the clarified monoclonal antibody solution.

The success of the trials was assessed by:

1. Obtaining acceptable antibody yield.
2. Achieving adequate flow rates during processing.
3. Ability to clean the cassettes after processing.

1. Background

The following filtration steps were performed to 1. separate the antibody from the cells, and 2. concentrate the antibody and change the buffer to that required for the following affinity chromatography step.

2. Materials and Methods

Starting material:

2 liters of clarified monoclonal antibody solution.

Crossflow system:

Sartoclon Alpha system in ultrafiltration setup with rotary lobe pump connected to jacketed feed vessel cooled with chilled water (temperature maintained below 10°C).

Membrane:

Hydrosart 30 kD,
Catalog no. 3051445901E--SG,
Membrane area 0.1 m².

2.1. Flushing the Membrane, Determining the Water Flux Rate

After assembling the system and clamping the cassettes, the system was rinsed with 10 litres of purified water ($p_{\text{in}} = 2$ bar, $p_{\text{out}} = 0.5$ bar, and $p_{\text{per}} = 0$), this was done to remove the preservative (20% ethanol) from the new cassette.

A fresh 5 litres of purified water was then circulated around the system for five minutes. The water flux rate was determined ($p_{\text{in}} = 2$ bar, $p_{\text{out}} = 0.5$ bar, and $p_{\text{per}} = 0$).

This water was drained out of the system and replaced with buffer which was then circulated around the system for five minutes.

Microfiltration

0.2 μm Hydrosart

Concentration and washing of NS0 Cells.
Clarified antibody passes into permeate.
Low Trans Membrane Pressure (TMP) to minimize damage to cells.



Ultrafiltration

30kD Hydrosart

Concentration of permeate pool from above step.
Antibody is retained in the retentate.
Buffer exchange for subsequent chromatography step.
High TMP to increase flow rate and reduce processing time.

2.2. Optimization

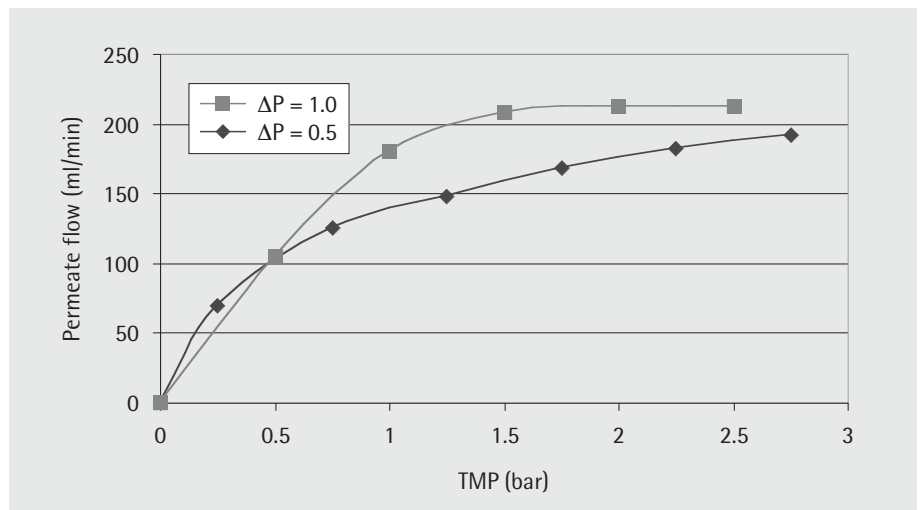
Clarified monoclonal antibody was introduced into the system and permeate channel returned the permeate to the retentate tank (complete recirculation and no concentration).

During the optimization procedure the permeate flow rate was measured at various Trans Membrane Pressures (TMPs) and crossflow velocities (see Graph 1).

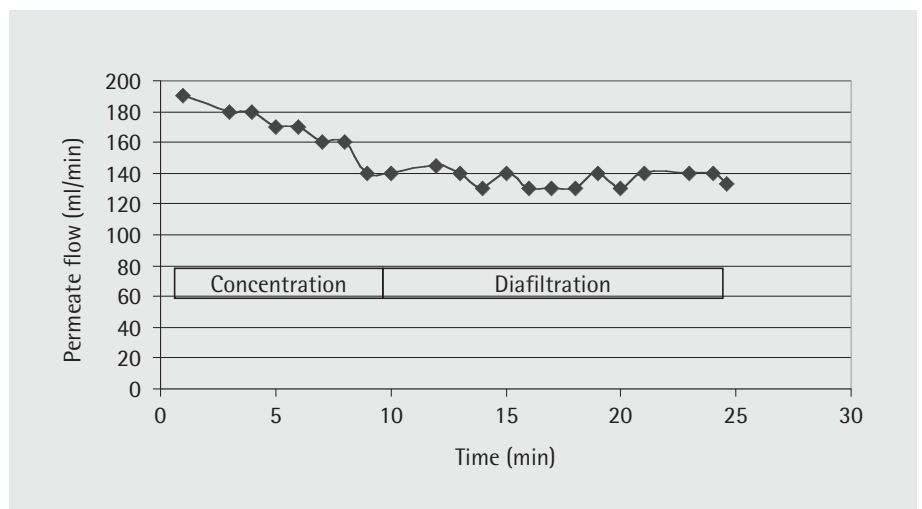
The TMP is the average pressure across the membrane. The crossflow velocity is equivalent to ΔP (Inlet pressure minus outlet pressure). The results showed that at higher ΔP (1.0 compared with 0.5 bar) the permeate flow was greater. This is probably due to more efficient disruption of the gel layer that forms on the membrane surface during filtration.

The results also showed that at a ΔP of 1.0 bar the higher the TMP, the greater the permeate flow rate up to a TMP of 1.5 bar. Above this TMP the flow rate did not continue to increase. Previous experience has shown that processing of antibody preparations at high pressures (and therefore high shear forces) does not cause significant loss of activity.

Given the low sensitivity to shear force degradation of antibody preparations and the results of the optimization, it was decided to run the concentration | diafiltration at a TMP of 1.5 bar [2.0 bar inlet and 1.0 bar outlet (retentate) pressure] and ΔP of 1.0 bar.



Graph 1: Optimization of antibody concentration



Graph 2: Concentration and diafiltration of monoclonal antibody.

2.3. Run Details

The run details are presented in Graph 2.

The results showed that the permeate flux progressively decreased during the concentration of the antibody; this flux rate decrease is most likely due to an increasing protein concentration resulting in an increasingly viscous solution. During the diafiltration stage of the process, the retentate volume and therefore antibody concentration was held constant. During this process the permeate flux rate remained relatively constant at 80.0 L/hr. m², indicating no fouling of the membrane during buffer exchange.

Remark

Maintaining a relatively high ΔP or crossflow velocity helps to reduce the build up of a gel layer at the membrane surface.

The concentration step achieved a 7.7-fold concentration of the monoclonal antibody at an average flux rate of 84 L/hr. m². The diafiltration step achieved an 8 volume buffer exchange and would have also removed low molecular weight contaminants present in the growth media.

3. Antibody Recovery

The recovery of the monoclonal antibody after the concentration and diafiltration was measured and found to be 99%, an excellent antibody recovery demonstrating the suitability of the 30kD Hydrosart membrane for this application.

Optimization 30 kD Hydrosart

Pressure Inlet bar	Outlet bar	TMP bar	ΔP bar	Retentate Volume ml	Permeate flow ml/min	flux litres/hr. m ²
0.5	0.0	0.25	0.5	1920	70	42
1.0	0.5	0.75	0.5	1920	126	76
1.5	1.0	1.25	0.5	1920	148	89
2.0	1.5	1.75	0.5	1920	168	101
2.5	2.0	2.25	0.5	1920	182	109
3.0	2.5	2.75	0.5	1920	192	115
1.0	0.0	0.5	1.0	1920	105	63
1.5	0.5	1	1.0	1920	180	108
2.0	1.0	1.5	1.0	1920	208	125
2.5	1.5	2	1.0	1920	212	127
3.0	2.0	2.5	1.0	1920	212	127

Concentration | Diafiltration of Monoclonal Antibody 30 kD Hydrosart

Time min	Pressure Inlet bar	Outlet bar	TMP bar	volume ml	Retentate conc. factor	volume ml	Permeate flow rate ml/min	flux rate litres/hr. m ²
Concentration								
1	2	1	1.5	1920	1.0	190	190	114
3	2	1	1.5			550	180	108
4	2	1	1.5			730	180	108
5	2	1	1.5			900	170	102
6	2	1	1.5			1070	170	102
7	2	1	1.5			1230	160	96
8	2.1	1.1	1.6			1390	160	96
9	2.1	1.1	1.6			1530	140	84
10	2.1	1.1	1.6	250		1670	140	84
Diafiltration								
12	2.1	1.1	1.6			290	145	87
13	2.1	1.1	1.6			430	140	84
14	2.1	1.1	1.6			560	130	78
15	2.1	1.1	1.6			700	140	84
16	2.1	1.1	1.6			830	130	78
17	2.1	1.1	1.6			960	130	78
18	2.1	1.1	1.6			1090	130	78
19	2.1	1.1	1.6			1230	140	84
20	2.1	1.1	1.6			1360	130	78
21	2.1	1.1	1.6			1500	140	84
23	2.1	1.1	1.6			1780	140	84
24	2.1	1.1	1.6			1920	140	84
24.6	2.1	1.1	1.6	270	7.1	2000	133	80

4. Cleaning Procedure

The system and the cassette were cleaned with sodium hydroxide solution (NaOH). 5 litres of 1 N NaOH was recirculated ($P_{in} = 2$ bar, $P_{out} = 0.5$ bar, and $P_{per} = 0$) through the system for 30 min. The used caustic solution was rinsed out and replaced with a fresh 5 litres of 1 N NaOH and recirculated for an additional 30 minutes. The total recirculation time was 60 min. The caustic solution was drained out of the system and replaced with purified water.

The clean water flux rate after cleaning was measured using the same conditions as described in section 2.1. The water flux was found to be greater than 90% of that of the new cassette indicating excellent cleanability of the membrane material.

The relative ease of cleaning was further evidence that the Hydrosart (30kD) ultrafiltration membrane did not display gel formation or fouling during the run

5. Conclusion

This investigation further demonstrates the outstanding performance of Hydrosart ultrafilter membranes, as shown by:

- ... Excellent product recovery (virtually no loss of product).
- ... Very good flow rates
- ... No fouling of the membrane.
- ... Excellent cleanability of the membrane.

Note:

The major benefit of optimization of crossflow applications is the reduction of processing time or reduction of the number of cassettes needed to perform the task. Optimization of all crossflow applications should be considered. If assistance to perform the optimization is required, please contact a Sartorius Stedim Biotech representative.

It should be kept in mind that optimization of a process must consider not only filtration parameters but also product recoveries. Sometimes high flow rates will result in significant product losses due to denaturation of the product by high shear forces or loss of product due to membrane fouling or gel formation.

The current application note further demonstrates the excellent performance of Hydrosart membranes and its suitability for processing antibody preparations.

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