



Cell-Clarification of a Monoclonal Antibody Broth with Microfilter Membranes



#15

Application
Note

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Cell-Clarification of a
Monoclonal Antibody
Broth with Microfilter
Membranes
with Hydrosart 0.2 μm
membrane in
Sartocon Slice format

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Abstract

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

The cell clarification (removal of cells) was performed using a 0.2µm Hydrosart Cross-flow step. **This application note describes the microfiltration procedure which incorporates the cell clarification in crossflow mode.**

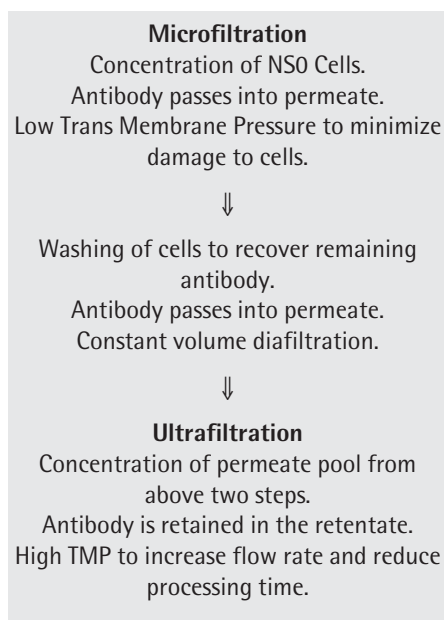
The purpose of the trial was to demonstrate the suitability of the Sartococon system and cassettes to separate | harvest the NS0 cells from the monoclonal antibody solution.

The success of the trials was assessed by:

1. Obtaining acceptable antibody recovery.
2. Achieving adequate flow rates during processing.
3. Ability to clean the cassettes after processing.
4. Cell recovery and cell viability were also assessed but these were less important in determining the success of the trial.

1. Background

The following steps were performed to maximize the recovery of antibody.



2. Materials and Methods

Starting material:

Initial volume of 2 liters of a NS0 cell containing broth solution. Clarification was performed with a 0.2 µm Hydrosart micro-filter crossflow cassette.

Crossflow system:

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

Membrane:

Hydrosart 0.2 µm,
order no. 3051860701E--SG,
membrane area 0.1 m².

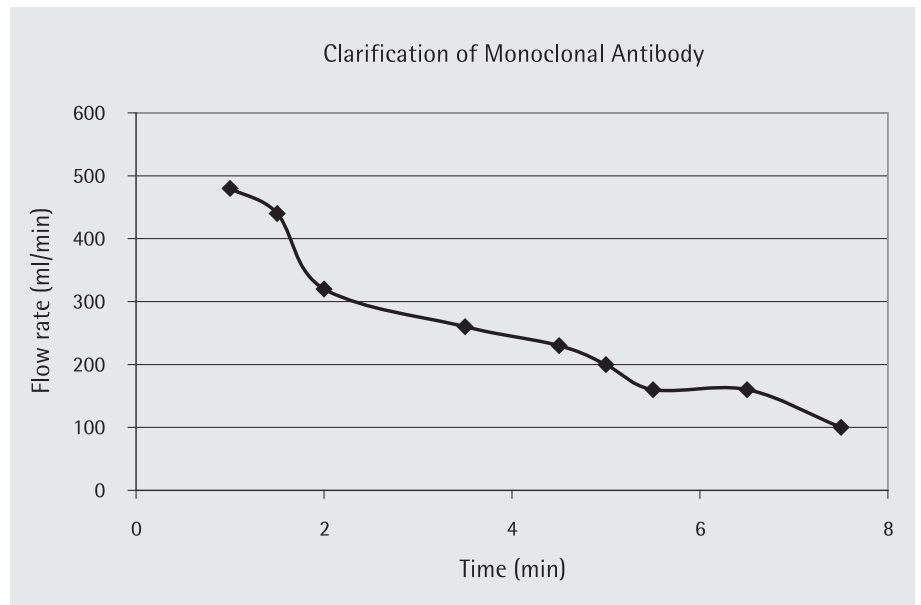
2.1. Flushing the Membrane, Determining the Water Flux Rate

After assembling the system and clamping the cassettes, the system was rinsed with purified water ($p_{in} = 2$ bar, $p_{ret} = 0.5$ bar, and $p_{per} = 0$) to remove the preservative (20% ethanol) from the new Cassette. Fresh purified water was then circulated around the system for five minutes. The water flux rate was determined ($p_{in} = 2$ bar, $p_{ret} = 0.5$ bar, and $p_{per} = 0$).

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

2.2. The Clarification Step

During clarification, a low Trans Membrane Pressure (TMP) was selected to support high cell viability. This prevents cell rupture and the release of unwanted substances out of the cells. The permeate flow rate was measured at low TMP. (see Graph 1).



Graph 1: Clarification of monoclonal antibody broth

2.3. Run Details

The run details are presented in Table 1.

The results show the typical performance of microfiltration: the permeate flux progressively decreases as a result of increasing cell concentration and increasing viscosity in the feed vessel.

During start-up with cell-contained solution, the secondary boundary layer must be developed slowly. This prevents unwanted fouling effects and reduces cleaning-in-place issues after the end of filtration. With microfiltration, low TMPs are recommended to avoid blockage or fouling of the microporous membrane skin.

Remark

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

After optimization during the initial part of the run, the inlet pressure was maintained at 0.7 bar and the outlet (retentate) pressure at 0.0 bar (TMP 0.35 bar). The permeate flow rate declined slowly while the cell mass concentration increased.

The concentration step achieved a 4.8-fold concentration of the cell broth at an average flux rate of 157 L/hr. m².

3. Antibody Recovery

The recovery of monoclonal antibody after clarification and concentration was measured and found to be 99% – an excellent antibody recovery rate – demonstrating the suitability of the 0.2 μm Hydrosart membrane for this application.

4. The Diafiltration Step

The diafiltration step was performed under constant volume. The antibodies were released into the permeate for further ultrafiltration steps.

Time min	Pressure Inlet bar	Pressure Outlet bar	TMP bar	volume ml	Retentate conc. factor	volume ml	Permeate flow rate ml/min	flux rate litres/hr. m ²
0				2000	1.0			
1	0.7	0	0.35			480	480	288
1.5	0.7	0	0.35			700	440	264
2	0.7	0	0.35			860	320	192
3.5	0.7	0	0.35			1250	260	156
4.5	0.7	0	0.35			1480	230	138
5	0.7	0	0.35			1580	200	120
5.5	0.7	0	0.35			1660	160	96
6.5	0.7	0	0.35			1820	160	96
7.5	0.7	0	0.35	420	4.8	1920	100	60

Table 1: Cell clarification of monoclonal antibody broth.

5. Cleaning Procedure

The system and the cassette were cleaned with sodium hydroxide solution (NaOH). 5 liter of 1N NaOH was recirculated ($P_{in} = 2$ bar, $P_{ret} = 0.5$ bar, and $P_{per} = 0$) through the system for 30 minutes. The used caustic solution was rinsed out and replaced by fresh 5 liters 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 in the same range as the initial determination (within the specification).

The relative ease of cleaning was further evidence that the Hydrosart 0.2 μm micro-filtration membrane did not display gel formation or fouling during the run.

6. Conclusion

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

- ... Excellent monoclonal antibody release into the permeate (near 100%).
- ... Low product binding capacity
- ... Very good flow rates
- ... 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.

Within cell clarification procedures, low TMPs are recommended to avoid membrane fouling and cell rupture. These events cause the release of unwanted cell contaminants into the broth solution, making filtration and cleaning more difficult.

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

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