



## Concentration and Diafiltration of IgG



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Application  
Note

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Purification by  
Crossflow Filtration  
Hydrosart® 30 kD

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## Abstract

Due to the outstanding filtration performance of Hydrosart® 30kD Ultrafiltration membrane in an IgG process the yield improvement could be significantly increased. Hydrosart®, which is a stabilized cellulose based membrane material, additionally perform in easy cleanability (high caustic resistance) combined with lowest product adsorbtion.

## 1. Background

The target is the processing of an IgG-eluate in 600 kg batches are processed with a crossflow ultrafiltration system (Sartoflow 20 with total membrane area of 12 m<sup>2</sup>) in the final purification step.

The main purpose is to remove sodium chloride (NaCl) by sodium acetate (NaAc) as the chloride leads to decomposition of the IgGs. The whole process consists of a concentration factor of 6 (down to 100 kg) within a duration of 1 hour and a subsequent diafiltration against NaAc buffer (duration 2 h) until a final conductivity of 1.2 µS/cm is reached. After use, the system is cleaned with 0.1 or 0.5 M NaOH which takes about 2 hours.

## 2. Materials and Methods

### Properties Medium

8368 g pre-filtered (Sartoclear® S5P [0.3µm nominal]) eluate, at a concentration of 1% IgG (NMWCO 146 kD) in NaCl solution containing sorbitol and PEG 6000 as well, pH = 5–6. The density was supposed to be 1 g/ml.

### Crossflow system

Sartocon Slice ultrafiltration setup, rotary lobe pump Unibloc PD 250, 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® 30 kD,  
order no. 3051445901E–SG,  
lot no. 01030090–016,  
membrane area 0.1 m<sup>2</sup>.



### 2.1. Flushing the Membrane, Determining the Water Flux Rate

After assembling the system and clamping the cassettes, 10 l WFI were rinsed at  $p_{in} = 2$  bar,  $p_{out} = 0.5$  bar, and  $p_{per} = 0$  through the entire system to flush it and to remove the preservative (20% ethanol) out of the new Cassette.

The rinsed liquid was replaced by 2 l fresh WFI and circulated for five minutes in the system. The permeate water flux rate was determined (under following process parameters:  $p_{in} = 2$  bar,  $p_{out} = 0.5$  bar, and  $p_{per} = 0$ ).

The initial clean water flux rate was determined with 114 l/hm<sup>2</sup>.

This water was drained out of the system.

### 2.2. The Concentration Step

During start-up with protein contained solution the secondary boundary layer must be developed slowly. This avoids unwanted fouling effects and reduces cleaning in place issues after the end of filtration. Slowly increasing inlet pressure or an optimization procedure (under total recirculation condition – permeate connected to the feed tank –) avoid an increased deposition of substances on the membrane surface.

The membrane was first equilibrated with 1.5 l, 15 mM NaAc buffer circulating for 5 min at  $p_{in} = 2$  bar,  $p_{out} = 0.5$  bar, and  $p_{per} = 0$  through the system. The buffer was drained out for disposal, and the IgG-eluate was allowed to circulate at the same pressure conditions as with buffer. After 5 min, the permeate was collected in a vessel.

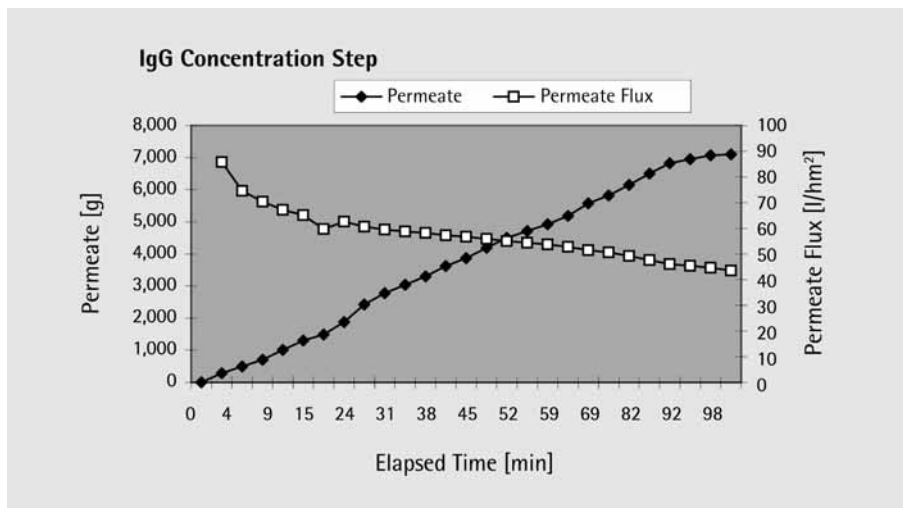
The permeate volume was counted by balance control. The crossflow rate were kept constant throughout the experiment, resulting in a Trans Membrane Pressure (TMP) of 1.25 bar.

The average permeate flow was performed with 57.2 LMH.

The temperature of the cooling agent WFI was 6.0°C.

The graph shows the typical performance of an ultrafiltration: the permeate flux decreases continuously as a result of an increasing protein concentration (viscosity change develop a gel polarization near the membrane skin) in the feed vessel.

In 98 min, 8,368 g product were concentrated to 1,263 g (concentration factor  $C_G = 6.6$ ).



Results of the concentration trial.

Elapsed Time [min]	Permeate [g]	Permeate Flux [l/hm <sup>2</sup> ]	Temperature [°C]
0	0		6.0
2	286	85.8	8.8
4	497	74.6	
6	703	70.3	
9	1,008	67.2	8.0
12	1,302	65.1	7.3
15	1,492	59.7	7.8
18	1,878	62.6	6.8
24	2,423	60.6	6.2
28	2,777	59.5	
31	3,036	58.8	6.7
34	3,298	58.2	
38	3,625	57.2	7.1
41	3,872	56.7	
45	4,190	55.9	
49	4,494	55.0	6.0
52	4,712	54.4	
55	4,922	53.7	
59	5,188	52.8	6.5
65	5,576	51.5	6.4
69	5,819	50.6	
75	6,150	49.2	5.9
82	6,501	47.6	
89	6,823	46.0	
92	6,951	45.3	
95	7,069	44.6	
98	7,105	43.5	

### 2.3. The Diafiltration Step

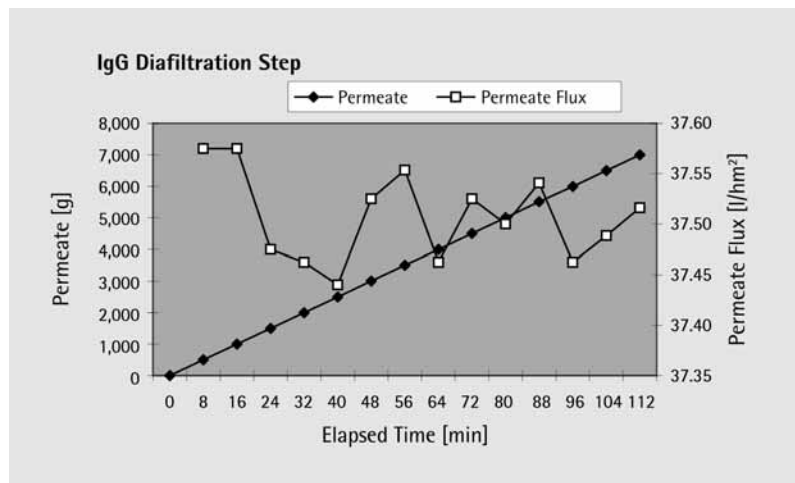
In order to reduce the ionic strength, NaCl was replaced by dia-filtration against a 15 mM NaAc buffer (conductivity 1.13 mS/cm). The starting volume was 1,263 g.

The dia-filtration was established in 500 ml steps and subsequently processed until these 500 ml released the membrane into the permeate.

This procedure was continued until the conductivity of the IgG-solution in the feed vessel reached a conductivity of 1.2  $\mu$ S/cm.

The filtration proceeds virtually linear. This is due to constant protein concentration within the feed vessel proceed the dia-filtration in 500 ml steps (in process procedures the "constant volume-wash" procedure is preferred). The permeate flux is averaged with 37.5 l/hm<sup>2</sup>.

Elapsed Time [min]	Permeate [g]	Permeate Flux [l/hm <sup>2</sup> ]	Temperature [°C]
0	0		
8	501	37.6	
16	1,002	37.6	
24	1,499	37.5	9.0
32	1,998	37.5	9.8
40	2,496	37.4	10.4
48	3,002	37.5	8.0
56	3,505	37.6	8.3
64	3,996	37.5	8.3
72	4,503	37.5	7.5
80	5,000	37.5	8.0
88	5,506	37.5	7.5
96	5,994	37.5	7.1
104	6,498	37.5	6.9
112	7,003	37.5	6.4



Results of the dia-filtration trial.

### 3. Investigation Results

Samples of the original eluate as well as after the concentration process and in addition samples of both permeates (concentration and dia-filtration step) have been taken for analytical purposes. The results are summarized in the table (see beside).

Very important is the analysis of the IgG subclass distribution. The results of this trial meet the requirements and Hydrosart® has no negative influence on the original distribution.

The total amount of protein after concentration|diafiltration is higher than regularly achieved with competitor PESU Cassettes (57 vs. 41 g/l). This results in a higher level of concentration during our test filtration.

Unfortunately, the anti complementary activity is higher than previous used cassettes which indicates an increased impact of proteins which courses aggregate formations. This might be caused by the test conditions as the concentration factor in our experiments is higher (see above).

The permeate after ultrafiltration step showed an IgG content of 0.004 g IgG/l indicating the protein retention of Hydrosart® 30 kD cassette being excellent. The protein loss is low.

Thus, there is no need to change to a 10 kD membrane. Just to compare, the IgG content with the existing large scale Crossflow system is 0.014 g IgG/l.

	Before UF DF	After UF DF	
		Vendor A	Hydrosart® 30 kD
<b>Subclass distribution</b>			
(55–65%) IgG <sub>1</sub> %	55	54	56
(30–40%) IgG <sub>2</sub> %	41	41	39
(2–5%) IgG <sub>3</sub> %	3	4	4
(1–4%) IgG <sub>4</sub> %	1	1	1
Total protein g/l	11	41	57
<b>Amount of complement ≤50%</b>	22.9	36.6	47.2
<b>A<sub>280</sub></b>		Permeate UF 0.014	Permeate UF 0.004
<b>Protein concentration g/l</b>		Permeate DF 0.004	Permeate DF 0.001

### 4. Cleaning Procedure

After product recovery out of the system the cassette was cleaned with sodium hydroxide solution (NaOH). Two liter of 1N NaOH was recirculated at  $p_{in} = 2$  bar,  $p_{out} = 0.5$  bar, and  $p_{per} = 0$  through the system for 10 min. The warm caustic solution (26°C) was drained out for disposal and replaced by fresh two liter 1 N NaOH. Total recirculation was required for 50 min.

After a total cleaning time of 1 hour, the caustic solution was drained drained out of the system and neutralized rinsed with WFI water.

The clean water flux rate – after use and CIP – was determined with the result of 118 l/hm<sup>2</sup>. The CIP efficiency is above 100%.

### 5. Conclusion

The permeate flow performance and cleaning advantages of Hydrosart® 30 kD have been demonstrated.

To handle multiple 600 kg batches (8.4 kg target product) it is recommended to establish at least 9 m<sup>2</sup> filter area (which refers to 15 Hydrosart® cassettes 0.6 m<sup>2</sup> each) to finalize the concentration and dia-filtration step within 3.5 hours. With the Sartoflow 20 holding device the filter surface area can be upgraded to 12 m<sup>2</sup>. This leads to an initial volume increase to 800 liter batch volume produced in the same time.

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