



## Concentration and Diafiltration of Recombinant Protein



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Application  
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Purification by  
Crossflow Filtration  
Hydrosart® 5kD

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## Abstract

The recombinant protein of interest was solubilized in 8M Urea. The purpose of the filtration step being trialed was to concentrate the protein and remove the urea to prepare the product for the next step of re-folding the protein.

### 1. Background

A 3400 ml sample of genetically engineered protein in 8M Urea (Tris HCl buffer pH 3.8) was supplied for the trial. The liquid was free of visible particles, therefore, it was decided that pre-filtration was not necessary.

The protein of interest has a molecular weight of 20kD. The membrane of choice for the trial was a 5kD Hydrosart® ultrafilter. A protein with MW of 20kD should be readily retained on a 5kD ultrafilter membrane.

The main aim of the current trial was to investigate leakage of protein into the permeate during diafiltration seen in the previous trial.



## 2. Description of Trial Procedure

Wash out the preservative solution (0.1 M NaOH) from the cassette using RO water. pH at end of this step was measured to be approximately 7.



Determination of the clean water flow rate.



Equilibration membrane by recirculation of 8M Urea in Tris-HCl buffer pH 3.8 for 10 minutes.



Concentrate 3400 ml of material to about 600 ml using a 5kD Hydrosart® ultrafilter cassette.



Dia-filtration against approximately 14 volumes of re-fold buffer (Tris-HCl pH 3.8).



Protein leakage (as assessed by Coomassie blue R250) and specific protein assay of retentate (chemical reaction followed by absorbance at 280 nm) was determined at various times during diafiltration.



After finalisation of dia-filtration the target product was collected with retentate



Wash membrane with 200 ml of re-fold buffer for product harvest.  
Add wash solution to retentate.  
Measure recovery of specific protein.



CIP followed by Determination of the clean water flow rate.

### 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® 5 kD  
order no. 3021442906E--SG,  
membrane area 0,6 m<sup>2</sup>.

The details of the run are presented in **Table 1** and **Graph 1**.

#### 3.1 Performance improvement of Hydrosart® membrane

First 20 minutes of this investigation the permeate was returned to the feed vessel. During total recirculation the concentration and temperature of material in the feed vessel should remain constant. If the permeate flow drops during this period it indicates that material in the feed is being adsorbed or bound (fouling effects) to the membrane. This process is usually completed in the first 20 minutes of a run.

The results showed that the flow rate did not drop during this period, indicating that the constituents of the starting were not adsorbed onto the membrane. Lowest adsorption is a consistent finding with Hydrosart® ultrafiltration membranes.

#### 3.1.1 Concentration

The permeate flow rate was constant for the first 50 minutes of the concentration step. It was then increased (greater than two fold) by restricting the retentate tube (increasing the Trans Membrane Pressure (TMP)) and reducing the pump speed.

The increased restriction on the retentate tube and reduced cross flow velocity did not result in fouling of the membrane. In fact, the permeate flow rate increased during the remainder of the concentration, probably due to a slight temperature influence of the feed solution.

The final concentration factor was 6. At this concentration the permeate flow rate was not affected by the product concentration. The concentration of the urea is described in the next section.

The concentration procedure was performed in 78 minutes. The volume of collected permeate was 2,490 ml. The average flow rate was therefore 32 ml/min (3.2 liters/hr.m<sup>2</sup>). This is underestimate due to the initial operating conditions and a more realistic figure for the flow rate would be 75 ml/min (7.5 liters/hr.m<sup>2</sup>).

#### 3.1.2 Diafiltration

Diafiltration was performed by maintaining a constant feed volume and adding re-fold buffer at the same rate as permeate was being removed (Constant volume wash procedure).

The operating conditions (pump speed and TMP) remained constant during diafiltration. The permeate flow rate however increased to a peak of 170 ml/min then dropped and stabilized at about 130 ml/min.

The initial increase in flow rate was due mainly to the reducing concentration of urea and therefore decreasing viscosity.

The changing urea concentration also affects the solubility of the constituents remaining in the feed vessel. The flow rate reduction mid way in the diafiltration was probably due to reduced solubility of one or more components of the feed solution as a result of the lower urea concentration.

Approximately 14 volume changes of re-fold buffer were achieved during the diafiltration. Using the formula for concentration remaining after a given number of volume changes and starting concentration, the final urea concentration was calculated to be  $1.6 \times 10^{-5} M$ .

The diafiltration procedure was performed in 88 minutes and the volume of permeate collected was 9,200 ml. The average flow rate was therefore 105 ml/min (10.5 liters/hr.m<sup>2</sup>).

#### 3.3 Product Recovery

After 22 minutes of diafiltration it was observed that something was passing into the permeate that was reacting with Coomassie blue 250. A likely explanation for this is that in the reduced urea concentration (after 22 minutes diafiltration) the material passing through the membrane had become more soluble or had dissociated from a larger protein.

The retentate was assayed for the specific protein of interest and it was found that it was all present in the retentate and none had passed into the permeate. Diafiltration was continued and the retentate was assayed always after 4 litres of buffer until the end of the trial. No target protein loss into the permeate was detected.

### 3.4 Cleanability of Hydrosart® Membrane

After the concentration | diafiltration the membrane was rinsed with buffer or saline solution for approximately 60 minutes. The clean water flow rate were determined. The flow rates are presented in **Table 2**. The results showed that the water flow rates were recovered to the initial clean water flow rate. This result underline the excellent cleanability of the Hydrosart® ultrafilter membrane.

### 3.5 Scale up based on Investigation Results

Based on the results obtained in the current trial the following predictions can be made:

Average flux rate during concentration: 7.5 l/hr.m<sup>2</sup>.

Average flux rate during diafiltration: 10.5 l/hr.m<sup>2</sup>.

Volume of Starting Material: 300 litres

Permeate volume during concentration: 250 litres

Permeate volume during diafiltration: 700 litres (14 vol.changes × 50 l)

Processing Time: 6 hours

### Recommended filter surface area: 18 m<sup>2</sup>. (30 cassettes).

If this surface area was used the concentration time would be 1.9 hours and the diafiltration time would be 3.7 hours.

### 4. Summary

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.
- ... Removal of Urea to acceptable levels (calculation must be confirmed by assay for urea).
- ... Partial purification by removal of low molecular weight impurities.

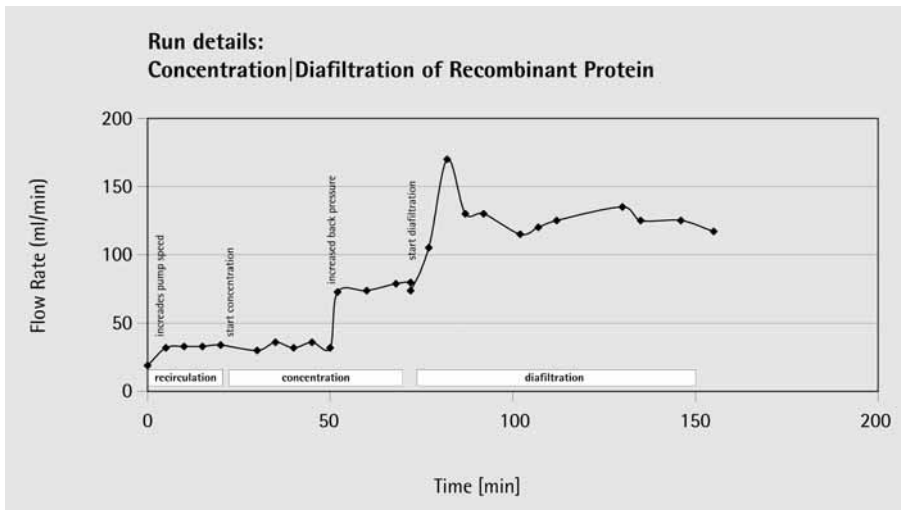
#### Note:

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.

**Table 1: Summary of the results appears in the following table.**

Permeate volume (ml)	Approx. Urea conc. (M)	Volume of Retentate (ml)	Conc. of Protein (g/liter)	Total Protein (g)	% Recovery from start	% Recovery from 22 liters
<b>Starting material</b>		8	3,400	0.97	3.3	
2,490	2.3×10 <sup>-1</sup>	680	5.27	3.6	109	
4,000	2.7×10 <sup>-2</sup>	720	4.85	3.49	106	97
9,200	1.6×10 <sup>-5</sup>	845	4.13	3.49	106	97

The results above clearly showed outstanding rejection (no loss) of the protein of interest.



Graph 1: Flow rate performance through the entire process

**Table 2: Water flow rates**

	Pressure			Flow Rates	
	Inlet bar	Outlet bar	TMP bar	Retentate ml/min	Permeate ml/min
<b>Before run</b>	1.3	0.6	0.95	7,620	130
<b>After run</b>	1.3	0.5	0.9	7,800	130

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