Optimization of concentration and diafiltration of a bovine γ-globulin solution

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This application note describes the development of a cross flow filtration process for concentration of a bovine γ-globulin solution. The goal was to optimize process parameters for concentration and diafiltration (buffer change) of an 80 L bovine γ-globulin solution, keeping the process time below four hours. Process optimization and verification were conducted in small scale using the ÄKTA™ flux s cross flow filtration system. To show scalability, the process was scaled up to medium scale using the larger ÄKTA flux 6 system. As bovine γ-globulin has a molecular weight (M_r) of 150 000, filter cassettes with a 50 000 membrane nominal molecular weight cut-off (NMWC) were used.

Introduction

The high flux rates that can be obtained with cassettes make these filters well-suited for applications such as concentration of protein solutions. The membrane pore size plays a critical role in the performance of the process. If using a membrane with a larger pore size than needed, the target product might pass the filter, with a reduction in yield as a result. The use of a membrane with smaller pore size than required, on the other hand, can result in a low flux, with a consequential extended process time and costly system size increase.

For cross flow filtration applications, the membrane pore size selection is based on the size of the target product. When selecting membrane for concentration applications, a rule of thumb is to use a membrane with a pore size three to five times smaller than the target product. Hence, for concentration of a bovine γ-globulin solution, filter cassettes with 50 000 NMWC was chosen.

This application note describes the development of a process for concentration and diafiltration of a bovine γ-globulin solution. The goal was an optimized process for an 8× concentration and a buffer exchange of an 80 L bovine γ-globulin solution in less than four hours. Process optimization and verification were performed in small scale using the ÄKTA flux filtration system, while considering the requirements of the large-scale process. The cross flow filtration rate was controlled by the differential pressure (ΔP), and a transmembrane pressure (TMP) was selected to optimize flux. To show scalability, the process was scaled from concentration and buffer exchange of a 340 mL bovine γ-globulin solution using the small-scale ÄKTA flux s to concentration and buffer exchange of a 9.57 L γ-globulin solution using the medium-scale ÄKTA flux 6.

Material and methods

ÄKTA flux s, installed with a Kvick Lab™ packet cassette with a membrane pore size of 50 000 NMWC and area of 0.01 m^2, was used in the optimization and verification of an 8× concentration and diafiltration (exchange factor 6) process for a γ-globulin solution. Resulting data were automatically logged and saved on a USB stick, enabling export to Microsoft® Excel® software for analysis.

Optimization of TMP

The flux is directly related to TMP. A low TMP can result in a low flux, with the need for a greater membrane area and a larger system size. A high TMP, on the other hand, can cause fouling of the filter. An optimal TMP is typically selected based on TMP/flux curves at specific flow rates, here controlled by the ΔP. For TMP optimization, ΔP settings of 2.5, 2.0, and 1.5 bar were used and the flux was measured at five TMP settings. TMP/flux curves were generated and used to select TMP and ΔP for the process. TMP optimization was performed using γ-globulin solutions of 15 g/L and 120 g/L. For this process, a ΔP of 2.0 bar and a TMP of 1.0 bar were selected.

Preliminary calculation of sample load

Kvick Lab packet cassettes can be linearly scaled to the larger Kvick Flow™ cassettes. To calculate the sample volume required for optimization of diafiltration time, sample load (L/m²) for the large-scale process was calculated based on selected ΔP and TMP. The average flux achieved at a ΔP of 2.0 bar and a TMP of 1.0 bar was 44 L/m²/h. To achieve an overall process time of four hours, the concentration step was estimated to one hour. A starting volume of 80 L of γ-globulin solution was used for calculation of the membrane area:

$$A = 1.2 \times \frac{V_{conc}}{flux_{ave} \times t}$$

Where

A = membrane area (m²)

t = concentration time (h)

V_{conc} = volume processed during concentration (L)

$flux_{ave}$ = average flux (L/m²/h)

A size margin of 20% (1.2 in the equation) was used in the calculation of membrane area.

The membrane area was calculated to 2.2 m². Hence, by using a 2.32 m² Kvick Flow cassette, the concentration time is expected to be approximately one hour. Based on the selected starting volume (80 L) and membrane area (2.32 m²), the sample load was calculated to 34 L/m² (= 80 L/2.32 m²). From the calculated sample load, the sample volume required for the small-scale verification process was calculated to 340 mL. These preliminary process parameters are summarized in Table 1.

Using the selected parameters for the small-scale process, estimations about process parameters at large scale can be made. In small scale using Kvick Lab packet cassettes, the achieved flow rate at ΔP 2.0 bar and TMP 1.0 bar was approximately 0.100 L/min (read from display), which corresponds to 18.4 L/min in large scale using the Kvick Flow cassettes.

Table 1. Summary of preliminary process parameters

	Large scale	Small scale
Start volume	80 L	340 mL
Final volume	12 L (10 L before recovery)	53 mL (43 mL before recovery)
Start conc. γ-globulin	~ 15 g/L	~ 15 g/L
Target final conc. γ-globulin	120 g/L	120 g/L
Filter area	2.32 m²	0.01 m²
ΔP	2.0 bar	2.0 bar
TMP	1.0 bar	1.0 bar

Optimization of diafiltration time

To minimize the requirements on vessel volume when scaling, the process can be run in fed-batch mode. Hence, the optimization of the diafiltration time was performed in fed-batch mode, starting with 200 mL in the recirculation vessel. The 8× concentration of 340 mL to 43 mL was performed using the parameters selected in the optimization of TMP. The flux obtained at a given concentration factor was multiplied by the same concentration factor and the results were plotted against the concentration factor. From this plot, the concentration degree, at which to perform diafiltration, was optimized for a short process time. A diafiltration exchange factor of 6 was selected for sufficient buffer exchange from the initial 20 mM sodium phosphate, 0.15 M NaCl, pH 7.4 buffer to the final 0.1 M glycine, 0.1 M NaCl, pH 4.5 buffer. The results are summarized in Table 2.

Table 2. Results from optimization of concentration and diafiltration of a bovine γ-globulin solution

Process stage	Max. flux (L/m²/h)	Min. flux (L/m²/h)	Average flux (L/m²/h)	Permeate volume (L)
Concentration factor 3	86	32	59	57
Diafiltration exchange factor 6	32	32	32	137
Concentration factor 2.7	32	5	19	13

Small-scale process verification

To verify the selected process parameters, the full concentration and diafiltration process was run using a starting volume of 340 mL with a γ-globulin concentration of approximately 15 g/L. The γ-globulin concentration was increased to 120 g/L in the concentration step (8× concentration). After product recovery with a final buffer flush, a γ-globulin concentration of 100 g/L was achieved.

Final calculation of sample load

Based on data obtained from optimization of diafiltration time, a more accurate calculation of sample load based on the large-scale filters was done, assuming a starting volume of 80 L γ-globulin solution. Calculation was based on a 3× concentration (average flux 59 L/m²/h), followed by diafiltration (exchange factor 6, average flux 32 L/m²/h) and a final 2.7× concentration (average flux 19 L/m²/h). For the calculation, an overall process time of four hours was selected:

$$A = 1.2 \times \left(\frac{V_{conc1}}{flux_{ave1}} + \frac{V_{dia}}{flux_{dia}} + \frac{V_{conc2}}{flux_{ave2}} \right) / t_{tot}$$

Where:

A = membrane area (m^2)

t_{tot} = total process time (h)

V_{conc1} = permeate volume from first concentration (L)

V_{dia} = permeate volume from diafiltration (L)

V_{conc2} = permeate volume from second concentration (L)

$flux_{ave1}$ = average flux during first concentration (L/m^2/h)

$flux_{dia}$ = average flux during diafiltration (L/m^2/h)

$flux_{ave2}$ = average flux during second concentration (L/m^2/h)

A size margin of 20% (1.2 in the equation) was used in the calculation of membrane area.

The filter area was more accurately calculated to 1.79 m^2. By using four 0.46 m^2 Kvick Flow cassettes, with a total filter area of 1.84 m^2, the overall process time would be approximately four hours. Using this filter area, the sample load can be calculated to 43 L/m^2 (= 80 L/1.84 m^2). As this sample load was greater than previously calculated, the sample volume at small scale could be increased to 443 mL (Table 3).

Table 3. Summary of final process parameters

	Large scale	Small scale
Start volume	80 L	443 mL
Final volume	12 L (10 L before recovery)	65 mL (55 mL before recovery)
Start conc. γ-globulin	~ 15 g/L	~ 15 g/L
Target conc. γ-globulin	120 g/L	120 g/L
Conc. after recovery	100 g/L	100 g/L
Filter area	1.84 m^2	0.01 m^2
ΔP	2.0 bar	2.0 bar
TMP	1.0 bar	1.0 bar

Final process verification in small scale

A 3× concentration followed by diafiltration (exchange factor 6) and a final 2.7x concentration was performed at a ΔP of 2.0 bar and a TMP of 1.0 bar. Starting with a 443 mL bovine γ-globulin solution (run in fed-batch mode, starting with 277 mL in the reservoir), the final volume was 55 mL. Protein concentration was determined spectrophotometrically at 280 nm.

Process scaling

To show scalability, the process was scaled up to concentration and buffer exchange using ÄKTA flux 6 equipped with two Kvick Lab cassettes (0.22 m^2) with a membrane pore size of 50 000 NMWC. A 3× concentration followed by diafiltration (exchange factor 6) and a final 2.7× concentration was performed at a ΔP of 2.0 bar and a TMP of 1.0 bar. Starting with a 9.57 L bovine γ-globulin solution (run in fed-batch mode, starting with 5.97 L in the reservoir), the final volume was 1.20 L. Resulting data were automatically logged and saved on a USB stick and exported to Microsoft Excel software for analysis. Protein concentration was determined spectrophotometrically at 280 nm.

Results

TMP optimization

From the TMP/flux curves shown in Figures 1 and 2, a ΔP of 2.0 bar was selected. As the ΔPs of 2.0 bar and 2.5 bar resulted in a similar flux, the lower ΔP was selected to reduce the requirements for feed pump capacity in the large-scale process. A TMP of 1.0 bar was selected, as a higher TMP did not significantly increase the flux and a higher TMP also generated fouling of the filter.

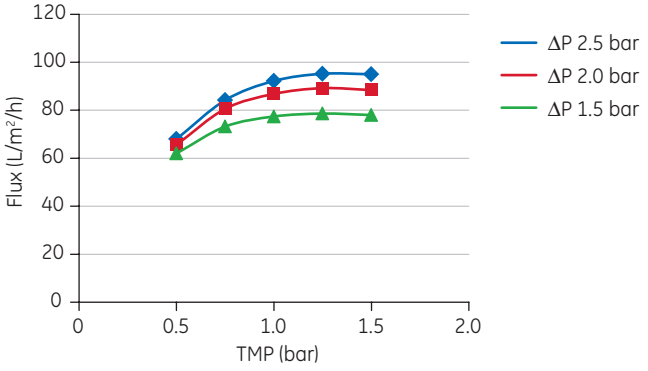

Fig 1. Flux plotted against TMP using a 15 mg/mL bovine γ-globulin solution.

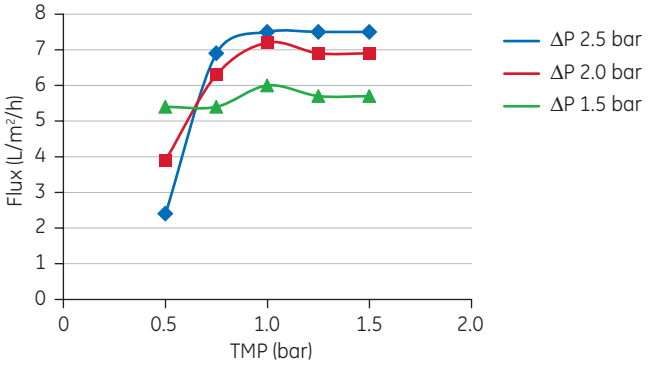

Fig 2. Flux plotted against TMP using a 120 mg/mL bovine γ-globulin solution.

Optimization of diafiltration time

For optimization of the diafiltration time, the flux at a given concentration factor was multiplied with the concentration factor and plotted against the same concentration factor (Fig 3). At a ΔP of 2.0 and a TMP of 1.0 bar, the shortest processing time was achieved by performing the diafiltration step after an initial 3× concentration. A final 2.7× concentration was conducted to achieve an overall 8× concentration of the sample.

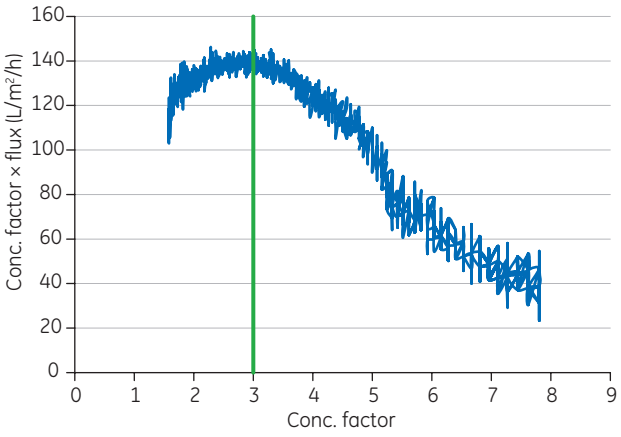

Fig 3. Optimization of diafiltration time at a ΔP of 2.0 bar and a TMP of 1.0 bar. The shortest process time was obtained when diafiltration was performed at concentration factor 3, indicated by the green line at the maximum of the curve.

Process verification and scale-up

Results from verification and scale-up of the overall concentration and diafiltration process are summarized in Tables 4 and 5. Process verification data were exported from the ÄKTA flux systems on a USB memory stick, for visualization using the Microsoft Excel program installed on a separate computer (Fig 4 and 5). In the overall small-scale process using ÄKTA flux s, 443 mL γ-globulin solution was 8× concentrated, giving a final volume of 62 mL with 98% recovery of the target protein in 3.1 h. In the medium-scale process using ÄKTA flux 6, 9.57 L γ-globulin solution was 8× concentrated, giving a final volume of 1.41 L with 100% recovery of the target protein in 3.2 h.

Table 4. Recovery of bovine γ-globulin in small scale using ÄKTA flux s

	Volume (mL)	Protein conc. (g/L)	Protein amount (g)	Recovery (%)
Start material	443	14	6.3	
Retentate	50	114	5.8	92
Flush	11	34	0.4	6
Permeate	1280	0	0	0
Retentate + flush	62	99	6.1	98

Table 5. Recovery of bovine γ-globulin in medium scale using ÄKTA flux 6

	Volume (L)	Protein conc. (g/L)	Protein amount (g)	Recovery (%)
Start material	9.57	14.9	143	
Retentate	1.20	115	137	96
Flush	0.22	19.5	4.20	3
Permeate	27.6	0.01	0.30	0
Retentate + flush	1.41	101	142	100

Filter	Kvick Lab packet cassette (50 000 NMWC, 0.01 m² membrane area)
Sample	bovine γ-globulin solution (14.2 g/L)
Sample volume	433 mL
Sample load	43.5 g/L
Wash buffer	0.1 M glycine, 0.1 M NaCl, pH 4.5
Concentration factor	8
Diafiltration exchange factor	6
ΔP	2.0 bar
TMP	1.0 bar
Throughput	128 L/m²
Process time	3.1 h

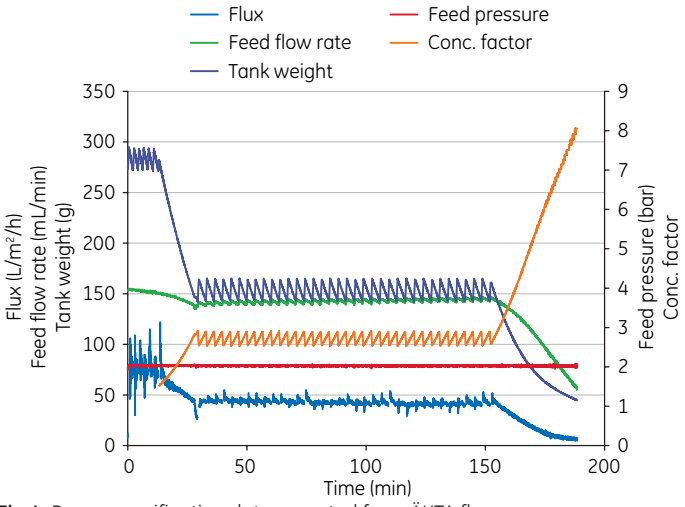

Fig 4. Process verification data exported from ÄKTA flux s.

Filter	Two Kvick Lab cassette (50 000 NMWC, 0.22 m² membrane area)
Sample	bovine γ-globulin solution (14.2 g/L)
Sample volume	9.57 L
Sample load	43.5 g/L
Wash buffer	0.1 M glycine, 0.1 M NaCl, pH 4.5
Concentration factor	8
Diafiltration exchange factor	6
ΔP	2.0 bar
TMP	1.0 bar
Throughput	125 L/m²
Process time	3.2 h

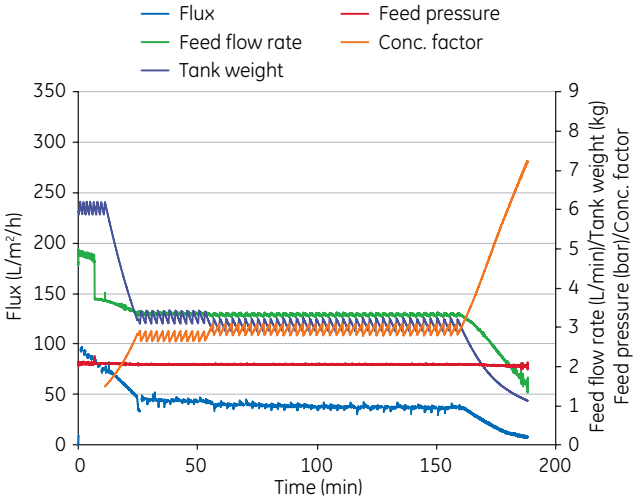

Fig 5. Process scale-up data exported from ÄKTA flux 6.

Summary

A concentration and diafiltration process for a bovine γ-globulin solution was successfully developed and verified in small scale, using ÄKTA flux s installed with a Kvick Lab packet cassette (0.01 m^2) with a membrane pore size of 50 000 NMWC. The goal was an optimized process for concentration and diafiltration of an 80 L bovine γ-globulin solution in less than four hours. The ΔP and TMP settings were evaluated and diafiltration time was optimized for a short overall process time. The selected process parameters were verified in a small-scale run on ÄKTA flux s, where a 443 mL γ-globulin solution was concentrated eight times. To show scalability, the process was scaled up to an eight-time concentration of a 9.57 L γ-globulin solution using the medium-size ÄKTA flux 6 equipped with two Kvick Lab cassettes (0.22 m^2) with a membrane pore size of 50 000 NMWC. The overall process resulted in a 98% γ-globulin recovery in a final volume of 62 mL over a process time of 3.1 h in small scale, and a 100% γ-globulin recovery in a final volume of 1.41 L over a process time of 3.2 h in medium scale.

Order information

Product	Description	Quantity	Code number
ÄKTA flux s	Cross flow filtration system	1	29-0384-37
ÄKTA flux 6	Cross flow filtration system	1	29-0384-38
Kvick Lab packet cassette	50 000 NMWC, 0.01 m^2	1	56-4112-10
Kvick Flow cassette	50 000 NMWC, 2.32 m^2	1	56-4113-41
Kvick Flow cassette	50 000 NMWC, 0.46 m^2	4	56-4113-52
Kvick Lab cassette	50 000 NMWC, 0.1115 m^2	1	56-4113-28

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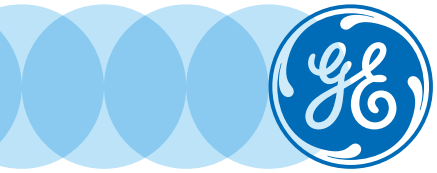

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