Process development for optimized recovery of a domain antibody (Dab) from *E. coli* using cross flow filtration

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This application note describes the development of a clarification process (removal of cells and cell debris) with optimized recovery of a Dab from an *E. coli* extract using cross flow filtration (CCF). CFF is suitable for applications involving viscous or high-solid feeds. Hollow fiber filter cartridges are commonly used for the CFF step. Because of their open channel structure, hollow fiber filters are well-suited for microfiltration applications such as recovery of proteins expressed in bacteria. In the optimization of the clarification process, the filter pore size and operating conditions were selected for retaining solids in the retentate, while achieving high recovery of the target protein in the permeate.

Introduction

Following the success of monoclonal antibodies (MAbs), antibody fragments such as Fab, scFv, and Dab are gaining interest as protein-based therapeutics (1). Antibody fragments possess advantages suitable for a range of diagnostic and therapeutic applications. For example, fragments are smaller than MAbs and, thus, can more easily penetrate tissue. A Dab has a molecular weight (M_r) of approximately M_r 13 000, to be compared with the molecular weight of a MAb, which is approximately M_r 150 000 (Fig 1).

This application note describes the development of a clarification process for a Dab-containing *E. coli* extract using CFF. The goal was a process with optimized Dab recovery that requires less than four hours to perform. An additional requirement was that the optimized small-scale process would be scalable to clarification in a larger scale.

The Dab was expressed in the periplasm of *E. coli* and released into the culture medium through heat treatment. Optimization of the clarification process involved selecting a filter pore size and operating conditions suitable for retaining solids in the retentate, while yielding high recovery of the target protein in the permeate. Two serially connected Start AXM 50 cm² hollow fiber cartridges were used to obtain sufficient filter area for rapid clarification of a 300 mL *E. coli* extract. Shear rates were selected to be suitable also for clarification of a 50 L *E. coli* extract, using two parallel-connected size 9A hollow fiber cartridges. Process optimization was performed on the ÄKTAcrossflow™ filtration system, and the final process was verified using the ÄKTA™ flux s filtration system. To show scalability, the process was scaled up to Dab recovery from 2.53 L *E. coli* extract using the ÄKTA flux 6 system equipped with a Xampler™ 850 cm² hollow fiber filter cartridge.

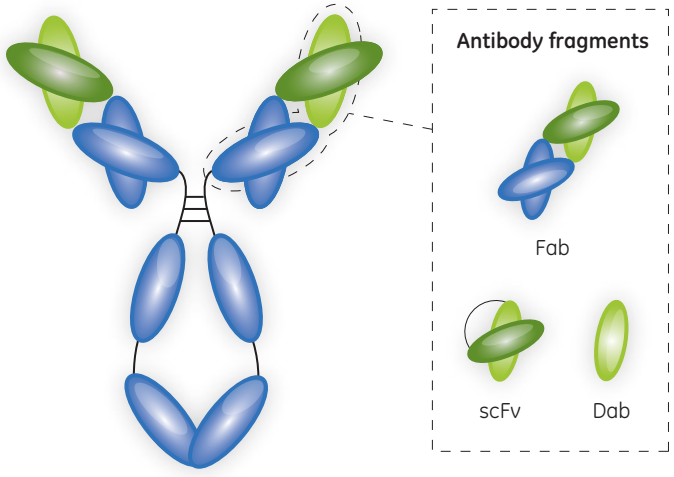

Fig 1. Structure of MAb and antibody fragments. As fragments, such as Fab, scFv, and Dab, are relatively small, production in bacterial expression systems is easier and more cost-effective compared with the mammalian cell systems used for production of full-size MAbs. Fab = fragment, antigen-binding, scFv = single-chain fragment, variable, and Dab = domain antibody.

Material and methods

Dab production

Seed cultures of *E. coli* strain RV308, started in 50 mL shaker flasks, were further cultured in 10 L bioreactors with 10 L fermentation broth. After 15 hours at OD 80, the cultures were induced for Dab production. Second inductions were performed after an additional three hours. After five hours of induction, the *E. coli* cultures were terminated at OD 110 by heat treatment (48°C for 3 h) to release the target protein. The Dab concentration was determined to approximately 2.5 g/L on a 1 mL HiTrap™ Protein L column using purified Dab as reference. The *E. coli* extracts were stored at 4°C before use.

Screening of membrane pore size

Three membranes, with pore sizes 750 000 nominal molecular weight cut-off (NMWC), 0.1 µm, and 0.2 µm, were screened. The experimental conditions were:

Hollow fiber filters:	two Start AXM 50 cm^2 in series
System:	ÄKTAcrossflow filtration system
Sample volume:	300 mL
Shear rate:	6000 s^{-1}
Concentration factor:	2.5
Diafiltration exchange factor:	3 (in three wash steps)
Flux:	15 L/m^2/h
Permeate:	collected and assayed for Dab recovery

As the highest Dab recovery was obtained with the 750 000 NMWC hollow fiber filter, this membrane was selected for screening of shear rates.

Shear rate screening

Three shear rates, 4000 s^{-1}, 6000 s^{-1}, and 8000 s^{-1}, were screened. The experimental conditions were:

Hollow fiber filters:	two Start AXM 50 cm^2 with 750 000 NMWC in series
System:	ÄKTAcrossflow filtration system
Sample volume:	300 mL
Tested shear rates:	4000, 6000, and 8000 s^{-1}
Concentration factor:	2.5
Diafiltration exchange factor:	3 (in three wash steps)
Flux:	15 L/m^2/h
Permeate:	collected and assayed for Dab recovery

When controlling a process using a flux setting, the permeate pressure should always be kept at > 0 bar to prevent fouling of the filter membrane. During the membrane pore size screening, a flux of 15 L/m^2/h was used to obtain a permeate pressure of 0.3 to 0.4 bar. Increasing the flux would reduce the permeate pressure to below 0 bar.

Required parameters for a large-scale process

Required parameters for a large-scale process were:

Sample volume:	50 L
Shear rate range:	4000 to 8000 s^{-1}
Concentration factor:	2.5
Diafiltration exchange factor:	3
Flux:	15 L/m^2/h
Process time:	< 4 h
Max. feed pump flow rate:	30 L/min

From the required parameters, the total permeate volume (V_p) for the large-scale process can be calculated:

$$V_p = (V_s - V_s/C_f) + (V_s/C_f) \times D_f$$

Where:

V_p = total permeate volume (L)

V_s = start volume (L)

C_f = concentration factor

D_f = diafiltration exchange factor

Using the total permeate volume, the filter area (A) needed to achieve target processing time can be calculated:

$$A = V_p / (\text{flux} \times t)$$

Where:

A = filter area (m^2)

V_p = total permeate volume (L)

flux = permeate flux (L/m^2/h)

t = target process time (h)

From the total permeate volume, calculated to 90 L, the required filter area was calculated to 1.5 m^2. For the large-scale process, two parallel-connected size 9A hollow fiber cartridges can be used, as together they give a filter area of 1.68 m^2. For two parallel-connected size 9A filters, the required flow rates for shear rates in the desired range (4000 to 8000 s^{-1}) are listed in Table 1.

Table 1. Shear rates with corresponding flow rates for two parallel-connected size 9A filters

Shear rate	Pump flow rate
8000 s^{-1}	49.0 L/min
6000 s^{-1}	36.8 L/min
4000 s^{-1}	24.5 L/min

Small-scale verification run

The optimized process was verified on ÄKTA flux s filtration system. Dab recovery was determined after the concentration step and after each diafiltration wash step.

Process scaling

A process scale-up run was performed on the medium-size ÄKTA flux 6 system. The experimental conditions were:

Hollow fiber filter:	Xampler size 4X2MA 850 cm^2 with 750 000 NMWC
System:	ÄKTA flux 6
Sample volume:	2.53 L
Shear rate:	8000 s^{-1}
Concentration factor:	2.5
Diafiltration exchange factor:	3
Flux:	15 L/m^2/h
Permeate:	collected and assayed for Dab recovery

Results

The objective of the membrane screening was to identify a suitable pore size for high Dab recovery. In Table 2, the results from the membrane screening experiments are summarized. As highest recovery was obtained using the membrane with a pore size of 750 000 NMWC, this membrane was selected for further investigation.

Table 2. Results from the screening of pore sizes for optimized Dab recovery

Membrane pore size	Dab recovery
750 000 NMWC	87%
0.1 µm	85%
0.2 µm	81%

For screening of shear rates, the first objective was to find a suitable shear rate for a stable process and optimized Dab recovery using the two serially connected hollow fiber filter cartridges with 750 000 NMWC. The results from the shear rate screening experiments are summarized in Table 3. The permeate pressure was more stable at the higher shear rates. Hence, a shear rate of 8000 s^{-1} was chosen for process verification on the ÄKTA flux s system.

Table 3. Results from the screening of shear rates for optimized Dab recovery

Shear rate	Dab recovery
8000 s^{-1}	83%
6000 s^{-1}	87%
4000 s^{-1}	86%

Results from process verification and scale-up runs are summarized in Table 4. During the concentration step using ÄKTA flux s, 41% of the total Dab content passed in the permeate. The corresponding figure for the ÄKTA flux 6 process was 44%. In the ÄKTA flux s process, three wash steps increased the Dab yield to a total of 83% in a total collected permeate volume of approximately 600 mL. The total Dab yield of the ÄKTA flux 6 process was 82% in a collected permeate volume of 4.56 L. Increasing the number of wash steps would contribute to a higher recovery. However, increased protein recovered should be balanced against sample dilution as well as the additional time required for the extra wash steps. In this study, the overall process time was 3.5 h for the ÄKTA flux s process and 3.1 h for the ÄKTA flux 6 process.

Table 4. Dab recovery in the collected permeate

Permeate	Dab recovery	
	ÄKTA flux s	ÄKTA flux 6
Concentration	41%	44%
Wash 1	22%	18%
Wash 2	12%	12%
Wash 3	7%	7%
Total recovery	83%	82%

Process data was exported from ÄKTA flux s and ÄKTA flux 6 on USB memory sticks, for visualization using the Microsoft® Excel® program installed on a separate computer (Fig 2 and 3).

Hollow fiber filters	Two Start AXM 50 cm^2 with 750 000 NMWC in series
System	ÄKTA flux s filtration system
Sample	*E. coli* feed containing Dab (2.61 g/L)
Sample volume	300 mL
Sample load	30 g/L
Wash buffer	Phosphate buffered saline (PBS), pH 7.4
Concentration factor	2.5
Diafiltration exchange factor	3 (in three wash steps)
Shear rate	8000 s^{-1}
Flux	15 L/m^2/h
Throughput	54 L/m^2
Process time	3.5 h

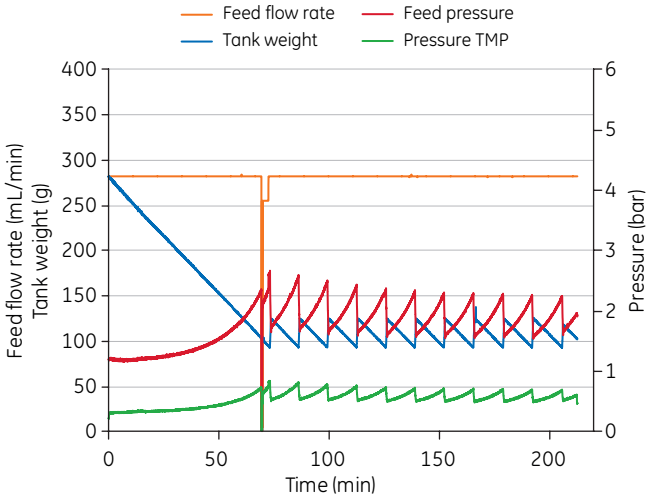

Fig 2. Process verification using ÄKTA flux s filtration system.

Hollow fiber filters	Xampler 850 cm2 with 750 000 NMWC
System	ÄKTA flux 6 filtration system
Sample	*E. coli* feed containing Dab (2.61 g/L)
Sample volume	2.53 L
Sample load	30 g/L
Wash buffer	PBS, pH 7.4
Concentration factor	2.5
Diafiltration exchange factor	3 (in three wash steps)
Shear rate	8000 s^{-1}
Flux	15 L/m^2/h
Throughput	54 L/m^2
Process time	3.1 h

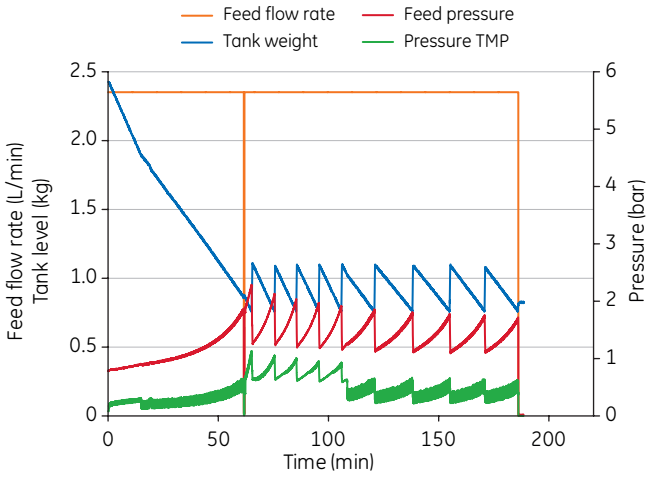

Fig 3. Process scale-up using ÄKTA flux 6 filtration system.

Summary

The aim of this work was to develop a clarification step with optimized Dab recovery from a 300 mL *E.coli* extract, while keeping the process time below four hours. An additional requirement was that the optimized process should be scalable to clarification of a 50 L Dab-containing *E. coli* extract. Two serially connected Start AXM 50 cm^2 hollow fiber cartridges with 750 000 NMWC were selected to obtain a sufficient membrane area for a short process time. The selected shear rate was 8000 s^{-1}. The optimized process, verified using the ÄKTA flux s cross flow filtration system, resulted in an overall process yield of 83% in a process time of 3.5 h To show scalability, the process was scaled-up to Dab recovery from 2.53 L using the medium-size ÄKTA flux 6 system. The results from the scaled-up process were similar to those from the small-scale verification process, with a Dab yield of 82% in a process time of 3.1 h.

Reference

1. Nelson, A.L. and Reichert, J.M. Development trends for therapeutic antibody fragments. Nature Biotechnology 27, 331–337 (2009)

Order information

Product	Description	Quantity	Code number
ÄKTA flux 6	Cross flow filtration system	1	29-0384-38
ÄKTA flux s	Cross flow filtration system	1	29-0384-37
ÄKTAcrossflow	Cross flow filtration system	1	18-1180-00
Start AXM 50 cm^2	750 000 NMWC	2	11-0005-50
Start AXM 50 cm^2	Pore size 0.1 µm	2	11-0005-51
Start AXM 50 cm^2	Pore size 0.2 µm	2	11-0005-52
Hollow fiber cartridge size 9, 0.84 m^2 (UFP-750-E-9A)	Ultrafiltration cartridge, 750 000 NMWC	2	56-4103-08
Xampler hollow fiber cartridge, 0.085 m^2 (UFP-750-E-4X2MA)	Ultrafiltration cartridge, 750 000 NMWC	1	56-4102-20

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