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

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 (Mₐ) of approximately Mₐ 13,000, to be compared with the molecular weight of a MAb, which is approximately Mₐ 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 filtration system.
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 cm² 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/m²/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

Two shear rates, 6000 s⁻¹ and 4000 s⁻¹, were screened. The experimental conditions were:

- **Hollow fiber filters:** two Start AXM 50 cm² with 750 000 NMWC in series
- **System:** ÄKTAcrossflow filtration system
- **Sample volume:** 300 mL
- **Tested shear rates:** 4000 and 6000 s⁻¹
- **Concentration factor:** 2.5
- **Diafiltration exchange factor:** 3 (in three wash steps)
- **Flux:** 15 L/m²/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/m²/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 6000 s⁻¹
- **Concentration factor:** 2.5
- **Diafiltration exchange factor:** 3
- **Flux:** 15 L/m²/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:

\[
A = \frac{V_p}{(flux \times t)}
\]

Where:

- A = filter area (m²)
- \(V_p\) = total permeate volume (L)
- flux = permeate flux (L/m²/h)
- t = target process time (h)

From the total permeate volume, calculated to 90 L, the required filter area was calculated to 1.5 m². 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 m². For two parallel-connected size 9A filters, the required flow rates for shear rates in the desired range (4000 to 6000 s⁻¹) are listed in Table 1.

<table>
<thead>
<tr>
<th>Shear rate</th>
<th>Pump flow rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>6000 s⁻¹</td>
<td>36.8 L/min</td>
</tr>
<tr>
<td>4000 s⁻¹</td>
<td>24.5 L/min</td>
</tr>
</tbody>
</table>

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.
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

<table>
<thead>
<tr>
<th>Membrane pore size</th>
<th>Dab recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>750 000 NMWC</td>
<td>87%</td>
</tr>
<tr>
<td>0.1 µm</td>
<td>85%</td>
</tr>
<tr>
<td>0.2 µm</td>
<td>81%</td>
</tr>
</tbody>
</table>

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 Dab recovery was found to be higher at the higher shear rate. However, to lower the requirements for feed pump capacity in the large scale, the lower shear rate 4000 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

<table>
<thead>
<tr>
<th>Shear rate</th>
<th>Dab recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>6000 s⁻¹</td>
<td>91%</td>
</tr>
<tr>
<td>4000 s⁻¹</td>
<td>88%</td>
</tr>
</tbody>
</table>

Results from process verification using ÄKTA flux s are summarized in Table 4. During the concentration step, 49% of the total Dab content passed in the permeate. Three wash steps increased the Dab yield to a total of 86% in a total collected permeate volume of approximately 550 mL. 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 3.1 h.

Table 4. Dab recovery in the collected permeate

<table>
<thead>
<tr>
<th>Permeate</th>
<th>Dab recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration</td>
<td>49%</td>
</tr>
<tr>
<td>Wash 1</td>
<td>21%</td>
</tr>
<tr>
<td>Wash 2</td>
<td>11%</td>
</tr>
<tr>
<td>Wash 3</td>
<td>6%</td>
</tr>
<tr>
<td>Total recovery</td>
<td>86%</td>
</tr>
</tbody>
</table>

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 cm² hollow fiber cartridges with 750 000 NMWC were selected to obtain a sufficient membrane area for a short process time. To suite the requirements of a large-scale process using two parallel-connected size 9A hollow fiber cartridges, the selected shear rate was 4000 s⁻¹. The optimized process, verified using the ÄKTA flux s cross flow filtration system, resulted in an overall process yield of 86% in a process time of 3.1 h.

Reference
## Order information

<table>
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<th>Product</th>
<th>Description</th>
<th>Quantity</th>
<th>Code number</th>
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</thead>
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<tr>
<td>AKTA flux s</td>
<td>Cross flow filtration system</td>
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<tr>
<td>AKTAcrossflow</td>
<td>Cross flow filtration system</td>
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<td>18-1180-00</td>
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<td>Start AXM 50 cm²</td>
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<td>11-0005-50</td>
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<tr>
<td>Start AXM 50 cm²</td>
<td>Pore size 0.1 µm</td>
<td>2</td>
<td>11-0005-51</td>
</tr>
<tr>
<td>Start AXM 50 cm²</td>
<td>Pore size 0.2 µm</td>
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<td>11-0005-52</td>
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<td>Pilot-scale ultrafiltration cartridge, 750 000 NMWC</td>
<td>2</td>
<td>56-4103-08</td>
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