Concentration and buffer exchange using the automated, single-use ÄKTA readyflux system

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This work describes the capabilities of ÄKTA readyflux when used in ultrafiltration/diafiltration (UF/DF) applications. The process was conducted in an automated manner based on methods created using phases predefined in the UNICORN™ system control software. Concentration of 5 g/L bovine serum albumin (BSA) was performed using a 0.46 m² filtration cassette with a M_r 10 000 nominal molecular weight cut-off (NMWC) pore size, with process parameters fixed at a transmembrane pressure (TMP) of 1.2 bar and a feed flow of 4 L/min. Protein recovery was ≥ 99%, after 5-times concentration and 5-fold diafiltration. The process was performed according to set specifications for a tangential flow filtration (TFF) process, with the added benefits of the single-use technology and consistency through automation.

Introduction

Clinical production operation and contract manufacturing organizations typically produce many different drug substances, and the productions can differ in scale and be based on both microbial fermentation or cell culture. For such facilities, there is a continuously increasing interest in single-use technologies to gain flexibility in production capacity and decrease changeover rate between production campaigns. Today, single-use technologies are used from upstream production to downstream purification of biomolecules.

ÄKTA readyflux is a single-use TFF system intended for use in both upstream culture harvest and downstream product concentration and buffer exchange operations, ranging from pilot to small-scale commercial manufacturing. The system uses a single-use flow path that prevents cross-contamination between batches, shortens batch changeover time, reduces utility requirements, and makes cleaning-in-place (CIP) and steam-in place (SIP) validation redundant. To suit a broad range of applications, the system can be used with both hollow fiber filter cartridges and filter cassettes.

This work describes the use of ÄKTA readyflux in automated UF/DF operations for reproducibility and minimized hands-on time. Automated TFF equipment are operated based on current conditions of the process and, therefore, can save time and increase process consistency. Automation provides sophisticated control for TFF operations, where two interdependent control modes are included for the recirculation and permeate side, respectively.

Materials and methods

A BSA solution of 5 g/L in purified water was used as sample for concentration (UF) and buffer exchange (DF) into phosphate buffered saline (PBS).

Determination of minimum working volume

ÄKTA readyflux was installed with a Flow Kit plus TC flow path. The filter was bypassed and the feed and retentate section were formed into a closed loop. A 2D bag was filled with a 5 L of BSA solution (5 g/L) using the transfer pump. The feed line valves XV005 and XV006 and the retentate line valves XV051 and XV052 were opened, and the feed pump was started at a low flow rate to remove air from the circuit. Once the feed flow was stabilized, the feed flow rate was set at 2 L/min and the flow was allowed to stabilize. During the run, the third port on the 2D bag was opened and solution was collected in a beaker until air bubble started to pull towards the feed line or was observed at the start of bag feed port. Thereafter, the feed pump was stopped and the minimum working volume was determined. The procedure was repeated for feed flow rates of 2, 6, 10, 14, and 18 L/min.

System setup

For this study, a 0.46 m^2 filter cassette was used. Water, 0.5 M NaOH CIP solution, and 0.1 M NaOH storage solution were connected, using 3/8 inch tubing, to the Flow Kit valves XV001, XV002, and XV003, respectively. PBS equilibration buffer and sample were connected at XV082 and XV083, respectively. A 3/8 inch tubing was connected at XV071 and placed in the recovery reservoir. The 3/8 inch tubing connected at XV031 and at the UV outlet (XV034) were directed to the waste reservoir. A summary of the process is given in Table 1.

Table 1. Process summary

Start feed volume	14 L
Concentration factor	5
Diafiltration exchange factor	5
Feed flow rate	4 L/min
Cassette filter area	0.46 m^2
TMP	1.2 bar

Sample collection and analysis

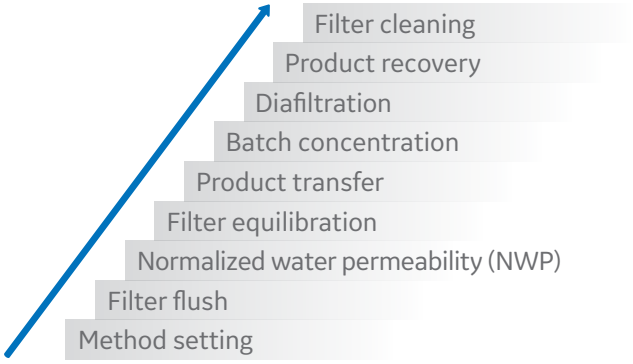
After the run was completed, samples were collected according to Table 2. Collected samples were analyzed spectrophotometrically at 280 nm for protein content and the recovery was calculated.

Table 2. Sample collection

Sample stage	Sample volume
Initial protein sample	10 mL
Final recovered protein	10 mL
Buffer flush sample	10 mL

Method creation

Preprogrammed phases were used to create the automated method in the ***Method Editor*** of the software, by first choosing ***Create New Method***, and then selecting ***Empty Method***. From the phase library, individual phases were drag-and-dropped to the method outline, starting from method settings:



In ***Phase properties*** under each phase selected, process parameters were entered. The created method was named and saved. To run the process, the method was accessed from ***Method navigator*** under ***System control***.

Results

Minimum working volume was determined to avoid concentration to lower volume than allowed for the system (Table 3).

Table 3. Minimum working volume determined at different feed flow rates

Feed pump flow rate (L/min)	Volume recovered (mL)	Minimum working volume (mL)
2	4460	540
6	4270	730
10	3880	1120
14	3540	1460
18	3270	1730

Run data exported from the ***Evaluation*** module of the UNICORN software are shown in Figures 1 to 4.

Pressure is the driving force for any filtration process, and stable pressure signals are key during TMP control to maintain a stable concentration process. Figure 1 shows the stability of the pressure signals for all the three sensors, resulting in a highly stable TMP during the concentration and diafiltration process.

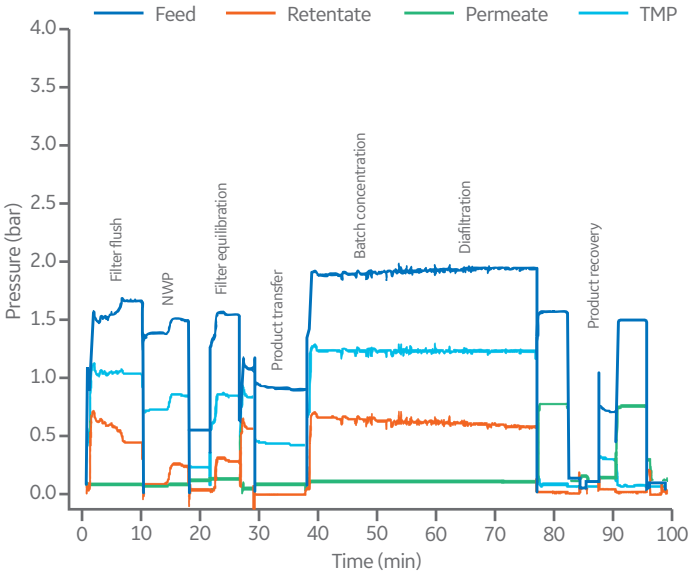


Fig 1. Pressure profile: maximum pulsation during product phases was 0.07 bar.

The system software can calculate the exact concentration and diafiltration factors to control the process. Figure 2 demonstrate the accuracy of the implemented calculation, which is used to end the phase based on concentration or diafiltration factors.

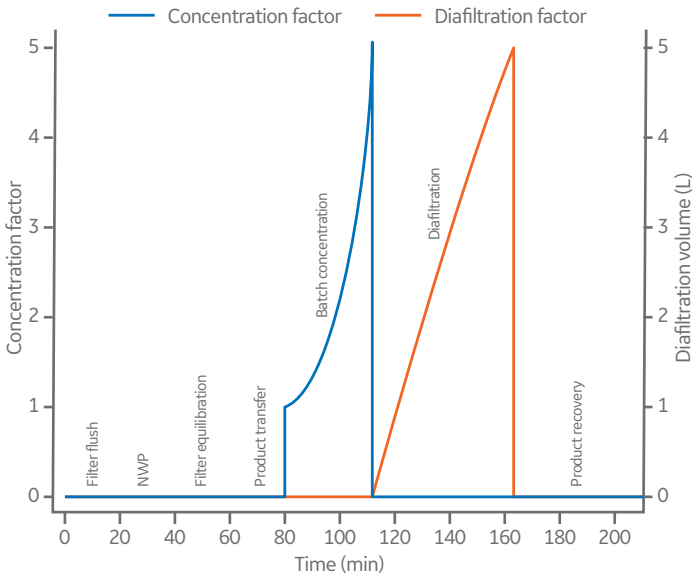


Fig 2. Accurate concentration and diafiltration factors.

The conductivity curve during diafiltration shows that the buffer exchange is sufficient at the end of the step. Figure 3 shows the efficiency of the diafiltration step by the increase in conductivity during buffer exchange.

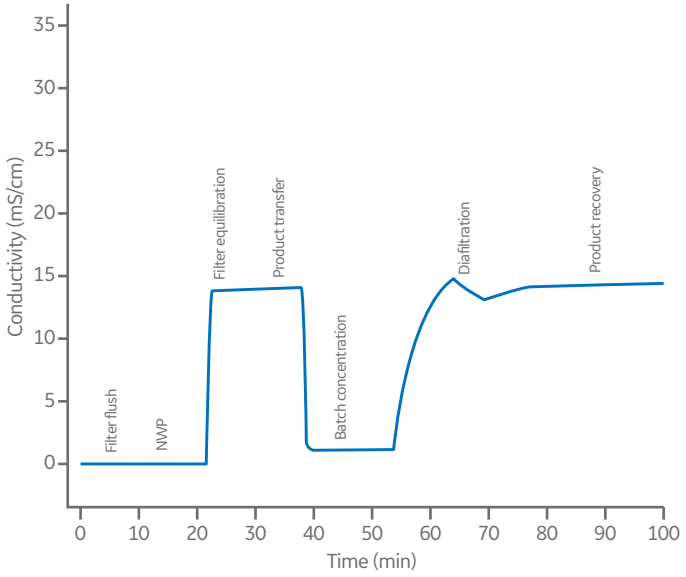


Fig 3. Conductivity curve for the diafiltration process.

The load cell associated with the 2D bag can control the bag weight with high accuracy and stability. Figure 4 shows the feed bag weight during the process. Using the feed pump, product was recovered primarily through the lowest drain port. Total recovery of BSA after a final 1 L buffer flush was found to be ≥ 99% for the UF/DF process (Table 4).

Table 4. Summary of results

Sample	BSA g/L	Volume (L)	Total (g)	Recovery (%)
Feed	4.347	14	60.87	-
Final concentration	20.757	2.79	57.91	95.14
Buffer wash	2.54	0.98	2.489	4.09
Overall recovery				**≥ 99%**

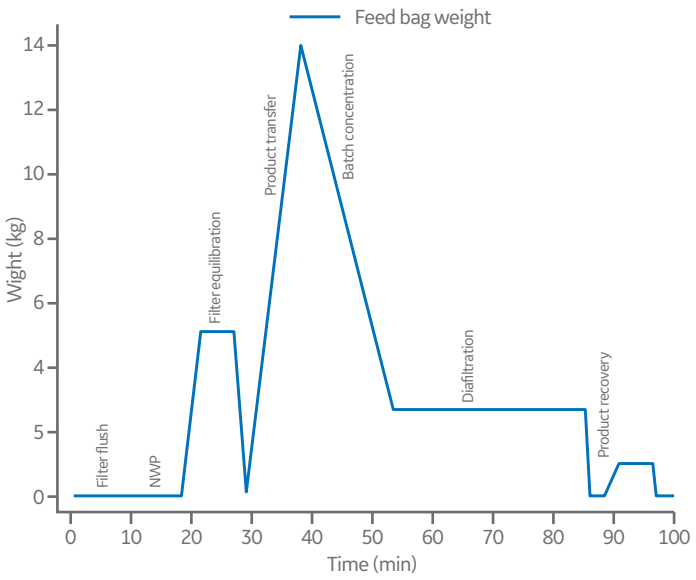


Fig 4. Feed bag weight and product recovery profile.

Discussion

We observed that using ÄKTA readyflux and its disposable flow path helps minimize the cross-contamination risk and reduce the time spent on cleaning and sanitization. Turnaround time between batches or products can be improved and uptime maximized, facilitating multiproduct and flexible manufacturing in an environment with an ever-increasing need for higher and more effective facility utilization.

ÄKTA readyflux is suitable for use in a cGMP environment, and the system can be connected to other single-use equipment within the ReadyToProcess™ platform. ÄKTA readyflux can, for example, be connected to the Xcellerex™ XDR stirred-tank bioreactor systems or the rocking WAVE Bioreactor™ systems for use in clarification of cell culture feed. Used together with the ÄKTA ready chromatography system, ÄKTA readyflux can be used for concentration and buffer exchange in downstream applications (1).

The use of ReadyMate™ disposable aseptic connections, presanitized columns, disposable chromatography system flow path together with the filtration assemblies contributes to ensuring maximum product safety and to maintain high process hygiene. The major advantage comes in terms of a small foot print, flexible usage of facility (multi-products), and saving time.

Conclusion

This application note describes an UF/DF process performed in an automated manner using the ÄKTA readyflux filtration system. Easy method creation and flexible automation methods require no programming expertise and free up time for other activities. Methods were easily created in the UNICORN software by dragging and dropping predefined phases from a phase library. Using the described process, BSA was concentrated and buffer exchanged with a recovery of more than 99%.

Reference

1. Application note: A flexible antibody purification process based on ReadyToProcess products. GE Healthcare, 28940348, Edition AC (2012).

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