Connected polishing and concentration under one automation method

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Chromatography and tangential flow filtration (TFF) steps are traditionally performed as separate unit operations in downstream bioprocessing. This work aims to demonstrate the capability of the UNICORN™ system control software to integrate these unit operations to eliminate intermediate hold steps thereby releasing resources and reducing overall process time. Here, the ÄKTA™ ready chromatography system was directly connected to the ÄKTA readyflux filtration system, for polishing and concentration of a target molecule. One automation method was created for control of both systems. Single-use process components were used to add flexibility to the process.

Introduction

For biomanufacturing operations, production processes are typically performed in a series of separate unit operations. Included equipment is often controlled at an instrument-level. Such a setup demands intermediate handling of the bioprocess material, with manual product transfer between steps. Manual handling not only adds process time, but also increases the risk of operator errors.

Integration of the different unit operations streamlines the manufacturing process and reduces manual interaction between process steps. Here, we describe integration of a polishing step with the subsequent concentration step by direct connection of the ÄKTA ready chromatography system to the ÄKTA readyflux filtration system.

ÄKTA ready is a liquid chromatography system built for process scale-up and manufacturing. ÄKTA readyflux is a TFF system intended for both microfiltration and ultrafiltration applications in both upstream and downstream processes, ranging from pilot to small manufacturing scales. For flexibility and ease-of-use, ÄKTA ready and ÄKTA readyflux operate with single-use flow paths, and both systems are controlled through the UNICORN software, eliminating the need for a separate automation software. To suit a broad range of applications, ÄKTA readyflux can be used with both hollow fiber filter cartridges and filter cassettes.

For systems controlled by the UNICORN software, connected processing can be facilitated by the ***Method Queue*** function and ***Ready*** instruction. Here, the polishing and concentration process was based on individual methods executed using the ***Method Queue*** function. The ***Method Queue*** defines which systems are included as well as the order of individual methods and their initiator: at a set time point immediately after the preceding method or at the ***Ready*** instruction. Once a set stage in an individual method has been executed on one system, the ***Ready*** instruction initiates start of another individual method on another system.

Materials and methods

Model process

A process for polishing and concentration of bovine serum albumin (BSA) was used as model. For the polishing step, a ReadyToProcess™ DEAE Sepharose™ FF 1 L column was connected to the ÄKTA ready system. The column was operated in flow-through mode, where the flowthrough and wash fractions were directly transferred to ÄKTA readyflux. For the concentration step, ÄKTA readyflux was equipped with a hollow fiber filter size 6 with a M_r 10 000 nominal molecular weight cut-off (NMWC). In this step, BSA was five times concentrated and seven-fold diafiltered before final concentration and recovery.

System setup

ÄKTA ready and ÄKTA readyflux were connected to one computer each, both installed with UNICORN 7.1 software with instrument configurations for both systems. In a setup with two integrated systems in a method queue, one of the system computers needs to be set as the “controlling” computer (via ***Connect to system***), and the other should be set as “view only” to enable execution of the methods. Here, the ÄKTA ready system computer was set as the “controlling”, whereas the system computer of ÄKTA readyflux was set as “view only”.

A database is required to be installed on one of the system computers or on an external server. Here, the database was

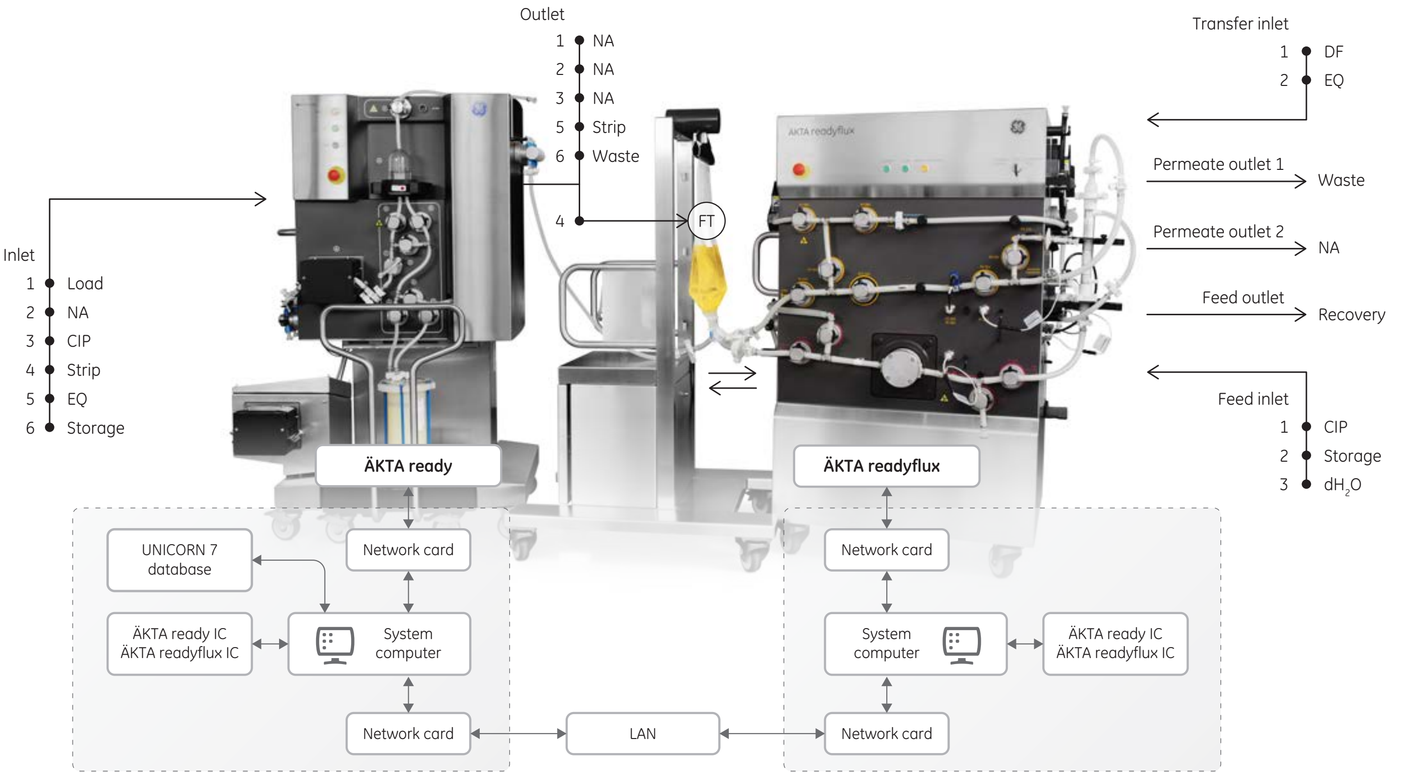

Fig 1. Setup of hardware and software for ÄKTA ready and ÄKTA readyflux. EQ = equilibration, FT = flowthrough, DF = diafiltration, IC = instrument configuration, LAN = local area network, NA = not applicable.

installed on the ÄKTA ready computer and the two systems were interlinked over a local-area network (LAN). The two computers were equipped with two network cards each, one for communication between the system and its computer and one for communication between the system and the database.

A schematic description over the setup is given in Figure 1. For transfer of the process material, process steps were interconnected via tubing from the ÄKTA ready outlet to the ÄKTA readyflux recirculation bag.

Creation of method queue

For polishing using ÄKTA ready, individual methods were created for column cleaning-in-place (CIP), equilibration, load, strip, and storage. For concentration using ÄKTA readyflux, individual methods were created for CIP, equilibration, batch concentration, diafiltration, final concentration, recovery, and storage using predefined methods in the ***Phase Editor***. The methods were executed using the ***Method Queue*** function of the UNICORN software (Fig 2).

The ***Ready*** instruction was used as initiator and included in two individual methods on ÄKTA ready: in the beginning of the CIP method to initiate CIP of ÄKTA readyflux and in the beginning of the strip method to initiate batch concentration on ÄKTA readyflux. ***Immediately after the preceding method*** was used as start initiator for all other individual methods. To avoid interruption in the execution of the individual methods, it is essential to define in the start protocol for each individual method where and how the created data is saved, and the start questions should be disabled.

It is also of importance that the system receiving the ***Ready*** instruction is in a passive mode to assure a secure handover of the instruction. The instruction is sent once to the "receiver" and there is no receipt to the "sender" if the instruction was received. The ***Ready*** instruction can be used in both directions: "controlling" to or from "view only". Here, the ***Ready*** instruction was programmed to be sent from the "controlling" computer (ÄKTA ready) to be received by the "view only" computer (ÄKTA readyflux).

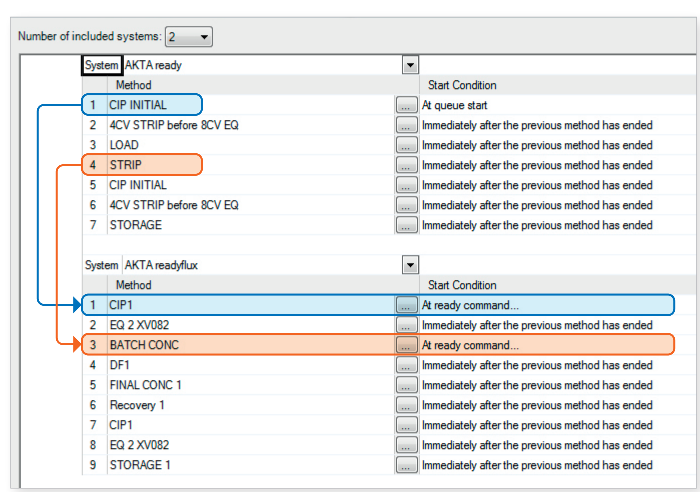

Fig 2. Screenshot showing the ***Method Queue*** function of the UNICORN software, listing methods to control the two systems: ÄKTA ready and ÄKTA readyflux. Subsequent methods are initiated immediately after the preceding method or by the ***Ready*** instructions.

For programming of the systems, parameter time was used as base, as it is recommended to use the same base for all methods included in the method queue. For ÄKTA ready, all methods were initially designed for a flow rate of 8 L/h. CIP was designed to have a contact time of 60 min and the equilibration step was designed to run for 30 min. The load and wash steps were merged into one method, denoted Load. The strip and storage methods were designed to both run for 22.5 min.

For ÄKTA readyflux, all methods were designed for a transmembrane pressure (TMP) of 1.5 bar and a feed flow rate of 2 L/min. CIP and rinse were merged into one method (CIP) designed with an intermediate pause for 30 min between CIP and rinse to reach a 60 min total contact time for cleaning. Equilibration was designed to add 6 L of equilibration buffer to recirculation bag and flush this through the filter. Batch concentration was designed to read the start volume of the bag and concentrate this volume 5 times. The diafiltration step was designed to read the bag volume at start and retain this volume by adding diafiltration buffer until the permeate conductivity was > 17 mS/cm or until > 7 diafiltration volumes were achieved. Final concentration was designed to concentrate the recirculation bag volume to < 2 L. Recovery was designed to recirculate the concentrate for 1 min, before emptying the system via the recovery valve until bag weight was < 0.2 L, after which 2 L of diafiltration buffer was added to the system and recirculated for 3 min before emptying the system again via the recovery valve until recirculation bag weight was < 0.2 L. Recovery was designed to be followed by recovery by air blow down for 2 min. Storage method was designed to add storage solution until 2 L permeate was collected.

When using predefined method phases for programming, it should be noted that the ***End Block*** instruction after the ***Watch*** functions are by default set to 9999 min. To avoid overconcentration, the default setting should be changed from 9999 min to an applicable time interval, where 0 min is directly after occurred watch condition.

Sample analysis

Sampling was performed at the load, intermediate, and final product steps of the polishing process as well as from the permeate at the concentration and diafiltration/final concentration steps of the filtration process to calculate the recovery over the individual process steps. Protein content was analyzed spectrophotometrically at A_{280}.

Results

In general, individual methods are developed in laboratory scale, after which the overall process is tested in cycles of iterations. Once the individual methods as well as the overall process is established, the process is transferred to larger scale, at which the process is again tested in iterated cycles. This is repeated for each increase in scale. The aim of this work was to demonstrate the ***Method Queue*** function and the ***Ready*** instruction of the UNICORN software when integrating two separate process steps. Hence, the model process used was not optimized or subjected to scale-up activities. Graphs showing execution of the individual methods are presented in Figure 3.

As the process was not fully optimized, some unexpected events occurred during the run. At the start of initial CIP, for example, the column was filled with storage solution (20% ethanol), with a different viscosity than the CIP solution (0.5 M NaOH). Therefore, the column delta-pressure (Δp) alarm (SP 1.2 bar) was activated by the software and the system was set in "pause". In the method, the flow rate needs to be lower for the part where the storage solution (20% ethanol) is flushed out of the column to avoid overpressure when working with high-viscosity fluids. The user was alerted by

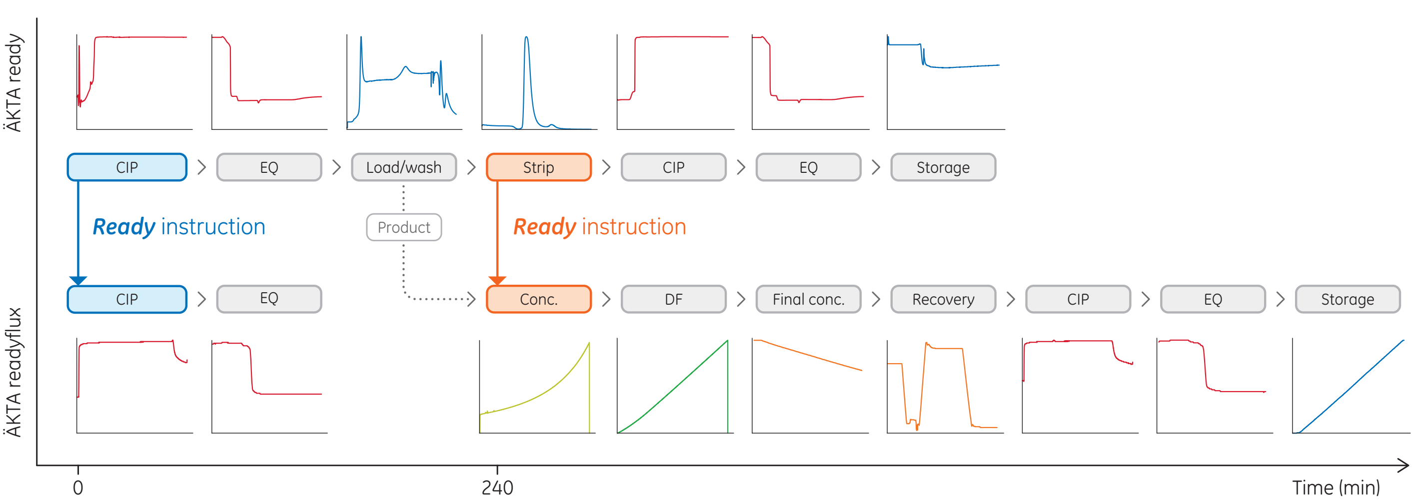

Fig 3. Execution of individual methods on ÄKTA ready and ÄKTA readyflux using the ***Method queue*** function and the ***Ready*** instruction of the UNICORN software.

the alarm, enabling manual setting of the flow to 6 L/h to flush out the storage solution, and thereafter to hold. After the solution was flushed out from the column, the flow was set back to 8 L/h and the method could be executed as defined.

In the design of a chromatography process, it is recommended to study the pressure and flow characteristics for the fluid and column combinations as an iteration step. The influence of the difference in buffer viscosities will not be observed when testing the methods with water. Although process interruption and manual interaction during initial CIP on ÄKTA ready, the concentration and diafiltration steps on ÄKTA readyflux were executed as intended.

At the start of column wash, the system was paused due to flow deviation created by air entering the flow path from the load inlet when the system switched from load to wash. The bubble trap was set inline and the air in the flow was switched to the bubble trap manually, after which the bubble trap was set offline again and the wash method was allowed to continue.

Introduction of air into the flow path can be avoided by addition of an air sensor placed, for example, on a three-foot-long ReadyCircuit™ Jumper (12410118) connected to the tubing between the single-use buffer bag and the inlet of ÄKTA ready. Also, the single-use flow path needs to be align with the automation method. In our example, the inlet tubing length needs to correspond to the flow rates used.

To avoid situations where the buffer runs out, an air sensor can be connected to the feed inlet. For full control over the buffer consumption, methods should be designed to primarily be controlled by time or feed volume.

These UNICORN software alerts demonstrate how the user is notified in the case of any unexpected events that need to be addressed. Actions taken did not affect the proceeding of the automation method.

Conclusion

Here, we demonstrate integration of the single-use ÄKTA ready chromatography system and ÄKTA readyflux filtration system. Method creation was straightforward, using method phases predefined in the software. The polishing step and the subsequent concentration step were successfully executed in a consecutive manner using the ***Method Queue*** function and the ***Ready*** instruction. Alarm functions alerted if anything needed operator attention.

By integrating unit operation, resources can be released and overall process time reduced. Direct connection of unit operations minimized intermediate hold steps that requires manual interaction and enables operator to control several instruments from one computer. As both systems used in this work are controlled through the same software, the integrated process steps could be executed without the requirement of any additional automation software. The single-use flow paths omitted the need for costly and time-consuming cleaning and cleaning validation operations. Bringing flexibility and speed to operations, the described solution is especially beneficial for manufacturers operating in smaller scales, with many parallel productions.

Related literature

Product	Product code
Data file: ÄKTA ready chromatography system	28915986
Data file: ÄKTA readyflux filtration system	29214956
Data file: UNICORN 7 system control software	29135786

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