Scale-up and process economy calculations of a dAb purification process using ready-to-use products

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Purification of a domain antibody (dAb) in a chromatography capture step was successfully scaled from laboratory to pilot scale using ready-to-use equipment and ready-made buffers. The scaled-up process shows reproducible results over triplicate runs, indicating process robustness. Results from the scaled-up process were comparable with the process run at laboratory scale.

In addition, process economy of using ready-to-use equipment and ready-made buffers versus using reusable equipment and in-house prepared buffers was compared. A three-step dAb purification process was used as model. The results show that although operating expenses can be more extensive when scale and number of batches increase, ready-to-use equipment and ready-made buffers offer great time-savings. As less time is spent on equipment qualification and maintenance with these products, annual process time can be reduced by 3000 work hours, corresponding to two full-time equivalents (FTEs) per year by using ready-to-use equipment and ready-made buffers.

Introduction

With constant demands for higher productivity, biomanufacturers seek to develop more efficient processes for producing new drugs at lower cost in ever-shrinking time frames. Although processes based on ready-to-use equipment, disposables, and ready-made buffers are sometimes perceived as costly, such products can help simplify operations. Buffer manufacturing, for example, is one of the more resource-intense activities that can rapidly become a bottleneck in the overall workflow. Ready-made buffers can help free up time and contribute to better resource utilization. With products delivered ready for use, less time is spent on preparation and equipment qualification prior to startup as well as annual maintenance and requalification of the equipment. Consequently, great time-savings can be made with such products, contributing to that more batches can be produced per year than what is possible in facilities using stainless steel equipment. With a higher annual batch throughput comes an increased profit opportunity.

Fig 1. Setup with ready-to-use equipment.

ReadyToProcess™ columns are validated high-performance bioprocessing columns that are supplied prepacked, presanitized, and ready for use to save time in preparation for chromatography unit operations. The columns are available with a range of BioProcess™ resins in different sizes. To help release capacity constraints in buffer preparation, HyClone™ buffers delivered ready-made in easy-connectable single-use containers can be used. HyClone buffers are produced according to GMP guidelines from consistent raw materials for high lot-to-lot equivalence between batches.

Here, we demonstrate the feasibility of using a ReadyToProcess Capto™ L column, operated though the ÄKTA™ ready chromatography system, and HyClone buffers in a pilot-scale dAb capture step. ReadyCircuit™ assemblies were used for connectivity. Process performance was compared with that of a laboratory-scale process.

In a process economy simulation, the use of ready-to-use products was compared with the use reusable products in a three-step dAb purification process. In this simulation, ready-to use products comprise ReadyToProcess columns, operated through the ÄKTA ready chromatography system, ReadyCircuit assemblies, and HyClone buffers (Fig 1). Reusable products comprise user-packed, stainless steel columns, operated through the ÄKTAprocess™ chromatography system, and conventional, in-house prepared buffers. Scales of 100 L and 2000 L starting feed volumes were compared.

Materials and methods

Sample preparation

The dAb used in this study was expressed in *E.coli* and heat-released into the supernatant. The dAb-containing supernatant was clarified using ULTA™ Pure HC 0.2 um filter capsule prior to the capture step.

Capture step

For the laboratory-scale dAb capture step, a HiScale™ 16/20 column was packed to a 20 cm bed height with Capto L resin. The column was operated on an ÄKTA avant chromatography system installed with UNICORN™ system control software. As starting material, a filtered dAb-containing sample was used. The process was run in duplicate.

For pilot-scale dAb capture, a 2.5 L ReadyToProcess Capto L column was used. The column was operated on an ÄKTA ready chromatography system installed with UNICORN software. ReadyCircuit bags and tubing assemblies were used to create a closed system. A filtered dAb-containing sample was loaded onto the column. The process was run in triplicate using custom made HyClone buffers delivered in 10 to 200 L single-use containers that could be directly connected to the equipment. Process conditions are listed in Table 1.

Analytical methods

The dAb concentration was measured using a 1 mL HiTrap™ Capto L column. The dAb was adsorbed to the resin, washed, and eluted with 50 mM phosphate, pH 2.5. The elution peak was integrated and dAb concentration was determined using standards of known dAb concentrations.

Host cell protein (HCP) was measured with an ELISA method using Gyrolab™ Workstation LIF (Gyros AB) and antibodies from Cygnus Technologies.

Endotoxin concentration was measured with a chromogenic kinetic limulus method.

Purity was determined in 50 µL samples by size exclusion chromatography (SEC) using a Superdex™ 75 Increase 10/300 GL column. As mobile phase, 200 mM phosphate, pH 7.0 was used at a flow rate of 0.8 mL/min.

Process economy simulation

Included process equipment is listed in Table 2.

Table 1. Process conditions for dAb capture on Capto L resin

Step	Volume	Buffers and process liquids	Flow velocity	Comment
Equilibration	1 column volume (CV)	20 mM Na-citrate + 800 mM NaCl, pH 5.0	500 cm/h	
Sample load	12 g/L resin	N/A	300 cm/h	4 min residence time
Wash 1	5 CV	20 mM Na-citrate + 800 mM NaCl, pH 5.0	300 cm/h	
Wash 2 (small scale)	1 CV	20 mM sodium citrate, pH 5.0	300 cm/h	Wash without salt for salt-free elution
Wash 2 (large scale)	2 CV*			
Elution	5 CV	20 mM sodium citrate, pH 2.8	300 cm/h	
Wash 3 (small scale)	1 CV	20 mM sodium citrate + 800 mM NaCl, pH 5.0	300 cm/h	Wash to avoid direct contact between low-pH elution buffer and high-pH cleaning-in-place (CIP) solution.
Wash 3 (large scale)	2 CV*			
CIP	2 CV	15 mM NaOH	250 cm/h	
Re-equilibration	5 CV	20 mM sodium citrate + 800 mM NaCl pH 5.0	500 cm/h	

CIP = cleaning in place

* Extra column wash was performed in pilot scale to compensate for extra hold-up volume in air trap.

Table 2. Equipment included in process economy simulation

	Stainless steel equipment		Ready-to-use equipment	
	100 L	2000 L	100 L	2000 L
Chromatography system	1 × ÄKTAprocess	3 × ÄKTAprocess	1 × ÄKTA ready	3 × ÄKTA ready
Chromatography columns	1 × AxiChrom™ 70 mm 2 × AxiChrom 140 mm	1 × AxiChrom 300 mm 2 × AxiChrom 400 mm	1 × ReadyToProcess 1 L column 2 × ReadyToProcess 2.5 L column	3 × ReadyToProcess 20 L column
Buffer tanks	1 × 50 L buffer tank 1 × 200 L buffer tank	1 × 50 L buffer tank 1 × 200 L buffer tank 2 × 500 L buffer tanks	HyClone buffers in single-use bags	HyClone buffers in single-use bags

Cost categories

Costs included in the comparison:

- Hardware and facility for one (100 L) or three (2000 L) downstream and buffer utilities
- Qualifications and cleaning validation for hardware
- Annual maintenance and requalification
- Cost for production
- Cost for disposables, chemicals, resins, buffers, and similar

Costs not included in the comparison:

- Minor hardware (scales, tube welders, pumps, and similar)
- Minor connectors
- Cost for electricity
- Minor disposables (syringes, vials, and similar)
- Amortization for facility and hardware
- Supply-chain management, including activities such as buffer raw material characterization

Assumptions

The comparison includes:

- Three chromatography steps based on process modified from method developed in laboratory scale (1):
  - Capto L initial capture
  - Capto adhere ImpRes intermediate purification
  - Capto MMC ImpRes final polishing
- Buffer preparation

General assumptions:

- The cost of labor is set to 150 USD/h and one FTE equals ~ 1600 h/year (BioSolve™ Enterprise V5.0 process economic simulation tool from BioPharm Services).
- Costs for hardware are based on both GE Healthcare's list prices and BioSolve Enterprise V5.0.
- Cost for facility is based on estimated required foot print and price for foot prints from BioSolve Enterprise V5.0.
- Costs for qualification, validation, annual maintenance, and production are based on estimated number of work hours for each unit operation. The time estimations are based on in-house experience as well as from input from external sources.
- Cost of disposables and raw materials are based on GE Healthcare's list prices, BioSolve Enterprise V5.0, and external sources (e.g., official websites).
- Maximum number of batches/year: 40.
- One ÄKTA ready flow kit required per chromatography step and batch.
- Each ReadyToProcess column is used for a maximum of 10 chromatography cycles, bulk resin changed after 40 batches.
- Target: production for preclinical or clinical phase.

Results

Capture step

Figure 2 depicts overlay chromatograms from triplicate runs on 2.5 L ReadyToProcess Capto L columns using HyClone buffers. The nearly identical results indicate process robustness and reproducibility. The dAb purity in the eluate was analyzed by SEC (Fig 3). No aggregates or impurities could be observed.

Figure 4 compares the results from the pilot-scale process with results from the process run in laboratory scale. For both column sizes, a resin bed height of 20 cm was used. The results were comparable between the scales. HCP and endotoxin removal in the pilot-scale process was comparable with corresponding process performance attributes of the process run in laboratory scale (Table 3). The yield over the Capto L step was about 90% for both processes.

Column:	2.5 L ReadyToProcess Capto L columns
Equilibration:	20 mM Na-citrate + 800 mM NaCl, pH 5.0
Sample:	77 L clarified dAb containing cell culture supernatant
Sample load:	12 g/L resin
Wash 1	20 mM Na-citrate + 800 mM NaCl, pH 5.0
Wash 2:	20 mM sodium citrate, pH 5.0
Elution:	20 mM sodium citrate, pH 2.8
System:	ÄKTA ready

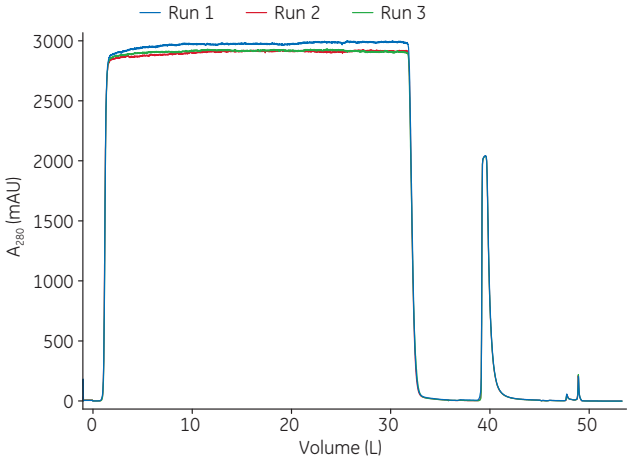

Fig 2. Overlay of chromatograms from triplicate dAb capture runs in 2.5 L ReadyToProcess Capto L columns.

Column:	Superdex 75 Increase 10/300 GL
Sample:	50 µL dAb containing fraction from capture step
Mobile phase:	200 mM phosphate, pH 7.0
Flow rate:	0.8 mL/min

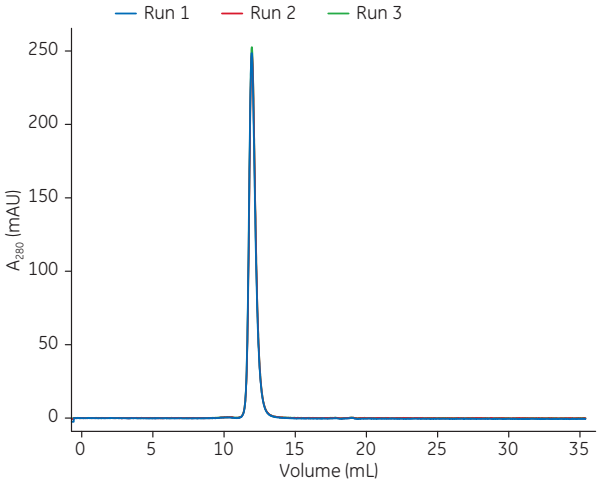

Fig 3. dAb purity determined in Capto L eluate by SEC.

Columns:	2.5 L ReadyToProcess Capto L columns 40 mL HiScale 16/20, bed height 20 cm
Equilibration:	20 mM Na-citrate + 800 mM NaCl, pH 5.0
Sample:	77 L clarified dAb containing cell culture supernatant (ReadyToProcess column) 1.25 L clarified dAb containing cell culture supernatant (HiScale column)
Sample load:	12 g/L resin
Wash 1:	20 mM Na-citrate + 800 mM NaCl, pH 5.0
Wash 2:	20 mM sodium citrate, pH 5.0
Elution:	20 mM sodium citrate, pH 2.8
Systems:	ÄKTA ready (ReadyToProcess column) ÄKTA avant (HiScale column)

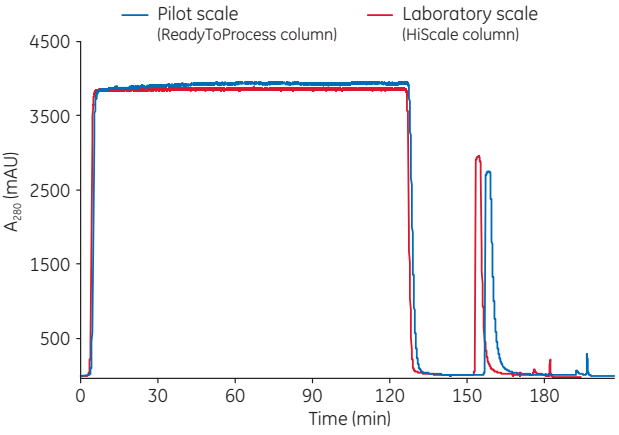

Fig 4. Comparison of dAb capture in pilot scale with the capture step performed on in laboratory scale. Curves are normalized against the UV280 signal for the laboratory-scale column. Compared with the chromatogram from the laboratory-scale process, the chromatogram from the pilot-scale process is slightly delayed due to the extended column wash phase that was implemented in pilot scale to compensate for hold-up volume in the air trap.

Table 3. Summary of dAb yield and purity for Capto L chromatography steps

Sample	dAb recovery (%)	HCP (ppm)	Endotoxin (EU/mg dAb)
Feed	100	300 000*	66 979
HiScale column, eluate†	> 93	151	Not analyzed
ReadyToProcess column, eluate‡	> 90	172†	2.5

*Approximate levels

†Average of duplicate runs

‡Average of triplicate runs

Process economy simulation

The process economy simulation assumes a single-product facility producing 40 batches of dAb per year, using either ready-to-use products or reusable products. Ready-to-use products comprise ReadyToProcess columns, operated though the ÄKTA ready system, ReadyCircuit assemblies, and HyClone buffers (ready-to-use process). Reusable products include user-packed stainless steel columns, operated through the ÄKTAprocess system, and buffers prepared in-house in stainless steel tanks (stainless steel process). As can be expected, column preparation for the stainless steel process is more time-consuming than for the ready-to-use process (Fig 5), as stainless steel columns need to be packed and subjected to cleaning and cleaning validation as well as performance testing before use.

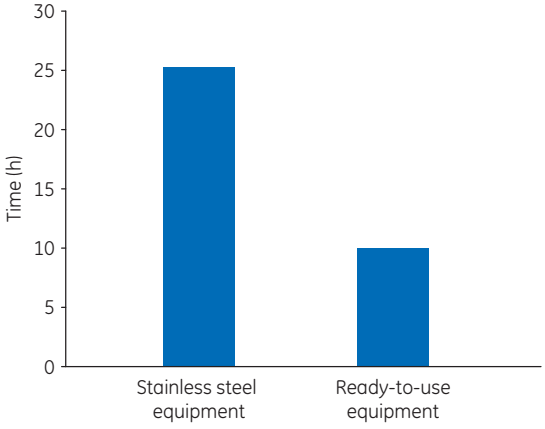

Fig 5. Column preparation time for a 100 L process, comparing stainless steel equipment and ready-to-use equipment.

Before manufacturing can start, all hardware must undergo installation, operational, and performance qualification (IQ/OQ/PQ), including cleaning validation. As shown in Figure 6, time spent on IQ/OQ/PQ for the stainless steel process is 1440 h, whereas only 100 h for the ready-to-use process. The difference in time spent on qualification work between the stainless steel and the ready-to-use processes can mainly be attributed to qualification of the stainless steel tanks used for buffer preparation. While requiring 12 weeks for the stainless steel process, qualification work only requires 1 week for the ready-to-use process. Qualifications of the ready-to-use equipment require about 0.84 FTE/year less than the stainless steel process.

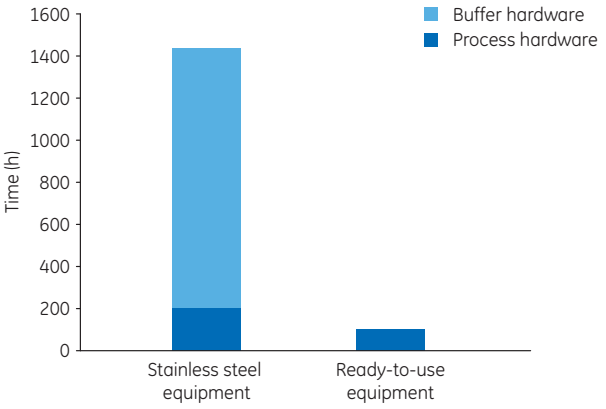

Fig 6. Equipment qualification time for a 100 L process, comparing stainless steel equipment and ready-to-use equipment.

As shown in Figure 7, time for annual equipment maintenance and requalification is also higher for the stainless steel process. Also here, the difference can be attributed to the stainless steel buffer tanks, which are not needed for the ready-to-use process that uses HyClone buffers. Compared with the stainless steel process, the ready-to-use process requires 80% less work hours for annual maintenance, corresponding to 0.2 FTEs/year.

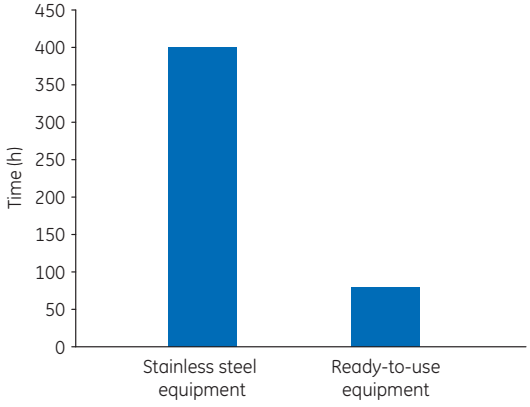

Fig 7. Annual maintenance time for a 100 L process, comparing stainless steel equipment and ready-to-use equipment.

Table 4. Summary of process economy simulation

	Stainless steel equipment		Ready-to-use equipment	
	100 L	2000 L	100 L	2000 L
IQ/OQ/PQ (h)	1440	2340	100	200
Annual equipment maintenance and requalification (h)	400	640	80	80
Manufacturing (h/batch)	208	270	132	144

The difference in manufacturing time per batch shown in Figure 8 is associated with the work hours per batch, which is 208 h/batch for the stainless steel process versus 132 h/batch for the ready-to-use process. Besides the actual chromatography process, the manufacturing working hours per batch also include preparation of equipment, including column cleaning, buffer preparation, and autoclaving of tubing and connectors when required. As the ready-to-use process includes equipment and buffers delivered ready for use, startup is quicker for this process.

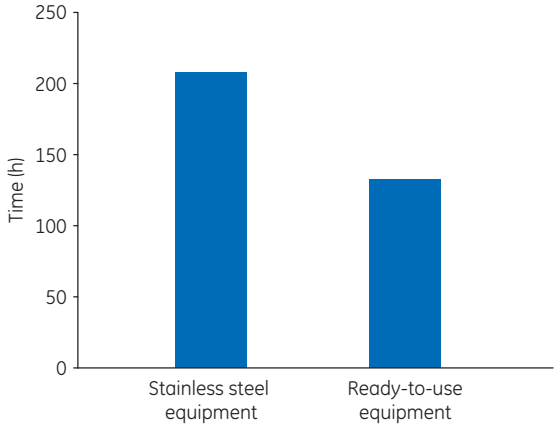

Fig 8. Production working hours for a 100 L process, comparing stainless steel equipment and ready-to-use equipment.

As shown in Figure 9, the total cost for consumables (single-use tubing and bag assemblies, buffers, etc.) is higher for the ready-to-use process. On the other hand, 37% less time is required for the dAb process using ready-to-use products. Assuming 40 annual batches, the process time can be reduced with 3000 work hours, corresponding to almost two FTEs, per year using these products.

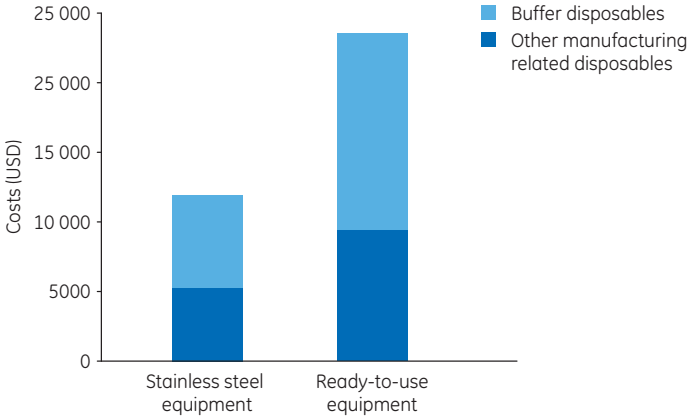

Fig 9. Consumables cost for a 100 L process, comparing stainless steel equipment and ready-to-use equipment.

Results shown for the 100 L process are even more prominent for the 2000 L process, requiring additional buffer tanks and associated equipment for buffer preparation. At 2000 L scale, however, the buffer cost increases significantly for the ready-to-use process due to the increased number of chromatography cycles required per batch using the listed equipment. The results from the process economy simulation are summarized in Table 4.

Conclusion

In this work, we have demonstrated a successful scale-up of a dAb capture step to pilot scale using ready-to-use equipment and ready-made buffers, with results comparable to those from the process run in laboratory scale. Similar results from triplicate runs indicate robust performance of the scaled-up process.

To further explore the usability of products delivered ready for use, the time required for a dAb purification process, run either with user-packed, stainless steel columns and in-house prepared buffers or with ready-to-use equipment and ready-made buffers, was assessed in a process economy simulation. At 100 L scale, the results show that time-savings of as much as 3000 work hours, corresponding to almost two FTEs, can be made per year with ready-to-use products. The time-savings can mainly be attributed to the eliminated need for buffer preparation and associated qualification and maintenance of required equipment. At 2000 L scale, however, more process cycles are required per batch for prepacked ReadyToProcess columns, increasing the buffer cost. However, ready-made buffers can be an attractive choice, especially under conditions of fast growth. Just-in-time HyClone buffers can help moderate the financial risk of large capital expenditures under periods of capacity constraints in buffer production.

As the difference in qualification and maintenance time was shown to be less prominent between chromatography columns and systems used for the processes, the results indicate that user-packed, stainless steel columns can be a cost-efficient option for processes including HyClone buffers under the conditions described here. This observation can have significant impact at larger scales, where stainless steel columns are more commonly used.

However, in multiproduct facilities, producing smaller batches, ReadyToProcess columns offer advantages associated with the reduced need for column preparation and qualification, enabling quick start-up and less need for cleaning and cleaning validation, for quick changeover between productions. These features allow for faster time to market and for more annual productions to be made, generating a greater profit opportunity. In addition, ready-to-use equipment and ready-made buffers offer the benefit of reduced cross-contamination risk.

Disclaimer

The results from the process economy simulation and conclusions presented in this application note are valid for this specific study. Other study conditions and assumptions could have significant impact on the outcome. The overall finding in this study is that ready-to-use equipment and ready-made buffers enable quick startup and changeover between productions, contributing to that more batches can be produced per year compared with using reusable equipment and in-house prepared buffers. Combined with the use of ready-made buffers, however, stainless steel columns can be a cost-efficient alternative for larger scales.

Reference

1. Application note: A platform approach for the purification of domain antibodies (Dabs). GE Healthcare, 29065541, Edition AB (2014).

Ordering information

Product	Description	Product code
ReadyToProcess Capto L column	2.5 L	29015989
HiScale 16/20 column	40 mL	28964441
Capto L resin	1 L	17547803
ULTA Pure HC filter capsule	0.2 µm	KMP-HC9204TT
HiTrap Capto L column	1 mL	29048665
Superdex 75 Increase 10/300 GL	24 mL bed volume	29148721

To order HyClone buffers, please contact your local sales representative.

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