Integration of continuous upstream and downstream operations in mAb production

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Integration of continuous upstream and downstream operations in mAb production

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Introduction

Process intensification is gaining interest as a strategy to reduce production costs, while improving product quality and throughput in the manufacturing of biopharmaceuticals. For a competitive production process, continuous or semi-continuous upstream and downstream processing can be employed. Compared with a process performed in batch runs, continuous processing allows for increased capacity utilization and eliminates or minimizes the need for intermediate hold-up steps. We describe the integration of a high-performing upstream cell culture process with downstream purification utilizing emerging technologies such as periodic counter-current (PCC) chromatography and straight-through processing (STP).

In this case study, we demonstrate the feasibility of integrated upstream and downstream mAb processing performed in a continuous manner. The developed process shows a performance equivalent to traditional processing performed in batch runs.

Results

Upstream: perfusion medium development

A high-performing cell culture medium supporting a low cell-specific pefusion rate (CSPR) is the key to developing a sustainable perfusion process. To develop a high-performing perfusion medium, we studied combinations in an existing medium platform, consisting of medium and feeds (Fig 1).

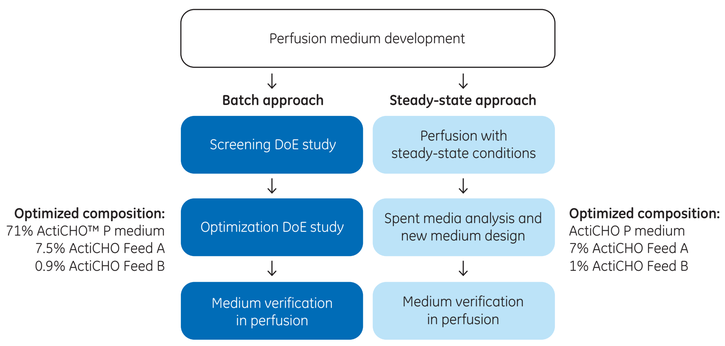

Fig 1. Study design for developing a high-performing perfusion medium from an existing fed-batch platform.

High-performing perfusion media can be developed from an existing medium platform, the perfusion rate can be used as a tuning fork for product quality (Fig 2 and Table 1).

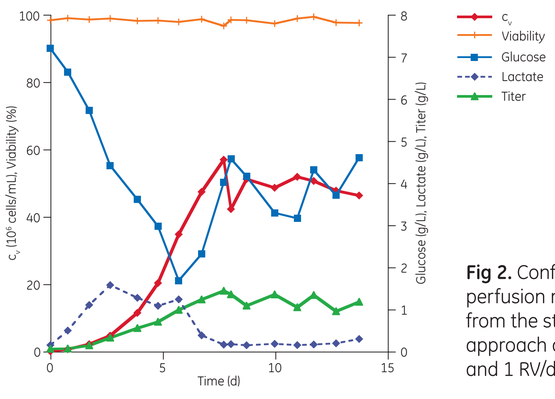

Fig 2. Confirmation of perfusion media formulation from the steady-state approach at 50 MVC/mL and 1 RV/d (= CSPR 20).

Table 1. Quality attributes impacted by the perfusion rate (quality attributes not impacted were: glycosylation profile and TNF-α binding)

Analytical technology	Analyte	Fed-batch	20 pL/c/d	43 pL/c/d	77 pL/c/d	90 pL/c/d
CIEX	Acidic variants	> 60%	25%	23%	19%	12%
	Alkaline variants	3%	2%	4%	4%	4%
SEC	Aggregate	1%	0.4%	0.3%	0.2%	0.5%

Downstream: capture by 3C PCC

The capture step was performed running on a 3C PCC setup using HiScreen™ columns prepacked with MabSelect SuRe™ chromatography resin. The principle is shown in Figure 3. The performance and robustness was evaluated, running 10 cycles, with respect to purity and yield. Loading was controlled by dynamic control, which enables system operation under process conditions where either feed concentration or chromatography medium capacity varies (Fig 4).

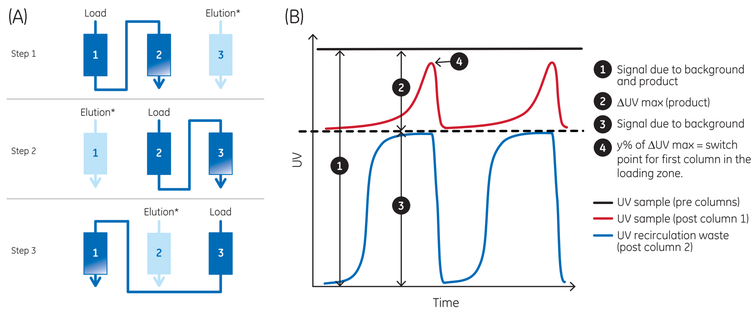

Fig 3. (A) Working principle of 3C-PCC; (B) schematic view of UV curves during loading.

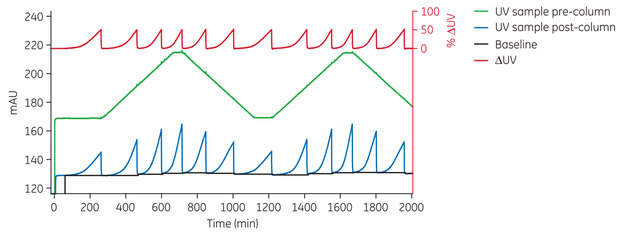

Fig 4. Demonstration of the dynamic control. The dynamic control was challenged by performing a feed gradient run from 1 to 2 g/L IgG. The breakthrough curves follow the gradient change closely, the amount of product eluted from each column remained constant (data not shown).

Robust performance and minimal variation in amount mAb, aggregate, and HCP between columns was determined (Fig 5). Results were comparable to results from batch protein A capture. A 56% increase in capacity compared with batch mode was observed (67 g/L, compared with 43 g/L, loaded onto each column).

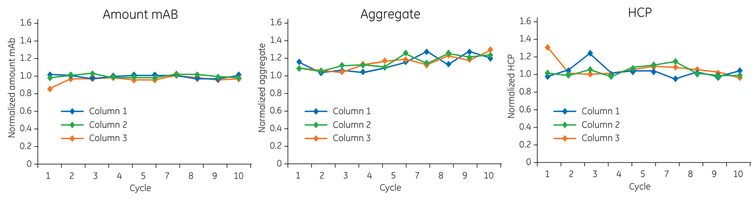

Fig 5. The elution pool from each individual column and cycle was analyzed with respect to the amount mAb, aggregate content, and host cell protein. Results from each column in the graphs are presented according to column number.

Downstream: polishing by STP

A polishing STP step was developed including the following steps:

- Individual studies on the different chromatography resins
- Conditioning study based on results from studies on Capto™ S ImpAct and Capto adhere resins
- Verification runs with Capto S ImpAct and Capto adhere connected in series with in-line conditioning between the steps

Figure 6 illustrates the set-up used. Table 2 shows the quality indicating analytitical data.

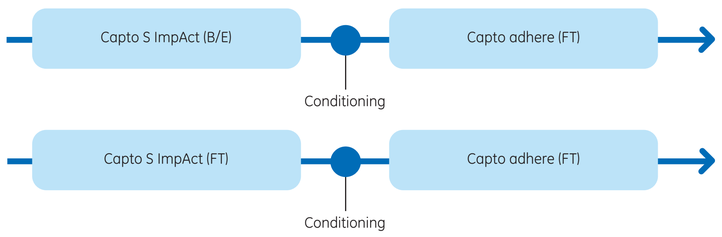

Fig 6. Process options investigated during verification runs.

Table 2. Results from the STP step: B/E-FT and FT-FT process alternatives. The starting material contained 2% aggregates, 1277 ppm HCP, and 3 ppm leached ligand.

Process step	Yield over two steps (%)	Aggregates (%)	HCP (ppm)	Leached ligand (ppm)
Capto S ImpAct (B/E)-Capto adhere (FT)	89	0.8	16	< 1
Capto S ImpAct (B/E)-Capto adhere (FT)	90	0.7	18	< 1
Capto S ImpAct (FT)-Capto adhere (FT)	88	0.7	40	< 1

Yield as well as amount aggregate, HCP, and leached ligand were comparable with results from batch runs.

Abbreviations

3C PCC	three-column periodic counter-current chromatography
B/E	bind-and-elute mode
CIEX	cation exchange chromatography
CSPR	cell-specific pefusion rate
DoE	design of experiments
FT	flow-through mode
HCP	host cell protein
PAT	process analytical technology
PCC	periodic counter-current chromatography
RV	reactor volume
SEC	size exclusion chromatography (gel filtration)
STP	straight-through processing

Conclusions

- The cell culture medium is critical for the development of high-performing perfusion processes.
- High-performing perfusion media can be developed from existing fed-batch platforms, allowing CSPR's of 20 pL/c/d.
- The perfusion rate can be used as a tuning fork for product quality.
- Continuous protein A capture increased productivity compared with a batch process and delivered robust performance.
- The dynamic control is an example of feed-back control and integrated PAT.
- An STP step with two columns connected with in-line adjustment in-between showed similar yield and purity as batch runs.

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