Horizon 2020 – nextBioPharmDSP
1000 L-scale implementation of fully connected, single-use, advanced DSP platform for mAb production

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Introduction

Within the biopharmaceutical industry, there is a significant shift toward higher productivity processes resulting in improved economics without compromising robustness. Therefore, integrated continuous production technologies are of greatest interest.

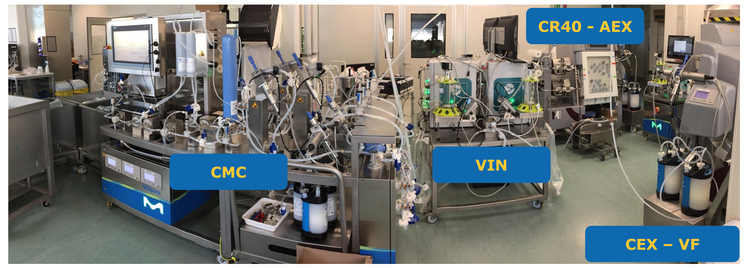
From 2016 to 2020 a collaborative 10.6-million-euro project funded by the European Union's Horizon 2020 program under Grant agreement No. 635557 was conducted jointly by the following partners: Lek Pharmaceuticals d.d. (coordinator), Sandoz GmbH (Austria), Millipore SAS (France), University of Natural Resources and Life Sciences (Austria), Karlsruhe Institute of Technology (Germany), National Institute of Chemistry (Slovenia) and National Systems srl (Italy).

The main objective of this "Next-generation biopharmaceutical downstream process" ("nextBioPharmDSP") project was the optimization and implementation of a fully integrated manufacturing platform for mAb based on continuous chromatography, in combination with single-use technology for all unit operations of DSP on 1000 L scale together with incorporation of advanced analytical tools.

Additional associated objectives were:

- Substitution of the standard process for primary separation with the utilization of flocculants (or Tangential Flow Filtration (TFF) or ATF for perfusion).
- Integration of continuous chromatography in the capture step and development of novel single-use equipment for larger scale. Development and evaluation of non-chromatographic capture step alternatives (precipitation reagents).
- Comparative evaluation of single-use equipment and technology over hard-piped equipment of the exemplary process (intermediate/polishing and UF/DF step), including the testing of new mAb flow-through template and membrane adsorbers.
- Development of advanced analytical tools for in-line monitoring of quality attributes.

This poster describes a full flow-through mAb template capable of robustly removing process and product related impurities from a fed-batch pool while achieving high product yields and quality. It highlights the technologies specifically developed within the framework of this project, including multi-column chromatography and continuous viral inactivation systems, and summarizes the results of the four 1000 L-scale technical runs performed.



Pre-treated harvest followed by high capacity depth filters

0.05% of **pDADMAC** polycationic polymer was injected into the 1000 L bioreactor to bind cells, HCP and DNA among other cellular debris. The harvest was then processed through 3.85m² **Clarisolve® 40MS** depth filters, specifically designed to handle large particles (≤40µm).

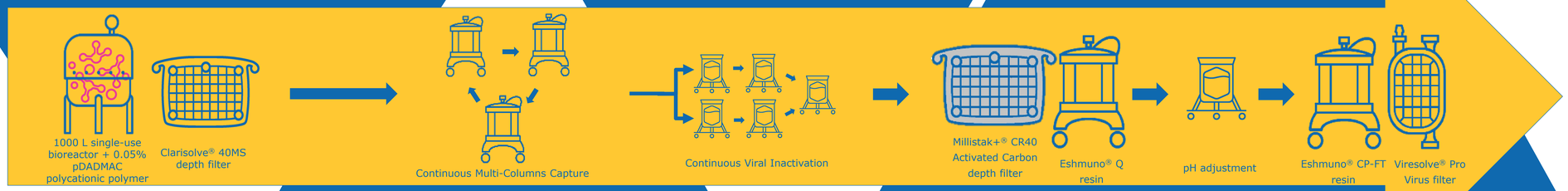
This innovative clarification strategy outperformed a classical cellulosic depth filtrations by enabling a lower filtration area (4.5x reduction), decrease in water consumption (4x), easier product recovery, and a 30% reduction in operation time.

Continuous Viral Inactivation (VIN)

To allow continuous DSP operations, continuous viral inactivation single-use skid was custom designed to adjust pH and maintain product during a targeted incubation time. This was done through 2 synchronized lines (lagged steps).

It was demonstrated that for a given pH, temperature and buffer type, 5 minutes at low pH would be efficient for viral clearance, as reliable as standard batch viral inactivation.

While effective in this study, Merck is pursuing a coiled flow design for continuous viral inactivation



Continuous Multi-Columns (CMC) Protein A capture

Clarified harvest was continuously loaded on protein A media (Amsphere A3, 3 x 0.55 L, 10 cm Ø x 7 cm B.H.), using a prototype Multi Column Capture system with the following features*:

- 3 columns operation system fully single-use and fully automated able to accommodate columns diam. from 7 to 25cm (8 to 120L/h per line)
- 2 zones (3 product inlets, 6 solvent inlets)
- CCP® 6 software driven
- Elution peak detection through UV280 nm*
- Protein breakthrough detection through UV shift*
- Mass balance monitored through FlowVPE*

* Since then, the system has been improved into the Mobius® Multi Column Capture system

Flow-through polishing (f/t)

Millistak+® CR40 (1.21 m²) are depth filters made of activated carbon retained in a rigid structure by a cellulose matrix, and encapsulated in the stackable Pod format. The mesoporosity of carbon, combined with the flow path tortuosity of cellulose, allows for effective clearance of small molecular weight HCPs.

Eshmuno® Q (3.2 L) is a strong AEX chromatography media designed for flow-through polishing of mAbs solutions. Eshmuno® Q resin was set inline with Millistak+® CR40 depth filter, and both technologies were operated at pH 7.0 and conductivity < 4.5 mS/cm.

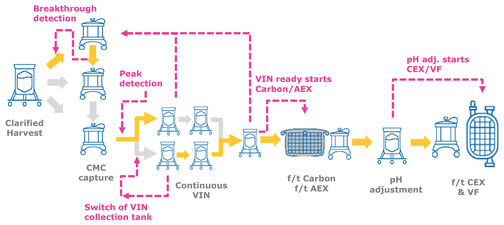
Eshmuno® CP-FT (3.2 L) is a new CEX chromatography media especially designed for mAb aggregates clearance in frontal chromatographic mode, which allows for a 12x gain of productivity compared to traditional B/E mode. Beyond HMW species clearance, Eshmuno® CP-FT resin contributed to remove HCPs and leached PrA.

Viresolve® Pro Virus Clearance filter (0.44 m²) was set inline with Eshmuno® CP-FT resin and operated at pH 5.5 and 4 mS/cm.

Connected, continuous DSP process

The DSP unit operations were performed by automated synchronized continuous systems based on:

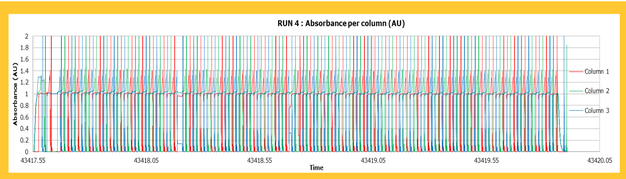
- Breakthrough detection for capture columns switch
- Peak detection and tank level for VIN tanks filling
- VIN skid tank level triggering Carbon and AEX purification
- pH adjustment skid controlling CEX and Virus Filtration (VF)



Results

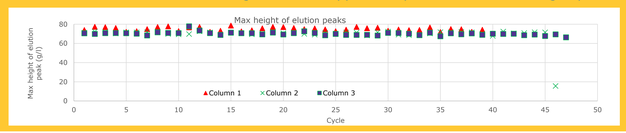
Four full-scale technical runs:

Attributes	Average results
Processed volume	850 – 950L of a mAb @ 3.95 – 4.20 g/L
Qty of mAb purified	2.5 – 3.4 kg in 2.5 days
Overall mAb recovery yield (all steps considered)	> 80%
mAb concentration of final pool	9.0 -10.5 g/L
Purity of final pool *(target)*	Aggregates < 0.2% *(< 1%)* HCP : 12-35 ppm *(<40 ppm)* Leached PrA 0.3-0.5 ppm *(< 5 ppm)*



Continuous capture was performed on protein A columns over 47 cycles with extreme regularity in impurities plateau and breakthrough detections. Short pauses in loading were due to software retro-control from VIN skid (regulation).

FlowVPE® live monitoring demonstrated the cycle-to-cycle consistency of 139 elutions, and confirmed the efficient cleaning conditions applied on protein A resins during capture.



Key Potential Benefits

- **Productivity**
  - Capable of processing up to 3000 L harvest in 24 hours (12 kg mAb)
- **Footprint**
  - Complete DSP in 30 m² (10-15x smaller)
  - Elimination of large intermediate hold tanks
  - Flexibility (mobile equipment)
- **High Safety Assurance Level**
  - Reduce bioburden risk
  - No carry-over issues
  - No cleaning, regeneration, steaming
  - Rapid change-over
- **Economics**
  - Facility investment: ↘ 35%
  - Running costs: ↘ 30%
  - Cost of materials: ↘ 50%
- **Environmental**
  - CO_2 emissions: ↘ 25%
  - Reduced water, buffer, resins consumption (water ↘ 10x)

Summary

The aim of this project was to develop and implement at 1000 L scale an innovative, fully connected, single-use DSP platform for mAb purification.

This new template allowed for producing more than 3.3 kg of mAb in 2.5 days, with similar product quality and purity than those obtained in a traditional batch process, in less than 30 m² with a significant improvement of productivity and a reduced environmental impact.

This proof of concept can be considered as "door opening" to the mAb manufacturing of tomorrow.

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