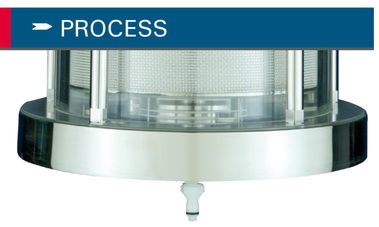

Flow-through mAb-aggregate removal using salt-tolerant cation exchange chromatography: A comparison of batch and continuous processing

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SMCC-experiment

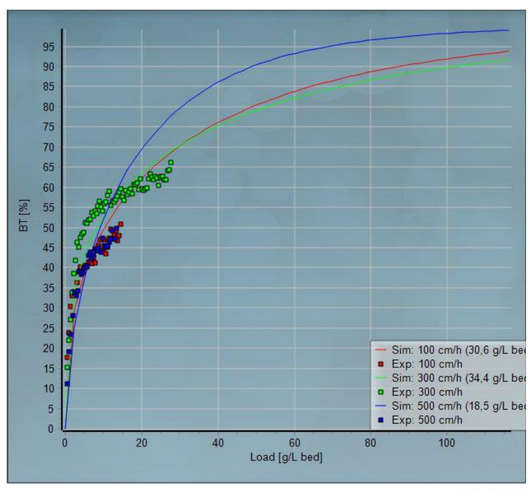
In our continuous approach we used a two column Sequential Multi Column Chromatography (SMCC) model, where both columns are connected in series. The breakthrough of the first column is collected by the second column. When column one is fully loaded, it is then eluted, regenerated and re-equilibrated and connected after column two, to bind breakthrough as soon as it occurs. In order to achieve a fully continuous process we focused on keeping the productivity constant and therefore improving purity and yield.

- Productivity is constant in all experiments
- Focus to improve yield and purity
- Acid aggregated Protein A eluate was loaded at different residence times and volumes

The conditions of the batch and continuous experiments are shown in table 1:

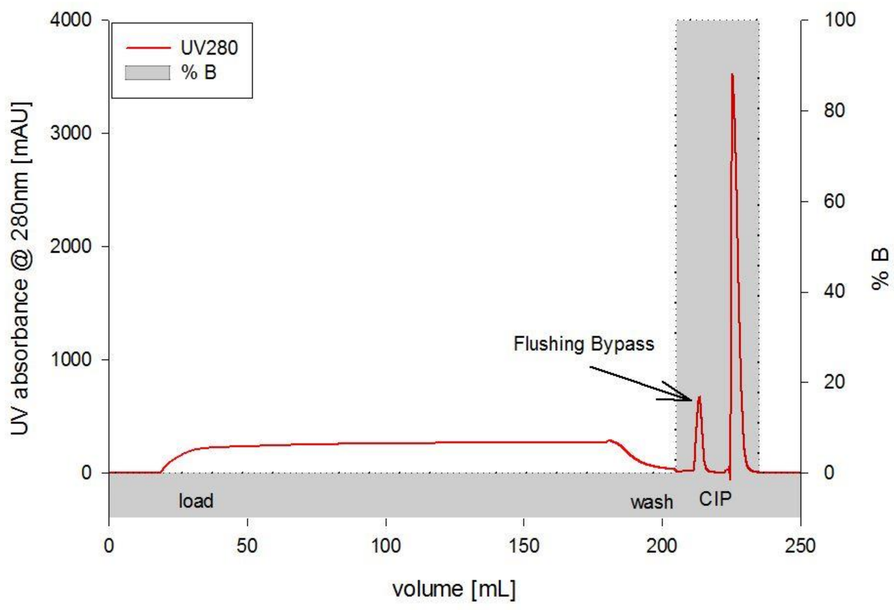
	Batch	Conti 8	Conti 16
Cycles	10	8	16
CV (Load)	40	40	20
CV (Equi)	3	3	3
CV (Wash)	8	8	8
CV (CIP)	5	5	5
Linear flow rate	300 cm/h	278 cm/h	343 cm/h
Buffer consumption	100%	100%	200 %

Breakthrough curves for aggregates and the corresponding BioSC Predict model at different flow rates are shown in figure 2.

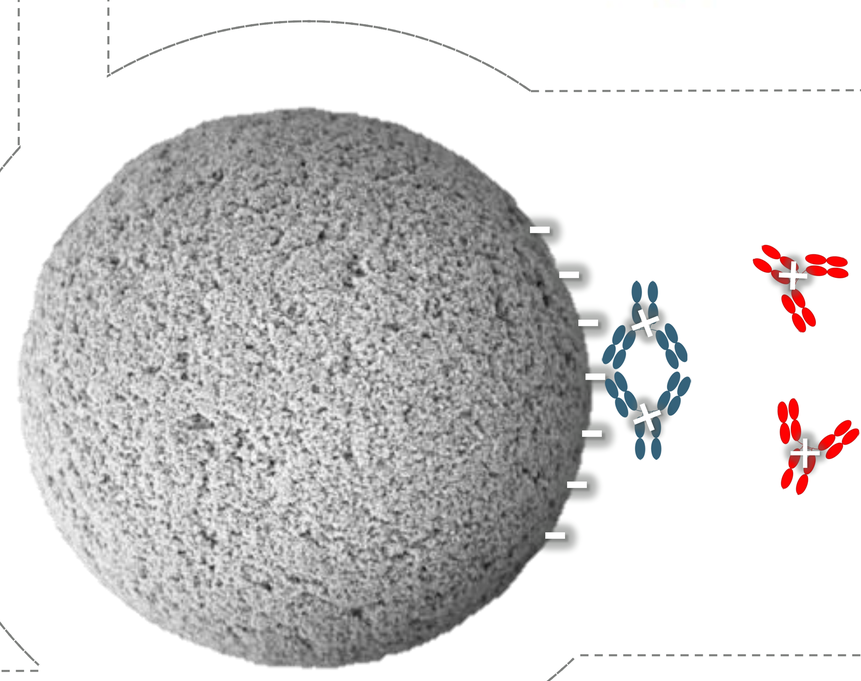


Batch-experiment

TOYOPEARL® Sulfate-650F is a salt-tolerant cation exchange chromatography resin based on 45 µm polymethacrylate particles. Figure 1 shows a chromatogram of a lab-scale batch-experiment run for mAb-aggregate removal from acid-aggregated Protein A eluate.



40 CV of the aggregated Protein A eluate were loaded on the column at 1 min residence time. Monomer flows through the column during loading. No elution of the bound aggregate was performed. Area of CIP-peak indicates the amount of bound aggregate.



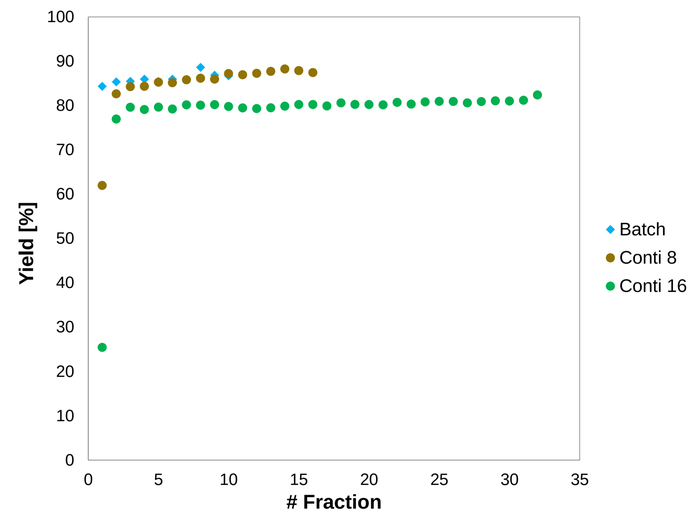
BioSC Lab

Acknowledgements: The BioSC Lab to perform the continuous experiments was kindly provided by Thomas Flouquet, Novasep.

Monomer yield

Determination of Monomer yield was conducted by comparing the monomer peaks of the feed and flow-through fractions. Yield is shown in figure 3.

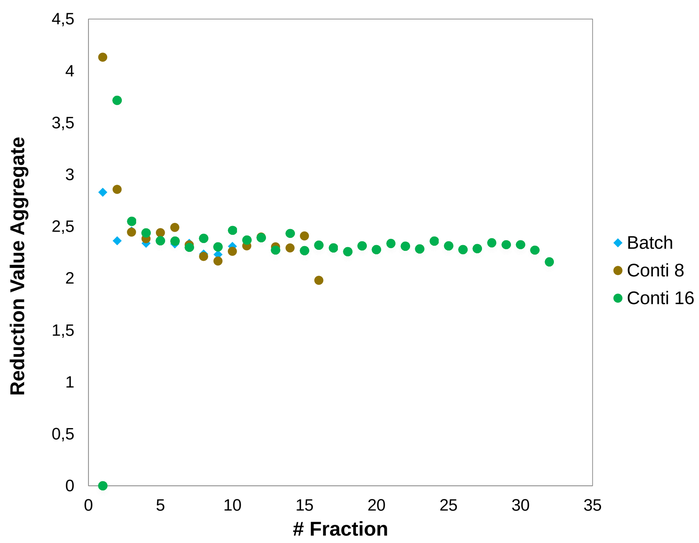
- Monomere yield of batch and conti 8 is comparable
- Slightly lower yield in conti 16



Reduction value for aggregates

Figure 4 shows the reduction value for aggregates after the flow-through experiments. mAb-aggregate content was analyzed with SE-UHPLC using TSKgel® UP-SW3000 (4.6 mm ID x 15 cm L)

- Reduction value for aggregates of around 2 for all experiments
- Loading and flow rate do not influence purity



Conclusion

Continuous chromatography shows highly advantageous results in Protein A chromatography. However, for the tested scenario using a salt-tolerant cation exchange resin in flow-through mode, no advantages of continuous processing compared to batch could be achieved. The parameters of the experiment were chosen to keep the productivity constant and therefore improving yield and/or purity. This could not be realised.

- Reduction value for aggregates is comparable in batch and continuous
- Monomer yield is slightly higher in batch chromatography