simAbs

Application note 1

Applying the MCSGP technique to separate bispecific antibodies: a case study.

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Abstract

In this application note we describe the potential of using the Multi-Column Countercurrent Solvent Gradient Purification (MCSGP) technique to accurately separate bispecific antibodies. Three monoclonal antibodies with different charge variants have been selected to mimic a heterogenous pool of difficult to separate bispecific antibodies. MCSGP is an innovative chromatographic process that tackles current drawbacks in difficult bio-separations and outperforms in terms of productivity, buffer consumption, yield and purity. Here, simAbs demonstrates the unique capability of the MCSGP platform to separate monoclonal antibodies (mAbs) with charge variants.

Introduction

In current bioprocessing, the golden standard for the purification of antibodies is Protein A based affinity chromatography. However, as protein A specifically recognizes and binds the common Fc region of an antibody, it is not capable of distinguishing bispecific antibodies containing variations in charge. The MCSGP technique on the other hand has the potential to separate very similar bispecific antibodies, that are often generated as unwanted by-products during the bioproduction process. The Contichrom® Cube30 equipped with the MCSGP technique offers a unique platform capable of continuously separating antibody charge variants. This results in an increased productivity compared to batch processes and overcomes the typical trade-off between high purity and high yield.

The increased productivity of the MCSGP technique compared to typical batch processes can be ascribed to the fact that the system is able to continuously feed one available column, while processing loaded material onto the other one. A higher product purity and yield is achieved by optimized internal recycling of the target product (Figure 1).

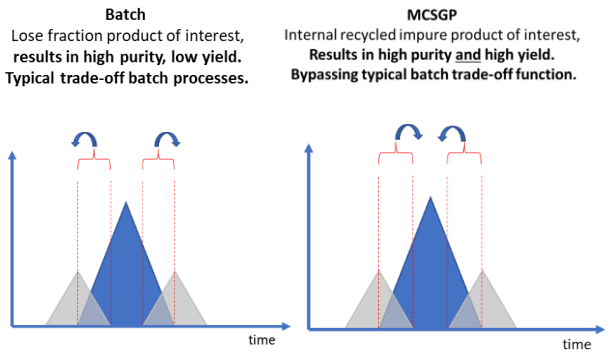

Figure 1: Presentation of the product purity versus yield trade-off, batch processing compared with the MCSGP technique. Arrow directions are indicating the part of product is lost in batch runs (grey-blue overlapping region); this overlapping region is being recycled in MCSGP to recover product and increase purity and yield.

This application note shows the separation of three mAbs with charge variants; Pertuzumab, Nivolumab and Pembroluzimab. The MCSGP technique results in a superior product purity and yield of the target product namely; Nivolumab. This technique can be applied to a wide range of antibodies, such as bispecific antibodies, fragmented antibodies (Fab), Nanobodies® and single chain variable fragments (scFv). Furthermore, it can easily be upscaled from preparative amounts to larger volumes.

Materials and Methods

The feed solution consists of protein A purified Pertuzumab, Nivolumab and Pembroluzimab mixed in a 25/50/25 ratio, respectively.

Batch gradient elution experiments were initially performed to determine the right conditions for setting up the salt elution gradient, flow rates and buffers used. Successful parameters are listed in table 1.

Mixed feed	
Concentration (mg/mL)	1.4
Conductivity (mS/cm)	4
pH	5.2
Buffers	
Buffer A	25mM sodium acetate buffer, pH 5.0
Buffer B	25mM sodium acetate buffer, pH 5.0, 1M NaCl
Column	
Columns	BioPro IEX, YMC, 100 X 8.0MM SCREENING COLUMN, 30µm

Method			
Buffer	CV/load	Buffer type	cm/h
Equilibration	5	Buffer A	300
Load Feed	Load 1 g/L	Pro A eluate, pH 6.0	300
Wash 1	5	Buffer A	300
Elution	10	Buffer A/ Buffer B 0-100%	300
CIP	3	0.1 M NaOH	300
Re-equil 1	1	Buffer B	300
Re-equil 2	4	Buffer A	300

Table 1: Overview of conditions and parameters used.

Conductivity of the mixed feed solution was lowered by diluting it with water. Lowering product conductivity is crucial to ensure an optimal binding of the antibodies to the cation exchange column. During the initial batch experiment a load of 1mg per ml resin was used. In the actual MCSGP experiments, the load was increased to 5mg per mL resin.

Results

The batch chromatogram (Figure 2) shows a well separated profile with a good resolution between the three peaks, representing the three antibodies present in the feed mix. The middle peak is the product of interest, Nivolumab. Buffers used in this run are ensuring a good absorption of the three different antibodies to the resin and the optimized elution gradient is able to discriminate the three differently charged antibodies (different iso-electric point; pI).

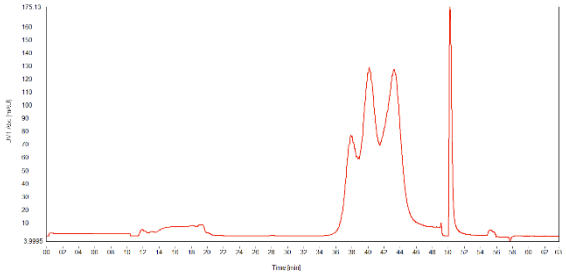

Figure 2: Batch chromatogram of mixed feed

The chromatogram using the MCSGP technique, is showing three out of five successful cycles with 50mg per cycle, with a total load of 250mg (Figure 3).

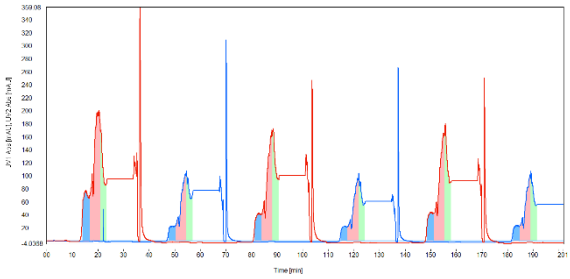

Figure 3: The MCSGP chromatogram of 3 cycles using the mixed feed.

The zoomed chromatogram in Figure 4 shows more details of the 1st cycle. The red highlighted area represents Nivolumab (our product of interest). The areas indicated in blue and green represent regions containing Pembroluzimab (blue) or Pertuzumab (green) (mimicking the unwanted by-products). These latter regions typically still contain small fractions of Nivolumab and are therefore recycled internally on the second, downstream column. This recycling loop is performed to obtain maximum purity and yield of Nivolumab.

Starting with the first switch of one cycle (red UV trace), elution of weekly absorbing impurities is happening on the upstream column (directed to waste, not containing product of interest). As a next step, the overlapping region (containing unwanted side product, Pembroluzimab, and product of interest, Nivolumab; blue region) is collected and recycled on the downstream column. Next, pure product of interest,Nivolumab, is being eluted and collected in fractionator (red region). At the same time, extra feed is added onto the downstream column. As a final step of the first switch, strong absorbing impurities (Pertuzumab), together with a

fraction of Nivolumab (both represented by green region), are transferred and recycled on the downstream column. As a last step, the strongest absorbing impurities are again eluted and redirected to waste. To complete 1 cycle, a second switch starts (blue UV trace). The downstream column becomes the upstream one and the process repeats.

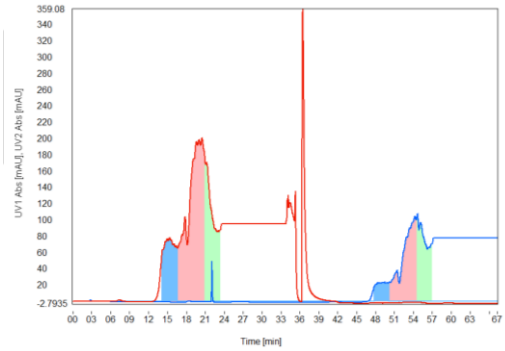

Figure 4: The MCSGP chromatogram using the mixed feed, zoomed on the first cycle. Red trace (1st switch), UV absorbance upstream column. Blue trace (2nd switch), UV absorbance downstream column.

Conclusion

The MCSGP technique tackles current limitations in difficult bio-separation and set new standards in terms of product yield and purity. Using the MCSGP technique, we were able to separate three monoclonal antibodies based on their isoelectric point (difference in net charge) using a gradient elution performed during a batch run. The obtained batch protocol was successfully extrapolated to the MCSGP platform. Multiple cycles were run, where continuous product feeding was applied in combination with an increased product purification. Overlapping regions between difficult to separate products were recycled and re-purified using the MCSGP technique increasing end-product purity and yield.

About the author

Gert Struys graduated as Master Biochemistry and Biotechnology at the KULeuven. He started working in industry right away. As first employee, he was responsible of the buildout of a GMP-ready facility for KBI Biopharma Belgium. Later on his focus changed to Method validations, qualifications, formulation studies and stability evaluations. At simAbs NV, he gained extensive expertise in continuous manufacturing and has knowledge about state of the art techniques like simulated moving bed purification (cSMB), Multi-Column Single Gradient Purification (MCSGP) and integrated batch polishing.

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