mAb Aggregate Removal Using Flow Through Chromatography on Fractogel® EMD Resin

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Abstract

Even though monoclonal antibody (mAb) platform processes are now well developed and characterized, the propensity for mAb aggregation and the need to remove product related impurities can still be a bottleneck and have economic repercussions both in process cost and development time. This poster describes the use of Fractogel® EMD COO- resin (weak cation exchanger) to remove mAb aggregates in flow through mode of operation. Previous work had shown differences in selectivity for aggregates, dimers and monomer in bind/elute mode, but it was not possible to achieve the required combination of purity and monomer recovery. The flow through method presented here relies upon adjustment of the feed conductivity to maximize the selectivity between monomers and aggregates/dimers. It was possible to over–achieve customer requirements by demonstrating 88% monomer recovery at a loading of 200 g/L resin with removal of product related impurities to the limit of the SEC detection method. Scale up robustness was shown from 1 to 10 cm resin bed height. Flow through purification methods are more easily adapted to continuous processing and in this case achieved a combination of purity, recovery and throughput not possible in conventional bind/elute mode. This technique makes highly efficient use of the chromatography media (200 mg/mL gel) and the absence of a high conductivity elution facilitates subsequent polishing steps.

Study Objective

The aim of these set of experiments was to elucidate a purification method that would separate monoclonal antibody from dimers and aggregates. The goal was to leave the monomer antibodies with as high level of purity as possible (>98%) with a recovery >80%.

Methodology

Standard scouting experiments were carried out using Fractogel® EMD COO- (weak cation exchange resin) (1). Fractogel® EMD media consists of synthetic methacrylate based polymeric beads capable of high flow rates. Fractogel® EMD COO- is comprised of a particle size range of 40-90 µm (M-type).

Initially at a 1 mL column volume, an AKTA® Avant system was used to perform scouting experiments for pH and conductivity. It was decided to utilize the Fractogel® EMD COO- resin in flow through mode as the dimers and aggregates (2) were binding with greater affinity compared to the monomer and therefore the efficiency of the column could be maximized. The feed material was a Protein A eluate stored at -70°C and filtered before use to 0.22 µm.

Results

Initial Bind and Elute Results

Purity levels over 98% and an economic recovery could not be achieved using the standard bind and elute technique (**see Figure 1**).

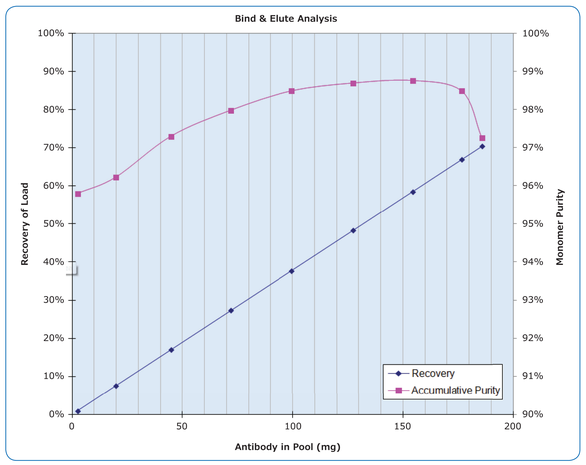

Figure 1: Showing the relationship between purity and recovery in bind elute mode

During these experiments, it was observed that at specific conductivities the monomer would not be bound by the cation exchanger. This non-binding could be utilized as a "flow through" technique. The Fractogel® COO- resin had been shown in previous internal work to be more selective in the binding properties, having a trade off in lower binding capacities than that of strong cation exchangers. The idea was to utilize this higher selectivity capabilities of Fractogel® EMD COO- resin to retain stronger binding dimers and aggregates, allowing the monomer to pass directly through the column (3).

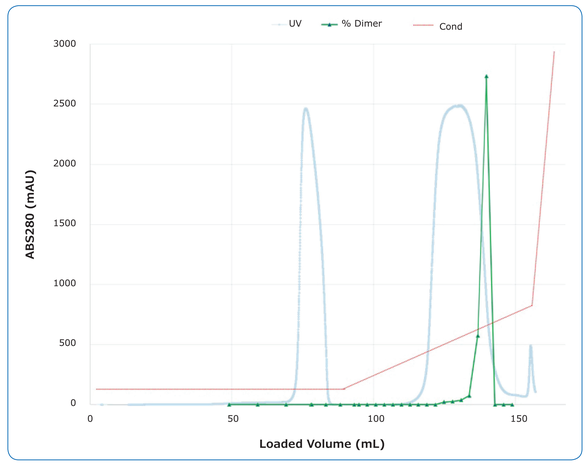

Figure 2: Showing separation of monomers and aggregates in an elution peak resulting from an increase in conductivity. This graphic indicates that during a standard bind and gradient elute it was observed that monomer elutes at a different salt concentration than the stronger binding dimer, allowing the potential use of flow through mode to completely separate the aggregates from the monomer pool.

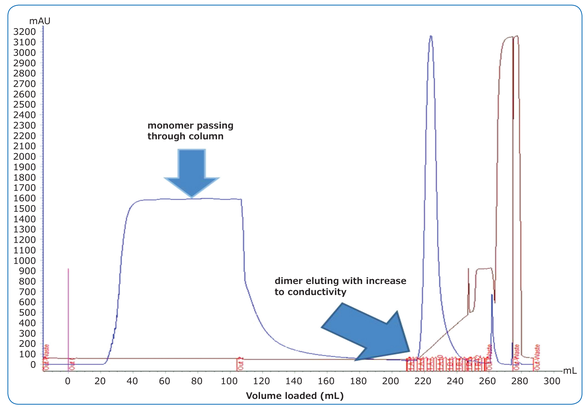

Figure 3: Showing that the conductivity (brown line) of the load pool can be adjusted to perform in flow through mode (4), allowing passage of the monomer through the column as unbound material. The dimer, stronger binding monomers and large aggregates are eluted with a standard salt gradient (far right hand peak)

Flow through Results

Scouting flow through experiments, with excursion at several pH and conductivities, were conducted to optimize the separation conditions:

pH	Conductivity	Recovery %	Aggregates in flow through %
5	10	84.3	0
5.8	10	96	1.4
5	12	97	1.9
5	10	85	0

Table 1: Results from flow through screening. This round of experiments indicated that a load material of 10 mS/cm at pH 5.0 would allow the maximum passage of monomeric antibody through the column without significant binding loss.

A determination of capacity experiment was carried out, on a 1 mL column, by pooling fractions and assaying for monomer and impurities in the flow through.

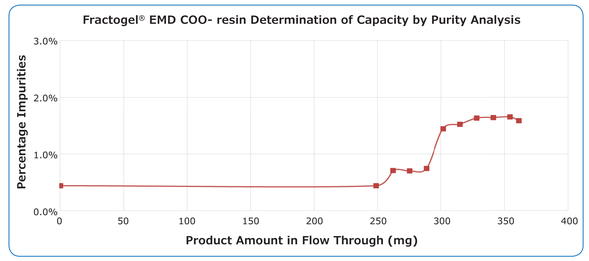

Figure 4: Showing an impurity breakthrough curve. The results indicate that an increase in the amount of impurities was observed at 250 mg mAb/mL gel. It was decided that with a safety margin, total of 200 mg of product could be purified per 1 mL of Fractogel® EMD resin using this technique.

Afterwards, the reproducibility of this technique was evaluated by loading different amounts of mAbs on a Fractogel® EMD COO- column. Results demonstrated a good consistency.

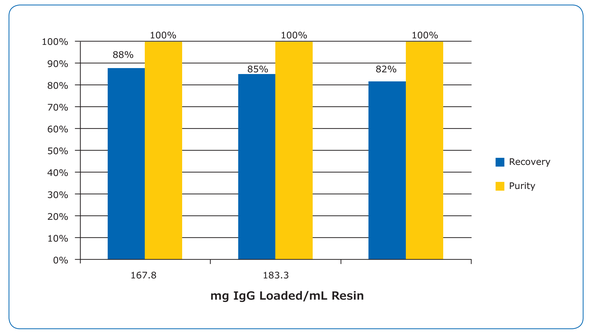

Figure 5: Showing repeated flow through experiments. Different loading amounts of starting mAb to confirm the high purity levels at flow through loadings of up to 200 mg.

Further experiments investigated performance on process relevant media bed heights. **Figure 6** shows that the final product recovery was reproducibly within specification limits at 10 cm bed height (b.h.). This also helps to determine the operation limits and therefore processing specification.

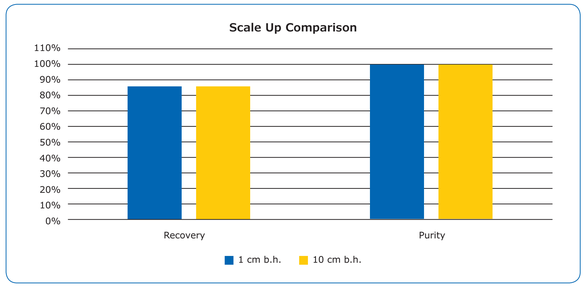

Figure 6: Showing data from repeated runs at each scale and the variance between runs (n=3 at each scale). A residence time of 4 minutes was kept constant across scales.

Reference List

1. J Chromatogr A. 2007 Jun 22;1154(1-2):121-31
2. Curr Pharm Biotechnol. 2009 Jun;10(4):421-6.
3. Journal of Chromatography. A [2008, 1200(2):156-165]
4. DOI: 10.1002/9783527673681.ch04

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Conclusion

Fractogel® EMD COO- resin is an efficient media for the elimination of aggregates, that can be used in specific cases in negative bind and elute mode (flow through mode). In this case the aggregate levels post protein A were over specification set out for the product.

The small scale 1 cm b.h. was able to be scaled up to 10 cm b.h. with a slight enhancement of the overall purity. More work would be needed to asses the overall procedure robustness and ease of transferability between mAbs.

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