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# Expanded bed adsorption in the purification of monoclonal antibodies: a comparison of process alternatives

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**Abstract**

Expanded bed adsorption (EBA) was examined as the initial capture/purification step in the purification of monoclonal antibodies from Chinese hamster ovary (CHO) cultures. Two process alternatives each using EBA were compared to a conventional Protein A process without EBA. One alternative used Protein A affinity EBA followed by packed-bed cation and anion-exchange steps. The other alternative used cation-exchange EBA as the capture step followed by packed-bed Protein A and anion-exchange steps. The process using Protein A EBA produced comparable purity (host cell protein, DNA, Protein A, antibody aggregate) to the conventional process. However, the Protein A EBA column showed a significant decrease in dynamic capacity with a limited number of cycles. The process using cation EBA achieved comparable levels of host cell proteins (HCP) and DNA but not antibody aggregate or leached Protein A compared to the conventional process.

## Introduction

Increasing numbers of monoclonal antibodies are being used as therapeutic agents. In many of these cases the doses are large and the therapy consists of repeated doses, requiring large quantities to be manufactured. Current recovery processes for monoclonal antibodies have demonstrated robust and reproducible manufacture of large batch quantities of protein. These processes have met the rigorous regulatory requirements with regard to product purity, removal of host cell proteins, DNA, antibody aggregate, small molecules, potential viruses, and leached Protein A. Furthermore these processes have excellent overall yields and high product throughput. Despite the outstanding performance of existing processes, increases in batch sizes and market requirements will increase the need to develop even more robust and cost efficient processes.

One new technology which has the potential to simplify and reduce costs in recovery processes is EBA. In EBA the harvest, initial capture, purification and concentration steps are combined in a single unit operation. This has the potential to decrease the number of unit operations thus reducing manufacturing cost of goods.

Successful application of EBA has been documented for the initial recovery of proteins from mammalian cells (Erickson et al., 1994), *E. coli* cells (Hansson et al., 1994), and *E. coli* homogenates (Barnfield Frej et al., 1994). There are two options for the use of EBA in a monoclonal antibody recovery processes. One option is using Protein A EBA (Thommes et al., 1996; Fahrner et al., 1999). A second option is the use of cation exchange EBA (Thommes et al., 1995; Zapata, 1999). Due to the high isoelectric point of most therapeutic antibodies cation exchange chromatography is ideal for binding these antibodies without going to extremes of pH.

The goal of this work was to compare monoclonal antibody recovery processes using Protein A EBA (followed by packed-bed cation-exchange and anion-exchange steps), cation exchange EBA (followed by packed-bed Protein A and anion-exchange steps), or a conventional recovery process (packed-bed Protein A, cation-exchange and anion-exchange steps). To be a viable option, the processes using EBA needed to demonstrate comparable purity and process performance with fewer unit operations than the conventional process.

## Materials and Methods

*Feedstocks*

Load material for EBA studies was as previously described (Fahrner et al., 1999). All studies except for the comparison of dynamic binding capacity (DBC) for different antibodies on Streamline SP XL, were done using the same antibody. The antibodies used in the comparison study represent several humanized, $IgG_1$ antibodies with similar molecular weights but different pIs ranging from 7.1 to 8.8.

*Resins*

Streamline Protein A, Streamline SP XL (cation exchange), SP-Sepharose FF, and Q-Sepharose FF media were from Amersham Pharmacia Biotech (Uppsala, Sweden). Prosep A media was from Millipore (Consett, England).

*Analysis*

Yield was determined as the percent of loaded antibody that eluted in the purified pool. Antibody concentration was determined by Protein A HPLC in crude feedstreams and absorbance at 280 nm in purified pools. DNA was measured using the Molecular Devices DNA Threshold Assay Kit. The amount of host cell proteins (Chinese hamster ovary proteins, CHOP) and leached Protein A were determined by ELISA. Antibody aggregate was measured by size exclusion HPLC.

*Protein A EBA*

Parameters for operation of Protein A EBA were according to Fahrner et al. (1999). The Protein A EBA re-use study was done with a single batch of cell culture fluid stored with sodium azide. Each cycle consisted of load (to the DBC) using cell culture fluid, wash, elution, regeneration, and equilibration. The elution pool was collected for each cycle for yield determination. At the completion of the re-use study a small sample of resin was removed from the EBA column and packed into a small scale column. An identical small scale column was packed with naive Streamline Protein A resin. Breakthrough curves using purified antibody were performed on each column. Breakthrough was determined by absorbance at 280 nm.

*Cation-exchange EBA*

Breakthrough curves with Streamline SP XL were performed using cell culture fluid. The pH of the cell culture fluid was adjusted to the indicated pH using acetic acid and the conductivity was adjusted with water. The DBC value represents breakthrough at 5%. Elution studies were performed with Streamline SP XL in a packed-bed column. Clarified cell culture fluid was adjusted to pH 4.5 and a conductivity of 10 mS, and loaded to capacity. After washing, the column was eluted with sodium acetate and either pH 4.5 or 5.0. The concentration of sodium acetate in the elution buffer ranged from 180 to 280 mm.

*Process comparison*

The packed bed Protein A column used Prosep A media and was loaded with clarified cell culture fluid previously described (Fahrner et al., 1999). The packed bed cation exchange column used SP-Sepharose FF with a bed height of 20 cm. Protein A pools were adjusted to a pH of 5.5 with Tris base and a conductivity of less then 9.0 mS with water. This adjusted pool was loaded onto the cation exchange column and the column washed with 20 mm MES, 80 mm NaCl, pH 5.5, until absorbance 280 nm reached baseline. The column was eluted with 20 mm MES, 145 mm NaCl, pH 5.5. The pool was approximately 4 bed volumes after the absorbance 280 nm reached 0.1 AU. The anion-exchange column used Q Sepharose FF packed to a bed height of 20 cm. The cation-exchange pool was adjusted to pH 8.0 with Tris base and a conductivity of less then 7.5 mS with water. The column was equilibrated in 25 mM Tris, 25 mm NaCl, pH 8.0. After equilibration the adjusted cation-exchange pool was loaded onto the column. Under these conditions the anion-exchange column functions as flow-through chromatography. The antibody flow-through pool is collected when the absorbance rises above 0.1 AU. Pooling is stopped when the absorbance drops below 0.1 AU. Purity was determined at the various steps as described above.

## Results and discussion

*Protein A EBA*

Previous work has suggested that there is a significant increase in the dynamic binding capacity when Protein A EBA is operated at slower flow rates (Fahrner

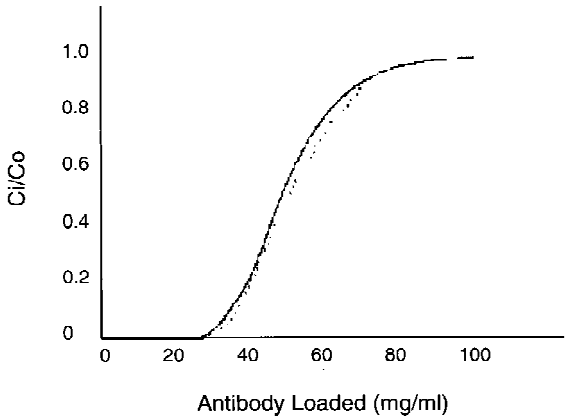


*Figure 1.* Breakthrough curves on Streamline Protein A using clarified cell culture fluid in packed-bed mode (solid line) or cell culture fluid in EBA mode (dashed line).

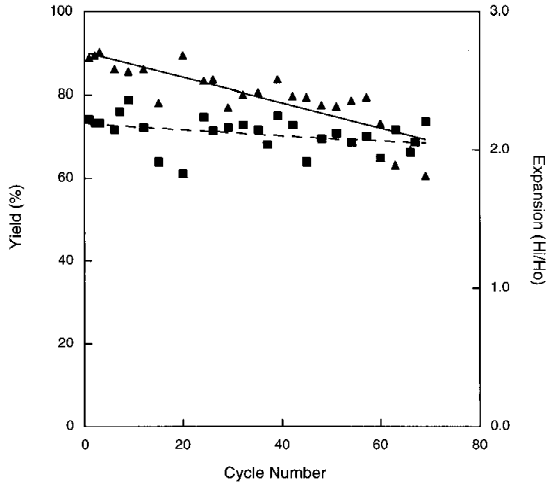


*Figure 2.* Re-use study on Streamline Protein A. Yield (triangles, solid line) and expansion (squares, dashed line). Expansion is expressed as the ratio $H_i/H_o$ where $H_i$ is the height of the bed during expansion and $H_o$ is the height of the bed before expansion. Lines represent a linear curve fit to the data points.

et al., 1999). Breakthrough studies were done on Streamline Protein A using purified antibody in the packed-bed mode and unclarified cell culture fluid in the expanded-bed mode (Fig. 1). The results show very similar DBCs under both operating conditions. Therefore process development can be done at small scale using purified antibody or clarified cell culture fluid in either packed-bed or expanded-bed mode. This considerably simplifies process development since experiments are not dependent upon fresh cell culture fluid.

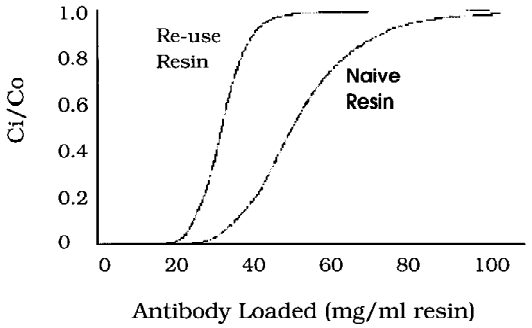


*Figure 3.* Breakthrough curves using clarified cell culture fluid on Streamline Protein A resin which is either naive or at the end of the re-use study in Fig. 2.

A critical element in Protein A chromatography is the number of cycles or re-uses that can be achieved. This is due to the relatively high cost of any type of Protein A resin. When loading clarified cell culture fluid on packed bed Protein A columns the number of cycles achievable is typically greater than 400. Thus for Protein A EBA to compete with packed-bed Protein A the EBA column must be able to be operated for hundreds of cycles. Cell culture fluid was loaded onto a Protein A EBA column and approximately 80 cycles of load, wash, elution, regeneration, equilibration were performed. The yield and degree of bed expansion were measured for each cycle (Fig. 2). While the bed expansion stayed constant over the 80 cycles there was a steady decrease in the yield over the course of the study going from ~90% in the initial cycle to <70% by cycle 80. The yield decrease over the course of the re-use study could be a result of several factors. These factors may include failure of all bound antibody to elute, interaction of the cell debris with the antibody and/or resin, or a decrease in the DBC. A breakthrough study was done with purified antibody using either naive Protein A EBA resin or Protein A EBA resin taken at the completion of the re-use study (Fig. 3). Use of purified antibody eliminates any debris or cell culture component interaction with the resin or antibody. The DBC on the naive resin was 31 mg/ml while that of the used resin was only 23 mg/ml. Thus the decrease in yield is due to a change in the DBC of the resin. Alternative cleaning procedures such as different regeneration solutions or more frequent cleaning may improve the yield with re-use.

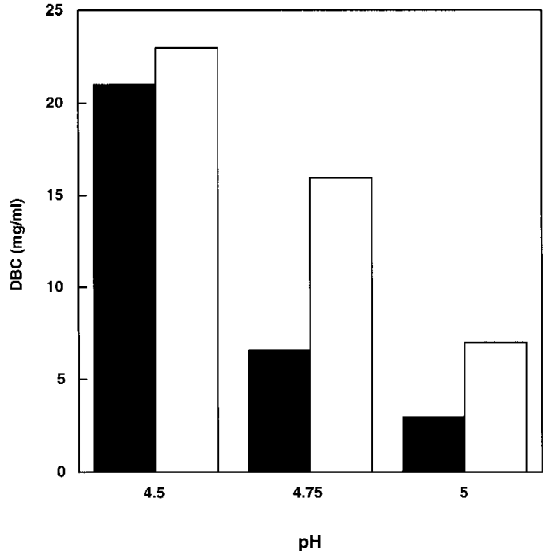


*Figure 4.* DBCs on Streamline SP XL loading cell culture fluid. Conductivity of the load was 10 mS (clear bars) or 14 mS (solid bars).

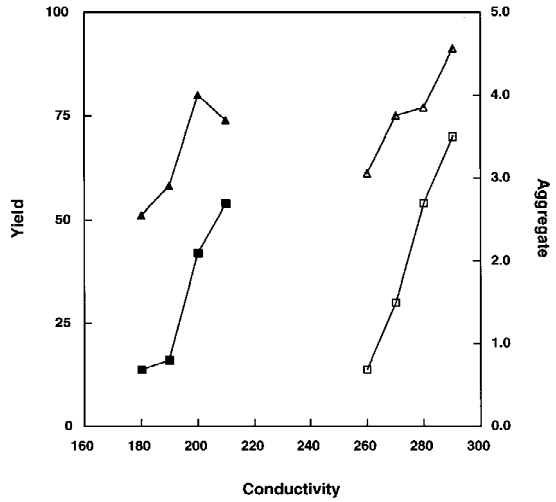


*Figure 5.* Effect of elution buffer pH and conductivity on the yield and aggregate level. Streamline SP XL was loaded with cell culture fluid and washed to remove the debris. The column was eluted with buffers of various conductivities at either pH 5.0 (solid symbols) or pH 4.5 (open symbols). The yield (triangles) and aggregate level (squares) in the elution pools were determined.

*Cation-exchange EBA*

The optimal pH and conductivity of the cell culture fluid load were established by performing breakthrough studies (Fig. 4). At a pH of 5.0 the DBC was very low at both conductivities examined (10 and 14 mS). Lowering the pH to 4.75 at 10 mS improved the DBC to 15 mg/ml. At pH 4.5 the DBC in both cases was $>$ 20 mg/ml. Lowering the pH further was not examined due to the potential for activating acid proteases. Activation of these proteases would limit the time cell culture fluid could be held during the load stage and would impose constraints in the manufacturing setting. The DBC may also be increased by decreasing the conductivity. However, further dilution of the load increases the overall load volume and thus load time. In addition, unless in-line dilution is performed a very large tank would be needed for the adjusted load material. A pH of 4.5 and conductivity of 10 mS are reasonable parameters for manufacturing operations and give an acceptable DBC of 23 mg/ml.

The cation exchange step in the conventional antibody process has several functions. This step removes charged antibody variants, host cell proteins, and low-molecular weight antibody variants, and antibody aggregates. Of particular concern with therapeutic antibodies is antibody aggregate. A key question in assessing the feasibility of cation exchange EBA was the ability of the EBA step to remove antibody aggregate. Antibody aggregates are more tightly bound than the antibody monomer and elute after the main peak. Elution conditions must be optimized to elute all the antibody monomer but not the aggregated antibody. Cell culture fluid was loaded onto to a cation exchange EBA column. After the column had been washed the elution conditions were examined using buffers at a pH of 4.5 or 5.0 and conductivities ranging from 170 to 290 mS (Fig. 5). At both pH 4.5 and 5.0 there was incomplete resolution of antibody aggregate from antibody monomer. As the level of aggregate went down the yield also decreased. Only at the lowest conductivities with each pH was the level of aggregate reduced to below 1.0%. However, this reduction in aggregate also resulted in yields of between 50 and 60%. By contrast a packed-bed cation-exchange column, following the Protein A chromatography step, typically has yield of greater than 80% and aggregate levels of less than 0.5%. Thus under the conditions tested acceptable levels of aggregate and yield were not achieved.

A key factor in a multi-product manufacturing plant is the ability to fit the recovery operation of several products into one recovery train (a combination of chromatography skids and tanks). Monoclonal antibodies are particularly suited for a single recovery train due to their homology and the similarity of their

*Table 1.* DBC of five antibodies on SP XL Streamline resin.

| Antibody | Dynamic binding capacity (mg/ml) |
|---|---|
| A | 33 |
| B | 41 |
| C | 28 |
| D | 31 |
| E | 31 |

DBC is the breakthrough at 5%.

recovery processes, which is driven in large part by the generic nature of the initial Protein A step. If cation exchange EBA were to be viable in such a multi-product environment, it must be able to have process parameters for several antibodies that are similar enough to allow a single capture system. A key for determining this similarity would be how similar the DBCs would be for multiple antibodies. The DBC is a key factor since it defines the column volume needed to process a batch which in turn determines the flow rate and tank sizes. To determine how much variability existed in the DBCs among different antibodies, breakthrough curves on Streamline SP XL were performed for five monoclonal antibodies produced at Genentech. The DBCs for these antibodies ranged from 28 to 41 mg/ml (Table 1). These results indicate that a single recovery train could be utilized for these antibodies and probably all other antibodies using a similar humanized framework. A single pH and conductivity were chosen and used for all five antibodies. No attempt was made to optimize the load conditions for each antibody. Thus it is possible that the DBCs of the antibodies on the lower end could be increased by optimizing the pH and conductivity.

*Process purity*

A process that utilizes EBA must achieve comparable levels of purity compared to a conventional packed bed process. In Table 2 three processes were compared for their ability to remove host cell proteins (CHOP, Chinese hamster ovary cell proteins), DNA, leached Protein A, and antibody aggregate. Although the recovery processes for therapeutic proteins must be validated to demonstrate removal of other impurities and contaminants the four impurities measured in this study are representative of process performance. Process 1 is a typical antibody recovery process using three packed-bed column steps in which clarified cell culture fluid is loaded on the initial Protein A column. Process 2 uses a Protein A EBA column and is loaded directly with cell culture fluid. The cation and anion exchange columns are identical to Process 1. Process 3 uses a cation-exchange EBA column as the capture step with the Protein A and anion columns are the same as in Process 1.

Processes 1 and 2 show similar levels of CHOP at each step in the process with levels below the level of detection after the anion exchange step. The cation EBA pool in Process 3 shows a higher level of CHOP compared to the other two processes that have the Protein A step first. However, despite the higher level of CHOP in the cation-exchange EBA pool, all processes had similar CHOP levels by the second column and the levels were undetectable by the third column.

All three processes reduced DNA to similar levels at each step in the process with DNA being less than detectable after the final step. The slight differences among the DNA levels after the second steps are within run to run and assay variation. In processes 1 and 2 Protein A which has leached from the initial Protein A column ($\sim$10 ppm) is completely removed by the cation-exchange step. In contrast in process 3 where the Protein A column is second and is followed only by the anion step there is no Protein A removal. The slight amount of leached Protein A from the Protein A column is present in the final material. In processes 1 and 2 where the packed-bed cation-exchange column follows the Protein A step, antibody aggregate is reduced from $\sim$4% in the Protein A pool to less than 0.1% in the cation-exchange pool. In contrast, when the cation-exchange column is operated in the initial step as EBA, aggregate is only reduced to 1.3%. This level of aggregate is not reduced during the remaining Protein A or anion-exchange steps.

The purity of material produced using Protein A EBA as the initial step is comparable to the purity of the conventional process. However, when cation exchange is the initial EBA step, the comparable levels of process purity were not achieved with respect to antibody aggregate and leached Protein A.

## Conclusion

The goal of this study was to examine the potential for either Protein A EBA or cation-exchange EBA to replace the harvest and initial capture step in an antibody recovery process. To do so the EBA process must

*Table 2.* Purity at each step of three antibody recovery processes

| | | CHOP (ppm) | DNA (pg/mg) | Protein A (ppm) | Aggregate (%) |
|---|---|---|---|---|---|
| Process 1 | Protein A | 842 | 520 | 10 | 3.7 |
| | Cation IEX | 96 | LTD | LTD | 0.07 |
| | Anion IEX | LTD | LTD | LTD | 0.05 |
| Process 2 | Protein A EBA | 904 | 451 | 11 | 4.2 |
| | Cation IEX | 96 | 47 | LTD | 0.04 |
| | Anion IEX | LTD | LTD | LTD | 0.05 |
| Process 3 | Cation EBA | 113 270 | 370 | NA | 1.3 |
| | Protein A | 25 | 13 | 2.9 | 1.3 |
| | Anion IEX | LTD | LTD | 2.7 | 1.3 |

CHOP chinese hamster ovary protein, the host cell protein; LTD, less than detectable; NA, not assayed; ppm, ng of impurity per mg of antibody product.

significantly reduce process costs (by eliminating at least one process step) and have equivalent purity and yield to a conventional process.

The load to EBA columns are some of the dirtiest in the bioprocess industry. A significant challenge with EBA is to successfully and repeatedly clean the column cycle after cycle. The ability to treat ion exchange resins with harsh cleaning treatments combined with the low raw material cost is an advantage for cation-exchange EBA. However, our data demonstrate that a three-step process with cation-exchange EBA as the capture step cannot remove antibody aggregate and leached Protein A to acceptable levels. Certainly a packed-bed cation-exchange column could be inserted after the Protein A column to remove aggregate and Protein A. This results in a process with the same number of steps as the conventional process, substituting cation exchange EBA for the harvest operation, in our case centrifugation. In this case, is EBA a less expensive operation than centrifugation? Although we have not operated EBA at manufacturing scale and thus do not have definitive operating costs, a reasonable estimate can be made using cost data from a packed-bed cation-exchange column. This approach gives an operating cost for cation exchange EBA that is at least 2.2× that of centrifugation. Therefore the process costs for the conventional process (centrifugation followed by three chromatography steps) is superior to an EBA process (cation-exchange EBA followed by the same three chromatography steps).

A three step process with Protein A EBA results in the same purity as the conventional process. This route has the advantage of placing the affinity step at the beginning to take full advantage of the purification power of affinity chromatography. The downstream steps can then focus on fine polishing. The traditional downside to an initial affinity chromatography step, an expensive resin that is usually sensitive to harsh cleaning treatments, becomes magnified given the nature of EBA loads. This drawback is confirmed by the data from the re-use study which showed significant fouling and product loss after only 70 cycles. This may appear to be a large number of cycles but when clarified cell culture fluid is loaded onto a packed bed Protein A column greater than 400 cycles are typically achieved. The high cost of Protein A necessitates the ability to re-use the column for a large number of cycles, regardless of whether the column is run in one cycle or multiple cycles per batch. Our conclusion is that the development of a ligand with the antibody binding properties of Protein A but with resistance to harsh cleaning agents will be needed to make Protein A EBA a viable option in the purification of therapeutic antibodies.

## References

Barnfield F, Hjorth R & Hammarstrom A (1994) Pilot scale recovery of recombinant anexin V from unclarified *Escherichia coli* homogenate using expanded bed adsorption. Biotech. Bioeng. 44: 922–929.

Erickson JC, Finch JD & Greene DC (1994) Direct capture of recombinant proteins from animal cell culture media using a fluidized bed adsorber. In: Berthold RE & Berthold W (eds.) Animal Cell Technology-Products for Today, Perspectives for Tomorrow (pp. 557–562) Butterworth & Heinemann, Oxford.

Fahrner RL, Blank GS & Zapata G. (1999) Expanded bed protein A affinity chromatography of a recombinant humanized monoclonal antibody: process development, operation, and comparison with a packed bed method J. Biotech. 75: 273–280.

Hansson M, Stahl S, Hjorth R, Uhlen M & Moks T (1994) Single-step recovery of a secreted recombinant protein by expanded bed adsorption. Bio/Technology 12: 285–288.

Thommes J, Halfar M, Lenz S & Kula M.-R (1995) Purification of monoclonal antibodies from whole hybridoma fermentation broth by fluidized bed adsorption. Biotechnol. Bioeng. 45: 205–211.

Thommes J, Baker A, Halfar M, Karau A & Kula M.-R (1996) Isolation of monoclonal antibodies from cell containing hybridoma broth using a protein A coated adsorbent in expanded beds. J. Chromatogr. A 752: 111–122.

Zapata G (1999) Cation exchange expanded bed chromatography — one step combining initial harvesting, purification and concentration applicable to humanized IgG antibodies and antibody fragments. Presentation at BioTherapeutics '99: Evaluating Primary Recovery Technologies. Washington, DC, USA.