Evaluation of Different Approaches for the Chromatographic Purification of Monoclonal Antibodies

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Summary

Purification of monoclonal antibodies is one of the major applications of chromatography in the life sciences. Protein A and Protein G supports provide an efficient means to purify various classes of immunoglobulins. However, the species or subclass specificities, high costs, and relatively harsh elution conditions associated with such supports are often incompatible with the overall purification strategy. Potential leakage of Protein A or G from these supports can also be problematic for antibodies destined for therapeutic applications.

The recently introduced series of analytical and preparative Bio-Scale chromatography columns provides several options for the purification of immunoglobulins in a cost effective manner under gentle elution conditions. The methods detailed in this study for monoclonal antibody purification include:

- Tandem use of DEAE Affi-Gel® blue mixed mode anion exchange/dye ligand followed by Bio-Scale CHT-I ceramic hydroxyapatite chromatography column
- Cation exchange chromatography using a Bio-Scale S strong cation exchange column
- Anion exchange chromatography using a Bio-Scale DEAE weak anion exchange column

All three methods provide cost effective alternatives to affinity based methods for monoclonal antibody purification. The results, advantages, and disadvantages of the different methods are discussed in this study.

Introduction

The Monoclonal Antibody Core Facility at the Memorial Sloan Kettering Cancer Center produces monoclonal antibodies used in the center's research of diseases such as AIDS and cancer. In an effort to develop a single step, non-Protein A based chromatographic monoclonal antibody (MAb) purification, three different approaches were investigated on three different MAbs.

Materials and Methods

All samples were filtered through a 0.22 µm filter and then dialyzed against the equilibration buffer for the column used. The system used was the BioLogic Chromatography Workstation. All of the columns were from Bio-Rad Laboratories. The columns used in Approach I: Tandem Columns for Human MAb, were a 5 ml Econo-Pac® DEAE blue cartridge (catalog number 732-0031) (mixed mode anion exchange/dye ligand) and a 2 ml Bio-Scale CHT2-I Column (catalog number 751-0021) packed with 10 µm Macro-Prep® ceramic hydroxyapatite Type I media. The column used for Approach II: Strong Cation Exchange for Human MAb, was the 2 ml Bio-Scale S2 column (catalog number 751-0011). This column is packed with a 10 µm Macro-Prep S strong cation exchange support. The column used for Approach III: Weak Anion Exchange for GK 1.5 Anti-mouse CD MAb and PC-61 Murine CD 25 (IL-2 Receptor P 55 alpha chain), was the 20 ml Bio-Scale DEAE20 column (catalog number 751-0037), packed with 10 µm Macro-Prep DEAE (diethylamine substituted methacrylate) support.

Approach I: Tandem Columns for Human MAb

The first approach evaluated was the use of tandem chromatography on an Econo-Pac DEAE blue cartridge and a Bio-Scale CHT-I column. This approach involves more than one chromatographic column, but was evaluated as a single step because the elution buffer from the Econo-Pac DEAE blue cartridge had the potential to act as a binding buffer for the Human MAb on the CHT-I column. The BioLogic Workstation was used to automatically load the peak from the Econo-Pac DEAE blue cartridge onto the CHT-I column. The peaks from the CHT-I column could then be collected via a fraction collector. The purification thus operated as a single step because no intervention was required to obtain purified MAb, after the method had been started from the BioLogic System controller (Figures 1 and 2).

When the peak from the Econo-Pac DEAE blue cartridge was collected in the Dynamic Loop, the valves were re-positioned as shown in Figure 2. This allowed the peak from the Econo-Pac DEAE blue cartridge, which had been collected in the Dynamic Loop, to be injected onto the CHT-I column.

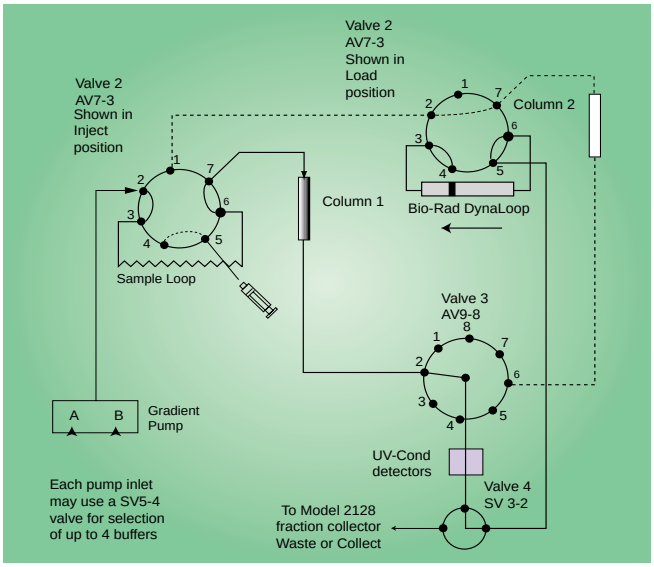


Fig. 1. *The protein sample is loaded onto column 1 via the loop of valve 1. The column is then eluted normally. The output of column 1 is routed through the detector. Valve 4 then diverts the flow either to the fraction collector or to the Bio-Rad DynaLoop ready for re-injection onto column 2. This diagram shows the liquid path from column 1 to the dynamic loop.*

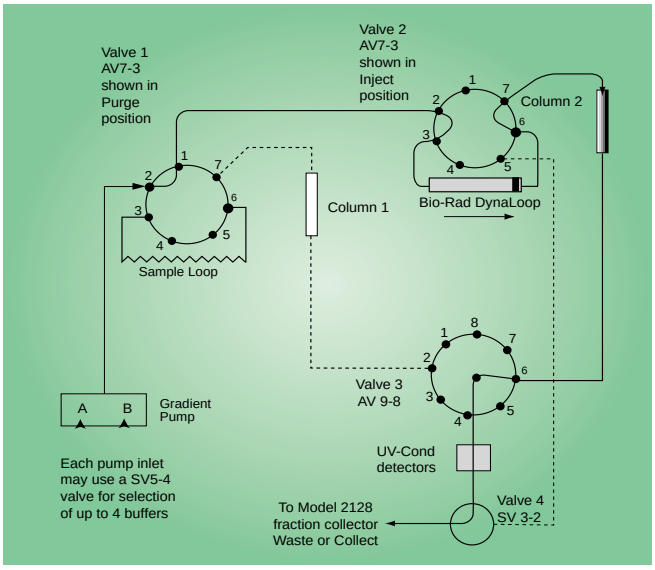


Fig. 2. *Valves are re-positioned as shown. Sample in the dynamic loop is injected onto column 2. Elution can then proceed, with fractions being collected into fraction collector.*

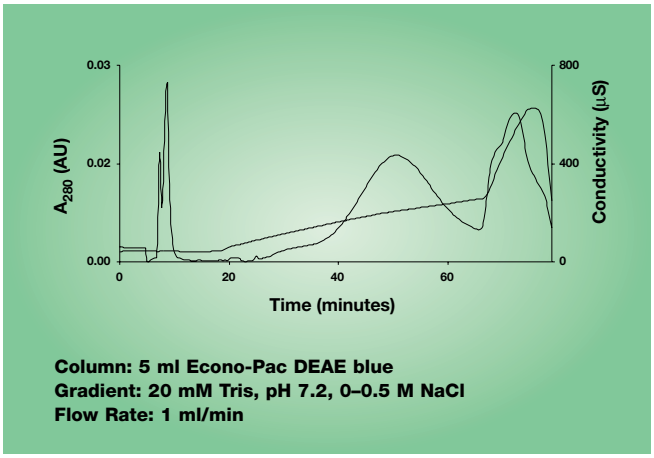


Fig. 3. *Separation of human monoclonal antibody using DEAE Affi-Gel blue gel.*

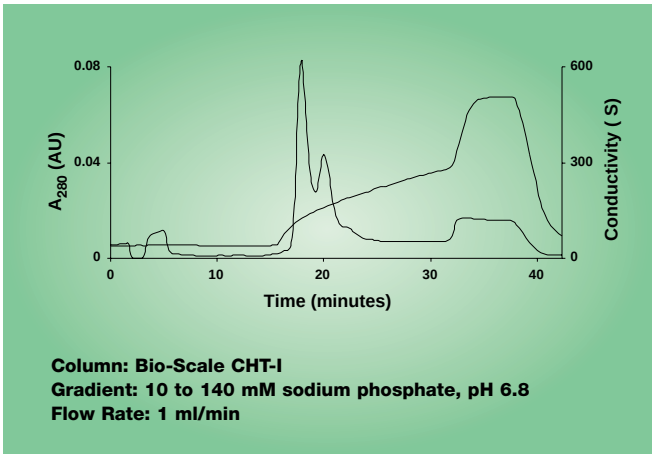


Fig. 4. *Separation of IgG containing fraction from Figure 3 using ceramic hydroxyapatite.*

This study was divided into two phases. The first phase involved the search for buffer conditions which would elute the human MAb from the Econo-Pac DEAE blue cartridge while still allowing the antibody to bind to the CHT-I column. The results shown in Figures 3 and 5 are from this phase of the project. The second phase involved the implementation of the automation.

The DEAE Affi-Gel blue gel is a bifunctional affinity gel containing Cibacron blue F3GA dye covalently attached to DEAE Bio-Gel® A agarose gel. The dye binds albumin and the DEAE group binds the remaining acidic proteins.[1] This gel offers several advantages over Protein A. These include the ability to bind all IgG subclasses, mild elution conditions, complete removal of all proteases, and significantly lower cost than Protein A. Under the elution conditions of a gradient from 0 to 0.125 M NaCl in 20 mM Tris-HCl, pH 7.2, the antibody eluted but the albumin remained bound to the column (Figure 3).

Fractions 16–24 from the Econo-Pac DEAE blue cartridge were pooled and loaded directly onto the Bio-Scale CHT2-I ceramic hydroxyapatite column. The pooled fractions bound to the CHT-I column, and were eluted using a 10 to 140 mM sodium phosphate gradient, pH 6.8 (Figure 4).
Figure 5 is the PAGE gel which was run on fractions 16–21 from the CHT-I column. It shows that the antibody has been significantly purified.

Approach II: Strong Cation Exchange for Human Monoclonal Antibody

The second approach developed for the purification of hMAb used the Bio-Scale S2 strong cation exchange column. This column is packed with a 10 µm methacrylate based bead. Since the same bead is available in a 50 µm bulk format, conditions developed for this separation could easily be scaled up to the 50 µm bead for large scale purifications.

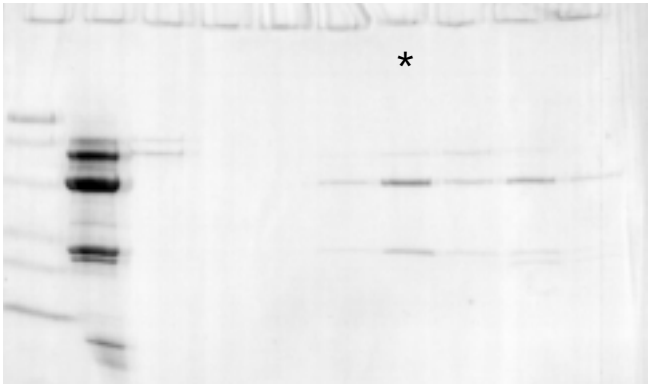


Fig. 5. *SDS-PAGE gel of fraction obtained from Figure 4. Lane labeled with the asterisk is fraction from chromatogram in Figure 4.*

Cation exchange was investigated at pH 5.5 in MES. At this pH, albumin (pI 4.5–5.0) is negatively charged and does not bind. The human MAb is positively charged at this pH and does bind to the column. Elution is done using a sodium chloride gradient (Figure 6).

A gel was run on Fractions 8–10. As Figure 7 clearly shows, fraction 8 displays heavy and light bands characteristic of antibodies analyzed via SDS-PAGE with no visible contaminating proteins.

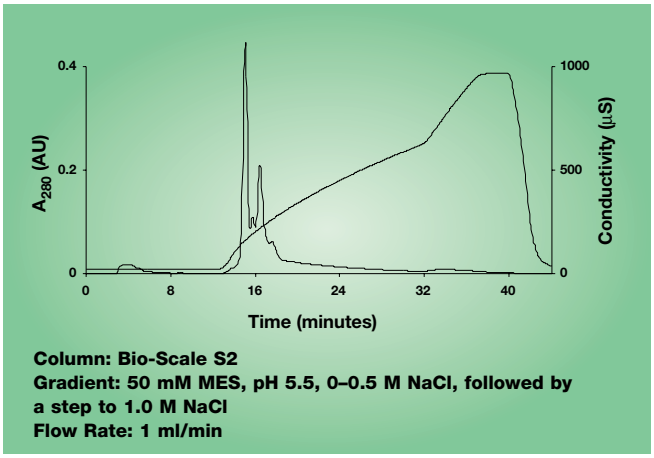


Fig. 6. *Strong cation exchange of human MAb.*

Approach III: Weak Anion Exchange for GK 1.5 Anti-mouse CD MAb and PC-61 Murine CD 25 (IL-2 receptor p 55 alpha chain)[4]

The purification of Mouse GK 1.5 anti-mouse CD MAb[3] and PC-61 murine CD 25 (IL-2 receptor p 55 alpha chain)[4] was evaluated on the Bio-Scale DEAE20 weak anion exchange column. This column contains 20 ml of 10 µm methacrylate based weak anion exchange support.

The 10 µm bead was chosen because of its high degree of resolution, which makes it easier to develop and optimize a purification method. DEAE was chosen over a Q-type strong anion exchanger for historical reasons, as earlier antibody purification work has used weak anion exchange. Most immunoglobulins have pIs in the range of 6–8, hence a pH of 7.5 was chosen for the work on the DEAE column. Most immunoglobulins should bind under these conditions and elute early in the gradient.

The load for the GK 1.5 anti-mouse CD 4 MAb was 120 mg. The gradient was from 0 to 160 mM NaCl. As shown in Figure 8, two small peaks, eluted on both sides of the main peak, which contains a shoulder. Figure 9 shows the SDS-PAGE analysis of the fraction 11 from the main peak.

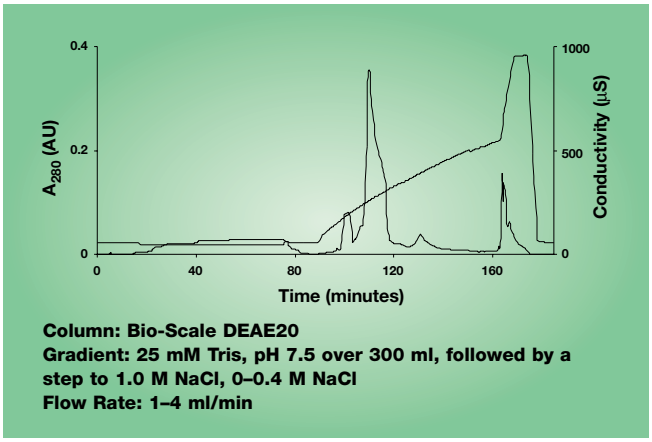


Fig. 7. *Separation of GK 1.5 anti-mouse CD4 MAb using Bio-Scale DEAE column.*

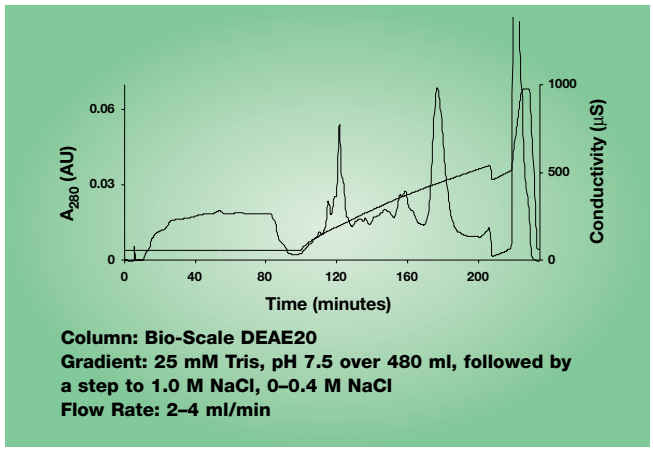
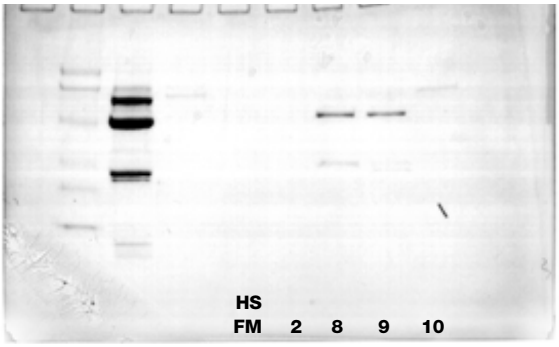


Fig. 8. *Weak anion exchange of PC-61 murine CD 25.[4]*

The load for the murine CD 25 (IL-2 receptor p 55 alpha chain) was 154 mg. The gradient was from 0 to 160 mM NaCl. As shown in Figure 10, peaks are eluting throughout the gradient. Figure 11 shows the purity.



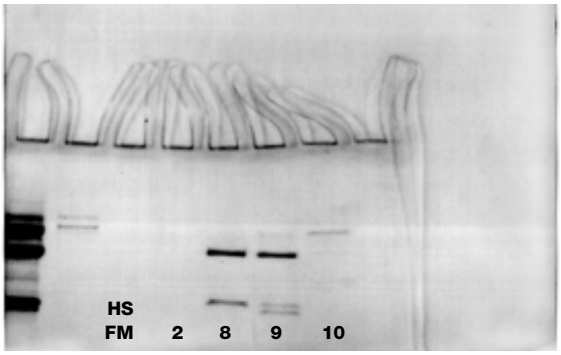


Fig. 9. *SDS-PAGE of fractions from Figure 6.*

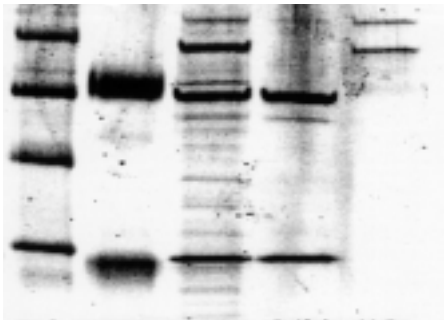


Fig. 10. *SDS-PAGE of separation in Figure 8. IgG from PC1.53.*

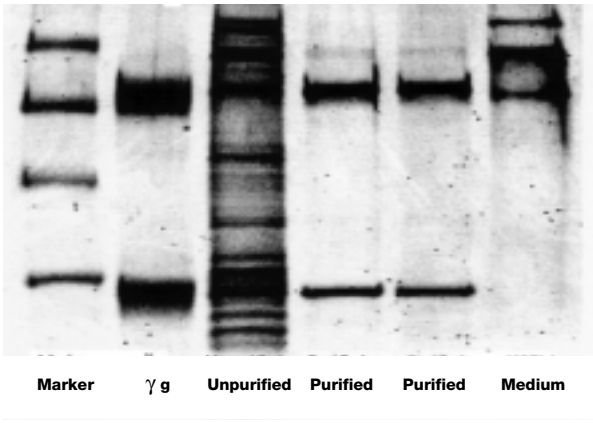


Fig. 11. *SDS-PAGE of fractions from Figure 10. IgG_1 from PC61.53.*

Conclusion

The purpose of this study was to develop single step, non-Protein A based MAb purification procedures. Three approaches were investigated, the tandem use of DEAE Affi-Gel blue followed by a Bio-Scale CHT-I column, the use of strong cation exchange on the Bio-Scale S2 column, and the use of weak anion exchange on the Bio-Scale DEAE20 column. The tandem use of DEAE Affi-Gel blue followed by Bio-Scale CHT-I is useful when the pI of the MAb is close enough to the pI of albumin to cause problems with ion exchange. In addition, the use of CHT-I could allow different idiotypes of the MAb to be separated. A two step procedure can be easily automated using the BioLogic system. The strong cation exchange approach yielded the purest antibody, as shown in the gel in Figure 7. This approach would be less effective in cases where the pI of the MAb is close to the pI of albumin.

The advantage of the weak anion exchange approach is the great number of literature references which employ this technique. The disadvantage of using any weak ion exchanger is the requirement for large equilibration volumes when changing pH, compared to strong ion exchangers which have no titratable moieties in the pH region where protein separations are conducted.

References

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4 *Ceredig,* et al., Nature, **314**, *98–100.*

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