Comparison of Radial and Axial Flow Chromatography for Monoclonal Antibody Downstream Processing at Bench and Pilot Scales

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ABSTRACT

Axial Flow Chromatography (AFC) is widely used for the purification of therapeutic Monoclonal Antibodies (MAbs). However, AFC columns can generate high pressure drops across the resin bed, preventing operation of the column at high flow rates especially at pilot or manufacturing-scales. Radial Flow Chromatography (RFC) was developed to provide lower pressure drops during chromatographic separations. In these studies, small and pilot-scale columns were used to evaluate purification of a MAb using both AFC and RFC technologies. A bench-scale, wedge RFC column (250 mL) was compared to a bench-scale AFC column at various linear velocities with resulting Residence Times (RT) using Protein A resin for the recovery of a monoclonal antibody. The bench RFC column was successfully operated at 4.5 min RT for equilibration and loading steps and 2 min RT for washing, elution and cleaning steps without compromising yield. The RFC column had approximately 50% lower pressure drop than the AFC column at similar flow rates. The process was then scaled-up to 5 L using a pilot-scale RFC column. The 5-L RFC column was operated at 4.5 min RT for equilibration and loading and 2 min. RT for washing, elution and cleaning with no loss of yield. However, pressure drop across the 5 L RFC column was higher than expected, but antibody recovery yields were similar for both column types. Subsequent investigations revealed a potential design issue with the RFC column. Overall, RFC has great potential to be used for pilot or manufacturing scale without high pressure drop concerns, which will certainly improve processing efficiency.

Keywords: Monoclonal Antibodies, Purification, Chromatography, Protein A, Axial Flow Chromatography, Radial Flow Chromatography

1. INTRODUCTION

Monoclonal Antibodies (MAbs) continue to gain importance since their introduction for therapeutic use in the 1980s. Their development is summarized by many researchers (Tami et al., 1986; Reichert, 2001; Nelson et al., 2010). The first human MAB was only approved by FDA in 2002. Currently there are almost 100 human MAbs under FDA review, in phase I, II, or III of clinical trials (Nelson et al., 2010). However, their commercial production including downstream processing presents many challenges.

After initial production, the antibody needs to be separated from the culture fluid or homogenates and subsequently purified. Chromatographic methods have been commonly used for downstream processing of
MAbs. Traditionally, chromatography systems use a cylindrically-shaped vessel. Buffers or solutions containing products are pumped onto the top of the column and collected from the bottom (Fig. 1), referred as Axial Flow Chromatography (AFC). A scale-up of monoclonal antibody purification using ion exchange chromatography was reported by Jungbauer et al. (2004). Gottschalk (2008) compared column with membrane chromatography for antibody separation. However, during processing, high pressure drops may occur, which sometimes prevent operation at high flow rates especially for large pilot-scale or manufacturing-scale systems. As an alternative, Radial Flow Chromatography (RFC) was developed to reduce pressure drops in the system while maintaining high flow rates (Fig. 1). In RFC, the column is also cylindrical; however the flow of mobile phase passes from the outside of the cylinder, through the resin bed to the inside of the cylinder; separation taking place in the axial direction (Fig. 1). The packed bed is supported between two cylindrical frits and the gap between the frits represents the bed height or column length. The outer frit is the column inlet and consequently the sample initially has a large area of stationary phase with which to interact. The cross-sectional area of packing decreases progressively as the solute moves towards the center (Fig. 2). Cabanne et al. (2007) evaluated chromatographic parameters such as efficiency, capacity factor, asymmetry and resolution between AFC and RFC. Dai et al. (2009) also studied the isolation and purification of polysaccharide from Nostoc flagelliforme by RFC.

The greatest advantage of RFC is operation with less pressure drop under the same flow rates. For cases where the absorption and desorption kinetics are not limiting, RFC can be used with higher rates to reduce processing times. Actually, this is not a new method as RFC may be dated back to 1950 based on the study of Lapidus and Amundson (1950). Long after that, there were some studies reported to evaluate RFC for various applications and to compare RFC with AFC. For example, Lee (1989) evaluated RFC for trypsin purification and developed mathematical models to describe the system. Kim and Lee (1996) compared AFC and RFC for protein separation and reported that 2 or 3 times higher flow rates could be achieved by using RFC, because increased back pressure was more pronounced in AFC. With all these studies, significant progress has been made. However, there is still a need for the application of RFC to MAbs purification and the potential for manufacturing scale processing. Therefore, this study was undertaken to evaluate a specially-designed, bench-scale RFC prototype (wedge shape) and subsequent increase to pilot scale.

![Fig. 1. Axial and radial flow chromatography flow patterns](image-url)
2. MATERIALS AND METHODS

2.1. Resin

Protein A resin (MabSelect, GE Healthcare, Biosciences Corp., Piscataway, NJ) is used in this study, which is a commonly used affinity resin for Monoclonal Antibody (MAb) purification. The resin is highly cross-linked agarose with an epoxy-based coupling chemistry. The particle size is 40-130 µm.

2.2. Buffers

The following buffers were used unless otherwise stated: (i) phosphate buffered saline (Buffer # 1, 6 mM NaPi + 100 mM NaCl, pH 7.2) used for packing, equilibration and washing steps; (ii) citrate buffer (Buffer #2, 0.1M, pH 3.5) used as the elution buffer; (iii) phosphoric acid solution (Buffer # 3, 0.1N); and alkaline buffer (Buffer # 4, 50mM NaOH + 1M NaCl) used as the regeneration and cleaning buffer, respectively.

2.3. Bench-Scale Chromatography

To represent Radial Flow Chromatography (RFC), a wedge-shaped column (250 mL), manufactured by Sepragen Corp. (Hayward, CA) was used for the study. This small wedge system was used to compare RFC with AFC. A wedge column is basically a fraction of a complete column (Fig. 3), which saves resin and buffers costs. The wedge column had a total volume of 250 mL and a 16 cm bed-length. For comparison, the AFC column had a volume of 325 mL, a 5 cm diameter and a bed-height of 16.5 cm. Although the volumes of both columns were different, the bed height or length was kept similar (~16 cm).

A BioCAD chromatography system (Model 250, Applied Biosystems, Foster City, CA) was used for packing and running the RFC process. The following protocol was used for column packing: A 25% resin
slurry was prepared in Buffer #1. The wedge column was filled with Buffer #1, the prepared resin slurry was pumped into the wedge column. Slurry pumping was continued until the wedge inlet pressure reached 5-10 psig (34-69 kPa). After closing the resin inlet port with a cap, the column was connected to the chromatography system. The wedge column was then equilibrated with 10 Column Volumes (CV) of Buffer #1 at a flow rate of 84 mL min⁻¹.

For comparison, AFC column with a 5 cm diameter x 50 cm length and a bed-height of 16.5 cm was used (325 mL, Model # HP-5, Waters Corp., Milford, MA). The same BioCAD chromatography system was used for packing and running the AFC process. The following protocol was used for packing the AFC column. A 50% resin slurry was prepared in Buffer #1. After connecting the column onto the BioCAD system, the column was filled with Buffer #1 and most of the resin slurry was pumped onto the column. After installing the top flow adaptor, the packing buffer was continued to pump at 108 mL min⁻¹ until the column bed height no longer changed. After the flow was stopped, the top flow adaptor was lowered to the top of the bed. Packing buffer was pumped through the column for an additional 10 CV at the same flow rate.

2.4. Salt Test

After packing the columns, a salt test using 5M NaCl solution was performed to evaluate the column’s performance according to the following protocol. The packed column was equilibrated with Buffer #1 at 54 mL min⁻¹ for 5 min and 2 mL of 5M NaCl solution was injected onto the column. Finally, the column was washed with Buffer #1 at 54 mL min⁻¹ for 15 min. Conductivity measurements were recorded. From the obtained conductivity chromatogram, plates/meter (N/m) and Asymmetry Factors (A/F) were determined according to the resin manufacturer (GE, 2010).

2.5. Evaluation of Columns for Pressure Drops

Both wedge RFC and AFC columns were evaluated at various flow rates using Buffer #1. Flow rates for each column were calculated to represent 6, 3, 2 and 1.5 min Residence Times (RT). Pressure drops were recorded for both columns at these selected flow rates.

2.6. Monoclonal Antibody

The Monoclonal Antibody (MAb) produced using cell culture was a proprietary product of Merck and Company. The concentration of MAb was about 2 mg mL⁻¹. Cell-free broth was prepared by first centrifuging at 14,000×g and filtered through a 0.45 µ filter.

2.7. Evaluation of Bench Scale RCF and AFC Columns

The following methods were used for both wedge RCF and AFC columns. Flow rates and conditions for equilibration/loading and washing/elution/cleaning steps are described below. Each column system was flushed with 2 CV of Buffer #1 at 100 mL min⁻¹, then equilibrated with 5 CV of Buffer #1 at the selected flow rates. Each column was loaded with 3.5 L of MAb broth (~2 mg mL⁻¹) at the selected flow rates and washed with 8 CV of Buffer #1. After elution with 8 CV of Buffer #2, the columns were washed with 5 CV of Buffer #1, regenerated with 5 CV of Buffer #3, cleaned with 8 CV of Buffer #4 and finally washed with 5 CV of Buffer #1. The evaluated flow rates were: 42, 56, or 84 mL min⁻¹ for the RCF column and 54, 72, or 108 mL min⁻¹ for the AFC column during equilibration and loading. Flow rates during washing/elution/cleaning were 84, 126, or 168 mL min⁻¹ for RCF column and 158, 164, or 221 mL min⁻¹ for the AFC column. During the runs, pressure drops were recorded at each step. Broth, non-bound and eluted product were filtered through 0.45 µ filters for HPLC analysis.

2.8. Pilot-Scale Chromatography

The scalability of RFC and comparison to AFC were evaluated by using pilot-scale RFC and AFC columns. For these studies, the entire RFC column was used instead of the wedge. RFC column was an experimental, variable volume unit with a total volume of 30 L manufactured by Sepragen Corp. (Hayward, CA). To keep the column volume at 5-L, a 25-L stainless steel donut-shaped disk was placed in the column as a spacer (Fig. 4). The RFC column had a total volume of 5-L and a 15 cm bed-length. For comparison, the AFC column had a volume of 4.7-L, a 20 cm diameter and bed-height of 16 cm. The volumes of both columns were different so that the bed height or length was approximately the same (15-16 cm). A Varian chromatography skid (Model SD-1 CCS-3200, Varian Inc., Palo Alto, CA) was used for packing and running the chromatography processes for both columns.

The following protocol was used for packing the RFC column. A 25% resin slurry was prepared in Buffer #1 and the column filled with the same buffer. The 25% resin slurry was pumped into the column at 2.5 L min⁻¹ until the column inlet pressure reached 5-10 psig (34-69 kPa). After connecting the column onto the chromatography
system, the column was equilibrated at 2.5 L min$^{-1}$ in both forward and reverse directions according to the users’ manual. Column preparation was completed by pumping 10 CV of Buffer #1 at 1,111 mL min$^{-1}$.

The AFC column packed as follows. A 50% resin slurry was prepared in Buffer #1. After connecting the column to the chromatography system, the column was filled with the same buffer. Then, the resin slurry was pumped into the column, top flow adaptor installed and packing buffer pumped at 2.5 L min$^{-1}$ until the column bed height no longer changed. After the flow was stopped, the top flow adaptor was lowered to the top of the bed. Ten CVs of buffer was pumped through the column at 2,500 mL min$^{-1}$.

2.9. Salt Test

After column packing was completed, each column’s performance was evaluated by performing a salt test with 5M NaCl. The packed columns were equilibrated with Buffer #1 at 1000 mL min$^{-1}$ for 1 min. Twenty mL of 5M NaCl solution were then injected into each column. The columns were washed with Buffer #1 at 1 L min$^{-1}$ for 7 min and conductivity measurements recorded. From the obtained conductivity chromatograms, plates/meter (N/m) and Asymmetry Factors (A/F) were determined according to the resin manufacturer (GE, 2010).

2.10. Evaluation of Columns for Pressure Drops

Pressure drops were determined for both column types using in-line pressure gauges at various flow rates (500-3,000 mL min$^{-1}$) using Buffer #1.

2.11. Monoclonal Antibody

Monoclonal Antibody (MAb) was produced in cell culture and was a proprietary product of Merck and Company. The concentration of MAb was 0.69 mg mL$^{-1}$. Approximately 100 L of cell-free culture broth was prepared for chromatography by centrifugation at 14,000×g followed by filtration through 0.45 µ filter.

2.12. Evaluation of RCF and AFC Columns at Various Flow Rates

For this scale-up study, 4.5 min Retention Times (RT) for equilibration/loading and 2 min RTs for washing/elution/cleaning were suggested by the bench-scale studies. Therefore, 1,111 and 2,500 mL min$^{-1}$ flow rates were used for the RCF during equilibration/loading and washing/elution/cleaning, respectively, whereas 1,046 and 2,355 mL min$^{-1}$ flow rates were used for the AFC during equilibration/loading and washing/elution/cleaning, respectively.

The following protocol was used for both RCF and AFC columns: The columns were equilibrated with 5 CVs of Buffer #1, then loaded with MAb broth at the selected flow rate and washed with 8 CV of Buffer #1. After elution with 5 CV of Buffer #2, the columns were washed with 5 CV of Buffer #1, regenerated with 5 CV of Buffer #3, cleaned with 8 CV of Buffer #4 and finally washed with 5 CV of Buffer #1. Pressure drops were recorded at each step. Broth, non-bound and eluted MAb product were filtered through 0.45 micron filter for HPLC analysis.

2.13. HPLC Analysis

MAb titers were determined at 280 nm using an HP Agilent 1100 HPLC (Agilent Technologies, Palo Alto, CA) and run at room temperature (22-26°C). The HPLC column was a Protein a HLD Disk (BIA Separations, Villach, Austria). Mobile phases were 50 mM NaPhosphate+100 mM NaCl, pH 7.2 (Buffer A) for equilibration/washing and 0.5M Acetic Acid (Buffer B) for elution. HPLC flow rate was 3 mL min$^{-1}$ with a total run time of 6 min, which included 2 min with Buffer A for equilibration, followed by 1 min Buffer B for elution and 3 min Buffer A for washing. Purified MAb was used as a standard.

2.14. Percent Recovery Yield

After HPLC analysis, the following equation was used to determine recovery yield:

\[
\text{Recovery yield (\%)} = \left(\frac{\text{MAb Broth Conc} \times \text{Broth Vol.}}{\text{MAb Conc in the eluent} \times \text{Eluent Vol.}}\right) \times 100
\]
3. RESULTS AND DISCUSSION

3.1. Bench Scale Chromatography

In our comparison of bench scale RFC vs. AFC, a 250 mL wedge column and 325 mL axial flow column were evaluated with Protein A resin under the same residence time (flow conditions). The volumes of both columns were kept different to maintain equivalent bed heights (15-16 cm). Based upon the salt tests, the average number of plates (N) and Asymmetry Factors (AF) were determined as 9,637 and 1.16 for the wedge column compared to 7,759 and 1.16 for the AFC column. AF values were exactly the same. Although the N values were not identical, they were not significantly different, demonstrating similar chromatographic performance. Also, it is worth to mention that adsorptive protein A separation is not very plate sensitive in comparison to other chromatographic methods such as size exclusion chromatography. Conversely, there was a significant difference in pressure drops for both columns. Figure 5 shows the pressure drops for RFC and AFC columns packed with Protein A resin and their residence times using equilibrium buffer. Pressure drops significantly increase for both columns as flow rates increased (i.e., RTs decreased). However, the AFC column showed a much higher pressure drop at any given flow rate. The difference is 50% or higher (for some cases up to 85%) depending on the flow rate. This clearly indicates that RFC column has significant advantages in terms of lower pressure drop, meaning that RFC columns can be operated at higher flow rates, with less pressure drops than AFC columns.

Then, bench scale RFC and AFC columns were evaluated with Protein a resin for MAb recovery (Fig. 5). Table 1 summarizes the RFC and AFC pressure drops at various loading and elution/washing steps and their recovery yields. The first attempt was to run at 6-min Residence Time (RT) for equilibration/loading and 3 min RT for washing/elution/cleaning. At these conditions, RFC demonstrated extremely low pressure drops (34 kPa for loading; 159 kPa for washing), whereas AFC pressure drops were 76 kPa for loading and 345 kPa for washing, nearly two-times higher for all steps (Table 1). The recovery for both RFC and AFC columns were very similar; 91.8 and 92.3%, respectively (Table 1).

When the RT for both column types was reduced to 3 min for all steps, The RFC column again demonstrated much lower, nearly two-fold, pressure drops (179 kPa for loading; 159 kPa for washing), whereas the pressure drops were 352 kPa for loading and 331 kPa for washing for the AFC column (Table 1). The recovery for both columns was slightly reduced due to an increase in unbound product in the spent broth; 88.8% for RFC and 89.4% for AFC, but they are significantly different from each other (Table 1). Therefore, it was decided to increase RT to 4.5 min to decrease unbound MAb in the effluent.

Table 1. Bench scale RFC and AFC column summary of pressure drop and MAb recovery yield at various loading and elution/washing flow rates

| Radial flow column (250 mL) | Loading flow Rate (residence time) | Elution and cleaning flow rate | Pressure drop (residence time) (kPa)-Step | Unbound MAb (%) | Total MAb Yield (%) |
|-----------------------------|-----------------------------------|--------------------------------|-------------------------------------------|-----------------|---------------------|
| 42 ml min⁻¹ (6 min)         | 84 ml min⁻¹ (3 min)               | 34-Load 159-Wash              | 1.6                                       | 91.8            |
| 84 ml min⁻¹ (3 min)         | 84 ml min⁻¹ (3 min)               | 179-Load 159-Wash             | 4.5                                       | 88.8            |
| 56 ml min⁻¹ (4.5 min)       | 84 ml min⁻¹ (3 min)               | 69-Load 159-Wash              | 1.7                                       | 90.8            |
| 56 ml min⁻¹ (4.5 min)       | 126 ml min⁻¹ (2 min)              | 69-Load 303-Wash              | 1.8                                       | 89.2            |
| 56 ml min⁻¹ (4.5 min)       | 168 ml min⁻¹ (1.5 min)            | 69-Load 552-Wash              | 3.1                                       | 86.0            |
| Axial Flow                  |                                    |                                |                                            |                 |
| Column (325 mL)             | 54 ml min⁻¹ (6 min)               | 108 ml min⁻¹ (3 min)          | 76-Load 345-Wash                          | 1.5             | 92.3                |
| 108 ml min⁻¹ (3 min)        | 108 ml min⁻¹ (3 min)              | 352-Load 331-Wash             | 4.1                                       | 89.4            |
| 72 ml min⁻¹ (4.5 min)       | 162 ml min⁻¹ (2 min)              | 379-Load 710-Wash             | 2.5                                       | 88.7            |

Table 2. Pilot Scale RFC and AFC column summary for MAb yield and pressure drops.

| Chromatography Type | Loading flow rate (residence time) | Elution and cleaning flow rate (res time) | Pressure (kPA) | Total MAb Yield (%) |
|---------------------|-----------------------------------|-----------------------------------------|----------------|---------------------|
| RFC                 | 1,111 ml min⁻¹ (4.5 min)          | 2,500 ml min⁻¹ (2 min)                 | 48 Load 103 Wash 103 Elution 352 Cleaning | 98              |
| AFC                 | 1,046 ml min⁻¹ (4.5 min)          | 2,355 ml min⁻¹ (2 min)                 | 69 Load 124 Wash 124 Elution 138 Cleaning | 92              |
Therefore, in the third experiment, RT was set at 4.5 min for loading while keeping RT for equilibration/loading at 2 min RT. For these conditions, the unbound MAb in the effluent again decreased to 1.7% for RFC and 2.5% for AFC. This resulted in increased recovery to 90.8% for RFC and 88.7% for AFC. This again demonstrated a more than two-fold decrease in pressure drops; 69 kPa for loading; 159 kPa for washing for the RFC, compared to the AFC column (352 kPa for loading and 331 kPa for washing) (Table 1).

To reduce the time for washing/elution/cleaning, RT was decreased to 2 min while keeping the RT for loading at 4.5 min. Even under these conditions the RFC column demonstrated significantly lower (more than twice) pressure drops (69 kPa for loading; 303 kPa for washing), compared to the AFC column (379 kPa for loading and 710 kPa for washing) (Table 1). The recovery yields for both RFC and AFC columns slightly decreased to 89.2% for RFC and 88.7% for the AFC column (Table 1). In the final bench scale experiment, the RFC column operating conditions were 4.5 min RT for loading and 1.5 min for elution and cleaning. These conditions resulted in 69 kPa for loading; 552 kPa for washing and a recovery yield of 86.0 (Table 1). It wasn’t possible to operate the AFC at these conditions, because the pressure drop was too high to operate. These studies clearly demonstrate that RFC permits high flow rates without causing high pressure drops. It can be concluded from these data that RFC columns can be operated at 4.5 min RT for equilibration/loading and 2 min RT for washing/elution/cleaning without creating system shutdown and compromising the recovery yield for MAbs.

### 3.2. Pilot-Scale Chromatography

Based on the salt test, the average number of plates (N/m) and Asymmetry Factors (AF) were determined to be 1227 and 1.97 for the 5-L RFC column and 2249 and 1.09 for the 4.7-L AFC column. These differences between the two column types are unexpected and could have resulted from inadequate packing of the RFC column due to its experimental variable volume system. This might not be a problem with full scale systems. Figure 6 shows the pressure drops for the pilot scale RFC and AFC columns. At all flow rates, there was a pressure drop difference between the RFC and AFC columns, however not as high as previously seen at the bench scale; only less than 50% (~30% average) for almost all flow rates for pilot scale, whereas, the difference was 50% or above depending on the flow rate for bench scale. Even though this clearly indicated that there is problem in the pilot scale RFC system, which contributed significantly to the pressure drop, there is still certainly an advantage of RFC over AFC column. Table 2 presents the column yields and pressure drops at various loading and elution/washing flow rates. Both columns were operated at 4.5 min RT for equilibration/loading and 2 min RT for washing/elution/cleaning as described earlier. Under these conditions, RFC demonstrated pressure drops of 48 kPa for loading; 103 kPa for washing/elution and 352 kPa for cleaning, whereas pressure drops for the AFC column were 69 kPa for loading and 124 kPa for washing/elution and 138 kPa for cleaning. The pressure differences between RFC and AFC were not as pronounced as the previous bench scale studies due to the unexpected high system pressure for the RFC column. This is evident by the extremely high pressure drop (352 kPa) during cleaning. A possible explanation for this is that aggregates generated during the cleaning step might plug the small perimeter gap around the vessel, which might need to be enlarged to prevent this problem (Fig. 4). According to the manufacturer, Sepragen Corp., this problem was improved their design to address this issue. Despite this potential design issue on the existing RFC system, it demonstrated 20% less...
average pressure drops during loading, washing and elution steps. Finally, the yields for both RFC and AFC columns were above 90%.

4. CONCLUSION

The bench scale wedge RFC column was successfully operated at 4.5 min RT for equilibration and loading steps and 2 min RT for the washing, elution, cleaning steps without compromising yield. In addition, the RFC column had at least 50% lower pressure drop compared to the AFC column under the same flow condition. The process was then scaled-up to 5 L using a pilot-scale RFC column which was operated at 4.5 min RT for equilibration/loading and 2 min RT for washing/elution/cleaning without compromising the yield. Pressure drops were higher than expected for the RFC column, suggesting that some mechanical modification is needed on the RFC column design. Overall, this study demonstrated that RFC can significantly help to reduce column chromatography processing times without compromising recovery yields.

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