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Predictable scale-up through column design and robust packing methodology

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Introduction

Demands to develop more rapid and cost-effective biopharmaceutical processes have led to an increased focus on minimizing downtime and non-value adding activities. Chromatography columns are a central part of any biopharmaceutical process and much time is spent optimizing the packing and performance of columns. Modern chromatography media have made it possible to operate at very high flow rates, leading to an increased demand on columns and supporting systems to fully utilize the potential of these media. To achieve optimal efficiency, columns need to be packed quickly and reliably at all scales, demanding minimal set-up and operator time. They also need to perform consistently at all scales throughout the process development procedure, in order to avoid costly surprises in late phases. The ability to predict the separation performance and operational parameters at the final production scale is vital for success. This study describes the characteristics and performance of AxiChrom™ columns spanning the 50 to 1000 mm diameter range.



Fig 1. AxiChrom 600 column and AxiChrom Master.



Column Packing

For a column to operate reliably and consistently over a long period of time it needs to be properly packed every time. Robustness is a key feature, requiring simplicity of packing. AxiChrom columns currently range from 50 to 1000 mm in diameter. The design and characterization of this column line is focused on maximizing robustness while increasing safety and operational simplicity. An axial compression type column was chosen for its versatility, proven performance, and ease of scale-up and scale-down (which was a focus of this study). The axial compression technique also simplifies the packing of the many high flow media on the market, where the technique of flow packing becomes quite difficult at the required high flow rates. Another primary benefit of axial compression is that very few packing parameters are needed – the only controlled variables are the packing adapter speed and the amount of mechanical compression applied to the bed. This enables a high degree of automation in the process. A pre-defined Packing Factor* ensures a high success rate and that the bed is consistently compressed to the optimal level. This technique reduces problems with pressure drop in hoses and hardware, as well as variations in bed height and diameter. The Packing Factor has proven to be the most vital parameter for the repeatability of robust packing, while the optimal adapter speed for most media depends mostly on the particle size and density of the particles. The other two variable parameters are slurry concentration and slurry buffer. The slurry concentration often has limited impact on the packing, while the specific buffer often plays a role in developing the most optimal packing method. With AxiChrom, all verified methods have been entered into a packing database, where built-in packing methods guide the user to achieve successful, robust packing at all scales.

*Packing factor (PF) is defined as:
$$\frac{\text{Consolidated bed height}}{\text{Packed bed height}}$$

Packing methodology

Axial compression packing consists of 4 main steps (Fig 2):

- 1) After the column is filled with slurry, consolidation begins, with the adapter pushing the slurry downwards, expelling excess liquid through the bottom screen
- 2) At some point all medium has been settled into a consolidated bed
- 3) When the adapter meets the consolidated bed, this is detected and/or confirmed by the user
- 4) The adapter is stopped when the correct Packing Factor is reached (i.e., when the bed is compressed to an optimal degree)

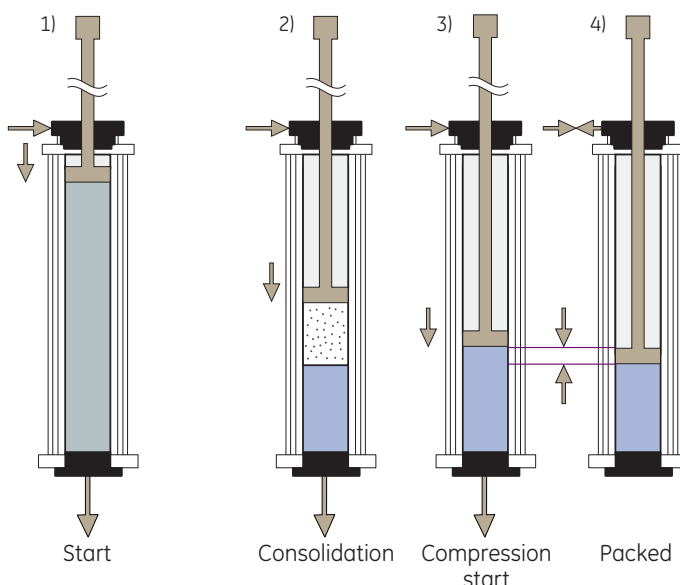


Fig 2. A schematic view of packing using axial compression.

Liquid distribution design

The liquid distribution system is a vital part of a chromatography column as it ensures that the incoming liquid is distributed and collected evenly over the entire cross-sectional surface of the column without back-mixing or stagnant zones. At the same time, the distribution system cannot create too much pressure drop as this may limit the operational velocities and choice of column and chromatography system. In addition, since the distribution needs to be the same or similar at all scales, the changes with column diameter must also be considered. Therefore, Computational Fluid Dynamics (CFD) tools were utilized to theoretically optimize a distribution system before the prototypes were manufactured and experimentally verified.

The evaluation of the designs was done by comparing the contribution of the distribution system to the overall band broadening, here characterized by the reduced plate height (HETP normalized by the average particle size of the medium). Results indicated that the largest particles were least affected by the distribution system, since the plate height is larger for large particles. Another factor is the bed height, where taller beds are less sensitive to the contribution of the distribution system. It was also obvious that the larger the column, the larger was the contribution from the distribution system (Fig 3). In the 50mm column the results only look questionable for 5 μm particles below a 10 cm bed height. For the 1 m column the lower limit appears to be near 30 μm particles, but only for bed heights of approximately 10 cm and below, which is acceptable. Similar effects are expected for asymmetry.

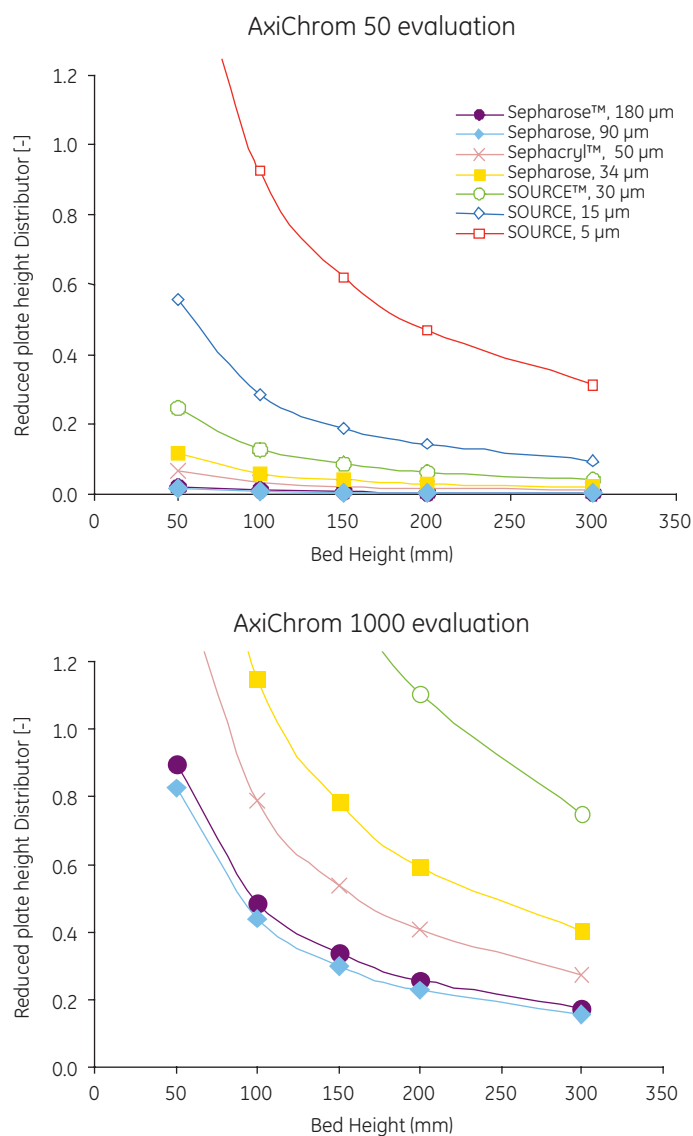


Fig 3. Evaluation of the distribution system in AxiChrom columns.

When a suitable general distribution system design had been identified it was necessary to develop and verify packing methods to ensure that the demands on efficiency, pressure drop, and scalability could be met for a variety of different media, all having different properties and demands. A verification plan with a media matrix was developed in order to ensure that the concept was fully functional, as shown below.

Column packing verification

Packing studies

- At least three packs per media/bed height/diameter were made
- 1–5 different ligands on each base matrix were tested
- The Packing Factor was varied from optimal to $\pm 2\%$
- Reproducibility and stability have been tested for each medium and bed height
- Various packing buffers were used: Water, 20% ethanol, and salt containing buffers

Requirements

- Reduced plate height < 3 ; $0.8 < A_s < 1.5$; less than 20% change after 16 h stability test at maximum operational flow rate
- All media pressure/flow criteria must be fulfilled

Table 1. Example verification matrix for AxiChrom 400, 600, and 1000 columns

Column diameter (mm)	Bed height 10 cm	Bed height 20 cm	Bed height 30 cm	Bed height 40 cm
400	Sepharose FF	MabSelect Xtra™	Sepharose FF	MabSelect SuRe™
	Sepharose HP	MabSelect SuRe		Capto™
	Capto	Capto		
600	Sepharose FF	Sepharose XL	Sepharose FF	Capto
	Sepharose HP	Sepharose HP	Sepharose HP	
		MabSelect Xtra		
		MabSelect SuRe		
		Capto		
1000	Sepharose FF		Sepharose FF	
	Sepharose HP	Sepharose HP	Sepharose HP	
	Capto	Capto	Capto	
	Sepharose Big		Sepharose Big	
	Beads		Beads	

Results

Table 2. Packing results for various media on AxiChrom columns

Media	Diameter (mm)	Height (cm)	Average N/m	Average A_s
Capto Q	50	20	6900	1.15
	50	40	7100	1.01
	70	20	6600	1.23
	70	30	6200	1.25
	400	20	7500	1.17
	400	40	7200	1.13
	1000	10	7500	1.30
MabSelect™	50	20	7900	1.05
MabSelect SuRE	400	20	8300	1.13
	400	35	8200	1.08
	600	20	8200	1.20
	50	10	6600	1.32
SP Sepharose Fast Flow	70	10	7100	1.32
	70	30	7200	1.04
	400	10	5500	1.35
	400	30	7100	1.20
	1000	10	5500	1.30
	1000	30	6000	1.20
SP Sepharose High Performance	600	10	13000	1.30
	600	20	18000	1.20
	600	30	16000	1.05

The packing results in Table 2 support the earlier theoretical studies, especially in terms of asymmetry.



Fig 4. AxiChrom 70 column.

Analyzing test conditions

Not only were all packed beds tested for optimal efficiency, but the effect of test conditions was also analyzed as this may significantly affect the results.

The speed at which the HETP test sample is applied and run through the bed is important for two main reasons. The first is that through increased mass transfer restrictions, the efficiency of the bed decreases. In this case, where Phenyl Sepharose Fast Flow, with an average particle size of 90 microns, is packed to 20 and 30 cm bed heights, the best efficiency is found at low velocities near 20 cm/h. When increasing the test speed, the peak will broaden and the plate height will increase. The other effect is that of sample distribution, whereby at low speed, the flow in the tubing is more susceptible to mixing. In addition, the sample is better distributed when the pressure drop in the column is higher. At low speed and low bed heights, asymmetry tends to be higher than at higher speed and in taller beds (where the pressure drop is higher, as illustrated by the asymmetry for the 20 cm bed in Fig 5).

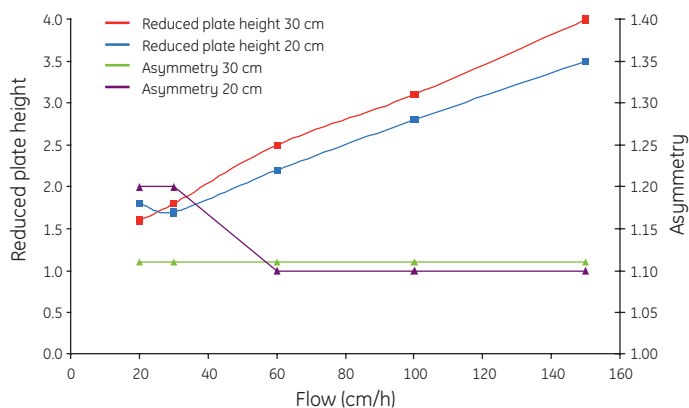


Fig 5. Test speed analysis showing changes in reduced plate height and asymmetry values with increased test speed.

The applied sample volume also affects the efficiency test results. As the applied sample volume increases, the peak will broaden due to the larger volume of sample. But the most notable effect is that on asymmetry. In this case, the very small 0.5% sample is very susceptible to mixing in the tubing and is already distorted when entering the packed bed. It is further distorted as it exits the column before being detected. In this case (Fig 6), where the bed height is only 10 cm, the extra-column effect is not completely negligible until the sample size is 4% of the column volume. Had the bed been taller, the asymmetry would have been lower at a smaller sample size.

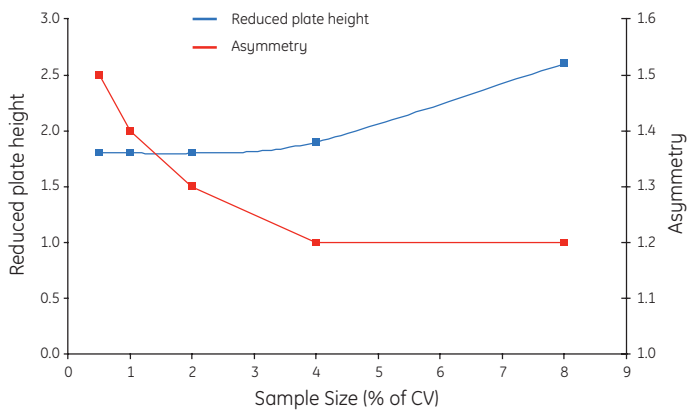


Fig 6. Sample size analysis on 10 cm Sepharose Fast Flow bed in an AxiChrom 600 mm column.

The analyses of test speed and sample size show that it is important to know what effects these two variables have on the efficiency and asymmetry of a packed bed. Acceptance specifications must be set with the test conditions in mind.

Column pressure/flow properties

In addition to being important characterization parameters, packed bed efficiency and stability are also important when designing a purification process as they can also affect the pressure and flow properties and how these may change with scale. Due to the wall support (through friction between the wall and the particles in the bed), a bed in a narrow column does not compress as much as in a larger column at a given liquid velocity. Therefore it is often possible to operate a smaller column at higher liquid velocities than in a wider column.

There are several scale-up models available that give a reasonably accurate estimate of large-scale pressure and flow properties by utilizing special measurement equipment or empirical data from smaller columns. One of the potential problems with these models is that they only predict results with an approximate accuracy of 10%–20%. Another problem is that the models do not take anything but the bed into consideration. Other effects such as pressure limitations in systems and column hardware must be accounted for, as must the dimensions of the hardware (such as inlet and outlet piping), distribution systems, and hoses. The packing buffer may also play an important role since some media require a specific packing buffer for best results. Many of these special packing buffers contain salt, which in some cases can alter the settling behavior of the media and therefore the properties of the packed bed (the latter is not commonly accounted for).

Therefore, it is quite difficult to estimate the pressure drop in a bed at a given velocity. It is also difficult to estimate the highest operational velocity that can be used in the purification process. Below, a few examples of common issues will be presented for two different media, Capto S and SP Sepharose Fast Flow. The first example (Fig 7) is from data obtained with Capto S in 50 and 400 mm AxiChrom columns at 20 and 40 cm bed height. The Packing Factor was higher in the small columns (1.15 compared to 1.10) due to the salt containing buffer, which caused the bed to settle less densely. The Compression Factor, which is the ratio between the gravity settled media (in water) and the packed bed height, is the same, which neutralizes the effect of the buffer.

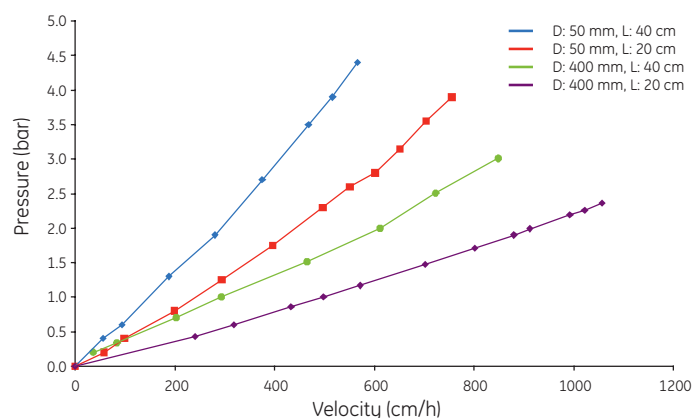


Fig 7. Pressure and flow data with column pressure drop included.

Surprisingly, the pressure drop in the smallest column is higher. In this example it is obvious that the hardware affects the overall pressure over the column and not only the packed bed. In this case the pressure is approximately twice as high in the small column. The 50 mm column is operated with a much smaller system than the large column. The hoses are thin and optimized for optimal bed efficiency in order to avoid the issues with sample volume and mixing in hoses (as described previously). However, both the small column and the system used for lab- and pilot-scale chromatography have higher pressure ratings and the pressure is far below this rating. This could pose a problem for scale-up. Assuming the same or even higher pressure drop in the process-scale column (where the pressure rating is often 3 bar), the flow rate would be limited to a maximum of 500–600 cm/h for the 20 cm bed instead of the 1000 cm/h that was achieved in the 400 mm column.

The most important aspect for process development and scale-up is that the same operational velocity can be used with equipment suitable for the scale in question without exceeding any pressure limits. In this case, both the 50 mm and the 400 mm column can be operated at 700 cm/h at 20 cm bed heights, which was the target.

In the second example (Fig 8), with SP Sepharose Fast Flow, the pressure drop by the column and system were subtracted and the curves display only the pressure drop over the packed bed. Here, models and theory agree better, since only the physical properties of the bed and the wall effect are considered. In this case, the results are more as expected since the pressure drop is larger in the larger column.

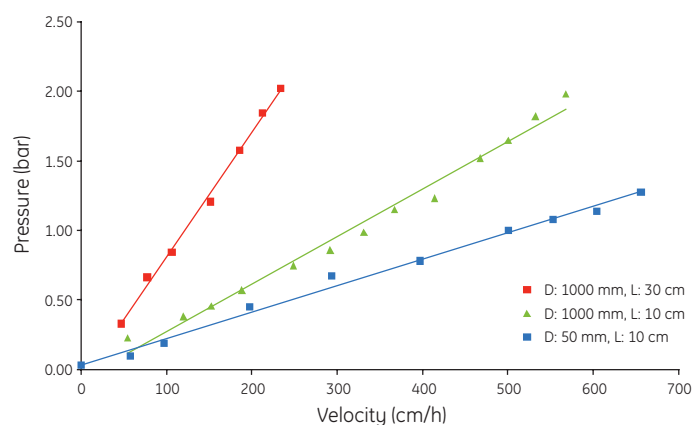


Fig 8. Pressure and flow properties over the bed.

In conclusion, it is vital for any purification process scale-up to assess the maximum operational velocity at large scale. It is also important to assess the column pressure drop (including the hardware) at all scales to ensure that no pressure rating is exceeded.

When the packing procedure has been developed and thoroughly verified, the test conditions have been identified, and the pressure/flow scale-up pattern has been established, the column can be tested under more realistic conditions with a protein separation.

Protein scale-up study

The purpose of this study was to show that the same results (i.e., bed efficiency and the pattern of the chromatograms) can be achieved in three different sizes of AxiChrom columns. Three columns (AxiChrom 70 connected to an ÄKTApilot™ system, AxiChrom 400 connected to an ÄKTApilot™ 1/2" system, and AxiChrom 1000 connected to an ÄKTApilot™ 1") were packed with SP Sepharose Fast Flow. Bovine Serum Albumin (BSA) and Lactoferrin were applied to the columns according to the method described below. Buffers and sample were made in large containers and then divided to the three columns, which were run in parallel. AxiChrom 70 was run three times on the same system, with the same buffers and sample in each run to investigate reproducibility.

Columns were packed according to the preprogrammed packing methods and the bed efficiency was tested. The results are presented in Table 3.

Table 3. Effects of AxiChrom column size on bed efficiency results

	AxiChrom 70	AxiChrom 400	AxiChrom 1000
Bed height	19.6 cm	19.5 cm	19.5 cm
N/m	7000	7450	7340
A_s	1.18	1.09	1.12

An overlay picture of the HETP peak profiles for the different columns is shown in Figure 9.

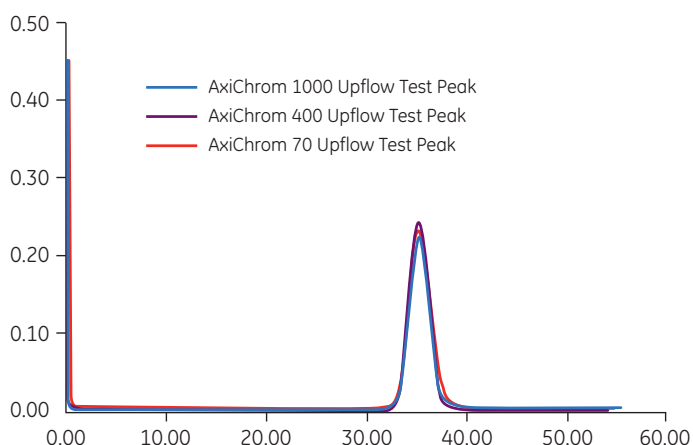


Fig 9. HETP overlay profiles.

Separation process

Buffer A: 50 mM Acetic acid pH 4.5
Buffer B: Buffer A + 1.0 M NaCl pH 4.5
Sample: 7.5 mg BSA + 2.5 mg Lactoferrin/ml in Buffer A
Direction: Up-Flow
Linear velocity: 150 cm/h at equilibration, sample application and wash. 100 cm/h at elution, CIP and re-equilibration
Equilibration: 5 column volumes (CV) 50 mM Acetic acid pH 4.5
Sample volume: 0.4 CV (approx. 4.2 mg protein/ml adsorbent)
Wash: 2 CV 50 mM Acetic acid pH 4.5
Elution: 0%–100% B in 10 CV, 2 CV at 100% B
CIP: 2 CV 1 M NaOH
Re-equilibration: 3 CV 50 mM Acetic acid pH 4.5

Protein scale-up study results

Table 4. Results of protein scale-up studies on three sizes of AxiChrom columns

Column	AxiChrom 70	AxiChrom 400	AxiChrom 1000
Elution time (min), BSA peak	46.3	45.8	45.6
Elution time (min), Lactoferrin peak	112.0	111.7	113.3
Peak distance (min)	65.7	65.9	67.7
Elution time in relation to AxiChrom 1000			
Elution time (min), BSA peak	+0.7	+0.2	-
Elution time (min), Lactoferrin peak	-1.3	-1.6	-
Peak distance (min)	-2.0	-1.8	-

The overlay chromatogram for all three runs is shown in Figure 10. The BSA peaks overlap each other almost perfectly, while there is a slight difference between the different columns for Lactoferrin.

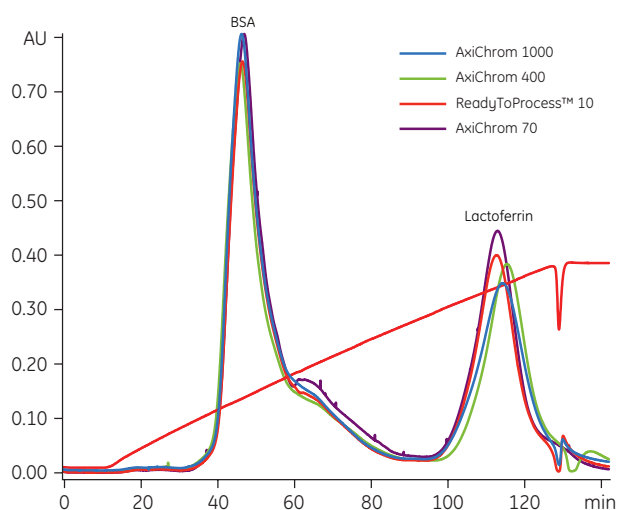


Fig 10. Chromatograms from scale-up study.

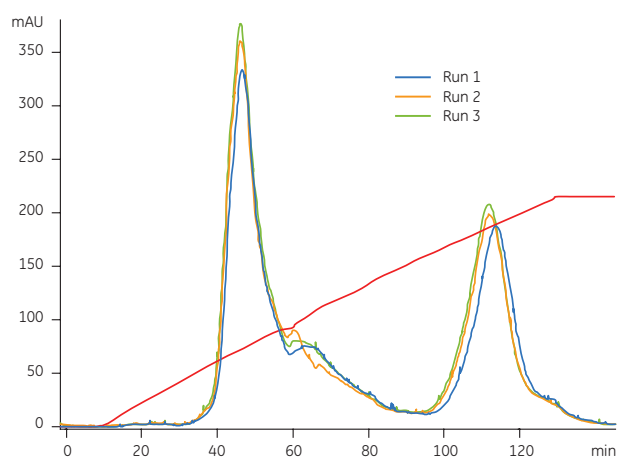


Fig 11. Chromatograms from AxiChrom 70 study.

The difference between columns in the chromatograms was probably related to the small difference in gradient slope and hold-up volumes in the systems. Consequently, two similar runs were made in the 70 mm column (Fig 11). All 70 mm results show the same type of shoulder on the BSA peak. In addition, the results in Table 5 show that the peak time for Lactoferrin varies somewhat.

Table 5. Elution time studies on AxiChrom 70 columns

	Run 1	Run 2	Run 3
Elution time (min), BSA peak:	46.7	46.2	46.3
Elution time (min), Lactoferrin peak:	113.8	112.2	112.0
Time (min) between peaks:	67.1	66.0	65.7

The table shows that the small variation seen between the columns of different size is no larger than the difference between repeated runs on the same column.

In conclusion, the small differences in bed efficiency between the different AxiChrom columns (N/m approx. 7000–7500 and Asymmetry 1.09–1.18) will not affect the performance for separation of BSA and Lactoferrin in this study.

The elution position for BSA and Lactoferrin is slightly different in all three columns (Fig 10). The differences can be explained by different hold-up volumes in the systems and possibly also to slight variations in gradient slope. In three consecutive runs on AxiChrom 70 columns, the elution position differed by 0.5 min for BSA and by 1.8 min for Lactoferrin, which is of the same magnitude as the differences in elution position between the three columns in Figure 10.

This study shows that the AxiChrom platform is scalable from at least 70 to 1000 mm. The same plate numbers and asymmetry are achieved when SP Sepharose Fast Flow is packed in the three column sizes. Furthermore, BSA and Lactoferrin can be separated on SP Sepharose Fast Flow with the same elution pattern (e.g., small shoulders present in all columns sizes). The BSA and Lactoferrin peaks are also eluted at the same positions in the gradient in all column sizes.

Conclusions

- The axial compression packing technique, governed by packing factor, ensures robust packing
- Utilizing a similar liquid distribution design across scales ensures scalability
- Effects of elution speed and sample volume are significant and must be considered
- Pressure drop depends on the scale of operation and the hardware contributions
- Similar bed efficiency throughout scale-up is a prerequisite for successful protein separation

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