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Columns up to 1600 mm in diameter packed with protein A chromatography medium using axial mechanical compression

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Introduction

To this day, different methods have been developed to pack a broad range of chromatography media (resins) in columns with axial mechanical compression. This includes packing of traditional BioProcess™ media, such as the Sepharose™ family, in addition to the more modern high-flow agarose media, like Capto™ and MabSelect™ family.

Figure 1 is an illustration of both specific medium and medium families that have been packed in AxiChrom™ columns, a chromatography column with axial compression. For most media the packing parameters have been verified and implemented in a software application referred to as Intelligent packing.

We present data showing scalability of AxiChrom columns up to 1600 mm diameter. A comparison of AxiChrom and Chromaflow™ 1000 column performance was also performed. Figure 2 illustrates the packing principles for the two column types.

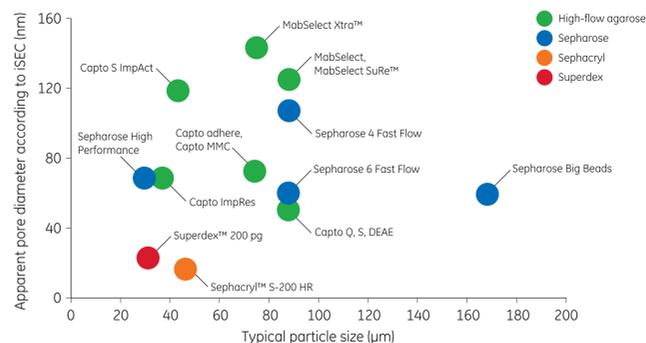


Fig 1. Illustration of apparent pore size vs particle size for some chromatography medium families. Pore diameter from inverted size exclusion chromatography (iSEC) and typical median particle size of volume weighted distribution.

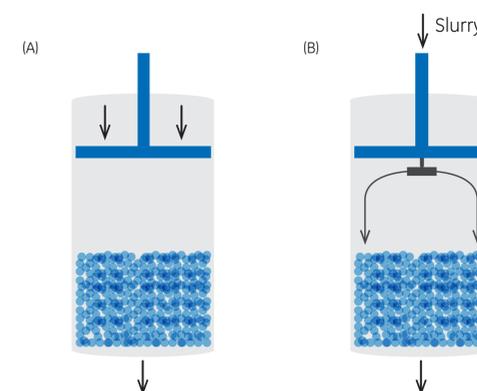


Fig 2. Illustration of the principles for (A) axial mechanical compression used in AxiChrom columns; (B) pack-in-place technique used in Chromaflow columns.

Materials and methods

Chromatography medium: MabSelect SuRe.

Chromatography systems: ÄKTApilot™ (AxiChrom 100 and 140) and ÄKTApilot™ (AxiChrom 300, 400, 600, 1000, and 1600)

MabSelect SuRe medium was packed to 20 cm bed height in AxiChrom 100, 140, 300, 400, 600, 1000, and 1600 according to packing parameters implemented in Intelligent packing wizard.

MabSelect SuRe medium was packed in Chromaflow 1000 with Pack Station 200 at maximum achieved packing flow velocity, 1100 cm/h.

Efficiency evaluation: reduced plate height (h) and peak asymmetry factor (A_s) was determined with pulse test at 30 cm/h.

Sample: 1% acetone or 0.8 M NaCl. **Eluent:** water or 0.4 M NaCl.

Bed stability was tested using purified water for 16 h at a given flow velocity.

Pressure-flow (P/F) properties were investigated to determine the maximum operating flow velocity.



Fig 3. (A) A selection of columns from the AxiChrom platform; (B) Unpacking a Chromaflow column

Results

Performance of columns packed with axial mechanical compression

P/F curves provide performance information of the packed columns (Fig. 4). Table 1 shows efficiency and bed stability results for AxiChrom columns with different diameters, packed to 20 cm bed height. All column sizes tested meet the criteria for the MabSelect SuRe medium.

Table 1. Efficiency results for AxiChrom columns

Column diameter (mm)	Reduced plate height (h)	Assymetry factor (A_s)	Flow velocity for stability test (cm/h)	Change after stability test (%)	
				h	A_s
100	1.4 to 1.5	1.0 to 1.0	500	3	8
140	1.4 to 1.6	1.1 to 1.2	500	11	11
300	1.4 to 1.5	1.1 to 1.1	500	2	4
400	1.4 to 1.5	1.1 to 1.3	500	6	10
600	1.3 to 1.5	1.1 to 1.2	500	8	5
1600	1.4 to 2.0	1.1 to 1.3	500	-5	8

Comparison between columns packed with axial mechanical compression vs pack-in-place technique

Table 2 shows the results of the study comparing three consecutive packings using either axial mechanical compression (AxiChrom) or a traditional pack-in-place technique (Chromaflow). Both columns had a column diameter of 1000 mm and were packed to 20 cm bed height with MabSelect SuRe medium. Efficiency results for both column types were within specification. In the bed stability test, the operational nominal fluid velocity specified for MabSelect SuRe (500 cm/h at 20 cm bed height) was only met in AxiChrom 1000.

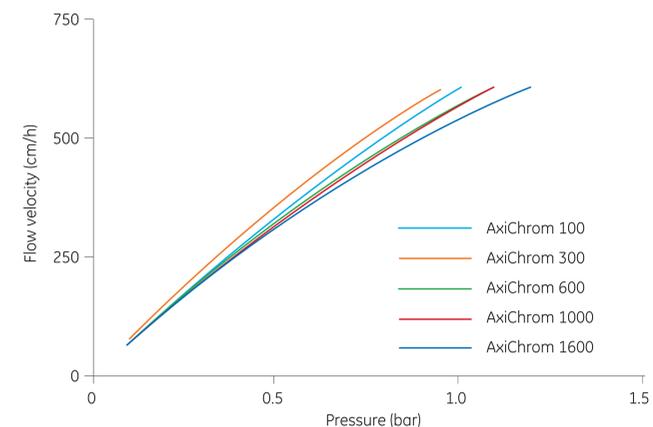


Fig 4. Regression curves of P/F tests for MabSelect SuRe medium in 20°C water at 20 cm packed bed height in AxiChrom 100, 300, 600, 1000, and 1600.

Table 2. Case study results

Column	Column diameter (mm)	Reduced plate height (h range*)	Assymetry factor (A_s range*)	Flow velocity for stability test (cm/h)	Change after stability test (%)	
					h	A_s
AxiChrom	1000	1.4 to 1.7	1.1 to 1.2	500	3	14
Chromaflow	1000	1.9 to 2.3	0.8 to 1.1	280	11	13
				360	29	45

* Average and ranges of up-flow and down-flow tests for at least three packings

Conclusions

- Axial mechanical compression gives efficient beds for MabSelect SuRe medium in all AxiChrom column sizes tested
- AxiChrom 1000 is a better choice than Chromaflow 1000 for high-flow agarose chromatography media
- Intelligent packing gives verified and highly robust methods as seen by low variation in column efficiency

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