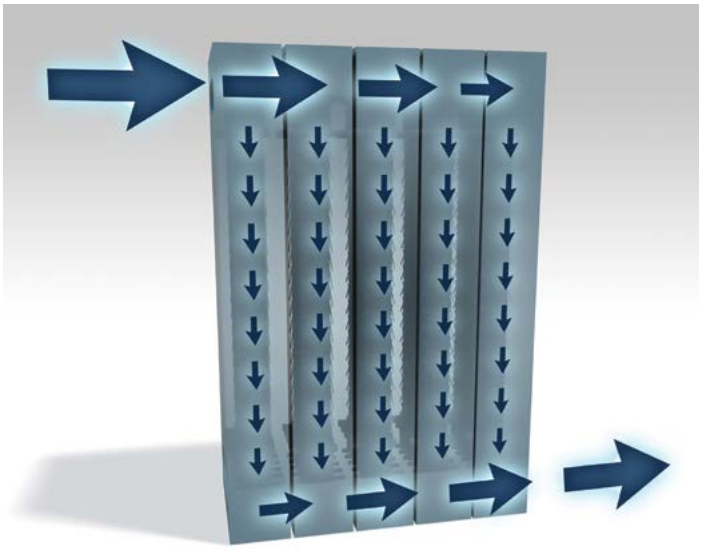
JSR Life Sciences

Chromassette®: Equivalent Performance of Individual and Stacked Cassettes

Introduction

Chromassette® is a stackable, pre-packed chromatography cassette with an internal lattice that supports the resin bed. It combines the separation capabilities of conventional chromatography resins with the benefits of a pre-packed, modular cassette format. The supported bed within the Chromassette allows very high flow rates to be used with all standard chromatography resins, including legacy and other "mechanically soft" resins which otherwise offer good capacity and selectivity. This enables up to 10-fold productivity improvements. In addition, Chromassette cassettes allow very flexible scaling to meet capacity requirements and provide high convenience. Standard cassettes are available at 20 and 6 cm bed height. W hile 20 cm bed height is often considered a bench mark value for Protein A chromatography columns, shorter bed heights allow for even greater increases in productivity and throughput. Chromassette also allows for easy scale-up of these short bed height cassettes, while large scale columns with such short bed height are very challenging to pack.



Scope of the document

In this application note, we present experimental data on the chromatographic performance of 4 cassettes packed with Amsphere A3™, both individually as well as stacked to obtain 4 times higher resin volume. Peak analysis (HETP and asymmetry), dynamic binding capacity (DBC), elution peak volumes and pressure-flow curves are shown for the 20 cm and 6 cm bed height Chromassette devices.

Abbreviation List

BT: Breakthrough
CIP: Cleaning In Place
CV: Cassette Volume
DBC: Dynamic Binding Capacity
HETP: Height Equivalent of a Theoretical Plate
PD: Process Development
RSD: Relative Standard Deviation
RT: Residence Time

Materials and Methods

The Amsphere A3 used was from a typical commercial lot. Chromatographic bed theoretical plate height (HETP) and asymmetry (As) were calculated in order to determine how well the cassette is packed. An injection pulse of 1.0 M NaCl in a mobile phase of 0.1 M NaCl in water and a linear flow rate of 300 cm/h were used. The obtained conductivity curves were fitted by an EMG model (c.f. Fehlinger[1]) and the HETP was calculated based on the moments of the fitted curve. Asymmetry was calculated by dividing the distance from the peak midpoint to the trailing edge of the peak, by that of the leading edge of the peak at 10% of peak height. For the reduced plate height, the HETP value is divided by the mean particle diameter.

Lyophilized human polyclonal IgG was used for DBC measurements. Standard buffers and protocol were as defined in the User Manual[2] for Amsphere A3 Protein A resin. Equilibration and wash steps were performed at 1000 cm/h, as Chromassette allows such high flow rates to be used. Elution flow rates were 400 cm/h for the 20 cm cassettes and 800 cm/h for the 6 cm cassettes.

Results

Pressure-flow Curves

Chromassette devices are made with less than 10% RSD of the pressure drop of the individual cassettes. The pressure flow curves (Figure 1) remain linear for the entire range tested (up to 1200 cm/h for the individual cassettes). The pressure drop of the stack of 4 Chromassette devices (diamond markers in figure 1) perfectly matches the theoretical prediction based on the individual pressure drops.

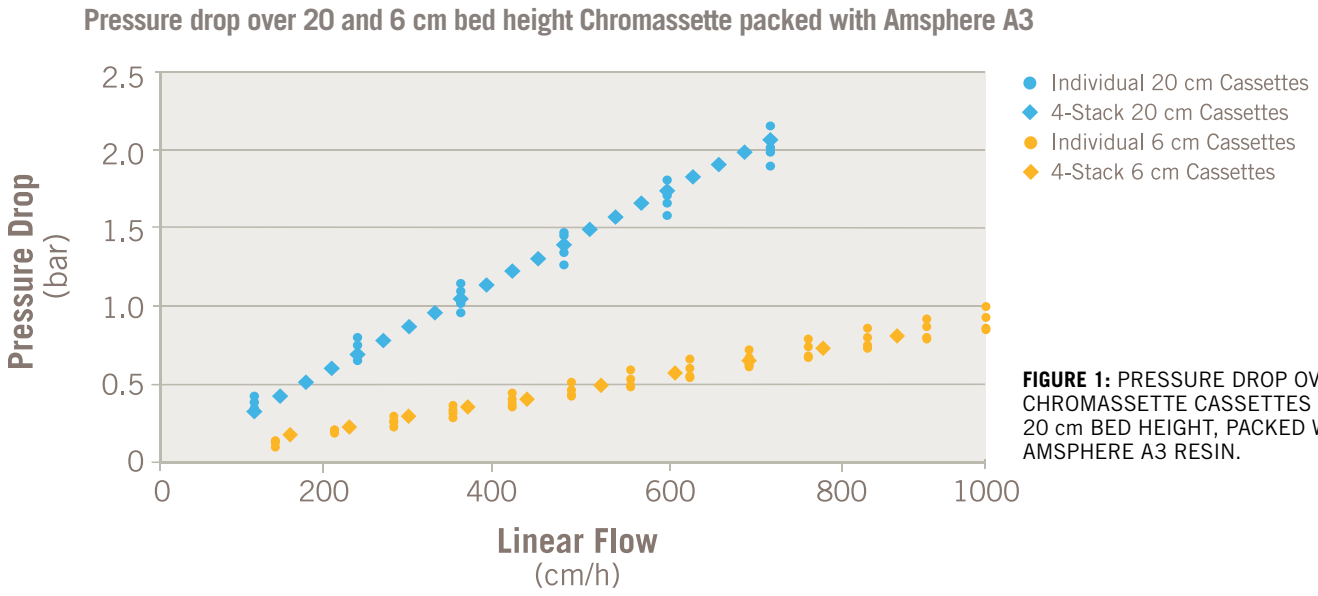


FIGURE 1: PRESSURE DROP OVER CHROMASSETTE CASSETTES WITH 20 cm BED HEIGHT, PACKED WITH AMSPHERE A3 RESIN.

In Figure 1, 0.1 M NaCl was used as mobile phase for the pressure-flow curves.

Packing Performance Testing

In order to characterize the chromatographic performance of the packed bed, the asymmetry and plate count for each individual Chromassette and the 4-stack of these individual Chromassettes were measured. It is worth noting that the 20 cm Chromassette devices used in this study were early prototypes and therefore the absolute value of As and HEPT are slightly higher and vary more than expected in commercial devices. Despite this caveat, the Chromassette 4-stack assembly shows very high robustness, a low range of variation, and provides good separation performance.

Figure 2 shows the measured peak asymmetries of the individual Chromassettes and the 4-stack assemblies. It can be seen that the 4-stack reliably averages out variations in the individually measured cassette performance.

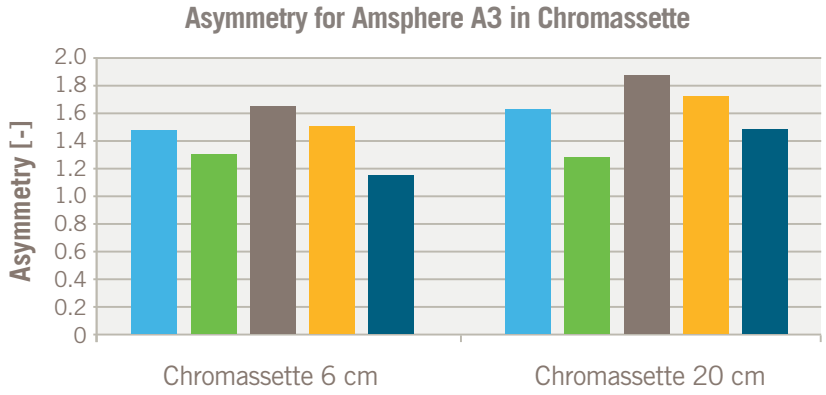


FIGURE 2: ANALYTICAL PEAK ASYMMETRY AT A LINEAR FLOW VELOCITY OF 300 cm/h FOR CHROMASSETTE CASSETTES WITH 6 AND 20 cm BED HEIGHT, PACKED WITH AMSPHERE A3 RESIN.

In Figure 3, the results for the measurement of the reduced plate height are shown. This figure underlines the robustness of the 4-stack Chromassette assemblies in regard to varying cassette performances with individual runs. The individual plate height value may seem relatively high, however these measurements have been done at a higher flow rate than usual (300 cm/h) with relatively low bed volumes and residence times of only 1.2 and 4 minutes.

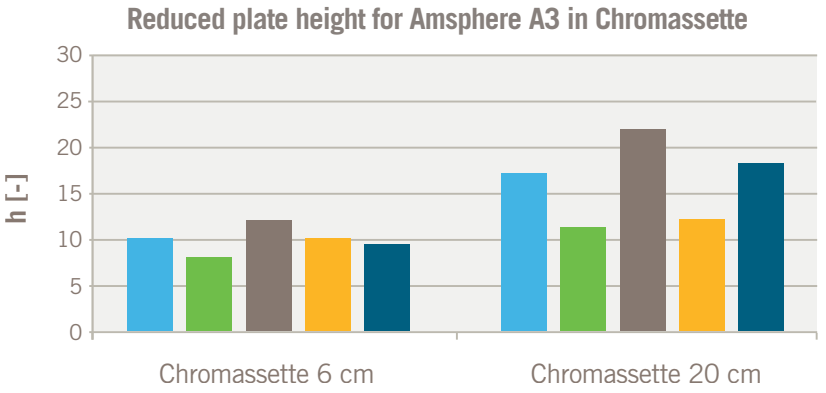


FIGURE 3: REDUCED PLATE HEIGHT AT A LINEAR FLOW VELOCITY OF 300 cm/h FOR CHROMASSETTE CASSETTES WITH 6 AND 20 cm BED HEIGHT, PACKED WITH AMSPHERE A3 RESIN.

Dynamic Binding Capacity (DBC)

The DBC at 10% breakthrough of polyclonal human IgG in a Amsphere A3 packed Chromassette is comparable to the values obtained in columns in other Amsphere A3 studies. Figure 4 shows DBC values at 3 residence times for the individual Chromassette devices and for the 4-stack assembly. The DBC of the stack matches the average of the values obtained for the individual cassettes. At the shortest residence times, experimental scatter of the DBC measurement is naturally larger than at longer residence times.

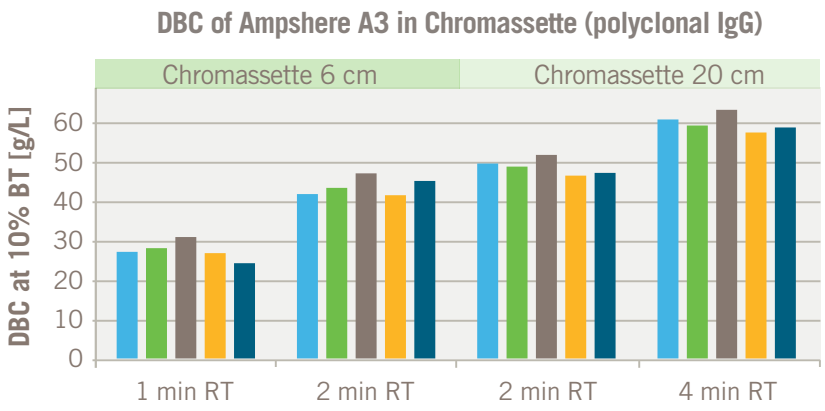


FIGURE 4: DBC VALUES AT DIFFERENT RESIDENCE TIMES FOR AMSPHERE A3 RESIN, PACKED IN 6 AND 20 cm BED HEIGHT CHROMASSETTE CASSETTES. DBC VALUES ARE FOR POLYCLONAL HUMAN IgG AT 10% BREAKTHROUGH.

Elution Volumes

Low elution volumes can be obtained with Chromassette. As shown in figure 5, stacking multiple cassettes does not lead to an increase of elution volume. Good flow distribution between the cassettes reduces peak broadening of the Amsphere A3 elution peaks in the 4-stack assemblies. The elution volume is smaller for the stack of the 6 cm cassettes, compared to the individual cassettes. This is due to the higher ratio of the hold-up volume in the holder to the total bed volume. This effect is naturally more prominent when using the 6 cm Chromassette devices.

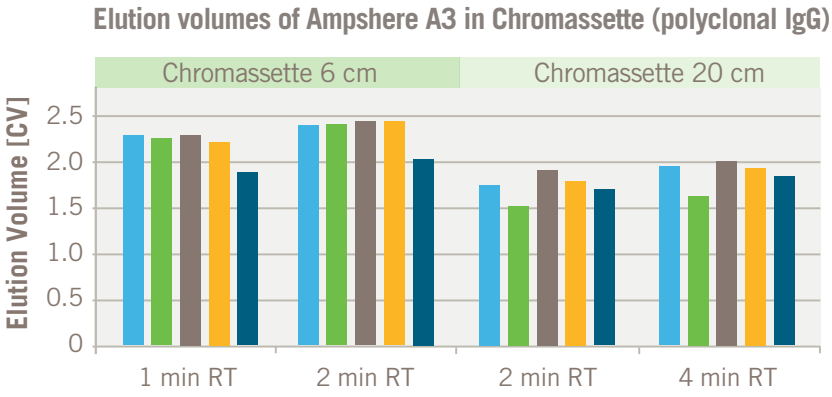


FIGURE 5: ELUTION VOLUMES FOR AMSPHERE A3 RESIN, PACKED IN 6 AND 20 cm BED HEIGHT CHROMASSETTE CASSETTES. POLYCLONAL HUMAN IgG WAS LOADED AT DIFFERENT RESIDENCE TIMES. 100 mM SODIUM ACETATE pH 3.2 WAS USED AS ELUTION BUFFER. ELUTION FLOW RATE WAS 800 AND 400 cm/h FOR THE 6 AND 20 cm BED HEIGHT CASSETTES RESP. ELUATE PEAKS WERE COLLECTED ABOVE 50 mAU UV280 nm ABSORBANCE.

Conclusions

Chromassette devices offer impressive individual performance. Stacking of Chromassette devices with 6 cm or 20 cm bed heights has been shown to have little or no impact on the process performance as determined by measuring several analytical (As, HETP) and process performance (DBC, EV) indicators. The stacking ability of matched Chromassette devices enables easy and quick adjustment of resin volumes in order to meet the capacity requirements of individual processes.

[1] A. Felinger (1998). Data Analysis and Signal Processing in Chromatography, Volume 21- 1st Edition.
[2] Amsphere A3 Application Note (AN6) Product Overview REV 4.4.17, JSR Life Sciences, JSR Corporation

JSR Life Sciences

NORTH AMERICA
JSR Life Sciences
JSR Micro, Inc.
1280 North Mathilda Avenue
Sunnyvale, CA 94089
408-543-8800

ASIA
JSR Corporation
1-9-2 Higashi-Shimbashi
Minatoku, Tokyo
105-8640 Japan
+81-3-6218-3557

EUROPE
JSR Life Sciences
JSR Micro NV
Technologielaan 8
3001 Leuven
Belgium
+32-16-668-721

For more information visit www.jsrlifesciences.com
EMAIL: chromassette@jsrlifesciences.com