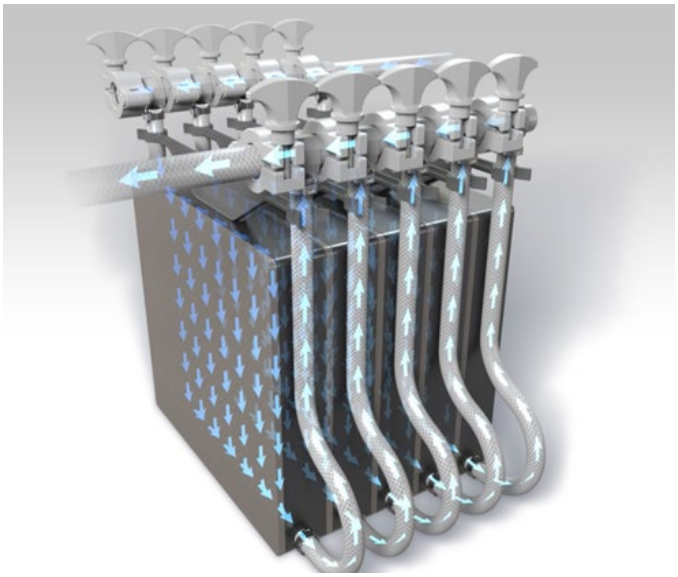
CHROMNEX™: EQUIVALENT PERFORMANCE OF INDIVIDUAL AND STACKED DEVICES

Introduction

ChromNeX™ is a stackable, pre-packed chromatography device 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 device format. The supported bed within the ChromNeX device allows 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, ChromNeX devices allow flexible scaling to conveniently and easily meet process capacity requirements. Standard devices are available at bed heights of 20 and 6 cm. While 20 cm bed height is often considered a benchmark value for Protein A chromatography columns, shorter bed heights allow for even greater increases in productivity and throughput. ChromNeX also allows for easy scale-up of these short bed height devices, while large scale columns with short bed heights are very challenging to pack.



Scope of the Document

In this application note, we present experimental data on the chromatographic performance of 4 devices packed with Amsphere™ A3 Protein A resin, both individually and stacked to obtain 4 times more 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 ChromNeX devices.

Abbreviation list

BT: Breakthrough
CIP: Cleaning In Place
DV: Device 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 device was 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 the peak height. For the reduced plate height, the HETP value is divided by the mean particle diameter.

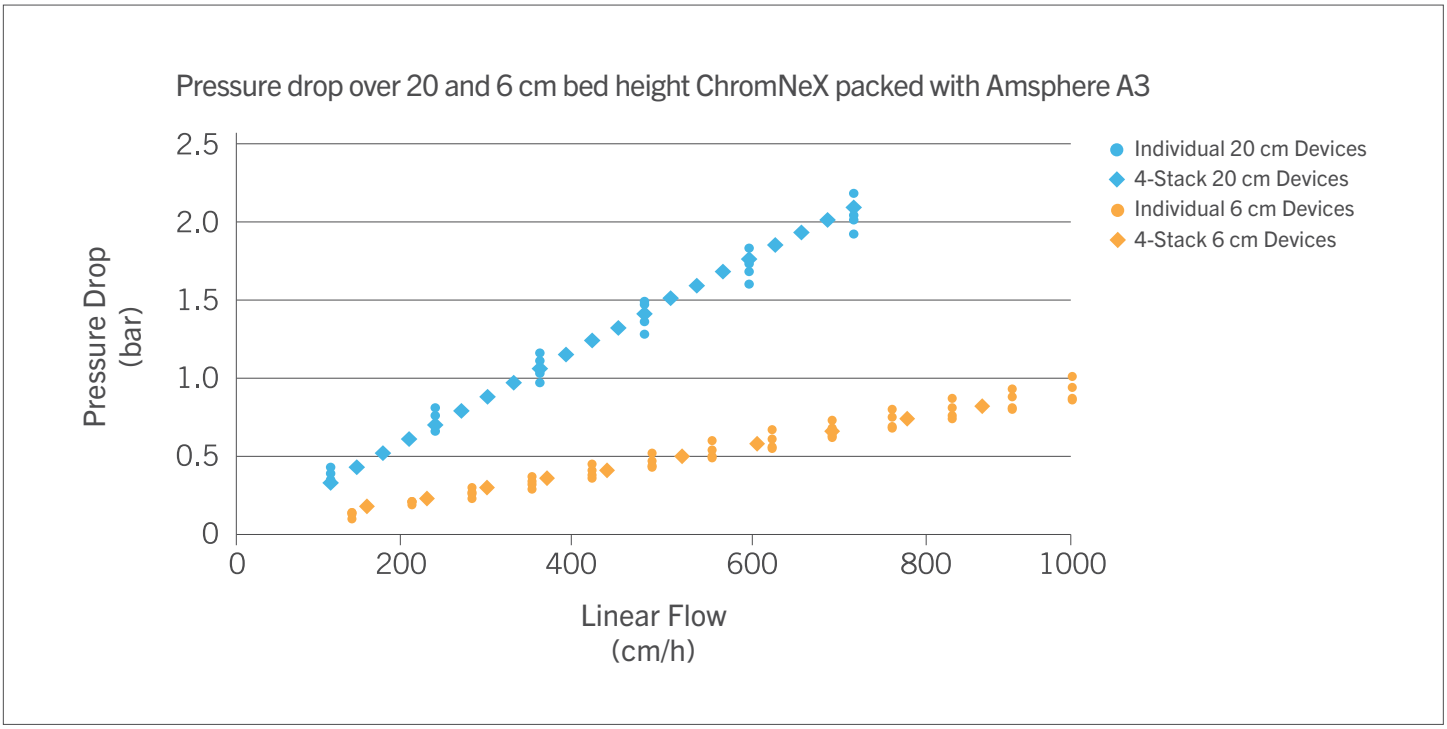
Lyophilized human polyclonal IgG was used for DBC measurements. Standard buffers and protocol were as defined in the User Manual[2] for Amsphere A3 Protein A resin. Equilibration and wash steps were performed at 1000 cm/h, as ChromNeX allows such high flow rates to be used. Elution flow rates were 400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

Results

Pressure Flow Curves

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

Figure 1: Pressure drop over ChromNeX devices with 20 cm bed height, packed with Amsphere A3



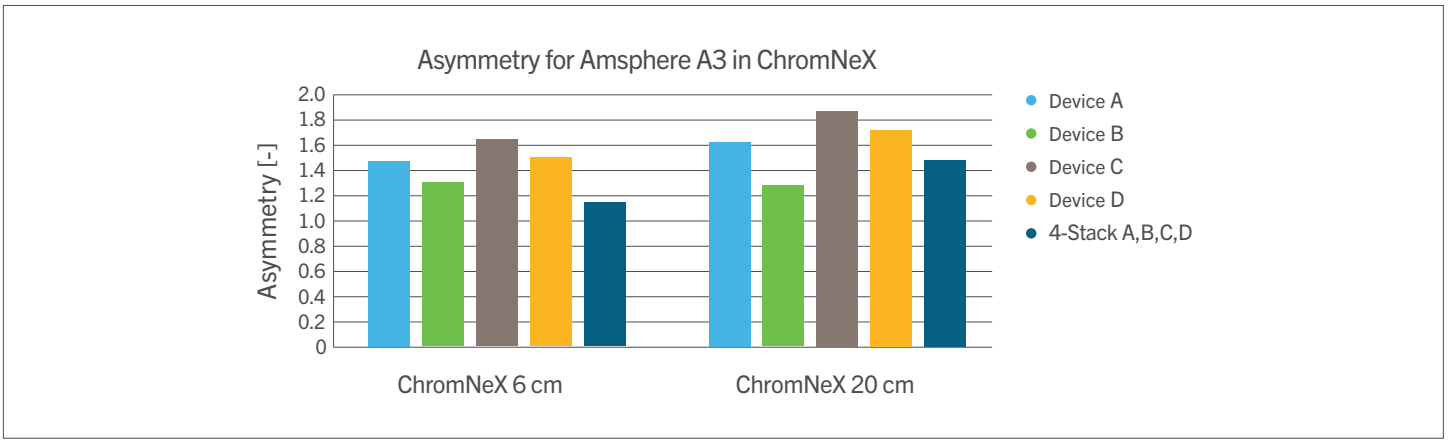
In Figure 1, 0.1 M NaCl was used as mobile phase for the pressure-flow curves.

Packing Performance Testing

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

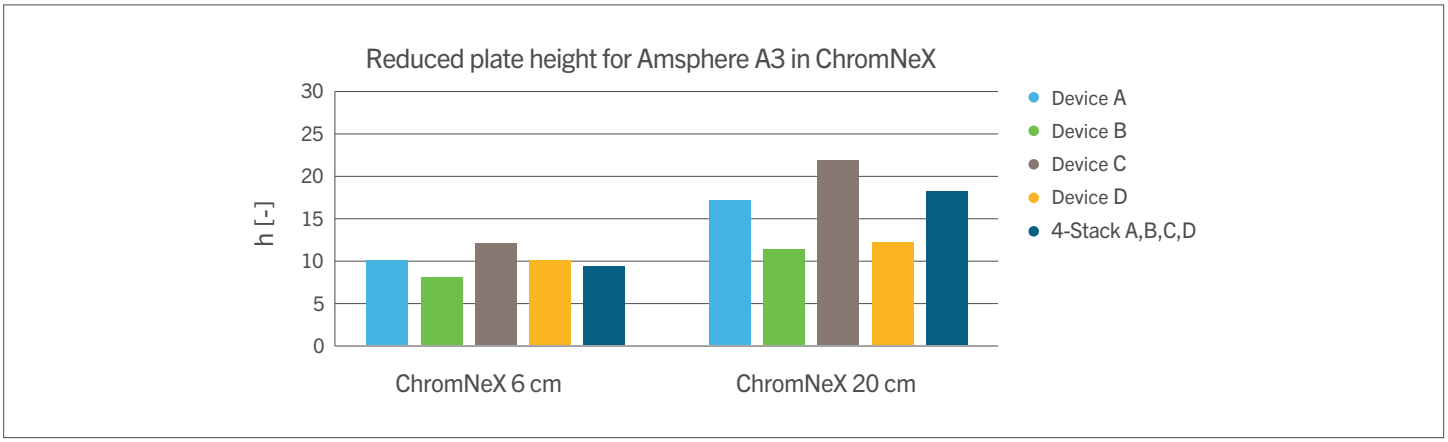
Figure 2 shows the measured peak asymmetries of the individual ChromNeX devices and the 4-stack assemblies. It can be seen that the 4-stack reliably averages out variations in the individually measured device performance.

Figure 2: Analytical peak asymmetry at a linear flow velocity of cm/h for ChromNeX devices with 6 and 20 cm bed heights, 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 ChromNeX assemblies in regard to varying device performances with individual runs. The individual plate height value may seem relatively high, however these measurements were 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 ChromNeX devices 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 an Amsphere A3 packed ChromNeX 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 ChromNeX devices and for the 4-stack assembly. The DBC of the stack matches the average of the values obtained for the individual devices. At the shorter residence times, experimental scatter of the DBC measurement is naturally larger than at longer residence times.

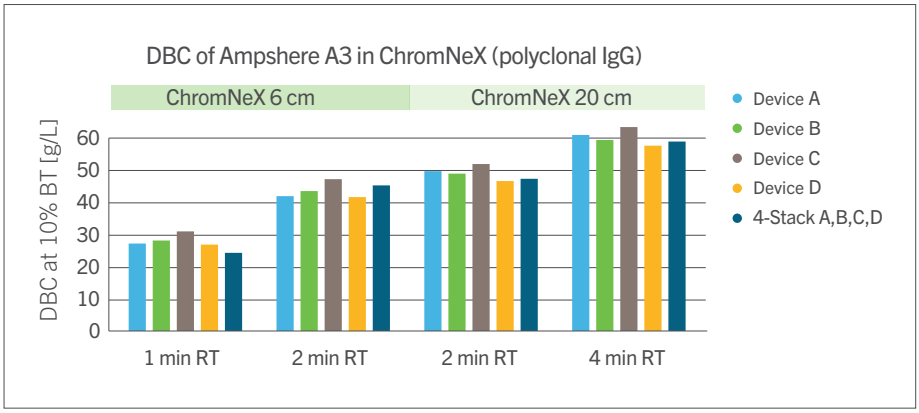


Figure 4: DBC values at different residence times for Amsphere A3 resin, packed in 6 and 20 cm bed height ChromNeX devices. DBC values are for polyclonal human IgG at 10% breakthrough.

Elution Volumes

Low elution volumes can be obtained with ChromNeX. As shown in figure 5, stacking multiple devices does not lead to an increase of elution volume. Good flow distribution between the devices reduces broadening of the Amsphere A3 elution peaks in the 4-stack assemblies. The elution volume is smaller for the stack of 6 cm devices, compared to the individual devices. 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 6 cm ChromNeX devices.

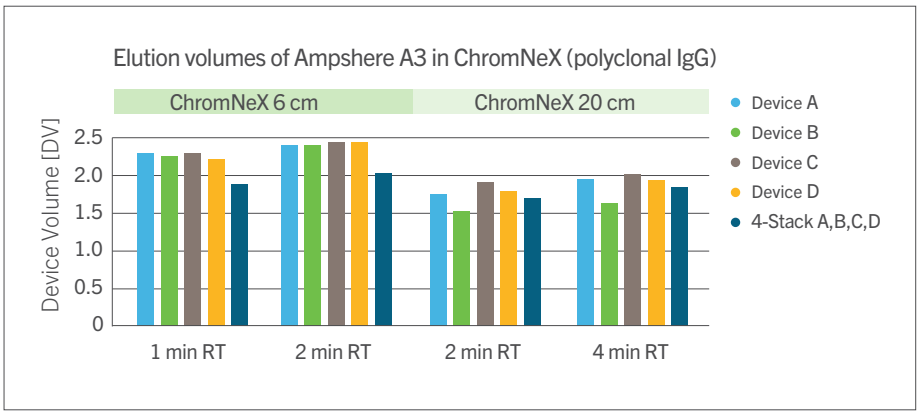


Figure 5: Elution volumes for Amsphere A3 resin, packed in 6 and 20 cm bed height ChromNeX devices. Polyclonal human IgG was loaded at different residence times. 100 mM sodium acetate pH 3.2 was used as the elution buffer. Elution flow rate was 800 and 400 cm/h for the 6 and 20 cm bed height devices, respectively. Eluate peaks were collected above 50 mAU UV280 nm absorbance.

Conclusions

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

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