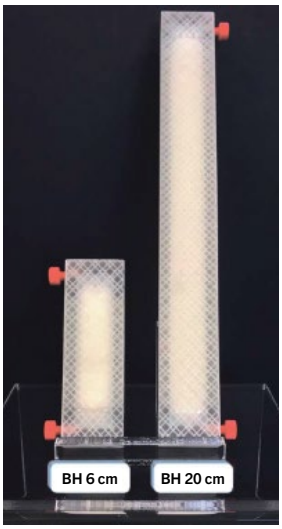
EVALUATION OF CHROMNEX™ PACKED WITH CATION EXCHANGE RESIN

Introduction

ChromNeX™ is a high performance, rectangular, stackable device that can be easily packed with any chromatography resin. Its unique supported bed offers the reliability and performance of a column together with improved pressure-flow properties which are not altered by scaling. As such, it readily meets today's bio-separation scale up demands for improved ease of use. Comparisons of ChromNeX with similar dimensioned columns indicate that ChromNeX can offer improved productivity. This application note is based on an internal comparison of a commercial cation exchange resin packed in a 1 cm diameter x 6 cm bed height column and a 6 cm bed height ChromNeX device. Results suggest comparable separation performance with the ability to be operated at much faster flow rates and thereby higher productivities.

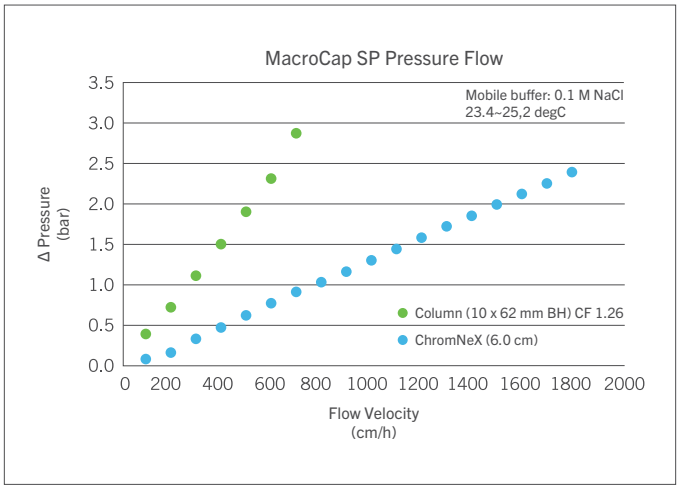
Materials and Methods



MacroCap® SP cation exchange resin was used for this study. This resin was packed into a 6 cm ChromNeX device (1 CV = 5 mL, 6 cm BH) and a conventional column, 10 x 100 mm Tricorn® (Cytiva) with a 6.2 cm bed height following vendor instructions. Operating conditions followed those in the MacroCap Data File (1). Column integrity, pressure-flow, break-through, dynamic binding capacity (DBC), carryover and resolution were examined. In keeping with the MacroCap Data File, lysozyme (Sigma-Aldrich, #L6876), cytochrome c (Sigma-Aldrich, #C2506), and RNase A (Sigma-Aldrich, #R6513) proteins at 0.5 g/L in 20 mM Phosphate buffer pH 6.8 were used as the model feed. Lysozyme at 5 g/L concentration was used for the dynamic binding capacity (DBC) evaluation.

Pressure-flow Performance

Figure 1: Delta pressure vs. flow velocity for column and Chromasette



MacroCap offers good selectivity and capacity, especially for larger MW targets (1), however it is a mechanically soft resin which is recommended to be used in a 30 cm diameter column with a 20 cm high bed at a maximum flow rate of only 120 cm/h if a back pressure of < 3 bar is desired (MacroCap Data File (1)). Faster flow rates are expected to be possible in smaller diameter columns with shorter bed heights (2). In the present study the 1 cm diameter, 6.2 cm high bed yielded a pressure of 2 bar at 500 cm/h (Figure 1). The unique internal scaffolding of the ChromNeX device enabled 1500 cm/h at 2 bar due to the fully supported bed. Unlike traditional columns, this performance is maintained when moving to larger ChromNeX devices. End users are therefore able to use the optimal size modular ChromNeX device for their process and facility specifications.

Breakthrough and Binding Capacities

Table 1: Processing Conditions

Operation	Buffer	# CV	Flow Rate (cm/h)
Sanitization	0.1 M NaOH (15 minute hold after 3 CV)	3	180
EQ	20 mM Phosphate buffer, pH 6.8	4	180
Load	5 g/L Lysozyme	N/A	N/A
Wash 1	20 mM Phosphate buffer, pH 6.8	5	90
Elution	20 mM Phosphate buffer, pH 6.8 + 0.4 M NaCl	5	90
Strip	2 M NaCl	3	180
Rinse	WFI or DI water	3	180
Sanitization	0.1 M NaOH (15 minute hold after 3 CV)	3	180
Equilibration	20 mM Phosphate buffer, pH 6.8	3	180

Table 1 shows the processing conditions used for chromatographic performance evaluation.

The result of lysozyme loading to 10% breakthrough in both column and ChromNeX devices can be seen in Figure 2. Dynamic binding capacity (DBC) results tested at various loading residence times can be seen in Figure 3. In both cases, similar chromatographic performance was observed.

Figure 2: Breakthrough curves at loading residence time of 4 minutes

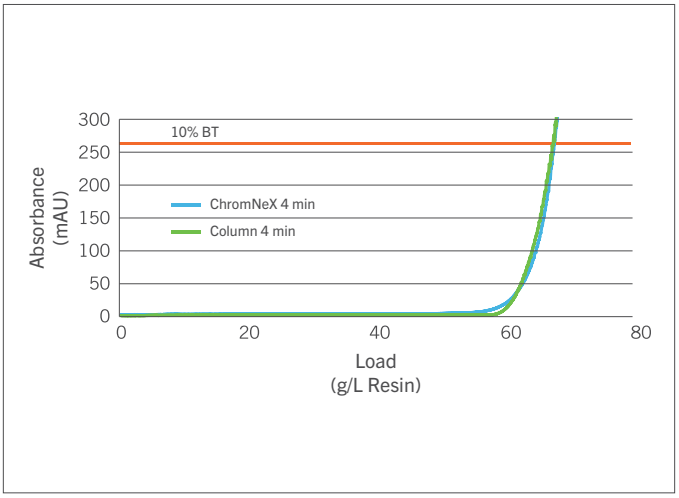


Figure 3: Lysozyme dynamic binding capacities

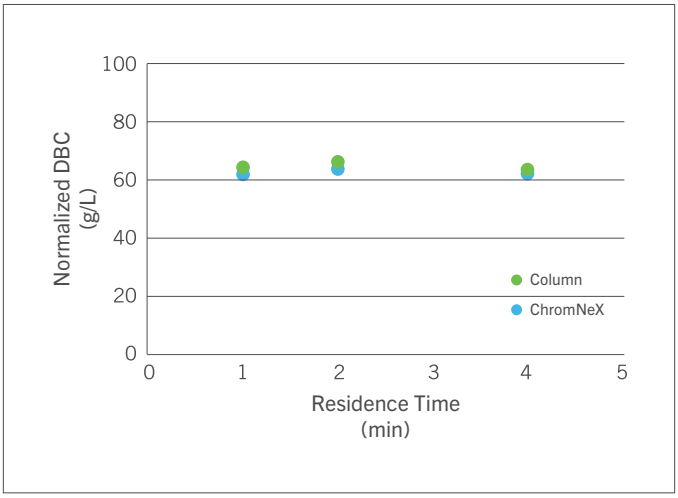


Figure 2 data was normalized in regard to device resin static volume.

Carryover Study

The carryover study was conducted using mock process conditions and the buffer sets shown in Table 2.

Table 2: Mock run process conditions

Operation	Buffer	# CV	Flow Rate (cm/h)
EQ	20 mM Phosphate buffer, pH 6.8	1.0	300
Elution	20 mM Phosphate buffer, pH 6.8 + 0.4 M NaCl	6.0	300
EQ	20 mM Phosphate buffer, pH 6.8	3.0	300

Figure 4: Carry over study mock process steps

Mock runs were conducted after loading purified lysozyme onto the column and ChromNeX devices. The sequence of process steps is shown in Figure 4 and the results in Table 3.

Table 3: Carryover study

Sample	Carry Over Amount (% of load)
ChromNeX 1st Mock Run	< 0.1%
ChromNeX 2nd Mock Run	< 0.1%
Column 1st Mock Run	< 0.1%
Column 2nd Mock Run	< 0.1%

The carryover characteristics of the MacroCap resin were not affected by use of a ChromNeX device or column. In both cases carry over was < 0.1% of the load amount.

Resolution

Resolution studies followed those noted in the MacroCap AA Data File (1) and involved a load buffer of 20 mM Phosphate, pH 6.8, containing 0.5 g/L each of lysozyme, cytochrome c and RNase A.

Table 4: Condition (reference 1)

Operation	Buffer	# CV	Flow Rate (cm/h)
EQ	20 mM Phosphate buffer, pH 6.8	4	180
Load	0.5 g/L lysozyme, 0.5 g/L cytochrome c, 0.5 g/L RNase A in EQ buffer	1	75
Elution	20 mM Phosphate buffer, pH 6.8 + 0.4 M NaCl, Linear gradient (0–100%)	15	75
Strip	2 M NaCl	3	180
Rinse	WFI or DI water	3	180
Sanitization	0.1 M NaOH (15 min hold after 3 CV)	3	180
Equilibration	20 mM Phosphate buffer, pH 6.8	3	180

Figure 5.1: Salt gradient elution resolution of a three-protein mixture for ChromNeX

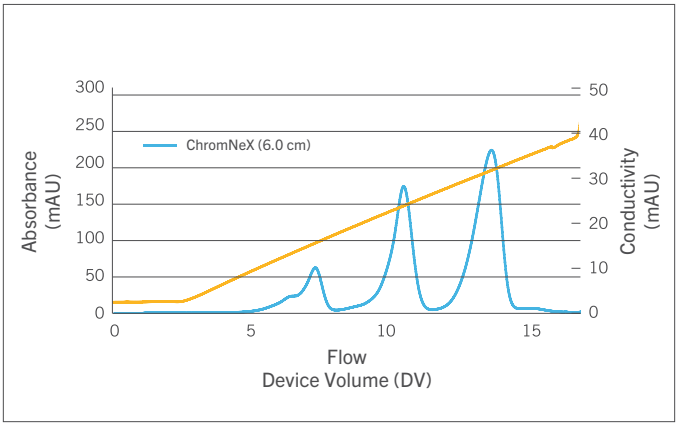
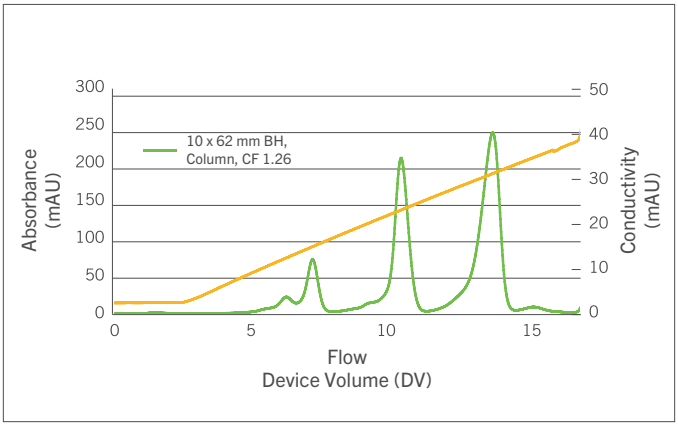


Figure 5.2: Salt gradient elution resolution of a three-protein mixture for column



Effective separation capability of the resin was maintained in ChromNeX. Peak centers occur in exactly the same position in the elution gradient. The peaks are somewhat narrower and higher in the column elution profile as it was packed at a higher compression factor (see below), however good baseline resolution was seen in both devices. To gain insight into these results, and the possibility that slightly different bed packing was responsible for them, the column and ChromNeX device bed integrity "height equivalent to a theoretical plate" (HETP) and asymmetry (As) were determined.

Bed Integrity Testing

ChromNeX bed integrity can be determined in the same manner as for a regular column system. For packing integrity testing, a mobile phase of 1 M NaCl buffer with an injection pulse of 2 M NaCl (2% CV) were employed at various linear flow rates (Table 5).

Table 5: Bed integrity comparison of column and ChromNeX

Flow Velocity	Column		ChromNeX		(HETP Ratio)^{½}
(cm/h)	As	HETP (cm)	As	HETP (cm)	
75	0.88	0.01421	0.85	0.03807	1.63
180	0.89	0.01911	0.81	0.04373	1.51
360	0.95	0.02520	1.02	0.04418	1.32

ChromNeX bed asymmetry was found to match that of the column, but ChromNeX exhibited a larger HETP. This is because the column was packed to a compression factor (CF) of 1.29 whereas the ChromNeX device was pre-packed at a CF of 1.03. This difference is revealed in the HETP difference (Table 5). One would expect the HETP difference to reflect slightly sharper peaks with base line width ratios comparable to the square root of the HETP ratio (2). This appears to be true (Figure 5), however in both cases good base line resolution of a three-protein mixture was obtained in a 6 cm high bed. Table 5 also suggests that, in keeping with its greater bed support, ChromNeX HETP is less affected by flow than column HETP. This should be advantageous at the higher flow rates ChromNeX can offer.

Conclusions

ChromNeX is a modular chromatography technology that has the same separation capabilities of conventional chromatography and is capable of handling any type of chromatography resin. In this application, we have seen that column-equivalent performance is obtained for target break-through, dynamic binding capacity, and carry-over; with similar chromatographic resolution even in beds that may have been packed somewhat differently with a relatively "mechanically soft" ion exchange resin. The differences should be readily reduced if both column and ChromNeX devices were packed to identical HETP parameters. The unique internal scaffold of ChromNeX enables higher flow rates while maintaining a linear pressure drop response. This enables increased operational flexibility to achieve high productivity processes even with large pore, soft resins such as MacroCap SP.

References
1. MacroCap Data File 28-4005-84 AA, 01/2006 and AB dated 09/2011, GE Healthcare Bio-sciences AB
2. Protein Chromatography: Process Development and Scale-Up, Giorgio Carta and Alois Jungbauer, Wiley-VCH, Weinheim, 2010. See page 288.

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