Investigating the Important Parameters for Packing Nuvia HR-S Resin at Process Scale

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Abstract

Process-scale production typically involves more than one column. The packing quality of each column affects the efficiency of the whole workflow and is an important validation criterion. Column packing efficiency itself depends on multiple criteria. Nuvia HR-S is a strong cation exchange resin for the high resolution of protein molecules during polish purification in downstream processing. Here, we investigate the effects of packing methods, packing solutions, compression factor, resin slurry concentration, and bed height on the efficiency of packed columns. General recommendations for packing process-scale Nuvia HR-S Columns are presented.

Introduction

Nuvia HR-S is a strong cation exchange resin built on proprietary UNOsphere* polymerization technology. Its macroporous matrix allows efficient mass transfer of biomolecules, leading to fast binding kinetics and excellent binding capacity. Nuvia HR-S particles have been optimized to allow for high resolution of biological molecules of similar properties, such as the fragments and aggregates of monoclonal antibodies (bulletins 6448 and 7027, Carta et al. 2017, Guo and Carta 2015, Guo et al. 2016, Ng et al. 2014, Reck et al. 2017). The chemical stability of Nuvia HR-S is remarkable under conditions commonly employed for protein separation and column cleaning-in-place, allowing it to be used repeatedly in manufacturing cycles (Table 1). It has been widely used for large-scale manufacturing of protein therapeutic molecules.

Well-packed and stable columns are crucial for successful bioprocessing as they provide the consistent chromatography performance needed for the production of high-quality active pharmaceutical substances. A long-lasting column will undoubtedly help reduce production cost as well. The primary goal of the present investigation is to identify parameters important for packing Nuvia HR-S into columns with the desired chromatography performance. Based on our data, we offer practical guidance for packing Nuvia HR-S Resin with commercial chromatography columns and packing hardware.

* U.S. patent 6,423,886 B1.

Table 1. Characteristics of Nuvia HR-S Resin.

Property	Description
Type of ion exchanger	Strong cation
Functional group	$–SO^{3-}$
Total ionic capacity	100–180 µeq/ml
Dynamic binding capacity*	≥70 mg/ml at 300 cm/hr
Shipping counterion	Na^+
Median particle size	50 µm
Recommended linear flow rate	50–200 cm/hr
Chemical stability	
1.0 N NaOH (20°C)	Up to 5 weeks (840 hr)
0.1 N NaOH (20°C)**	Up to 5 years
Compression factor	1.20–1.30 (settled bed volume/packed bed volume)
pH stability	
Short-term	2–14
Long-term**	4–13
Shipping solution	20% ethanol + 0.1 M NaCl
Regeneration	1–2 M NaCl
Sanitization	0.5–1.0 N NaOH
Storage conditions	20% ethanol or 0.1 N NaOH

* 10% breakthrough capacity determined with 5.0 mg/ml human IgG in 20 mM Na acetate, pH 5.0.
** Data derived under accelerated conditions at 60°C.

Materials and Methods

Process-Scale Columns and Control Station

A HiScale 50/20 Column (GE Healthcare), BPG 200/500 Column (GE Healthcare), VERDOT Ips² Chromatography Skid 03, or VERDOT Ips² InPlace Chromatography Column D446 H600 (ID 45 cm) or D200 H600 (ID 20 cm) (VERDOT Ips²) was used for packing Nuvia HR-S Chromatography Resin (Bio-Rad Laboratories, Inc.). All chemicals for the preparation of solutions and buffers used in this study were purchased from VWR International.

Preparation of Nuvia HR-S Resin Slurry in Packing Solution and Determination of Slurry Percentage

Nuvia HR-S Resin was washed with 3 bed volumes of ddH_2O followed by 3 bed volumes of packing solution to fully remove the storage solution. Appropriate amount of packing solution was added to prepare resin slurry at test concentrations. For the determination of exact slurry percentage, 10 ml of a Nuvia HR-S slurry in the packing solution was transferred into an Econo-Pac Column (catalog #7321010 or #7321011) and the packing solution was removed by draining. The final volume of settled resin (V_s) was recorded and used to calculate the slurry percentage (C_s) in the container using Equation 1:

C_s (%) = $100 \times V_s/10 = 10 \times V_s$ Eq. 1

The volume of resin slurry in packing solution (V_r) required for packing a column of known radius (R) with desired bed height (H_p) can be calculated using Eq. 2.

$V_r = 3.14 \times R^2 * H_p * CF / (C_s/100)$ Eq. 2

Evaluation of Column Efficiency

The packing quality of the column was subjected to an efficiency test, which typically includes the determination of height equivalent to a theoretical plate (HETP) or reduced height equivalent to a theoretical plate (rHETP), number of theoretical plates per meter (N/m), and asymmetry factor (A_s) (Figure 1).

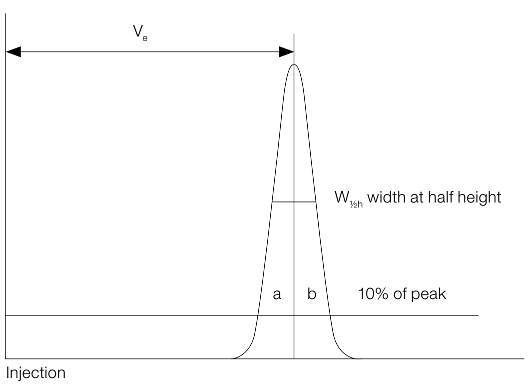

Fig. 1. Simulated chromatogram to illustrate the calculation of HETP, rHETP, and A_s values.

Number of theoretical plates $N = 5.54 * (V_e/W_{½h})^2$ Eq. 3

V_e = peak elution volume or time
$W_{½h}$ = peak width at peak's half height in volume or time
V_e and $W_{½h}$ should always be in the same units.

HETP = L/N Eq. 4
HETP = height equivalent to the theoretical plate
L = bed height (cm)
N = number of the theoretical plates

rHETP = 10,000 * HETP/average bead diameter (µm) Eq. 5

$A_s = b/a$ Eq. 6

a = front section of peak width at 10% of peak height bisected by line denoting V_e

b = latter section of peak width at 10% of peak height bisected by line denoting V_e

The column qualification tests were performed using 0.5 or 1 N NaOH as the probe (1–3% v/v) and 0.1 N NaOH as the eluent for the 45 x 20 cm and 20 x 20 cm columns. A 2% acetone solution was used as the probe for testing the HiScale 50/20 Columns. The packed columns were equilibrated with 0.5–1 column volume (CV) eluent at 100 cm/hr before the probe solution was injected.

Packing Methods

All packing experiments in this study were performed using open columns. Column and skid manufacturer's instruction manuals were followed for column assembly, air purging, and packing control. The system pressure limit was set at 3 bar or the maximum pressure specified by the hardware manufacturer. Three packing methods were evaluated:

1. Axial packing, in which the chromatography resin is consolidated and compressed to the target bed height by the downward movement of the column top piston at a set speed.
2. Flow packing, in which the chromatography resin is consolidated then compressed by a downflow of packing solution at a set flow rate and the top piston is then lowered to the final bed height.
3. Hybrid packing, which is a combination of axial piston movement and flow. The final packing speed is the sum of axial piston movement and downward flow rate.

All packed columns were conditioned at 300 cm/hr with 3 CV of packing solution in the direction of upflow followed by 3 CV in the downflow direction before they were subjected to qualification, pressure-flow properties, or a cycling stability test. Headspace and column backpressure, at which column headspace was formed, were recorded.

Pressure-Flow Test of Packed Columns

Flow was ramped up until the pressure reached 3 bar (often ≥300 cm/hr). Net column pressure drop and column headspace, if any, were recorded after 0.2 CV of 0.1 N NaOH was run through the column at a specific flow rate. After each pressure-flow test, a column was equilibrated with 0.5 CV of 0.1 N NaOH and retested for its efficiency to assess the stability of the column under stress.

Column Cycling Stability Test

Columns of two sizes, 45 x 20 cm and 20 x 20 cm, were packed at a compression factor of 1.25. They were subjected to five cycles of the following buffers sequentially:

1. 3 CV of 200 mM NaP_i,* pH 6.5
2. 10 CV of 10 mM NaOAc, pH 5.0
3. 10 CV of 50 mM NaOAc + 1.0 M NaCl, pH 5.0
4. 10 CV of 1.0 M NaOH

The column efficiency test was repeated at the completion of each cycling.

Results

Packing Nuvia HR-S Columns by Axial Compression: Effect of Compression Factor and Packing Speed

Columns (45 x 20 cm) were packed at various compression factors (1.15–1.30) by piston movement at a speed of 150, 200, or 300 cm/hr. All columns were then subjected to a pressure-flow test, in which the mobile phase flow rate was increased from 0 to 300 cm/hr. Headspace was observed at merely 194 cm/hr for the column packed at CF 1.15, which suggests that this column was underpacked. Columns packed with CF 1.20–1.25 were generally stable at up to 250 cm/hr without developing headspace (Figure 2). For the column packed with CF 1.30, headspace was not observed during the entire course of the pressure-flow test (Table 2). However, a more pronounced pressure drop was associated with columns packed at this compression factor.

All columns maintained their performance after pressure-flow tests, as reflected by their near constant N/m and A_s values. These results indicate that the additional column compression or headspace formation at higher flow rates is reversible. Piston movement speed, from 150 to 300 cm/hr, did not have significant impact on column packing quality or column stability (Table 2).

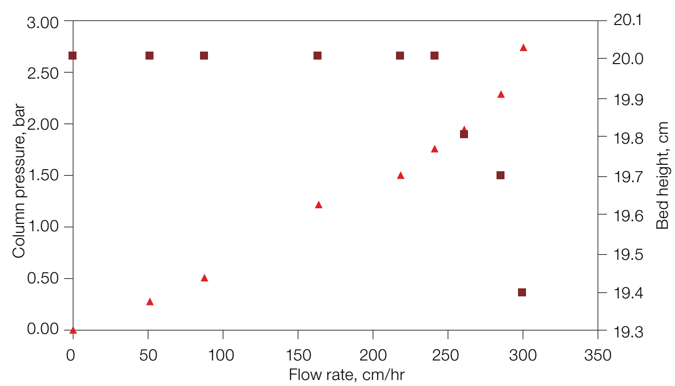

Fig. 2. The pressure-flow curve of Nuvia HR-S Resin packed in InPlace Columns (45 x 20 cm) at a compression factor of 1.25. The axial column piston movement speed was set at 200 cm/hr. The actual bed heights at specific flow rates are also plotted. Column pressure (▲); bed height (■).

Table 2. Effect of compression factor and packing speed on final column qualification and stability. Nuvia HR-S Resin in 0.1 N NaOH was packed in InPlace Columns (45 x 20 cm) by axial compression, followed by equilibration with 0.1 N NaOH. Column qualification tests were performed using 1 N NaOH as the probe.

Compression Factor (CF)	Packing Speed, cm/hr	Column Pressure when Headspace Formed, bar	Flow Rate at Which Headspace Formed, cm/hr	A_s		N/m	
				Before PFT	After PFT	Before PFT	After PFT
1.15	200	1.22	194	1.54	1.13	8,403	7,407
1.20	150	1.89	254	1.14	1.00	10,152	10,050
	200	1.85	246	1.19	1.14	10,753	10,471
	300	1.86	265	1.18	1.45	10,204	9,524
1.25	150	2.58	261	1.44	1.20	5,988	6,601
	200	2.50	262	1.09	1.06	7,663	7,752
	300	2.46	264	1.17	1.41	9,174	7,813
1.30	150	**	**	1.35	1.70	4,255	6,849
	200	**	**	1.31	1.17	6,494	5,831
	300	**	**	0.97	1.19	8,850	8,197

PFT, pressure-flow test.
** Not observed at 300 cm/hr.

* Sodium phosphate.

Similar trends were observed for smaller columns (20 x 20 cm) (Table 3). In an undercompressed column, such as one packed with CF 1.15, headspace was observed at 170 cm/hr; while compressing the column bed to CF 1.30 resulted in an overall higher pressure drop (Figure 3).

Table 3. Effect of compression factor and packing speed on final column qualification and stability. Nuvia HR-S Resin in 0.1 N NaOH was packed in InPlace Columns (20 x 20 cm) by axial compression, followed by equilibration with 0.1 N NaOH. Column qualification tests were performed using 0.5 N NaOH as the probe.

Compression Factor (CF)	Packing Speed, cm/hr	Column Pressure when Headspace Formed, bar	Flow Rate at Which Headspace Formed, cm/hr	A_s		N/m	
				Before PFT	After PFT	Before PFT	After PFT
1.15	200	0.78	170	1.00	1.00	5,435	5,510
1.20	200	1.30	220	0.91	0.93	6,079	5,510
	300	1.30	222	1.00	1.00	5,236	5,000
1.25	200	2.09	257	1.25	1.08	5,208	5,362
	300	2.07	258	1.15	1.00	5,333	5,376
1.30	200	1.96	254	0.72	0.76	1,887	2,028
	300	2.21	262	0.81	0.86	2,509	1,773

PFT, pressure-flow test.

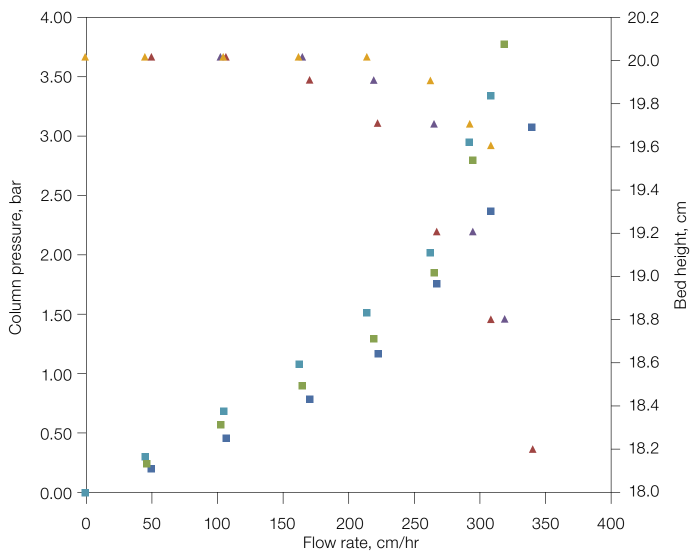

Fig. 3. The pressure-flow curve of Nuvia HR-S Resin packed in InPlace Columns (20 x 20 cm) at a compression factor of 1.15–1.30. The axial column piston movement speed was set at 200 cm/hr. The actual bed heights at specific flow rates are also plotted. CF 1.15 column pressure (■);CF 1.20 column pressure (■); CF 1.30 column pressure (■); CF 1.15 bed height (▲); CF 1.20 bed height (▲); CF 1.30 bed height (▲) .

Packing Nuvia HR-S Columns under Constant Flow Rate of Packing Solution: Effect of Compression Factor and Packing Speed

Nuvia HR-S Resin can also be packed into process-scale columns by a downflow of packing solution. Efficiency test results demonstrate excellent chromatography performance and stability for all columns packed under constant mobile phase flow rate in the range of 200–350 cm/hr. In the present testing ranges, no significant effect of compression factor or packing flow rate was observed (Tables 4 and 5, and Figure 4).

Table 4. Effect of packing speed on final column qualification and stability. Nuvia HR-S Resin was packed to the final bed height at CF 1.25 in InPlace Columns (45 x 20 cm) by a constant flow of 0.1 N NaOH followed by equilibration with the same solution. Column qualification tests were performed using 1 N NaOH as the probe.

Compression Factor (CF)	Packing Speed, cm/hr	Column Pressure when Headspace Formed, bar	Flow Rate at Which Headspace Formed, cm/hr	A_s		N/m	
				Before PFT	After PFT	Before PFT	After PFT
1.25	220	1.16	196	1.57	1.22	10,204	8,032
	300	1.77	240	1.03	1.25	10,638	10,811
	350	2.25	240	1.36	1.31	7,692	8,032

PFT, pressure-flow test.

Table 5. Effect of compression factor and packing speed on final column qualification and stability. Nuvia HR-S Resin was packed to the final bed height in InPlace Columns (20 x 20 cm) by a constant flow of 0.1 N NaOH followed by equilibration with the same solution. Column qualification tests were performed using 0.5 N NaOH as the probe.

Compression Factor (CF)	Packing Speed, cm/hr	Column Pressure when Headspace Formed, bar	Flow Rate at Which Headspace Formed, cm/hr	A_s		N/m	
				Before PFT	After PFT	Before PFT	After PFT
1.20	200	1.38	222	1.05	1.00	6,108	5,736
	250	1.42	218	1.05	1.00	5,144	5,048
	250	1.45	220	1.00	1.00	5,286	5,174
	300	1.44	218	1.00	1.00	5,736	5,541
1.25	250	2.15	258	1.07	1.00	5,082	4,859
	300	2.01	255	1.06	1.02	5,076	4,495
	350	2.05	260	1.10	1.20	5,731	5,571

PFT, pressure-flow test.

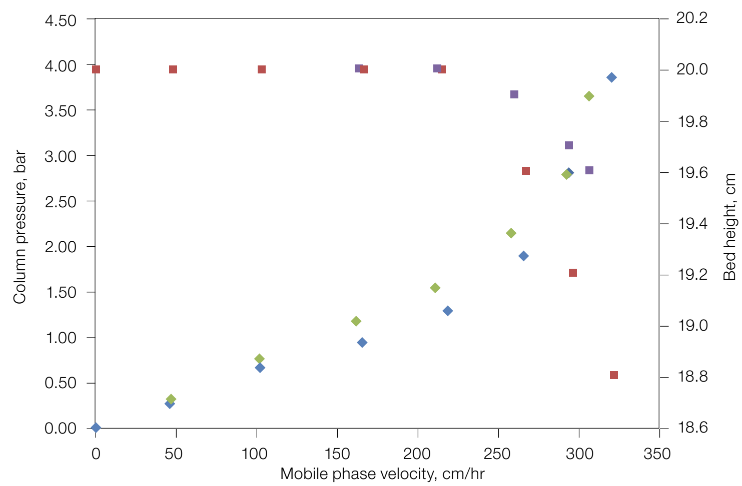

Fig. 4. Pressure-flow curves of Nuvia HR-S Resin packed in InPlace Columns (20 x 20 cm) by constant flow at 300 cm/hr to a compression factor of 1.2 or 1.25. The actual bed heights at specific flow rates are also plotted. CF 1.20 column pressure (◆); CF 1.25 column pressure (◆); CF 1.20 bed height (■); CF 1.25 bed height (■).

Packing Nuvia HR-S Columns with the Hybrid Method: Effect of Compression Factor and Packing Speed

Nuvia HR-S Resin can also be packed by the combination force of axial piston movement and mobile phase flow, or hybrid method, with excellent column efficiency, regardless of the column dimension. In the current testing ranges, no significant effect of compression factor or packing speed was observed (Tables 6 and 7, and Figure 5).

Table 6. Effect of compression factor and packing speed on final column qualification and stability. Nuvia HR-S Resin was packed to the final bed height in InPlace Columns columns (45 x 20 cm) by the combination of axial piston movement and flow. The actual packing speed was the sum of axial piston movement and the downward flow rate of 0.1 N NaOH. After equilibration with 0.1 N NaOH, columns were subjected to qualification tests using 1 N NaOH as the probe.

Compression Factor (CF)	Packing Speed, cm/hr		Column Pressure when Headspace Formed, bar	Flow Rate at Which Headspace Formed, cm/hr	A_s		N/m	
	Piston	Flow			Before PFT	After PFT	Before PFT	After PFT
1.20	75	75	1.90	246	0.85	1.14	8,403	8,000
	100	100	1.94	244	1.17	1.00	9,569	9,479
	150	150	1.98	243	1.24	1.00	10,204	11,050
1.25	75	75	2.79	249	1.74	1.44	6,920	6,098
	100	100	2.90	261	1.11	0.97	6,780	6,944
	150	150	2.89	259	1.27	1.00	7,692	8,197
1.30	75	75	*	*	1.61	1.47	4,878	4,057
	100	100	*	*	1.00	1.00	6,154	6,135
	150	150	*	*	1.17	1.08	6,289	5,540

PFT, pressure-flow test.
* Not observed at 300 cm/hr.

Table 7. Effect of compression factor and packing speed on final column qualification and stability. Nuvia HR-S Resin was packed to the final bed height in InPlace Columns (20 x 20 cm) by the combination of axial piston movement and flow. The actual packing speed was the sum of axial piston movement and the downward flow rate of 0.1 N NaOH. After equilibration with 0.1 N NaOH, columns were subjected to qualification tests using 0.5 N NaOH as the probe.

Compression Factor (CF)	Packing Speed, cm/hr		Column Pressure when Headspace Formed, bar	Flow Rate at Which Headspace Formed, cm/hr	A_s		N/m	
	Piston	Flow			Before PFT	After PFT	Before PFT	After PFT
1.20	125	125	1.40	198	0.98	0.98	6,383	5,986
	150	150	1.38	195	1.00	1.02	5,973	5,959
1.25	125	125	2.08	218	0.96	0.98	5,392	5,286
	150	150	2.14	220	1.05	1.00	5,352	5,246

PFT, pressure-flow test.

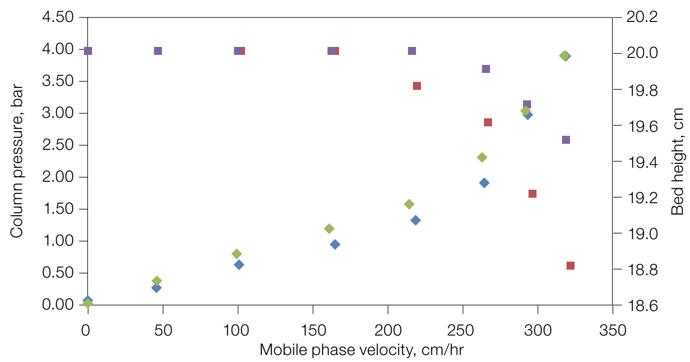

Fig. 5. Pressure-flow curves of Nuvia HR-S Resin packed in InPlace Columns (20 x 20 cm) using a hybrid of axial piston movement and flow at the combined speed of 300 cm/hr to a compression factor of 1.2 or 1.25. The actual bed heights at specific flow rates are also plotted. CF 1.20 column pressure (◆); CF 1.25 column pressure (◆); CF 1.20 bed height (■); CF 1.25 bed height (■)

Packing Nuvia HR-S Columns in Different Buffers

Nuvia HR-S Resin can also be packed in buffers commonly used for cation exchange chromatography, such as 10 mM sodium acetate and 20 mM sodium citrate (pH 5) for column equilibration and 50 mM sodium acetate + 1 M sodium chloride (pH 5) for column elution. This resin can also be packed in a solution as simple as 0.1 M sodium chloride with equivalent column performance. As shown in Tables 8–11 and Figures 6 and 7, columns packed in these buffers are equivalent to those packed in 0.1 N NaOH, in terms of column efficiency and stability. Therefore, Nuvia HR-S can be packed in a variety of solutions.

Table 8. Packing Nuvia HR-S in 10 mM sodium acetate (pH 5) — effect of compression factor and axial compression speed. InPlace Columns (45 x 20 cm) were packed to the final bed height by constant axial piston movements at 200 or 300 cm/hr with a compression factor of 1.20–1.30. They were equilibrated with 0.1 N NaOH prior to efficiency testing using 0.5 N NaOH as the probe.

Compression Factor (CF)	Packing Speed, cm/hr	Column Pressure when Headspace Formed, bar	Flow Rate at Which Headspace Formed, cm/hr	A_s		N/m	
				Before PFT	After PFT	Before PFT	After PFT
1.20	200	1.92	245	1.00	1.14	9,785	10,050
	300	1.91	245	1.19	1.00	9,434	10,152
1.25	200	2.44	263	1.00	1.00	10,050	9,950
	300	2.45	262	1.03	1.00	9,662	9,524
1.30	200	*	*	1.03	1.00	10,870	10,000
	300	*	*	1.07	1.07	10,929	9,302

PFT, pressure-flow test.
* Not observed at 300 cm/hr.

Table 9. Packing Nuvia HR-S in 50 mM sodium acetate and 1 M sodium chloride (pH 5) — effect of compression factor and axial compression speed. InPlace Columns (45 x 20 cm) were packed to the final bed height by constant axial piston movement at 200 or 300 cm/hr with a compression factor of 1.20–1.30. They were equilibrated with 0.1 N NaOH prior to efficiency testing using 1 N NaOH as the probe.

Compression Factor (CF)	Packing Speed, cm/hr	Column Pressure when Headspace Formed, bar	Flow Rate at Which Headspace Formed, cm/hr	A_s		N/m	
				Before PFT	After PFT	Before PFT	After PFT
1.20	200	1.98	244	1.00	1.19	9,569	10,526
	300	1.95	245	1.20	1.21	8,734	8,511
1.25	200	2.68	265	1.00	1.00	8,772	8,264
	300	2.65	264	1.00	1.04	10,471	10,638
1.30	200	*	*	1.14	1.10	8,929	8,230
	300	*	*	1.00	1.00	10,811	10,050

PFT, pressure-flow test.
* Not observed at 300 cm/hr.

Table 10. Packing Nuvia HR-S in 20 mM sodium citrate and 1 M sodium chloride (pH 5). Nuvia HR-S Resin was packed in a HiScale 50/20 Column to a final bed height of 19.2 cm by constant flow of buffer at 300 cm/hr. The compression factor was 1.30.

Compression Factor (CF)	Packing Speed, cm/hr	A_s		N/m	
		Before PFT	After PFT	Before PFT	After PFT
1.3	300	1.34	1.38	7,657	6,852

PFT, pressure-flow test.

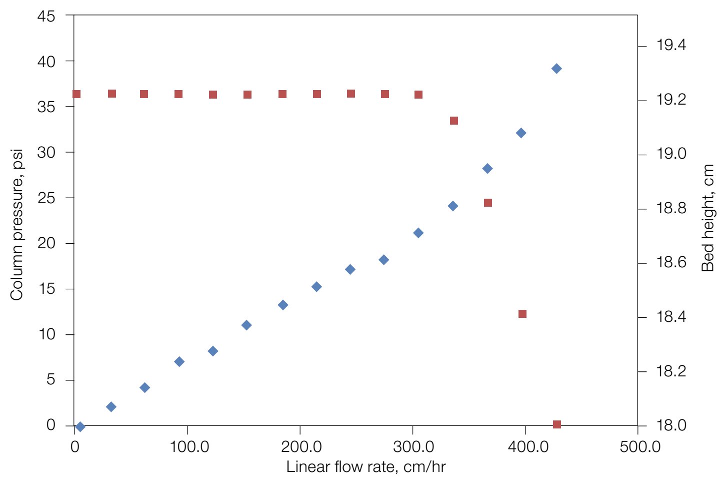

Fig. 6. Pressure-flow curves of Nuvia HR-S Resin packed in a HiScale 50/20 Column by constant flow of 20 mM sodium citrate and 1 M sodium chloride (pH 5) buffer at 300 cm/hr to a compression factor of 1.3. The actual bed heights at specific flow rates are also plotted. Column pressure (◆); bed height (■).

Table 11. Packing Nuvia HR-S in 0.1 M sodium chloride. Nuvia HR-S Resin was packed in a HiScale 50/20 Column to a final bed height of 19.2 cm by constant flow of buffer at 300 cm/hr. The compression factor was 1.30.

Compression Factor (CF)	Packing Speed, cm/hr	A_s	N/m
1.3	300	1.16	7,207

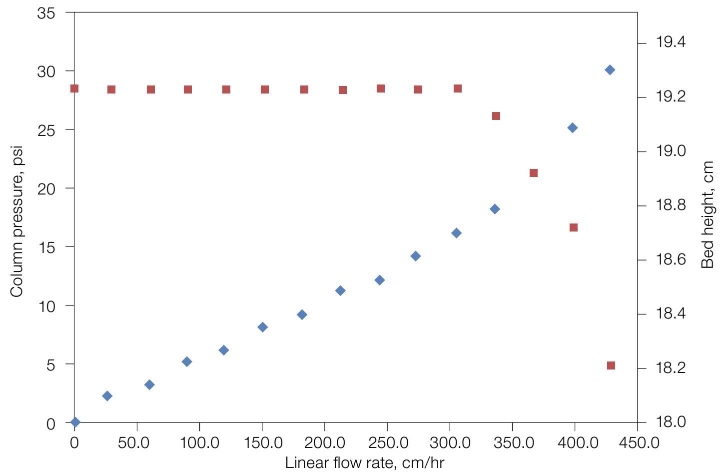

Fig. 7. Pressure-flow curves of Nuvia HR-S Resin packed in a HiScale 50/20 Column by constant flow of 0.1 M NaCl at 300 cm/hr to a compression factor of 1.3. The actual bed heights at specific flow rates are also plotted. Column pressure (◆); bed height (■).

Packing Nuvia HR-S in Other Column Dimensions

Columns of different dimensions are needed during downstream manufacturing to accommodate change of feed volume, target titer, and hardware availability. In general, increase of column backpressure is associated with longer columns. Nuvia HR-S Resin has been packed in an InPlace Column (ID 20 cm) with three bed heights, which all demonstrated decent stability after being challenged by mobile phase at different linear velocities (Table 12). The column pressure drop shows a linear correlation with bed height (Figure 8), while headspace would appear at lower mobile phase linear velocity for a longer column (Table 12). Data from packing Nuvia HR-S in a BPG 200/500 Column at constant flow of mobile phase are shown in Table 13 and Figure 9.

Table 12. Correlation of column pressure drop, headspace formation, and column height. Nuvia HR-S Resin in 0.1 N NaOH was packed in InPlace Columns (ID 20 cm) with different bed heights by axial compression at 200 cm/hr to a compression factor of 1.25. They were equilibrated with 0.1 N NaOH prior to efficiency testing using 0.5 N NaOH as the probe.

Column Height, cm	Column Pressure when Headspace Formed, bar	Flow Rate at Which Headspace Formed, cm/hr	A_s		N/m	
			Before PFT	After PFT	Before PFT	After PFT
14.8	1.35	309	1.00	1.00	6,075	5,819
14.9	1.29	299	0.98	0.98	5,579	5,655
14.9	1.28	305	1.04	1.02	6,092	5,656
19.4	1.45	251	1.09	1.08	6,705	6,565
19.5	1.42	243	1.00	1.05	6,942	6,069
19.5	1.39	252	1.05	1.15	6,872	6,329
24.1	1.50	196	1.22	1.35	6,005	5,845
24.3	1.50	198	1.28	1.35	5,095	5,095
24.3	1.56	190	1.20	1.26	5,411	5,324

PFT, pressure-flow test.

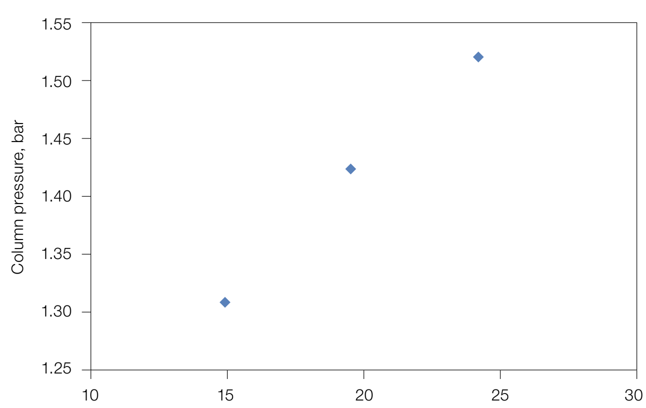

Fig. 8. Correlation of column pressure drop and bed height. Nuvia HR-S Resin in 0.1 N NaOH was packed at a compression factor of 1.25 in an InPlace Column (ID 20 cm). Packed columns were equilibrated with 1 CV of 0.1 N NaOH before pressure-flow tests. The column pressure drop at which headspace was observed was recorded and plotted against column bed height.

Table 13. Effect of compression factor and mobile phase flow rate on packing Nuvia HR-S Resin in a BPG 200/500 Column. Nuvia HR-S Resin was packed to the final bed height by a constant flow of 0.1 N NaOH followed by equilibration with the same solution. Column qualification tests were performed using 0.5 N NaOH as the probe.

Compression Factor (CF)	Packing Speed, cm/hr	Pressure during Conditioning, bar	A_s		N/m	
			Before PFT	After PFT	Before PFT	After PFT
1.25	300	1.93	1.32	1.13	2,951	3,167
	300	2.00	1.29	1.15	3,062	3,152
1.31	300	2.60	1.33	1.18	5,360	5,282
1.32	300	2.70	1.44	1.39	5,986	6,076
	300	2.57	1.31	1.23	5,469	5,057

PFT, pressure-flow test

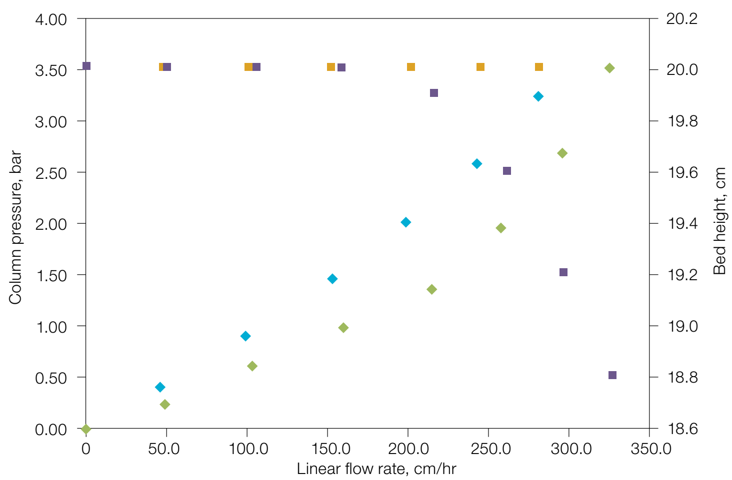

Fig. 9. Pressure-flow curves of Nuvia HR-S Resin packed in a BPG 200/500 Column by constant flow of 0.1 N NaOH to compression factors of 1.25–1.32. The actual bed heights at specific flow rates are also plotted. CF 1.25 column pressure (◆); CF 1.32 column pressure (◆); CF 1.25 bed height (■); CF 1.32 bed height (■).

Effect of Nuvia HR-S Slurry Percentage on Column Packing Quality

The effect of Nuvia HR-S Resin slurry concentration and column packing quality was also investigated. No significant impact was observed when the slurry concentration was in the range of 50–60% (Table 14).

Table 14. Effect of Nuvia HR-S resin slurry concentration on column packing quality. Nuvia HR-S Resin slurry in 0.1 N NaOH at various concentrations was packed in an InPlace Column (20 x 20 cm) to a compression factor of 1.28. The packed column was equilibrated with 0.1 N NaOH prior to efficiency testing using 0.5 N NaOH as the probe.

Slurry, %	Column Pressure when Headspace Formed, bar	Flow Rate at Which Headspace Formed, cm/hr	A_s		N/m	
			Before PFT	After PFT	Before PFT	After PFT
58.8	2.83	291	1.11	1.03	7,080	6,605
54.4	2.88	293	1.05	1.08	6,339	6,387
51.4	3.03	286	1.00	0.98	6,526	5,849

PFT, pressure-flow test.

Packed Nuvia HR-S Column Performance during Multiple Purification Cycles

During downstream process cycles, a column is likely in contact with various solutions, such as feedstream/equilibration buffers, elution buffers, sanitization solutions, and regeneration buffers. The integrity of a packed column is essential to the consistency and economics of production campaigns. The stability of Nuvia HR-S Resin packed in InPlace Columns (20 x 20 cm or 45 x 20 cm) was evaluated and the results are shown in Tables 15 and 16, respectively. Minor headspace appeared when these columns were exposed to a buffer/solution with a high concentration of salts. However, such column compression is reversible without substantial impact on column integrity. Practically, the headspace can be eliminated by a slight reduction in liquid velocity.

Table 15. Stability of Nuvia HR-S Resin packed in an InPlace Column (20 x 20 cm) during cycling at mobile phase linear velocity of 200 cm/hr. The column was packed by axial compression to CF 1.25 at 200 cm/hr. Column efficiency was tested before each cycle using 0.1 N NaOH as the mobile phase and 0.5 N NaOH as the test probe.

			Column Headspace during Run, mm			
Cycle	**N/m**	**A_s**	**200 mM NaP_i,* pH 6.5, 3 CV**	**10 mM NaOAc, pH 5.0, 10 CV**	**50 mM NaOAc, 1.0 M NaCl, pH 5.0, 10 CV**	**1.0 N NaOH, 10 CV**
Initial	6,011	1.16	0	0	0	0
1	6,364	1.05	2	0	4	4
2	6,267	1.02	2	0	4	4
3	6,248	1.00	2	0	4	4
4	5,891	1.00	2	0	4	4
5	6,100	1.00	2	1	4	4

* Sodium phosphate.

Table 16. Stability of Nuvia HR-S Resin packed in an InPlace Column (45 x 20 cm) during cycling. The column was packed by axial compression to CF 1.25 at 300 cm/hr. Column efficiency was tested before each cycle using 0.1 N NaOH as the mobile phase and 1 N NaOH as the test probe.

			Column Headspace during Run, mm			
Cycle	**N/m**	**A_s**	**200 mM NaP_i,* pH 6.5, 3 CV**	**10 mM NaOAc, pH 5.0, 10 CV**	**50 mM NaOAc, 1.0 M NaCl, pH 5.0, 10 CV**	**1.0 N NaOH, 10 CV**
Initial	9,479	1.00	0**	0**	0**	0**
1	10,638	1.00	1**	0**	1**	1**
2	11,050	1.00	1**	0**	1**	2**
3	10,695	1.00	1**	1**	1**	2**
4	10,582	1.00	4***	1***	3***	2**
5	10,638	1.00	4***	2***	4***	2**

* Sodium phosphate.
** At a linear velocity of 200 cm/hr.
*** At a linear velocity of 250 cm/hr.

Summary

We have demonstrated the packing of Nuvia HR-S Resin into process-scale columns with an ID of 20 or 45 cm, using axial, flow, and hybrid compression methods. Our data show that this cation exchanger can be effectively packed in common buffers/solutions used in chromatography separation and resin storage to different bed heights using the following parameters:

Compression factor: 1.20–1.30

Packing speed: 200–350 cm/hr

The concentration of Nuvia HR-S slurry does not have a significant effect on the performance of the packed columns. Nuvia HR-S process-column packing is robust, reproducible, and scalable. Users are recommended to thoroughly review the column manufacturer's instructions for designing a column packing procedure suitable for a specific manufacturing facility.

Troubleshooting Tips

Observation	Possible Cause	Correction
High rHETP	Underpacked column	Compress column further
	Clogged column screen/frit	Clean screen/frit
	Probe volume too large/unoptimized efficiency test condition	Modify injection loop to reduce probe volume; use alternative test probe
	Unevenly packed column	Compress column further or repack
Peak fronting	Channel(s) in column	Repack
	Overpacked column	Repack using a lower compression factor/packing factor
	Packing pressure/flow rate too high	Use a lower packing pressure/flow rate
Peak tailing	Probe volume too large/unoptimized efficiency test condition	Modify injection loop to reduce probe volume; use alternative test probe
	Interaction between test probe and resin	Use alternative test probe
	Column underpacked	Compress column further
	Air trapped under column adaptor/piston	Eliminate air
	Space between column adaptor/piston and bed	Adjust adaptor/piston
High column pressure	Clogged column screen/frit	Clean column screen/frit
	Presence of fine particles	Decant to remove fines
	Contaminated resin	Clean or replace resin
Split peak	Channels in column bed	Compress column further or repack
Shoulder peak	Interaction between test probe and resin	Use alternative test probe
	Plugged or contaminated resin	Clean or replace resin
Channeling when packing small ID columns	Hardware configuration	Use lower flow rate to consolidate the bed and then lower the adaptor to the desired bed height followed by conditioning with high flow rate

References

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Ng P et al. (2014). Improving aggregate removal from a monoclonal antibody feedstream using high-resolution cation exchange chromatography. Bio-Rad Bulletin 6439.

Reck JM et al. (2017). Separation of antibody monomer-dimer mixtures by frontal analysis. J Chromatogr A 1500, 96–104.

Christopher Foster and Payal Khandelwal generated the data for this application note. They are no longer employees of Bio-Rad Laboratories, Inc.

Ordering Information

Catalog #	Description
Prepacked Screening Tools	
732-4707	**Foresight Nuvia HR-S Plates**, 2 x 96-well, 20 µl
732-4831	**Foresight Nuvia HR-S RoboColumn Unit**, 200 µl
732-4832	**Foresight Nuvia HR-S RoboColumn Unit**, 600 µl
732-4723	**Foresight Nuvia HR-S Column**, 1 ml
732-4743	**Foresight Nuvia HR-S Column**, 5 ml
12009284	**EconoFit Nuvia HR-S Column**, 1 x 1 ml, 7 x 25 mm
Bulk Resin	
1560511	**Nuvia HR-S Media**, 25 ml
1560513	**Nuvia HR-S Media**, 100 ml
156-0515	**Nuvia HR-S Media**, 500 ml
156-0517	**Nuvia HR-S Media**, 10 L

Visit bio-rad.com/NuviaHRS for more information.

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