Reference Methods for Packing Columns with Nuvia™ IMAC Resin: From Lab-Scale to Process-Scale

Protocol

Immobilized metal affinity chromatography (IMAC) is an effective chromatography tool for preparing recombinant proteins naturally rich in histidine/cysteine residues, as well as those with a histidine residue cluster engineered to their *N*- or *C*-terminus via recombinant DNA technology. While a small quantity of protein prepared on a laboratory bench is often sufficient for structural and functional characterization studies, process-scale columns are needed in the manufacturing of recombinant proteins for therapeutic applications. We have developed effective protocols for packing Nuvia IMAC Resin in various column sizes to fulfill the needs of lab-scale to commercial production.

Product Characteristics

Nuvia IMAC is a rigid macroporous high-capacity metal affinity resin with good tolerance to a wide variety of chemicals employed for protein purification (bulletins 6859 and 6964). It is built on the robust UNOsphere™ base beads with nitrilotriacetic acid (NTA) as the chelating ligand for di- or trivalent metal ions. Its ligand density and particle size distribution are optimized to facilitate high dynamic binding capacity (DBC) while maintaining excellent pressure flow properties. Nuvia IMAC Resin is chemically stable under conditions commonly employed for protein preparation and column regeneration (Table 1).

Table 1. Properties of Nuvia IMAC Resin.

Property	Description
Ligand	Nitrilotriacetic acid (NTA)
Particle size	38–53 µm
Total ligand density	≥18 µmol/ml
Dynamic binding capacity*	>40 mg/ml at 300 cm/hr
Compression factor	1.20–1.30
Recommended linear flow rate	50–300 cm/hr
Pressure vs. flow performance	Under 2 bar at flow rate of 300 cm/hr in DI water (20 x 20 cm packed bed, 1.2 compression factor)
pH stability	2–14
Shipping solution	2% benzyl alcohol or 20% ethanol
Regeneration	50 mM EDTA, pH 8.0 (stripping) 1 N NaOH (CIP/SIP) 0.1 M Ni_2SO_4 (recharging)
CIP solution	1 N NaOH
Sanitization	1 N NaOH
Storage conditions	20% ethanol
Chemical compatibility**	Reducing agents (β-ME, TCEP, DTT) Denaturing agents (GnHCl) Detergents (Triton X-100, NP-40, CHAPS, CHAPSO) Additives (glycerol)
Chemical stability***	48 hr at 1 N NaOH 1 week at 0.01 N HCl
Shelf life	5 years

* 10% breakthrough capacity determined with 1.2 mg/ml of a 40 kD histidine-tagged protein in 50 mM sodium phosphate, 5 mM imidazole, 300 mM NaCl (pH 7.5).
** No recharge required. Refer to the instruction manual for a complete list.
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Abbreviations

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

A_s — asymmetry factor

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

C_s — resin slurry concentration

CF — compression factor

CV — column volumes

HETP — height equivalent to a theoretical plate

H_p — bed height/height of packed column

L — bed height (cm)

N — number of theoretical plates

V_e — peak elution volume or time

V_r — resin slurry in packing solution

V_s — settled bed volume

V_t — volume of resin transferred into a column

V_p — volume of packed column

R — radius of column

rHETP — reduced height equivalent to a theoretical plate

$W_{½h}$ — peak width at peak's half height in volume or time

Calculations

1 Determination of resin slurry concentration (C_s)

Method 1

- Transfer 10 ml of resin slurry [Nuvia IMAC Resin is provided as a 50% (v/v) slurry in 20% ethanol or 2% benzyl alcohol] into an Econo-Pac® Chromatography Column (catalog #7321010)
- Apply gentle suction at the column outlet to remove solution (**Note**: Avoid drying the resin)
- Add water to remove resin off the column wall. Stop the suction when a stable bed is formed
- Wait 5 to 10 min and record the settled bed volume
- Calculate C_s by dividing the settled bed volume (V_s) by 10 ml:

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

Method 2

- Transfer 10 ml of resin slurry into a graduated cylinder
- Allow resin to settle overnight
- Record the V_s
- Calculate slurry concentration using Eq. 1

Definition of compression factor (CF)

Eq. 2 $CF = V_t / V_p$

2 Determination of resin slurry in packing solution (V_r) needed to pack a column to desired bed height (H_p)

Eq. 3 $V_r = 3.14 \times R^2 \times H_p \times CF/(C_s/100)$

Eq. 4 $V_t = V_r \times C_s$

3 Preparation of resin in packing solution

- Transfer appropriate amount of resin slurry in storage solution into a column and drain to remove the storage solution
- Wash the resin with three column volumes (CV) of packing solution to fully remove the storage solution
- Add packing solution to resuspend the resin to make slurry with desired concentration

4 Evaluation of column efficiency

The packing quality of a column should be subjected to efficiency tests, which typically include determination of the height equivalent to a theoretical plate (HETP) or reduced height equivalent to a theoretical plate (rHETP), as well as the asymmetry factor (A_s). These tests should be repeated as necessary during the working life of a column to ensure that it meets the performance requirement for specific purification.

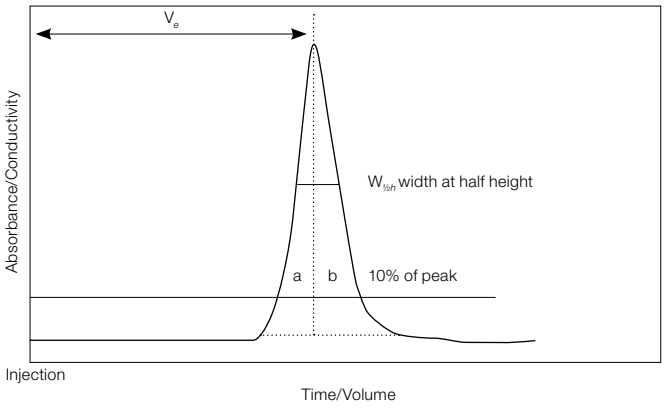

Fig. 1. A simulated chromatogram illustrating the calculation of HETP, rHETP, and A_s values.

5 Calculation of the number of theoretical plates

Eq. 5 $N = 5.54 \times (V_e/W_{½h})^2$

Eq. 6 $HETP = L/N$

Eq. 7 rHETP = 10,000 × HETP/average bead diameter (µm)

Eq. 8 $A_s = b/a$

Performing Column Qualification Tests

Method 1
Efficiency test with acetone as the probe

Probe solution composition:	2% v/v acetone in nanopurified water
Probe solution volume:	1% of V_p
Test velocity:	30 cm/hr
Eluent:	nanopurified water

Method 2
Efficiency test with sodium chloride (NaCl) as the probe

Probe solution composition:	0.8 M NaCl in nanopurified water
Probe solution volume:	1% of V_p
Test velocity:	30 cm/hr
Eluent:	0.4 M NaCl in nanopurified water

A packed column is equilibrated with 1.5 CV of eluent at test velocity before the probe solution is injected.

Reference Column Packing Protocols

Please review the column manufacturer's instruction manual thoroughly before performing any of the following lab-scale or process-scale procedures.

Lab-Scale Column Packing

Packing Nuvia IMAC Resin in a Vantage L Column (1.6 x 20 cm)

1. Calculate V_r (typically at a C_s of 45–65%) required for packing a column of desired H_p at a specific CF using Eq. 3.
2. Wet the bottom frit of the column with packing solution and remove air; then close the bottom outlet of the column.
3. Leave 1–2 cm of packing solution at the bottom of the column. Transfer an appropriate amount of resin (V_r) into the column. Use an extension tube or a packing reservoir, if needed, to ensure the entire resin slurry is transferred in one operation.
4. Rinse the interior wall of the column to wash down resin particles.
5. Allow the resin to settle in the column for 30 min or until a clear supernatant of 2–3 cm has developed above the resin bed.
6. Insert the top adaptor into the column and tighten the seal. Avoid trapping air between the adaptor and the supernatant.
7. Connect the column top adaptor to a chromatography system. Open the bottom outlet and consolidate the column bed by pumping packing solution through the column at 60 cm/hr.
8. When a stable bed is formed, stop the pump and close the bottom outlet.
9. Close the column top outlet and push the adaptor down slowly to compress the column to the desired H_p.
10. Connect the column to a chromatography system again and condition the column with 3 CV of packing solution at 600 cm/hr down flow.
11. Perform column qualification test.

Nuvia IMAC Resin can be packed in a Vantage L Column (Millipore Sigma) with excellent consistency, and post-packing conditioning improves column efficiency (Table 2). Packing at a CF higher than 1.2 may result in slight column fronting; however, this has little effect on the rHETP or the pressure flow properties of the packed columns (Table 3, Figures 2 and 3). For example, the integrity of a column packed with Nuvia IMAC Resin at a CF of 1.28 is well-maintained even at a linear flow rate of 800 cm/hr (Figure 3).

Table 2. Reproducibility of Nuvia IMAC Resin packed in a Vantage L Column (1.6 x 20 cm) using nanopurified water as the packing mobile phase.

	Column efficiency before conditioning		Column efficiency after conditioning	
Column	A_s	rHETP	A_s	rHETP
1	1.12	2.67	1.11	2.41
2	1.12	2.53	1.05	2.43
3	1.00	3.29	0.92	2.24
4	1.29	2.52	1.00	1.90
5	1.41	3.27	1.35	2.38

Column efficiency was evaluated using acetone as the probe (Method 1).

Table 3. Effect of CF on the qualification of Nuvia IMAC Resin packed in a Vantage L Column (1.6 x 20 cm) using nanopurified water as the packing mobile phase.

CF	A_s	rHETP
1.26	0.78	2.29
1.24	0.84	2.43
1.22	0.92	2.52
1.20	1.11	2.41

Column efficiency was evaluated using acetone as the probe (Method 1).

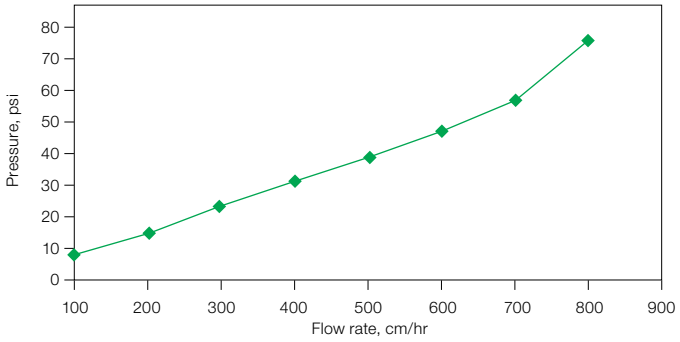

Fig. 2. Pressure flow curve of Nuvia IMAC Resin packed in a Vantage L Column (1.6 x 20 cm) at a CF of 1.2.

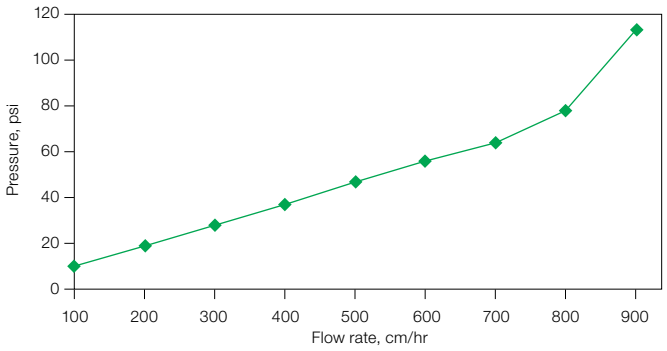

Fig. 3. Pressure flow curve of Nuvia IMAC Resin packed in a Vantage L Column (1.6 x 20 cm) column at a CF of 1.28.

Pilot/Process-Scale Column Packing

Packing Nuvia IMAC Resin in a BPG 100/500 Column (10 x 20 cm)

1. Calculate the V_r (typically at a C_s of 45–65%), required for packing a column of desired H_p at a specific CF using Eq. 3.
2. Wet the bottom frit of the column with packing solution. Remove air and then close the bottom outlet of the column.
3. Leave 1–2 cm of packing solution at the bottom of the column.
4. Transfer the appropriate amount of resin slurry (V_r) into the column.
5. Rinse the interior wall of the column to wash down resin particles. Allow resin to settle in the column for 30 min or until a clear supernatant of 2–3 cm has developed above the resin bed.
6. Lower the top adaptor to 1 cm below the liquid surface. Gently shake the adaptor to remove air trapped near the seal or underneath the adaptor.
7. Tighten the seal and lower the top adaptor further to remove any remaining air through the waste line of the top valve.
8. Connect the top valve to a pump and immediately open the bottom valve.
9. Consolidate the column bed by pumping packing solution through the column at 60 cm/hr.
10. When a stable bed is formed, stop the pump and close the bottom valve.
11. Loosen the seal slightly and lower the top adaptor to approximately 1 cm above the consolidated bed surface, and then tighten the seal again.
12. Close the top valve and open the bottom valve. Slowly screw down the top adaptor to compress the bed to the desired H_p.
13. Condition the column with 2 CV of packing solution at 320 cm/hr down flow, or at 43–54 psi, as hardware allows.
14. Perform column qualification test.

CF is crucial for packing Nuvia IMAC Resin in a BPG 100/500 Column (GE Healthcare Life Sciences) (Table 4). It appears that a CF of 1.28 provides the best packing quality among all compression factors tested. Similar to packing resin in a Vantage L Column, flow conditioning a packed column can further improve its efficiency (Table 5). The pressure flow property of Nuvia IMAC Resin packed in a BPG 100/500 Column (10 x 20 cm) is shown in Figure 4. The recommended maximum flow rate for performing protein purification with this column is 320 cm/hr.

Table 4. Effect of CF on packing Nuvia IMAC Resin in a BPG 100/500 Column (10 x 20 cm) using nanopurified water as the packing mobile phase.

CF	A_s	rHETP
1.25	1.35	4.72
1.28	1.16	3.91
1.3	1.21	4.86
1.34	0.99	4.77

Column efficiency was evaluated using sodium chloride as the probe (Method 2).

Table 5. Effect of post-packing conditioning of a column packed with Nuvia IMAC Resin (10 x 20 cm) at a CF of 1.28 using nanopurified water as the packing mobile phase.

		Column efficiency before conditioning		Column efficiency after conditioning	
Column	Column conditioning	A_s	rHETP	A_s	rHETP
1	Down flow 3 CV at 200 cm/hr, 23 psi	0.80	2.73	0.94	3.00
2	Down flow 2 CV at 367 cm/hr, 53 psi	0.86	2.40	1.05	2.42
3	Down flow 2 CV at 317 cm/hr, 54 psi	1.32	8.05	0.99	2.94

Column efficiency was evaluated using acetone as the probe (Method 1).

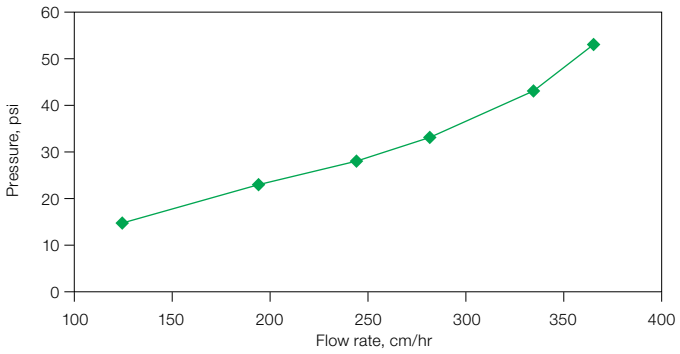

Fig. 4. Pressure flow curve of Nuvia IMAC Resin packed in a BPG 100/500 Column (10 x 20 cm) at a CF of 1.29.

Packing Nuvia IMAC Resin in an InPlace™ Column (Verdot Ips²) (20 x 20 cm) by axial compression

1. Calculate the V_r (typically at a C_s of 45–65%) required for packing a column of desired H_p at the specific CF using Eq. 3. Program the control console to set the desired H_p.
2. Wet the bottom frit of the column with packing solution and remove air, and then close the bottom outlet of the column.
3. Leave 1–2 cm of packing solution at the bottom of the column.
4. Transfer appropriate amount of resin slurry (V_r) to the column.
5. Rinse the interior wall of the column to wash down resin particles. Allow resin to settle in the column for 30 min or until a clear supernatant of 2–3 cm has developed above the resin bed.
6. Lower the top adaptor to 1 cm below the liquid surface. Gently shake the adaptor to remove air trapped near the seal or underneath the adaptor.
7. Inflate the seal to four bars and lower the piston at 300 cm/hr to remove any remaining air trapped inside the piston through the waste line of the top valve.
8. Close the column top valve and open the bottom valve.
9. Set the piston speed to 200 cm/hr to compress the resin to the desired H_p. Close the bottom valve when compression is completed. **Note**: Using a higher piston speed may cause column fronting.
10. Inflate the seal to six bars. Condition the column with 2 CV of packing solution at 200 cm/hr down flow.
11. Perform column qualification test.

While the CF is important for packing Nuvia IMAC Resin in an InPlace Column (Table 6), the resin C_s has no significant effect on column efficiency (Table 7). The pressure flow property of Nuvia IMAC Resin packed in an InPlace Column (20 x 20 cm) is shown in Figure 5. The recommended maximum flow rate for performing protein purification with this column is 300 cm/hr.

Table 6. Effect of CF on packing Nuvia IMAC Resin in an InPlace Column (20 x 20 cm) by axial compression.

CF	A_s	rHETP
1.2	1.03	2.84
1.25	1.19	1.80
1.3	0.77	4.97

Nanopurified water is used as the packing mobile phase and sodium chloride is the probe for column efficiency evaluation (Method 2).

Table 7. Effect of C_s on packing Nuvia IMAC Resin in an InPlace Column (20 x 20 cm) by axial compression.

C_s, %	A_s	rHETP
45	0.94	2.11
55	1.19	1.80
65	1.11	2.21

Nanopurified water is used as the packing mobile phase and sodium chloride is the probe for column efficiency evaluation (Method 2).

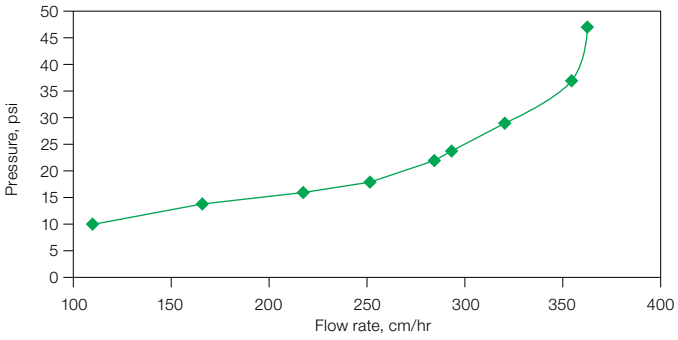

Fig. 5. Pressure flow curve of Nuvia IMAC Resin packed in an InPlace Column (20 x 20 cm) at a CF of 1.2.

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 or use an alternative test probe
	▪ Unevenly packed column	▪ Compress column further or repack
Peak fronting	▪ Channel(s) in column	▪ Repack
	▪ Over-compressed column	▪ Repack using a lower CF
	▪ 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 or use an alternative test probe
	▪ Interaction between test probe and resin	▪ Use an alternative test probe
	▪ Underpacked column	▪ 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
	▪ Over-compressed column	▪ Repack using a lower CF
Split peak Shoulder peak	▪ Channels in column bed	▪ Compress column further or repack
	▪ Interaction between test probe and resin	▪ Use alternative test probe
	▪ Plugged or contaminated resin	▪ Clean or replace resin
Channeling when packing small columns	▪ Hardware configuration	▪ Use slower flow rate to consolidate the bed and then lower the adaptor to the desired bed height followed by conditioning with high flow rate.

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