A Scalable Immobilized Metal Affinity Chromatography Resin for Process Purification

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Abstract

Downstream process purification of proteins requires a resin with optimized bead size for ideal pressure/flow properties and decent dynamic binding capacity (DBC) that provides production efficiencies and good process economics. Our newly developed metal chelate affinity resin — Nuvia™ IMAC — provides the mechanical strength, pore structures, ligand density, and particle size distribution required for an operation run at 300 cm/hr with a DBC of >40 mg/ml at <2 bar column backpressure. Protein purification can efficiently be scaled up from milligrams in the lab to kilograms in bioprocess manufacturing. The chemical stability of Nuvia IMAC ensures efficient column regeneration, reproducibility between purification runs, and extended column lifetime. It tolerates more than 100 clean-in-place cycles without significant change in DBC or product purity and is compatible with reagents typically employed for histidine-tagged protein purification. In summary, Nuvia IMAC overcomes the challenges associated with process purification of proteins using metal affinity chromatography.

Introduction

Immobilized metal affinity chromatography (IMAC) is a powerful purification technique that relies on a target molecule's affinity for metal ions immobilized on polymeric particles. It offers easy, single-step removal of the majority of contaminants irrespective of their chemical properties. IMAC has been used to purify many native histidine-rich proteins as well as recombinant histidine-tagged proteins (Block et al. 2009).

Nuvia IMAC Resin is a high-capacity affinity resin built on a robust and industry-proven base bead with nitrilotriacetic acid (NTA) as the chelating ligand for di- or trivalent metal ions. It has an optimized bead size for excellent pressure/flow properties and high DBC. The inert hydrophilic bead surface and the chemical structure of NTA ensure highly selective binding of recombinant histidine-tagged proteins when charged with Ni^{2+} or with other transition metals, such as Zn^{2+} or Cu^{2+}.

Features of Nuvia IMAC

High Binding Capacity at Fast Flow Rates

Nuvia IMAC Resin is designed with downstream process purification requirements in mind. Its large pore size ensures high DBCs even at the fast flow rates needed for process production efficiency (Figure 1), providing the flexibility needed to use this resin at any purification scale.

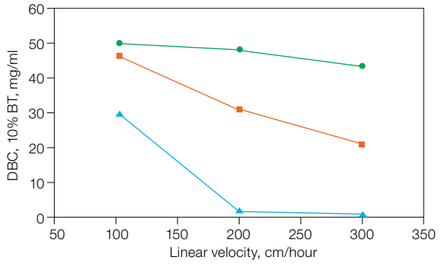

Fig. 1. DBC vs. flow velocity of Nuvia IMAC Resin. Comparison of DBCs between Nuvia IMAC (●), agarose IMAC resin A (■), and agarose IMAC resin B (▲). The resins were packed into 1 ml columns (0.5 x 5 cm). A 45 kD histidine-tagged protein (1.2 mg/ml) in 50 mM sodium phosphate, 5 mM imidazole, 300 mM NaCl (pH 7.5) was loaded onto each column until 10% breakthrough (BT) was observed.

Superior Pressure/Flow Performance

Nuvia IMAC Resin is designed with an optimal bead size distribution to achieve both laboratory- and process-scale purification of histidine-tagged proteins at high flow rates, without being limited by column pressure. This leads to an increase in protein purification productivity (Figure 2).

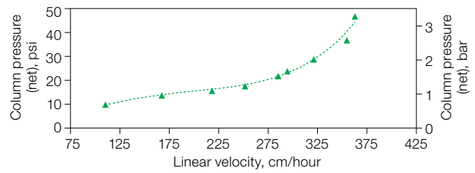

Fig. 2. Pressure/flow performance of Nuvia IMAC Resin. Uncharged Nuvia IMAC Resin slurry prepared in water was packed into a 20 x 20 cm column by axial compression using a compression factor of 1.2. The column pressure remained below 2 bar at a linear velocity of 300 cm/hr.

Purification of Histidine-Tagged Recombinant Protein

Figure 3 shows the purification of a 45 kD histidine-tagged protein from a crude *E. coli* lysate. The various fractions shown on the chromatogram were analyzed by SDS-PAGE (Figure 4). The purity of the purified bands was estimated by densitometry to be >90%.

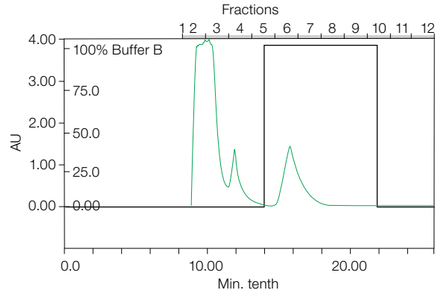

Fig. 3. Purification of a 45 kD histidine-tagged protein on a Nuvia IMAC Column. *E. coli* extract (2 ml) was loaded on a 0.46 x 5 cm column pre-equilibrated with 50 mM Na_3PO_4, 10 mM imidazole, and 300 mM NaCl, pH 7.5 (buffer A). The target protein was eluted with 10 CV 50 mM Na_3PO_4, 125 mM imidazole, 300 mM NaCl, pH 7.5 (buffer B). Column chromatography was monitored at OD 280. A_{280} (—); %B (—). AU, absorbance units.

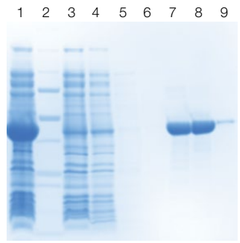

Fig. 4. Analysis of column fractions from the purification of a 45 kD histidine-tagged protein on a Nuvia IMAC Column. Fractions collected from the chromatography run shown in Figure 3 were analyzed by SDS-PAGE. Equal amounts (5 µl) were loaded onto the gel. Lane 1, *E. coli* lysate; lane 2, Precision Plus Protein™ Unstained Standards; lanes 3–9, fractions 3–9.

Excellent Recovery

Nuvia IMAC has an inert and hydrophilic bead surface that minimizes nonspecific binding due to charge or hydrophobic interactions. The pore structure of the beads is also optimized to allow efficient mass transfer under dynamic conditions. These features contribute to the excellent recovery of histidine-tagged proteins from Nuvia IMAC (Figure 5).

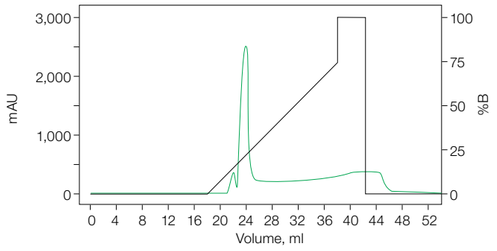

Fig. 5. Recovery performance of Nuvia IMAC Resin. A 45 kD histidine-tagged protein (10 mg) was loaded onto a 1 ml column of Nuvia IMAC (0.46 x 5 cm) at a flow rate of 300 cm/hr. The equilibration and post-loading wash was performed with buffer A (50 mM sodium phosphate, 300 mM NaCl, pH 7.5). The elution was performed in a 20 CV linear gradient with 0–75% buffer B (50 mM sodium phosphate, 300 mM NaCl, 1 M imidazole, pH 7.5) and then held at 100% buffer B for 5 CV. Recovery of target protein was 96.8%. A_{280} (—); %B (—).

Reproducible Performance and Reusability

Nuvia IMAC Resin shows no significant loss in its DBC for over 100 cycles of repeated use (Figure 6). It is produced by a validated manufacturing process to ensure batch-to-batch reproducibility.

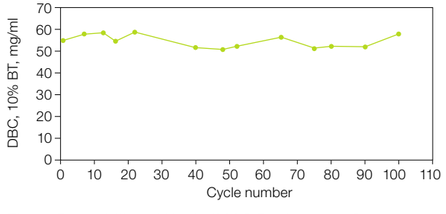

Fig. 6. Reusability of Nuvia IMAC Resin. A 45 kD histidine-tagged protein was loaded onto a 0.5 x 5.1 cm column packed with Nuvia IMAC with a compression factor of 1.25. The column was run at 300 cm/hr. The binding buffer was 50 mM sodium phosphate, 5 mM imidazole, and 300 mM NaCl (pH 7.5) and the elution buffer contained 250 mM imidazole. Solutions for column regeneration are listed in Table 1.

Wide-Range Chemical Stability and Compatibility

Nuvia IMAC Resin is stable in a broad range of chemicals (Table 1). It is compatible with all reagents commonly used for histidine-tagged protein purification, including reducing agents, detergents, denaturing agents, and additives.

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	1.20–1.25
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 (urea, 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 45 kD histidine-tagged protein in 50 mM sodium phosphate, 5 mM imidazole, 300 mM NaCl (pH 7.5).
** Recharging is not required. Refer to the instruction manual for a complete list.
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Conclusions

Nuvia IMAC meets the demands of scalability in process purifications. It delivers ease of use, value, and flexibility by providing process developers with:

- Robust scale-up
- High product quality
- High throughput
- High productivity

References

Block H et al. (2009). Immobilized-metal affinity chromatography (IMAC): a review. Methods Enzymol 463, 439–473.

Bulletin 6860 Ver A 16-1167 1216