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Application Note

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Vivaspin® Ultrafiltration Devices: Tools for Concentrating His-Tagged Proteins Eluted in High Imidazole Following Affinity Chromatography

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Abstract

Recombinant proteins are often engineered to include a his-tag at the N- or C-terminus and can be over-expressed in several systems, to account for over 30% of the total protein content in *E.coli* , for example. The his-tag on these proteins allows for a single affinity chromatography step to achieve high purity of the target protein. Elution from the affinity chromatography matrix is effected with imidazole, at concentrations up to 500 mM. Imidazole presents a challenge in downstream applications, where it can interfere in, for example, protein quantification. It is therefore important to remove imidazole but conventional techniques such as dialysis are time-consuming. Ultrafiltration is a much faster technique for both concentration of the purified protein and buffer exchange to remove imidazole. However, some ultrafiltration devices on the market are not recommended for use with high concentrations of imidazole, due to chemical incompatibility. In this study, we evaluated the resistance and ultrafiltration performance of Vivaspin® devices when used to process samples containing high imidazole concentrations.

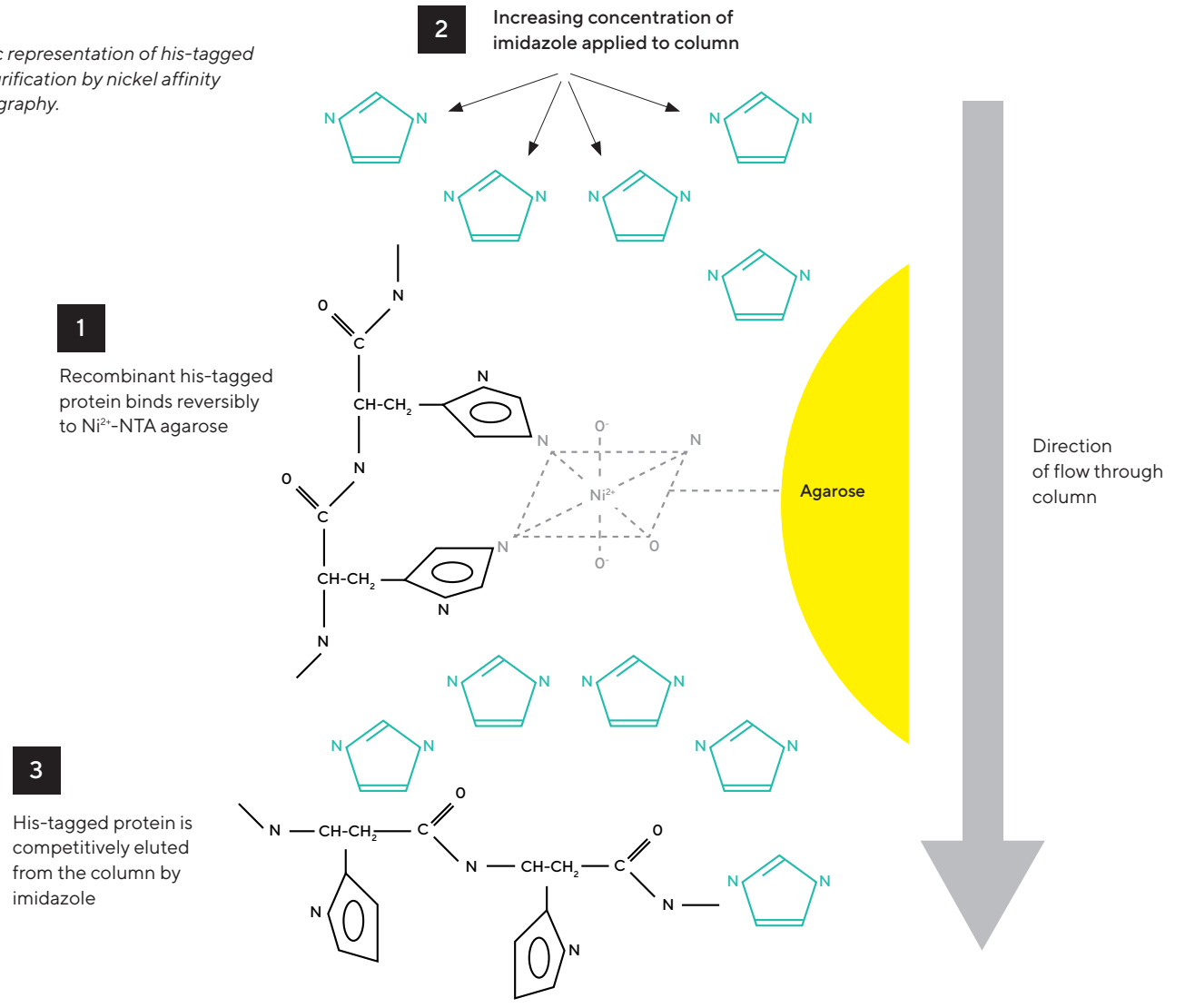
Introduction

Soluble recombinant proteins are produced using numerous expression systems, including mammalian and insect cells, yeast, filamentous fungi, bacteriophage and bacteria. Many of these proteins are engineered to contain 2-10 adjacent histidine residues at the N or C terminus. These so-called his-tags facilitate rapid protein purification based upon the selective affinity of proteins with polyhistidines for an immobilized metal chelate adsorbent, such as Ni^{2+} ions[1,2]. This binding occurs in physiological buffers that are suited to the protein of interest, and can be performed under native or denaturing conditions[3]. Strong denaturants such as urea can be used for efficient solubilization and purification of receptors, membrane proteins and proteins of limited solubility found in inclusion bodies. The interaction between the histidine residues and the Ni^{2+} ions is reversible and the bound protein can be eluted under mild conditions by increasing the concentration of imidazole (typically up to 500 mM) or by lowering the pH. Elution with imidazole tends to be the method of choice.

His-tagged proteins may be purified in either batch mode, using Ni^{2+}-NTA agarose pellets; or with spin, gravity flow or FPLC columns (Figure 1). Eluted proteins often require further concentration but the high imidazole content presents a chemical compatibility problem for some centrifugal ultrafiltration devices available on the market. Therefore, eluents require lengthy dialysis to remove imidazole prior to concentration, or dilution, which extends the processing time for ultrafiltration. In this study, we demonstrate the high resistance of Vivaspin® 500, 2 and 20 devices to imidazole at concentrations up to 500 mM, enabling efficient concentration and buffer exchange without the need to first remove or dilute imidazole in nickel affinity eluates.

Figure 1.

Schematic representation of his-tagged protein purification by nickel affinity chromatography.



Materials and Methods

His-tagged proteins are typically eluted from affinity columns in 50 mM Tris-HCl (pH 7.6), 200 mM NaCl, containing up to 500 mM imidazole. Chemical tolerance to imidazole was therefore determined by challenging Vivaspin® 10 kDa MWCO devices (Figure 2) with 0.05 mg/mL BSA (to mimic the recombinant protein) in this buffer, with imidazole concentrations from 0-500 mM for 2 hours prior to centrifugation at 12,000 *g* for 10 min (Vivaspin® 500), 5,000 *g* for 10 min in a 45° fixed angle rotor (Vivaspin® 2) or 3,000 *g* for 20 min in a swing bucket rotor (Vivaspin® 20).

The devices were inspected visually for any signs of plastic deformation and performance was measured using estimations of protein concentration by Bradford assay[4]. BSA (fraction V, essentially fatty acid free) was used for protein standards and it was confirmed by BIO-RAD Technical Support (Hemel Hempstead, UK) that concentrations up to 500 mM imidazole are compatible with the Bradford assay prior to beginning the study.

Figure 2.

Vivaspin® ultrafiltration devices tested in this study. From left: Vivaspin® 500, 2 and 20.

Results

Total compatibility with 0.5 M imidazole was observed. No visible defects of the centrifugal devices were observed after 2 hour exposure to 500 mM imidazole.

Table 1.

Process conditions used to concentrate BSA samples with imidazole concentrations from 0-500 mM, and mean protein recoveries determined by Bradford assay.

Device	Initial Volume	Mean Final	Concentration Factor	Process time and RCF	Mean recoveries at different imidazole concentrations (n=8)			
					0 mM	100 mM	250 mM	500 mM
Vivaspin® 500	500 µL	5 µL	100x	10 min @ 12,000 *g*	94%	93%	92%	96%
Vivaspin® 2	2,000 µL	66 µL	30x	10 min @ 5,000 *g*	95%	96%	92%	98%
Vivaspin® 20	10,000 µL	117 µL	85x	20 min @ 3,000 *g*	97%	98%	95%	94%

Rapid concentration of the protein sample was observed. The concentration factor is often a critical measure of performance and was independent of the imidazole concentration in all devices tested. When centrifuged at 5,000 *g* for 10 min, a 2 mL BSA sample processed in Vivaspin® 2 was concentrated 30-fold, with tightly clustered final mean volumes of 66 µL ± 3.9 SD (~30-fold concentration, Table 1).

High recoveries (in excess of 90%) of protein were observed at all concentrations of imidazole, further supporting the use of Vivaspin® devices for concentration of his-tagged proteins eluted with imidazole from immobilized metal chelate adsorbents.

Conclusion

Two hour exposure of Vivaspin® ultrafilters to 500 mM imidazole had no impact on device integrity. Furthermore, there were no deleterious effects on speed of concentration or recovery of the target protein tested in this study. Therefore, Vivaspin® 500, 2 and 20 are the ideal research tools for sample concentration following affinity purification, even with high imidazole content. In addition, diafiltration can be performed in the same device, to remove residual imidazole without the need for time and buffer-intensive conventional re-buffering techniques, such as dialysis or gel filtration chromatography.

References

1. Hochuli, E., Döbeli, H. and Schacher, A. (1987) New metal chelate adsorbents selective for proteins and peptide containing neighbouring histidine residues. Journal of Chromatography **411**, 177-184.

2. Hochuli, E. (1990) Purification of recombinant proteins with metal chelate adsorbent. p. 87-98. In: Genetic Engineering, Principle and Methods, vol. 12 (Setlow, J. K. ed.) Plenum Press, New York.

3. Schmitz, M. L. and Baeuerle, P. A. (1997) Bacterial expression, purification and potential use of His-Tagged GAL4 fusion proteins. Ch. 12. p. 129-137. In: Recombinant Protein Protocols: Detection and Isolation (Rocky S. Tuan ed.) Humana Press, New Jersey.

4. Bradford, M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry **72**, 248-254.

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