Waters™

Application Note

Robust Protein and Oligonucleotide Desalting with Sep-Pak™ Desalting Cartridges

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Abstract

Desalting is a critical step in preparing samples for downstream analysis. Waters™ Sep-Pak Desalting Cartridges employ advanced size-exclusion chromatography to efficiently purify, desalt, and buffer exchange samples. Designed for both manual and automated workflows, the Sep-Pak Desalting Cartridges ensure reproducible high sample recovery and effective impurity exclusion across a broad range of sample concentrations and volumes.

Benefits

- Sep-Pak Desalting Cartridges provide efficient purification, desalting, and buffer exchange.
- Consistent sample recovery and impurity exclusion across a wide range of sample concentrations and volumes.
- Optimized flow properties for reliable gravity-based flow that are compatible with both manual and automation workflows.

Introduction

Biomolecules are complex entities that require special considerations prior to their analysis. For example, sample preparation processes like desalting are often needed to reduce salts and/or change buffer components ahead of a downstream analysis. Given the ubiquitous nature of desalting, a device that can provide high recovery of biomolecules while effectively removing impurities is valuable across a wide range of applications. In addition to protein purification, desalt devices are also effective for nucleic acid clean-up, including single-guide RNA (sgRNA), which is commonly used in CRISPR-based gene editing workflows. With a relatively large size of ~100 nucleotides, sgRNA can be efficiently separated from smaller contaminants with devices that are also designed for desalting ≥5 kDa proteins. In this application note, the robustness and consistency of Sep-Pak Desalting Cartridges is demonstrated using a representative protein or oligonucleotide. These devices facilitate the removal of high ionic strength buffer compositions to levels less than 5 mM. Just as importantly, these devices have shown batch-to-batch reproducibility to achieve >83% recoveries with RSD values less than 3%.

Experimental

Two experiments were conducted to evaluate the performance of the Sep-Pak Desalting Cartridges: one using a model protein (bovine serum albumin, BSA) and the other using a synthetic oligonucleotide, sgRNA.

For obtaining analytical characteristics of the Sep-Pak Desalting Cartridges (Waters p/n: 186010127 < https://www.waters.com/nextgen/global/shop/sample-preparation--filtration/186010127-sep-pak-sec-desalting-cartridge-1-cc-5k-mwco.html>), the protocol described in the Care & Use (Literature Code: 720007983 < https://help.waters.com/help/global/support/library-details.html?documentid=720007983>) was performed with 1 mg/mL BSA in 6 M guanidine HCl. While using the 1cc Cartridge Stand (Waters p/n: 186010128 < https://www.waters.com/nextgen/global/shop/sample-preparation--filtration/186010128-1-cc-cartridge-stand.html>) to position the cartridges, the desalted sample was eluted with 100 mM Tris-HCl and the collected eluent was analyzed using a spectrophotometer at 280 nm.

For the demonstration of nucleic acid clean-up, sgRNA (mouse Cas9 GATA2 - Waters p/n: 186011357-1) in a concentrated aqueous ammonium hydroxide solution (28% NH_3 in water) was processed using the Sep-Pak Desalting Cartridges. After conditioning the cartridges with three aliquots of 400 µL of 10 mM Tris-HCl, a 100 µL volume of 140 ng/µL sgRNA sample was added to the cartridges. To generate conductivity and sample mass

profiles, two 250 µL aliquots of 10 mM Tris-HCl were added to the cartridges, and each eluted fraction was collected and analyzed with a conductivity meter and a microvolume spectrophotometer at 260 nm.

Results and Discussion

Sep-Pak Desalting Cartridges are engineered for the efficient purification of samples. The 1cc cartridge's flangeless design allows for seamless integration into both automation workflows (the cartridges are preprogrammed consumables in OneLab™ for automation on Andrew+™ systems) and manual workflows (the cartridges can be arranged in a standard 8 x 12 format for interfacing with single- or multi-channel pipettes). The cartridge's packing material facilitates particle-based size-exclusion chromatography (SEC) by using a crosslinked spherical dextran-based resin (Figure 1A) that is stable from pH 2–13. With optimized geometry and flow characteristics, the resin bed is designed for speed and analyte recovery. Given the particle's pore size properties, the size exclusion material is suitable for samples sized ≥5 kDa for globular proteins and 20 base pairs for nucleic acids (Figure 1C).

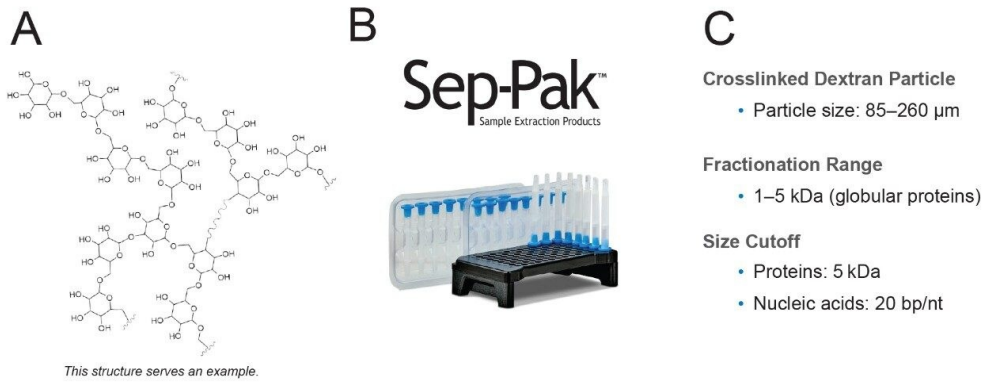

Figure 1. Overview of the physical and chemical properties of the Sep-Pak Desalting Cartridges.

Protein Desalting

The effectiveness of protein desalting was investigated using a 1 mg/mL BSA solution prepared in 6 M guanidine HCl. The applied protocol closely resembled the desalting step that is applied in the PeptideWorks Tryptic

Protein Digestion Kit. Percent recovery values achieved using devices fabricated from several different sorbent batches and unique manufacturing runs are depicted in Figure 2. Each analyzed lot was studied as a cohort of ≥16 cartridges. Across all test variables, protein recovery consistently exceeded 83% with <3% RSD (Figure 2A). Additionally, salt removal was highly effective; salt concentration ionic strength was reduced from 6 M to 2 mM ± 2 mM (Figure 2B).

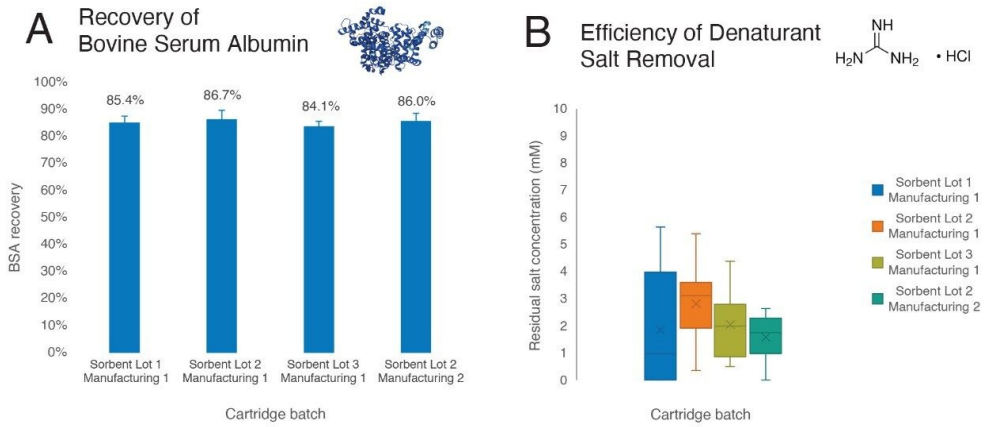

Figure 2. Percent protein recovery (A) and salt removal efficiency (B) from Sep-Pak Desalting Cartridges. Each analyzed lot (≥16 cartridges) was fabricated from different sorbent lots and manufacturing runs. Error bars represent one standard deviation.

The effectiveness of a desalting tool is intimately tied to the protocol with which it is used. For this reason, the elution behavior across increments of column volume as well as the load dependence of the Sep-Pak Desalting Cartridges was studied. These findings are presented in Figure 3. Robust protocol conditions can be observed across varying concentrations and volumes. A simplified average response model (Figure 3) indicated that sample concentration has a minimal impact on recovery. Consistent performance was observed with BSA concentrations ranging from 0.25 to 1.75 mg/mL. The 3D surface plot (Figure 3A) illustrated that recovery is primarily influenced by volume, with optimal recovery observed around 100 µL. This suggests that Sep-Pak Desalting Cartridges are reliable and effective in maintaining recovery rates across different sample concentrations, making them suitable for diverse experimental conditions.

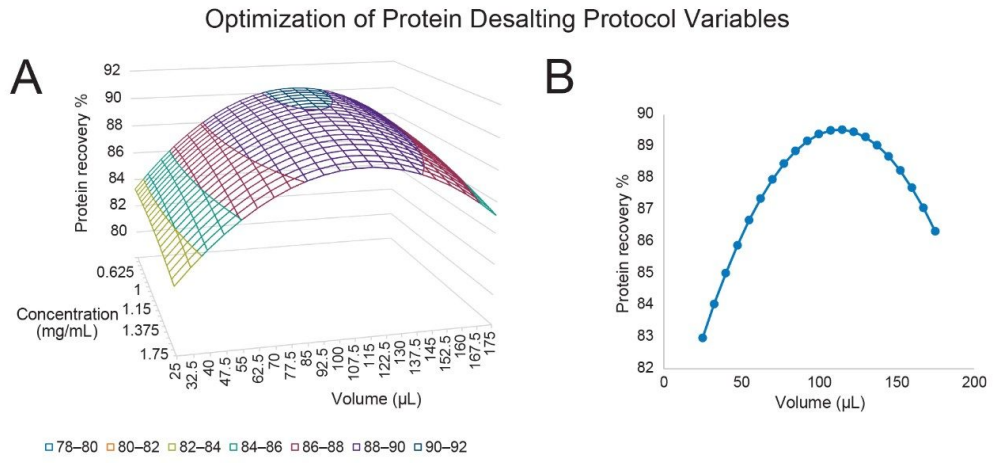

Figure 3. Results from a simplified average response model. The 3D surface plot (A) exemplifies that protein recovery is primarily influenced by volume; (B) demonstrates that optimal recovery is observed with a sample volume of approximately 100 µL.

Oligonucleotide Clean-up

Following oligonucleotide synthesis, residual impurities (deprotection reagents, hydrolyzed protecting groups, *etc.*) can interfere with downstream applications and analytical procedures. The Sep-Pak Desalting Cartridges provide an effective method for post-synthesis clean-up by enabling size-based separation of reaction components. Smaller molecules are efficiently separated from larger species, like the 100-mer sgRNA used here. In Figure 4, a mixture of sgRNA and ammonium hydroxide was processed using the Sep-Pak Desalting Cartridges and subsequently analyzed using a microvolume spectrophotometer. The traces demonstrate that the sgRNA, being a relatively large molecule, elutes earlier with lower conductivity fractions. As the separation progresses and most of the sgRNA has eluted from the resin bed, ammonium hydroxide begins to elute, as evidenced by the sharp rise in conductivity. This profile confirms the cartridge's ability to isolate high purity sgRNA from lower molecular weight species. This same separation power can also be employed to reduce salt concentrations encountered with ion exchange purified oligonucleotides.

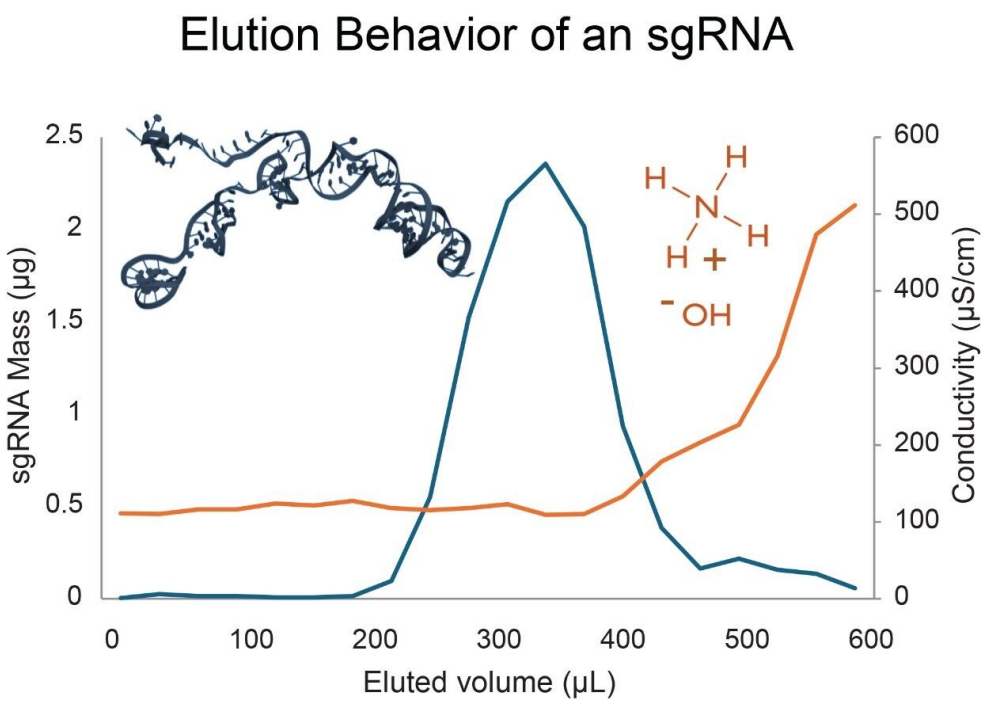

Figure 4. Elution profiles of oligonucleotide (sgRNA) mass (blue trace) and ammonium hydroxide conductivity (orange trace). The sgRNA elutes first in lower conductivity fractions.

Conclusion

- Sep-Pak Desalting Cartridges efficiently purify, desalt, and buffer exchange both proteins and oligonucleotides.
- Reliable gravity-based flow properties allow for smooth integration into manual and automated biomolecular workflows.
- Consistent recovery and impurity exclusion across a broad range of sample volumes and concentrations make these cartridges ideal for platform methods that require reliability and flexibility.

With demonstrated performance in both protein and oligonucleotide clean-up, the Sep-Pak Desalting Cartridges

offer a versatile solution for modern biomolecular workflows, including those supporting gene editing, proteomics research, and the development of biotherapeutics.

Featured Products

Sep-Pak Sample Extraction Products <https://www.waters.com/nextgen/global/products/sample-preparation/sep-pak-sample-extraction-products.html>

PeptideWorks Tryptic Protein Digestion Kits <https://www.waters.com/nextgen/global/products/application-kits/peptideworks-tryptic-protein-digestion-kits.html>

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