

Automated Desalting of Proteins With the Profinia™ Protein Purification System: Comparison to Manual Desalting by Dialysis

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Introduction

Protein desalting is the process of separating proteins in a solution from low molecular mass components. Desalting can be used for buffer exchange, to remove low molecular mass reagents, and to adjust the ionic strength of a protein solution. Two commonly used methods for desalting are dialysis and gel filtration. Dialysis uses a membrane to retain large molecules, such as proteins, while allowing diffusion of smaller molecules into a solvent. Gel filtration uses size exclusion to separate proteins from small molecules: Small molecules permeate the pores of the gel filtration media and are retarded, while proteins larger than the molecular weight cutoff are excluded from the pores and elute first.

The Profinia protein purification system is an automated low-pressure chromatography system that can be used to desalt proteins by gel filtration. Optimized methods are preprogrammed and can be used with the buffers and prepacked cartridges available in Profinia purification kits (Figure 1). The preplumbed system is also easily maintained through automated self-cleaning protocols. When used with Profinia software, the system displays run data in real time and allows the generation and printing of reports containing chromatograms, method steps, and run information.

The present study compares the performance of the Profinia system to dialysis, the most commonly used method for protein desalting. Performance parameters tested include the time required for complete desalting, exchange buffer consumption, product yield, and sample dilution.

Methods

Bovine serum albumin (BSA) fraction V (Sigma-Aldrich) was dissolved in 300 mM KCl and 50 mM potassium phosphate to a final concentration of 3 mg/ml in preparation for desalting. A 2 ml sample and a 10 ml sample were then desalted using the Profinia system or by dialysis. The progress of desalting was followed by measuring sample conductivity, and protein concentration and yield were determined spectrophotometrically using the sample absorbance at 280 nm and the known absorbance of a 1 mg/ml BSA solution. Three desalting runs were performed with each method.



Fig. 1. Workflow from protein sample to desalted protein using the Profinia system.

Desalting With the Profinia System

To desalt a sample of BSA by gel filtration, the appropriate method (either “10 ml Desalting” or “50 ml Desalting”, depending on the sample size) was selected from the menu of Profinia chromatography methods. The required reagents, sample loop, and Bio-Scale™ Mini Bio-Gel® P-6 desalting cartridge were installed as instructed by the instrument display

screen. To desalt the 2 ml sample, a 2 ml sample loop and a 10 ml desalting cartridge were used. To desalt the 10 ml sample, a 10 ml sample loop and a 50 ml desalting cartridge were used. Profinia software was used for real-time monitoring of the UV absorbance and conductivity of the sample during the desalting run and for collection and analysis of run data. Buffers and desalting cartridges are part of the Profinia desalting kit, while sample loops can be obtained as an accessory for the desalting-only application.

Desalting by Dialysis

The dialysis protocol described by Zumstein (1994) was followed using a Spectra/Por dialysis membrane with a molecular weight cutoff of 14 kD (Spectrum Laboratories) and a 1:500 volume of phosphate buffered saline (PBS). During desalting, PBS was mixed continuously using a magnetic stirrer, and conductivity of the BSA sample was monitored at 15 min intervals using a MeterLab CDM210 conductivity meter (Radiometer Analytical). Dialysis was complete when the conductivity of the sample reached equilibrium with that of the exchange buffer.

Results and Discussion

Using the Profinia system, UV absorbance and conductivity data were automatically collected and plotted to demonstrate the performance of this desalting method (Figure 2). The Profinia system also automatically delivered the desalted protein product to a collection tube in 15 min, regardless of the sample size being desalted. In contrast, the dialysis method required manual conductivity measurements to determine when buffer exchange was complete.

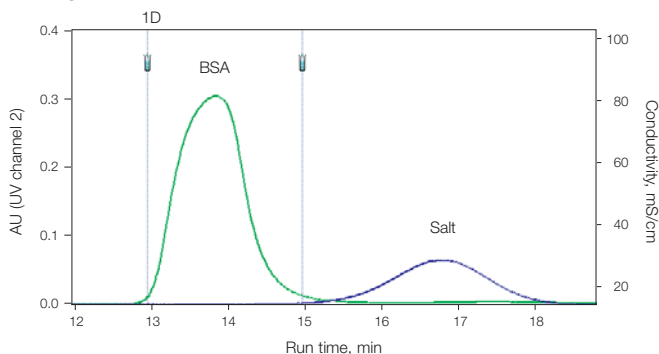
Both methods were capable of desalting protein samples to completion; however, there were notable differences in performance (Table 1). The time required to complete desalting with the Profinia system was 8- to 10-fold less than the time required for dialysis, depending on the size of the sample. Although both methods resulted in >90% yield, the final concentration of the dialyzed protein sample was 2-fold higher. Finally, the Profinia system used 20-fold less desalting buffer than was required for dialysis.

Table 1. Performance comparison of the two desalting methods.

Average values from three replicate desalting runs (n = 3) are shown.

Method	Total Time Required	Final Protein			Yield	Buffer Used
		Concentration (mg/ml)	Recovery (mg)			
2 ml Sample						
Profinia system	15 min	1.4	5.6	93%	50 ml	
Dialysis	120 min	2.8	5.6	93%	1,000 ml	
10 ml Sample						
Profinia system	15 min	1.4	28.0	93%	250 ml	
Dialysis	150 min	2.7	28.3	94%	5,000 ml	

2 ml BSA



10 ml BSA

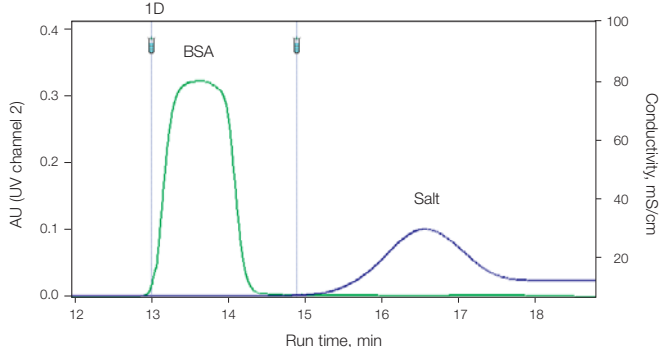


Fig. 2. UV absorbance and conductivity profiles of desalting runs on the Profinia system. Absorbance at 280 nm and conductivity readings of BSA samples were collected over time. (—), UV absorbance at 280 nm; (—), conductivity trace. The two vertical lines mark the collected protein peak (1D), which is resolved completely from the salt peak.

Conclusions

Dialysis and gel filtration chromatography are two commonly used methods for desalting protein solutions. As the mechanism of buffer exchange is not the same, there are advantages to each method. Dialysis will usually produce a more concentrated product than gel filtration, though some sample dilution should still be expected. Desalting by gel filtration using the Profinia protein purification system offers substantial time savings as well as the significant advantages of greatly decreased buffer consumption and an automated, scalable process.

Reference

Zumstein L, Dialysis and ultrafiltration, appendix 3C.1 in Current Protocols in Molecular Biology (Ausubel FM et al., eds), Wiley, New York (1994)

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