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Application Note

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Vivaspin® 20 Diafiltration Cups: A Rapid Alternative to Buffer Exchange by Dialysis

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Abstract

Many typical protein purification workflows will include a buffer exchange of the protein sample. This may be important to provide the appropriate conditions for the next purification step, prepare the protein of interest for use in downstream applications, or ensure stability of the purified protein. A conventional buffer exchange process may be performed by dialysis. However, this method is time consuming, requires large volumes of the exchange buffer, and increases the potential for degradation of the target protein by proteases in the sample. Diafiltration (DF)—a process using ultrafiltration devices for the same purpose—ensures a much faster, effective, and safer buffer exchange. Here, we demonstrate the increased efficiency of buffer exchange when using Vivaspin® 20 centrifugal ultrafiltration devices with DF cups. Unique to Sartorius, these DF cups enable a gradual change to the sample buffer composition, ensuring a gentle but still more efficient buffer exchange.

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Introduction

During the preparation of biological samples, buffer exchange is an essential step, as it prepares the sample for downstream applications or enables subsequent long-term storage.[1,2] It can be performed by dialysis or diafiltration. Diafiltration with Vivaspin® 20 and Sartorius DF cups is a well-established method in protein science laboratories for buffer exchange and desalting steps. To only highlight a few examples, it has been applied by Read *et al.*[3] in the preparation of fusion proteins for a linking reaction to affinity purification columns. Here, GST fusion proteins were purified by glutathione-agarose affinity chromatography and subsequently the buffer was exchanged to a coupling buffer (0.2 M $NaHCO_3$, 0.5 M NaCl, 2 M urea, pH 8.3) using the DF cup.[4] Aziz *et al.*[5] performed a desalting step prior to crystallization of the receiver domain of a putative response regulator, BPSL0128. Here, 0.2 M NaCl, 50 mM Tris pH 8.0 was exchanged for 10 mM Tris pH 8.0 using the DF cup. Tovar-Herrera *et al.*[6] desalted the expansin protein ScExlx1 prior to activity assays (20 mM NaH_2PO_4, 20 mM imidazole, 0.5 M NaCl pH 7.4 against 50 mM NaOAc, pH 5). Finally, Guccione *et al.*[7] desalted active site subunit of methylmenaquinol: fumarate reductase (Mfr) prior to enzymatic assays (1.5 M NH_4SO_4, 50 mM Tris, pH 8.0 against 50 mM Tris, pH 8.0).

The dialysis process traditionally used for buffer exchange in biological samples relies on passive diffusion. It is therefore time consuming and requires large volumes of dialysis buffer.[8] Here we present an approach based on diafiltration with Vivaspin® 20 centrifugal concentrators. In combination with Sartorius DF cups, these devices offer a fast, efficient, and reliable way to exchange protein sample buffers. The gradual buffer exchange by diafiltration allows for gentle salt removal from protein samples prone to precipitating at high salt concentrations and thus keeps them in solution. In addition, the short processing time helps prevent degradation of the protein of interest by proteases.

Materials and Methods

To assess the effectiveness and performance of diafiltration in comparison to the conventional dialysis approach, Sartorius Vivaspin® 20 products were used in parallel to dialysis cassettes. A dialysis utilizing these cassettes was performed according to the instructions given by the manufacturer, following an overnight procedure. The aim was to reduce the salt concentration by 99%.

The Vivaspin® 20 operating conditions for buffer exchange were optimized with and without a DF cup, using a BSA model solution and CHO cell culture supernatant (salt reduction from 1 M to 0.01 M).

Optimal conditions for > 99% salt reduction were:
- 4,000 *g* in a swing bucket rotor
- 15 mL exchange buffer

Centrifugation time for each sample type was determined (Table 1) for two spins to reach the dead stop volume, with an addition of exchange buffer in between.

Table 1

Centrifugation Times for BSA and CHO With and Without DF Cup

	BSA	CHO
Vivaspin® 20 without DF cup	2 × 8 min	2 × 45 min
Vivaspin® 20 with DF cup	2 × 6 min	2 × 45 min

After optimizing the Vivaspin® 20 desalting conditions, the diafiltration procedure was compared to dialysis. The desalting process was more efficient with Sartorius DF cups.

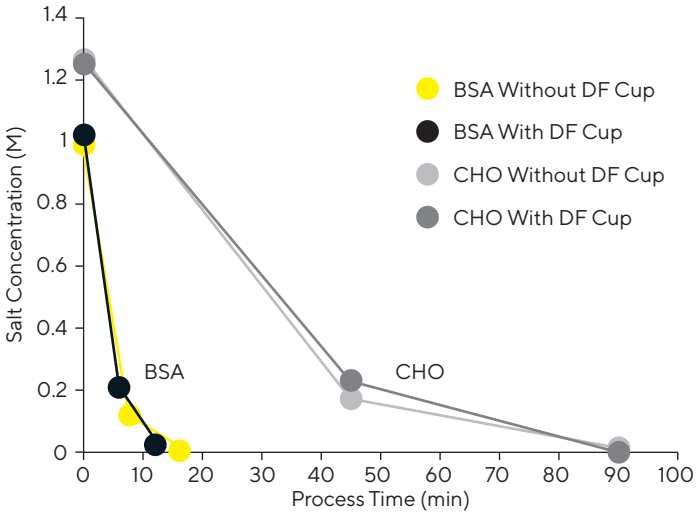
Following the buffer exchange, the integrity of all protein samples was checked by SDS-PAGE. Salt concentrations were assessed by conductivity measurement.

Results

Comparison of Buffer Exchange Using Vivaspin® 20 and Dialysis Cassette

The desalting process was performed using Vivaspin® 20, with and without a DF cup. For this experiment, two samples were used: 2 mL BSA model solution and 2 mL CHO culture supernatant. Figure 1 shows the salt concentration measured for each sample plotted against the time taken to achieve > 99% buffer exchange.

Figure 1
Diafiltration With Vivaspin® 20



Note. Salt concentration during diafiltration in Vivaspin® 20 (30 kDa MWCO) with BSA solution (yellow | black lines; 1 mg/mL solved in 1 M NaCl/0.25 mM NaOAc) and CHO cell culture supernatant (gray lines), deionized water was used as exchange buffer.

Buffer exchange by dialysis using a conventional, pre-assembled dialysis cassette was performed in parallel with the same samples. In accordance with the manufacturer's instructions, the dialysis buffer was changed after 2 hours and 4 hours and the sample was recovered after a final overnight dialysis step. The whole dialysis procedure took approximately 24 hours. In comparison, Vivaspin® 20 devices enable buffer exchange to the desired salt concentration significantly faster than dialysis cassettes (Figures 1 and 2, Table 3).

Comparison of Process Times

The time required for buffer exchange was up to 140 times shorter when using Vivaspin® 20 compared to the method using dialysis cassettes (Table 2 and Figure 2).

Table 2
Time Needed for Each Process Step to Perform Buffer Exchange Using Vivaspin® 20 or Dialysis Cassettes

Buffer Replacement	Vivaspin® 20 Without DF Cup		Vivaspin® 20 With DF Cup		Dialysis Cassette
	BSA	CHO	BSA	CHO	BSA | CHO
1	8 min	45 min	6 min	45 min	120 min
2	8 min	45 min	6 min	45 min	120 min
3	-	-	-	-	1,200 min (20 h)
Total	16 min	90 min	12 min	90 min	1,440 min (24 h)

Figure 2
Comparison of Time Needed for a Complete Buffer Exchange Using Vivaspin® 20 or Dialysis Cassettes

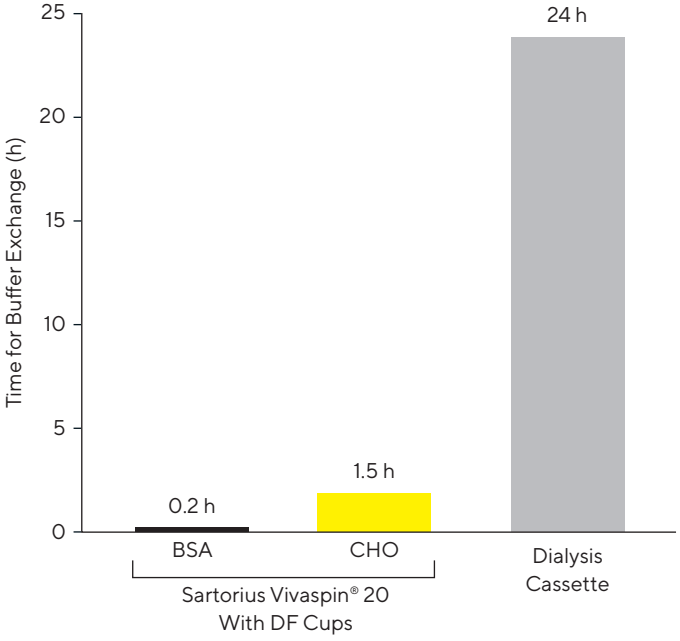


Table 3
Comparison of Salt Concentration Reduction and Process Times for Buffer Exchange of BSA and CHO Cell Culture Supernatant by Diafiltration With Vivaspin® 20 or Dialysis

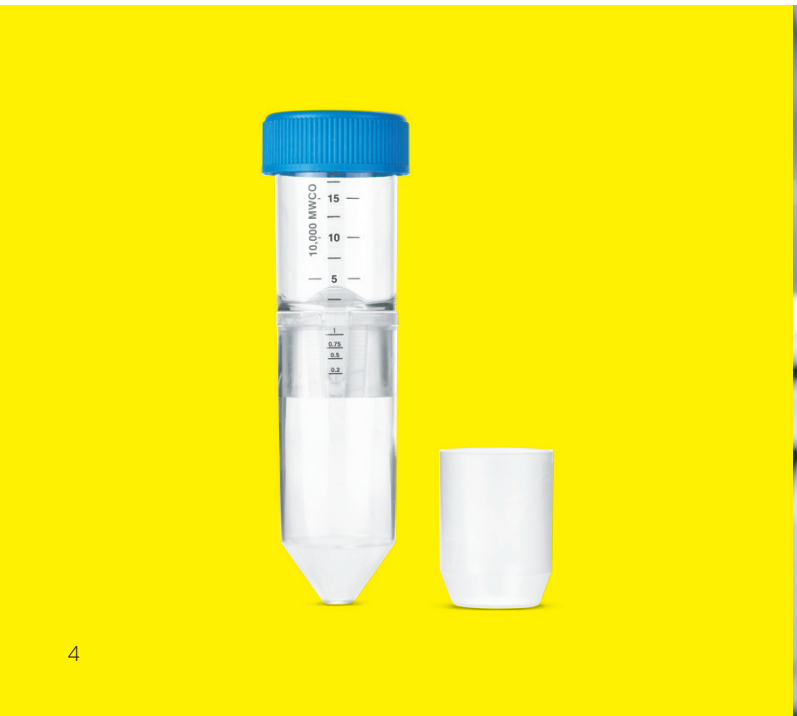
	Before DF		Diafiltration (DF)			After DF
	Device	Salt Conc.	Buffer Exchange Amount	Hands-On Time	Process Time	Salt Conc. (% original salt conc. remaining)
BSA	Vivaspin® 20 without DF cup	1 M	35 mL	45 min	16 min	0.01 M (0.9%)
	Vivaspin® 20 with DF cup	1 M	30 mL	45 min	12 min	0.01 M (1.6%)
	Dialysis cassette	1 M	1,500 mL	60 min	1,440 min	0.02 M (0.0%)
CHO cell culture supernatant	Vivaspin® 20 without DF cup	1.26 M	35 mL	45 min	90 min	0.02 M (1.83%)
	Vivaspin® 20 with DF cup	1.26 M	30 mL	45 min	90 min	0.01 M (0.95%)
	Dialysis cassette	1.26 M	1,500 mL	60 min	1,440 min	0 M (0%)

Conclusion

Diafiltration using Vivaspin® 20 concentrators allows fast buffer exchange. In combination with the Sartorius DF cups, a gradual buffer exchange can be performed. This gentle buffer exchange ensures a decrease in salt concentration prior to concentration of the target molecule down to the dead-stop volume. This way, proteins prone to precipitation at higher salt concentrations are more likely to remain soluble. The DF cups also help to shorten the process time and allow a more efficient decrease in salt concentration (Figure 1). The spin times should be optimized for each sample by measuring the salt content after each diafiltration step. When the sample is concentrated down to the dead-stop volume prior to each buffer exchange, two spin cycles are typically sufficient to achieve a 99% reduction in salt concentration.

The approach using Sartorius DF cups in Vivaspin® 20 concentrators is superior to traditional dialysis methods due to increased process speed, reduced buffer volume requirements, and ease of use. In contrast, dialysis takes substantially longer and requires more hands-on time. Since buffer exchange with Vivaspin® 20 is much faster, an additional benefit is that the target proteins are largely protected from proteases. Furthermore, dialysis leads to dilution of the sample during buffer exchange and a final concentration step would be necessary to reach the required final concentration. Utilizing Vivaspin® 20 with DF cups enables simultaneous desalting and concentration of the sample and therefore efficiently prevents sample dilution.

Diafiltration with Vivaspin® 20 and DF cups allows for time-efficient recovery of highly concentrated samples in virtually any buffer of choice.



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