sartorius

Vivaspin® 20 with Sartorius Diafiltration Cups: Comparing Their Performance in Buffer Exchange to Conventional Dialysis Cassettes

Application Note

#04
#05
#06
#07
#08

turning science into solutions

Marlene Völler,
University of Applied Sciences Osnabrück

Hannes Landmann,
Sartorius Lab Instruments

Richard McRae,
Sartorius Stedim Lab Ltd

Ben Williams,
Sartorius Stedim Lab Ltd

1. 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 diafiltration 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.* in the preparation of fusion proteins for a linking reaction to affinity purification columns.[3] Here, GST-fusion proteins were purified by glutathione-agarose affinity chromatography [4] and subsequently the buffer was exchanged to a coupling buffer (0.2M $NaHCO_3$, 0.5M NaCl, 2M urea, pH 8.3) using the diafiltration cup. Aziz *et al.* performed a desalting step prior to crystallization of the receiver domain of a putative response regulator, BPSL0128, (0.2 M NaCl, 50 mM Tris pH 8.0 was exchanged for 10 mM Tris pH 8.0 through the diafiltration cup). [5] Tovar-Herrera *et al.* desalted the expansin protein ScExlx1 prior to activity assays (20 mM NaH_2PO_4, 20 mM Imidazole, 0.5M NaCl pH 7.4 against 50 mM NaOAc, pH 5).[6] Guccione *et al.* desalted active site subunit of Methylmenaquinol : fumarate reductase (MfrA) prior to enzymatic assays (1.5M NH_4SO_4, 50 mM Tris, pH 8.0 against 50 mM Tris, pH 8.0 respectively).[7]

The dialysis process traditionally used for buffer exchange in biological samples relies on passive diffusion. It is therefore very time consuming and requires a large amount of dialysis buffer.[8] Here we present an approach based on diafiltration with Vivaspin® 20 centrifugal concentrators. In combination with Sartorius diafiltration cups (DF cups) they offer a fast, efficient, and reliable way to exchange buffer in protein samples. This gradual buffer exchange 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.

2. Method

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

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

Optimal operational conditions for > 99% salt reduction were found to be:
- 4,000 × g in a swing out rotor
- 15 ml exchange buffer
- Spin time was determined for each sample (concentration to dead-stop volume and with a buffer change in between):

	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 the dialysis procedures. It was demonstrated that the use of Sartorius diafiltration cups results in an ideal desalting process.

3. Results

3.1 Comparing buffer exchange using Vivaspin® 20 and dialysis cassette

The desalting process was performed with the Vivaspin® 20, with and without a diafiltration cup. For this experiment, two samples were used: 2 ml of a BSA model solution and 2 ml CHO culture supernatant (Figure 1). The diagram in Figure 1 shows the salt concentration measured for each sample at the time of buffer exchange.

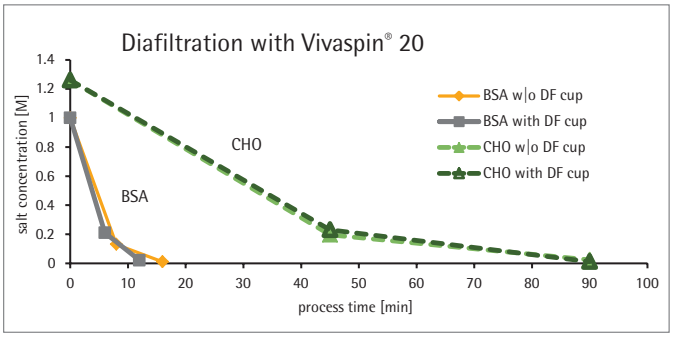

Figure 1: Salt concentration during diafiltration in Vivaspin® 20 (30,000 MWCO) with BSA solution (yellow lines; 1 mg/ml solved in 1 M NaCl/0.25 mM NaOAc) and CHO cell culture supernatant (green dashed lines), deionized water was used as exchange buffer.

The buffer exchange by dialysis in a conventional preassembled dialysis frame was performed in parallel with the same samples. In accordance with the manufacturer's instructions the dialysis buffer was changed after 2 h and 4 h and the sample was harvested after a final overnight dialysis step. The whole dialysis procedure took approximately 24 h. The Vivaspin® 20 devices reach the desired salt concentration faster than the dialysis cassettes (see Figures 1 and 2).

3.2 Process times in comparison

The time needed for the buffer exchange was up to 140 times shorter with the Vivaspin® 20 method compared to the approach using dialysis cassettes (see Table 1).

Table 1: Time needed for every process step in min for the individual devices investigated.

Buffer replacement	**Vivaspin® 20 w | o 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	–	–	–	–	1200 min (O | N)
Total	16 min	90 min	12 min	90 min	1440 min (24 h)

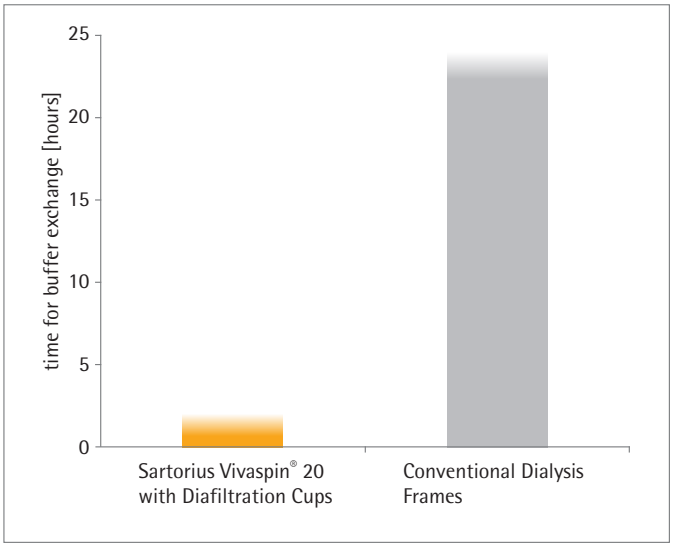

Figure 2: Comparison of the approximate time needed for a complete buffer exchange.

After the desalting step the integrity of all protein samples was ensured by SDS-PAGE. The salt concentration was assessed by conductivity measurement.

Table 2: Comparison of salt concentration as well as process time parameters for BSA and CHO cell culture supernatant in Vivaspin® 20 and dialysis cassette.

	Before DF		Diafiltration (DF)			After DF
	Device	**Salt conc. [M]**	**Buffer exchange amount [ml]**	**Hands-on time [min]**	**Process time [min]**	**Salt conc. [M] (%: remaining end conc.)**
BSA	Vivaspin® 20 without DF cup	1.00	35	45	16	0.01 (0.9%)
	Vivaspin® 20 with DF cup	1.00	30	45	12	0.01 (1.6%)
	Dialysis cassette	1.00	1500	60	1440	0.00 (0.0%)
CHO cell culture supernatant	Vivaspin® 20 without DF cup	1.26	35	45	90	0.02 (1.83%)
	Vivaspin® 20 with DF cup	1.26	30	45	90	0.01 (0.95%)
	Dialysis cassette	1.26	1500	60	1440	0.00 (0%)

Conclusion

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

The approach using Sartorius diafiltration cups in Vivaspin® 20 concentrators is superior to traditional dialysis methods in terms of speed, amount of buffer needed, and ease of use. The dialysis process with a dialysis cassette takes substantially longer and requires more hands-on time compared to the diafiltration method. As the buffer exchange with Vivaspin® 20 is much faster, the target proteins are largely protected from proteases. 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 diafiltration cups leads to simultaneous desalting and concentration of the sample and therefore efficiently prevents dilution of the sample.

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

References

1 Cartwright I. J. &tHiggins, J. A. "Direct Evidence for a Two-step Assembly of ApoB48-containing Lipoproteins in the Lumen of the Smooth Endoplasmic Reticulum of Rabbit Enterocytes" J. Biol. Chem.276, 48048–57 (2001).

2 Große, C. *et al.* "Transcriptional Organization of the czc Heavy-Metal Homeostasis Determinant from Alcaligenes eutrophus" J. Bacteriol. 181, 2385–2393 (1999).

3 Read, A.J., Gauci, C.G., Lightowlers M.W. Purification of polyclonal anti-conformational antibodies for use in affinity selection from random peptide phage display libraries: A study using the hydatid vaccine EG95 J. Chromatog. B, 877, 1516–1522 (2009).

4 Smith, D.B., Johnson, K.S. Single-step purification of polypeptides expressed in Escherichia coli as fusions with glutathione S-transferase. Gene 67, 31-40 (1988).

5 Aziz A.G.A., *et al.* Crystallization and preliminary X-ray analysis of the receiver domain of a putative response regulator, BPSL0128, from Burkholderia pseudomallei. Acta Cryst. F68, 917–922 (2012).

6 Tovar-Herrera *et al.* A Novel Expansin Protein from the White-Rot Fungus Schizophyllum commune. PLoS ONE 10, e0122296 (2015).

7 Guccione, E. *et al.* Reduction of fumarate, mesaconate and crotonate by Mfr, a novel oxygen-regulated periplasmic reductase in Campylobacter jejuni Environmental Microbiol. 12, 576-591 (2009).

8 Blatt, W.F.; Robinson, S.M.; Bixler, H.J. "Membrane Ultrafiltration: The Diafiltration Technique and Its Application to Microsolute Exchange and Binding Phenomena". Analytical Biochemistry. Elsevier. pp. 151–173 Oct (1968).

Sartorius Lab Instruments
GmbH & Co. KG
Otto-Brenner-Strasse 20
37079 Goettingen, Germany

Phone +49.551.308.0
Fax +49.551.308.3289
www.sartorius.com

USA Toll-free +1.800.635.2906
UK +44.1372.737159
France +33.1.70.62.50.00
Italy +39.039.4659.1
Spain +34.913.586.095
Russian Federation +7.812.327.53.27
Japan +81.3.3740.5408

Publication No.: SL-4076-e160901 · Order No.: 85037-556-08 · Ver. 09 | 2016