Concentration and recovery of proteins from cell culture media using Vivaspin sample concentrators

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Ultrafiltration provides a simple and efficient technique to concentrate proteins from dilute solutions. We used Vivaspin sample concentrators to obtain sample solutions with concentrations high enough for robust quantitation of IgG1. Starting directly from a CHO cell culture, IgG1 concentration was too low to be detected by affinity chromatography unless the sample had first been concentrated using Vivaspin.

Introduction

Samples recovered from chromatographic processes are frequently too dilute for immediate use in the next purification step or for detection in biological assays. The initial concentration of a target protein can also be too low to quantitate.

Vivaspin sample concentrators efficiently remove buffer and low molecular weight (LMW) components while retaining the proteins in higher concentrations. Up to 30-fold sample concentration can be achieved with a recovery of the target molecule typically exceeding 95%. The entire process is performed in a single tube with an upper compartment containing sample and a lower compartment separated by a semipermeable membrane with a defined molecular weight cutoff (MWCO). Centrifugation is applied to force the solvent through the membrane, leaving a more concentrated sample in the upper chamber.

In this study, we concentrated samples from *E. coli* fermentation broth and from culture supernatant from a mammalian cell line with Vivaspin. In the first experiments, two representative model proteins were spiked into *E. coli* fermentation broths. One is protein L, an intracellular-expressed protein and the other ZätA, an extracellular-expressed periplasmatic protein. Intracellular-expressed proteins usually require mechanical treatment, such as sonication to break the intracellular cell wall. In the case of periplasmic protein, heat treatment is often used to cause leakage of the protein directly into the medium. In both cases, contaminant proteins usually follow the target protein into the medium. When optimizing the factors influencing extraction, purification, and characterization of such proteins, the content of fermentation broth needs to be analyzed.

In a final experiment, IgG1 was concentrated and recovered from an extremely diluted CHO cell culture feed. Purification of IgG1 from CHO feed requires several purification steps and sample concentration can be required several times during the process. Affinity chromatography was used to highlight the ability of Vivaspin to remove unwanted LMW components and concentrate the target protein in the samples.

Methods

All equipment was used according to the manufacturer's recommendations. The initial absorbance was measured and protein solutions were loaded and the tubes weighed. After concentration in Vivaspin, the recovered concentrates were diluted to the original weight, and the absorbance measured.

Table 1. Results from concentration experiments with Vivaspin sample concentrators

Sample	Vivaspin concentrator	Volume loaded	Centrifugation	Conc. Factor*	Adjusted conc. (mg/ml)	Expected conc. (mg/ml)	Recovery† (%)
E. coli fermentation broth spiked with protein L	Vivaspin 6 MWCO 10 000	4 ml	15 min 4000 rpm swing out	3 ×	1.2	1.5	75
	Vivaspin 2 MWCO 10 000	2 ml	10 min 4000 rpm swing out	8 ×	3.4	4	86
E. coli fermentation broth spiked with ZätA	Vivaspin 6 MWCO 10 000	6 ml	15 min 4000 rpm swing out	3 ×	0.25	0.21	86
CHO cell culture supernatant containing IgG1	Vivaspin 6 MWCO 50 000	6 ml	20 min 4000 rpm swing out	23 ×	0.012	Too low to quantitate; below detection limit of the method	Not determined

* *Conc. Factor = starting volume/recovered volume*

† *Recovery (%) = $Abs_{recovered} \times 100/Abs_{start}$*

Chromatography was performed using ÄKTAexplorer™ system with UNICORN™ software, v5.01. Recovery and concentration factor (CF) were calculated and compared.

Bacterial cell culture samples

E. coli BL21 cells were grown at 37°C overnight on a sterile 2TY culture medium containing 16 g/l peptones, 10 g/l yeast extract, and 5 g/l NaCl, pH 7.0. The cells were harvested by centrifugation for 10 min, 10°C, after which the supernatants were collected and refrigerated until use. To achieve a full quantitative comparison before and after Vivaspin concentration, the broth was spiked with known concentrations of protein L (0.5 mg/ml) or ZätA (0.07 mg/ml), respectively. Samples were then concentrated with Vivaspin 2 and Vivaspin 6, MWCO 10 000.

Absorbance was measured at 280 nm for protein L and at 273 nm for ZätA, before and after concentration. After concentration, samples were purified by affinity chromatography on a 1 ml HiTrap™ IgG Sepharose™, or 1 ml HiTrap MabSelect SuRe™ column. Protein concentration was determined by the standard addition method (STD-add). Briefly, sample was split into five aliquots after which the standard protein was spiked into each aliquot in increasing concentration. This was used instead of a calibration curve to help with the matrix effect problem. A minimum of 1.5 ml sample was needed for the STD-add method, so the recovered sample from the Vivaspin 2 concentrator was diluted to 1500 µl before sample application.

Mammalian cell culture samples

A clarified filtered CHO cell culture supernatant supplied by Polymun Scientific (Austria) containing less then 0.2 µg IgG1/ml and colored by phenol red for pH indication was concentrated with Vivaspin 6, MWCO 50 000. The concentrate was purified with analytical affinity chromatography on a 1 ml HiTrap MabSelect SuRe column to enrich the IgG1 for concentration determination. The recovered sample was diluted four-fold to obtain enough volume for the chromatographic step.

Results

Table 1 shows the result of concentration by Vivaspin and the following affinity chromatography analysis for protein L, ZätA, and IgG1. For the CHO feed, recovery could not be calculated since the original concentration was too low to quantitate. The result of affinity chromatography of protein L and ZätA, respectively, on HiTrap IgG Sepharose FF after concentration is shown in Figures 1 and 2. The corresponding results for IgG1 run on HiTrap MabSelect SuRe are shown in Figure 3.

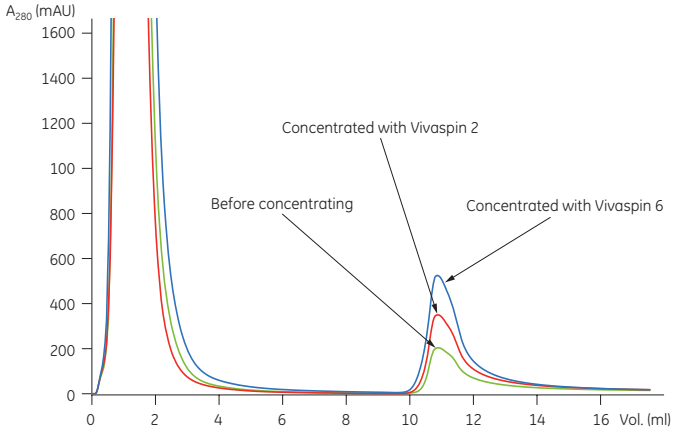

Fig 1. *E. coli* fermentation broth spiked with protein L (0.5 mg/ml), concentrated with Vivaspin 2 or 6. Affinity chromatography on HiTrap IgG Sepharose FF.

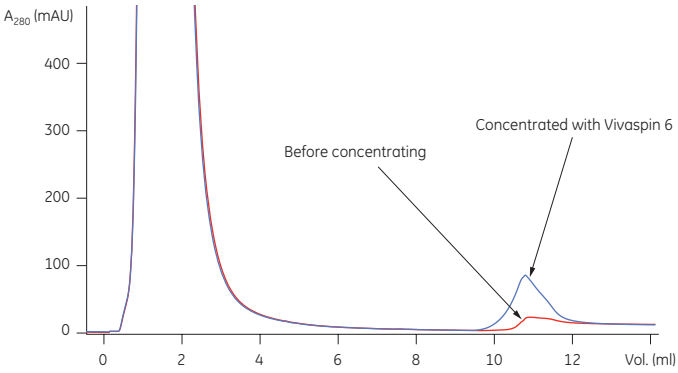

Fig 2. *E. coli* fermentation broth spiked with ZätA protein (0.07 mg/ml), concentrated with Vivaspin 6. Affinity chromatography on HiTrap IgG Sepharose FF.

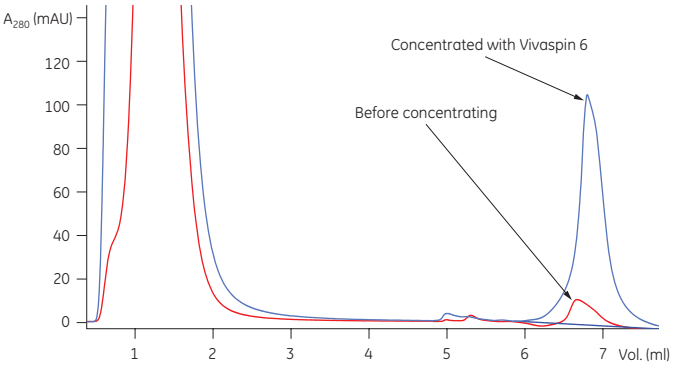

Fig 3. Concentration of IgG1 from CHO cell culture feed. Concentration by Vivaspin 6 and affinity chromatography on HiTrap MabSelect SuRe .

Conclusion

Vivaspin 2 and Vivaspin 6 were used for protein concentration in fermentation broth and mammalian cell culture medium. Using Vivaspin, most of the interfering components in the broth were removed by centrifugation in one step while the volume was reduced. Further analysis of the samples on the affinity columns could be performed without excessive signal noise and normal affinity chromatography curves were obtained. Further, using Vivaspin for concentration, IgG1 from an extremely diluted CHO cell culture could be quantitated by affinity chromatography.

Vivaspin reduced the complexity of the samples, made further analysis convenient, and ensured reliable results.

Ordering information

Product	Code number
Vivaspin 2, MWCO 10 000	28-9322-47
Vivaspin 6, MWCO 10 000	28-9322-96
Vivaspin 6, MWCO 50 000	28-9323-18
HiTrap MabSelect SuRe, 5 × 1 ml	11-0034-93

For more information on our range of Vivaspin sample concentrators, visit www.gelifesciences.com/sample prep