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High Recovery of Cationized Protein using Vivaspin® Filtrate

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Abstract

Mammalian cell membranes comprise a high proportion of negatively charged molecules. It can therefore be challenging to deliver anionic drug candidate proteins into cells where they are needed for effective disease treatment. Protein cationization offers a means to enhance the permeability of drug candidates. However, since cationized proteins may ultimately be produced at low initial concentrations, it is necessary to perform a concentration step to enable downstream analytics, such as MALDI mass spectrometry, dynamic light scattering and zeta potential measurements. An improved method for concentration of cationized proteins using Vivaspin® Filtrate is described. In comparison to conventional techniques such as lyophilization, ultrafiltration shows increased processing speeds and high cationized protein recovery.

Introduction

Protein cationization is a method used in biochemical research whereby a protein of interest (POI) is chemically modified to produce a protein with an excess of overall positive charge compared to its native form; sometimes referred to as a 'super-charged' protein. Protein cationization can be used for a variety of purposes; a well-documented use is of cationized BSA due to its greatly enhanced immunogenicity compared to non-cationized BSA.

Here, a 38 kDa protein was isolated and purified from *E. coli* cell lysates but at a low concentration of 0.1 mg/mL. Freeze drying (lyophilization) was used initially to increase concentration, due to its high yield results. However, since a dialysis step is required upstream to remove unwanted salts, protein was lost due to some aggregation. Lyophilization is also a time consuming process involving multiple steps.

Use of a standard PES ultrafiltration device, although a much faster process, proved insufficient for the level of protein recovery required. Vivaspin® Filtrate provided the ideal solution to this issue, with its highly non-specific binding cellulose triacetate (CTA) membrane and novel self-cleaning method of concentration, giving both a fast process speed compared to lyophilization methods and a high recovery compared to alternative centrifugal ultrafiltration devices.

Materials and Methods

Protein isolated and purified from *E. coli* was cationized with N,N'-Dimethyl-1,2-propanediamine (DMPA) using 1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide (EDC) activation. The cationized protein was dialyzed into a 10 mM phosphate buffer (pH 7) using BioDesign dialysis tubing (Fisher Scientific). 2 mL of the dialyzed, cationized protein was loaded into each Vivaspin® Filtrate 10 kDa MWCO CTA device (13239-E, Sartorius). Vivaspin® Filtrate devices were centrifuged at 2,500 *g* for 30 min (Allegra X12-R with SX4750 swing out rotor, Beckman Coulter). Protein recovery was determined by UV-vis spectrophotometry using a Cary-60 spectrophotometer (Agilent).

Further analysis included MALDI mass spectrometry for protein identification, peptide quantitation and determination of cationization efficiency (% sites modified). Particle size and charge of cationized proteins were also determined, using dynamic light scattering (DLS) and zeta potential measurements, respectively.

Results

Figure 1 outlines the workflows for conventional lyophilization and concentration using Vivaspin® Filtrate. Protein recovery of 90 % was obtained when using Vivaspin® Filtrate for the protein concentration process. The resulting higher sample concentrations (relative to the conventional lyophilization method) made additional downstream analysis possible, such as MALDI mass spectrometry for confirming protein presence, and dynamic light scattering and zeta potential measurement for determining the particle size and charge, respectively.

Conclusion

The Vivaspin® Filtrate device used in this study demonstrated its suitability as a low process time, high yield concentrator for a purified cationized protein. By using Vivaspin® Filtrate instead of conventional lyophilization methods, process time was compressed and by using a CTA instead of a PES membrane, protein yield was increased. Selection of the correct ultrafiltration product for a given sample type is critical and, when the optimal device is chosen, can dramatically improve the process workflow and results
for a given sample in laboratory research.

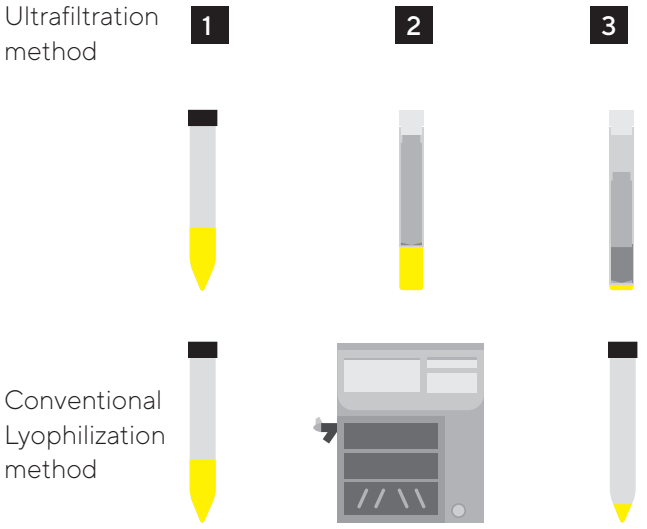
Vivaspin® Filtrate was a very simple to use device. We were impressed with the ease of retentate retrieval: pipettes fit into both the concentrator and floating filtrate tubes, with no narrow sections to retain protein. Furthermore, sample recovery was excellent compared to other concentrators we have used.

Notes

Details of this protein, the method and results are proprietary information. We respect the authors' right to reserve certain information. Additional information on cationization and similar methods can be found in the references provided.

Vivaspin® Filtrate is part of the Vivaspin® product family. Literature published up to c.2022 may reference the use of Centrisart 1, which is a name previously used for the same centrifugal ultrafilters. When these devices were renamed, there was no change made to fit, form or function, so results collected using Centrisart 1 devices remain valid also for Vivaspin® Filtrate.

Figure 1:



Note: Schematic comparison of sample preparation by ultrafiltration or lyophilization. 1: Purified sample is obtained; 2: Sample is concentrated using Vivaspin® Filtrate or lyophilization equipment; and 3: Concentrated sample is available for downstream analytics.

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