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Application Note

Circa 2012

Keywords or phrases:
Protein purification, concentration, reducing sample volumes, lyophilization, ultrafiltration

Using Ultrafiltration with Vivaspin® 500 as an Alternative to Sample Volume Reduction by Lyophilization

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Abstract

Protein purification workflows are common to laboratories researching potential drug targets, novel biotherapeutics and diagnostic markers. These workflows often demand the use of techniques to reduce sample volumes to improve stability of a purified protein, increase the concentration of a protein prior to further analysis, or simply to economize on storage or transport space. Lyophilization is a common technique to reduce sample volumes but is time-consuming, and risks denaturation, aggregation and reduced yields. An improved method of sample volume reduction is described using ultrafiltration with Vivaspin® 500. In comparison to conventional lyophilization, ultrafiltration is a faster technique that also enables higher recoveries of target proteins due to the reduced risk of denaturation and aggregation.

Introduction

Lyophilization, the removal of the majority of the water in a sample under conditions of low temperature and vacuum – otherwise known as freeze drying – is a widely used technique in the areas of protein purification, protein reagent preparation, and the manufacture of protein biomolecules for therapeutic and diagnostic applications. It can be performed on a sample to increase stability, reduce the volume, increase efficiency for storage or transport, and various other applications. Proteins are highly diverse and whereas a single chain protein with a highly ordered tertiary structure may freeze dry with little difficulty, a multimeric protein with multiple domains and hydrophobic proteins will pose a far greater challenge to achieve successful lyophilization.

Lyophilization can be divided into three process stages – freezing, primary drying at lower temperatures when most of the water is removed, and secondary drying at ambient or higher temperatures to minimize the final unbound water content. During the freezing process, water crystallizes to ice and the excluded excipient salts increase to local concentrations far higher than those in the original liquid state. This can in itself have implications for the stability of the protein(s) present, which may be destabilized and denature because of the change in ionic strength. In addition, if buffer salts, such as mixed phosphates are present, the selective crystallization or precipitation of one of these salts at a higher temperature to the other may result in localized pH shifts. This again may induce denaturation of the proteins. Such denaturation can lead to exposure of normally buried residues and an increase in aggregation, and may be irreversible on reconstitution.

Other proteins may be satisfactorily immobilized in the lyophilized state but undergo changes that result in aggregation on reconstitution. Membrane bound proteins may pose special problems when undergoing lyophilization – cell membranes, for instance, are particularly prone to disruption during the dehydration process and so membrane-associated proteins will also be at risk.

In contrast to lyophilization, ultrafiltration (UF) separates-dissolved particles and molecules according to size and configuration by flowing a solution that contains these molecules through a membrane with ultra-fine pore sizes under a driving force.

The driving force most commonly applied in research and development laboratories is centrifugal; though may alternatively be generated by solvent absorption (static concentration), positive pressure or tangential flow filtration. The membrane will retain most particles and molecules above its retention rating, while allowing smaller molecules, including solvent and salts, to pass through. Because UF membranes have the ability to retain large macromolecules, they have been historically characterized by a molecular weight cut-off (MWCO) rather than by a particular pore size. The concept of the MWCO expressed in kilodaltons (kDa) is a measure of the removal characteristic of a membrane in terms of atomic weight rather than size. Therefore, UF membranes with a specified MWCO are presumed to act as a barrier to compounds or molecules with a molecular weight exceeding the MWCO.

Various membranes have been commercialized for ultrafiltration, including polyethersulfone (PES), cellulose triacetate (CTA), regenerated cellulose (RC) and Hydrosart®. Each of these materials have their own importance. For example, where PES is recommended for the fastest concentrations, CTA is typically chosen for protein removal applications. Stabilised cellulose materials (RC and Hydrosart®) typically serve special applications, when highest recovery of Ig fractions is demanded. For maximum recoveries, it is recommended to select a MWCO that is at least 50% smaller than the size of the molecule to be retained. Ultrafiltration is preferred over other methods when high and easy recovery of protein is required without causing any structural or functional changes; in the shortest time.

Suggested Method

1. Select a Vivaspin® 500 with an appropriate MWCO (in this case, 5 kDa MWCO PES, as the protein of interest was around 12 kDa).
2. Fill the Vivaspin® 500 with 500 µL of protein sample, and ensure the cap is fully sealed.
3. Centrifuge for the recommended amount of time at an appropriate speed for the device, membrane and MWCO. We performed centrifugation with 10,000 rpm for 20 min in a fixed angle rotor.
4. Empty the filtrate container and refill the concentrator with additional sample if required.
5. Centrifuge as before, repeating the process until the entire sample has been concentrated to the desired final volume.
6. Recover the concentrate from the dead-stop pocket using a suitable pipette.

Test Sample

- Cell Line: BL21 (Invitrogen, CA, USA)
- Media: LB Broth (Luria Bertini)
- Protein of Interest: Recombinant mutated lectin (12 kDa MW)

Equipment

- Sartorius Vivaspin® 500, 5 kDa MWCO PES
- Sorvall MC12V Centrifuge with fixed angle rotor
- Lab scale Operon freeze dryer (–55 °C)
- Standard Eppendorf pipettes and tips
- Bio-Rad polyacrylamide gel apparatus

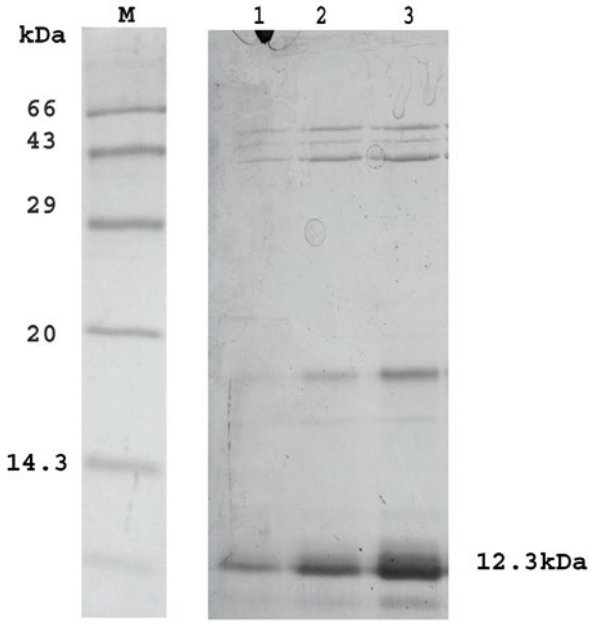
Results and Conclusions

Recombinant mutated lectin, purified by Ni-NTA (nickel nitrilotriacetic acid) affinity chromatography, was concentrated using Vivaspin® 500 or lyophilization. The degree of concentration was analyzed in 15% SDS-PAGE, with proteins visualized by straining with Coomassie brilliant blue. Samples concentrated by both methods were electrophoresed on 15% SDS polyacrylamide gels, alongside the original Ni-NTA purified protein. Equal sample volumes were loaded in each lane. A clear band corresponding to the 12 kDa lectin was observed in all samples (Figure 1).

The result clearly indicates the fundamental advantage offered by ultrafiltration over lyophilization. The protein was present in much high concentration when using Vivaspin® centrifugal ultrafiltration devices, with minimal loss (Lane 3), when compared with lyophilization. Moreover lyophilization was – as expected – much more time-consuming, compared to ultrafiltration which took not more than 20 minutes. Overall, we have demonstrated a clear indication of the efficiency of ultrafiltration over lyophilization.

Figure 1:

SDS-PAGE analysis of samples concentrated by ultrafiltration or lyophilization. Lane M, molecular weight marker; lane 1, Ni-NTA purified protein; lane 2, protein concentrated by lyophilization; lane 3, protein concentrated by ultrafiltration. The molecular weights (kDa) of the protein markers are shown at the left. 12.3 kDa label indicates the recombinant protein band.



Testimonial

"Ultrafiltration is a smarter, one step process to concentrate protein samples in less time, with high yield, in comparison to lyophilization. I can directly store my protein sample at –80 °C with no further processing for future use. I will prefer this technique for my further protein work" – Prithwi Ghosh, Senior Research Fellow (SRF), Division of Plant Biology, Bose Institute.

Abbreviations	
MWCO	Molecular Weight Cut-Off
Ni-NTA	Nickel Nitrilotriacetic Acid
PAGE	Polyacylamide Gel Electrophoresis
UF	Ultrafiltration

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Status: 09 | 2021