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High Recovery of Cationised Protein in Centrisart® I

Application Note

#07
#08
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turning science into solutions

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Abstract | Brief
Protein cationisation 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; sometime referred to as a 'super-charged' protein. Protein cationisation can be used for a variety of purposes; a well-documented use is of cationised BSA due to its greatly enhanced immunogenicity compared to non cationised 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 (lyophilisation) was used initially due to its high yield results to increase concentration, but as a dialysis step is required upstream to remove unwanted salts, protein was lost due to some aggregation. Lyophilisation 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. The Centrisart® I provided the ideal solution to this issue with its highly non-specific binding CTA membrane and novel self-cleaning method of concentration, giving both a fast process time compared to lyophilisation methods and a high recovery compared to alternative centrifugal ultrafiltration devices.

Equipment

- Centrisart® I, 10K CTA (Sartorius, 13239-E)
- Allegra X12-R with SX4750 swing out rotor (Beckman Coulter)
- Cary-60 (Agilent)
- BioDesign dialysis tubing (Fisher Scientific)

Method

1. The isolated protein was cationised with N,N'-Dimethyl-1,3-propanediamine (DMPA) using 1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide (EDC) activation.
2. The cationised protein was dialysed into a 10 mM phosphate buffer (pH7).
3. 2 mL of dialysed cationised protein was loaded into each Centrisart 1® 10K CTA device.
4. Centrisart devices were centrifuged at 2,500 xg for 30 min.
5. Protein recovery was quantified by UV-vis.

Results and discussion
Protein recovery of 90 % was obtained using the Centrisart® I for the protein concentration process. At higher sample concentrations, additional downstream analysis was possible, such as MALDI mass spectrometry for confirming protein presence, dynamic light scattering and zeta potential for determining the particle size and charge, respectively.

Summary
The Centrisart® I device demonstrated its use as a low process time, high yield concentrator for a purified cationised protein. By using the Centrisart® I over traditional lyophilisation methods process time was compressed and by using CTA over PES membrane increase protein yield. The Centrisart® I device demonstrates that choice of the correct ultrafiltration product for a given sample is critical and if correct can dramatically improve the process workflow for a given sample in laboratory research.

Notes

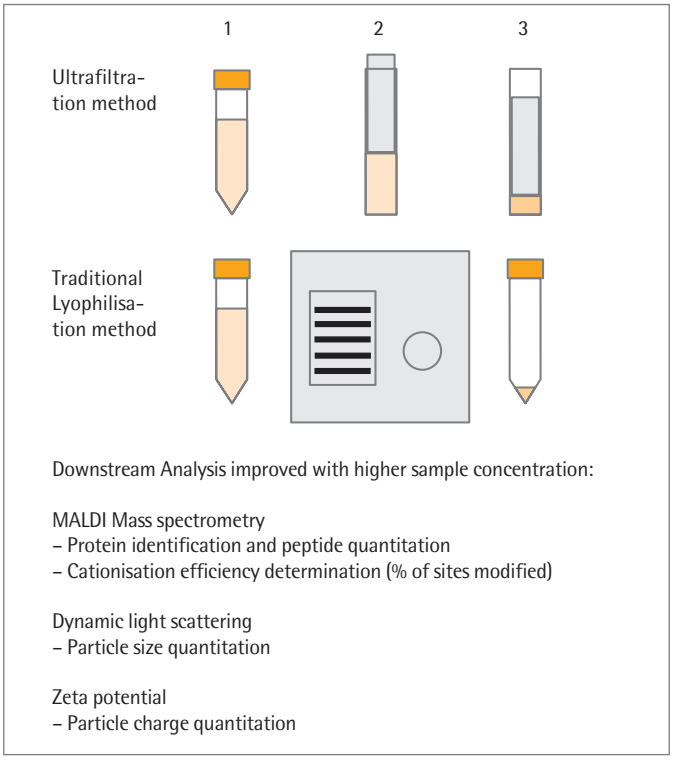
Details of this protein and the exact method are proprietary information. We respect the individual's right to reserve certain information. However, additional information on cationisation and similar methods can be found in the references section.

Testimonial

"Very simple to use."

"Impressed with ease of recovery: pipettes fit into both main compartment and floater, no narrow sections to consume protein."

"Excellent sample recovery compared to other concentrators I've used."



Above: Schematic use of ultrafiltration in standard laboratory sample preparation compared to traditional method.
1. Purified sample is obtained.
2. Sample is loaded into lyophilisation equipment or Centrisart® I for centrifugation.
3. Sample is concentrated to aid downstream analytics.

References

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