Protein Separation

Application Note

VIVASPIN 2 devices

Use of VIVASPIN Ultrafiltration concentrators to separate a 12.3 kDa protein from a 45 kDa protein.

Rik McRae
Vivascience LTD, Stroud Business Centre, Stonehouse, Gloucestershire, GL10 3RQ, UK.

Background

Ultrafiltration is most often used for the concentration or desalting of one molecular species at a time. Rarely is thought given to using centrifugal ultrafilters to separate mixtures of species into size graded fractions. One reason for this is that most suppliers of ultrafiltration devices recommended that to be assured of separation, molecules must differ in size by at least a 10 × difference in molecular weight. While this may be true for ultrafilters from some manufacturers, the advanced design of the VIVASPIN range from VIVASCIENCE allows species much closer in size to be separated.

Materials and Methods

VIVASPIN 2 devices with a 30 kDa MWCO Regenerated Cellulose membrane, (Product number VS02L1), were used, to show that molecules with only a 3–4 × difference in molecular weight can be quickly and easily separated. They were filled with 2 ml of a 1:1 mixture of cytochrome c, (12.3 kDa), and ovalbumin, (45 kDa), each at 0.1 mg/ml in **deionized water***. The devices were centrifuged for 15 minutes at 5,000 g in a 25° fixed angle rotor, filled with 2 ml deionized water and centrifuged a second time. All devices reached final volumes of less than 20 µl. The filtrate was subsequently concentrated using VIVASPIN 2 devices with a 5 kDa MWCO polyethersulfone membrane, (Product number VS0211). Both retentate and filtrate solutions were analyzed by SDS-PAGE. See Figure 1 on page 2.

* **arium 611DI recommended for application**
Type I water purification system for low TOC and low endotoxin applications. Water quality to 18.2 MΩ × cm, TOC level <4 ppb, product flow rate up to 2.0 l/min.

Conclusion
As solution passes through most ultrafilters, retained molecules build up on the membrane surface to form a polarized layer or gel layer. This gel layer can be so highly concentrated that small molecules, which should normally pass through the membrane, are trapped by the layer and retained. The vertical membrane configuration in VIVASPIN devices ensures that the centrifugal force acts across the membrane surface. As large molecules are retained, the centrifugal force across the membrane moves them down to the bottom of the device away from the membrane surface. This reduces the formation of a gel layer, so that small molecules pass freely through the membrane. This allows molecules with only a 3–4 × difference in molecular weight to be separated using VIVASPIN devices.

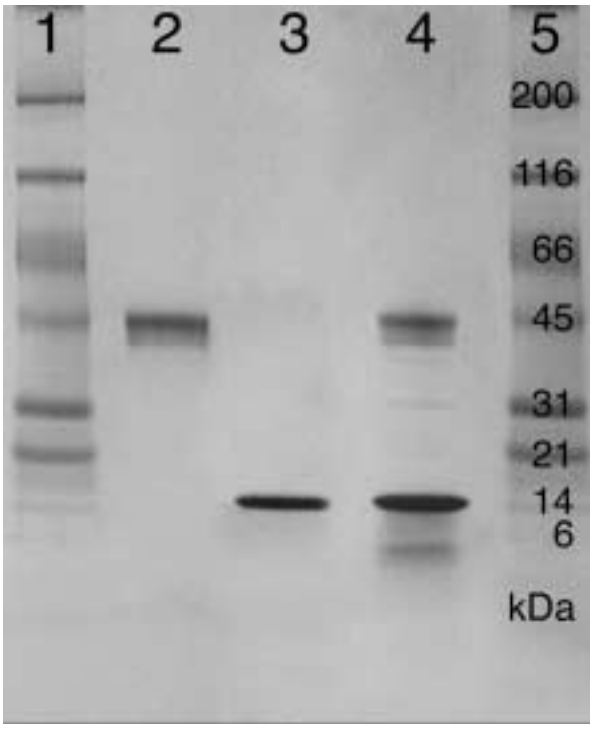

Fig 1. SDS-Polyacrylamide gel showing complete separation of cytochrome c and ovalbumin.

1. Molecular weight standards marker.
2. Ovalbumin/cytochrome c before separation.
3. Retentate, only ovalbumin is present.
4. Filtrate, showing cytochrome c only.
5. Molecular weight standards marker.

Sartorius AG
Weender Landstrasse 94–108
37075 Goettingen, Germany

Phone +49.551.308.0
Fax +49.551.308.3289

www.sartorius.com/arium

USA +1.800.3687178
UK +44.1372.737100
France +33.1.69192100
Italy +39.055.505671
Spain +34.91.3588566
Japan +81.3.33295533

Specifications subject to change without notice.
Printed in Germany on paper that has been bleached without any use of chlorine.
W/sart-000 · G
Publication No.: SLG2013-e01041
Order No.: 85030-514-74