Protein dialysis with automated liquid handling devices

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Introduction and Overview

Introduction: Desalting, rebuffering, renaturation, precipitation, resolving of proteins are necessary to purify and to characterize molecules of interest. The most gentle method under preservation of protein activity is the dialysis of protein samples. scienova have developed dialysis tools known as Dialyzer Family in combination with Deep Well Microplates suitable for automated liquid handling. Guanidine hydrochloride (GndHCl) is one of the most often used compounds to dissolve proteins from inclusion bodies. Conductivty is a very promising method to determine GndHCl in samples in combination with disposables suitable for automation.

Overview:

- Dialysis results with volume and protein recoveries using scienova dialyzer with liquid handling devices are shown
- First results for exact determination of Guanidine hydrochloride through conductivity in dialysis samples are shown

Volume recovery with Hamiltons STARlet

Main point for the selection of a method and device for protein sample preparation is the sample recovery. Hamilton Robotics STARlet are very often used liquid handling devices in daily laboratory tasks. Established members of the scienova Dialyzer Family MD100 (10–100µl), ED300 (50–300µl), and MD1000 (150–1000µl) were tested for sample volume recovery and an example for dialysis efficiency of ED300 for cut off's 3.5 kDa, 6–8 kDa and 12–14 kDa with protein samples.

Materials: PBS: Phosphate Buffered Saline pH 7.2, pNP: 1 mM 4-Nitrophenole solved in PBS, BSA: 1 mg/ml Bovine Serum Albumin solved in PNP **Measurment:** 405 nm Spectra Max Plus (Molecular Device)

Fig. 1: **Hamilton STARlet**

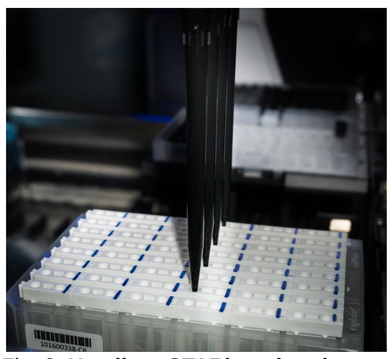
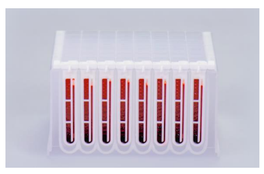


Fig. 2: **Hamilton STARlet, pipetting** of samples from MD1000

Volume recovery (Vol.R.)

MD100

V in µl	Vol. R. %	Vk%
10	75%	7,96
50	85%	3,26
100	82%	1,02



ED300

V in µl	Vol. R. %	Vk%
50	78%	2,45
150	86%	2,31
300	94%	0,68

MD1000

V in µl	Vol R. %	Vk%
150	83%	3,62
500	91%	2,60
1000	93%	2,81

Fig. 3: **Volume recovery (Vol.R.):** unwetted dialyzer without dialysis buffer outside, cut off 6–8 kDa: test solution 1 mM pNP, dialysis buffer PBS, n=8

Equilibrium dialysis ED300

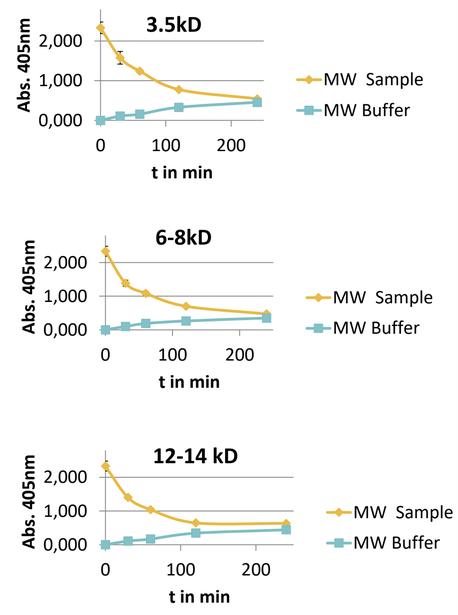


Fig. 4: **Equilibrium dialysis:** Sample BSA 250 µl, Dialysis Buffer 1400 µl PBS, n=8, -ambient temperature

BSA recovery with Analytik Jena Cybios Cybi®-FeliX

To demonstrate the suitability of dialysis samples for high throughput applications in liquid handling devices 48 BSA samples (concentrations between 10 and 100 µg/ml) were dialyzed parallel in scienova Xpress Dialyzers and were handled with Analytik Jenas liquid handling device Cybi®-FeliX.

Fig. 5 *Xpress* Micro Dialyzer MD100 GridKit48

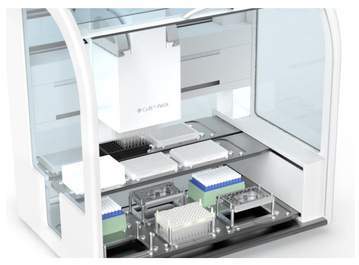


Fig. 6 Analytik Jena AGs liquid handling device Cybio® FeliX.

Material and Methods. Dialysis device: scienova *Xpress* Micro Dialyzer MD100 GridKit48 MWCO 6–8 kDa. Samples were dialysed against 4.4 ml dialysis buffer (A. deion.) for 2 hrs. at room temperature. Liquid handling device: CyBi®-FeliX multi-channel/single-channel pipettor with Head R96/250 µl. Measurement: Photometer Tecan Sunrise, 590 nm (measurement), 450 nm (reference). Protein determination according to Bradford.

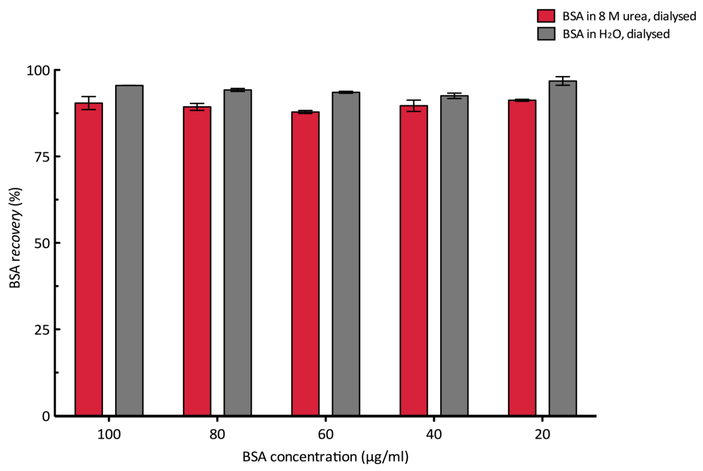


Fig. 7 BSA recovery after dialysis for 2 hrs. at room temperature. BSA concentration (between 20 and 100 µg/ml in water or 8M urea) was determined before and after dialysis (n=6 for each concentration).

Conductivity : Guanidin Hydrochloride Determination in Dialyzed Samples

Guanidine hydrochloride (GndHCl) concentration of 6 M was reduced by dialysis in ED300 and MD1000 using a dialysis buffer with 0.75 M GndHCl. Conductivty was used to determine GndHCl concentration in samples and dialysis buffer.

Material and Methods

The *Xpress* Equilibrium Dialyzer ED300 (scienova) MWCO 6–8 kDa, was used with 300 µl sample in a 96 well deep well plate (Ritter AG) and the *Xpress* Mini Dialyzer MD1000 (scienova) MWCO 6–8 kDa, was used with 1 ml sample in a 48 well deep well plate (Ritter AG). All experiments were performed five times. The sample of 20 mM Tris pH 8, 6 M guanidine hydrochloride, 100 mM imidazole was dialyzed against 50 mMTris pH 8.5, 9.6 mMNaCl, 0.4 mMKCl, 1 mM EDTA, 750 mM guanidinehydrochloride, 0.05 % PEG2000, 1 mM DTT and 500 mM arginine.

Dialysis buffer was changed every 30 minutes in the deep well plates. Conductivity was measured in all samples using the ÄKTA avant 150 (GE Healthcare, United Kingdom).

Fig. 8 Kinetic of guanidine hydrochloride concentration during dialysis a) in ED 300 and b) in MD1000

Conclusion

- Protein sample purification performed by automated liquid handling devices (Analytik Jena Cybio®-FeliX and Hamilton) with low sample losses and low scattering demonstrated (volume and concentration recoveries)
- Measurment of guanidin hydrochloride by conductivity shows the chance for online measurement of conductivity to track the dialysis processes in dialysis disposables

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Video demonstration:
scienova *Xpress* Equilibrium Dialyzer ED300 and Analytik Jena Cybi® FeliX

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