Meet Unagi: Completely hands-free benchtop buffer exchange

Introduction

Buffer exchange and concentration of biologic and gene therapy samples is a critical and often manual, time-consuming process. For proteins and mAbs, efficient exchange into final formulation buffers is necessary for maintaining conformational, chemical, and colloidal stability. Altering buffer salts, pH, ionic strength, excipients, and surfactants may increase or decrease the stability of a molecule.

Existing methods for buffer exchange have various limitations, often requiring hands-on time and delivering low sample recovery. Automated buffer exchange solutions enable more uniform sample handling and degrees of process control that are otherwise impractical by manual methods. Unagi was developed to address gaps in biomolecule sample buffer exchange and concentration (Figure 1).

Unagi uses a pressure-based ultrafiltration/diafiltration (UF/DF) method to remove buffer. During each exchange or concentration cycle, each sample is gently mixed, ensuring that protein cannot accumulate at the membrane surface, while keeping flow more uniform and faster than dead-end filtration methods. Unagi automates the buffer exchange process, reduces hands-on time while delivering ≥96% sample recovery. Unagi also enables sample concentration to a new target after the exchange is complete.

Buffer exchange with Unagi is customizable and adaptable, allowing for buffer exchange of up to 8 unique biomolecule samples in a single experiment. Unchained Labs developed a single use consumable for this process. The Una is a consumable sample vessel with a 10 kDa regenerated cellulose membrane and is designed to withstand 60 psi pressurization during the buffer exchange process (Figure 2). Unagi can process up to 8 samples in Unas ranging in volume from 0.5-8 mL, or up to 48 mL for sample concentration, in a single run.

Figure 1: Unagi is your automated benchtop buffer exchange solution.

The automated pressure-based UF/DF buffer exchange technology on Unagi was designed to maximize sample recovery in an otherwise unpredictable process (Figure 3). The exchange rate is highly dependent on solution viscosity, which is a function of the sample concentration, formulation, volume, and temperature. Different concentration samples and formulations would be expected to exchange at different rates. To maintain uniform

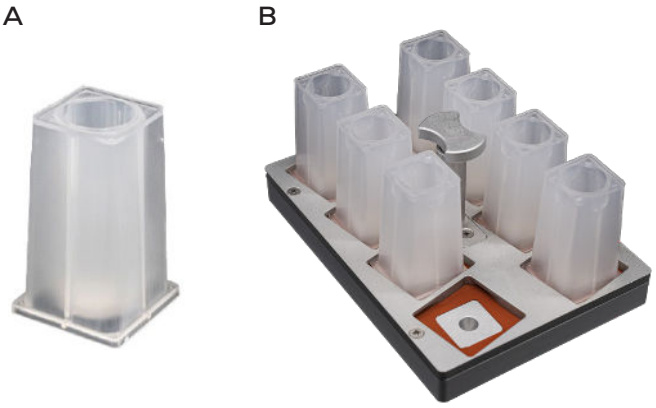

Figure 2: (**A**) Unagi processes samples in up to eight Una consumables. Each Una has a working volume range of 0.5 – 8 mL. (**B**) The sample rack holds 1 – 8 Unas and can be used to transport samples to and from Unagi. The sample rack locks into place in the buffer exchange chamber on Unagi.

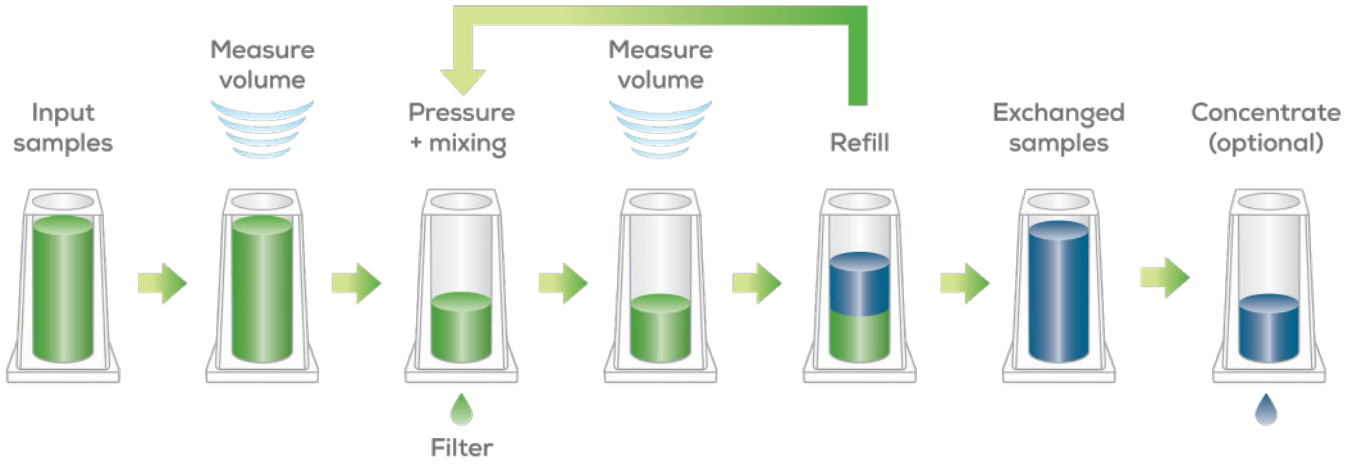

Figure 3: Unagi's automated pressure-based UF/DF technology uses ultrasonic volume measurement of each sample to monitor flow rate and adapt the pressure cycle time accordingly. The sample volume measurement enables precise target exchange percentages and concentration values, since the amount of retentate is known at every step.

flow, an ultrasonic sensor measures the volume for each sample at the start of a run and in every buffer exchange or concentration cycle. This measurement is used to calculate the amount of volume removed, and the amount of the new buffer to be added back before the next cycle. The actual volume removed in each Una is compared to the user-defined target, and the pressurization time is adjusted, optimizing buffer exchange in real time.

Sample volume measurement on Unagi has the added benefit of providing a level of control that would be otherwise unavailable by manual methods. The degree of exchange per cycle, or percent buffer removal, ensures a protein is not over concentrated during the exchange and also guards against making rapid changes to the formulation that would cause other aggregation events. The user can control the level of the exchange based on their application needs. With low protein concentrations, or exchange into similar buffers, the % removal per cycle can be set high to finish the exchange faster. With high concentrations of protein, proteins sensitive to aggregation, or large formulation changes, a lower % removal can be used. This would increase the exchange time, but also ensure a quality protein and recovery at the end of the process.

Unagi automates and minimizes hands-on time. Before the run, the Una is filled with protein to be exchanged and placed in the exchange chamber, and new buffers contained in Falcon tubes are placed in the buffer rack. During the run, Unagi alternates between filtration, volume measurement, and new buffer addition (Figure 3).

Unagi has a reduce sample volume application for larger volume sample concentration (Figure 4). This concentration application can concentrate starting sample volumes up to 48 mL down to 8 mL, with the ability to run a subsequent concentration experiment to a final volume of 0.5 mL. Initial sample volumes exceeding 8 mL are stored in Falcon

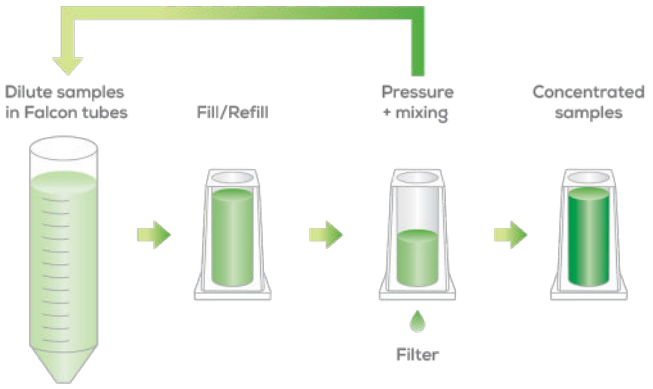

Figure 4: The reduce sample volume application on Unagi utilizes the on-deck Falcon tubes to concentrate larger volumes of samples, up to 48 mL, down to 8 mL in a single run. Subsequent concentration experiments will bring the final volume down to as low as 0.5 mL, for a 96x final sample concentration factor.

tubes contained in Unagi's on deck buffer rack. The same UF/DF technology is used to concentrate up to 8 samples in parallel, with the same guarantee against sample loss afforded by the cyclic sample volume measurement.

In this application note, proof-of-concept experiments were run on Unagi, demonstrating the ability to perform buffer exchange and sample concentration to precise target and recovery values that would be impossible to do with manual methods.

Methods

Protein and buffer preparation

Human IgG (hIgG) was nominally prepared at 10 mg/mL or 100 mg/mL in PBS, pH 6.8. Proteins were manually pipetted into 10 kDa Unas before the start of each buffer exchange. Proteins were buffer exchanged into PBS, pH 6.8.

Protein concentration

Lunatic was used to analyze the concentration of all samples before and after buffer exchange. Protein concentration was determined with the A280 application on Lunatic using the E1% specific to hIgG. Final concentrations were measured for each Una and are reported as the average concentration for all Unas ± standard deviation.

Buffer exchange capabilities

Unagi enables the operator to set a total % exchange up to 99%. For all experiments in this application note, the exchange % was set to 96% total exchange. Unagi allows the operator to choose the % volume removal per cycle. All experiments used 66% removal per cycle.

Other capabilities for buffer exchange methods are described within their respective experiments. Unagi software was used for experimental design and execution. Results were logged, and further concentration analysis was performed offline.

Results

Buffer exchange

A stock of hIgG (10.5 mg/mL) was buffer exchanged into PBS, pH 6.8. 4 mL was manually transferred to each Una. The target percent exchange of 96% for each was reached in 3 cycles of approximately 20 minutes each. The average percent exchange was 96.5% (Table 1). The total run time to complete the buffer exchange of eight proteins exchanged into one buffer in Unas was 2.3 hours.

Final fill volume per well was like the initial fill volume per well at 4.04 ± 0.03 mL, showing consistency and no significant difference between the initial and final fill volume per Una. Following buffer exchange the average concentration for each sample in each Una was 53.4 ± 1.0 mg/mL (Table 1).

Buffer exchange and 8x concentration

For this experiment, protein was exchanged into buffer and then concentrated 8-fold after exchange was completed. A stock of hIgG (9.9 mg/mL) was buffer exchanged into PBS pH, 6.8. 8 mL was manually transferred to each Una. The target percent exchange of 96% was reached in 4 cycles of approximately 16 minutes each. The duration of each cycle was adjusted automatically so the average volume removed per cycle was approximately the target of 66% exchange per cycle. The

Variable	Initial	Target final	Actual final
Conc. (mg/mL)	52.3	52.3	53.4 ± 1.0
Una fill vol. (mL)	4.0	4.0	4.04 ± 0.03
% exchange	-	>96	96.5 ± 0.5
% recovery	-	-	96.2 ± 0.5

Table 1: Unagi buffer exchanges human IgG into PBS at 4 mL per Una.

Variable	Initial	Target final	Actual final
Conc. (mg/mL)	9.9	79.2	79.3 ± 1.6
Una fill vol. (µL)	8,000	1,000	989.3 ± 10.8
% exchange	-	>96	97.5 ± 0.2
% recovery	-	-	96.7 ± 0.7

Table 2: Unagi buffer exchanges and concentrates human IgG into PBS at 8 mL per Una.

average percent exchange for each sample in each Una was 97.5% (Table 2).

Human IgG was concentrated 8-fold after buffer exchange, with a target final concentration of 79.2 mg/mL. Following buffer exchange, 1 concentration cycle was needed to concentrate the protein to the target of 79.2 mg/mL. The concentration cycle was about 1 minute long.

The final average concentration across all samples was 79.3 ± 1.6 mg/mL, slightly above target. Final fill volume per well was targeted to 1 mL, due to the 8-fold concentration step. The final fill volume per Una was similar to the target at 989.3 ± 10.8 (Table 2).

The total run time to complete the buffer exchange of eight samples exchanged into one buffer followed by a 8-fold concentration step on Unagi was 3.3 hours.

Reduce sample volume

Reduce sample volume is identical to the concentration application in that they both concentrate samples, but has the added functionality of starting with a higher volume, up to 48 mL, of sample.

A stock of hIgG (0.25 mg/mL) was concentrated 6x (Table 3). 8 mL was manually transferred to each Una, with an additional 40 mL transferred to Falcon tubes. The target concentration of 6x was determined by the target final concentration of 1.47 mg/mL.

The final fill volume per well was similar to the target at 8.15 ± 0.02 mL. Following reduce sample volume, the average measured concentration for the samples in the Unas was 1.4 ± 0.01 mg/mL, right on target with a sample recovery of 95.5 ± 0.4 (Table 3).

Conclusion

Unagi allows for automated buffer exchange of up to 8 samples of 0.5-8 mL in a single experiment. It is the only benchtop buffer exchange designed to accommodate both low- and high-volume samples.

Unagi is capable of conducting automated buffer exchange with minimal hands on time, with optional sample concentration run in parallel or separately. It allows for flexible buffer exchange procedures by letting users select their desired percent exchange, volume removed per cycle, buffer exchange method, and final concentration. Initial and final protein conditions, such as concentration, sample volume, and percent exchange showed consistency across 10 kDa Unas in all three experiments conducted here.

Variable	Initial	Target final	Actual final
Conc. (mg/mL)	0.25	1.47	1.4 ± 0.01
Una fill vol. (mL)	48	8	8.15 ± 0.02
% recovery	-	-	95.5 ± 0.4

Table 3: Unagi concentrates 48 mL of human IgG 6x using the reduce sample volume application.

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