Volume and concentration control for automated buffer exchange

Stephen Lambert, Robin Sweeney, Donna Chen, Amrita Yadav, Sowmya Tummala, Kirsten Jackson, Joe Barco

PURPOSE

Buffer exchange is an essential step for all biologics preparatory work. Conventional exchange methods are labor intensive, prone to recovery challenges, and difficult to manage in larger numbers. We developed Big Tuna, an automated, flexible buffer exchange system, to address these challenges (Figure 1).

Figure 1: Big Tuna automates buffer exchange.

Big Tuna uses a combination of pressure-based ultrafiltration/diafiltration (UF/DF) and orbital mixing (Figure 2). An integrated ultrasonic sensor measures volumes during the exchange process without making physical contact with samples.

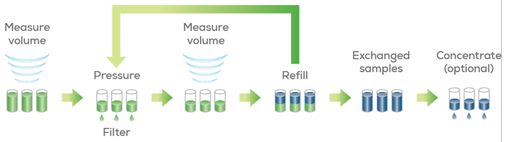

Figure 2: Big Tuna uses a pressure-based ultrafiltration/diafiltration method with gentle orbital mixing to buffer exchange proteins.

Big Tuna can buffer exchange up to 96 samples from 100-450 µL each or up to 24 samples from 0.45-8 mL each using Unfilters (Figure 3).

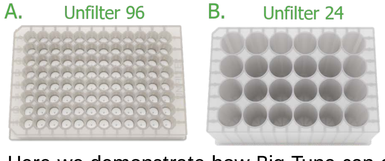

Figure 3: 96- and 24-well formats enable exchanges between 100 µL and 8 mL per sample.

Here we demonstrate how Big Tuna can exchange multiple proteins into multiple buffers with uniform results and high protein recovery.

METHODS

Protein and buffer preparation

- Four stock monoclonal antibodies (mAbs), nominally at 10 mg/mL, were prepared in their stock buffers and transferred to an Unfilter 24
- 8 mL of each mAb was automatically exchanged into 6 formulations (Table 1)

Buffer exchange

- Automated buffer exchange was completed on Big Tuna in an Unfilter 24
- Key buffer exchange parameters were user defined (Table 2)
- Pressurization cycle duration was adjusted in real time by Big Tuna

Concentration to target

- Big Tuna was used to concentrate protein to 50 mg/mL after buffer exchange
- Initial and final protein concentration measured in duplicate on Lunatic

Protein stability

- Stock proteins and buffer exchanged proteins analyzed on Uncle
- DLS measured at 20 °C and 95 °C
- T_m and T_{agg} measured to assess formulation stability (data not shown)

Condition	Variable(s) tested
Buffer	10 mM Histidine
pH	6.0
Excipients	None, 150 mM NaCl, 150 mM sucrose, 75 mM mannitol, 150 mM arginine, 100 mM glycine
Antibodies	mAb A, mAb B, mAb C, mAb D

Table 1: Formulation candidates exchanged on Big Tuna. A total of 24 conditions were exchanged.

Parameter	Setting
Target exchange percentage	96%
Target volume removed per cycle	67%
Initial concentration	10 mg/mL
Initial well volume	8 mL
Target final concentration	50 mg/mL
Target final well volume	1.6 mL

Table 2: Key buffer exchange parameters were user defined in the Big Tuna software. Pressurization cycle duration was automatically adjusted to reach a maximum of 67% volume removed from any well.

RESULTS

Buffer exchange

- 10 buffer exchange cycles to hit 96% exchange per well target
- Actual percent exchange ranged from 96.9% to >99.9% (Table 3)
- Average cycle duration of 27 minutes, range of 24-33 minutes
- Total run time for buffer exchange was approximately 8.5 hours
- Initial well volume was 7958 ± 60 µL (target: 8000 µL)
- Final well volume was 1623 ± 126 µL (target: 1600 µL)

Concentration to target

- 3 concentration cycles on Big Tuna immediately following buffer exchange to hit 5-fold concentration target
- Total time for protein concentration was about 1.5 hours (100 min)
- Average concentration cycle duration of 20 minutes, range of 10-38 minutes
- Actual final protein concentration was highly accurate (Table 4)

Protein	No excipient	150 mM NaCl	150 mM sucrose	75 mM mannitol	150 mM arginine	100 mM glycine
mAb A	99.5	97.5	98.9	99.5	98.0	99.6
mAb B	97.8	99.0	96.9	97.5	99.0	97.8
mAb C	>99.9	99.5	>99.9	>99.9	99.7	>99.9
mAb D	99.9	98.6	99.4	99.8	99.2	99.9

Table 3: Actual percent exchange for each well after automated buffer exchange on Big Tuna range from 99.0% to >99.9%. Target percent exchange per well was user-defined as 96%.

Protein	Initial conc. (mg/mL)	Final conc. (mg/mL)						Average final conc. (mg/mL)
		No excipient	150 mM NaCl	150 mM sucrose	75 mM mannitol	150 mM arginine	100 mM glycine	
mAb A	10.0	51.0	42.4	54.1	56.6	48.8	56.0	51.5
mAb B	10.0	51.6	55.0	42.4	51.9	57.3	58.7	52.8
mAb C	9.9	52.1	52.9	58.8	56.8	57.6	55.1	55.6
mAb D	9.7	50.5	54.3	52.9	55.6	57.9	56.8	54.7

Table 4: Protein concentrations of each mAb formulation before and after buffer exchange.

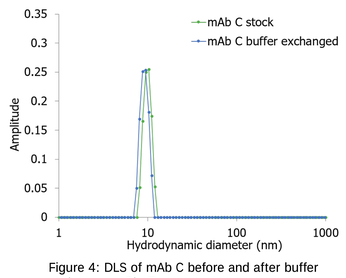

Figure 4: DLS of mAb C before and after buffer exchange on Big Tuna.

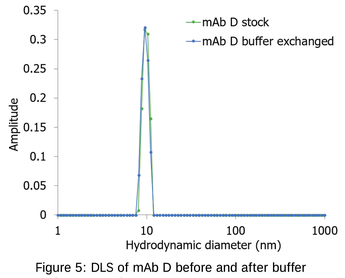

Figure 5: DLS of mAb D before and after buffer exchange on Big Tuna.

RESULTS CONT.

Protein stability

- Automated buffer exchange did not affect protein quality
- Protein quality was assessed by DLS on Uncle after buffer exchange on Big Tuna
  - DLS measurements for mAb C stock were compared to those for mAb C buffer exchanged into 10 mM histidine pH 6.0 with 100 mM glycine (Figure 4)
  - DLS measurements for mAb D stock were compared to those for mAb D buffer exchanged into 10 mM histidine pH 6.0 with no excipient (Figure 5)
  - No significant differences in size before and after buffer exchange and no aggregation after exchange

CONCLUSIONS

- Big Tuna is capable of conducting high-throughput buffer exchange with multiple proteins, into multiple buffers
- Buffer exchange rates are viscosity-dependent, which are dependent on formulation, protein, and concentration.
- Big Tuna optimizes the pressurization cycle duration in real time to ensure that all samples reach the exchange target.
- With the UF/DF method, the operator can control % volume removed per cycle which could be used to control the buffer exchange process and prevent drying or over-concentration of samples.
- Initial and final protein conditions showed consistency across the Unfilter 24 despite protein and formulation differences.
- Multiple exchange formats at lower volumes enables more formulations or conditions to be generated and screened simultaneously. This enables higher buffer exchange throughput which is often a bottleneck to formulation or other biologic studies.

Unchained Labs 6870 Koll Center Parkway, Pleasanton, CA 94566

unchainedlabs.com