Automated platform buffer screening for multiple proteins on Big Kahuna

Introduction

The critical process of screening formulation buffers to optimize stability is labor-intensive and time-consuming, which is often a limiting factor in biologics development. The conformational, chemical, and colloidal stability of a protein are strongly influenced by the buffer solution. Altering buffer salts, pH, ionic strength, excipients, and surfactants may increase or decrease the stability of a molecule. To alleviate some of the time requirements in developing a new biologic molecule, a platform buffer screen is typically used to screen common formulation conditions to quickly narrow down optimal buffer conditions. A platform buffer screen analyzes the stability of a new molecule with common buffers, excipients, and surfactants in common pH ranges.

Conventional exchange methods are labor intensive, prone to inconsistency, and difficult to manage in larger numbers. Automated buffer exchange systems can provide a more uniform sample handling approach and degrees of process control that are otherwise inaccessible by manual methods. Automating a platform buffer screen can further cut down the time required to optimize buffer conditions for new biologic molecules. Big Kahuna with buffer exchange automates the process, reducing hands-on time and enabling increased throughput.

Figure 1: Big Kahuna with buffer exchange automates buffer exchange for up to 96 unique samples with the Unfilter 96.

Big Kahuna uses a pressure-based ultrafiltration/diafiltration (UF/DF) method to exchange up to 96 samples in a single run (Figure 1). Big Kahuna uses Unfilters, which are filter plates designed to withstand pressurization at 60 psi during the buffer exchange process (Figure 2). Unfilter 96 can process up to 96 samples ranging from 100-450 µL; Unfilter 24 can process up to 24 samples ranging from 0.45-8 mL. The Unfilter is transferred automatically between a pressure chamber, an acoustic volume sensor,

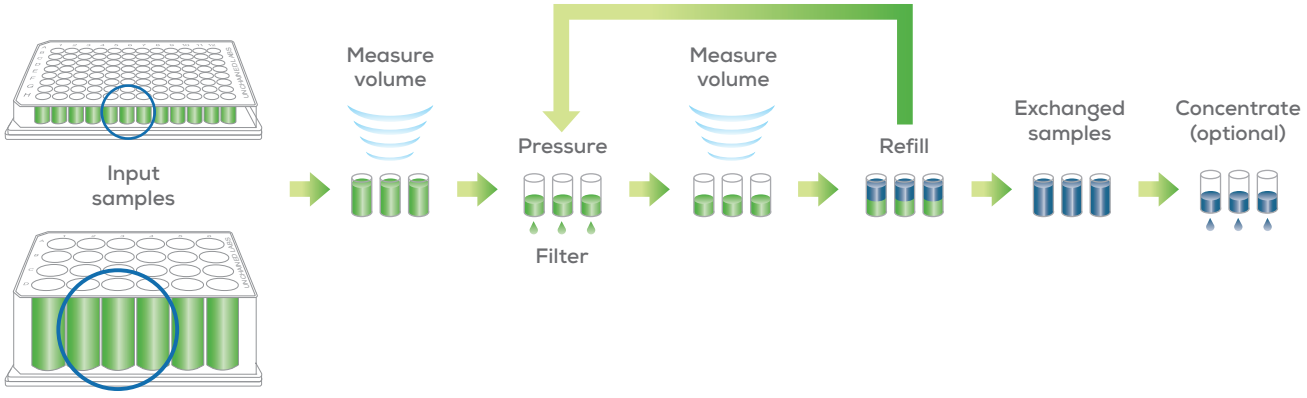

Figure 2: Big Kahuna uses a pressure-based UF/DF method with gentle orbital mixing to buffer exchange proteins with the Unfilters.

and onboard liquid handling to buffer exchange proteins. During an experiment, data from an acoustic volume sensor is used to calculate the volume of buffer removed. The actual volume removed in each well is compared to the user-defined target, and the pressurization time is adjusted for each cycle, optimizing buffer exchange in real time. Buffer exchange with Big Kahuna is highly customizable and adaptable, allowing for buffer exchange of up to 96 unique proteins and formulations on a single Unfilter 96.

In this application note, Big Kahuna is used to conduct a platform buffer screen on four monoclonal antibodies (mAbs) in 12 unique buffer formulations. The platform buffer screen performed on Big Kahuna consisted of a single base buffer with one of five excipients at two different pH levels for each of the 4 mAbs. Exchange was completed in approximately 5 hours.

Methods

Protein and buffer preparation

Big Kahuna was configured with an acoustic volume sensor, 6-channel disposable tips, and a pressurization chamber. Four stock mAbs (mAb A, mAb B, mAb C, and mAb D) were prepared at 10 mg/mL in their stock buffers and manually transferred to a multi-trough reservoir plate. A base buffer of 10 mM histidine was prepared at either pH 6.0 or pH 6.2 with one of five excipients: 150 mM NaCl, 150 mM sucrose, 75 mM mannitol, 150 mM arginine, 100 mM glycine, and no excipient. Each of the 12 unique prepared buffers was manually pipetted into two 2 mL 96-well plates and placed on the Big Kahuna deck.

Each of the four stock mAbs was automatically pipetted by Big Kahuna into 24 wells of a 10 kDa Unfilter 96 (450 µL/well), where they were exchanged into the 12 buffers prepared for the platform buffer screen in duplicate. Duplicate wells were pooled following buffer exchange, resulting in 48 biologic formulations consisting of 4 mAbs each in 12 unique buffers (Table 1).

Buffer exchange

The buffer exchange protocol was set to 96% total exchange per pool with a target volume removal per cycle of 67%. To maintain a final concentration of 10 mg/mL, final well volume was targeted at 450 µL. Throughout automated buffer exchange, pressurization cycle duration was automatically adjusted by Big Kahuna to have the maximum volume removed per well approximately equal to the user defined target volume removed per cycle of 67%.

The LEA software suite was used for experimental design, execution, and analysis of results. The experimental design was created in Library Studio with Design Creator and was executed in Automation Studio. Average cycle duration, initial and final well volumes, and final percent exchange per pool were calculated in Excel using the LEA Analysis Add-in.

Condition	Variable(s) tested
Buffer	10 mM Histidine
pH	6.0, 6.2
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 conditions studied on Big Kahuna. A total of 48 conditions were run in duplicate.

Protein	Initial conc. (mg/mL)	pH	Final concentration (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	6.0	10.1	10.1	10.1	9.9	10.5	10.3	10.2
		6.2	10.2	10.3	10.3	9.9	10.3	10.1	
mAb B	9.1	6.0	9.2	9.3	9.3	9.1	9.2	9.2	9.2
		6.2	9.2	9.2	9.3	9.2	9.1	9.2	
mAb C	9.9	6.0	9.9	10.2	10.1	9.9	9.9	9.9	10.0
		6.2	9.9	10.2	10.1	10.0	10.0	10.1	
mAb D	10.5	6.0	10.8	10.8	10.8	10.7	10.5	10.4	10.7
		6.2	10.8	10.7	10.7	10.6	10.5	10.8	

Table 2: Protein concentrations of each mAb formulation before and after buffer exchange as determined by the A280 application on Lunatic.

Protein concentration

Lunatic was used to analyze the concentration of each of the four stock mAbs and each of the 48 formulations that resulted from buffer exchange on Big Kahuna. Protein concentration was determined with the A280 application on Lunatic using the E1% specific to each of the four mAbs. All proteins were measured in triplicate and average concentration is reported in all cases. Initial and final protein concentrations and well volumes were used to calculate percent mass recovery following buffer exchange on Big Kahuna.

Results

Stock protein formulations

Each of the four stock mAbs was prepared in their stock buffer at a target concentration of approximately 10 mg/mL. Table 2 shows the actual concentration of each mAb before and after buffer exchange as measured by Lunatic.

Buffer exchange

The target percent exchange of 96% for each pool was reached in 7 cycles that ranged from 8.5 to 10.1 minutes per cycle with an average duration of 9.3 minutes per cycle. The duration of each cycle adjusted automatically so the maximum volume removed from any well per cycle was approximately the target of 67% exchange per cycle. The total run time to complete the buffer exchange of four proteins each in twelve buffer formulations on a single Unfilter 96 was approximately 5 hours (307 minutes).

Initial fill volume per well as measured by the on-deck volume check was 430 ± 11 µL. Final fill volume per well as measured by the on-deck volume check was similar to the initial fill volume per well at 426 ± 15 µL, showing consistency and no significant difference between the initial and final fill volume per well across the Unfilter 96.

A target percent exchange of 96% was set per pool, meaning that every pool must reach a minimum of 96% exchange. Because flow rate of solutions may vary, some pools exchanged to >96% to ensure that all pools exchanged to at least 96%. The average percent exchange per pool across the Unfilter 96 was 98.4%, with a minimum percent exchange of 96.7% and a maximum of over 99.9% (Table 3). Each of the four mAbs did exchange at slightly different rates, but despite these differences buffer exchange was successful across the Unfilter 96 due to Big Kahuna optimizing the pressurization cycle duration in real time.

Protein	pH	No excipient	150 mM NaCl	150 mM sucrose	75 mM mannitol	150 mM arginine	100 mM glycine
mAb A	6.0	99.3	97.6	98.9	99.2	98.7	99.4
	6.2	99.0	97.5	98.5	98.9	97.7	99.0
mAb B	6.0	97.1	98.3	96.7	97.1	98.5	97.4
	6.2	98.4	99.2	97.2	97.0	98.5	97.1
mAb C	6.0	>99.9	98.8	97.2	99.9	99.0	>99.9
	6.2	99.9	98.8	99.9	99.9	99.1	99.9
mAb D	6.0	98.5	96.7	97.7	98.2	97.7	98.7
	6.2	98.4	97.1	97.5	98.2	97.5	98.4

Table 3: Actual percent exchange for each pool after automated buffer exchange on Big Kahuna range from 96.7% to >99.9%. Target percent exchange per pool was user-defined as 96%.

Protein	pH	No excipient	150 mM NaCl	150 mM sucrose	75 mM mannitol	150 mM arginine	100 mM glycine
mAb A	6.0	99	100	100	97	100	100
	6.2	100	100	100	98	100	100
mAb B	6.0	99	100	100	99	100	100
	6.2	100	100	100	99	98	100
mAb C	6.0	100	100	100	100	100	100
	6.2	100	100	100	100	100	100
mAb D	6.0	98	100	99	99	98	97
	6.2	99	99	99	98	98	100

Table 4: Percent mass recovery for each of the 48 protein formulations after automated buffer exchange on Big Kahuna. Mass recoveries include ±3% error based on variability in the volume measurements.

Final protein concentration

During buffer exchange on Big Kahuna, the target final concentration was equal to the initial concentration of each protein, approximately 10 mg/mL. Table 2 shows the actual concentration of each mAb before and after buffer exchange as measured by Lunatic. Actual final concentrations were approximately equal to actual initial concentrations with only a slight and consistent increase in concentration in every pool across the plate.

Percent mass recovery was calculated based on initial and final volumes and concentrations per pool. The minimum percent mass recovery for any pool on the Unfilter 96 was 97%, meaning that nearly no protein was lost during buffer exchange on Big Kahuna (Table 4).

Conclusion

Big Kahuna is capable of conducting high-throughput platform buffer screens of multiple proteins and multiple buffer formulations with minimal hands on time. Big Kahuna exchanged

four mAbs into 12 buffer formulations in duplicate in about five hours in the platform buffer screen described above.

Initial and final protein conditions, such as well volume, concentration, target percent exchange, and percent mass recovery showed consistency across the Unfilter 96 despite differences in proteins and formulations. The ability of Big Kahuna to adjust pressurization cycle duration after each cycle provides efficiency and prevents over-concentration during the exchange process.

To further automate this process, physical or virtual integration of Lunatic with Big Kahuna can eliminate the need for manual steps to determine protein concentration. Automation of buffer preparation and titration on the deck of Big Kahuna would also further automate this process by eliminating the need for manual buffer preparation.

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