A fully integrated Big Kahuna workflow to automate the entire biologics formulation development process

Introduction and background

Therapeutic proteins can be inherently prone to degradation and instability, and they often pose challenges to drug developers. These developers perform screening and stability studies to identify suitable formuations from preformulation all the way through the formulation development process. The number of formulations that can be screened for each project is constrained by short timelines and the availability of material and resources. Scientists that automate formulation screening and stability studies significantly increase their productivity and improve throughput without additional resources.

Unchained Labs developed the Big Kahuna system configured for biologics formulation to automate the entire formulation development process: liquid and solid dispensing, preparation and stressing of samples, high-throughput pH, viscometry and enhanced visual inspection with visible particle counting – all on a single platform. The powerful and intuitive software, Lab Execution and Analysis (LEA), controls the Big Kahuna system and integrates third-party analytical and processing equipment. LEA collects all processing information and analytical results in a shared database. The database links sample composition information, stress conditions and analytical results. A wide range of Unchained Labs' analytical systems and third-party analytics, including DLS, UV/Vis and HPLC can be integrated into the Big Kahuna system.

Figure 1: The Big Kahuna system configured for biologics formulation. The robot on the right prepares formulations and measures pH, viscosity and visual inspection. The left robot integrates with the Wyatt DynaPro II dynamic light scattering (DLS) instrument and Molecular Devices SpectraMax microplate reader. A low-bioburden HEPA enclosure encapsulates the system.

A fully-integrated automation workspace

Designed with collaborators from R&D divisions of leading pharmaceutical and biotechnology companies, the Big Kahuna system automates many workflows common to formulation development and analytical testing labs. Robots and instruments are connected and configured for each customer workflow. Every Big Kahuna system removes manual steps to speed up lab work and liberate scientists to focus on more valuable tasks (Figure 1). One robot prepares the formulations and measures pH, viscosity, visible particles, color and turbidity. The other robot performs sample processing, stressing and analysis (DLS and UV/Vis, etc.). The Big Kahuna system handles a variety of formats from 96-well microplates to 20 mL serum vials. Powerful LEA software enables instrument control, data collection, data management and reporting. By combining the aforementioned activities, the Big Kahuna system supports the entire formulation development process from preformulation to late stage formulation for virtually all proteins, peptides and other biotechnology products.

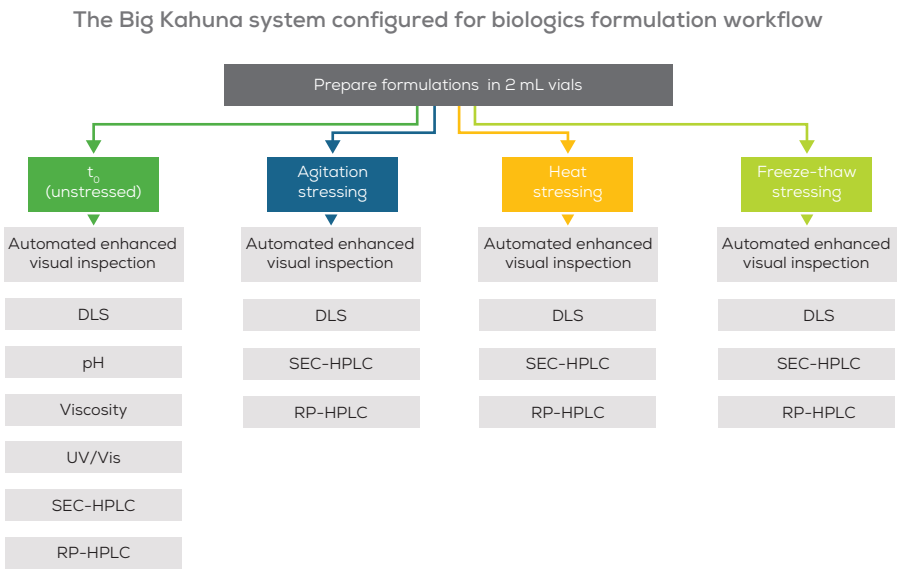

Figure 2: A schematic flowchart summarizes the automated study. The Big Kahuna system automatically prepared all formulations in serum vials, then t_0 samples were analyzed by nine analytical techniques using this system. Automated enhanced visual inspection: particles, color, turbidity. Three sets of vials were subjected to agitation, heat or freeze-thaw stressing on the Big Kahuna system, and then the samples were tested by six analytical methods.

Case study: formulation screening and stability studies can be automated

In this study, we applied a Big Kahuna system to complete a formulation screening and stability study.

To demonstrate the productivity improvements made possible with the Big Kahuna system, we automated the buffer and excipient screen for a protein, and the stressing and analysis of each sample (Figure 2).

- Using bovine serum albumin (BSA) as a surrogate drug substance, a single scientist was able to prepare and screen 48 formulations in 2 mL vials
- 48 total formulations (Table 1) with BSA concentrations of 10 mg/mL were prepared using five buffer systems (with pH values from 4.5–7.0) and four excipient combinations. All formulations were prepared in 2 mL (2R) serum vials.
- Three sets of formulations were subjected to three different stress conditions: agitation, heating and freeze-thaw cycles. An additional set of formulations was used for initial time point (t_0) measurements. Initial time point (t) samples were analyzed by both system analytics and third-party analytics, which were directly integrated into the Big Kahuna system via LEA software.
- Each formulation was made from sterile stock solutions using automated liquid handling (air displacement and positive displacement pipetting) together with the cooled storage bay and vortexer deck elements of the Big Kahuna system.
- After compounding, automated protocols aliquoted and diluted sample material from each formulation into the appropriate vials and 96-well plates to complete the t_0 testing.

The Big Kahuna system analytics were pH, viscosity and visible inspection (color, turbidity and visible particle detection). Integrated third-

Form	Buffer	pH	Excipients
1	Sodium citrate	5.0	Sorbitol, NaCl
2			Arginine, NaCl
3			Aspartic acid, NaCl
4			Aspartic acid, Arginine, NaCl
5		5.5	Sorbitol, NaCl
6			Arginine, NaCl
7			Aspartic acid, NaCl
8			Aspartic acid, Arginine, NaCl
9	Sodium phosphate	6.5	Sorbitol, NaCl *
10			Arginine, NaCl *
11			Aspartic acid, NaCl *
12			Aspartic acid, Arginine, NaCl *
13		6.5	Sorbitol, NaCl
14			Arginine, NaCl
15			Aspartic acid, NaCl
16			Aspartic acid, Arginine, NaCl
17		7.0	Sorbitol, NaCl
18			Arginine, NaCl
19			Aspartic acid, NaCl
20			Aspartic acid, Arginine, NaCl
21	Sodium acetate	4.5	Sorbitol, NaCl
22			Arginine, NaCl
23			Aspartic acid, NaCl
24			Aspartic acid, Arginine, NaCl
25		5.0	Sorbitol, NaCl
26			Arginine, NaCl
27			Aspartic acid, NaCl
28			Aspartic acid, Arginine, NaCl
29	Sodium phosphate-citrate	6.0	Sorbitol, NaCl
30			Arginine, NaCl
31			Aspartic acid, NaCl
32			Aspartic acid, Arginine, NaCl
33		6.5	Sorbitol, NaCl
34			Arginine, NaCl
35			Aspartic acid, NaCl
36			Aspartic acid, Arginine, NaCl
37	L-Histidine	6.0	Sorbitol, NaCl
38			Arginine, NaCl
39			Aspartic acid, NaCl
40			Aspartic acid, Arginine, NaCl
41		6.5	Sorbitol, NaCl
42			Arginine, NaCl
43			Aspartic acid, NaCl
44			Aspartic acid, Arginine, NaCl
45		7.0	Sorbitol, NaCl
46			Arginine, NaCl
47			Aspartic acid, NaCl
48			Aspartic acid, Arginine, NaCl

Table 1: Compositions of the 48 formulations prepared by the Big Kahuna system are summarized. Each formulation includes sorbic acid (0.1%), except control formulations denoted with asterisks. BSA concentration is 10 mg/mL in each formulation.

party analytics were Wyatt DynaPro™ for protein aggregation, Molecular Devices SpectraMax® for protein concentration (A280nm) and Agilent 1100 HPLC for reversed-phase (RP) and size-exclusion chromatography (SEC). After t_0 analysis, each formulation was analyzed by all analytics, except pH and A280nm. The Big Kahuna system generated agitation and heat stressed samples on the robotic work space. All sample stressing was automated by the Big Kahuna system, except freeze-thaw stressing. Analytical results were automatically linked to appropriate sample composition and stress conditions via the LEA software and stored in its database. Data series were viewed and organized in LEA, and results were then exported to Microsoft Excel for generation of tables and plots.

Results from the automated study

At t_0, the Big Kahuna system automatically prepared samples and performed color, turbidity, visible particle count, viscosity, pH, DLS, UV and HPLC. Initial turbidity measurements for all formulations were between 5–13 nephelometric turbidity units (NTU), which corresponded to clear solutions by manual visual inspection. Visible particles (particulates) counted by the automated visual inspection station ranged from 0–4 in the vials at t_0. The initial viscosities for all formulations were consistently low (around 1.0 cP), as expected for such protein solutions. All formulations had less coloration than the BY7 (brown-yellow 7) standard, except for formulations 1, 2 and 4, which were all equivalent to BY7. Formulations containing sorbic acid had interfering absorbance at 280 nm, so this technique was only used to measure control formulations (9–12), which did not contain sorbic acid. Additional t_0 results are shown in Table 2. Results from t_0 and after stress exposure are presented for DLS (Figure 3) and HPLC (Figure 4).

Figure 5 shows visible particle counts for all formulations across stress conditions compared to t_0. Small increases in visible particles (~1) were observed compared to t_0 samples after freeze-thaw and agitation stressing. Heat stressing led to significant increases in the number of visible particles for many formulations. Heat stressing also induced color changes for formulations 2, 4, 26 and 37–48 from ≤ BY7 (nearly colorless or colorless) to BY5 or darker (noticeable brown-yellow tint).

Modest increases in turbidity (Figure 6) were detected in the samples after freeze-thaw and agitation stressing compared to their t_0 values. Heat stressing led to significant changes in the turbidity of the formulations, usually an increase of 100 NTUs or more.

The results of DLS measurements of all formulations and conditions are shown in Figure 3. At t_0, the polydispersity percentage of formulated BSA ranged from 20%–24%. For most formulations, heat stressing was correlated with no change or decreases in the polydispersity compared to the t_0 samples. Agitation generally did not significantly alter the polydispersity of samples, except in the case of formulation 23, which showed a significant increase in polydispersity.

Formulations with low pH values (4.5–5.0), such as formulations 1–4 and 21–24 that were exposed to freeze-thaw stressing had significant increases in the polydispersity and the actual polydispersity values were not determined

All formulations were analyzed by RP and SEC for purity and colloidal stability, respectively, using an Agilent HPLC that was virtually integrated with the Big Kahuna system. Chromatograms for each formulation were compared to those of an unstressed BSA reference standard in 50 mM sodium phosphate, pH 7.0. Results from both RP and SEC HPLC indicate that the stability of the protein is largely unaffected by these stress conditions.

Placebo formulations with the same compositions, with BSA excluded were analyzed at t_0 with all analytics except HPLC (SEC and RP), DLS and A280.

Form	Automated pH	Automated turbidity (NTU)	Automated particulates	Automated viscosity (Cp)	Automated color	Automated A280
1	5.5	8	1	1.3	BY7	ND
2	5.5	10	1	1.2	BY7	
3	5.6	7	0	1.2	Colorless (<BY7)	
4	5.5	7	0	1.2	BY7	
5	5.9	8	0	1.3	Colorless (<BY7)	
6	5.9	7	3	1.2		
7	6.1	8	0	1.2		
8	5.9	8	0	1.2		
9	6.5	8	2	1.3		7.0
10	6.5	7	2	1.2		7.6
11	6.6	11	2	1.2		7.4
12	6.5	11	0	1.3		8.0
13	6.5	12	0	1.3		ND
14	6.5	9	1	1.3		
15	6.6	8	0	1.2		
16	6.4	8	1	1.1		
17	6.4	13	0	1.2		
18	6.5	7	0	1.2		
19	6.6	7	0	1.5		
20	6.4	7	0	1.1		
21	4.8	9	0	1.4		
22	4.8	9	0	1.3		
23	5.0	7	0	1.2		
24	4.8	7	1	1.2		
25	5.1	8	0	1.2		

Form	Automated pH	Automated turbidity (NTU)	Automated particulates	Automated viscosity (Cp)	Automated color	Automated A280
26	5.2	7	1	1.1	Colorless (<BY7)	ND
27	5.2	6	1	1.2		
28	5.2	7	1	1.3		
29	6.2	6	0	1.4		
30	6.2	7	1	1.2		
31	6.3	6	1	1.3		
32	6.2	6	0	1.0		
33	6.6	11	1	1.3		
34	6.6	9	0	1.3		
35	6.7	6	1	1.4		
36	6.6	6	0	1.4		
37	6.1	5	1	1.4		
38	6.2	8	0	1.2		
39	6.3	7	2	1.2		
40	6.2	5	0	1.3		
41	6.7	8	0	1.3		
42	6.7	7	1	1.3		
43	6.8	5	0	1.1		
44	6.7	7	4	1.2		
45	7.4	11	0	1.1		
46	7.4	8	0	1.1		
47	7.5	7	1	1.1		
48	7.3	6	1	1.3		

Table 2: Summary of results from six analytics collected at t_0. ND: not determined.

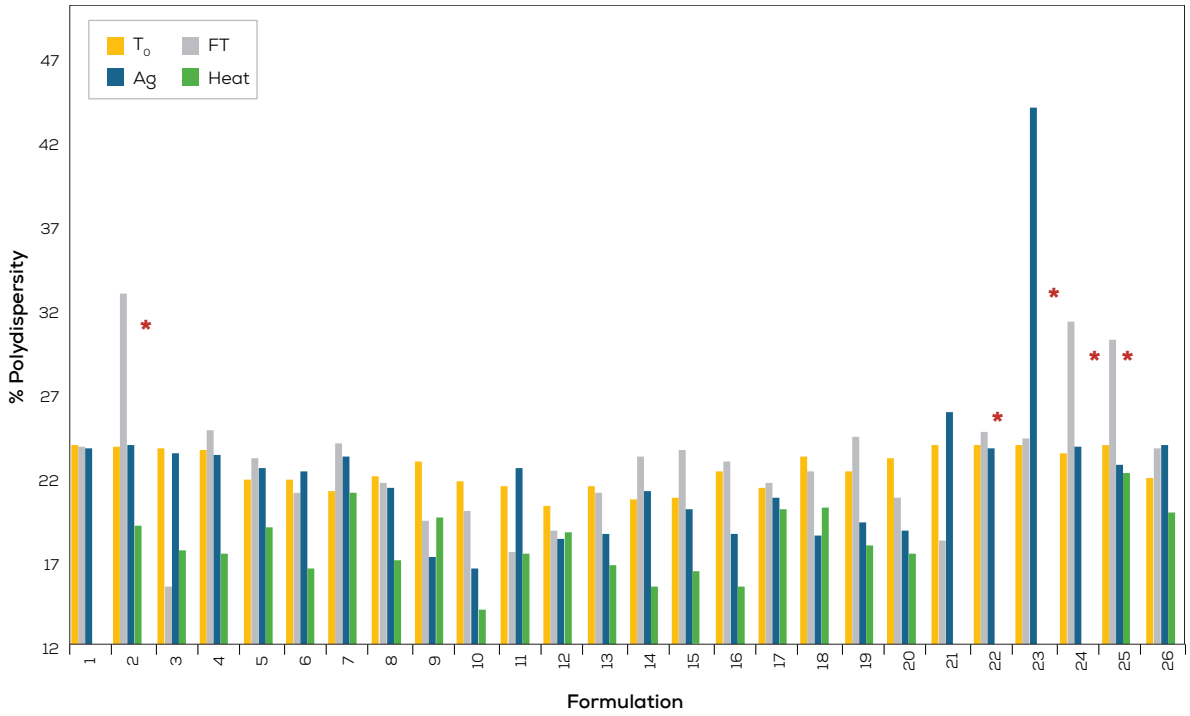

Figure 3: A graph of the DLS results shows that stressing by heat (Heat), freeze-thaw (FT) and agitation (Ag) caused increases in percent polydispersity. Increased polydispersity could indicate formation of particles. Formulations with pH values around 5 had significant increases in polydispersity to the point where many samples were not measurable (highlighted by the asterisks*).

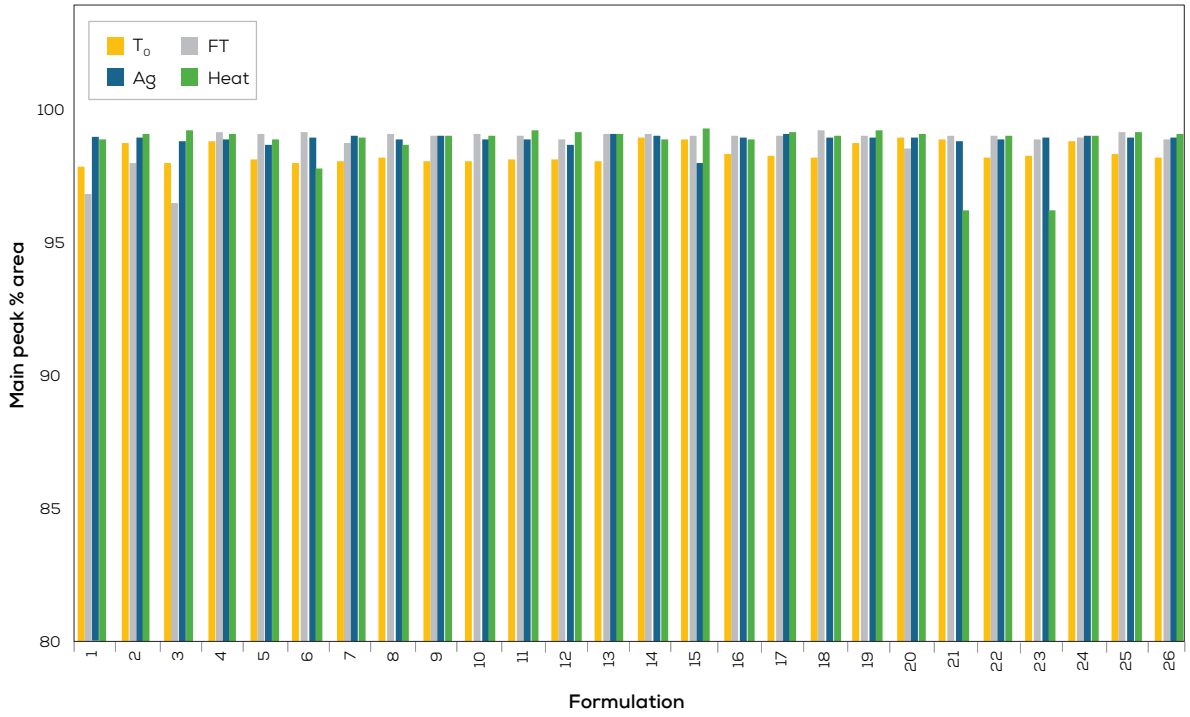

Figure 4: A graph of the RP HPLC results shows that the BSA is highly pure in all formulations at t_0 and that purity is maintained in all formulations throughout each stress condition.

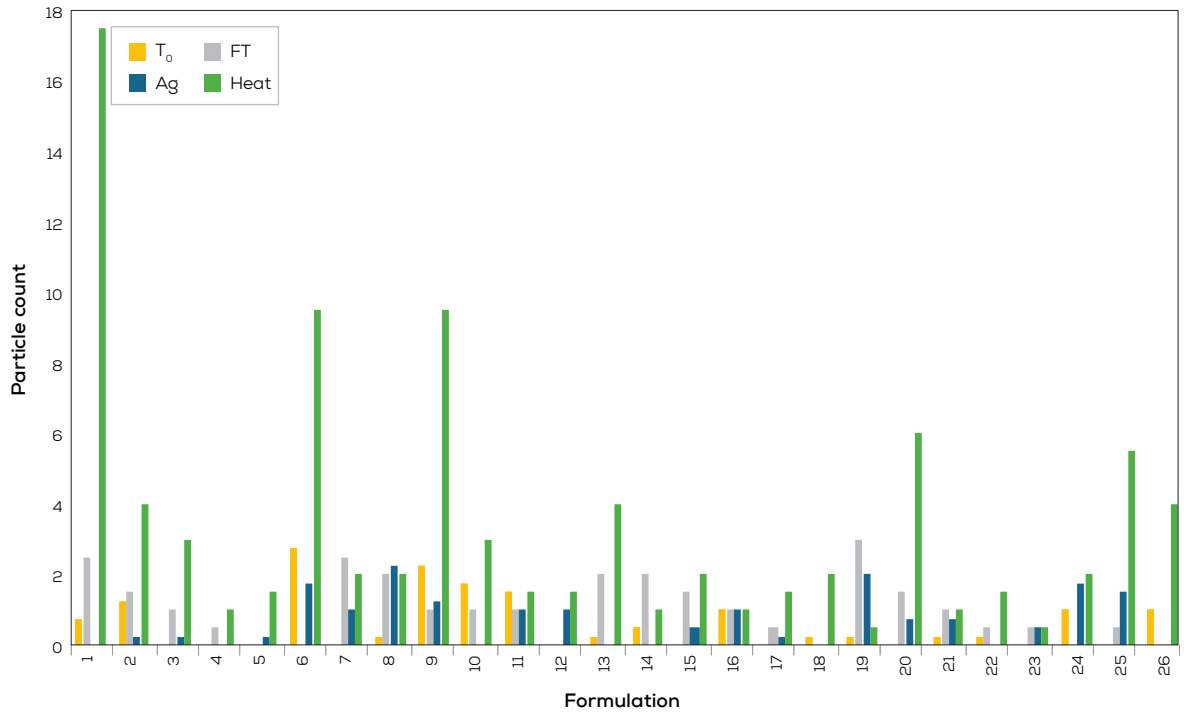

Figure 5: A graph of the visible particle counts is shown. Particle counting was performed by Unchained Labs' visual inspection station. Heat stressing (Heat) led to marked increases in particles in most formulations. Freeze-thaw (FT) and agitation (Ag) caused modest increases in particle counts in most formulations.

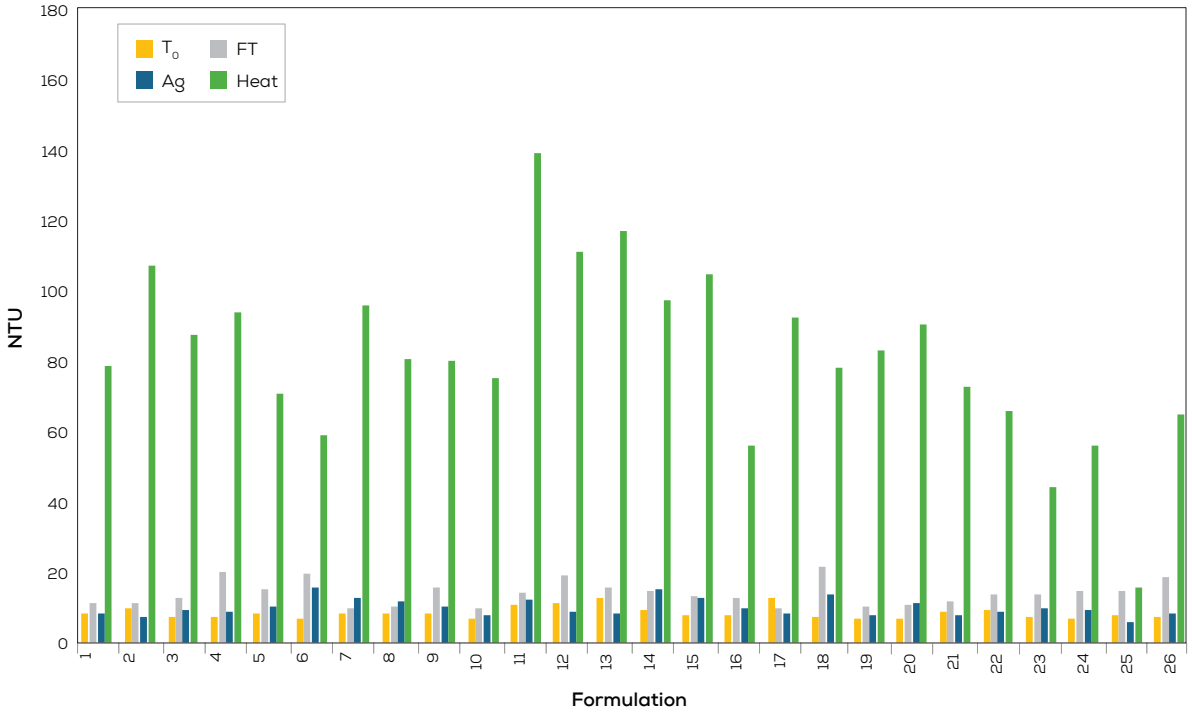

Figure 6: A graph of turbidity values shows that heat stressing (Heat) led to marked increases for most formulations. Freeze-thaw (FT) and agitation (Ag) lead to only modest increases in turbidity for most formulations.

Process	Attended time using the Big Kahuna system for 48 formulations (h)	Attended time using manual methods for 48 formulations (h)
Formulation preparation by liquid compounding*	0.5	2.5
Analytical sample preparation for six methods*	0.5	2.0
Visual inspection	0.3	0.5
DLS	0.3	0.3
pH	0.3	2.4
Viscosity	0.3	12.0
UV/Vis	0.1	0.1
SEC-HPLC*	0.8	0.8
RP-HPLC*	0.8	0.8
HPLC analysis and reporting	1.0	3.0
Lab notebook entries	1.5	5.5
Total attended time (h)	6.1	29.7

Table 3: A chart of turbidity values shows that heat stressing (Heat) led to marked increases for most formulations. Freeze-thaw (FT) and agitation (Ag) lead to only modest increases in turbidity for most formulations. * Not including preparation of stock or mobile phase solutions.

The results were nearly identical to those of the respective protein formulations (results not shown).

Conclusion

We developed and performed an automated procedure for the preparation and analysis of protein formulations suitable for a buffer and excipient screen. This demonstrated the productivity improvements that are possible with the Big Kahuna system configured for biologics formulation. Automated procedures significantly reduced the time scientists were working in the lab while efficiently providing highly informative results. Data from visual inspection and DLS reveal to the scientist strong relationships between buffer systems and pHs to the overall performance of the formulation. Formulations with lower pH values (4.5–5.0) demonstrated lower stabilities compared to those with higher pH values (5.5 to 7.0).

Generally, excipient conditions with sorbitol and NaCl were correlated with less stable formulations. Sorbic acid had little influence on the stabilities of the sodium phosphate formulations, as indicated by the control formulations (no sorbic acid). Results from HPLC (RP and SEC) testing indicated that BSA maintained a high purity and colloidal stability, which suggests that the stability indicators for each formulation may be due to components of the formulation exclusive of the protein. For this study, the results suggested that the performance of each formulation was independent of the stability of BSA, and that the stability for each formulation may be due to the buffer and excipients components.

To complete this study, approximately 28 hours of scientist time was required. A similar study performed by current manual procedures would require 110–170 hours of scientist time (Table 3).

By using the procedure automated by the Big Kahuna system, a 4X–6X reduction in scientist time was achieved. Without the benefit of automated execution of formulation compounding, analytical sample preparation analytics and data aggregation, evaluating such a broad range of formulation and stress conditions would be a significant commitment for a single scientist, or even a team of experts. Additional benefits of automating include:

- Robust automation enabled consistency and standardization of sample handling across all conditions
- Sample tracking for all time points ensures data integrity
- Raw data linked to appropriate samples and sample conditions in a user-friendly centralized database reducing interpretation time and effort
- Intuitive software allows scientists to rapidly review, reprocess and report

This case study demonstrates that high-throughput automation offered with the Big Kahuna system improves efficiency and productivity of formulation scientists. Unchained Labs' proprietary automation technologies enable a broad range of formulations to be screened with reduced time and labor requirements. Analytical results were collected in a central database, allowing scientists to reach meaningful conclusions quickly and confidently. Additionally, the use of automation provided consistency of sample handling while the automated data collection and tracking features of LEA increased confidence in data integrity and accelerated the time from experimental completion to reporting.

Approximately 28 hours of scientist time was required to complete this study using the Big Kahuna system compared to 110-170 hours, which would have been required with manual procedures.

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