Comparison of manual versus automated protein formulation development workflow on a Big Kahuna

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Application

Formulation development of biopharmaceuticals can be a challenging and time consuming process. To streamline development activities, many pharmaceutical companies have implemented laboratory automation for analytical sample preparation, formulation screening and forced-degradation studies. While automation can increase lab efficiency and throughput, it must also provide results comparable to manual methods. Recently, we performed a study with the French biopharmaceutical group LFB to compare manual and automated procedures for protein drug formulation. Recent updates to regulatory requirements are increasingly demanding studies with a larger number of samples. In this respect, LFB was interested in evaluating an automated system that could increase both experimental capacity and number of formulations evaluated, as well as enabling innovation in drug development. Here we present results for seven protein drug formulations after stressing and analysis by both manual and automated procedures.

Automation designed for formulation development

Automated procedures described in this application note were performed on Unchained Labs' Big Kahuna system configured for biologics formulation. The Big Kahuna system is a comprehensive automation platform that increases capabilities, productivity and repeatability in formulation development of biopharmaceuticals. The Big Kahuna system automates many tasks such as formulation and analytical sample preparation along with sample processing and stressing. It was designed to increase productivity while maintaining comparability to the procedures and analytical techniques currently used in formulation laboratories. The size and scope of the Big Kahuna can be tailored to the specific needs of a formulation workflow and laboratory. The platform used for this study consisted of two Big Kahuna systems connected by a carousel enclosed in a low-particulate workspace (Figure 1). The Big Kahuna system on the right side has multiple arms and tools that perform liquid handling and vial and plate transfers. These arms can access the deck, which includes heating, cooling and mixing (vortex or magnetic stir bars) stations as well as proprietary analytical instruments for visual inspection and viscosity measurement. The Big Kahuna system on the left side physically integrates with an incubated plate shaker, dynamic light scattering (DLS) system and UV/Vis plate reader. The two systems are connected by a carousel that provides storage for samples and consumables (pipetter tips, microplates, vial rack, etc.) and facilitates the movement of samples through the system. This unique set of capabilities allows scientists to prepare, stress and analyze formulations in a complete and uninterrupted workflow. The Big Kahuna is compatible with a variety of containers and formats including

> When using the 96-well plate format, the Big Kahuna system can screen more than 300 formulations in a single day.

Figure 1: Unchained Labs' Big Kahuna system configured for biologics formulation. The Big Kahuna system on the right prepares formulations and analytical samples, measures pH and viscosity, and performs visual inspection. The Big Kahuna system on the left accesses the Wyatt DynaPro® Plate Reader II Dynamic Light Scattering (DLS) instrument and Molecular Devices SpectraMax® Microplate Reader. A low-bioburden HEPA enclosure encompasses the entire system and work space.

ANSI/SLAS microplates (e.g., 96-well plates) and serum vials from 2–20 mL.

In this particular study, we used this dual system to perform stressing followed by analysis to determine the robustness of multiple biopharmaceutical formulations. High-throughput enhanced visual inspection (visible particles, color and turbidity), UV/Vis and DLS analyses were integrated onto the Big Kahuna deck. Size-exclusion chromatography (SEC) was performed using a UPLC instrument that was virtually integrated using Unchained Labs' software to simplify data interpretation, analysis and reporting.

Case study

Traditional process

Two confidential protein drug products, Molecule 1 and Molecule 2, were provided by LFB for this study. Three confidential formulations of Molecule 1 (A–C) and four formulations of Molecule 2 (A–C and B') were evaluated by traditional, largely manual processes. All formulations of Molecule 1 and Molecule 2 were manually prepared at the laboratory scale (approximately 200 mL) and then transferred to 2 mL vials and 5 mL tubes. All formulations were subjected to a series of stress conditions in vials or tubes and then tested for evidence of degradation. Molecule 1 formulations were stressed by stirring at 300 rpm using magnetic stir bars for 24 hours, rotational agitation at 35 rpm for 24 hours (5 mL tubes only) and heating at 48 °C for 3 hours. Molecule 2 formulations were stressed by stirring at 500 rpm for 8 hours using magnetic stir bars, rotational agitation at 35 rpm for 8 hours (5 mL tubes only) and heating at 57 °C for 3 hours. Analytical results from UV/Vis (protein content and turbidity), DLS and SEC of the stressed material were used to determine the relative robustness of each formulation for both molecules. All processes were performed using individual laboratory devices and instruments without further automation (Figure 2).

Automated process

The same confidential formulations of Molecule 1 and Molecule 2 were evaluated using the dual Big Kahuna system. Stress parameters for each molecule were optimized according to the capabilities of the robot. Rotational agitation, performed as part of the manual process, was replaced by vortexing. Vortexing speeds were adjusted to achieve a similar degree of agitation of the solution to that observed in rotational agitation. Stirring intensities were adjusted to the size of the stir bar in the vials and to the solution volume in order to achieve intended stressing of the sample.

The dual Big Kahuna system automated formulation stresses, all analytical sample preparation and analyses of stressed formulations except SEC, which was virtually integrated (networked via a shared database) to the Big Kahuna. Three confidential formulations for Molecule 1 and four confidential formulations for Molecule 2 were manually transferred to 2 mL serum vials inside the low bioburden Big Kahuna workspace. All formulations for T_0 testing and each stress condition were prepared in triplicate. After stressing, the heated and agitated formulations were analyzed by automated enhanced visual inspection (color, turbidity and visible particle counting). The stirred formulations were not tested by enhanced visual inspection due to interference by the stir bars. The dual Big Kahuna system was then used to aliquot and/or dilute protein formulations

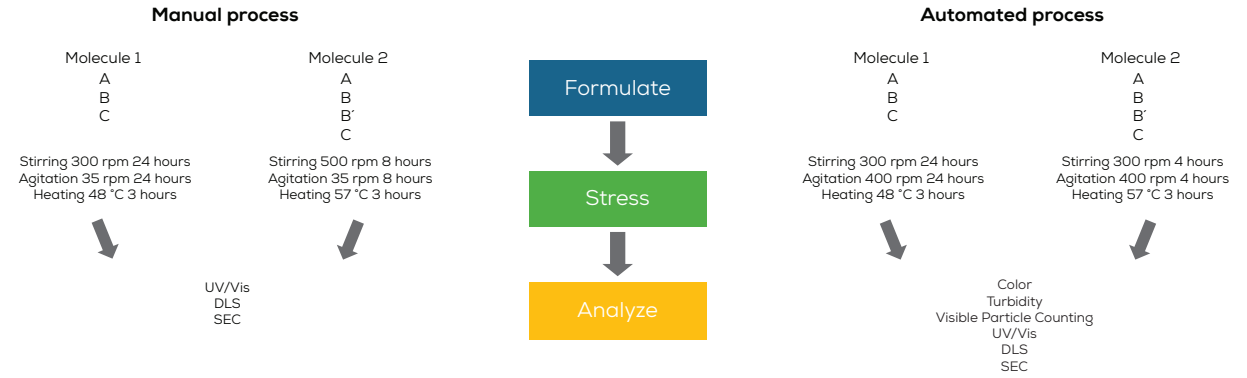

Figure 2: Formulation study design. Two protein drug molecules were formulated, stressed and analyzed by a traditional manual process and an automated process to assess if the automated process provided comparable results.

into 96-well plates for analysis by DLS, UV/Vis (A_{400} and A_{280}) and SEC.

Molecule 1

The dual Big Kahuna system subjected formulations A-C of Molecule 1 to stress conditions similar to the traditional processes described above. The experimental procedures for controlling the Big Kahuna were generated using Unchained Labs' Library Studio software. After the scientist set up the Big Kahuna with starting solutions and consumables, the experimental design was loaded and procedures were performed by the system automatically. Formulations of Molecule 1 in 2 mL serum vials were stressed by stirring at 300 rpm with magnetic stir bars for 24 hours, shaking (vortexing) at 400 rpm for 24 hours and heating at 48 °C for 3 hours. In addition to the study described above, 250 µL of each Molecule 1 formulation was transferred, in triplicate, to a 96-well microtiter plate and then stored at 48 °C for 3 hours in an Inheco incubator physically integrated with the dual Big Kahuna system. Formulations were incubated in a 96-well plate to investigate whether similar results would be obtained between serum vials and the microtiter plate formats. All stressing procedures (magnetic stirring, vortexing, heating and incubation) were performed in parallel on the dual Big Kahuna system.

Molecule 2

All Molecule 2 formulations were prepared in 2 mL serum vials, in triplicate, by manually dispensing the liquids into vials. Formulation B′ was generated by adding an excipient to formulation B. A Junior with solid dispensing capabilities added 2.2–2.5 mg (target was 2.6 mg) of excipient into three vials, then 2 mL of formulation B′ was manually dispensed into each vial. Vials of formulation B′ were mixed by the vortexer module of the dual Big Kahuna system. The purpose of formulation B′ was to test automated powder dispensing and also to investigate the stability of Molecule 2 when dry excipients were added to the formulation. Note that formulation B′ is not part of the traditional formulation assessment, and that in the end B and B′ have the exact same composition.

One set of the Molecule 2 formulations (A–C and B′) remained unstressed, T_0, while the remaining vials were exposed to the following stress conditions: stirring at 300 rpm for 4 hours using magnetic stir bars, shaking at 400 rpm for 4 hours and heat stress at 57 °C for 3 hours. Stressing procedures were performed in parallel on the Big Kahuna.

Results

Traditional process

The rank orders for the stability of these formulations were established using data derived from previously-conducted manual experiments including analysis by DLS, UV/Vis (A_{400} and A_{280}) and SEC. For Molecule 1, formulation B was the best performing followed by C and A (B > C > A). For Molecule 2, formulation C was the most stable followed

by B (includes B′), and formulation A was the least stable (C > B > A). These results provided the benchmark for comparison with the automated process.

Automated process: Molecule 1

DLS

Results from DLS measurements (summarized in Tables 1–5) provided insights into the comparative performance for each formulation of Molecule 1. Representative regularization graphs for all formulations of Molecule 1 after stirring stress are shown in Figures 3–5. Peaks with radii between 3–9 nm were assigned as monomers, and all larger peaks were considered aggregates. Intensities of monomer peaks were used to qualitatively assess the amount of monomer in each formulation. Tables 1–5 summarize DLS results for all formulations and conditions studied for Molecule 1. Decreases in percent intensity of monomer provided evidence that protein aggregation had occurred, and these data were used to assess the robustness of protein formulations. Stirring stress was so damaging to the protein that monomer peaks had average intensities between 0% and 4% in every formulation (Table 2). Heating in vials showed small but meaningful differences in the stabilities of all formulations (Table 3). Shaking stress was not optimized and did not permit clear discrimination of the robustness of the formulations (Table 4). Heating in vial or in microplate showed some variations that may be explained by the difference in heat transfer performance (Table 3 and Table 5). Specifically, heat transfer was far more efficient in the 96-well plates when in an Inheco incubator compared to heating vials in a vial rack made of aluminum.

Average monomer radii and percent intensity results indicated formulation B was the most robust in all stress conditions except heat stressing in a 96-well plate using the Inheco incubator. Formulation C was the second most robust formulation in all conditions except for at T_0 and heating in the 96-well plate, where this formulation had effectively the same amount of monomer as formulation B. Formulation A had the lowest monomer content in all formulations and conditions, except heating in 96-well plates. However, after heating in the 96-well plate, formulation A showed a small

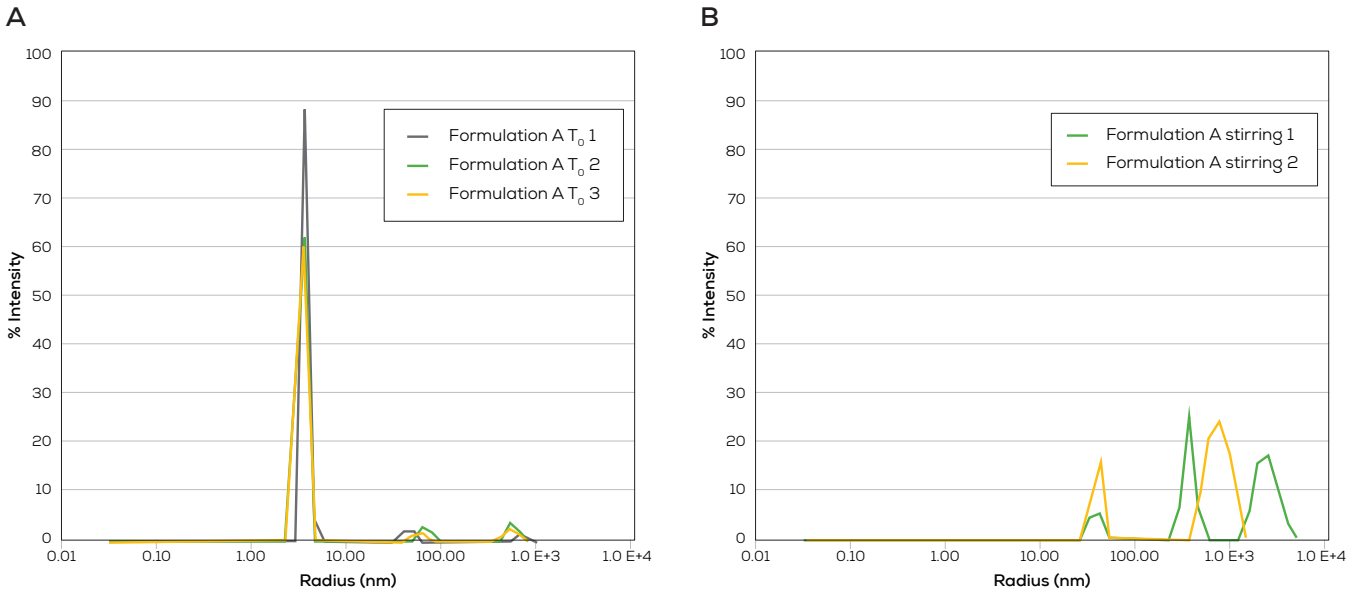

Figure 3: (**A**) Regularization chart of DLS results for Molecule 1 formulation A at T_0 with is shown. (**B**) Regularization graph is presented for Molecule 1 formulation A after stirring stress. One replicate was not stirred as intended and was removed from the analysis.

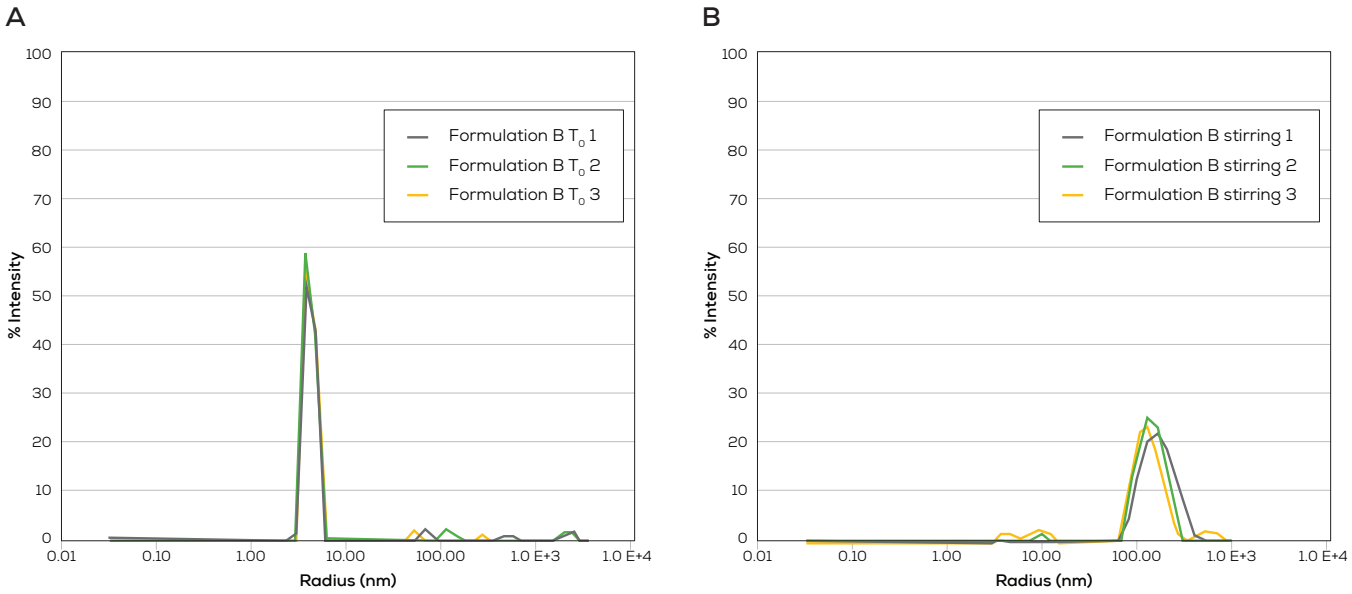

Figure 4: (**A**) Regularization chart of DLS results for Molecule 1 formulation B at T_0 is presented. (**B**) Regularization graph is shown for Molecule 1 formulation B after stirring stress.

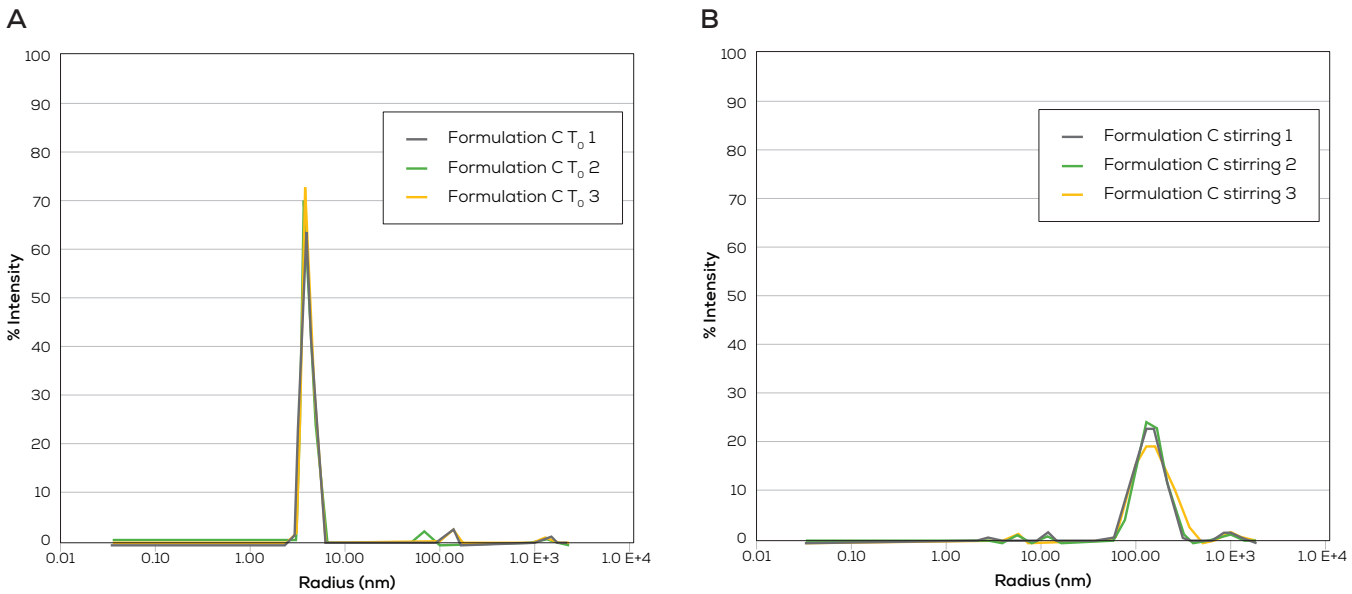

Figure 5: (**A**) Regularization chart of DLS results for Molecule 1 formulation C at T_0 is presented. (**B**) Regularization graph is shown for Molecule 1 formulation C after stirring stress.

Formulation	Preparation	Molecule 1 T_0			
		Monomer radius (nm)	Average monomer radius (nm)	Monomer intensity (%)	Average monomer intensity (%)
A	1	3.6	3.4	91.6	92.0
	2	3.3		91.6	
	3	3.4		92.7	
B	1	4.2	4.1	96.0	95.7
	2	4.1		94.2	
	3	4.2		96.9	
C	1	4.0	4.1	96.6	96.7
	2	4.2		97.5	
	3	4.0		96.0	

Table 1: Summary of DLS results for Molecule 1 at T_0.

Formulation	Preparation	Molecule 1 stirring			
		Monomer radius (nm)	Average monomer radius (nm)	Monomer intensity (%)	Average monomer intensity (%)
A	1	NA	NA	NA	NA
	2	NA		NA	
	3	NA		NA	
B	1	4.0	5.5	0.3	3.6
	2	5.2		1.3	
	3	7.2		9.1	
C	1	8.5	7.2	2.2	2.2
	2	7.4		2.5	
	3	5.7		1.9	

Table 2: Summary of DLS results for Molecule 1 after stirring stress. NA: not applicable; no monomer detected.

Formulation	Preparation	Molecule 1 heat stress in vial			
		Monomer radius (nm)	Average monomer radius (nm)	Monomer intensity (%)	Average monomer intensity (%)
A	1	3.9	4.1	94.6	93.7
	2	3.8		89.1	
	3	4.6		97.4	
B	1	6.1	7.7	97.3	98.6
	2	8.6		99.2	
	3	8.4		99.3	
C	1	4.3	4.5	96.8	96.2
	2	4.4		95.4	
	3	4.6		1.9	

Table 3: Summary of DLS results for Molecule 1 after heat stressing in vials.

Formulation	Preparation	Molecule 1 shaking			
		Monomer radius (nm)	Average monomer radius (nm)	Monomer intensity (%)	Average monomer intensity (%)
A	1	4.3	4.5	91.9	93.6
	2	4.7		96.0	
	3	4.4		92.9	
B	1	9.1	8.6	100.0	98.2
	2	7.3		96.3	
	3	9.5		98.4	
C	1	8.6	7.6	99.3	94.6
	2	8.2		100.0	
	3	5.9		84.6	

Table 4: Summary of DLS results for Molecule 1 after shaking stress in vials.

Formulation	Preparation	Molecule 1 heat 96-well plate			
		Monomer radius (nm)	Average monomer radius (nm)	Monomer intensity (%)	Average monomer intensity (%)
A	1	2.7*	7.0	0.4*	96.5
	2	8.5		96.3	
	3	5.5		96.7	
B	1	5.1	6.5	76.9	85.2
	2	6.9		84.3	
	3	7.5		94.4	
C	1	5.6	5.9	97.0	96.8
	2	6.6		100.0	
	3	5.5		93.3	

Table 5: Summary of DLS results for Molecule 1 after heat stressing in a 96-well plate using Inheco incubator.
*: Outlier data were omitted from averaged results.

increase in main peak radius compared to the other formulations. Taken together, the DLS results for Molecule 1 suggest that formulation B was the most robust followed by formulations C and then A (B > C > A).

SEC

Molecule 1 formulations were also evaluated by SEC. The monomer and aggregate peak area % are shown in Figures 6–7. The low molecule weight peak data is not shown. Monomer peak area percentages for Molecule 1 (Figure 6) show that formulation B best stabilized the protein across all stress conditions. Formulation B also had the least aggregation followed by formulations C and then A (Figure 7) (B > C > A).

UV/Vis (A_{400} and A_{280})

All formulations and conditions for Molecule 1 showed no significant changes in protein content or turbidity as measured by absorbance at 280 nm and 400 nm, respectively (data not shown).

Automated enhanced visual inspection

Results from automated enhanced visual inspection including color, turbidity and visible particle counting indicated no significant changes across the formulations stressed by heat and agitation for Molecule 1 (data not shown). Automated visual inspection was not performed on stirring stress samples due to the presence of stir bars in the vials.

Combined analysis and assessment for Molecule 1

Results from DLS, SEC and absorbance at 400 nm indicated that the most robust formulation for Molecule 1 was B followed by formulation C, and finally the least stable formulation was A (B > C > A).

Automated process: Molecule 2

DLS

Results from DLS (Tables 6–9) provided an understanding of the comparative robustness for all formulations of Molecule 2. Representative regularization graphs with peak percent intensity and radii results for all formulations of Molecule 2 after heat stressing are shown in Figures 8–11. Peaks with radii of 2-15 nm were considered as monomers. Intensities of the monomer peaks were used to qualitatively assess the amount of monomer in the formulations.Tables 6–9 summarize the various formulations and conditions studied

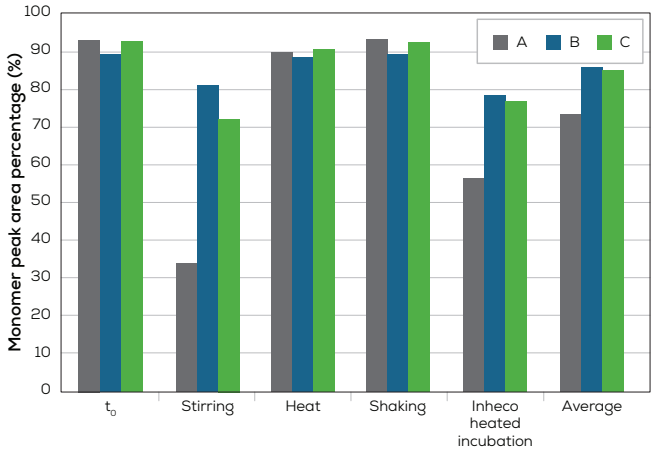

Figure 6: Automated Process Molecule 1 SEC results. SEC results for aggregate peak percentages for all Molecule 1 formulations and conditions.

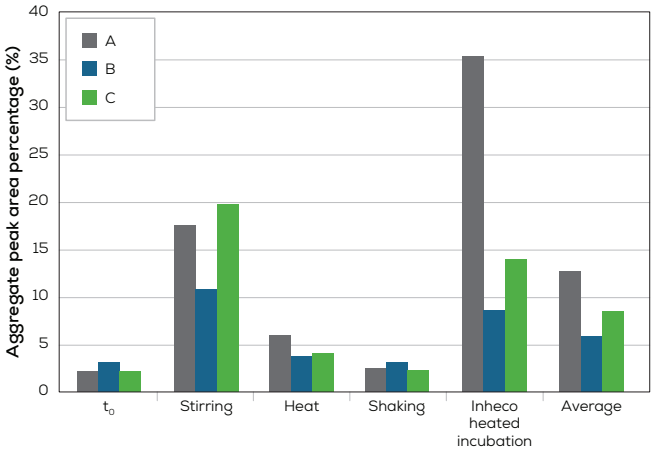

Figure 7: Automated Process Molecule 1 SEC results for aggregate peak percentages for all Molecule 1 formulations.

for Molecule 2. Decreases in monomer percent intensity provided evidence that protein aggregation had occurred, and these data were used to assess the robustness of protein formulations. As expected, at T_0 all formulations had similar average monomer radii and percent intensity results (Table 6). After stirring, formulation C was the most robust, when monomer intensities were averaged for formulations B and B′ (13.2% average monomer intensity) (Table 7). Formulation A was the least stable after stirring stress. Heat stressing in vials clearly showed that formulation A was the least robust and that formulations B and C had very similar stabilities (Table 8). Shaking stress did little to help determine the relative robustness of the formulations (Table 9). Taken together the DLS results for Molecule 1 suggest that formulation C was the most robust, followed by formulations B/B' and then A (C > (B ≈ B′) > A).

SEC

Significant changes in the area percentages for both the main peak and aggregate peak values in formulation A suggest that this formulation was comparatively the least robust (Figure 12). According to the SEC results, formulation C was the most stable, and formulations B and B′ were nearly equal in robustness (C > (B ≈ B′) > A).

UV/Vis (A_{400} and A_{280})

Absorbance values at 400 nm added to the understanding of comparative performances for each formulation (Figure 13). Average A_{400} intensity values were essentially unchanged after heating and shaking stresses; however, stirring stress led to significant increases in A_{400} values for formulations A and B. Increased A_{400} values suggested formation of particles and/or aggregates in formulations A and B and suggest that these formulations are less stable than C and B′. Overall, the A_{400} data indicate that formulation B′ is the most stable, followed by C, B and A (B′ > C > B > A).

Automated enhanced visual inspection

No significant changes in enhanced visual inspection (turbidity, color and particles) were observed in all Molecule 2 formulations (A–C, B′) stressed by heat and agitation (data not shown). Automated

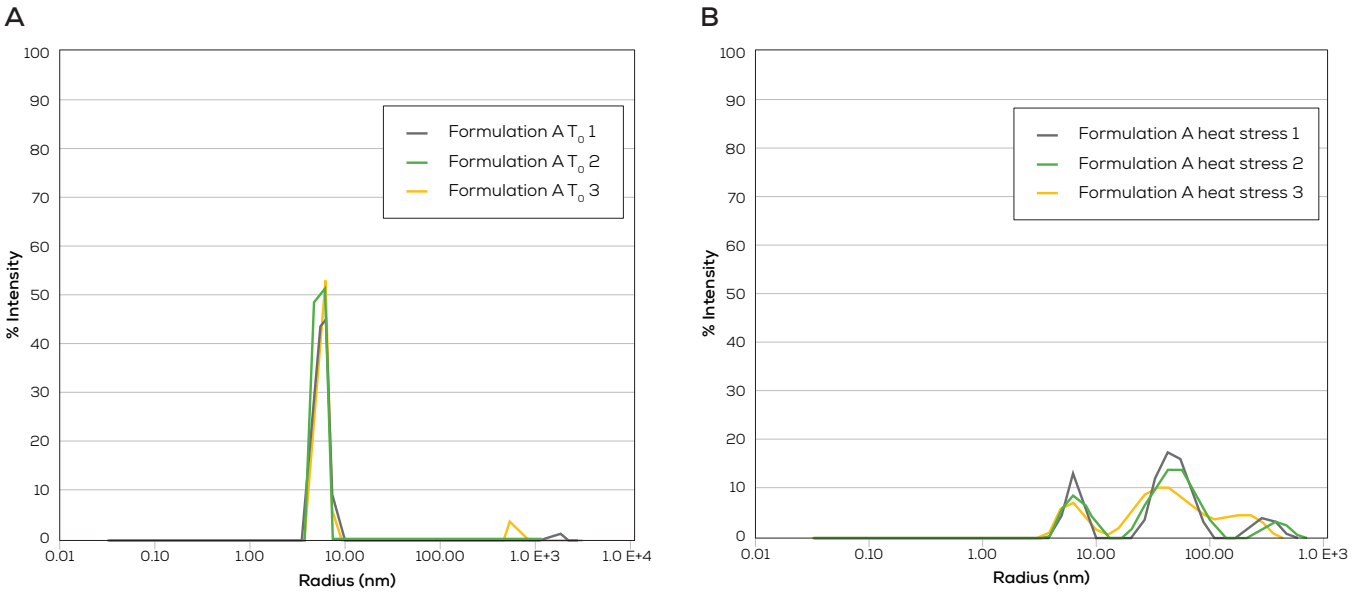

Figure 8: (**A**) Regularization chart of DLS results for Molecule 2 formulation B at T_0 is presented. (**B**) Regularization plot is shown for Molecule 2 formulation A subjected to heat stress in vials.

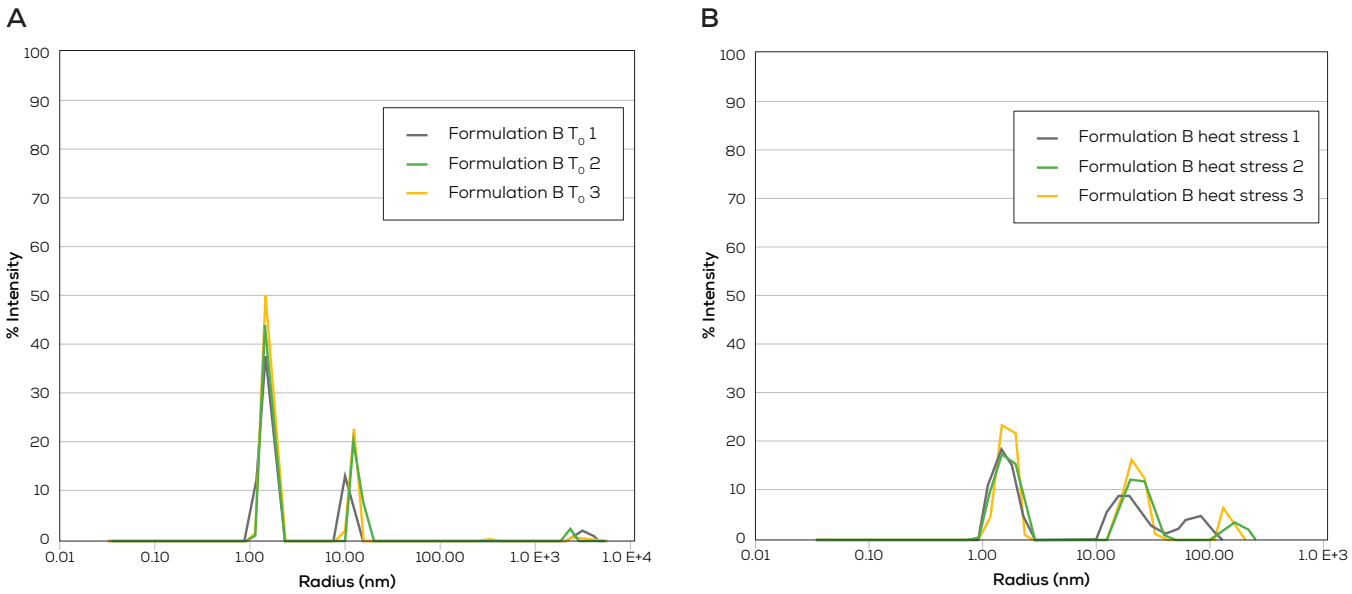

Figure 9: (**A**) Regularization graph for Molecule 2 formulation B at T_0 is presented. (**B**) Regularization graph is shown for Molecule 2 formulation B subjected to heat stress in vials.

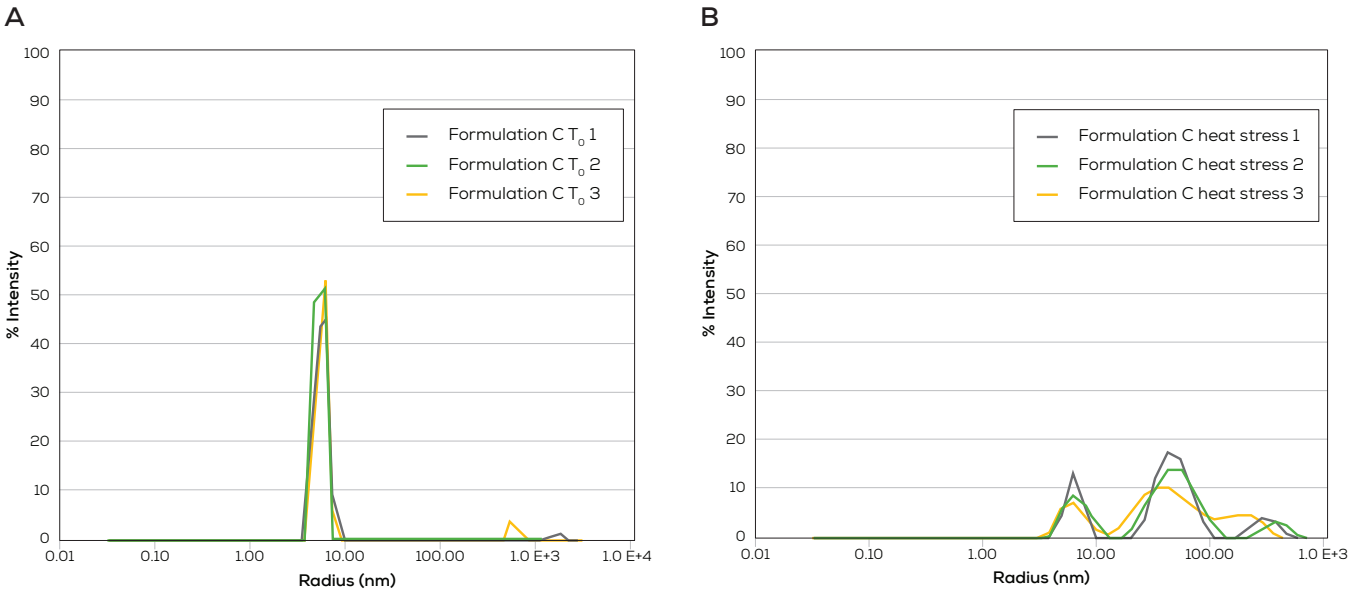

Figure 10: (**A**) Regularization graph for Molecule 2 formulation C at T_0 is presented. (**B**) Regularization graph is shown for Molecule 2 formulation C subjected to heat stress in vials.

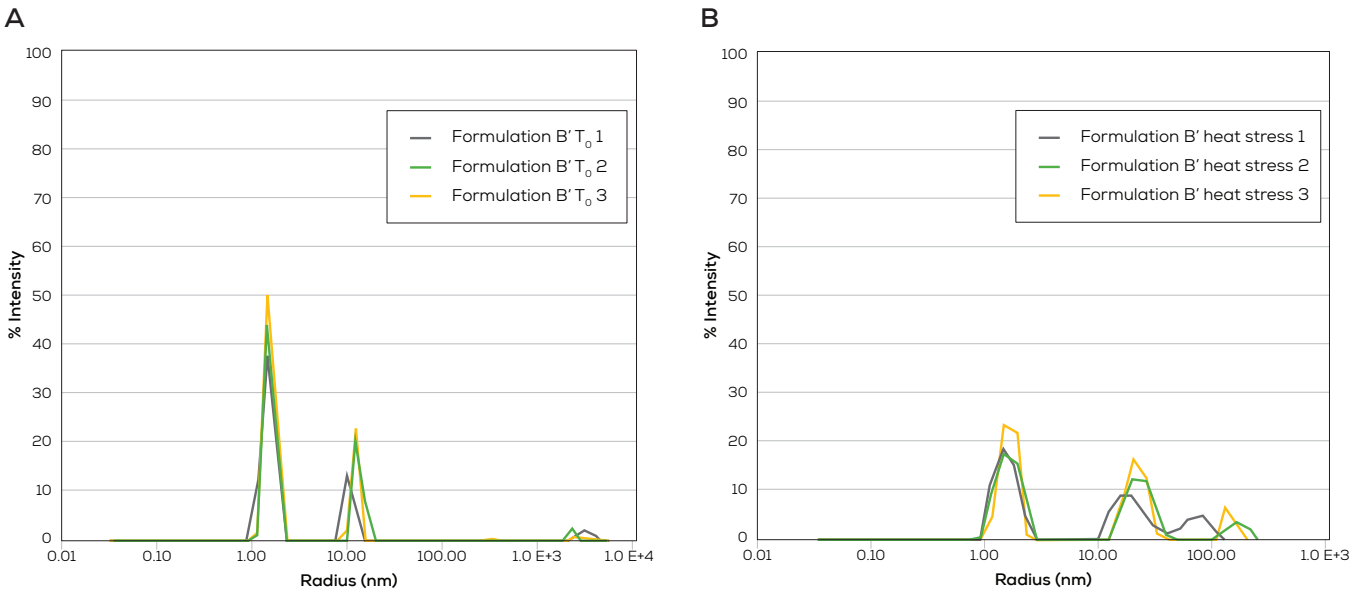

Figure 11: (**A**) Regularization graph for Molecule 2 formulation B′ at T_0 is presented. (**B**) Regularization graph is shown for Molecule 2 formulation B′ subjected to heat stress in vials.

Formulation	Prep	Molecule 2 T_0			
		Monomer radius (nm)	Average monomer radius (nm)	Monomer intensity (%)	Average monomer intensity (%)
A	1	5.8	5.7	96.5	96.6
	2	5.7		96.8	
	3	5.8		96.5	
B	1	6.1	6.7	96.1	97.3
	2	7.4		97.3	
	3	6.8		98.6	
C	1	8.2	7.5	94.8	92.8
	2	7.3		94.7	
	3	7.1		88.9	
B´	1	8.2	7.5	96.6	95.8
	2	7.2		95.3	
	3	7.2		95.4	

Table 6: Summary of DLS results for Molecule 2 at T_0.

Formulation	Prep	Molecule 2 stirring			
		Monomer radius (nm)	Average monomer radius (nm)	Monomer intensity (%)	Average monomer intensity (%)
A	1	4.7	4.7	10.3	10.3
	2	5.3*		71.8*	
	3	5.8*		68.8*	
B	1	NA*	6.9	NA*	7.3
	2	6.9		5.4	
	3	7.0		9.2	
C	1	8.6*	8.2	67.3*	15.1
	2	NA*		NA*	
	3	8.2		15.1	
B´	1	NA*	10.2	NA*	19.0
	2	9.6*		80.2*	
	3	10.2		19.0	

Table 7: Summary of DLS results for Molecule 2 after stirring stress. NA: data not acquired.
*: samples not stirred as intended; DLS data were removed from averaged results.

Formulation	Prep	Molecule 2 heat stress in vials			
		Monomer radius (nm)	Average monomer radius (nm)	Monomer intensity (%)	Average monomer intensity (%)
A	1	6.3	6.4	25.4	23.9
	2	6.5		24.7	
	3	6.3		21.5	
B	1	1.6	1.6	NA	51.3
	2	1.6		51.5	
	3	1.6		51.7	
C	1	1.7	1.8	57.7	57.2
	2	1.8		57.5	
	3	1.8		56.3	
B´	1	1.7	1.7	52.0	52.7
	2	1.7		52.6	
	3	1.7		53.4	

Table 8: Summary of DLS results for Molecule 2 after heat stressing in vials.

Formulation	Prep	Molecule 2 shaking			
		Monomer radius (nm)	Average monomer radius (nm)	Monomer intensity (%)	Average monomer intensity (%)
A	1	5.4	5.4	95.5	97.1
	2	5.3		100.0	
	3	5.4		95.7	
B	1	7.2	7.1	94.0	93.5
	2	7.7		95.2	
	3	6.4		91.4	
C	1	8.3	7.8	95.2	94.0
	2	7.1		93.3	
	3	7.9		93.5	
B´	1	9.1	9.4	96.4	95.5
	2	9.2		95.3	
	3	9.9		94.8	

Table 9: Summary of DLS results for Molecule 2 after shaking stress in vials.

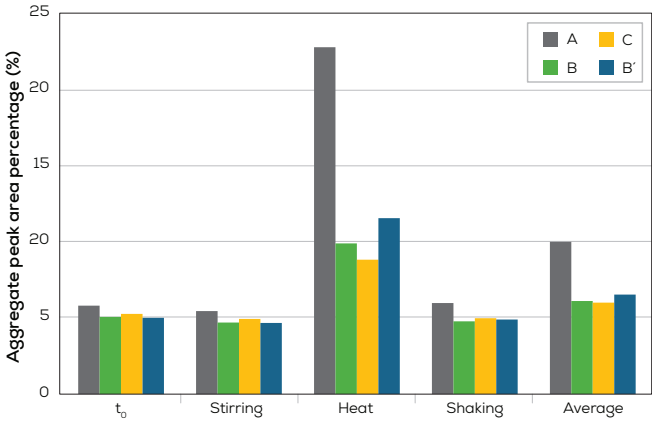

Figure 12: Automated process Molecule 2 SEC results. SEC results for aggregate peak percentages for all Molecule 2 formulations and conditions.

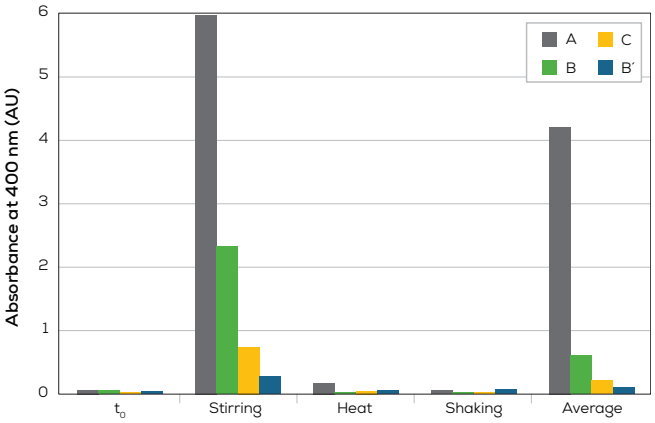

Figure 13: Automated process Molecule 2 turbidity result. Turbidity (A_{400}) values for all formulations and conditions for Molecule 2. Absorbances greater than 6 absorbance units (AU) are not shown.

	Molecule 1		Molecule 2	
	Traditional process	Automated process	Traditional process	Automated process
Most stable	B	B	C	C
↓	C	C	B, B′	B, B′
Least stable	A	A	A	A

Table 10: Summary of rank order comparison of formulation stability evaluated by both the traditional process and an automated process. The automated process provides the same results as the traditional manual process.

visual inspection was not performed on stirring stress samples due to the presence of stir bars in the vials.

Combined analysis and assessment

Results from A_{400}, DLS and SEC all indicated that formulation A was the least robust. Taken together, the other analytical results suggested that the rank order of the formulations was as follows from most to least robust: C, B≈B′ and then A (C › (B ≈ B′) › A).

Conclusion

Rank order of formulation stability

Table 10 shows the rank order of stability for all formulations for both manual and automated processes. The results demonstrate a high-throughput workflow can provide the same formulation rank orders as the manual workflow and procedures performed at LFB. For both automated and traditional processes, the rank order of Molecule 1 formulations from most to least stable was B, C and A. Results from the Molecule 2 study show a rank order from most to least stable: C, B≈B′, A . This study demonstrates that the Big Kahuna platform and automated high-throughput procedures can generate comparable results to traditional manual methods.

Comparability of formulation stability in 96-well plates to previous results

In this study, automation was used to prepare and stress formulations in both a final container/closure system and 96-well microplates. Testing formulation robustness in 96-well plates has tremendous advantages by reducing materi-

al consumption while increasing the number of formulations that can be screened in parallel. The SEC results from heat stressing Molecule 1 in a 96-well microtiter plate show comparability to final container/closures and traditional results (Figures 6–7). These results support the justification of using the microtiter plate in forced degradation studies of some protein formulations. Using a 96-well plate format on the Big Kahuna system enables scientists to screen more than 300 formulations in a single day.

Benefits of automation

Fully integrated automation, including both hardware and data integration for formulation studies can increase lab productivity and efficiency by eliminating manual, tedious steps such as formulation preparation, sample stressing and sample preparation for SEC and DLS while simplifying data analysis and compilation. Automation also provides an opportunity for scientists to focus on higher value activities including experimental design, evaluation of new and novel formulations, interpreting results and more extensive biophysical characterization.

This study proved to LFB that the automated system could provide comparable results while enabling time and material savings. The majority of time savings were realized from sample preparation, such as dilutions and also in regards to data compilation. LFB also expects to see improvement in reproducibility with an automated robotic system when compared to the manual process, specifically in regards to the visual inspection analysis and sample preparation. With a simple and logical design space, LFB scientists found the LEA software and specifically, Library Studio, easy to use. While system handling requires expertise, it shows tremendous promise for future use at LFB and other biopharmaceutical companies.

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