Speed up and simplify formulation screens with Big Tuna and Stunner

Introduction

Developability and formulation screening of biologics is labor-intensive and time-consuming. Buffer exchange is part of the problem because conventional methods are prone to inconsistency, and difficult to manage in larger numbers. Many workflows use multiple tools to determine protein concentration, sample quality, and stability, which takes up more time and more sample. Analytical tools with too high sample volume requirements make problems with buffer exchange worse. The best way to get out of this cycle is with versatile, high-throughput, hands-off techniques to speed up and simplify formulation and developability screens.

Automated buffer exchange systems can enable more uniform sample handling and degrees of process control that are impractical and inaccessible by manual methods. Big Tuna is a hands-free, low volume, high-throughput buffer exchange system that's perfect for biomolecules (Figure 1A). Buffer exchange with Big Tuna is highly customizable and adaptable, allowing for buffer exchange of up to 96 or 24 unique proteins and formulations in a single experiment, depending on the plate. Wells in the Unfilter 96 plates can process up to 96 samples ranging from 100-450 µL; Unfilter 24 plates can process 24 samples ranging from 0.45-8 mL per well. Each plate's design has several options of molecular weight cut-off (MWCO) membrane filters so it's no problem finding the right one for any situation.

Big Tuna's pressure-based ultrafiltration/diafiltration (UF/DF) method removes buffer while gently mixing samples, ensuring that protein cannot accumulate at the membrane surface. This also keeps flow more uniform and faster than dead-end filtration methods. Big Tuna automates the buffer exchange process, reduces hands-on time and increases throughput. Big Tuna also can add or remove buffer from a sample to set the sample concentration to a new target after the exchange is complete.

A

B

Figure 1: Big Tuna (**A**) automates buffer exchange for up to 96 unique samples. Stunner (**B**) is the only plate-based system that pulls together UV/Vis and DLS data on the same 2 µL sample.

UV/Vis absorbance is a classic way of determining protein concentration in a solution or % recovery from a process, like buffer exchange. Dynamic light scattering (DLS) is the quintessential method for non-destructively determining if a protein has aggregated. Stunner takes sample quant and quality one step further

by combining UV/Vis with DLS to give you protein concentration, size, and polydispersity of a single 2 µL sample on 1 instrument (Figure 1B). Just like with Big Tuna, Stunner uses a 96-well format plate which makes automation and high-throughput screening a breeze. All it takes is about a minute per sample without the need for dilutions, standards, or dyes.

DLS and UV/Vis combined can be a great starting point for stability studies. DLS results from a dilution series of a protein let you determine the diffusion interaction parameter (k_D). Static light scattering (SLS), which can be collected alongside DLS, gets you the second virial coefficient (B_{22}). These metrics are helpful for determining aggregation propensity - large positive values indicate that a protein is unlikely to aggregate in a formulation as the concentration of the protein increases. B_{22} and k_D can be determined at concentrations typical of early discovery stages, about 10 mg/mL, but indicate how a protein is likely to behave as it moves through the development pipeline and into manufacturing. That way you can spot potential issues before they become serious problems. Stunner's combination of UV/Vis and DLS on the same sample makes measuring B_{22} and k_D fast and easy.

In this application note, we used Big Tuna to screen 4 monoclonal antibodies (mAbs) in 10 mM histidine, pH 6.0, with 0.001% polysorbate 80 (PS80) and 4 common excipient combinations. Big Tuna monitored the buffer exchanges and ensured that no solutions dried out and everything reached its final concentration and exchange target. Stunner was used to determine % recovery, size, polydispersity, B_{22}, and k_D of each of the formulations.

Methods

Monoclonal antibody 1 (mAb1), monoclonal antibody 2 (mAb2) and adalimumab at stock concentrations of 26 mg/mL were diluted to 10 mg/mL and centrifuged at 14,000 x g. The supernatant was filtered through a 0.1 µm syringe filter. 400 µL of each antibody were added to a 10 kDa MWCO regenerated cellulose Unfilter 96 plate and buffer exchanged, in triplicate wells, into 10 mM histidine buffer, pH 6 with 0.001% polysorbate 80 (PS80) and either 0.9% NaCl, 80 mg/mL sucrose, 10 mg/mL arginine, or a combination of 80 mg/mL sucrose and 10 mg/mL arginine with Big Tuna (Figure 2). The samples were then concentrated to 100 mg/mL. 200 µL of trastuzumab at an initial concentration of 8.5 mg/mL were exchanged into the same formulations in single wells, then concentrated to 10 mg/mL. All buffer exchange and concentration steps were performed using the default parameters for protein solutions between 0.5–50 mg/mL. Initial and final protein concentrations, size, and PDI were checked in quadruplicate on Stunner with buffer blanks.

Dilution series of all 4 antibodies in each of the 4 formulations were generated from 2–10 mg/mL. The B_{22} & k_D app on Stunner was used on 2 µL of each concentration in quadruplicate to determine the B_{22} and k_D with buffer blanking of the 16 samples using default DLS acquisitions settings and E1%s of 14.7, 14.4, 16.4, and 15 for mAb1, mAb2, adalimumab, and trastuzumab, respectively.

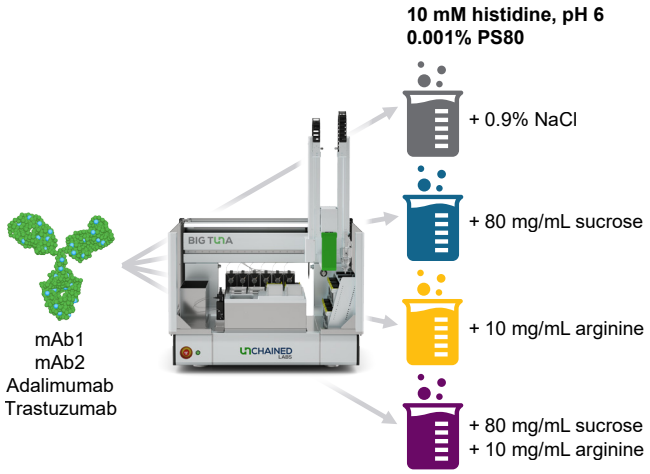


Figure 2: 4 monoclonal antibodies (mAb1, mAb2, adalimumab and trastuzumab) were exchanged into 10 mM histidine buffer, pH 6 with 0.001% PS80 and either 0.9% NaCl, 80 mg/mL sucrose, 10 mg/mL arginine, or a combination of 80 mg/mL sucrose and 10 mg/mL arginine.

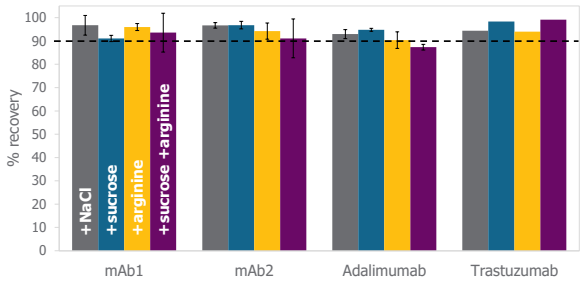
Results

Manual buffer exchange and concentration takes a lot of hands-on time and often has low recovery, even in the relatively small screen depicted here of 4 antibodies and 4 excipients. Big Tuna's buffer exchange was able to achieve better than 90% recovery for 15 of the 16 formulations evaluated in this screen (Figure 3A). There were no obvious trends to the % recovery amongst the samples and all the samples reached the target % exchange and concentration. However, recovery is only part of the story. As in every mAb processing step, quality matters as much as quantity.

DLS is a quick way to check and see if a particular process or buffer causes a mAb to aggregate. A non-aggregated mAb has a hydrodynamic diameter of 10–12 nm and a polydispersity index (PDI) ≤ 0.1. Stunner combines DLS with UV/Vis quantification to give a fast check on the overall quality of proteins in any formulations. All the tested mAbs in NaCl and arginine-alone formulations had diameters and PDIs that were consistent with monodisperse proteins (Figure 3B). Adalimumab in the sucrose-containing formulation had a diameter of 71 nm with a PDI of 0.14, both higher than is normal for a monodispersed mAb. All 3 of the buffer exchanges showed similar results, indicating that adalimumab aggregated in the combination of histidine with sucrose. The other mAbs in sucrose had PDIs exceeding 0.1, but their hydrodynamic diameters were as expected of non-aggregated proteins.

DLS can also be used for distribution analyses to show the different populations of particles in a solution. These intensity distributions can be used to help determine why a sample has a larger z-average or higher PDI. The peak at 10 nm in the distributions from mAb1 in NaCl and sucrose correspond to the non-aggregated antibody, but the smaller peak in the sucrose formulation at 0.8 nm is most likely due to the sucrose itself (Figure 4).[1] The other mAbs in sucrose-containing formulations had similar intensity distributions. Most of the buffer exchanges performed by Big Tuna resulted in mAb solutions with the desired concentrations

A



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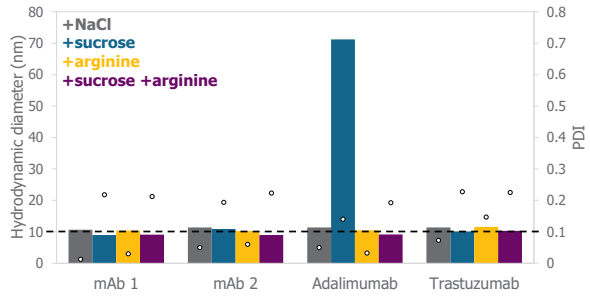

Figure 3: Average percent recovery of 3 wells of each combination of mAb and formulation (**A**), based on Stunner's initial and final concentration measurements, with dashed line indicting a 90% recovery acceptance criterion. Hydrodynamic diameter (bars, left y-axis) and PDI (dots, right y-axis) of 16 mAb formulations (**B**) from Stunner DLS with a dashed line at 10 nm diameter and 0.1 PDI. Error bars are 1 standard deviation.

and no significant aggregation. The positive pressure UF/DF and gentle orbital mixing used by Big Tuna was well-tolerated, so long as the mAb was compatible with the final formulation.

When you combine DLS data taken at different protein concentrations in the same formulation, something magical happens! It indicates the likelihood of aggregation of a protein in a formulation when you increase its concentration. That's useful for when you transition from the lower protein concentrations typical of early research and discovery stages to higher concentrations that are more common in manufacturing and delivery. Some proteins are prone to aggregation at high concentration even if they're stable at low concentrations, so testing this behavior early, for example in pre-formulation screening, can eliminate a problem before it worsens.

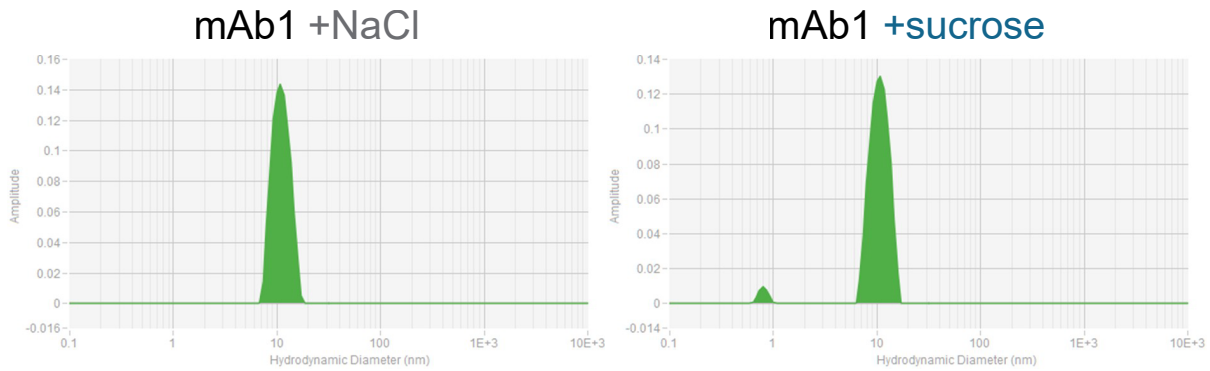

Figure 4: DLS intensity distributions of mAb1 in 10 mM histidine, pH 6, with 0.001% PS80 and either 0.9% NaCl (left) or 80 mg/mL sucrose (right).

Attractive intermolecular forces make protein molecules diffuse more slowly, as concentration increases, while repulsive forces make them diffuse faster. Proteins molecules that attract each other are more likely to aggregate and those that repel each other, less likely.[2] Formulation components, for example salts, sugars, and buffers have significant impact on these protein-protein interactions. The relationship between concentration and diffusion can be expressed as k_D. A positive k_D indicates repulsive intermolecular forces and low aggregation propensity, negative indicates the opposite. DLS measures diffusion, and since Stunner combines DLS with concentration measurements by UV absorbance, it's the simplest way to find k_D.

The B_{22}, or self-interaction parameter, of a protein is a thermodynamic measure of protein self-associations in solution.[3] It's determined from the linear regression of light scattering versus concentration, known as a Debye plot. Positive values of B_{22} indicate repulsion and negative values indicate attraction, just like with k_D. The main difference between the two parameters lie in how they're measured: B_{22} is based on static light scattering and k_D on DLS. However, Stunner can determine both in the same experiment.

MAb1 in 10 mM histidine, pH 6 with 0.001% PS80 and sucrose had a positive correlation between its concentration and diffusion coefficients and a positive slope in its Debye plot (Figure 5). The slopes were either negative or near-zero for all other tested formulations. The positive slope with sucrose indicates that mAb1 will be least prone to aggregation and most stable in a formulation which contains sucrose but not NaCl or arginine.

Screening dilution series of the other mAb formulations shows different effects of the excipients. MAb2 had the highest B_{22} and k_D values with a combination of sucrose and arginine while trastuzumab had the highest values with sucrose alone (Table 1). Adalimumab had negative k_D and B_{22} in all the tested formulations. This indicates none of the tested excipients would stabilize adalimumab at higher protein concentrations. Efficient screening of protein formulations based on colloidal stability is key to reducing risks and getting the best candidates through the development pipeline.

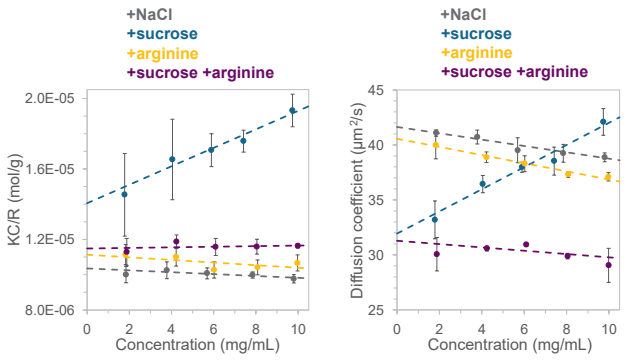

Figure 5: Debye plot (left) and diffusion coefficient vs. concentration (right) of dilution series of mAb1 in 10 mM histidine, pH 6, with 0.001% PS80 and either 0.9% NaCl, 80 mg/mL sucrose, 10 mg/mL arginine, or 80 mg/mL sucrose and 10 mg/mL arginine, measured in quadruplicate with linear regression (dashed lines). Error bars are 1 standard deviation.

mAb	Excipients	B_{22} (mL x mol / g^2)	k_D (mL/g^2)
mAb1	+NaCl	-2.7e-5	-6.9
	+ sucrose	2.6e-4	32
	+arginine	-3.7e-5	-9.1
	+ sucrose + arginine	8.0e-6	-4.8
mAb2	+NaCl	-2.6e-5	-12
	+ sucrose	-3.3e-5	-18
	+arginine	9.0e-5	4.5
	+ sucrose + arginine	1.2e-4	6.4
Adalimumab	+NaCl	-2.9e-5	-14
	+ sucrose	n.d.	n.d.
	+arginine	-9.5e-6	-13.8
	+ sucrose + arginine	-2.3e-5	-8.6
Trastuzumab	+NaCl	2.3e-6	-5
	+ sucrose	3.0e-4	22
	+arginine	2.8e-5	-10.7
	+ sucrose + arginine	2.5e-5	-13

Table 1: Second virial coefficient (B_{22}) and diffusion interaction parameter (k_D) of mAb1, mAb2, adalimumab, and trastuzumab in in 10 mM histidine, pH 6, with 0.001% PS80 and either 0.9% NaCl, 80 mg/mL sucrose, 10 mg/mL arginine, or 80 mg/mL sucrose and 10 mg/mL arginine. The largest positive B_{22} and k_D for each antibody is highlighted in green.

Conclusion

Finishing high-throughput formulation screens faster identifies the biologics with the best developability profiles more quickly and helps you discover which combinations of buffers and excipients stabilize them most effectively. Hands-free automated buffer exchange on Big Tuna lets scientists spend less time on tedious sample preparation and more time on more critical work. Stunner has the tools necessary to determine protein concentration, the % recovery from a buffer exchange or other process, spot aggregates, and even dig up aggregation propensity. Combining these solutions into a single workflow simplifies screening of formulations and candidates.

References

1. Measuring sub nanometre sizes using dynamic light scattering. M Kaszuba, et al. Journal of Nanoparticle Research. 2008; 10(5):823–829.
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3. Colloidal Behavior of Proteins: Effects of the Second Virial Coefficient on Solubility, Crystallization and Aggregation of Proteins in Aqueous Solution. W Wilson, et al. Current Pharmaceutical Biotechnology. 2006; 6(6):427–436.

Unchained Labs
4747 Willow Road
Pleasanton, CA 94588
Phone: 1.925.587.9800
Toll-free: 1.800.815.6384
Email: info@unchainedlabs.com

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