Sail through formulation screening on Uncle and Honeybun

Introduction

Monoclonal antibodies (mAbs) and other biologics experience all kinds of stresses and need to be stable in lots of different environments and at a range of concentrations. High concentration preparations introduce additional complexity, often in the form of elevated viscosities and reduced stability. Developability assessments or formulation screening can spot issues early, but only if they use the right tools. While methods like differential scanning calorimetry, size-exclusion chromatography, and traditional viscometry and rheometry are powerful, they demand a lot of time, sample, and expertise to run. Screening, on the other hand, needs high-throughput solutions that use only tiny volumes of your precious mAbs and provide clear insights. Ideally, the same analytical technique can follow a drug all the way from early discovery to manufacturing.

Uncle is the original all-in-one stability platform with full-spectrum fluorescence, static light scattering (SLS), and dynamic light scattering (DLS) to completely profile proteins and gene therapy vectors from 9 µL of sample in any formulation and at any concentration (Figure 1A). Temperature control (15–95 °C) and sealed sample holders make for great flexibility in how that profiling can be performed. Multiple parameters, including fluorescence, aggregation, size, polydispersity, and thermal unfolding, can be assessed from the same sample volume in just one experiment, allowing you to obtain orthogonal and complementary information. Uncle can measure up to 48 samples at a time, enabling quick access to a wide range of results when doing high-throughput characterizations in early discovery, process development, or manufacturing.

A

B

Figure 1: Uncle (**A**) is the original all-in-one biologics stability platform. Honeybun (**B**) is the only rapid microvolume viscometer and rheometer that uses no more than 35 µL of sample and can read up to 10 samples in minutes.

Viscosity is a critical parameter at every stage of the development pipeline. In discovery and early development, a high viscosity indicates the protein molecules interact either with each other or with solvents and excipients. As a biologic moves into later development stages, its viscosity and stability impact manufacturability and even safety and delivery to patients. Picking the right

formulation early keeps these issues under control from the beginning to the end of a drug's pipeline.

Despite it being such a useful parameter, it's surprisingly hard to obtain all the viscosity information you need. Classic techniques require hours of hands-on time in super slow, one-sample-at-a-time instruments that use up high volumes of sample. More modern tech uses expensive components that create a one-by-one bottleneck and need lots of cleaning and calibration.

Honeybun (Figure 1B) is the only rapid microvolume viscometer that combines low sample volume requirements (35 µL in default and 15 µL in low volume mode) with the ability to run up to 10 samples in parallel. Using Honeybun is easy. Simply pipette your analyte into the Bun, insert it into Honeybun, and in minutes get your super accurate and precise viscosity results. Honeybun's dynamic range (0.5–150 cP) covers most biological therapeutics, even at high concentrations.

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 (to remove sedimenting aggregates). 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 then buffer exchanged in triplicate 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, using Big Tuna. The samples were then concentrated to 100 mg/mL. In case of trastuzumab, 200 µL 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 using Big Tuna 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. The viscosities of each formulation were determined using Honeybun.

The proteins were diluted to 1, 10, or 100 mg/mL (as applicable) in their respective buffers and each sample was loaded into a Uni (an array of sixteen 9 µL quartz cuvettes, sealed by silicone gaskets) in triplicate. Protein unfolding and aggregation experiments were performed with the 'T_m & T_{agg} with DLS' application on Uncle. Samples were ramped in Uncle from 15–95 °C at a rate of 0.3 °C/minute and excited at 266 and 473 nm while fluorescence emission and SLS were recorded. Uncle Analysis software determined the melting temperature (T_m) from the barycentric mean (BCM) of the fluorescence intensity curves from 300–430 nm and the onset of aggregation temperature (T_{agg}) from the intensity of light scattered at 266 nm. T_ms and T_{agg}s are shown as the average of triplicates. The average hydrodynamic diameter before and after the thermal ramp was measured by DLS, with 4 acquisitions of 5 seconds each.

The viscosities of the protein solutions were determined in triplicate at 100 mg/mL from 35 µL at 20 °C with Honeybun's 'default' mode.

Results

Thermal stability screening accelerates developability testing of proteins and formulations and thus reduces the time it takes to bring novel, life-saving therapies to patients.[1] Key elements in this screening are using intrinsic fluorescence to find T_ms and SLS to show T_{agg}s. In this case study, adding either sucrose or arginine to mAb1 in 10 mM histidine, pH 6, improved its conformational stability, increasing the T_m (relative to the NaCl condition) from 67 °C to 70 °C (Figure 2). However, sucrose and arginine did not have a combinatorial effect as the addition of both together had a similar T_m as either excipient alone.

Even though sucrose increased the T_m of mAb1 relative to NaCl, the antibody had similar T_{agg}s with both excipients at 72.1 °C. Arginine alone had the highest T_{agg} of the tested excipients at 76 °C. However, NaCl and arginine showed much higher overall SLS signal than sucrose alone. Since SLS

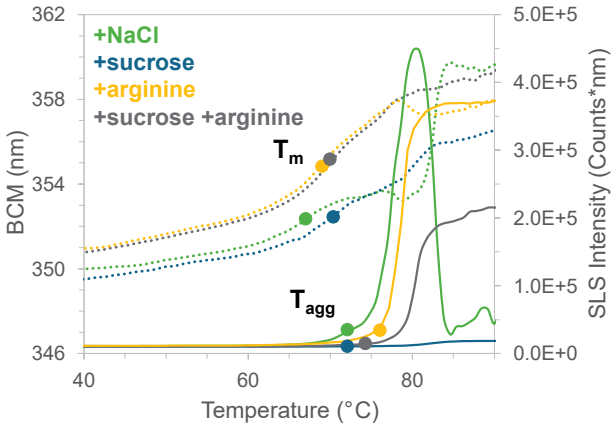

Figure 2: BCM (dotted lines, left y-axis) and SLS (solid lines, right y-axis) of mAb1 in 10 mM histidine buffer, pH 6, with 0.001% PS80 and either 0.9% NaCl (green), 80 mg/mL sucrose (blue), 10 mg/mL arginine (yellow), or 80 mg/mL sucrose and 10 mg/mL arginine (gray). T_ms and T_{agg}s are the average of triplicate wells.

is directly proportional to size and abundance of particles in solution, this indicates that even though aggregation may have begun at slightly lower temperatures with sucrose, it was more extensive in the presence of arginine and NaCl.

DLS is an orthogonal light scattering technique which can determine the sizes of the protein particles with each excipient to confirm the SLS results and additionally help find out about the size and polydispersity of the aggregates after heating. In the sucrose condition, mAb1 had singular peaks at 15 and 95 °C in the DLS intensity distributions with average hydrodynamic diameters of 8 and 17 nm, respectively (Figure 3A). The DLS peak was narrower at 15 than at 95 °C. With the addition of both sucrose and arginine, mAb1 was still monodisperse at 15 °C with an average size of 11 nm. At 95 °C, the distribution of particles shifted to larger sizes such that the hydrodynamic sizes were distributed over a wide range from below 100 to above 1,000 nm. MAb1 with NaCl or arginine alone exhibited much the same DLS behavior (data not shown). Based on the SLS and DLS results, mAb1 was more prone to forming large aggregates during thermal stress in the presence of either arginine or NaCl, compared to sucrose alone.

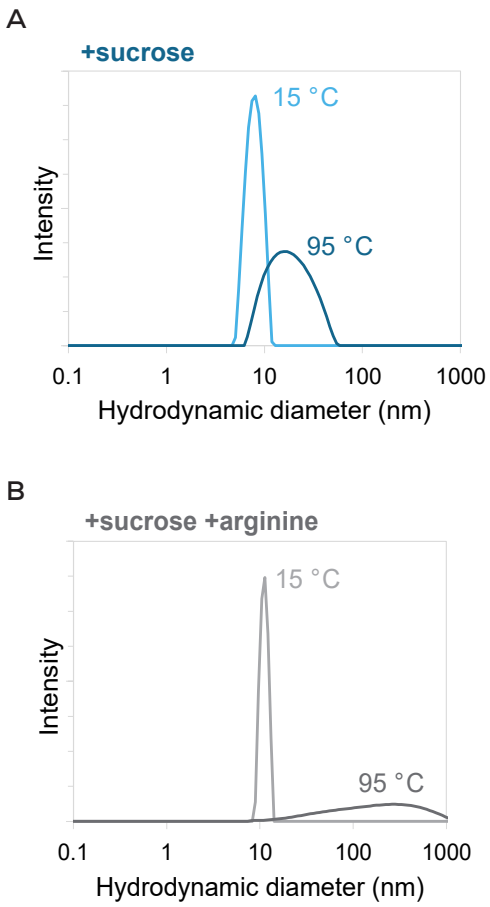

Figure 3: DLS intensity distributions of mAb1 at 15 °C (light) and 95 °C (dark) in 10 mM histidine, pH 6, with 0.001% PS80 and 80 mg/mL sucrose (**A**) or 80 mg/mL sucrose and 10 mg/mL arginine (**B**).

Every aspect of a formulation, from the protein's structure to its concentration to the choice of excipient, can impact stability. However, sometimes different antibodies can have similar conformational and colloidal stability properties. In the example described here, mAb1 and trastuzumab had similar T_ms and T_{agg}s across multiple formulations and concentrations (Figure 4). Adalimumab tended to have lower T_{agg}s but similar T_ms to mAb1 and trastuzumab while mAb2 had lower T_m and T_{agg} overall and is therefore less stable.

Changing a protein's concentration can impact its stability, just like adding excipients. Testing high concentrations requires more sample per volume, which makes it even more important to test these impacts using small volumes of sample. In the set of formulations used here, sucrose had the largest stabilizing effect on mAb1 and trastuzumab at

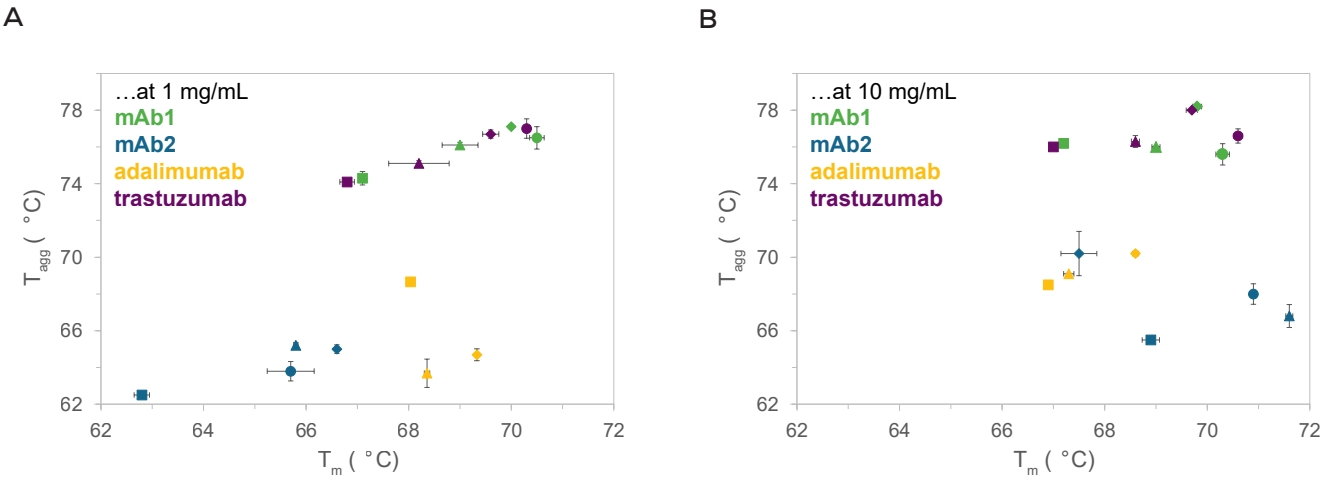

Figure 4: T_m vs. T_{agg} of mAb1 (green), mAb2 (blue), adalimumab (yellow), and trastuzumab (purple) at 1 (**A**) and 10 mg/mL (**B**) in 10 mM histidine buffer, pH 6 with 0.001% PS80 and 0.9% NaCl (squares), 80 mg/mL sucrose (circles), 10 mg/mL arginine (triangles), or a combination of 80 mg/mL sucrose and 10 mg/mL arginine (diamonds). Error bars are 1 standard deviation of triplicates.

Protein	Concentration (mg/mL)	T_{agg} (°C)			
		+ NaCl	+ sucrose	+arginine	+sucrose + arginine
mAb1	100	65.7	73.7	69.3	71.3
mAb2	100	64.6	66.1	68.3	68.9
adalimumab	100	65.7	n. d.	66.4	68.3

Table 1: T_{agg}s of 100 mg/mL mAb1, mAb2 and adalimumab in 10 mM histidine buffer, pH 6 with 0.001% PS80 and 0.9% NaCl, 80 mg/mL sucrose, 10 mg/mL arginine, or 80 mg/mL sucrose and 10 mg/mL arginine. The top performing excipients are highlighted in green for each antibody. The T_{agg} of adalimumab with sucrose was not determined (n. d.) due to its aggregation at room temperature in this condition.

1 and 10 mg/mL while NaCl destabilized mAb1, trastuzumab, and mAb2 (Figure 4A & B). NaCl had a noticeable impact on T_m and T_{agg} of these 3 mAbs at 1 mg/mL, but at 10 mg/mL it had a larger impact on T_m in the case of mAb1 and trastuzumab, while in case of mAb2, its effect was on T_{agg}.

Many mAbs are less stable at higher concentrations.[2] However, this is not always the case. When prepared at 10 mg/mL, mAb2 had better conformational and colloidal stability than it did when prepared at 1 mg/mL in the same formulation, as shown by the higher T_ms and T_{agg}s (Figure 4A vs. 4B). Adalimumab showed increased T_{agg}s under similar conditions. However, this concentration-dependent stabilization did not persist when the proteins were prepared at 100 mg/mL (Table 1).

Denaturation is less of a concern with higher concentration antibodies, for example those prepared for subcutaneous administration, but they tend to aggregate at lower temperatures. The right choice of excipient can greatly reduce this risk. Adding sucrose only to 100 mg/mL mAb1 in 10 mM histidine, pH6, with 0.001% PS80 increased its T_{agg}, especially relative to a NaCl spike-in (Table 1). The addition of sucrose and arginine combined delayed heat-induced aggregation of mAb2 and adalimumab at 100 mg/mL and had the largest stabilizing effect. Intrinsic fluorescence and SLS are flexible techniques, applicable to most protein concentrations, buffers and excipients, which

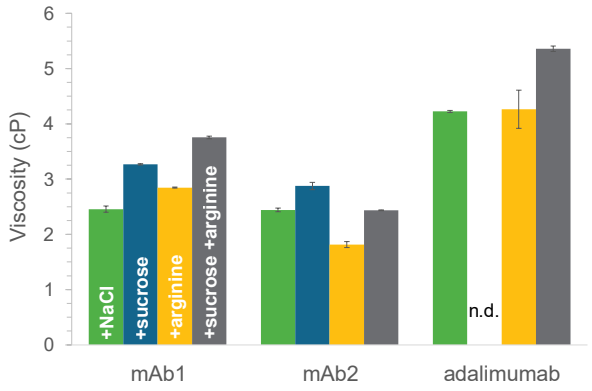

Figure 5: Viscosities of 100 mg/mL mAb1, mAb2 and adalimumab in 10 mM histidine buffer, pH 6 with 0.001% PS80 and 0.9% NaCl (green bars), 80 mg/mL sucrose (blue bars), 10 mg/mL arginine (yellow bars), or 80 mg/mL sucrose and 10 mg/mL arginine (gray bars). Error bars are 1 standard deviation of triplicates.

make it fast and easy to screen the stability of formulations for any application.

Viscosity is another key concern for high concentration mAb therapeutics and has consequences for delivery, developability, and manufacturability.[3,4] Many proteins display a "hockey-stick" pattern to their viscosity as concentration increases. The exact concentration at which the exponential increase begins varies depending on the protein, but the right formulation can reduce it. It's rare for viscosity assessment to be part of early-stage, high-throughput screens because most viscometers are slow, require substantial amounts of sample, or are difficult to use.

Honeybun is an easy-to-use viscometer and rheometer which needs as little as 15 µL of sample to measure up to 10 samples in parallel with no clean-up. Adding viscosity measurements to existing formulation screening workflows with Honeybun couldn't be smoother and it takes only minutes to get results. The viscosities of sucrose-containing formulations of 100 mg/mL mAbs tested here tended to be higher than the salt-only formulations (Figure 5). Arginine can weaken hydrophobic interactions between protein molecules, which makes it a common viscosity-lowering excipient of high concentration mAbs.[5] However, if hydrophobic interactions are not driving increased viscosity, arginine can have the opposite effect. As an example of both, adding arginine and sucrose together to mAb2 decreased its viscosity, relative to sucrose alone, but adding arginine to mAb1 increased its viscosity.

Balancing all the key attributes for a formulation, and doing it quickly, is the goal of formulation screening.

Sucrose as an excipient had the best stability profile for mAb1 and trastuzumab for all the tested protein concentrations when taking into account T_m, T_{agg}, and DLS results (Table 2). Generally, the combination of sucrose and arginine was optimal for mAb2 and adalimumab, but there were concentration-specific top performers as well. Adalimumab performed best with NaCl alone when the protein was at 1 mg/mL while mAb2

Antibody	Top excipients at...			
	1 mg/mL	10 mg/mL	100 mg/mL	Overall
mAb1	+ sucrose	+ sucrose	+ sucrose	+ sucrose
mAb2	+ sucrose + arginine	+ arginine	+ sucrose + arginine	+ sucrose + arginine
adalimumab	+ NaCl	+ sucrose + arginine	+ sucrose + arginine	+ sucrose + arginine
trastuzumab	+ sucrose	+ sucrose	n. d.	+sucrose

Table 2: Top performing excipients for mAb1, mAb2, adalimumab and trastuzumab at 1, 10, and 100 mg/mL, based on thermal stability results (T_m, T_{agg}, and DLS) and overall performance (includes viscosity results).

preferred arginine alone when prepared at 10 mg/mL. All in all, considering the data presented here, sucrose was the clear top performing excipient for mAb1 and trastuzumab while a combination of sucrose and arginine was best for mAb2 and adalimumab.

Conclusion

Optimizing formulations and eliminating potential problems with stability or viscosity as quickly and efficiently as possible, and as early as possible, is the goal for any formulation screen. Flexible, high-throughput, low volume tools make it easier to meet this goal. Intrinsic fluorescence, SLS, and DLS combined give a rich picture of a protein's response to thermal stress, be it denaturation, aggregation, or both. That makes it simple to spot the best excipients or formulations and minimize risks. When optimizing for subcutaneous administration, testing an antibody concentration relevant to this type of administration yields the most applicable data to select a formulation. Combining stability from Uncle with high-throughput viscosity from Honeybun opens up a whole new world of formulation and construct screening.

References

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