

# Rapid viscosity screening on Uncle

## Introduction

High-concentration biologics formulations present numerous challenges for manufacturing and efficacy. Two key challenges are protein aggregation and viscosity. Aggregation risk can be assessed early in characterization and screening by many methods, including interaction parameters  $k_D$  and  $B_{22}$ . For viscosity determinations, traditional viscometers consume large amounts of sample, which usually necessitates later characterization. A higher throughput method with low sample volume requirements would allow earlier assessment of formulation effects, and establish when higher concentrations of proteins start to impact viscosity. The dynamic light scattering (DLS)-based method on Uncle enables you to get around those limitations and get a read on viscosity at an earlier stage in your process.

This application note guides a user through the Viscosity application. Uncle can measure up to 48 samples simultaneously, so you can screen for formulation and concentration effects on viscosity earlier in characterization and development.

Uncle is an all-in-one stability platform that enables 12 different applications with one instrument. Fluorescence, SLS and DLS detection methods are used to characterize protein stability. Temperature control (15–95 °C) and sealed samples provide greater flexibility in how that characterization can be performed. Multiple measurements, such as thermal melting, aggregation and sizing are possible with the same set of samples, so you can thoroughly characterize more biologics and formulations earlier and more easily than before.

## Methods

For experiment development, 100 nm beads (Thermo Fisher #3100A) in PBS or PBST (PBS with 0.025% Tween-80) and 100 mg/mL of BSA

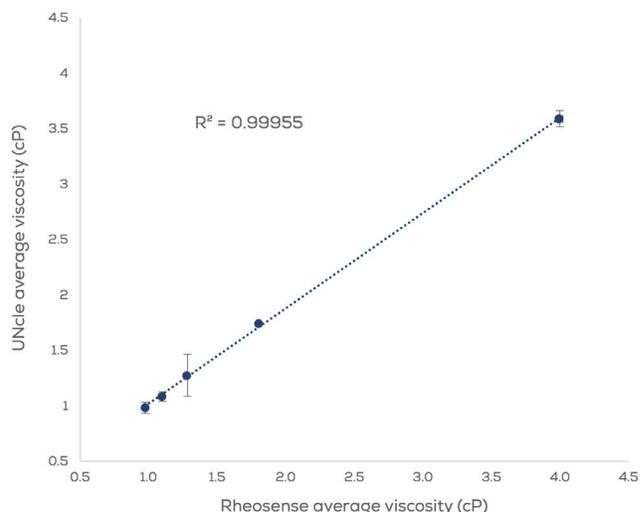


Figure 1: Correlation between the average viscosity measurements from a viscometer and Uncle, for a set of similar samples ranging from 0–300 mg/mL of BSA.

in either PBS or PBST were run in duplicate with the Sizing and Polydispersity application on Uncle, with four DLS acquisitions of 5 seconds each.

For the viscosity experiment, BSA was prepared at 400 mg/mL and dilutions were made to 300, 200, 100 and 50 mg/mL in PBS. Samples were centrifuged for 5 minutes at 14,000 rpm, and 50  $\mu$ L of each were added to 1  $\mu$ L of 1.25% Tween-80 and 1  $\mu$ L of beads and vortexed. Nine  $\mu$ L of each sample were loaded in triplicate in a Uni, and the Viscosity application was run at 24 °C twice, 30 minutes apart.

The default setting for the Viscosity application in the Uncle Analysis Software filters out peaks with a hydrodynamic diameter smaller than 70 nm, so the peak from BSA will not interfere with the automatic calculations. Uncle Analysis Software calculates the viscosity based on the reference bead size measured in buffer alone. For comparison, 300  $\mu$ L of each BSA concentration in PBST was used for triplicate measurements on a Rheosense *micro*VISC™ viscometer at 23 °C.

## Results

The speed at which a particle moves through a solution depends on the viscosity of that solution. If the size of a particle is known, DLS can be used to calculate the viscosity by rearranging the Stokes-Einstein equation:

$$\text{Viscosity} = \frac{kT}{6\pi DR_H}$$

At a given temperature, the diffusion coefficient (D) of a given particle depends only on its size and on the viscosity of the solution. Uncle employs standard polystyrene beads as reference particles with a known size, and uses DLS to measure their 'apparent' size in a protein solution with unknown viscosity. The viscosity of the protein solution can then be easily calculated by comparing the bead measurement in a solution with known viscosity (water or PBS, for example):

$$\text{Viscosity}_{\text{unknown}} = \text{Viscosity}_{\text{reference}} \times \frac{R_{\text{apparent}}}{R_{\text{reference}}}$$

Provided that the size of the beads is significantly larger than the protein molecules in your solution, the light scattering signal from the protein can be filtered out such that it does not interfere with the signal from the large (100 nm) beads.

## Experiment development

Before proceeding with a viscosity experiment, it is important to verify that your samples meet three criteria, by running the Sizing and Polydispersity DLS application and measuring:

- The size of the beads alone: confirms that the beads are monodisperse and establishes the reference size of the bead in buffer.
- The protein alone: confirms that there are no aggregates already present in the protein solution, especially at higher concentrations.

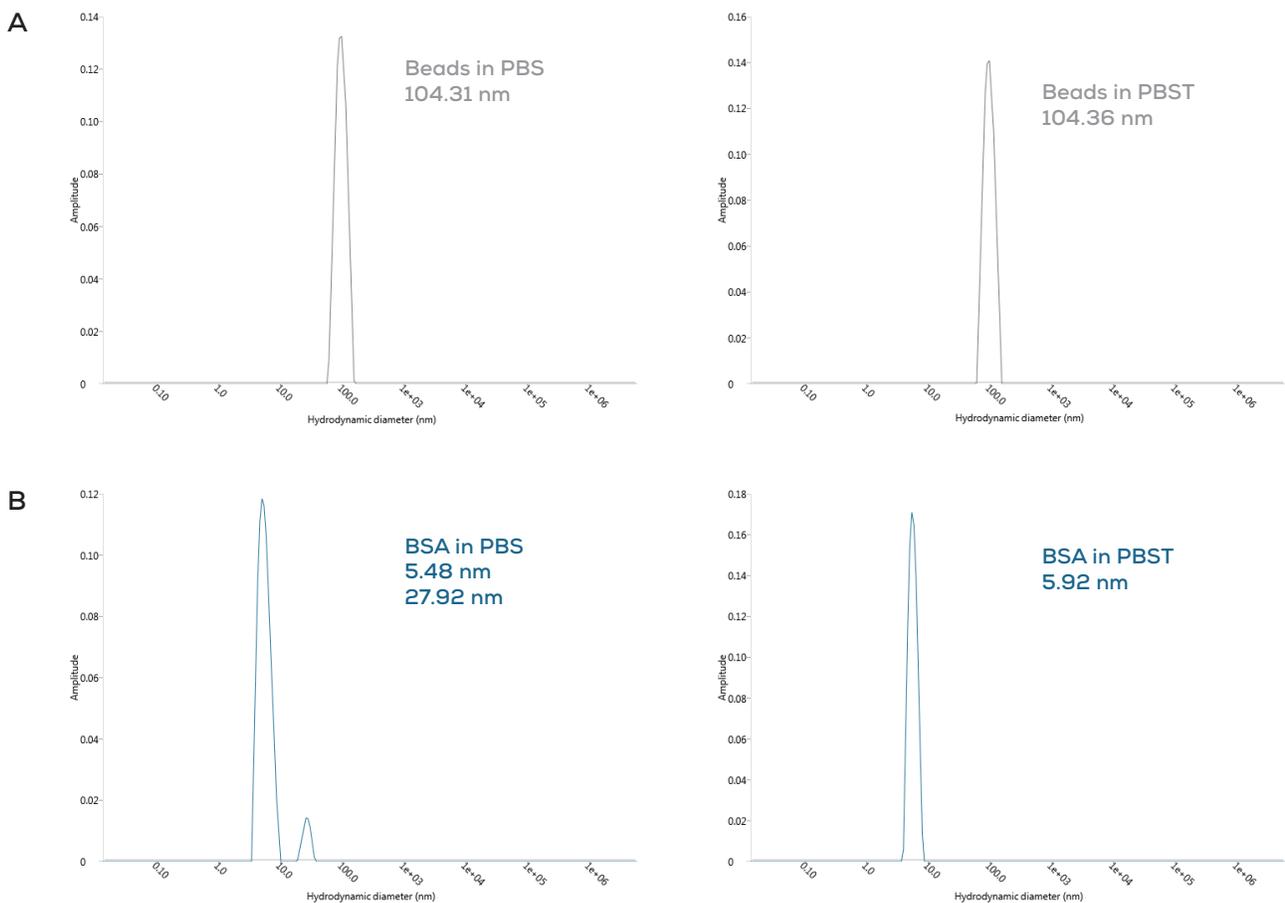


Figure 2: **A:** Intensity distributions from recommended pre-experiment to ensure that beads are not self-associating or aggregating. **B:** Intensity distributions from recommended pre-experiment to find a condition where the protein sample alone does not contain aggregates.

- The protein + bead, two independent measurements: confirms that the bead size, measured at  $t_0$  and again 30 minutes later, does not increase due to the protein binding non-specifically to it. This can be run in a separate experiment, or can simply be run on the Unis for your formulation a second time within the Viscosity application.

In this experiment, the beads in buffer were measured to have a diameter of 104 nm, which is consistent with the certified size of  $102 \pm 3$  nm reported by the manufacturer. The same measurement was obtained when beads were prepared in PBS or PBST, indicating that surfactant was not required for maintaining a monodisperse bead sample (Figure 2A).

To determine if there were aggregates present prior to the viscosity measurement, BSA was measured in buffer at 100 mg/mL in the absence of beads. A small amount of aggregation in PBS was observed, which can be seen as a small peak at 28 nm (Figure 2B, left). If there is a low level of aggregation, it can often be removed by centrifugation or filtration, or by adding a small amount of surfactant to the sample. In this case, the aggregate disappeared in the presence of 0.025% Tween, where only the monomer peak is seen (Figure 2B, right). This indicates that PBST is a better formulation for this protein, and was used for the subsequent viscosity experiment.

As the method follows an increase in bead size to determine viscosity, it is important to ensure that

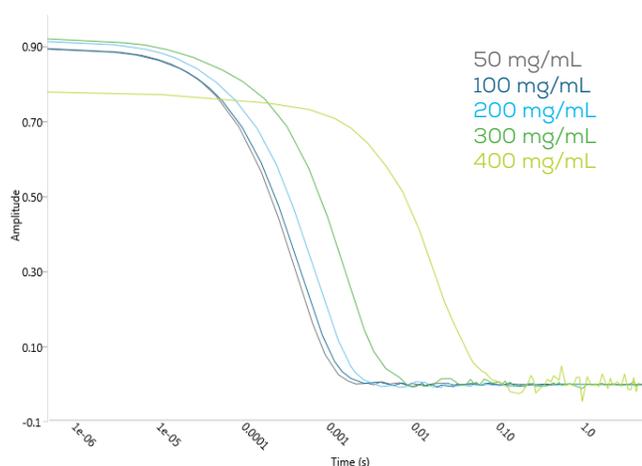


Figure 3: Correlation functions of viscosity determinations for BSA at 50–400 mg/mL in the presence of polystyrene beads.

the protein is not directly sticking to the beads, which would also result in a larger apparent bead size. When these types of protein-bead interactions do occur, they are likely to occur quickly, or within 30 minutes. The first time you run a new combination of beads and protein, we recommend running the Viscosity application immediately upon mixing, and then simply run it again 30 minutes later. If the measured size of the beads does not change significantly between the two time points, you can be confident that the data is valid. In this example, there was no change in the measured size of the beads after 30 minutes in the presence of 100 mg/mL BSA (data not shown), so we proceeded with the viscosity experiment in PBST.

### Viscosity experiment

We ran a concentration series of BSA, from 50–400 mg/mL and measured the viscosity of each solution. The correlation functions, which have been overlaid for each concentration, depict the raw data from this experiment (Figure 3). The correlation functions show similar initial amplitude, with the exception of the 400 mg/mL BSA sample, which also exhibits a noisier baseline. These changes are indicative of a DLS measurement beyond the specification (1,000 nm) and cannot be considered a quantitative sizing measurement.

The resulting overlaid intensity distributions display the calculated size of the bead peak in each sample (Figure 4). As the concentration of BSA increases, the average size of the particles

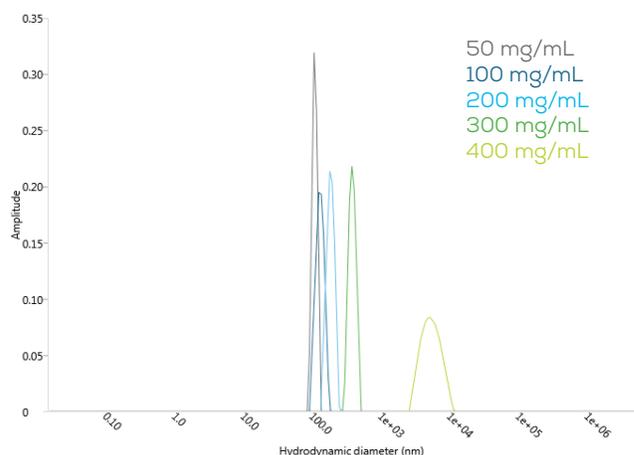


Figure 4: Graph of bead peak intensity distributions for each indicated BSA concentration.

BSA concentration	Apparent bead size
0 (PBS)	101 nm
50 mg/mL	112 nm
100 mg/mL	131 nm
200 mg/mL	180 nm
300 mg/mL	370 nm
400 mg/mL	>1,000 nm

Table 1: Apparent size of 100 nm beads measured by Uncle in the presence of increasing concentrations of BSA.

increases gradually, until they undergo a sharp increase between 300 and 400 mg/mL (Table 1). As previously stated, the Uncle DLS measurement is not quantitative above 1,000 nm. Therefore, Uncle Analysis Software has a quality filter such that it simply reports “>1,000 nm” if the measured size of the beads is too large, and “>14 cP” if the viscosity is too high for an accurate measurement. The specific protein concentration at which this drop-off occurs depends on a few factors, including the molecular weight of the protein and the inter-protein associations that occur in that particular formulation. Despite the lack of quantitative measurements in the 400 mg/mL BSA sample, a qualitative rank order of viscosity is still possible from the correlation functions and intensity distributions, where relative differences are apparent.

The measured, averaged viscosity values for each of the BSA samples tested are reported in Table 2, and a high correlation can be seen with values measured in a traditional viscometer for similar samples, for all samples except the highest concentration (Figure 1). For screening many candidate proteins or formulations, this is a valuable

BSA concentration	Viscometer measurement	UNcle measurement
0 (PBS)	0.98 cP	0.98 cP
50 mg/mL	1.10 cP	1.08 cP
100 mg/mL	1.28 cP	1.27 cP
200 mg/mL	1.81 cP	1.74 cP
300 mg/mL	4.00 cP	3.59 cP
400 mg/mL	9.85 cP	>14 cP

Table 2: Comparison of average viscosity measurements from a viscometer and Uncle for a similar set of samples.

method that can enable you to measure viscosity earlier and at lower volumes.

## Conclusion

In this note, we describe how to use the Viscosity application on Uncle to screen samples. Measuring viscosity earlier, and on more formulations, can reduce development risk and save time. Traditional viscometers can consume large amounts of concentrated protein samples and the measurement process is often labor-intensive. The DLS-based application on Uncle provides a fast and easy tool for measuring viscosity earlier in your process, to ensure that you are developing winning formulations.

## References

1. Connolly BD, et al. Weak interactions govern the viscosity of concentrated antibody solutions: high-throughput analysis using the diffusion interaction parameter. *Biophysical Journal* 103(2012): 69–78.
2. Gilroy E, et al. Viscosity of aqueous DNA solutions determined using dynamic light scattering. *Analyst* 136(2011): 4159–4163.



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