# MONITORING PROTEIN AGGREGATION USING DYNAMIC LIGHT SCATTERING

**The study of protein aggregation encompasses a broad range of interactions and mechanisms. Many studies into this subject area investigate the aggregation of misfolded proteins, which is thought to be responsible for many degenerative diseases. Since aggregation typically leads to a physical change in protein size, particle size analysis has proven to be a useful experimental technique in this field. Dynamic light scattering (DLS) is now widely used to study protein aggregation as described in this application note.**

## Introduction

Aggregation is a general term that encompasses several types of interactions or characteristics. Aggregates of proteins may arise from several mechanisms and may be classified in numerous ways, including soluble/insoluble, covalent/noncovalent, reversible/irreversible, and native/denatured. For protein therapeutics, the presence of aggregates of any type is typically considered to be undesirable because of the concern that the aggregates may lead to an immunogenic reaction (small aggregates) or may cause adverse events on administration (particulates).

Soluble aggregates refer to those that are not visible as discrete particles and that may not be removed by a filter with a pore size of 0.22 µm. Conversely, insoluble aggregates may be removed by filtration and are often visible to the unaided eye. USP <788> (1) provides clear guidelines and limitations on the number of particles ≥10 µm and ≥25 µm in size that may be present in pharmaceutical preparations. Covalent aggregates arise from the formation of a chemical bond between 2 or more monomers. Reversible protein aggregation typically results from relatively weak noncovalent protein interactions. The reversibility is sometimes indicative of the presence of equilibrium between the monomer and higher order forms.

There are also many environmental factors that can lead to protein aggregation. Conditions such as temperature, protein concentration, pH, and the ionic strength may affect the amount of aggregate observed. Stresses to the protein such as freezing, exposure to air, or interactions with metal surfaces may result in surface denaturation, which then leads to the formation of aggregates.

## Experimental

A study of protein aggregation was performed using lysozyme (see Figure 1). The concentration of lysozyme used was 10mg/ml. The protein was dissolved in 0.01N HCl at a pH of 2. Samples were incubated at 55°C and were subsequently frozen at -20°C. Incubation period for each sample is as represented in Table 1. The samples were transported frozen to the HORIBA Applications Lab where they were thawed and analyzed for particle size analysis.

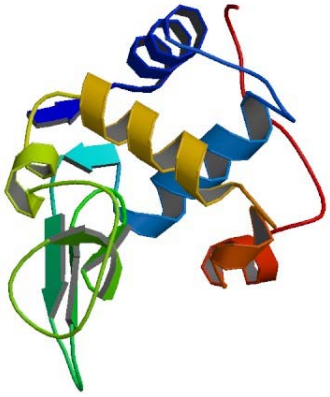

*Figure 1: Lysozyme protein*

The particle size of the proteins were measured on the HORIBA LB-550 DLS system (Figure 9). The sample preparation and measurement conditions are shown on the following page.

1. Mix the samples well.
2. Add the sample into 1-ml measurement cell.
3. Wait until sample temperature readings are stabilized.
4. Take 3 readings with 600 scans, and then calculate the average of the 3 readings.

**Results**

The results from the study are summarized in Table 1 below showing median size in nm as a function of time in hours.

| Sample | Hours | PS |
|---|---|---|
| A | 0 | 8.2 |
| J | 96 | 9.4 |
| K | 120 | 11.2 |
| L | 144 | 88.3 |
| M | 168 | 120.6 |
| S | 313 | 184.5 |

*Table 1: Protein size in nm vs. time in hours*

A graph of the data as a function of time is shown in Figure 2.

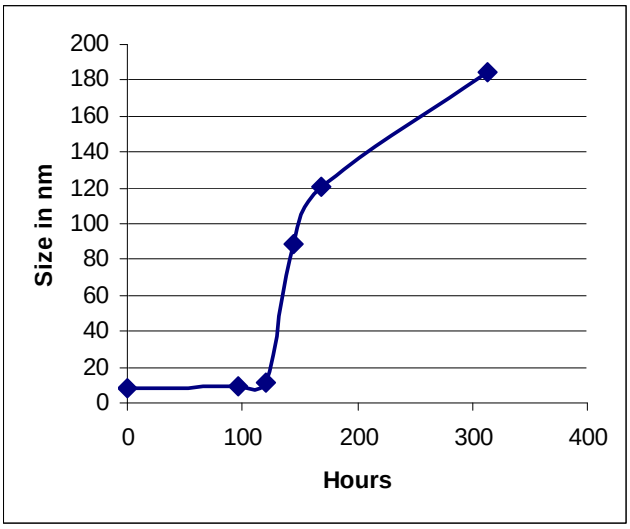


*Figure 2: Protein size in nm vs. time in hours*

More complete results from these experiments are shown in Figures 3-8 below.

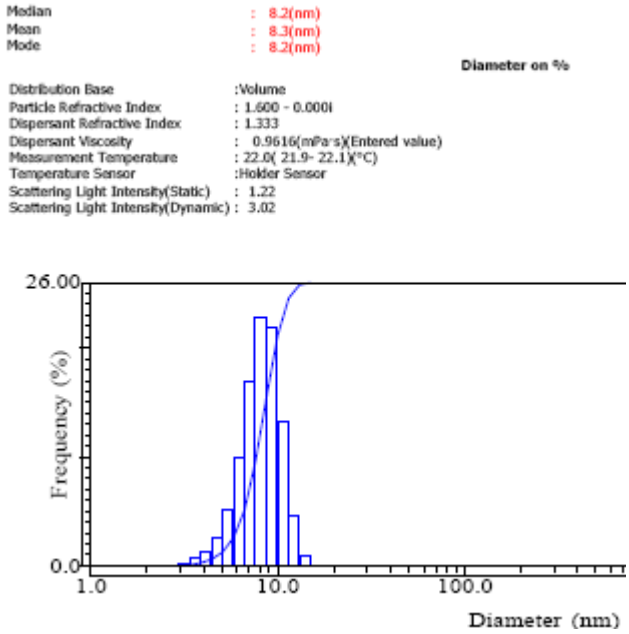


*Figure 3: Initial state*

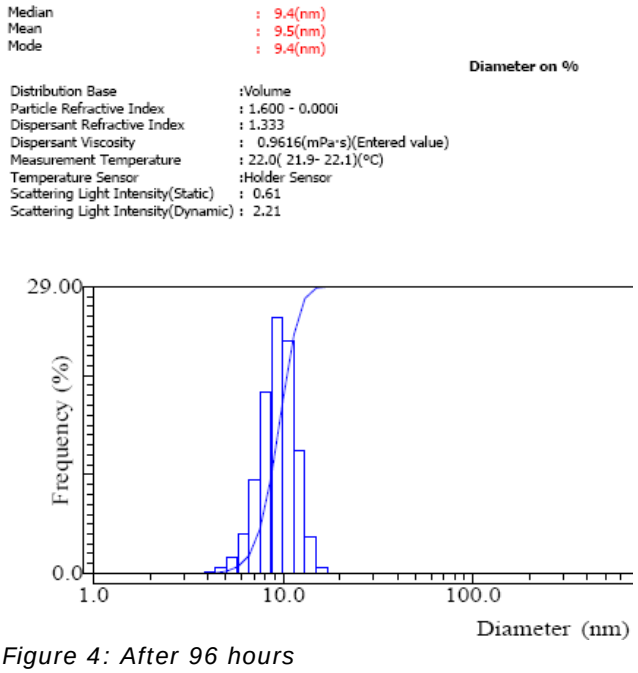


*Figure 4: After 96 hours*

Median : 11.2(nm)
Mean : 11.5(nm)
Mode : 10.9(nm)

Diameter on %

Distribution Base :Volume
Particle Refractive Index : 1.600 - 0.000i
Dispersant Refractive Index : 1.333
Dispersant Viscosity : 0.9615(mPa·s)(Entered value)
Measurement Temperature : 22.0( 21.7- 22.2)(°C)
Temperature Sensor :Holder Sensor
Scattering Light Intensity(Static) : 1.53
Scattering Light Intensity(Dynamic) : 3.74

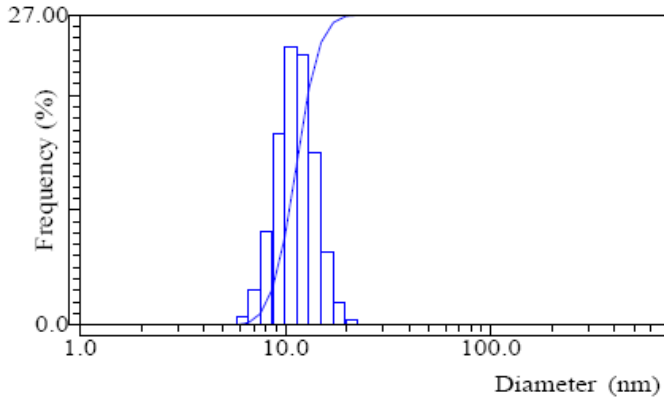


*Figure 5: After 120 hours*

Median : 88.3(nm)
Mean : 127.6(nm)
Mode : 71.6(nm)

Diameter on %

Distribution Base :Volume
Particle Refractive Index : 1.600 - 0.000i
Dispersant Refractive Index : 1.333
Dispersant Viscosity : 0.9616(mPa·s)(Entered value)
Measurement Temperature : 22.0( 21.7- 22.2)(°C)
Temperature Sensor :Holder Sensor
Scattering Light Intensity(Static) : 2.75
Scattering Light Intensity(Dynamic) : 5.89

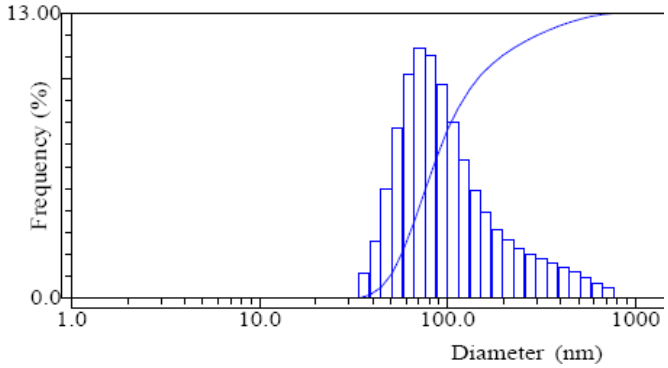


*Figure 6: After 144 hours*

Median : 120.6(nm)
Mean : 166.9(nm)
Mode : 106.6(nm)

Diameter on %

Distribution Base :Volume
Particle Refractive Index : 1.600 - 0.000i
Dispersant Refractive Index : 1.333
Dispersant Viscosity : 0.9615(mPa·s)(Entered value)
Measurement Temperature : 22.0( 21.8- 22.1)(°C)
Temperature Sensor :Holder Sensor
Scattering Light Intensity(Static) : 5.19
Scattering Light Intensity(Dynamic) : 9.76

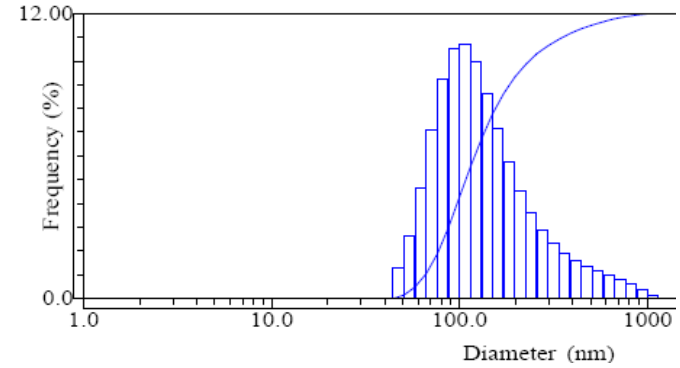


*Figure 7: After 168 hours*

Median : 184.5(nm)
Mean : 281.0(nm)
Mode : 160.5(nm)

Diameter on %

Distribution Base :Volume
Particle Refractive Index : 1.600 - 0.000i
Dispersant Refractive Index : 1.333
Dispersant Viscosity : 0.9635(mPa·s)(Entered value)
Measurement Temperature : 21.9( 21.9- 21.9)(°C)
Temperature Sensor :Holder Sensor
Scattering Light Intensity(Static) : 13.73
Scattering Light Intensity(Dynamic) : 25.18

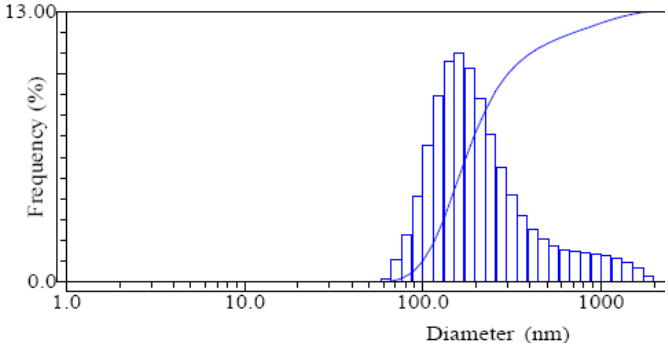


*Figure 8: After 313 hours*

## Discussion and Conclusion

The protein samples aggregated quickly after approximately 120 hours. The aggregated samples showed the expected broadening in the distribution. The LB-550 (Figure 9) could easily track protein aggregation as a function of time. Many pieces of fundamental research in the protein/macromolecule field of biotechnology are accomplished using size analysis equipment. Monitoring protein aggregation using dynamic light scattering is only one example, although an important one, of the growing significance particle characterization has in biotech and pharmaceutical research.


*Figure 9: The LB-550 Macromolecule Size Analyzer*

## References

1. USP<788> Particulate Matter In Injections

Note: HORIBA wishes to thank Lisa Cole and Ben Burnett at the Florida Institute of Technology for sharing the data shown in this application note.


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