0.1 mg/mL Lysozyme

Nicomp® DLS System

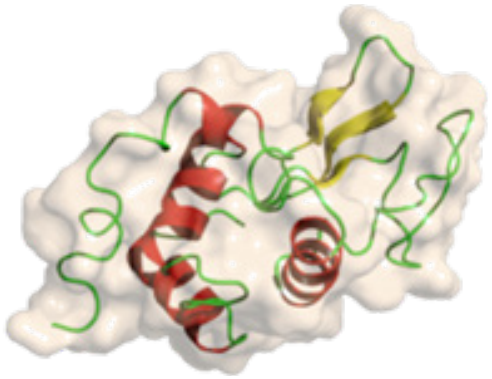

Figure 1. Lysozyme protein

The specifications for dynamic light scattering (DLS) systems are typically sample dependent. But many DLS systems specify the lower concentration limit as the ability to measure the protein lysozyme at a concentration of 0.1 mg/mL. This technical note documents proof that the Nicomp® DLS system is capable of repeatedly measuring 0.1 mg/mL lysozyme and provides details on how the measurements were performed.

INTRODUCTION

The performance of any DLS system is sample dependent. For example, the upper size limit may be >5 µm for an emulsion, but <1 µm for a high density particle since the analysis loses accuracy upon onset of sedimentation. The upper concentration limit may be >40 vol. % for particles <10 nm, but closer to 1% for particles ~1 µm. Be warned as well that the instrument will generate a result for every experiment performed, but that does not guarantee every result is equally accurate.

The lower size and concentration limits for the Nicomp DLS systems are both sample and configuration dependent. For low concentration measurements of small particles a higher power laser and high sensitivity APD detector may be required. Please communicate with your local Entegris representative when configuring a system for specific applications.

The lower concentration limit for the Nicomp DLS system is the ability to measure lysozyme at the concentration of 0.1 mg/mL. Lysozyme is a single chain polypeptide of 129 amino acids cross-linked with four disulfide bridges. The molecular weight is 14,307 Da and the reported particle size by DLS is typically ~3.6 nm.

MATERIALS

Previous experience had suggested the lysozyme monomer form is most stable at low pH values near pH 2.2[1]. A KCl buffer at pH 2.2 was created using the following recipe.[2]

- 0.2 M KCl solution was prepared by dissolving 14.91 g of KCl in 100 mL filtered DI water.
- 0.2 M HCl solution was created by adding 8.58 mL concentrated HCl into 491.4 mL water.
- 50 mL of the 0.2 M KCl solution was added into a 200 mL clean bottle.
- 7.8 mL of the 0.2 M HCL was slowly added to the KCL solution.
- Filtered DI water was added to fill up to 200 mL total volume.

This buffer solution was always filtered by a 0.2 µm syringe filter for any subsequent steps.

The lysozyme used purchased from sigma aldrich, catalog number L6876, lysozyme from chicken egg while supplied as a lyophilized powder. Protein content by UV absorbance is 90%, with the remainder being buffer salts such as sodium acetate and sodium chloride. Therefore, all reported concentrations in this document are actually slightly lower by a factor of about 10% since no concentration corrections were made.

The base 10 mg/mL lysozyme sample was prepared by adding 0.1 g lysozyme powder to 100 mL filtered buffer solution and mixing for one hour. This was diluted 10:1 with filtered buffer solution to create 1 mg/mL concentration. This sample was then diluted 10:1 with filtered buffer to create the 0.1 mg/mL concentration sample.

INSTRUMENT SETTINGS

An off the shelf Nicomp 380 DLS system equipped with a 50 mW laser at wavelength 658 nm and an APD detector was used for these experiments. All measurements were made at 90°.

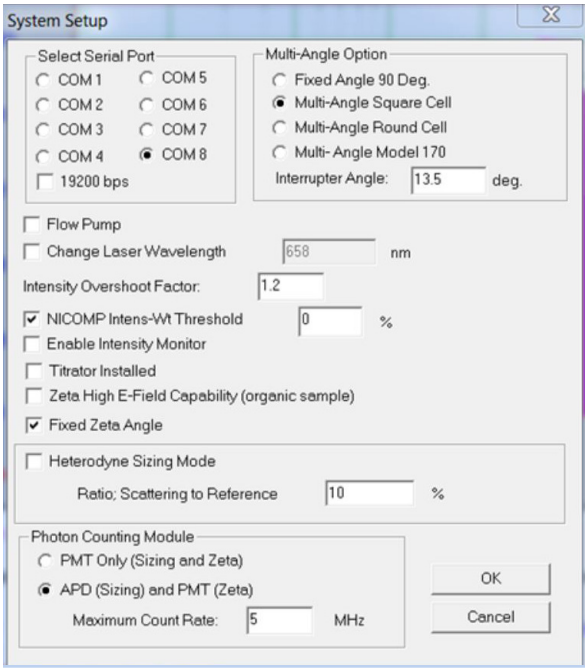

Figure 2. System setup dialog settings

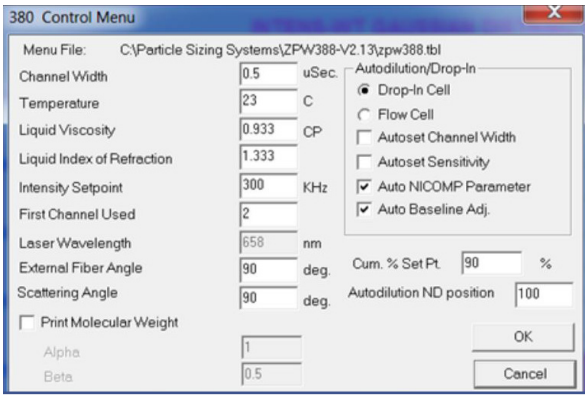

Figure 3. Particle-sizing control menu settings

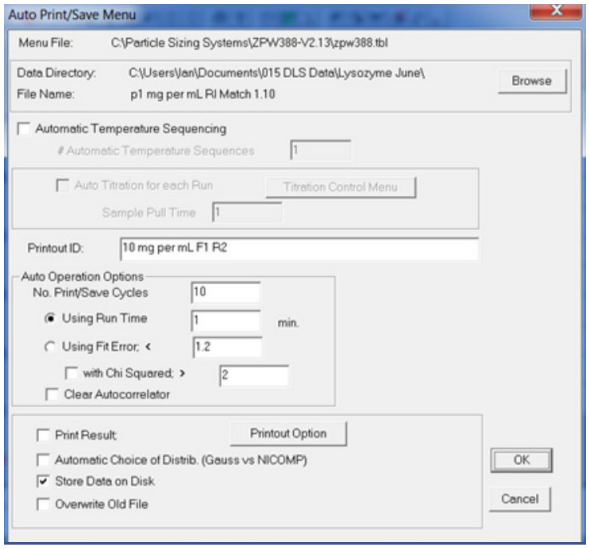

Figure 4. Auto print/save menu settings

RESULTS

The 10 mg/mL lysozyme sample was placed into a clean square glass cuvette after passing through a 0.2 µm syringe filter. The sample was analyzed using the settings described in this document and a typical result is shown in Figure 5.

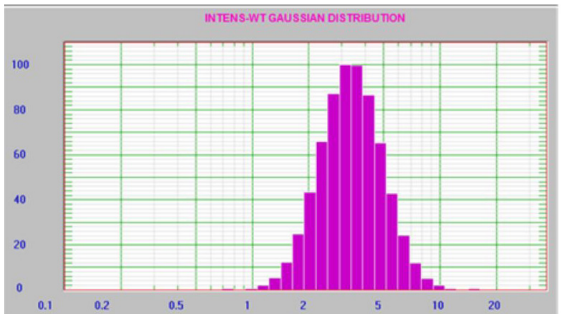

Figure 5. 10 mg/mL lysozyme result

Mean diameter	3.6 nm
Coefficient of variation	0.384
Standard deviation	1.389 nm
Normalized standard deviation	0.384
Variance (PI)	0.147

For clarification: Mean Diam = 3.6 nm,
Std Dev = 1.389 nm, PI = 0.147

Note: This small PI value is expected for a well prepared sample with count rate ~160 kcps.

The 1 mg/mL lysozyme sample was placed into a clean round glass cuvette after passing through a 0.2 µm syringe filter. The cuvette was then centrifuged at 8000 rpm for 10 minutes to separate aggregates. The sample was analyzed using the settings described in this document and a typical result is shown in Figure 6.

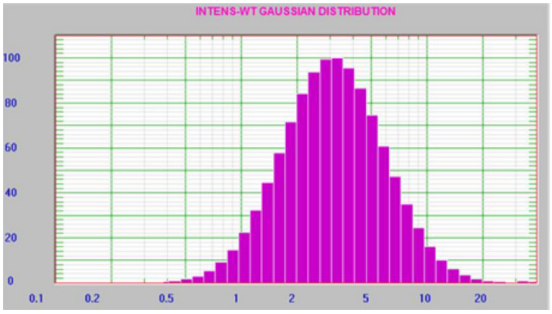

Figure 6. 1.0 mg/mL lysozyme result

Mean diameter	3.8 nm
Coefficient of variation	0.632
Standard deviation	2.404 nm
Normalized standard deviation	0.632
Variance (PI)	0.399

For clarification: Mean diameter = 3.8 nm,
Standard deviation = 2.404 nm, PI = 0.399

Note: This PI value is higher than expected for a well prepared sample, indicating possible aggregates and is not unusual for a sample at low count rate (~30 kcps).

The 0.1 mg/mL lysozyme sample was placed into a clean round glass cuvette after passing through a 0.2 µm syringe filter. The cuvette was then centrifuged at 8000 rpm for 10 minutes to separate aggregates. The cuvette was then placed in a square cell containing a matching refractive index (RI) oil. This additional sample preparation step improved the repeatability of the lowest concentration measurements. The sample was analyzed using the settings described in this document and a typical result is shown in Figure 7.

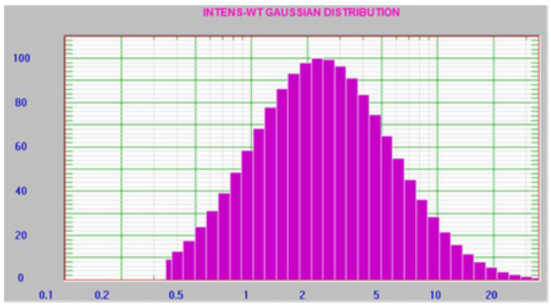

Figure 7. 0.1 mg/mL lysozyme result

Mean diameter	3.5 nm
Coefficient of variation	0.877
Standard deviation	3.048 nm
Normalized standard deviation	0.877
Variance (PI)	0.769

For clarification: Mean diameter = 3.5 nm,
Standard deviation = 3.048 nm, PI = 0.769

Note: This PI value is higher than expected for a well prepared sample, indicating possible aggregates and is not unusual for a sample at low count rate (~19 kcps). Using the RI matching cell appeared to improve the count rate for the lowest concentration experiments.

The 0.1 mg/mL sample was measured multiple times to check repeatability. Nine consecutive results are plotted in Figure 8.

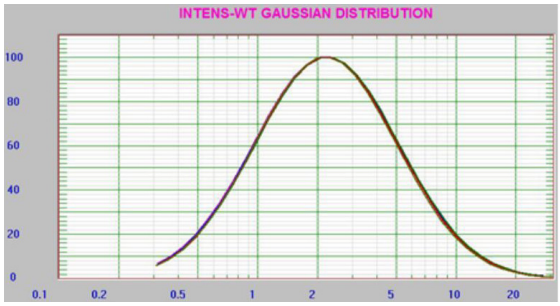

Figure 8. Nine consecutive results at 0.1 mg/mL concentration lysozyme

CONCLUSIONS

The Nicomp DLS system is quite capable of measuring lysozyme at the specified 0.1 mg/mL concentration. All results here are reported in intensity distribution, using the Gaussian distribution results. No other changes/conversions were used to generate the results shown.

References

[1] Nicoli, D. and Benedek, G., Study of Thermal Denaturation of Lysozyme and Other Globular Proteins by Light Scattering Spectroscopy, Biopolymers, Vol 15, 2421- 2437 (1976)

[2] http://labmanual.net/docs/FTP/files/Preparation% 20of%20 frequently%20used%20%20solutions.pdf

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