Automatic Dilution on the Nicomp® DLS System

Eliminates operator error and improves accuracy

Figure 1. Nicomp DLS system.

OVERVIEW

Dynamic light scattering (DLS) can be an easy or challenging technique depending on the sample. Sometimes the challenge comes from data interpretation[1] and sometimes from sample preparation.[2] The dilution step in sample preparation is where some users make mistakes that lead to poor accuracy, repeatability, or both. The Nicomp automatic dilution option eliminates the chance of operator error and facilitates generating optimum results.

Figure 2. Flow pump and inlet/outlet tubing.

INTRODUCTION

Classical DLS experiments are conducted at low concentrations for several reasons including the avoidance of concentration effects and the ease of using the solvent viscosity. Concentration effects include multiple scattering and particle-particle interactions. The theory and algorithms used in DLS systems assume that light interacts with a single particle and then scatters onto the detector. As concentrations increase, the probability that the scattered light interacts with additional particles before reaching the detector increases. This effect reduces the reported particle size. Higher concentrations also influence particle-particle interactions and can cause restricted diffusions – also introducing errors into the final result. Measuring at lower concentrations also means the viscosity of the solvent/diluent can be used rather than needing to perform a viscosity measurement prior to the particle size analysis.

The lower concentration limit of the Nicomp DLS system (Figure 1) is influenced by the scattering properties of the sample; see Entegris Technical Note – 0.1 mg/mL Lysozyme.[3] Measurements at very low concentrations can be noisy, reducing accuracy and repeatability. The upper concentration limit depends on both the size and concentration of the sample, with better high concentration measurements possible for very small than for larger size particles.
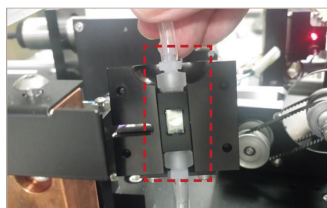

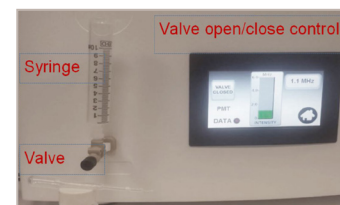Figure 3. Flow through cell.

Figure 4. Injection syringe and valve on front panel.
Note: the button on the left of the display is used to open and close the sample valve.

Ideally, the sample concentration is adjusted so that the count rate on the detector is approximately 300 kHz. This scattering intensity is "the sweet spot" for standard DLS size measurements. For many samples, the Nicomp can automatically adjust the neutral density (ND) optical filter to adjust incident laser intensity to tune the count rate to the optimum range. But for other samples a pre-dilution step may be required to adjust to the ~300 kHz range. A word of caution to users, just because the ND filter reduced counts to the proper range, this does not guarantee that all concentration effects have been eliminated from the experiment.

⬡ Entegris

NICOMP AUTODILUTION OPTION

The Entegris Nicomp is the only DLS system to offer an autodilution option. The fluidics that enable the autodilution include a flow pump (Figure 2), diluent source, mixing chamber, and flow through cell (Figure 3). In addition, a multi-position valve and syringe is mounted in the front of the unit to facilitate sample introduction (Figure 4).

EXPERIMENTAL

A high concentration pharmaceutical emulsion was used to test the Nicomp autodilution feature. These emulsions are frequently tested to determine if they meet the size criteria given in USP 729, globule size distribution in lipid injectable emulsions.[4,5] The samples are labeled "sample 1" for the newer sample and "sample 2" for the older sample. Both samples were over one year beyond the shelf life date on the container packaging.

The Nicomp settings used for the measurements are shown in Figures 5, 6, and 7.

Before measurements were made the flow pump was initialized and all tubing was checked for bubbles (none found). The syringe seen in Figure 4 was filled with 5 mL of filtered DI water. Drops of the emulsion samples were added to the open end of the syringe. First one drop, then two drops, three drops, (etc). The valve open/close control button was turned on until 2 mL of sample was dispensed into the system. The start autodilution icon was clicked and the system began diluting the sample. The protocol is to first exceed the desired count rate by about 20% (intensity overshoot factor in Figure 6) and then dilute down to achieve the intensity setpoint of 300.

This procedure was followed for sample 1, adding one through nine drops of emulsion and sample 2, adding one through four drops of emulsion. Drops were transferred using a standard disposable pipette. Two measurements were made for each sample and number of drops. The intensity mean and Polydispersity Index[1] (PI, or variance) was recorded for each sample.
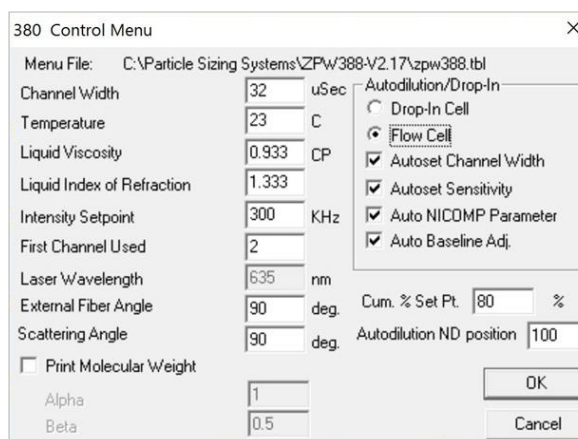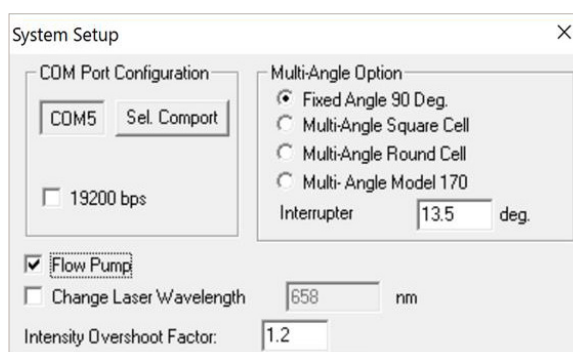

Figure 5. Nicomp control menu.
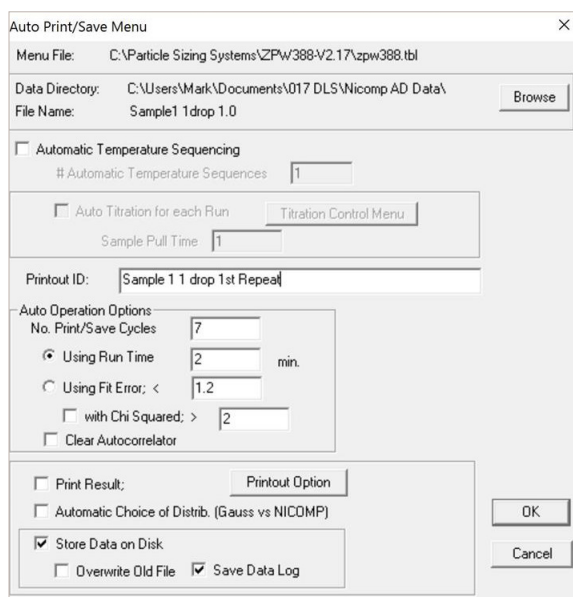

Figure 6. System setup menu.

Figure 7. Nicomp auto print/save menu.

RESULTS

A plot of five overlays from sample 1 is shown in Figure 8 and a table of the recoded values is shown in Table 1.

NOTE: overall repeatability/reproducibility is calculated by showing the coefficient of variation (COV) defined as:

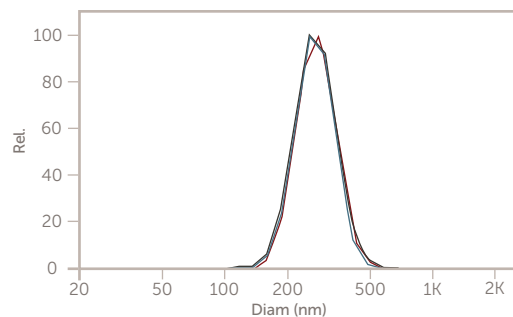**COV= (st dev/mean)*100**

Intens-WT gaussian distribution

Figure 8. Overlay of five results from sample 1.
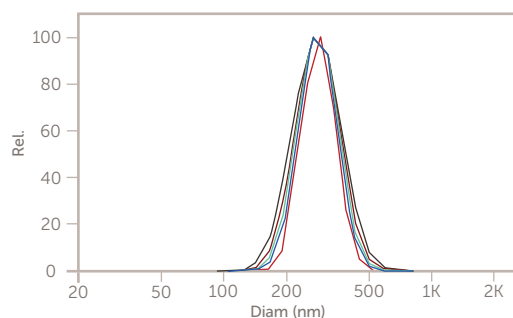
Intens-WT gaussian distribution

Figure 9. Overlay of five results from sample 2.

A plot of five overlays from sample 2 is shown in Figure 9 and a table of the recoded values is shown in Table 2.

Sample 1	Mean	PI
1 drop R1	282.3	0.032
1 drop R2	281.3	0.049
2 drops R1	277.3	0.036
2 drops R2	278.0	0.049
3 drops R1	280.2	0.047
3 drops R2	281.1	0.069
4 drops R1	280.7	0.042
4 drops R2	281.3	0.044
5 drops R1	279.3	0.06
5 drops R2	280.4	0.034
6 drops R1	280.2	0.037
6 drops R2	279.3	0.04
7 drops R1	280.2	0.064
7 drops R2	280.0	0.056
8 drops R1	278.2	0.05
8 drops R2	279.3	0.066
9 drops R1	280.6	0.083
9 drops R2	278.8	0.065
Mean	279.92	
Standard deviation	1.29	
COV	0.46	

Table 1. Intensity mean and PI for sample 1.

Sample 2	Mean	PI
1 drop R1	289.8	0.042
1 drop R2	288.4	0.056
2 drops R1	289.0	0.048
2 drops R2	290.7	0.035
3 drops R1	287.0	0.043
3 drops R2	285.9	0.05
4 drops R1	286.8	0.049
4 drops R2	287.0	0.043
Mean	288.08	
Standard deviation	1.67	
COV	0.58	

Table 2. Intensity mean and PI for sample 2.

References

[1] Entegris Technical Note - DLS Data Interpretation

[2] Entegris Technical Note - DLS Sample Preparation

[3] Entegris Technical Note - 0.1 mg per mL Lysozyme

[4] USP 729—Globule Size Distribution in Lipid Injectable Emulsions

[5] Entegris Application Note - USP 729

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