DLS Method Development and Validation

Nicomp® DLS System

Dynamic light scattering (DLS) is becoming an increasingly popular particle size analysis technique in the pharmaceutical industry for drug products in the submicron region. This technical note attempts to provide guidance to customers using this technique for release testing that requires method development and validation.

INTRODUCTION

While DLS is used extensively in the pharmaceutical industry very little has been published on the topics of method development and/or validation. The only compendia test referring to DLS is USP <729>, globule size distribution in lipid injectable emulsions.[1] Therefore this general chapter provides some insight into utilizing DLS in the pharmaceutical industry.

USP <729>

This standard preparation test requires that the DLS system be verified using three PSL standards at 100, 250, and 400 nm. The author of this document does not understand how/why these sizes were chosen, especially since the pass/fail criteria is 500 nm. Many expert DLS users believe just testing at one size should be acceptable to verify the system is performing properly. These standards are to be measured three times, and the intensity weighted mean diameter and standard deviation should coincide with the expected values within acceptable errors. Latitude is given with respect to "within acceptable errors." Perhaps a range of +/-10% for the mean diameter is reasonable. The system suitability section states that the reproducibility when analyzing the standards meet the criteria if the CV does not exceed 10%. Passing the USP <729> test requires that the intensity mean diameter be less than 500 nm (0.5 µm) and the chi-squared (χ^2) parameter remain "acceptably low." The Nicomp® user manual states that a good Gaussian result has a chi-squared value below 2 – 3. Therefore a chi-squared value <3 should indicate a passing result.

A NOTE ON SPECIFICATOINS

While the USP <729> test is the only compendial monograph utilizing DLS and is therefore worth considering, the choice of pass/fail criteria is not necessarily the perfect approach for all drug substances. A more common, approach to specifications would be to define a value and range at the intensity mean and some calculation associated with the width of the distribution. The ISO standard for DLS[2] suggests a focus on the intensity mean and the polydispersity index (PI). Some DLS specifications seen by the author include values for the D10, D50, and D90. These values probably come from the widespread use of laser diffraction for particle size analysis in the pharmaceutical industry. The USP <429> test light diffraction measurement of particle size[3] makes frequent use of the D10, D50 and D90. One possible approach to setting specifications that customers may be tempted to lift from USP <429> are those associated with reproducibility. The reproducibility ranges for the volume based distribution found in USP <429> given in the Replicates section are:

- D50 = 10%
- D10, D90 = 15%
- Below 10 µm, these maximum values can be doubled

Following the spirit of these guidelines, a DLS specification (by definition sizes below 10 µm) could be reproducible ±20% for the intensity mean and ±30% for PI, or other calculated result indicating distribution width. For some drug products, this may be an acceptable range but beware of the problem of the statistics of small numbers. If the mean size is 500 nm, the range would be 400 – 600 nm, which is not so bad. But for a protein with mean size of 10 nm, the range becomes 8 – 12 nm, a much more difficult span to live with.

While at first glance it may seem reasonable to transfer the D10, D50, and D90 approach from laser diffraction over to DLS, remember that the primary calculated result from DLS is based on intensity, not the volume distribution. Transformations from intensity to volume distributions involve calculations specific to instrument make, and have not been harmonized through ISO documentation.

METHOD DEVELOPMENT

Prior to developing a method, the issue of sample preparation requires investigation. This can range from simple (pipette the sample into a cuvette), to complex (disperse with surfactant and ultrasound). The topic of sample preparation is beyond the scope of this document, but has been addressed in a previous technical note.[4] Some advice to consider when preparing samples for analysis using DLS include:

- Test the effect of concentration. Measure, dilute, and measure again.
- Test the effect of measurement duration. Measure for 3, 5, and 10 minutes.
- Assure any diluent is adequately filtered. WFI may not be clean enough.
- Assure any cells used are adequately clean.
- Should a filter be used to remove large particles in the sample?
- Which sample cell is appropriate.
- Investigate any instrument setting that could affect results.

After the sample preparation steps and instrument settings have been optimized, it would be a good time to test robustness. Investigate if small changes in analysis time have any significant effect on results. If the method calls for 5 minutes analysis time, test at 4 minutes 30 seconds and 5 minutes 30 seconds. Check if the results are the same.

Once the method is optimized, the next step is to document the method following the standard FDA guidance.[5] The goal is to describe the procedure with sufficient detail, so another operator could perform the same test and generate the same results. The essential information that must be included is listed:

- **Principle/Scope**: Basic principles of the analytical test/technology.
- **Apparatus/Equipment:** Instrument type, laser, detector, angle of detection, and cell type.
- **Operating Parameters:** Temperature, viscosity, analysis time, and channel width.
- **Reagents/Standards:** Polystyrene latex (PSL) used to verify performance. PSS recommends a 90 nm PSL (See Entegris Technical Note - System Verification).
- **Standards control solution preparation:** Diluent, dilution.[6]
- **Procedure:** Step by step description of the procedure. Place prepared sample in cuvette, orientation into system, define parameters, and measure.
- **System Suitability:** Test to ensure system will function properly at time of use.[6]
- **Calculations:** All result calculations are typically made directly in the DLS system operating software.[8] Additional statistical calculations may be made in a spreadsheet.
- **Data Reporting:** Presentation of numeric data, format, significant figures. Entegris recommends results focus on the intensity weighted mean diameter.

A NOTE ON REFERENCE STANDARDS AND MATERIALS

As stated above, we believe testing at a single size is adequate to verify the system is operating properly. Many particle size standards are available from several vendors, and the customer may choose the sample they wish to use. However, Entegris has experience with two PSL standards, and these are the samples we typically suggest customers use to verify their Nicomp[6]. The Thermo Fisher 3000 series NIST traceable 90 nm nominal PSL standard is often used to verify Nicomp performance. The sample is catalog number 3090A with a certified value of 92 ± 3 nm. Another sample often used is Thermo Fisher catalogue number 5009A with a value of 90 nm. This sample is not NIST traceable but we have sufficient experience to recommend the use of this sample, and it is less expensive than the 3000 series products.

NOTE: DLS is a first principle technique that does not require calibration. The system is verified using one or more PSL standards. If the system fails the verification step there is no adjustment possible to bring the result into the expected range. Verification results failure could stem from either the system is not working properly and service is required, or more often the standard was not prepared properly and should be prepared again before attempting another measurement.

METHOD VALIDATION

Analytical method validation is the process of demonstrating that an analytical procedure is suitable for its intended purpose[5]. Not all validation characteristics are appropriate for particle size analysis. The older FDA Guidance for Industry (Not for Implementation) draft document[7] included a section specific to particle size analysis. Although the newer published document replaces the older draft guidance, the older section on particle size analysis provides insight into how particle size analysis differs from other techniques such as HPLC. The following statement in the older document helps focus the method validation efforts:

"The methods validation usually involves evaluation of intermediate precision and robustness. Assurance should be provided that the data generated are reproducible and control the product's quality."[7]

With this comment in mind, suggestions for addressing method validation for DLS are provide below:

- **Specificity:** N/A, DLS detects changes in size but is not sensitive to different chemical species.
- **Linearity:** N/A, There is nothing linear about DLS.
- **Accuracy:** N/A, Accuracy of the instrument is verified using a standard but lack of an accepted quantitative referee method (SEM/TEM microscopy) excludes accuracy determination. Including an SEM/TEM image of the sample to support the method validation may be appropriate.
- **Precision (repeatability, intermediate precision, and reproducibility):** This is where to focus efforts. Typically accepted definitions of these terms may be different in the realm of particles size analysis than for other techniques. The comments below come from the realm of particle size analysis:

  Repeatability: Measure the sample multiple times.

  Reproducibility: Prepare the sample, measure, discard, clean, repeat. A suggested approach would be to make five sample preparations and analyze each sample five times.

- **Intermediate precision:** This activity involves a second analyst, second instrument, or both. If all testing is at one location analyze samples from the same batch on different days by different operators on the same system. If testing is to be performed at multiple locations, the same sample (or batch) is analyzed by different operators on different systems in different locations.
- **Range:** N/A, Just work in the operating range of system used. No need to test or document this.
- **Quantitation limit:** N/A, This is just testing particle size.
- **Detection limit:** N/A, Method development should have assured the sample is within detection limit of system.

Once a method has been validated the procedure should be used for the life cycle of the product. The method should be reevaluated and revalidated if repeated adjustments are required.

EXAMPLE RESULTS

A drug substance (propofol emulsion) was analyzed on two Nicomp DLS systems by two operators. The sample was well past the expiration date but still showed passing results per USP <729>. The instrument settings are shown below:

Instrument A:

Instrument type	Nicomp 380ZLS
Age	5 years old
Laser wavelength	958 nm
Laser power	35 mW
Detector	PMT
Detector angle	90°
Software version	ZPW388 V2.17.0215

Instrument B:

Instrument type	Nicomp ZLS3000
Age	new
Laser wavelength	935 nm
Laser power	15 mW
Detector	PMT
Detector angle	90°
Software version	ZPW388 V2.17.0215

METHOD DEVELOPMENT

First a 92 nm PSL standard was analyzed to assure proper system performance. The measured result was within 98% of the expected value. This verification test is recommended before the beginning of such an effort.

Next a quick study was made to test for the effect of concentration (dilution). See results in Figure 1.

Conditions	Mean	PI
10 drops in 15 mL	212.4	0.082
10 drops in 15 mL	213.3	0.075
2:1 dilution	215.4	0.069
2:1 dilution	209.7	0.094
1 drop in 15 mL	208.7	0.092
1 drop in 15 mL	208.9	0.072
1 drop in KCL	204.9	0.064
1 drop in KCL	203.2	0.079

Figure 1. Data from method development

Ten drops of sample were added to 15 mL DI water and analyzed. The same preparation was repeated. These samples were then diluted 2:1 and analyzed. The sample appeared too cloudy and results were changing, so next one drop was added to 15 mL DI water. The sample appeared slightly cloudy and these results were acceptable. Sometimes a dilute salt solution is a better diluent than pure DI water. The next step was to add one drop of sample into 10 mL of filtered 10 mM KCl solution. These results appeared better, so the 10 mM KCl solution was used for all other measurements in this study.

While performing the dilution study the time of analysis was also varied from three to ten minutes. The time plots for a three, five and ten minute analysis time are shown in Figures 2 – 4. Red = intensity mean, blue = volume mean, teal = number mean.

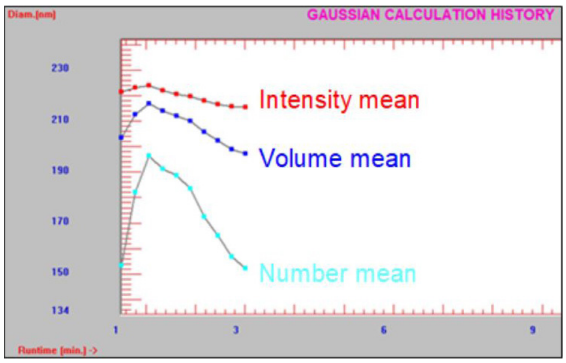

Figure 2. Time history plot for 3 minutes analysis time

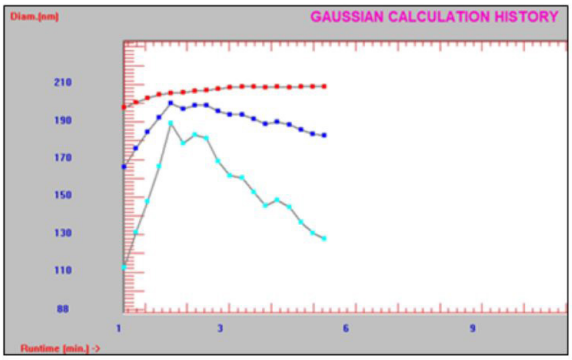

Figure 3. Time history plot for 5 minutes analysis time

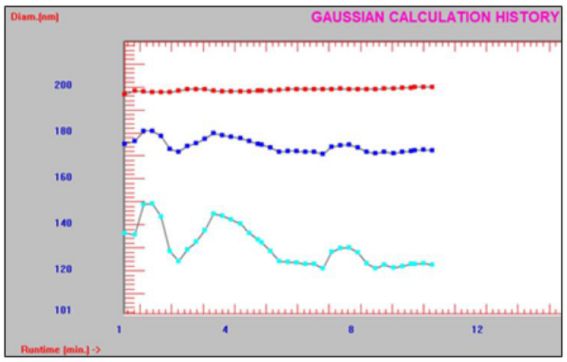

Figure 4. Time history plot for 10 minutes analysis time

A five minute analysis time was chosen for the method. The intensity mean result had stabilized before the five minute analysis time was completed. Notice how the volume and number weighted mean results were still changing until about nine minutes. This is another reason to work only in intensity weighted results when using DLS. This could be a good point in method development to test for robustness. In this case, perhaps next test with analysis times of four and a half, five, and five and a half minutes. This step was not performed during this study, and it is worth pointing out this entire method development and validation study was completed in under eight hours. A more rigorous approach for final release testing method development and validation would most likely take at least several days.

SAMPLE PREPARATION

- Fill clean bottle with 10 mL filtered KCl solution
- Use a syringe with needle to remove 0.5 mL propofol
- Inject 1 drop propofol into bottle with 10 mL KCl solution
- Hand swirl until well mixed (10 seconds)
- Use disposable pipette tip to pipette 200 µL sample into round disposable glass cell
- Place glass cell into black cell holder
- Open cell sample lid on Nicomp
- Insert cell and holder into Nicomp system with cell holder back oriented to left side of Nicomp
- Close cell sample lid on Nicomp

Assure instrument settings match values shown below:

Temp	23°C
Viscosity	0.933 cP
Liquid index of refraction	1.333
Intensity setpoint	300 KHz
First channel used	2
External fiber angle	90°
Scattering angle	90°
Print molecular weight	unchecked
Autoset channel width	checked
Autoset sensitivity	checked
Auto nicomp parameter	checked
Auto baseline adjust	checked
Cum % set point	10%
Autodilution ND position	N/A
Number of print/save cycles	5
Using run time	5 minutes

Using fit error	unchecked
Clear autocorrelator	unchecked
Print result	unchecked
Automatic choice of distribution	unchecked
Store data on disk	checked
Overwrite old file	unchecked
Save data log	checked

- Click on green "G" icon to start measurement
- Print and record mean diameter, standard deviation, PI D10, D50, and D90
- Enter results into Excel spreadsheet
- Calculate average and coefficient of variation for the five analyses
- Compare values for the two data sets

RESULTS

Samples were independently prepared and analyzed on the A and B systems. Each sample was analyzed five times to check repeatability. Example results from the two systems are shown in Figures 5 and 6.

Run A	Mean	PI
1	200.8	0.094
2	200.5	0.088
3	199.9	0.088
4	199	0.091
5	199.3	0.094
Sum	999.5	0.455
Mean	199.9	0.9
Standard deviation	0.76	0.003
COV	0.38	0.330

Figure 5. Example results from system A

Run B	Mean	PI
1	204.1	0.078
2	206.7	0.079
3	203.6	0.071
4	205.5	0.066
5	207.5	0.069
Sum	1027.4	0.363
Mean	205.5	0.0726
Standard deviation	1.66	0.006
COV	0.81	7.828

Figure 6. Example results from system B

The summary of the entire result set is tabulated and shown in Figures 7 and 8. An overlay of five results is shown in Figure.

	Mean	Standard deviation	COV
Mean	200.7	1.62	0.81
PI	0.085	0.012	13.87
Standard deviation	58.385	3.858	6.61
D10	134.9	4.671	3.46
D50	200.6	1.662	0.83
D90	284.9	5.691	2.00

Figure 7. Summary results from system A

	Mean	Standard deviation	COV
Mean	207.1	1.82	0.88
PI	0.1	0.019	29.16
Standard deviation	52.910	8.715	16.47
D10	146.7	9.749	6.65
D50	207.1	1.187	0.88
D90	282.5	12.955	4.59

Figure 8. Summary results from system B

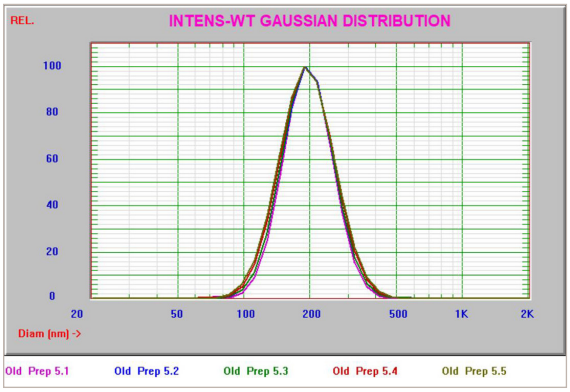

Figure 9. Overlay of five results

CONCLUSIONS

Reviewing the combined results leads to several observations:

- Even two systems built several years apart generate similar data
- There is a clear 3% bias between the two systems
- The PI calculation may not be the best value to use to define the width of the distribution
- If the PI is used, a value of 30% spread seems appropriate
- The D10, D50, D90 results show better reproducibility and may be easier for the pharmaceutical industry to live with

Based purely on this data set the specifications for this drug product based on three measurements could be something like:

- Intensity mean = 200 nm ±20%, COV = less than 20%
- D10 = 140 nm ±30%, COV = less than 30%
- D90 = 284 nm ±30%, COV = less than 30%

Or

- PI = 0.09 ±30%

The above suggested specifications just come from the observed reproducibility and might not have any influence on drug safety or efficacy. The actual pass/fail criteria in USP <729> is simply that the size must be under 500 nm and have a low χ^2 value. Another approach to specifications might be to focus on the size where efficacy and/or safety become an issue. The intent of the data collected for this technical note is to give a feel for the repeatability/reproducibility the DLS technique is capable of for an easy sample.

References

[1] USP <729>, Globule Size Distribution in Lipid Injectable Emulsions, http://www.usp.org/

[2] ISO 22412 Particle size analysis — Dynamic light scattering (DLS), https://www.iso.org/home.html

[3] USP <429>, Light Diffraction Measurement of Particle Size, http://www.usp.org/

[4] Entegris Technical Note, DLS Sample Preparation

[5] Analytical Procedures and Methods Validation for Drugs and Biologics, July 2015, https://www.fda.gov/media/87801/download

[6] Entegris Technical Note, DLS System Verification

[7] Guidance for Industry, Analytical Procedures and Methods Validation, Draft Guidance, July 2000. No longer available for download at FDA website.

[8] Entegris Technical Note, DLS Data Interpretation

Note: The advice and opinions given in this document are strictly the opinions of the author (Mark Bumiller, Technology Manager, Particle Sizing Systems, an Entegris Company) and do not indicate any agreement by regulatory agencies.

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