Poster# W4110

AAPS Annual Meeting 2010

Validation of a Particle Size Determination Method for Nano-particles by Dynamic Laser Light Scattering

J. Brunotte, M. Darter, J. Vaughn and V. Kulkarni
DPT Laboratories, San Antonio, Texas

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INTRODUCTION

Analysis of particle size is of paramount importance in pharmaceutical product development. Although there are several methods for size characterization of particles, light scattering and optical microscopy methods are typically employed for particle size analysis in pharmaceutical product development and quality control activities.[1,2] Because the light scattering technique for size measurement is rapid, reproducible, easy to use and suitable for both wet and dry particles, and because the software is compliant with regulatory requirements of quality control/assurance laboratories, it is a preferred technique in pharmaceutical product development and stability studies. For wet particles smaller than 1 µm, dynamic laser light scattering (DLS), also known as photon correlation spectroscopy (PCS), is commonly used.[3]

The transfer of methods for size determination from a method development laboratory to a quality group necessitates validation in accordance with FDA regulations.[4] The current study focuses on method development and validation of a size determination method for drug products comprised of nano-particles by DLS and focuses on the following validation parameters: 1) Linearity, 2) Robustness, 3) Specificity, 4) Reproducibility, 5) Intermediate Precision and 6) Limit of Detection.[5]

In order to evaluate specificity, samples of emulsions and dispersed solid particles were characterized using both DLS and laser diffraction techniques. Robustness was evaluated by monitoring the effects of dispersant electrolyte concentration, sample concentration, measurement duration and temperature.[6] Linearity and reproducibility were determined using certified sizing standards, and intermediate precision was evaluated using a second analyst. LOD was determined by sample concentration and correlating it with attenuation index, turbidity and transmittance.

RESULTS

Linearity

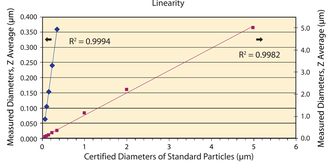

Fig. 1: Measured diameters of standard particles are plotted against the certified values. A good correlation (R^2 >0.999) is seen for the particles in size range of 60 nm to 350 nm and R^2 of 0.998 for range of 60 nm to 5 µm. However, large deviation (≥10%) from the standard value is observed for the large particles.

Robustness

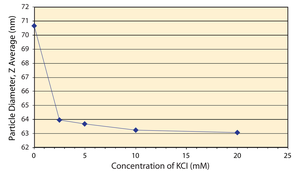

Fig. 2: Influence of an electrolyte in the media (KCl) on the measured diameters for standard latex particles of 60 nm.

Robustness

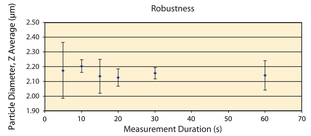

Fig. 3: Effect of measurement duration on 2 µm standard particles. The error bars indicate variation of ±1 standard deviation in the measured diameters.

Robustness

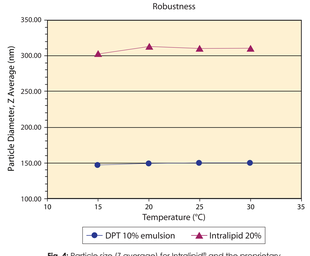

Fig. 4: Particle size (Z average) for Intralipid® and the proprietary nano-emulsion were measured at different temperatures. Data show that, for both products, particle size is not influenced by the sample temperature in the range of 15-30°C.

Reproducibility

Table 1: Data given in the Table shows particle size (Z average, nm), polydispersity index and mean intensity peak (nm) for 100 nm standard particles. Measurements were made on triplicate sample preparations with six replicate measurements per sample preparation. Average, median, standard deviation (SD), percent relative standard deviation (% RSD), minimum and maximum values determined from the 18 measurements are shown in the Table.

	Z Average (nm)	Polydispersity Index	Mean Intensity Peak (nm)
Standard Particles of 100 nm	104	0.006	104.7
	103.3	0.003	104.1
	103.8	0.015	104.1
	102.7	0.002	103.5
	103.9	0.006	104.8
	103.5	0.007	104.7
	104.4	0.006	105.1
	102.6	0.011	103.8
	104.3	0.032	104.2
	104	0.015	104.3
	103.9	0.011	104.4
	104.2	0.017	104.4
	103.4	0.005	104.7
	103.6	0.013	103.9
	102.6	0.011	103.9
	103.2	0.004	103.9
	103.5	0.012	104.9
	103.8	0.016	105.6
Average	**103.6**	**0.011**	**104.4**
Minimum	**102.6**	**0.002**	**103.5**
Median	**103.7**	**0.011**	**104.4**
Maximum	**104.4**	**0.032**	**105.6**
SD	**0.55**	**0.01**	**0.53**
% RSD	**0.53**	**66.60**	**0.51**

Intermediate Precision

Table 2: Particle size (Z average, in nm) data for Intralipid® obtained by two analysts is shown.

	Analyst I	Analyst II
	Particle Size (nm)	
Measurement of Intralipid®	295.8	312.4
	296.7	316.2
	300.9	315.7
	295.8	314.6
	296.7	314.7
	300.9	313.8
Average	297.8	314.6
SD	2.4	1.4
% RSD	0.8	0.4
Pooled Average	306.2	
Pooled SD	8.97	
% RSD	2.93	

Limit of Detection

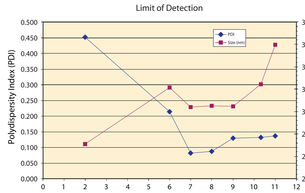

Fig. 5: Trend of polydispersity index and particle size with the attenuator (inversely related to the sample concentration) for Intralipid®

Limit of Detection

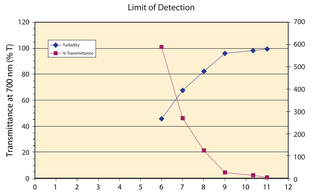

Fig. 6: The trend shows that for Intralipid® with increasing attenuator, transmittance of light increases while turbidity decreases. Both transmittance and turbidity reach equilibrium for attenuators 9-11.

Limit of Detection

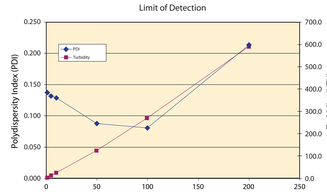

Fig. 7: The graph shows dependence of polydispersity index (PDI) and turbidity on the concentration of the sample of Intralipid®

Limit of Detection

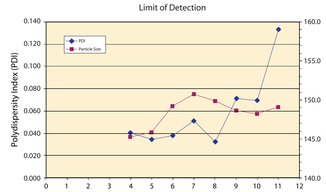

Fig. 8: Trend of polydispersity index and particle size with the attenuator (inversely related to the sample concentration) for a nano-emulsion.

Limit of Detection

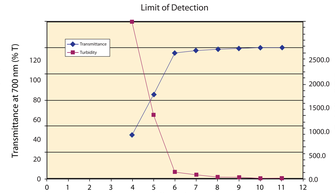

Fig. 9: The trend shows that for the nano-emulsion, with increasing attenuator, transmittance of light increases while turbidity decreases. Both transmittance and turbidity reach equilibrium for attenuators 6-11.

System
Temperature (°C): 25.0 — Duration Used (s): 20
Count Rate (kcps): 332.9 — Measurement Position (mm): 4.65
Cell Description: Disposable sizing cuvette — Attenuator: 8

Results

	Size (d.nm)	% Intensity	Width (d.nm)
Z Average (d.nm): 315.8	Peak 1: 344.3	100.0	92.41
PdI: 0.070	Peak 2: 0.000	0.0	0.000
Intercept: 0.924	Peak 3: 0.000	0.0	0.000
Result Quality: Good			

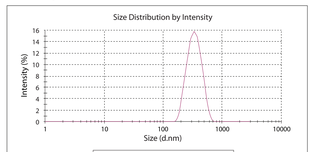

Fig. 10: At an appropriate dilution, Intralipid® showed a single sharp peak with low polydispersity index (0.07) and attenuator of 8.

System
Temperature (°C): 25.0 — Duration Used (s): 20
Count Rate (kcps): 237.0 — Measurement Position (mm): 4.65
Cell Description: Disposable sizing cuvette — Attenuator: 11

Results

	Size (d.nm)	% Intensity	Width (d.nm)
Z Average (d.nm): 390.9	Peak 1: 353.0	90.0	76.95
PdI: 0.203	Peak 2: 2528	9.0	183.5
Intercept: 0.864	Peak 3: 0.000	0.0	0.000
Result Quality: Refer to quality report			

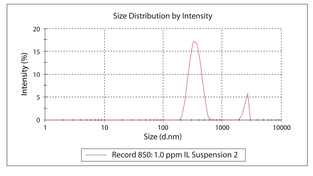

Fig. 12: A very dilute sample of Intralipid® showed two peaks and high polydispersity index (0.2) at attenuator of 11, suggesting that the sample concentration is too dilute for accurate size analysis.

Specificity

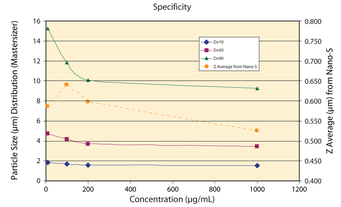

Fig. 14: Comparison of size distribution data obtained from Mastersizer and that from the Nano-S for Kaolin.

System
Temperature (°C): 20.0 — Duration Used (s): 70
Count Rate (kcps): 230.9 — Measurement Position (mm): 0.85
Cell Description: Disposable sizing cuvette — Attenuator: 2

Results

	Size (d.nm)	% Intensity	Width (d.nm)
Z Average (d.nm): 277.1	Peak 1: 479.5	89.8	240.4
PdI: 0.452	Peak 2: 71.68	8.5	21.55
Intercept: 0.743	Peak 3: 22.25	1.7	4.240
Result Quality: Good			

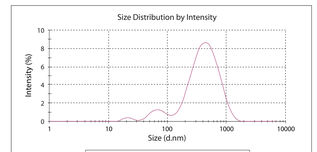

Fig. 11: A very concentrated sample of Intralipid® showed multiple peaks with high polydispersity index (0.45) at attenuator of 2, suggesting that the sample concentration is too high for accurate size analysis.

System
Temperature (°C): 25.0 — Duration Used (s): 20
Count Rate (kcps): 78.3 — Measurement Position (mm): 1.25
Cell Description: Disposable sizing cuvette — Attenuator: 11

Results

	Size (d.nm)	% Intensity	Width (d.nm)
Z Average (d.nm): 3933	Peak 1: 2478	100.0	210.9
PdI: 0.984	Peak 2: 0.000	0.0	0.000
Intercept: 0.124	Peak 3: 0.000	0.0	0.000
Result Quality: Refer to quality report			

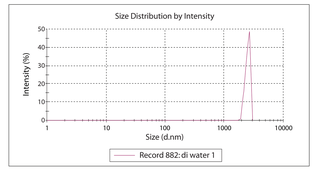

Fig. 13: A sample of deionized water showed polydispersity index of 0.98 and attenuator of 11, suggesting that when attenuator of 11 is reached, sample dilution is too high to accurately measure the size.

System
Temperature (°C): 20.0 — Duration Used (s): 70
Count Rate (kcps): 197.7 — Measurement Position (mm): 4.65
Cell Description: Disposable sizing cuvette — Attenuator: 7

Results

	Size (d.nm)	% Intensity	Width (d.nm)
Z Average (d.nm): 636.0	Peak 1: 305.7	66.0	47.37
PdI: 0.560	Peak 2: 2617	33.0	113.6
Intercept: 0.887	Peak 3: 0.000	0.0	0.000
Result Quality: Refer to quality report			

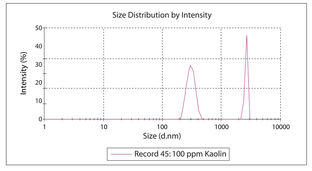

Fig. 15: A sample of 100 ppm Kaolin showed multiple peaks and high polydispersity index of 0.560.

MATERIALS AND METHODS

1. Particle Size Analyzer: Zetasizer Nano S (ZEN 1600) and Mastersizer 2000 (both from Malvern Instruments, Westborough, MA).
2. Turbidimeter, 2100N (Hach Company, Loveland, CO) and UV Spectrophotometer (UV-1700, Pharma Spec, Shimadzu Scientific Instruments, Columbia, MD).
3. Suspensions of Standard Particles of 60 nm to 5 µm diameter (Thermo Scientific Inc., Fremont, CA).
4. Intralipid® 20% (Baxter Healthcare Corp., Deerfield, IL) and Proprietary Nano-emulsion.
5. Kaolin Colloidal Powder (hydrated aluminum silicate), USP (Mineral and Pigment Solutions Inc., South Plainfield, NJ).

DISCUSSION

Specificity of the method was evaluated by using different types of particles, including nano-emulsions and suspended particles. Attenuation index data from the Zetasizer was correlated to the turbidity of the sample and transmittance at the visual wavelength range of 600-700 nm. The studies indicated that the size of standard nano-particles determined by dynamic light scattering technique showed out-of-specification-high results in the dispersant without a strong electrolyte, whereas in the presence of an electrolyte the size data passed the specifications, suggesting that the presence of small amounts of strong electrolyte is essential for accurate size measurements. This observation is consistent with previously reported data.[7] Results obtained for standard particles >1 µm showed >10% deviation from certified value, indicating that additional sample manipulation may be required, including density matching.[8]

CONCLUSIONS

- Data showed that measured value of diameters of standard particles was linear in the range of 60 nm to 350 nm; however, for particles of ≥1 µm, deviation of >10% from the certified value is observed, suggesting that the method is not suitable for large particles.
- The concentration of the particles in suspension is critical for accurate measurement of size by dynamic light scattering.
- A sample of pure deionized water showed the presence of particles, suggesting that it is critical to review other parameters, including polydispersity index and attenuation index, when interpreting the data obtained from the instrument.
- The dynamic light scattering technique is not suitable for the particles that have a tendency to settle. For the product comprised of particles of >1 µm, it is important to study effect of "measurement duration" during method development.
- The study indicates that particle size determination method using a dynamic laser light scattering technique can be validated in accordance with the FDA guidelines.

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