HORIBA Scientific

Application Note

Wet Method Development
AN151

Wet Method Development for Laser Diffraction Particle Size Measurements

Developing an appropriate testing method for measuring particle size distribution in liquids using laser diffraction requires a structured approach. A number of parameters of the test method must be evaluated including sampling, solvent, refractive index, dispersion, system settings, and concentration. These parameters may have a significant effect on the reported results, so each must be carefully evaluated.

Introduction

Developing an appropriate method for measuring particle size distribution in liquids using laser diffraction requires a structured approach. Samples analyzed as liquid dispersions include suspensions, emulsions, and solids dispersed in liquid. These three general categories of samples have a few unique considerations, but the majority of the content in this document will be applicable to all.

Suspensions and emulsions can sometimes be easily measured using the continuous phase of the original sample as the diluent in the analyzer. Proper dispersion of powders into a liquid may require the additional efforts of wetting and stabilization.

In all cases the basic goals remain the same; decide what the purpose of the measurement is, place a representative sample into the analyzer, disperse the sample in a liquid that does not dissolve or alter the particles, choose appropriate system settings for the measurement, and test for repeatability and reproducibility.

Sampling

It is rare that the entire sample brought into the lab is measured in the instrument. More typically a sub-sample of the total is analyzed, creating the need to consider the sampling technique.

Several references (1,2) can provide both background information and practical suggestions on proper sampling techniques. Too many scientists simply shake a suspension or tumble a powder sample and then remove a portion for analysis. Ignoring the sampling component of the method is inappropriate for several reasons:

- Accepted standards stress the importance of sampling. ISO 13320 (3) and USP <429> (4) both advise that a representative sample be prepared using a sample splitting technique. (5)

- One of the goals of proper method development is to minimize the total error. If sampling is ignored the developer doesn't know which portion of the total error comes from the sampling.

Care must be taken with sampling when dispersing a powder into a liquid. Many methods call for pre-dispersing the powder in a beaker and then pipetting the sample into the analyzer. When following this approach it is better to mix a concentrated paste in the beaker in order to minimize sampling bias during the transfer to the analyzer.

Choosing the Solvent (diluent)

Suspensions and emulsions can sometimes be easily measured using the continuous phase of the original sample as the diluent in the analyzer. When powders are dispersed in liquid the solvent must meet the following criteria:

- Negligible reactivity with powder, defined as:
  - Does not swell or shrink particles by more than 5% in diameter.
  - Solubility must be less than 5g powder in 1 kg liquid
- Have a refractive index (RI) different than the sample
- Be free from bubbles & particles
- Have suitable viscosity to enable recirculation
- Be compatible with materials in the analyzer

Dispersing powders in liquid can often present challenges. The ISO 14887 standard (6) provides useful insight into this realm. Among the suggestions in ISO 14877 is to prepare the sample on a slide and look at it under a microscope. Determine if you are looking at individual particles or clumps. See if exposing the sample to an external ultrasonic probe eliminates the clumps.

Surfactants are often required to wet the powder for proper dispersion. ISO 14887 provides a comprehensive listing of commercially available dispersing agents. The HORIBA applications lab makes frequent use of many surfactants including Micro 90 solution (also good for cleaning the instrument), Triton X-100, Igepal CA630, Tween 80 and lecithin.

Once a powder wets, it sometimes helps to add a stabilizer (or admixture) to the sample, such as sodium hexametaphosphate. The stabilizer alters the charge on the surface of the particles, avoiding re-agglomeration.

Stability Testing

After the dispersing liquid or mixture has been chosen, test the system for stability by measuring multiple times as a function of time. Measuring the recirculating sample should generate extremely reproducible results.

Measure the sample at least three times over a time frame of several minutes. Check the coefficient of variation (COV) for the multiple runs and question data when the COV > 1% at the D50.

A steady decrease in particles size alongside an increase in transmission may indicate dissolution. An increase in particle size may indicate agglomeration or swelling. Random variations are more difficult to interpret but could arise from thermal fluctuations or poor mixing.

Determine the Refractive Index

Although this document will not address this subject in detail, it is important to enter an appropriate refractive index (RI) value for the sample and diluent. Methods to obtain the sample RI include literature and internet searches, use of an Abbe refractometer, RI matching liquid and Becke line testing, and sending samples to third party laboratories.

Choosing an imaginary RI value can be facilitated by varying the imaginary component until the residual R value calculated in the LA-960 software is minimized. It is better to determine the RI early in the method development process in order to understand the general shape of the distribution before conducting other tests.

Effect of Ultrasound

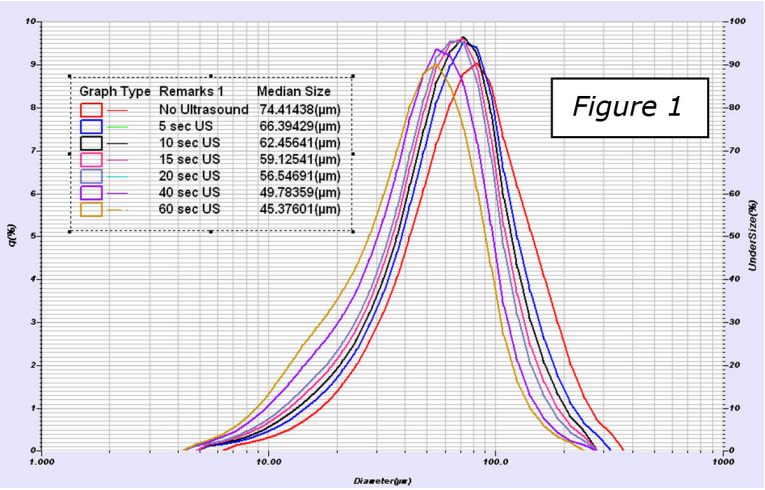
Ultrasound can be used to add energy into the system in order to disperse agglomerates into individual particles. Many HORIBA liquid samplers include an internal ultrasonic probe for this function. Difficult samples may require the use of a high energy external probe.

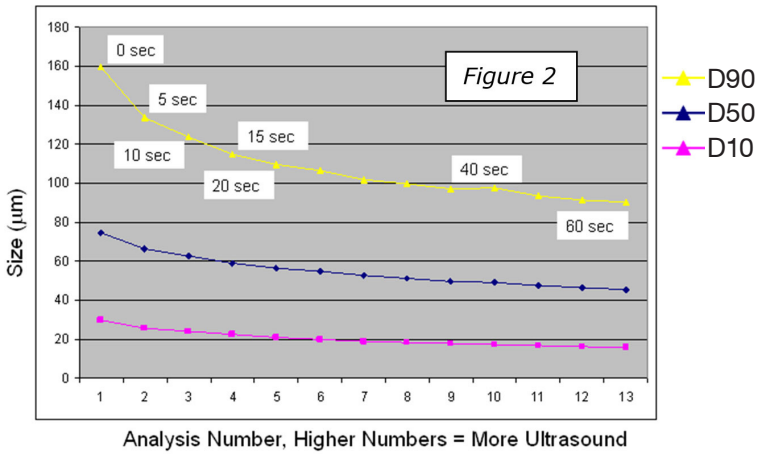
Either approach requires a study of the effect of ultrasound on measured particle size distribution. This is typically accomplished by exposing the sample to ultrasound for varying lengths of time. As an example, the method developer could follow this protocol:

- Analyze sample prior to adding ultrasound.
- Turn on the ultrasonic probe in the sampler for 5 seconds
- Turn off the probe and analyze the sample.
- Turn on the probe for 5 more seconds – turn off and measure again.
- Repeat this procedure until either the results begin to stabilize or an indication of particle breakage is observed.
- Note: the 5 second interval of ultrasound may vary depending on the sample sensitivity to additional energy input.

Performing this kind of study will generate the data required to make an informed decision on how to best use ultrasound. A plot of particle size vs. exposure time to ultrasound will hopefully guide the method towards the proper amount of exposure required to disperse the sample without causing breakage of the particles.

Figures 1 and 2 below depict how results varied when microcrystalline cellulose (MCC) was exposed to ultrasound within the LA-960.





Sample Concentration

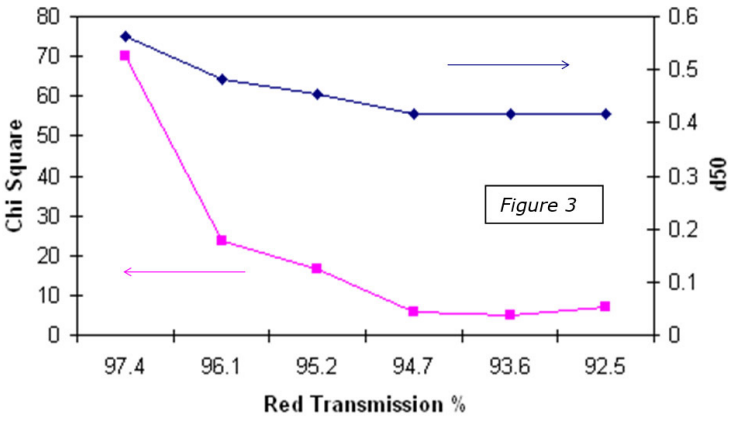
The instrument will report concentration in terms of percent transmission: the percent of the incident laser beam passing through the systems with the sample added. The transmission is typically kept in the range of 95 – 80%, but can vary depending on the sample.

Smaller particles are often measured at higher transmission levels in order to avoid multiple scattering. Larger particles can be measured at lower transmission levels to improve the sample quantity (number of particles) analyzed, thus improving the precision.

A plot of D10, D50, and D90 vs. transmission should indicate any sensitivity to concentration. A decrease in reported particle size as transmission decreases may indicate the onset of multiple scattering, especially with submicron samples. An increase in particle size as transmission decreases could indicate the need to measure more sample in order to analyze the entire distribution of a broad sample.

The Chi Square calculation is an indicator of the quality of a measurement. This value may also help determining the minimum sample concentration (displayed as transmission).

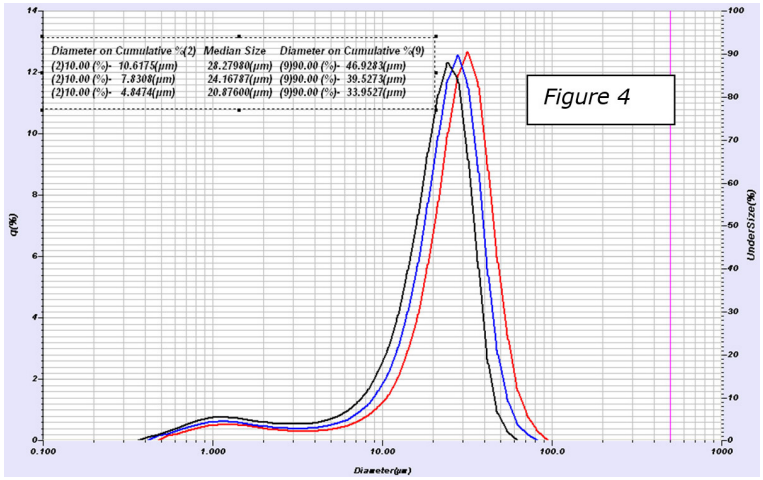
The data shown in Figure 3 below plots transmission of the red laser vs. Chi Square value on the left y axis and d50 on the right y axis for an emulsion sample. Once the red transmission reaches 94.7% both the Chi square and the d50 values stabilize, indicating an appropriate concentration has been reached.



System Settings

The pump and stirrer speeds for the liquid sampler may also have an effect on results and need to be investigated during the method development process. The goal is to keep the system well mixed so that all of the particles are analyzed while keeping the agitation below the level that creates bubbles or entrains air.

Large, heavy particles require higher pump and stir speeds. More gentle agitation may be better for samples containing surfactant in order to avoid bubble formation. Figure 4 below shows how the results vary for a dense sample when the stirrer/agitation settings change from 8 (red) to 4 (blue) to 2 (black).



Check Reproducibility

Once all of the important components of the method have been established it is time to check for reproducibility. Both ISO 13320 (3) and USP <429> (4) establish reproducibility goals at the d10, d50, and d90 based on the coefficient of variation (COV, the standard deviation/mean) for multiple measurements as described below:

Standard	COV@d50	@d10&d90
ISO13320	<3%	<5%
USP<429>	<10%	<15%

Note: These limits can be doubled if the d50<10 µm

These calculations are now automated in the LA-960 software, facilitating the process of both method development and daily pass/fail determination. Table 1 shows the results and COV calculations for microcrystalline cellulose (MCC) measured six times on the LA-960 using 15 sec of ultrasound for dispersion.

File Name	Sample Name	d50	d10	d90	Comment
200707161438232.NGB	Avicel PH-101	59.13	22.22	114.93	15 sec US
200707161500243.NGB	Avicel PH-101	60.13	22.66	119.12	15 sec US
200707161507246.NGB	Avicel PH-101	59.89	22.39	117.45	15 sec US
200707161516249.NGB	Avicel PH-101	60.42	22.96	119.97	15 sec US
200707161523252.NGB	Avicel PH-101	60.19	22.77	117.29	15 sec US
200707161531255.NGB	Avicel PH-101	59.98	22.83	116.59	15 sec US
Mean		59.96	22.64	117.56	
Standard Deviation		0.45	0.28	1.80	
COV (SD/mean)*100		0.74	1.23	1.53	

Conclusions

A systematic and comprehensive approach should result in a reproducible and robust method for wet particle size analysis. Selecting appropriate surfactants and diluents may involve trial and error for unknown samples. Optimizing other parameters such as sampler settings, concentration, and amount of ultrasound isn't difficult following the procedures described in this application note. In addition, the HORIBA support team is ready to help customers create methods for their samples.

References

1. T. Allen, Particle Size Measurement, Chapman and Hall, 4th Edition, 1993

2. ISO 14488, Particulate materials -- Sampling and sample splitting for the determination of particulate properties, available at webstore.ansi.org

3. ISO 13320, Particle size analysis -- Laser diffraction methods -- Part 1: General principles

4. USP <429> Light Diffraction Measurement of Particle Size, USP30, NF25

5. For sample splitting devices see www.retsch.com or www.quantachrome.com

6. ISO 14887, Sample preparation – Dispersing procedures for powders in liquids