



UTILIZING USP <429> "LIGHT DIFFRACTION MEASUREMENTS OF PARTICLE SIZE"

Pharmaceutical companies are beginning to refer to the USP <429> test as a guideline when using laser diffraction for particle size analysis. This document provides useful background material and offers specific advice on testing reproducibility and verifying accuracy using polydisperse standards.

Introduction

Laser diffraction has become the most widely used particle characterization technique in the pharmaceutical industry. The USP <429> test is the first attempt by this industry to provide guidance on using this technique. The origin of USP <429> begins with the ISO 13320 standard "Particle size analysis — Laser diffraction methods — Part 1: General principles" (1).

From there, the EP (the lead group for this effort) published test EP 2.9.31 "Laser Diffraction Measurement of Particle Size". The first appearance in the US came in Pharmacopeial Forum 28, Number 3 2002. The method is now published in USP 28, NF 25 and is in stage 4 of the harmonization process as of mid-2007.

This application note attempts to help pharmaceutical companies plan to incorporate USP <429> into their current and future particle size analysis methods.

Preparation of the Sample

The statement "Prepare a representative sample of suitable volume for the measurement by an adequate sample splitting technique." conveys a bigger message than might be apparent.

The standard practice in many pharmaceutical labs for powder samples is to tumble the bottle several times and then scoop some out. For a narrow particle size distribution this

may work in practice, but proper sampling will be critical for other samples with a broader distribution and a greater tendency to segregate.

Several references (2, 3) can provide guidance on proper sampling techniques for particle size measurements and vendors offer sample splitting devices (4, 5) such as the spinning riffler shown in Figure 1. The effect of sampling should be investigated for any particle size analysis method and steps should be taken to minimize the error from this component of the total procedure.



Figure 1

The USP <429> properly suggests that "The dispersion procedure is adjusted to the purpose of the measurement: for example, whether agglomerates should be detected or broken down to primary particles." During method development the challenge is often to disperse the agglomerates to the primary particle size without fracturing individual particles.



When measuring powders in the dry state this involves analyzing the sample at multiple pressures and interpreting the size vs. pressure results. For suspensions the use of ultrasound requires a systematic study of the effect of energy added to the system vs. reported particle size.

Comparison of wet vs. dry results for powders as suggested in ISO 13320 is another useful technique for selecting appropriate levels of dispersion. Excellent advice on dispersing powders into liquids can be found in ISO14887 "Sample preparation – Dispersing procedures for powders in liquids."

Concentration

The concentration level in any sample must be high enough to provide an adequate signal to noise ratio, but low enough to avoid multiple scattering. These levels are specific to both system used and the size of the particles analyzed.

When using the HORIBA *Partica* LA-950 analyzer (Figure 2), optical transmission levels through the measurement cell should typically be between 80 – 95%. The transmission should be higher for samples with submicron populations since these experience multiple scattering at lower concentrations. The effect of concentration should be part of any method development effort.

Selection of an Appropriate Optical Model

Most experts agree that Mie theory should be used for all samples analyzed using laser diffraction, not just those below 25 μm as suggested in USP <429>. It is easy to create a new refractive index (RI) file in the LA-950 software, or a default value around 1.60 and 0.01 is a good starting point for a white powder.

Setting a separate value for the Blue Index is not required for anything other than submicron samples showing significant wavelength-dependence of RI values. Various methods for determining the RI are available including testing at outside laboratories, so lack of RI data should never be an excuse to not use Mie theory.



Figure 2
HORIBA Partica LA-950



Replicates

Particle size measurements should always be replicated to look at data precision. USP <429> calls for analyzing three samples from the same batch and then calculating the coefficient of variation (COV = standard deviation/mean, also called RSD) for the measurements.

In order to pass the test expectation the COV at the D50 must be less than 10% and less than 15% at the D10 and D90. These values can be doubled when the D50 is less than 10 µm, presumably taking into account the greater degree of difficulty with dispersion for smaller particles.

Results from replicate measurements of magnesium stearate analyzed as a dry powder on the LA-950 are shown in Figure 3. Note the extremely low COV values. The LA-950 software can automatically calculate the COV for replicated measurements and apply the pass/fail criteria suggested in USP <429>. Users can also select the pass/fail criteria suggested in ISO 13320, or create their own criteria.

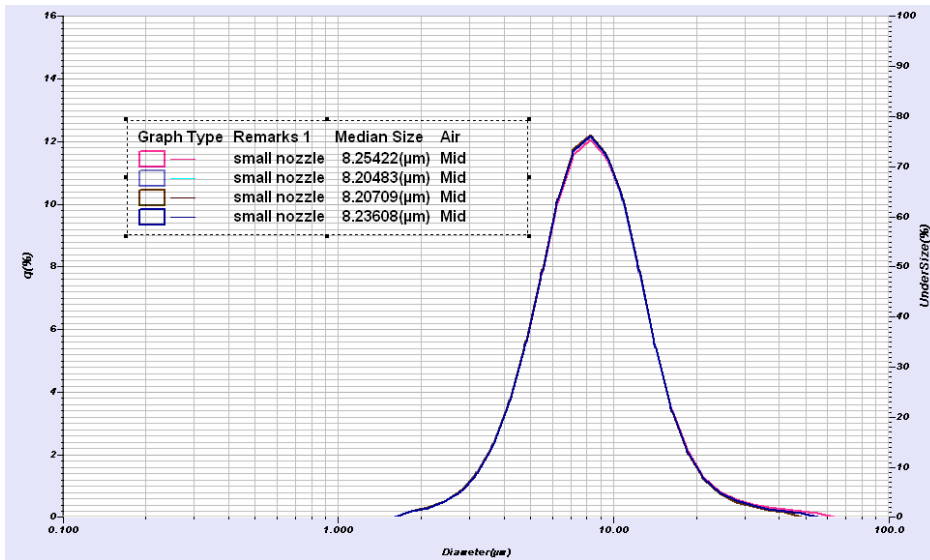


Figure 3 – Replicate Measurements

		D50	D10	D90
200707180858106.NGB	Mg Stearate	8.254	4.58	14.898
200707180859107.NGB	Mg Stearate	8.205	4.568	14.678
200707180900108.NGB	Mg Stearate	8.207	4.579	14.583
200707180901109.NGB	Mg Stearate	8.236	4.595	14.722
Mean		8.226	4.581	14.720
Standard Deviation		0.024	0.011	0.132
COV (st dev/mean)*100		0.288	0.242	0.896

**Qualification, Accuracy and Repeatability**

Laser diffraction systems are not calibrated, but a regular verification test is highly recommended. No time frame between verification tests is suggested in USP <429> but industry practice ranges from weekly to annually.

The verification test described in USP <429> calls for using a polydisperse, spherical standard (5) such as those used in the LA-950 OQ procedure. Three independent measurements (separate preparations) are performed and subjected to the following pass/fail criteria:

- Accuracy: the measured result must not deviate from the expected value by 3% at the D50 and 5% at the D10 & D90.
- Repeatability: The COV for the D50 < 3%, D10 & D90 < 5%.

Although these values can again be doubled when measuring below 10 μm , there are few standards in that size range and they are more challenging to disperse.

The LA-950 software includes routines that will automatically apply the USP<429> pass/fail criteria for both accuracy and reproducibility.

Conclusion

Many pharmaceutical companies will begin to use USDP <429> as a guideline for making particle size measurements using laser diffraction. Hopefully more methods will include procedures for minimizing sampling errors.

Following the USP <429> guidelines for sample preparation provides a basis for more robust method development. Checking measurement reproducibility is always worthwhile, and is now automated in the LA-950 software. Regular system verification is highly recommended and is now also automated to facilitate this procedure.

References

1. ISO 13320, Particle size analysis -- Laser diffraction methods -- Part 1: General principles.
2. T. Allen, Particle Size Measurement Chapman and Hall 4th Edition 1993
3. ISO 14488, Particulate materials -- Sampling and sample splitting for the determination of particulate properties.
- 4, 5. See www.retsch.com and www.quantachrome.com

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