

# A Guide to Determination of Particle Size – Making an Effective and Reliable Measurement

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## Introduction

When any material of one phase is dispersed into a second phase, an “interface” is created between the two phases (1). The *total extent* of this interface, which will depend on exactly how the dispersion is made (2), controls the fundamental behavior (stability and structure) of the dispersion. And, it applies equally to particles in air as to particles in liquids.

The overwhelming majority of manufactured industrial products and increasingly those for health-care purposes involve, either in the final state or at some stage of their production, suspensions of particulate materials; indeed, nanoparticulate dispersions are typically created *in situ*. The importance of the process of dispersion and its profound effect on the economics and quality of the subsequent product has long been recognized (3, 4). Thorough characterization of particulate suspensions is, therefore, paramount in obtaining optimum performance features and cost effective benefits from such systems.

## The Importance of Particle Size

The simplest, most straightforward way to characterize the interfacial extent is by the measurements of particle size (PS) and size distribution (PSD) and, sometimes in addition, particle shape (and morphology). The PSD affects properties of suspensions in many important ways. For example, in industrial applications it determines the setting time of cement, the hiding power of pigments, the activity of catalysts, the taste of food and the sintering shrinkage of metallurgical compositions.

In the pharmaceutical industry the PS/PSD of suspensions of active pharmaceutical ingredients (API) is known to strongly affect the stability and aesthetics of the formulation. Importantly, the PS/PSD directly impacts performance behavior characteristics such as total bioavailability, dissolution rate (5) as well as toxicity. In addition, regulatory agencies are becoming increasingly aware of the importance of the PSD and are requiring developers to have a greater control and understanding of this aspect of their drug products.

Because of perceived concerns regarding the safety of so-called nanoparticles (NP), especially because of a less than well-informed public and organizations with their own agendas, regulatory bodies both in the USA [[www.fda.gov/nanotechnology/](http://www.fda.gov/nanotechnology/)] and the EU [[ec.europa.eu/nanotechnology/](http://ec.europa.eu/nanotechnology/)] have recently presented “guidelines” regarding the manufacture, the use and the application, of “nanotechnology” as it relates to cosmetics, personal care and pharmaceuticals. Hence, it is absolutely critical that a rigorous measurement and analysis of PS/PSD be carried out.

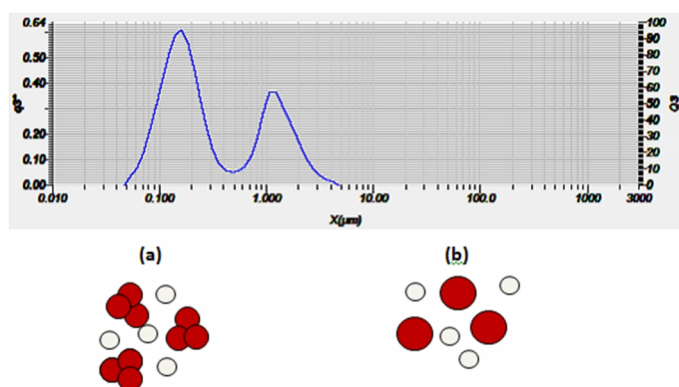
## Choosing a particle size analyzer

Choosing the correct device or technique for a given sizing need is not easy (6). There are a host of commercially available instruments and the sales literature claims of specification and performance have become highly inflated. The idea that one single instrument will suit every particle sizing need, and hence solve all problems, is simply not supported in practice.

Unfortunately, the various particle size analysis techniques can be applied inappropriately. A simple example can be used to illustrate the problem facing

the analyst. Consider, as shown in Figure 1, a bimodal, volume-weighted, distribution determined using a non-imaging, ensemble averaging device, such as Fraunhofer Diffraction (FD) or Dynamic Light Scattering (DLS). There is no way to determine if the modes arise from **(1a)** singlet particles plus a second fraction comprising agglomerates of those particles, or **(1b)**, a simple mixture of two different fractions of singlet particles each of a different size.

**FIGURE 1:** A bi-modal, differential, volume-weighted PSD



Microscopy (optical, TEM and SEM) is considered a primary technique for size analysis; it is used in conjunction with image analysis for size and shape distributions. While visual microscopy/image analysis can easily distinguish between a mixture of two separate modes and agglomerates, unfortunately, these latter techniques are only applicable to sizes greater than 500 nm. The presence of any smaller sizes will be completely missed (not detectable) and so the analyst might erroneously assume 100% of the material's particle sizes have been measured. It is also often overlooked that image analysis requires calibration.

Particle size analysis techniques are often misused because of a lack of understanding of their underlying principles. The theoretical basis for many of the "classical" and "modern" techniques has been extensively reviewed (7-9) and an excellent practical guide has been published by NIST (10). A study of methods used for PS/PSD analysis of API powders is available (11) as well as a paper that addresses the

use of FD to size sub-micron API particles and highlights the problems involved (12).

## The Pathway to an Effective Particle Size Measurement

When sizing an unknown material it is necessary to ask, and then respond to, very general questions and so, ultimately, create a "flow chart" of how to approach various scenarios where there is reason to suspect what the different particle size analyzers may (or may not) be telling the analyst. In this current paper, the range of techniques will be limited to: Visual microscopy/image analysis Ensemble averaging devices based on light scattering (FD and DLS) Disc centrifugation (XDC and DCP)

These techniques are fairly widely employed in industrial, personal care, food, agricultural and pharmaceutical applications.

### General statements

1. There is no substitute for a single particle counting device when absolute concentration as a function of size is required. And, with few exceptions, when shape information is required, there is no substitute for an image analyzer.
2. Many real-world particles (especially crystalline materials) are far from round or uniform. All non-imaging techniques (such as the light scattering devices) yield equivalent spherical diameters (ESD). This is the diameter of a sphere that would give the same result as the actual particle; it may not correlate with any single dimension of the particle. Different techniques may yield a very different ESD for the same particle; the more asymmetric the particle, the bigger can be the difference in ESD (13).

## The Pathway

### STEP 1: VISUAL OBSERVATION!

Is the sample a dry powder or a suspension?

**1A:** If a dry powder, is it amorphous (A) or crystalline (C)?

If A: The particle size distribution (PSD) will be comprised of small primary particles and larger aggregates and agglomerates all with indistinct features.

If C: The particles will have distinct features, e.g. needles, plates, cuboids....

Gently pour a sample out and observe if there are any airborne “fines”. These may be lost in any subsequent handling of the material (e.g. in the preparation of a liquid suspension).

**1B:** If a dispersion, do the particles settle? Yes, or No?

If YES: The particle size (PS) is large OR the particle size is small but the material is dense (unlikely with most API), or both.

How long for the suspension to settle? In minutes or in more than one hour?

If minutes or less: The PS is extremely large, OR the particles are very dense.

If more than one hour: The PS will be very small OR the particles are large but have a density almost that of the suspension fluid (see Appendix 1).

If NO: It is fairly certain that the particle size is submicron.

Is the supernatant clear, or is there a “haze”?

If clear: There are no submicron particles OR their concentration is very small.

If hazy: It suggests the presence of (potentially) submicron size particles.

*In all cases, retain a sample of the supernatant for STEP 4B.*

### STEP 2: MICROSCOPIC EXAMINATION

**2A:** With dry crystalline material, observe the *shape* of the particle.

*Note:*

- i. The aspect ratios of rod- and needle-like particles (the ratio of length to width),
- ii. The appearance of jaggedness (particles that have sharp projecting points - “saw shaped”)
- iii. Any fractal nature of aggregated particles (where they show similar repeating patterns of the aggregate shape – like a “snowflake”).

Examine the material under both normal and polarized light. The latter will allow the analyst to more clearly discriminate between the different shape features.

Fraunhofer diffraction (also known as laser diffraction) devices cannot distinguish between these different characteristics of crystalline materials.

*Determine a preliminary PSD using Image Analysis. Estimate an approximate “size range” based on “rod length” or “maximum length”.*

**2B:** With suspensions, dilute the sample so that it is possible to discriminate between particles.

*As in 2A, determine a preliminary PSD using Image Analysis and estimate an approximate “size range”.*

Observe if there is any background “Brownian motion” (the background will appear to “shimmer”) which is indicative of submicron size particles.

**STEP 3:**

**3A:** For powder materials, now prepare a suspension (see Appendix 2) in a suitable fluid and then go to STEP 1B and then STEP 2B.

**STEP 4: INSTRUMENTAL PSD MEASUREMENT**

**4A:** Using *Fraunhofer Diffraction* (with, or without high angle detectors), measure the PSD of a sample suspension. *Observe if there is any fraction below one micron.*

For materials whose particle size extends to below one micron, the Refractive Index (RI) of the material (both the real and imaginary parts) and the RI of the medium are all needed for accurate results when making (Mie) light scattering corrections (see Appendix 3).

Check that these RI parameters are correct. *Note:* typically, the default RI settings (most commercial instruments) are for *polystyrene in water*.

Compare the PSD results from STEP 2 with that from STEP 4A. Do they appear consistent? Yes, or No?

If YES: Refer to STEP 1B and STEP 4B to confirm the presence, or absence, of sub-micron particles in the suspension supernatant.

*If there is no evidence of sub-micron sizes, then accept the PSD results.*

If NO: First question the FD results.

Even for materials where the shape is fairly regular, e.g., needle-like or plate-like, the PSD distribution from an FD measurement is smeared out. It is not possible to know the difference between that and a truly broad distribution of spheres. This is why the preliminary image analysis to see shapes and rough estimates of size range is important.

Check that the RI data is correct!

*If there is evidence of sub-micron sizes then go to STEP 4C.*

**4B: Dynamic Light Scattering**

In addition to non-settling suspensions, it is imperative that all supernatants from suspensions that do settle also be measured using DLS.

Check that the RI parameters are correct. *Note:* typically, the default RI settings (most commercial instruments) are for *polystyrene in water*.

**4C: Disc Centrifugation**

IF DLS measurement indicates submicron sizes and they are NOT seen in the FD measurement then it is necessary that the material suspension sample should be measured using Disc Centrifugation for validation (see Appendix 4).

A Schematic of the Pathway is given in Appendix 5.

**Summary:** Understanding and performing relatively simple steps prior to measurement will ensure more reliable PSD results. Care taken in sampling and sample preparation are as important as the instrument used for PSD determination.

**APPENDIX 1: Settling of Polystyrene latex**

Polystyrene has a density of 1.05 g/cc. Observations of settling for three NIST traceable “monodisperse” PSL after 24 hours are:

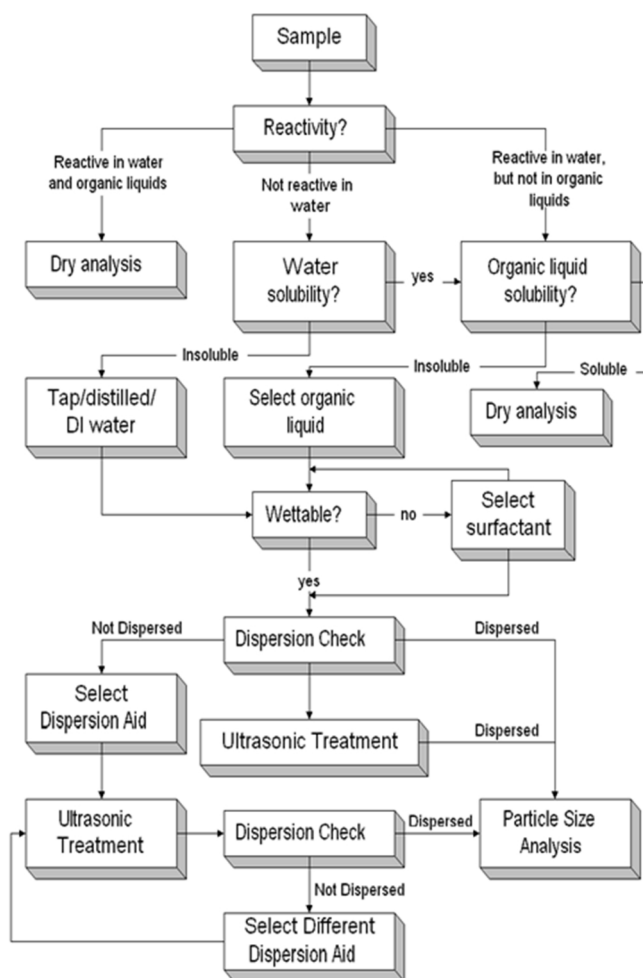
10 micron - completely settled; 2 micron - clear liquid in the top 20%-30%; 1 um - clear layer in the top 10%.

**APPENDIX 2: Preparation of Suspensions**

The formulation of any suspension requires that a stable (non-agglomerating) and *reproducible* PSD be achieved. The fundamentals of dispersion of powders in liquids are beyond the scope of this paper; such information can be sought from the

literature (2, 14-16). However, no matter what protocol is used it is imperative that it be consistent. Figure 2 is an *aide-memoir* for developing a suitable SOP.

**FIGURE 2:** Dispersing a Powder in a Liquid



### APPENDIX 3: The Refractive Index

A major, often overlooked, complication in light scattering devices is that for materials whose particle size is below a few microns, the Refractive Index (RI) of the material (both the real and imaginary parts) and the RI of the medium are all needed for accurate results when making the necessary (Mie) light scattering corrections (17). The effect is illustrated in Figures 3(a) and 3(b). With most commercial instruments, the typical default RI

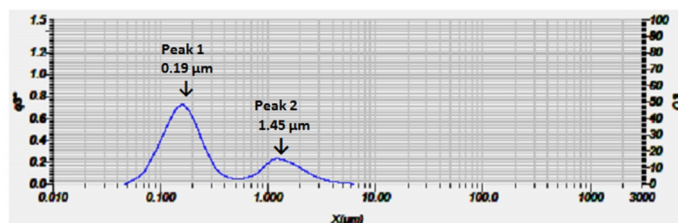
settings instruments are for *polystyrene in pure water* and Figure 3(a) shows a PSD determined for an aqueous API suspension using those values. However, the API in question was colored (yellow) and, moreover, the suspending liquid contained surfactants and polymers. Figure 3(b), in contrast, shows the PSD using the correct RI values and the differences in the PS data are summarized in Table 1.

Note:

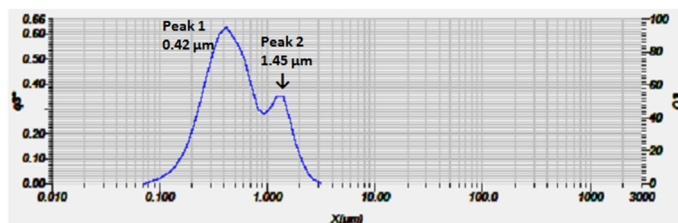
1. Not only does the “size” change (X-axis) but also the relative amounts at the given size (Y-axis).
2. The PSD is bimodal and so there are two values (peak 1 and peak 2) shown in the Table 1.
3. In both examples there is a minor fraction with a modal value at approximately 1.45 micron (peak 2) and this value is *not* affected by the change in RI.

**FIGURES 3(a) and 3(b):** Effect of Refractive Index on a PSD from a Fraunhofer Device (with high angle detectors)

**(a) Particle RI: Real 1.59, Imaginary 0.01; Liquid RI: 1.33**



**(b) Particle RI: Real 1.50, Imaginary 0.10; Liquid RI: 1.38**





**TABLE 1**

Comparison of PS data from Figures 3(a) and 3(b)

	Particle Size (microns)		
	Mean	Median	Modes
(3a)	0.53	0.16	0.19/1.45
(3b)	0.67	0.50	0.42/1.45

#### APPENDIX 4: Light Scattering Devices and Disc Centrifugation

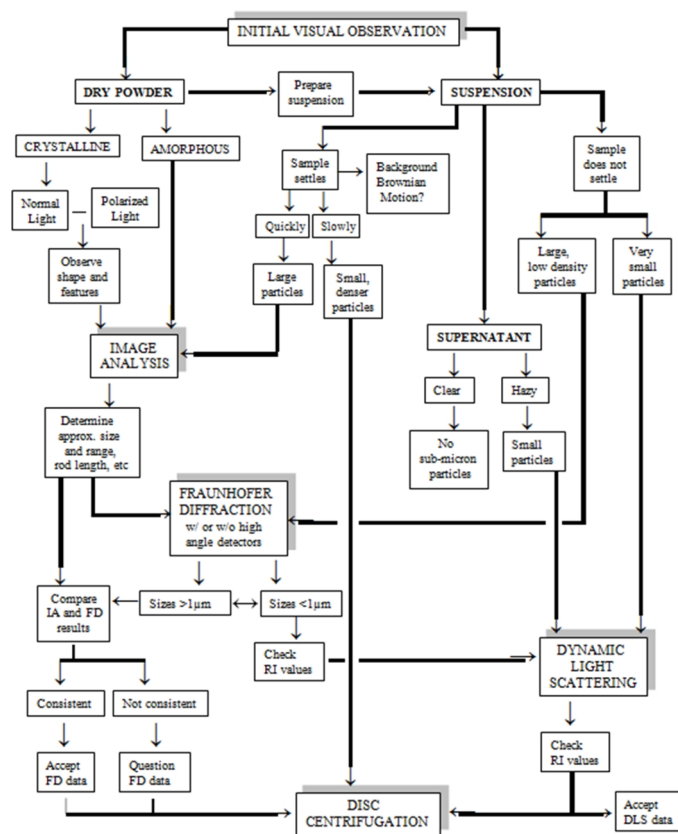
It is important to recognize that the light scattering devices, DLS and FD, are “ensemble averaging techniques”. What this means is that the raw data signal, from which the size distribution is calculated, is a sum over all the signals from all the particles during the entire measurement. Importantly, the particles are neither counted nor physically separated. Instead, over the duration of the entire measurement, particles of all sizes contribute to the final signals. Then the signals are mathematically deconvoluted, mathematically separated to produce size distribution information. The result then depends on the mathematical algorithms used, especially in the conversion of the raw data to give a volume-weighted PSD, as is necessary in pharmaceutical applications.

Disc centrifugation is a device that is based on centrifugal sedimentation. It fractionates according to size and density prior to size determination. The time to reach the detector is inversely proportional to the square of the particle diameter; there is no calibration. There are two variations of the technique. *DCP* devices are based on *photosedimentometry* and do require an optical correction in the form of the extinction coefficient (18). Such corrections become unnecessary when the detection is based on X-rays rather than visible radiation (*XDC*). X-rays are absorbed in direct proportion to the total mass of particles present. However, X-ray detection is not suitable for organic

materials (such as API) but is ideal for ceramics and metal oxides in general (19).

With both DCP and XDC devices, calculation of the volume-weighted PSD is more straightforward compared with the FD and DLS techniques. Importantly, for such devices even if a few of the larger particles are missed, the volume-weighted diameter is not much affected.

#### APPENDIX 5: The Pathway



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