Static Image Analysis of Size Standard

AN193

MEASURING 10 MICRON PSL ON THE PSA300

Jeffrey Bodycomb, Ph.D.

Static image analysis provides fast, accurate, and repeatable particle size and shape measurements of pharmaceutical API's, metal and oxide powders, and other materials. The PSA300 is a static image analysis instrument that is ideal for particle samples that are difficult to disperse or particles suspended in slurries or pastes. Here, a standard sample is characterized with the PSA300 in order to show the accuracy of the technique.

Figure 1: Static image analysis becomes turnkey with the PSA300.

Introduction

Particle image analysis refers to the collection of and computerized analysis of micrographs of particles to determine particle size and shape. Image analysis is an exciting technique for particle characterization because it can be used to determine a range of size and shape parameters. Since the images show data from each particle individually, number average properties can be obtained. Furthermore, high resolution size (and shape) distribution information is also available. For these reasons, image analysis has become more popular, particularly as increased computer power lowers the cost and time required for analysis and refined designs improve the user experience.

Polystyrene latex beads are a popular size standard for particle analysis since they are well characterized and relatively inexpensive. Thus, they are often the starting point of any instrument qualification process. In this note, we show the ability of the HORIBA PSA300 to characterize these beads to within close to theoretical limits.

First, I briefly review the expected uncertainty in bead size and how it is obtained. Then I briefly discuss the limits of the measurement technique. Finally, I review the measurement of these beads with the PSA300.

Manufacturer Characterization of PSL

Large latex beads are typically characterized by optical microscopy in addition to other techniques. Since these materials are spherical and have an extremely narrow size distribution, they can be characterized by the array method. In this method, an array of beads is created on the microscope slide. The length of this array is then determined. The number average bead size can then be readily obtained by dividing the array length by the number of beads in the array. This technique has the profound advantage of spreading any uncertainty of the bead edge location over many beads. That leads to significantly improved precision for the determined size [1].

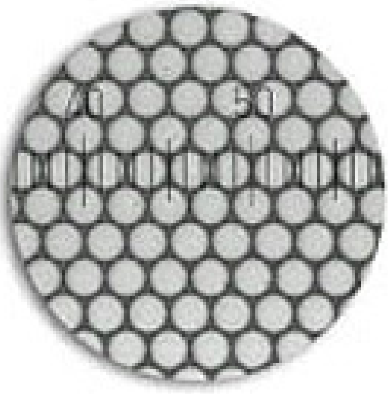

Figure 2: Polystyrene latex can be produced to have very narrow size distributions.

Of course, when characterizing real particles, the array technique is not appropriate since real particles are rarely spherical and can rarely be prepared as a neat array. Therefore, for this study, only individual beads were characterized, leading to a higher uncertainty. However, this higher value is useful since it represents a case closer to that of analysis of real, unknown samples.

The Diffraction Limit

Due to the wave nature of light, diffraction effects will affect measurements. These effects are trivially small and therefore ignored when considering macroscopic objects. However, in microscopy, they become important. One approach to mitigate this problem used in the PSA300 is to use short wavelength light. Even so, at the magnification used here (a 50x objective), diffraction limits resolution to about 0.5 microns. And this limit serves as a reasonable expectation of sizing resolution.

Materials and Methods

10 micron polystyrene latex spheres (part number #4210A, lot #6910) were purchased from Thermo Scientific [2]. They have a mean diameter of 10.00 +/- 0.08 microns and a size distribution with a standard deviation of 0.09 microns. The sample suspension was spread on a glass slide and allowed to dry thoroughly before measurement.

Images were collected and analyzed with the HORIBA PSA300 Static Image Analysis system at 500x magnification. The PSA300 was calibrated automatically with a NIST traceable calibration slide. A representative image is shown in Figure 3.

Aggregates and particles cut by the measurement frame were ignored. Since the size distribution was extremely narrow, Miles-Lantuejoul [3] [4] factors were not used to correct for the effect of particle size on the probability of the particle touching the image frame.

Images from 261 particles were analyzed; this is more than twice the number of particles required to analyze the number mean diameter to within 5% for this narrow size distribution [3].

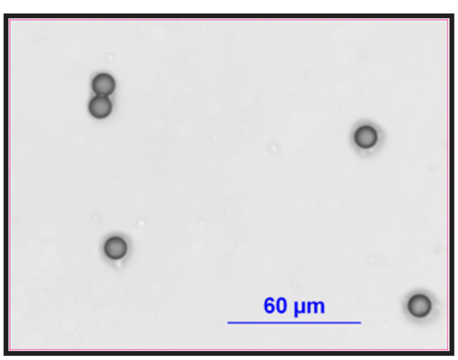

Figure 3: 10 µm PSL dispersed on PSA300 slide

Results and Discussion

The circular diameter, also known as the area equivalent or Haywood diameter, of the particle is reported. The “circular diameter” refers to the diameter of a circle with the same area as the image of the particle. For the case of PSL spheres, the image is a circle, and the measured circular diameter is the diameter of the PSL sphere.

In addition, two Feret diameters are reported. The Feret diameter is the size as would be measured by a caliper. Naturally, this value depends on caliper orientation for any shape besides a circle; the results of two different orientations are reported there. The first is the orientation giving the longest Feret diameter, also known as the length. This is the longest dimension of the particle that can be measured with a caliper. The second is the orientation giving the shortest Feret diameter, also known as the width. This is the shortest dimension of the particle that can be measured with a caliper.

Finally, the aspect ratio, also known as the shape factor, is reported. This quantity is the length (longest Feret) over the width (shortest Feret). For perfect spheres, the value is expected to be one. The aspect ratio is calculated for each particle and then averaged.

The results of each size measure are shown in Table 1

All of the results are within the diffraction limit of about 0.5 microns. The circular based diameter can be considered more reliable because it is derived from the area which means that many more pixels were evaluated and the effect of one extra or one missing pixel is relatively small compared to the effect of one missing pixel on the determined length or width, which is derived from a line of pixels.

Parameter	Expected Value	Measured Value with 95% confidence interval
Circular Diameter (microns)	10.00 +/- 0.08	10.08 +/- 0.02
Length (microns)	10.00 +/- 0.08	10.26 +/- 0.03
Width (microns)	10.00 +/- 0.08	10.01 +/- 0.02
Aspect Ratio	1.00	1.02 +/- 0.002

Table 1: Measurement results for 10 µm PSL

Conclusions

The results of these measurements show that the PSA300 can be used to measure the absolute particle size to within a few percent without resorting to the array method for enhanced precision. The deviations from expected value can be explained by the uncertainty introduced by diffraction effects and the reduced precision since the array method is not used. One pragmatic application for the image analyst is that the PSA300 can use certain PSL materials for internal equipment validation. In some situations this may have value as a second check or for peace of mind.

References

[1] Duke, Stanley, Layendecker, Ellen, "Improved Array Method for Size Calibration of Monodisperse Sphereical Particls by Optical Microscope" Thermo Technical Note TN-018.03.

[2]http://www.thermoscientific.com/wps/portal/ts/products/detail?navigationId=L10397&categoryId=87439&productId=11961526.

[3] "Particle Size Analysis – Image Analysis Methods—Part 1: Static Image Analysis Methods," ISO 13322-1:2004(E)

[4] "Test Method for Particle Size Analysis through Image Analysis," AFNOR NF X11-696: 1989.