AN OVERVIEW OF MICRON-RANGED PARTICLE SIZING TECHNIQUES

Amy Sabin

INTRODUCTION

Given the wide range of particle sizing technologies and instrumentation on the market, the choice of measurement technique can be daunting, especially if one is unfamiliar with the options available. Once introduced and familiar with an individual technique, it can be easy to continue to choose it by default. While this is certainly understandable, it may prove a disservice when the technique is not optimal for the sample type.

When the particle size of a sample needs to be determined, there are many factors that influence the choice of technique that is used. If the sample type has been analyzed previously, it is likely that the same technique will be employed again, especially when a method has been established in a regulated environment. However, if the sample type has never been analyzed, the choice of technique may be influenced by a variety of factors. One of these factors may include instrument availability; for example, if a company only has access to laser diffraction instrumentation, it may be strongly inclined toward laser diffraction analysis. In a related sense, familiarity with methodology may play a key role; if a company is accustomed to working with basic sieves, it may be a challenging leap for them to choose the more complex electrozone technique. When there is a choice of technique, the cost, ease, and speed of analytical methodology may represent important factors in the decision-making process from a business standpoint. While these factors hold importance and are very much justifiable in a practical sense, they may overlook the optimum choice of technique for the given sample type from a scientific perspective.

This discussion addresses several particle size characterization techniques for micron-ranged particle size distributions. It describes some of the considerations behind choosing each technique by providing examples of their potential benefits and disadvantages for certain sample types. By no means is this article all-inclusive. Rather, it contains a brief overview of a few of the more common techniques commercially available. It presents specific situations where the chosen technique adversely or positively affects the results with respect to the sample properties, and it aims to serve as an introduction to the decision-making process toward choosing the appropriate particle sizing instrumentation and methodology.

SIEVING

Sieving is one of the oldest and most rudimentary particle sizing methods. Sieves are easy to use and provide a simple and reliable method of defining particle size. Sieves may be used for particles ranging from several microns to several millimeters, although particles smaller than approximately 75 microns need extra consideration (1). They are available in a broad range of screen sizes (mesh). As measurement devices, they are most commonly applied in increments anywhere from 45 microns to 11.2 millimeters (1, 2). A sieve may be used individually for a single measurement, or several may be combined in a stack of a progressive series of sizes to obtain multiple measurements. A sample can be sieved by hand agitation, or the sieve(s) may be used in conjunction with a mechanical device to facilitate the process through oscillation, vibration, or air entrainment. These sieving apparatuses will generally aid in increased reproducibility of the method.

Sieving provides data on a mass-basis directly. The principle is simple: a known weight of sample is applied to the sieve, particles smaller than the screen aperture dimensions fall through the sieve, and particles greater than the screen size are retained and weighed. Sieves are a practical choice when a very discrete measurement of a sample is required (e.g., when 90% of the weight of a material must be smaller than 100 microns after milling). When the mean (average) particle size of a sample is in the range of several millimeters, the sieving technique can be invaluable, as this range is beyond the upper detection limit of many of the other common particle sizing techniques.

As with all particle characterization techniques, representative sampling is extremely important for sieve work. Sieving may require a larger quantity of sample per measurement than other techniques, so it may not be feasible when the sample is in limited supply. Sieves can be especially influenced by challenging powder characteristics involving static or moisture interactions. While there are ways to minimize these problems, extreme cases may necessitate a different choice of technique. Particle shape is also a consideration, as elongated particles tend to pass through the mesh on their shortest dimension. Depending on the scope of the method, sieving can prove labor intensive. Even when multiple sieve sizes are employed, typical sieve data are not terribly resolute when compared to other available options.

Despite these limitations, there are sample types that are ideal for sieve-based methods. Figure 1 is a photograph of a sample type that was subjected to multiple particle size techniques. It may look like a common powder, but it posed a deceptively unique challenge. Two lots of this sample type were submitted for comparative analysis; one represented a problematic lot, while the other represented an ideal lot. The problematic lot was causing issues in the production system; therefore, the goal was to determine any differentiation between the two lots in terms of particle size so that future problematic lots could be identified prior to product utilization. After observing the samples under a microscope, it was noted that the primary particles from both lots were of similar general morphology (i.e., shape, size, texture) and were appropriate for micron-ranged instrumentation. The samples were first analyzed using laser diffraction, by both liquid and dry (compressed air) dispersion methods. The results for both lots were virtually identical on a volume-weighted basis. The samples were then analyzed using light obscuration in order to determine if there were any differences in the number-based distributions for each lot. Again, there was little difference between the lots. It was noted in handling, however, that one of the lots contained large, soft agglomerates that readily dispersed in liquid carriers and under low dry dispersion air pressures. Based on this observation, the samples were then sieved using a gentle technique designed to maintain the integrity of the soft agglomerates. The results showed a stark difference between the lots. After further testing of additional lots proved successful at differentiating between the two qualities of sample, a sieving method was incepted.

Figure 1: This sample type benefited from sieve analysis due to the presence of soft agglomerates that indicated lot acceptability.

SEDIMENTATION

Sedimentation is another classic particle sizing technique. This method comes in many forms, from the simple hydrometer, to the pipette method, to more sophisticated techniques employing x-rays, photoelectric measurement, disc centrifuge, and more. For an excellent introduction to sedimentation technique, as well as many others, Allen's book is strongly recommended (3).

Based on Stokes' settling equation, gravitational sedimentation techniques relate the measured settling time or settling velocity of particulate to a calculated settling diameter. Basically, sedimentation relies on the principle that large particles generally settle more rapidly through a fluid than small particles. The technique requires a known particle density, fluid density, and fluid viscosity. It assumes that the particles consist of smooth, rigid spheres of equal density, and that they settle without disturbance. Sedimentation has a working range from a fraction of a micron to a couple hundred microns, depending on the particle density, the fluid viscosity, and the exact method utilized. In most suspensions, particulate below about a half a micron does not settle at a measureable rate due to its inability to overcome frictional drag of the surrounding fluid (although centrifuge techniques have a much lower range than gravitational techniques), and particulate in the hundred-plus micron range settles too rapidly for accurate measurement (unless extremely viscous fluids are utilized).

The accuracy of the measurement can be inhibited by particle shape or surface texture. For instance, platy or highly textured particles may settle more slowly than the predicted rate of a smooth, sphere, leading to an undersized result. Depending on the method, the analysis may be lengthy and labor intensive, and may require relatively large amounts of sample compared to other sizing techniques. Sedimentation may offer an ideal particle size technique for slurries or for any product where settling could be a concern (these may include highly concentrated suspensions of pigment or fillers). This is because the technique itself not only measures the size of the particles, but also may be reflective of the characteristics of the final product.

The characteristics of the final product are an important consideration when choosing a particle size technique. In the realm of particle technology, it is easy to disengage the particle size methodology from the application in which the sample is utilized. For example, if a drug product were to be administered as a dry powder, it would be ideal to have the particle size of this product determined using a dry dispersion method. This would likely give a better depiction of the particle size of the final product in its "real world state" than if it were suspended in a liquid dispersion, simply

because it would not expose the sample to conditions beyond its intentions for application. It is not always practical or feasible to replicate end product conditions during a particle size analysis, but should be considered whenever possible.

LASER DIFFRACTION

Laser diffraction is a common and versatile method of particle size measurement. Laser diffraction instruments have the reputation of being easy to use, producing results quickly and efficiently, and effectively highlighting lot-to-lot variability on a volume-weighted basis. Depending on the system configuration, these instruments have the capacity to analyze particles in a liquid dispersion, a dry (compressed air) dispersion, and can even measure the droplet size of sprays and aerosols. They have a broad measurement range, from several nanometers to a few millimeters, depending on the instrument model. Due to its versatility, laser diffraction is often the default technique chosen for the analysis of new sample types. There are, however, some situations in which laser diffraction is not the best-suited technique for the analysis.

Laser diffraction instruments are ensemble analyzers; they measure a cloud of particulate in suspension and correlate the resulting signal to that of equivalent spherical diameter on a volume basis. There are specific obscuration or transmission ranges that are recommended for analysis, depending on the particle size to be measured and the model of instrument used. These ranges relate to the concentration of particles in suspension. This should not be confused with a concentration measurement in terms of particles per volume of fluid because the laser diffraction technique does not count individual particles. In many cases it may be possible to achieve optimal obscuration or transmission requirements by simply diluting or dispersing the sample appropriately, but there are cases where this is not entirely feasible. For example, when a sample consists of particles in an extremely dilute liquid suspension, it is unlikely that optimal obscuration will be achieved (laser diffraction is usually ineffective when the sample is as transparent as drinking water). A particle counter (i.e., an instrument capable of the measurement of individual particles) is recommended for samples fitting this description.

Figure 2 shows another example of a particle type that is generally not recommended for laser diffraction analysis. Particles with extreme aspect ratios (e.g., elongated, acicular, or fiber-like particles) are not ideal for laser diffraction, or for any technique that provides data on a spherically equivalent basis. Simply put, this is because a rod-shaped particle is not best described by a spherical-based particle value. Instead, image analysis is recommended for these types of particles because it can characterize this particle shape in its absolute form. Several studies have been conducted on this issue (4, 5).

Figure 2: Acicular particles are not recommended for laser diffraction analysis.
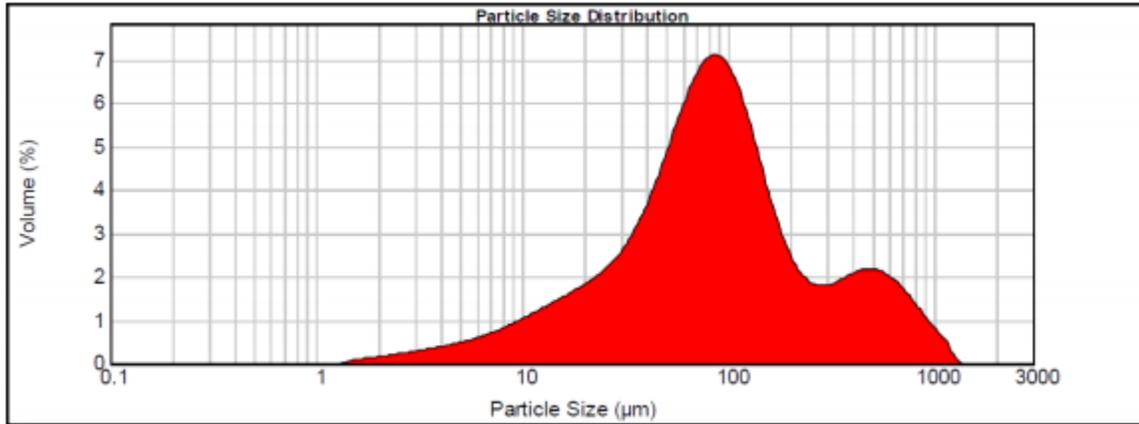*Image analysis is a better option for particle characterization.*

Figure 3: Laser diffraction results of particles depicted in Figure 2. Somewhat bimodal distributions are not uncommon for acicular particulate in laser diffraction analyses; the modes may be generally influenced by the length and width of the particles.

Another issue observed in laser diffraction methods is the inappropriate use of sizing statistics. While it is common to report laser diffraction results in terms of x10, x50, and x90 (i.e., the spherical diameter at which 10%, 50%, or 90% of the particles in the sample are smaller or larger on a volume basis), laser diffraction should not be used for determining the extreme limits of a particle size distribution. For example, while Figure 3 appears to clearly show that the largest particles in the sample are no more than approximately 1500 microns, it needs to be understood that this value should be treated with great caution. According to ISO13320:2009, "Characteristic size values below x5 and above x95 are likely to be vulnerable to additional uncertainty and systematic error, as a result of sampling problems as well as by limitations of laser diffraction. Quotation of an x100 value by laser diffraction is specifically deprecated by this International Standard" (6).

Similarly, one might ask if the sample represented by Figure 3 contains any submicron particulate. Beyond the contraindications as stated by ISO13320 previously, this can be quite the loaded question. First, laser diffraction measurement of fine particulate is especially sensitive to the choice of optical model (i.e., refractive index and absorption values) (6). Unless these values are known to be accurate, the resulting data should be handled with extreme caution. Furthermore, laser diffraction technology cannot determine the lack of particulate in a system; rather, it reports the relative volume of particles. So even though the results do not show particles below 1 micron, this may only mean that the submicron particles in the sample are insufficient in relative volume to command presence in the resulting distribution. As discussed later in this article, a particle counter would be better suited to make this type of assessment, however, not without its own caveats.

The resolving limitations of laser diffraction technology can mask some critical particle distribution attributes. For example, Figures 4 and 5 show the particle size results from a sample consisting of two distinct size populations: two narrow sieve cuts of a sample type had been combined (see Figure 6 for a photograph of the particles). Figure 4 shows the laser diffraction analysis result for this sample, while Figure 5 shows the electrozone result. While the laser diffraction results show the suggestion of two populations, the electrozone results greatly emphasize two clearly distinct populations. While there are ways to optimize laser diffractor software settings for multimodal, narrow distributions, this example shows how the "general purpose" settings for each instrument type produces greatly different results. It should also be noted that, for many reasons, it is not uncommon for different particle sizing techniques to produce different particle size results, especially when the particles in question are not smooth, monodisperse spheres.
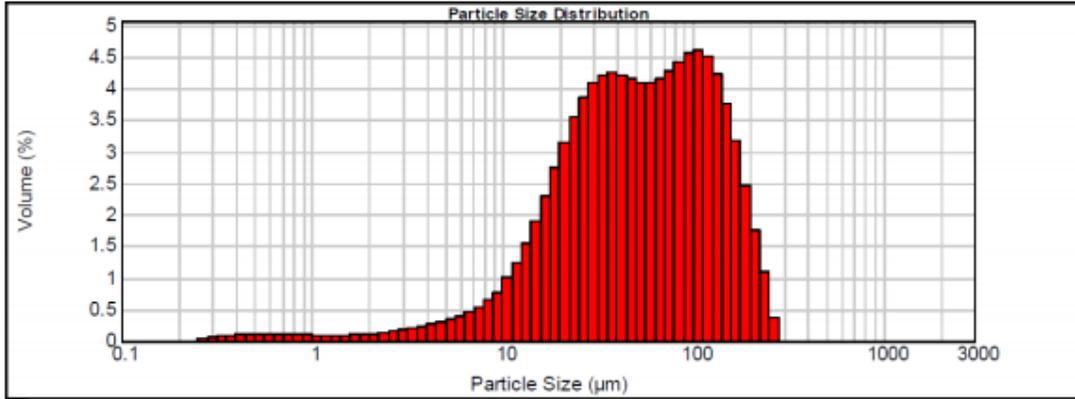
Figure 4: Laser diffraction analysis results of a multimodal, narrow distribution. Note that the two primary populations are difficult to discern. See Figure 5 for the electrozone results of the same sample.
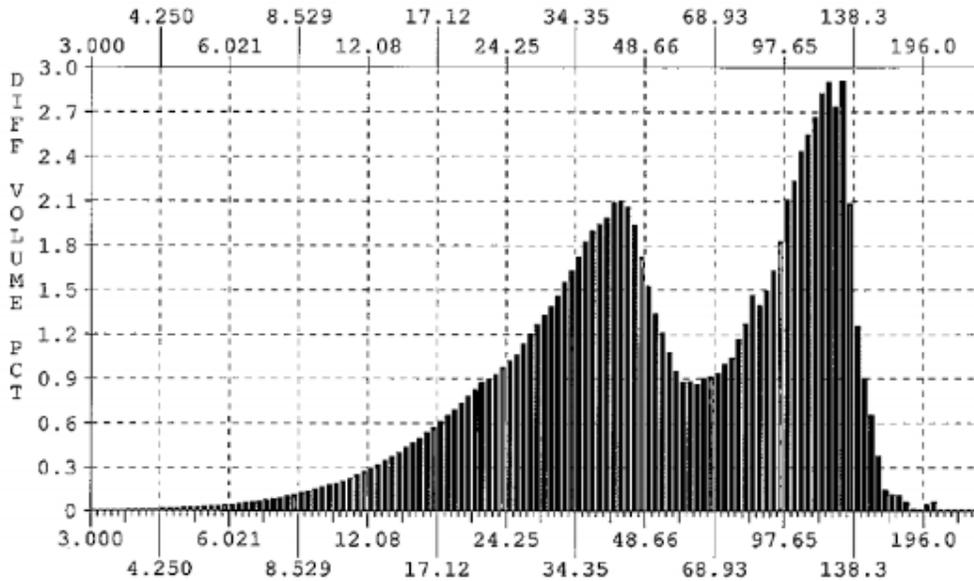

Figure 5: Electrozone analysis results of a multimodal, narrow distribution. Note that the two primary populations are very distinct. See Figure 4 for the laser diffraction results of the same sample.
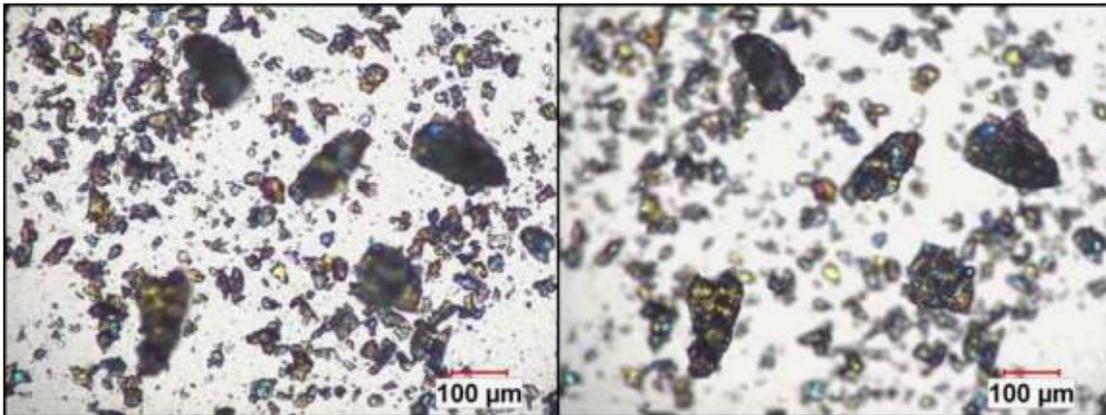

Figure 6: Photographs of the particles from Figures 4 and 5. Two planes of focus were used to show the two populations.

ELECTROZONE

The electrozone sensing (or Coulter counter) technique yields some of the most resolute data available among common, high speed particle analyzers. Electrozone instruments are particle counters that provide data on both a number (count) and volume (mass) basis. They operate by measuring individual particles in a conductive liquid to which a current is applied as they pass one-by-one through an orifice aperture of a known diameter. In doing so, each particle creates a voltage pulse that is detected by the instrument and correlated to an equivalent spherical particle size based on a calibration curve. This technique is commonly used for the measurement of particle sizes ranging from approximately a micron to a few hundred microns.

The electrozone technique is especially useful for the analysis of samples with a narrow particle size distribution. As a particle counter, it requires a minimal quantity of sample and provides data of high resolution. Electrozone sensing is a completely non-optical measurement technique; as such, it is a unique and useful complement to laser or light-driven methodologies. This can be an advantage when the sample particulate is highly reflective, opalescent, absorptive, or when the particle and the carrier have very similar refractive indices.

Electrozone technology may have a somewhat greater learning curve than other common particle characterization methods. From an operator's perspective, this technique can prove cumbersome and labor intensive when working with samples of broad particle size ranges (e.g., 2-3 decades on a logarithmic scale). The technique requires a liquid dispersion in a conductive electrolyte solution. While suitable organic carriers may be utilized in special cases, aqueous carriers are by far the most common. Saline solution is generally the carrier chosen by default. This obviously calls for concern if the sample is even minimally water-soluble.
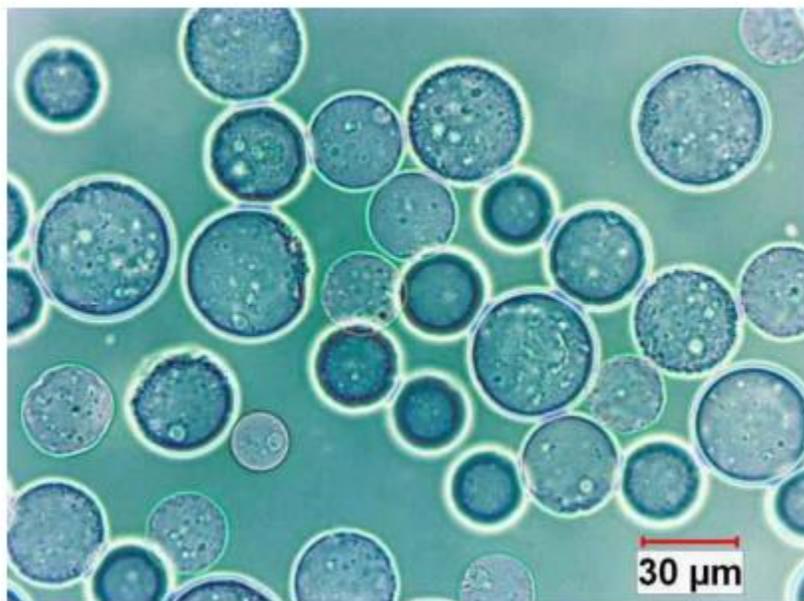
Figure 7: Porous particles need special consideration when using the electrozone sizing technique.

Figure 7 shows particles with a feature that is important to be knowledgeable of when considering the electrozone technique. These particles are particularly porous in nature, and without attention to and correction for this characteristic, the instrument will produce undersized results. This undersizing is a byproduct of this technique of data acquisition. If the particle's pores are filled with a conductive carrier, the voltage pulse produced by the particle during analysis will reflect a smaller displaced volume than its "true" outer dimensions (7). It can be corrected with a sample-specific calibration setting, but it can be easy to overlook the necessity for this step, because the preliminary results might seem otherwise plausible. It is important to observe the sample under a microscope in order to have a general particle size approximation for comparison against the instrument output. Pore structures can be misinterpreted as surface texture under low magnification, so it may be difficult to identify porous particles by microscopic observation alone. Therefore, it is especially helpful to have a background knowledge of the material properties prior to analysis.

LIGHT OBSCURATION

Also known as SPOS (single particle optical sensing) or photozone technique, light obscuration instruments are also particle counters. As such, they routinely supply data on a number and on a volume basis (which is calculated based on the number data). The measurement takes place as a single particle passes through a laser or laser diode light beam and casts a shadow upon a detector. This shadow is registered as a decreased voltage signal, which is correlated to a voltage of a known spherical particle size using a calibration curve. Light obscuration techniques may operate using dual measurement techniques: light extinction (or light blockage) for particles greater than approximately 2 microns and light scattering for particles less than approximately 2 microns, depending on the calibration and detector. Utilizing both methodologies, light obscuration covers particle size ranges from a fraction of a micron to several hundred microns. However, it is important to note that the measurement is influenced by the optical properties of the material used to create the calibration curve, and optical artifacts may result depending on the properties of the particles analyzed.

Like the electrozone technique, light obscuration instruments offer extremely resolute data. Since particle counters require minimal sample amount (insofar as the system is clean and electronically noise-free), light obscuration is useful when sample is extremely limited or if the sample consists of a very dilute suspension.

Light obscuration is used to measure the concentration of particles that may represent potential largeparticle contamination to an otherwise "particle-free" sample or a sample containing sub-detectable primary particle sizes, such as parenteral liquids or samples for injection (8). Special care must be taken to ensure a clean environment for this type of work. There is a fine balance in making sure that a sample is well mixed prior to analysis without introducing bubbles to the suspension. Like many particle sizing techniques, light obscuration cannot differentiate a bubble from a particle, and this may represent a considerable challenge during data interpretation.

As a particle counter, the light obscuration technique has a maximum concentration limit necessary for the purpose of minimizing coincidence in the system. Coincidence refers to the measurement of more than one particle at the same time, thereby resulting in inflated particle sizes and count loss. It may take surprisingly little sample to reach the maximum coincidence level, and for certain sample types this might represent a potential source of sample bias. Missing a few large particles in a sample by number can significantly bias volume-weighted results. This makes a direct comparison difficult between a particle counter such as light obscuration and an ensemble analyzer such as laser diffraction.

Figure 8: Samples 1 and 2 required light obscuration analysis due to their dilute nature. Samples 3 and 4 were also analyzed using light obscuration in order to acquire results comparable to samples 1 and 2 on both a number and volume weighted basis.

Figure 8 shows a set of samples that were analyzed using a light obscuration technique. Bottles 1 and 2 are ideal candidates for light obscuration, as they contain an extremely dilute suspension of particulate. Bottles 3 and 4 contain a much more concentrated suspension of particulate, and while light obscuration analysis is possible (following extreme dilution), sampling bias is a risk. The particulate loading of bottles 3 and 4 makes laser diffraction a very compelling option. Why choose light obscuration? Bottles 1 and 2 are far too dilute for laser diffraction analysis; they

would need to be analyzed using a particle counting technique, such as light obscuration. While bottles 3 and 4 could be analyzed using laser diffraction, it would not be recommended for the purpose of comparing the full set of results from all four samples. Both light obscuration and laser diffraction produce results on a volume-basis, but since the method of data acquisition is different, the comparison of the results is not exactly "apples to apples".

Although complementary techniques are useful to verify the legitimacy of a given data set, there is a possibility that certain nuances would be missed if the results of different analytical techniques were compared for a series of samples. When a sample set spans a range of potential measurement techniques, the best choice for individuals might not be optimal for the whole set, yet it might represent the most feasible methodology overall for the sake of data comparison. In this case, it was decided that the risk of bias for bottles 3 and 4 using light obscuration was far less significant than the risk of obtaining questionable results using laser diffraction for bottles 1 and 2.

IMAGE ANALYSIS

Image analysis is an extremely versatile particle characterization technique. The basic setup consists of a microscope, a camera, and a computer. The equipment may sound simple, but the data reduction can be quite involved. The image of a dispersed sample is evaluated using a thresholding process to determine the boundaries between particle and background. This process can be manual or automated. Specialized software converts the data into various shape and size parameters using a set calibration. These commonly include, but are not limited to, aspect ratio, circularity, convexity, and solidity, along with circular equivalent diameter on both a number and volume basis (9). Unlike the other particle sizing techniques, image analysis offers the advantage of reporting the "true" particle dimensions, rather than a spherical equivalent alone. This is important, as most real-world samples do not contain perfectly spherical particles, and the ability to quantify this property can offer great insight into the nature of the sample. While image analysis offers useful information for any particle shape, it is especially critical for the characterization of particles with extreme aspect ratios (e.g., acicular particles, platy particles, fibers), as reporting these in a simple spherical terms can be a gross misrepresentation of the actual particle properties. Image analysis serves as a frequent resource and reference in the realm of particle sizing technology.

Image analysis instruments are classified as particle counters, since they measure each particle individually. Since they operate using a small amount of sample, they can be vulnerable to sampling-related bias. It is important that a sufficient number of particles are measured to ensure statistical relevance (9). This process takes time, and it is not uncommon for typical image analysis methods to span several hours.

CONCLUSION

There are many varieties of particle sizing techniques available, and while this article only touches on a few, the careful evaluation of the appropriateness of a technique for a given sample type is a good habit whenever possible. It is important to investigate and understand the properties of the sample, from particle morphology to end-use product application. There are situations where it is not possible or practical to analyze a given sample type using a given technique; but more insidious is when it is possible to analyze a sample by a technique for which it is ill-advised. While historical precedence can be a driving force, be it in the form of technique familiarity or prior method establishment, this should not overshadow the scientific relevance of a technique for a sample type. It is optimal that the technique be thoroughly investigated in light of the sample characteristics before a method is initially established. It may not be realistic to expect that every modern technique is a viable consideration. Furthermore, there may be more than one way to determine or describe what is optimal for a given sample type. Nevertheless, it is important to evaluate the available particle sizing options for their suitability and applicability to the sample within the context of its properties, its end-use, and its relevant data representation.

REFERENCES

1) United States Pharmacopeia, "Particle Size Distribution Estimation by Analytical Sieving," USP 34-NF 18, 2011.

2) Pope, Lawrence R., and Charles W. Ward. Manual on Test Sieving Methods. West Conshohocken, PA: ASTM, 1998.

3) Allen, Terence. Particle Size Measurement. London: Chapman and Hall, 1981.

4) Kelly, Richard N., and Jacqueline Kazanjian. "Commercial Reference Shape Standards Use in the Study of Particle Shape Effect on Laser Diffraction Particle Size Analysis." AAPS PharmSciTech 7.2 (2006): E126-E137.

5) H.R. Prasanna, E.H. Jefferson, J.S. Taylor, A.S. Hussain, R.F. Karuhn, R.C. Lyon. "Comparative Analysis of Common Particle Sizing Techniques for Pharmaceutical Powders." FDA Science Poster, Feb. 2001.

6) ISO, "Particle Size Analysis - Laser Diffraction Methods," International Standards Organization ISO 13320:2009.

7) Horák, Daniel, Jan Peška, František Švec, and Jiří Štamberg. "The Influence of Porosity of Discrete Particles upon Their Apparent Dimensions as Measured by the Coulter Principle." Powder Technology 31.2 (1982): 263-67.

8) United States Pharmacopeia , "Particulate Matter in Injections," USP 34-NF 18, 2011. 9) ISO, "Particle Size Analysis - Image Analysis Methods" International Standards Organization ISO 13322-1:2004.