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▲〈1430.2〉 ANALYTICAL METHODOLOGIES BASED ON SCATTERING PHENOMENA—LIGHT DIFFRACTION MEASUREMENTS OF PARTICLE SIZE

1. INTRODUCTION

Light scattering measurements may be used for the determination of particle size distributions because light scattered from particles has an angular dependence on the size of the particles. In principle, this application makes use of the same phenomenon as the low-angle light scattering (LALS) and multi-angle light scattering (MALS) techniques. The difference in the particle size measurement method lies in the higher spatial or angular resolution needed for the detection of scattered light. Light diffraction measurements allow for the derivation of a full-size distribution, with moderate to high resolution, rather than a single characteristic size. Specialized instrumentation and software have been developed for the specific needs of this application. Additional details on method and instrument requirements are provided in *Light Diffraction Measurement of Particle Size* 〈429〉.

Light scattered by particles in the near-forward direction is analogous to diffraction of light through an aperture. As this application makes use of the full pattern of diffracted light, it is properly described as light diffraction. It is also often referred to as laser diffraction due to the almost universal use of laser light sources.

The range of application, speed, minimal sample restrictions, and ease of use has made this the most broadly applied and commonly used technique for particle size analysis. Distributions with a resolution on the order of 100 independent channels are common. Measurements can be performed in seconds or less. Depending on the instrument configuration, the method is applicable to sizes ranging from about 0.01 µm to greater than 3000 µm. Particles may be measured in almost all matrices through which light can be transmitted, including liquids, gases, vacuums, and, potentially, solids.

The discussion in this chapter will focus on the application of laser light diffraction to materials of pharmaceutical interest. This still covers a wide range of materials, including excipients, active pharmaceutical ingredients (APIs), intermediates, starting materials, raw materials, in-process formulations, and drug products. Materials may be liquid, solid, inorganic, organic, crystalline, amorphous, single- or multi-phase, and combinations of these materials.

2. THEORY

The complete description of light scattering by spheres is given by the Mie theory, which is based on the solution to Maxwell's equation for the interaction of light with an optically homogeneous sphere. This accounts, not only for light diffracted by the particles, but also for light passing through the particles, i.e., refracted light. The Fraunhofer model represents a special case of the Mie theory that does not account for refraction. The assumptions here are that the refractive index (RI) of the particle material relative to the matrix and the absorptivity are high, so that effectively no light passes through. It also assumes that particles are large relative to the wavelength of the incident light. Thus, it is most properly applicable to particles greater than several micrometers for visible light sources. Though the Fraunhofer model can be applied when these conditions are not met, it can produce known artifacts and errors. However, it can still be useful as a default when the RI is unknown. The errors are often negligible for larger particles. A more detailed description of the Mie and Fraunhofer models can be found in ISO 13320:2009—*Particle size analysis—Laser diffraction methods*.

Some limitations in Mie theory are that it assumes (among other conditions) that particles are spheres with smooth surfaces and that they are optically homogeneous. These conditions are not generally met for most real particles of pharmaceutical materials. An additional complication is that most measurements are performed with the particles in motion. Thus, non-spherical particles may present a range of aspects to the incident light axis during the measurement, in effect appearing as multiple particles. Consequences of these deviations from theory are discussed in *7. Data Interpretation and Reporting*.

RI includes both real and complex components. The real component is commonly determined by either a microscopy method (Becke line technique) or from the RI of solutions of known concentration.

In the Becke line technique, particles are immersed in oils of known RI and observed with transmitted light. As the focus is moved above the plane of the particle, a bright ring may be observed moving into the region of higher RI, i.e., either into or away from the particle boundary. Using different oils, the particle RI may be determined well within the needed accuracy. Using polarized light, the RI of the individual crystalline axes may also be determined. In that case, an averaged value may be used in the Mie model.

In the solution method, an Abbe refractometer is used to measure the RI of a solution for which the concentration of the material is known. Extrapolating the solution RI, from a series of known concentrations of the material, to that of the pure substance yields a good approximation. This may require knowledge of the true density of the material.

The imaginary component of the RI is the absorptivity value for the wavelength of light used in the light diffraction measurement. For most non-colored materials, this will be very low. This value is not typically measured.

For both components, the values should be considered as initial approximations. Although the Mie model results are not especially dependent on the chosen values, it can sometimes yield unrealistic results. The sensitivity of the measurement to small changes in the assumed values should be assessed. This requires only the recalculation of an existing measurement using different RI values. Final RI and absorbance values should be chosen to avoid being too near an area of high sensitivity. The resulting size distributions should always be evaluated to assure that they realistically represent the sample.

3. INSTRUMENTATION

Instrumentation consists of light source(s), a measurement region, optics to collect the scattered and unscattered light, multi-element detector(s), and necessary hardware and software for the computations. Often more than one light source may be used. Light sources are usually lasers, though not always. Sources of different wavelengths are mainly used to extend the size range, especially to better handle samples in the submicron region.

The measurement region is where the sample is measured. Depending on the specific application, this is typically in a well-defined position relative to the detector and optics, and comprises windows that physically contain the sample dispersion. Associated sample dispersion equipment is also part of the instrument. (An exception would be for instruments specifically designed for sprays or on-line use.)

The detectors must provide adequate spatial resolution needed to best define the diffraction pattern. To best resolve the variations in diffraction patterns in the applicable size range, most elements are within a relatively low forward angle. Additional detectors at wider angles are often incorporated to extend the range to smaller sizes. Depending on the instrument design, different lenses may be used for specific size ranges. This is not common with the reverse Fourier configuration.

Further descriptions and illustrations of common instrumentation configurations are shown in 〈429〉 and ISO 13320.

4. APPLICATIONS

In principle, the light diffraction technique may be applied to any particulate system that allows for sufficient transmission of light: solid in liquid, liquid in liquid, gas in liquid, solid in gas, and liquid in gas. However, it is most commonly applied to solids dispersed in either liquids or gas (air), and to sprays of liquids or solids in air.

For solid powders, dispersion in either liquid or gas both offer advantages and disadvantages. Dispersion in liquids generally allows better control of the degree of dispersion of the material, which is important for understanding the desired characteristics of the powder. It also offers the advantage of being able to sample the dispersion's particles from the instrument dispersion outflow for observation by microscopy. Microscopy permits observation of the particles in the same state they exist during the light diffraction measurement. Obtaining particles from a dry dispersion as they exist in the dispersion would be difficult, if not impossible.

Disadvantages of liquid dispersion include possible changes in the material characteristics due to dissolution or flocculation. Selection of the dispersant system, including use of surfactants, is critical to developing a suitable method.

The advantages of dry or air dispersion of solid powders include the absence of solubility concerns and minimal sample preparation. A significant disadvantage is decreased control of particle dispersion. Though a range of dispersion energy can be applied by controlling the shearing air pressure, there is typically a reduced ability to separate deagglomeration from particle fracture processes. The occurrence of artifacts appearing as larger particles has also been observed, possibly due to material flaking from the surfaces of the dispersion unit. Larger sample sizes may also be needed.

Dry dispersion also requires adequate filtration and containment of the vacuum exhaust. Highly potent or toxic materials may not be suited for this analysis.

The use of dry dispersion is most appropriate for materials used in a similarly dispersed state, for example, the analysis of liquid or powder sprays from drug products and devices.

5. METHOD DEVELOPMENT

The fundamental step in starting the development of a method is to define the desired state of dispersion for the sample. Almost all powder samples will exhibit some degree of agglomeration. Attractions due to dispersion or van der Waals forces, electrostatics, or physical attachment, etc., are present in any sample. The strength of the attachments varies widely and depends on the particles' size, composition, and prior processing.

The terms agglomerate and aggregate will not be defined here. Within the scope of pharmaceutical materials, there can be an essentially continuous range of particle association strengths. Agglomerates or associations can range from those that are very weak and easily disrupted with minimal agitation, to those that are very strong and physically bound particles. Hence, the terms agglomerate and aggregate are sometimes used interchangeably, depending on the process used to create the material.

It is critical that the intended use of the particle size information be understood to determine the appropriate degree of dispersion. For example, measurement of primary, i.e., fully deagglomerated particles may be more informative for understanding the particle formation process or inherent dissolution behavior of a powder. Alternatively, including agglomerates in the measured distribution may be more useful for understanding issues of content uniformity and powder flow. A good understanding of the intended use of the material and expected forces encountered during further processing will help to determine the appropriate dispersion. Optical microscopy is an important tool to characterize the sample dispersion.

A typical dispersion study (assuming a solid in liquid dispersion) would first consist of analyzing a suspension created with minimal dispersion energy, e.g., by stirring or vortexing. Subsequent analyses of the suspension are performed after application of increasing dispersion energy, e.g., with added sonication times. If this is not possible with a single preparation, multiple preparations may be used. At each stage, the stability of the sample should be examined, e.g., with a stirring study. Comparing the resulting distributions with consideration of the sample behavior and supporting microscopy reveals the suitability of the dispersion conditions.

For dry dispersions, analyses using different shear air pressures and feed rates are done. This is often referred to as an air pressure titration study.

Once the intended state of dispersion is determined, method development will focus on how to best achieve and maintain that state reproducibly and reliably. For powders, dispersion either in air or a liquid is necessary. For liquids, the dispersant must support the dispersion of the particles to the desired extent, with no dissolution or other physical change or re-agglomeration. Though dissolution is not an issue for air or gas dispersion, achieving the proper degree of dispersion may be a greater issue.

The amount of incident light scattered or absorbed by the sample, expressed in terms of obscuration, transmittance, or optical concentration range of the sample, is related to the sample concentration and serves as a convenient proxy for relative concentration. It is expected that this will affect the measured size, though a range of values where the sensitivity of the results is low should be possible. This is generally a function of the overall instrument design and not material-specific. The lowest concentration must be sufficient to minimize the effects of noise, and the upper limit is needed to minimize the effects of multiple scattering. Suitable ranges may be recommended by the instrument manufacturer. Restricting the range may improve reproducibility.

6. METHOD VALIDATION STRATEGY

Chapter 〈429〉 provides guidance to demonstrate the required accuracy and precision for the instrument and methods of the laser light diffraction technique.

The International Council for Harmonisation (ICH) guidelines on method validation do not fully account for characterization methods such as particle size analysis. However, the fundamental concerns about method precision and robustness still apply.

As described in *Validation of Compendial Procedures* 〈1225〉, precision should be addressed by assessing repeatability and intermediate precision. Additional assessments of reproducibility could include multiple instruments and laboratory location.

The robustness assessment should address the sensitivity of the method to other likely sources of variation and should establish a range of acceptable conditions. Factors to consider will depend on the type of dispersion. For liquid dispersions, in addition to the classic stability considerations, this should include an assessment of the stability of the dispersion in the instrument over potential analysis times. Sample concentration, as indicated by obscuration, is an important factor, as it is most likely to have some effect. Other typical factors could include stability of the predispersion, recirculation or stir rate, dispersion energy, equilibration or stir time before initiating the measurement, and measurement time. For dry dispersion, measurement time, sample amount, air pressure, and feed rate to achieve target obscuration may be most important. These factors may be examined using an experimental design. For example, two or more factors could be evaluated by using high and low values at about the nominal value and determining the main effects.

Overall, the greatest sources of variations in the light diffraction technique are the sample properties, the sample preparation, and sampling. These far exceed any variations in the measurement process.

Given the fundamental aspects of the measurement process and the technique-dependent definition of size, the assessment of the accuracy is not simple. Comparison to an orthogonal technique is not always possible, especially given the different effects the particle shapes may have. A subjective assessment using optical or electron microscopy is recommended to compare the range of particle sizes observed with the reported light diffraction results.

7. DATA INTERPRETATION AND REPORTING

The ease of acquiring particle size distribution information by light diffraction belies potential pitfalls in its interpretation. Other than for perfect spheres, the concept of accuracy in describing the size of a single particle, much less a collection of varied particles, is elusive. The chosen optical model, particle shapes, and optical properties of the sample all influence the results of the measurement.

Calculations are based not on the sizing of any single particle but rather on the average diffraction pattern from the ensemble of particles passing through the measurement region over time. During this time, the particles can present multiple orientations.

The calculated size distribution is also not directly calculated from the diffraction pattern. Rather it is the result of comparing the cumulative theoretical patterns of individual size fractions, calculated using the chosen optical model, to the measured diffraction pattern. The relative proportions of the individual size are varied until the resulting calculated pattern best matches the measured one.

Thus the size distribution of a sample is best described as the size distribution of spheres with defined optical properties that produces a diffraction pattern equivalent to that of the sample. Interpreting the measured size distribution in terms of specific physical dimensions, e.g., length or width, is a mistake. Doing so has important implications for the interpretation of the reported size distribution, especially when considering the effects of particle shape on the results.

In some cases, the distribution of the modeled spheres may be restricted to a certain defined shape, e.g., a normal, log-normal, or Rosin–Rammler distribution. This was more common during the early stages of the instrument development, when computational abilities were more limited. This approach is not commonly used today, though the option may be available in the instrument software.

Because of how the results are derived, the particle size distribution from light diffraction measurement is best considered as the cumulative fraction of volume (or mass, assuming a uniform density) that is less than the defined sizes. This cumulative distribution is the basis for deriving the most commonly used statistics. These include percentile sizes such as ×50 and ×90 (the sizes for which 50% and 90%, respectively, of the sample volume are less than), as well as size quantiles such as Q10 (the fraction less than 10 µm). These are derived easily by interpolation from the cumulative distribution. For other statistics, such as various mean sizes, central values of the individual size bins must be assumed.

In plotting particle size distributions, if represented as a scatter plot, it should be realized that it is more properly considered as a histogram, with the relative height of each band representing the volume fraction within the size band, i.e., between the upper and lower limit. Unless the bins are evenly distributed on the size scale, the distribution density function versus size is what should actually be plotted. The distribution density is the volume fraction divided by the width of the size bin. This allows for the properly proportioned representation of a distribution when the size bands are of different widths. It will also permit measurements from different sources, with different numbers of bins to be overlaid on the same plot. One caution is that this quantity will change if the scale basis of the size axis is changed, e.g., from linear to logarithmic.

Further descriptions of particle size distribution statistics can be found in ISO 9276-1:1998—*Representation of results of particle size analysis—Part 1: Graphical representation* through ISO 9276-6:2008—*Representation of results of particle size analysis—Part 6: Descriptive and quantitative representation of particle shape and morphology*.▲ (USP 1-Dec-2019)

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