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Rapid Communications

Micrometer-Scale Particle Sizing by Laser Diffraction: Critical Impact of the Imaginary Component of Refractive Index

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Purpose. This study evaluated the effect of the imaginary component of the refractive index on laser diffraction particle size data for pharmaceutical samples.
Methods. Excipient particles 1–5 μm in diameter (irregular morphology) were measured by laser diffraction. Optical parameters were obtained and verified based on comparison of calculated vs. actual particle volume fraction.
Results. Inappropriate imaginary components of the refractive index can lead to inaccurate results, including false peaks in the size distribution. For laser diffraction measurements, obtaining appropriate or "effective" imaginary components of the refractive index was not always straightforward. When the recommended criteria such as the concentration match and the fit of the scattering data gave similar results for very different calculated size distributions, a supplemental technique, microscopy with image analysis, was used to decide between the alternatives. Use of effective optical parameters produced a good match between laser diffraction data and microscopy/image analysis data.
Conclusions. The imaginary component of the refractive index can have a major impact on particle size results calculated from laser diffraction data. When performed properly, laser diffraction and microscopy with image analysis can yield comparable results.

KEY WORDS: image analysis; imaginary component of refractive index; laser diffraction; microscopy; particle size; refractive index.

INTRODUCTION

The pharmaceutical importance of particle size, and its analysis, is manifest in recent events such as the 2003 AAPS Workshop on Particle Size Analysis, cosponsored by the Food and Drug Administration (FDA), Product Quality Research Institute (PQRI), and United States Pharmacopeia (USP). It is well-known that particle size distribution affects key pharmaceutical properties, such as dissolution, flowability, and injectability. There are also numerous reports (e.g., 1–4) on particle size measurement that compare and criticize particle size techniques, such as microscopy with image analysis (IA), other counting methods and laser diffraction (LD; also called angular light scattering). Etzler, in a recent article (1), has criticized LD methods for lack of agreement with each other as well as with data derived from counting methods. Problems with LD can arise from numerous sources. For particles in the size range where Mie theory is required [in general, for particles <25 μm (5)], appropriate optical parameters for the particles are needed (5), and should be stated (5,6), but these have rarely been given in the pharmaceutical literature. These include the real and imaginary components of the refractive index of the particle, n_p and k_p, respectively. A usable k_p is not directly measurable with the available instruments, and the current approach is to infer a range of appropriate values (which we will term *effective k_p*) based on the accuracy of the calculated concentration for the sample (5,7), as well as the level of error in the fit of the LD result to the scattering data. In our experience, these procedures may not be sufficient for small (<10 μm) particles—similar optical models (i.e., complex refractive indices) with similar concentration matches and error may produce radically different particle size distributions [also see (5)]; special caution is needed when the result indicates an extra mode in the size distribution below 1 μm. Other methods may be needed to decide which range is correct.

In this paper, we show the crucial impact of the imaginary component of refractive index on LD particle size results for model pharmaceutical excipients in the micrometer size range. In particular, an inappropriate imaginary refractive index can lead to anomalies such as false, or artifactual, peaks that have been described elsewhere (5). We discuss some of the challenges in arriving at appropriate optical parameters. When effective optical parameters are used, good agreement

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ABBREVIATIONS: d(0.1), particle diameter corresponding to 10% of the cumulative undersize volume distribution; d(0.5) particle diameter corresponding to 50% of the cumulative undersize volume distribution (median particle diameter); d(0.9), particle diameter corresponding to 90% of the cumulative undersize volume distribution; IA, microscopy with image analysis; k_p, imaginary component of the particle refractive index; LD, laser diffraction; n_p, real component of the particle refractive index.

was found between results for LD and for an orthogonal (independent) method: microscopy with image analysis (IA).

MATERIALS AND METHODS

Materials

NaCl and bovine serum albumin (BSA; Sigma, St. Louis, MO, USA) particles were prepared by spray-drying aqueous solutions with a Buchi 190 Mini Spray Dryer (Flavil, Switzerland). Sedisperse A-12 (a hydrocarbon fluid consisting of white mineral oil with surfactants) was from Micromeritics (Norcross, GA, USA). Water, isopropanol and hexane were HPLC grade from Burdick and Jackson (Muskegon, MI, USA).

Size Analysis: LD

Background

When light interacts with a particle in suspension, it is attenuated by scattering and absorption (5). Scattering includes diffraction at the contour of the particle, reflection at the exterior and interior surfaces, and refraction of light passing through the particle (5). The angular light scattering pattern for cases where all these processes occur is complex; various intensity maxima and minima occur at different angles, and Mie theory is recommended (5). Mie theory was originally the application of the Maxwell electromagnetic equations to the interaction of light with an isotropic, homogeneous sphere: the intensity of the scattered radiation depends on the angle of detection, the relative index of refraction (of the particle and the dispersant), and a size parameter. Unlike Fraunhofer and Rayleigh scattering, which are essentially subsets of this theory, it is not limited to certain size ranges and thus is most widely applicable (8,9). However, it is limited to a few particle geometries (like the Fraunhofer approach)—for most purposes, including the studies reported here, spherical geometry is assumed. Bohren and Huffman (8) summarize the applicability of Mie theory for particles of arbitrary size and shape and conclude that they scatter similarly to area-equivalent spheres at low angles; differences tend to increase at higher angles.

LD measurement of particle size entails the reverse of the Mie calculation. Instead of predicting the intensity/angle relationship based on the particle size distribution (of spheres), the intensity/angle relationship is "deconvoluted" by an iterative process that finds the distribution of equivalent spherical scatterers with the best fit of its calculated intensity/angle relationship to the measured one. This process minimizes the error in the fit of the Mie-calculated intensity angle curve (the residual) and may not have a unique solution. Assumptions include spherical geometry, optical homogeneity, field independence of electromagnetic properties, random orientation in the flowing medium, and a concentration where multiple scattering is not significant. Because refraction occurs at the interface of the suspending medium and the particle, the relative refractive index is required. To account for the overall extinction of light, the effective k_p is needed. Often referred to as the imaginary component of the refractive index, the term *imaginary* may be misleading because it is in fact a "real," physical quantity. Complex numbers are often used as a mathematical representation for describing the time-dependent properties of a wave. The parameter k_p is generally understood as energy conversion due to absorption of light. However, the use of a bulk absorption value to obtain an effective k_p only works for measurements of smooth, homogeneous spheres. Surface roughness may result in loss of light (5) that is not accounted for by the scattering detectors, and these losses may be increased by nonspherical geometry as well. (For a discussion of reflections off rough surfaces, see Ref. 10.) Therefore, the effective k_p differs from absorption from an optical transition from one energy state to another. Adjustment of the effective k_p to correct for such effects has been recommended (5). Failure to use an effective k_p can result in the appearance of a false peak in the size distribution (5).

We are not aware of any commercially available instrument to measure the imaginary component of particles, and even if there were, adjustments in the available LD software would be needed to incorporate such information. Literature reports of such data for solid particles are sparse. Note that the listing in Annex D of (5) has few k_ps of pharmaceutical relevance. Thus, there is a strong likelihood that this parameter is neglected, the Fraunhofer approximation is used, and large size error may result. Also, if this parameter is outside an effective range, the particle size distribution will have large error. This may be manifested in the appearance of an extra mode in the size distribution. Broad ranges for effective k_p may be estimated as follows (5,8): $k_p = 0$ for perfectly smooth, homogeneous spheres; $k_p \sim 1$ or greater for particles that absorb near the laser wavelength (i.e., colored particles); and $k_p \sim 0.01–0.10$ for irregular (in terms of shape or surface) nonabsorbing particles. An effective k_p may be inferred from LD measurements when a sample of known volume concentration is run. A volume concentration is calculated (by instrument software) from the light obscured by the equivalent spherical cross-sectional areas of the particles using Mie theory combined with the Beer-Lambert relationship. The match of the calculated volume concentration to the actual is a criterion for selecting the effective k_p (7); however, this relationship assumes spherical particles, and the match may not be as good for irregular ones. As described in the results below, sometimes the concentration match and residual alone do not provide a sufficient basis for choosing an effective k_p. However, if the volume concentration match is not done, confirmatory methods (e.g., microscopy) are even more crucial.

LD Method

LD was performed on a Mastersizer 2000 (Malvern Instruments, Malvern, UK) (633-nm red laser supplemented by blue light of 466 nm from a solid state source) with a Hydro2000μP Cell. The dispersant was hexane for NaCl and isopropanol for BSA. The n_p of the spray-dried particles was obtained by the Becke line test (11,12): 1.544 for NaCl, 1.510 for BSA. The n_ps at 589 nm were used. It was found that correcting for the small changes in n_p at 633 nm and 466 nm had negligible impact on results. For colorless materials, there is little change in n_p across the visible wavelength range (12,13). Mastersizer measurement of a sample of known volume concentration was used to determine the effective k_p. A known mass of sample was suspended in a known volume of dispersant in the cell. Because the density of the particles had been measured (see below), the prepared volume concentration could be calculated. The measurements described here were run at concentrations corresponding to obscurations of 5–15% (obscuration is the fractional loss of light intensity when compared with the intensity taken during a background measurement). Concentration ("loading") studies had confirmed that obscurations <15% led to stable size results. A general purpose irregular model (default) was used for both types of particles. In this model, the measured scattering distribution is adjusted to account for the irregular light scattering prior to analysis (based on the spherical geometry assumption) to arrive at equivalent sphere diameters. The effective k_p for the initial run was generally chosen in the range 0.000–0.010 for colorless particles such as ours. A process of trial and error was used to arrive at the effective k_p—the results were recalculated with different effective k_p values until the volume percent calculated by the Mastersizer software was within 20% of the prepared volume percent concentration (based on weight and density of sample in suspension).

LD instruments generate a variety of size data. Here we focus on the volume-based size frequency distribution (frequency vs. diameter; Mastersizer 2000 used default size bands—100 size bands, spanning 0.01 to 10,000 μm, logarithmically spaced instrument code uses 77 bands over .02–2000 μm), percentile diameters [d(0.1), d(0.5), and d(0.9)], and indicators of data quality such as particle volume fraction and residual.

Sample Density

The density was determined by solvent displacement using a calibrated Wilmad cylindrical microcell (Sigma-Aldrich, Milwaukee, WI, USA), which required a minimal amount of material (10–20 mg).

Size Analysis: Microscopy with Image Analysis

Scanning Electron Microscopy

A small amount of dry sample was spread evenly on the adhesive surface of carbon conductive tabs on specimen mounting stubs (Pella)—any excess was tapped off. The sample was sputter-coated with gold palladium (60/40 alloy) using a Pelco SC-7 (Redding, CA, USA) sputter coater then examined with a Philips XL ESEM TMP SEM (Philips, FEI Company, Hillsboro, OR, USA). Images were captured with the XL Docu software.

Light Microscopy

Light microscopy was performed on Sedisperse A-12 suspensions of the sample particles with a Nikon Eclipse E800 (Tokyo, Japan) microscope with a 100× (oil immersion, phase contrast) objective and a 10× ocular lens. Immersion oil, when used, was Nikon Type A. The microscope was equipped with a Hamamatsu CCD color chilled 3 CCD camera and controller (Hamamatsu City, Japan), a Sony monitor (Tokyo, Japan), and a computer connection. For microscope images of suitable contrast, images were acquired with Adobe ImageReady and Photoshop and analyzed by the Image-Pro Plus version 4.5 software. A list of the diameters of all the particles was generated and placed in a Microsoft Excel file. The particles were sorted in ascending order by size, and for each size, the cumulative volume calculated at each level. Diameters with cumulative volumes at 10%, 50%, and 90% were compared to the d(0.1), d(0.50), and d(0.9) obtained by the Mastersizer.

Certain literature (14,15) has indicated that the number of particles that need to be counted (i.e., sample size) can be quite high, especially for volume-based median diameter [d(0.5)] when the geometric standard deviation of the sample is high (e.g., ≥1.6). Based on the observation that most particle size distributions are log normal, Annex A of the draft ISO document on IA (14) indicates that one would need a sample size of hundreds of thousands of particles for a size distribution like the one studied here (geometric standard deviation of 2.5) for results with 5% error and 95% confidence. Other literature (e.g., Ref. 16) has found much smaller sample size to be satisfactory or has proposed measuring particles until the data converges at some average value (2) for a plot of observed diameter vs. sample size (count). In our study, this convergence was observed at ~1000 particles rather than hundreds of thousands.

RESULTS AND DISCUSSION

LD Data and the Occurrence of Artifactual Peaks

Spray-Dried NaCl Particles

As described above, a typical procedure for arriving at the effective imaginary component of the refractive index (effective k_p) is to iteratively recalculate the distributions with different effective k_ps to find the one that gives the best match of volume concentration to the actual concentration of the sample. Figure 1 shows the influence of effective k_p on apparent particle size of spray-dried NaCl particles, as reflected in the various percentile diameters. Note the concentration for this measurement was 0.0037% v/v and resulted in an obscuration of 8.4%, well below the level where multiple scattering occurred (>15%). For a broad range of effective k_p values of >0.017, the apparent size is stable. There is a marked and abrupt transition in the observed size when effective k_p is reduced by only 0.001, to 0.016. This transition is accompanied by the appearance of another mode in the size frequency distribution centered at 100 nm, near the lower measurement limit of the instrument. There is a consequent dramatic de-

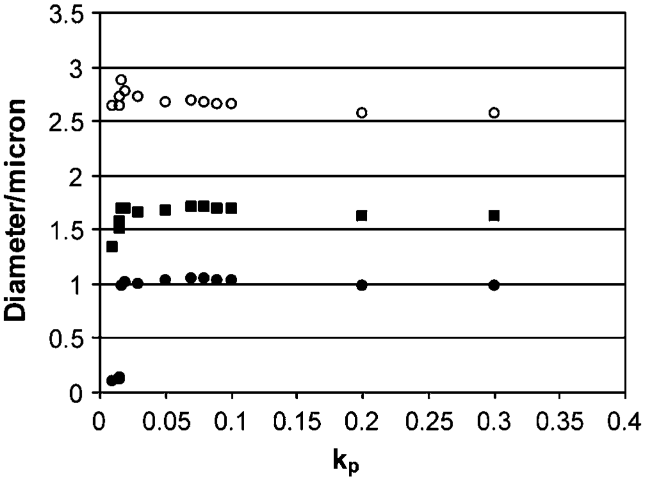

Fig. 1. Effect of effective k_p on size results for LD of NaCl particles. The value of n_p was set at 1.544 for all measurements: (●) d(0.1), (■) d(0.5), (○) d(0.9).

crease in the d(0.1) from 1.03 to 0.13 μm; the higher percentiles exhibit a lesser effect; for example, d(0.5) changes from 1.70 to 1.58 μm. Figure 2 shows that the concentration match is in an acceptable range (within 20%) for effective k_ps from 0.010 to 0.200 and is closest at an effective k_p of 0.015–0.016. The same figure indicates that the residuals were very low (less than 1% is highly acceptable) up to an effective k_p of 0.050 and acceptable (<1%) up to 0.100.

A strict volume concentration matching procedure by itself would lead to the selection of an effective k_p that would result in the extra mode in the distribution. The existence of such an extra particle population is suspect, as there is no physical reason it should appear. Under the light microscope with an oil immersion lens, these particles appeared 1–2 μm in diameter with an irregular shape; SEM of dry samples showed no obvious large population of ~100-nm-diameter particles. Although the hypothetical subpopulation was in a range just below 200 nm, the limit of resolution where particles appear as discrete entities by light microscopy, we have been able to observe particles as small as 100 nm in diameter as moving, wavy streaks (Duke standard nanospheres, in suspension). Such streaks were not observed here. We concluded that the subpopulation that resulted when LD data were calculated with effective k_p values below the crucial level was artifactual (a *ghost peak*). An effective k_p of 0.050 was selected based on low residual, an acceptable match (i.e., within 20%) of the volume fraction results, and its position centered in an effective k_p range where the size percentiles were stable (Fig. 1). When volume-based cumulative frequency distributions of LD and IA diameter results were compared, the match was quite acceptable: within 5% for d(0.1) and d(0.5) and within 10% for d(0.9). Another lot of these particles also exhibited an excellent match of LD and IA data (lot no. 2 in Fig. 3, at concentration of 0.0063% v/v, obscuration 12.0%—below the level for multiple scattering). These particles were somewhat larger [d(0.50) = 2.16 instead of 1.68 μm] and the critical value of the effective k_p in terms of the appearance of artifactual peaks was 0.040. So there can be batch-to-batch variations in effective k_p, possibly because this quantity is sensitive to surface roughness (5). Another difference between the two batches of particles was that the artifactual peak occurred at 15–16 μm—larger than the principal mode instead of smaller.

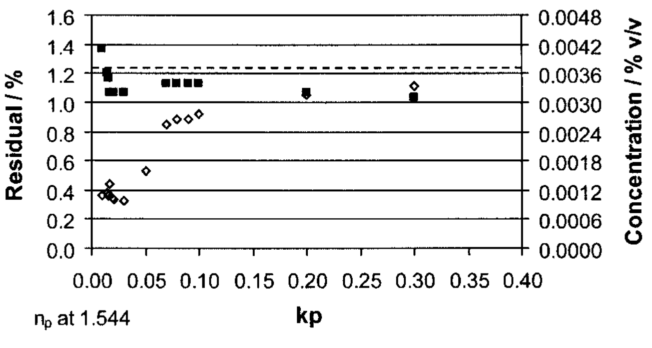

Fig. 2. Effect of effective k_p on residual and volume concentration. (◆) Residual, (■) concentration, (- - -) actual concentration.

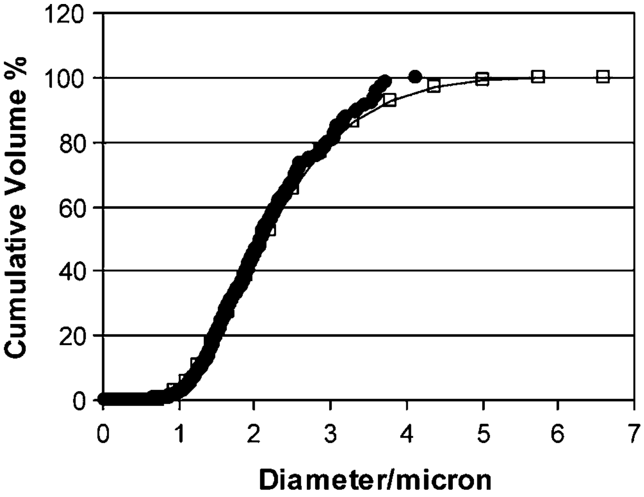

Fig. 3. Particle size distribution for NaCl (lot no. 2) by LD and IA. (●) IA with n = 934. (□) LD with n_p = 1.542, k_p = 0.040.

Spray-Dried BSA Particles

Spray-dried BSA particles were measured by light scattering at 0.0064% v/v (14.8% obscuration) in isopropanol. By light microscopy, these particles were in the 1–5-μm range, but the LD distributions exhibited a subpopulation in the <550-nm range as well. The volume % of particles in this subpopulation varied depending on the effective k_p used in the calculation: it was quite significant when effective k_ps of 0.001 and 0.010 were used; the total percent of particles <550 nm was 52% and 10%, respectively. When the effective k_p was decreased from 0.001 to 0.000 (again, a very small change), the population <550 nm dropped to 2.5%. For an effective k_p of 0.100 and higher, the apparent size of the <550-nm population also decreased, with the LD volume % concentration approaching the prepared value (from the weight and density of sample in the suspension) when the effective k_p was 1.000 to 1.250. Size results were stable over this range with d(0.1), d(0.5), and d(0.9) of 1.1, 2.7, and 5.4 μm, respectively. Although these particles were not transparent (k_p = 0), a value of ≥1 seems atypically high. This material would not be expected to absorb at visible wavelengths. SEM of these particles showed them to have an intricate, convoluted surface containing voids, where light might be in effect lost, perhaps accounting for the high effective k_p (5). The arrangement of instrument detectors may not have permitted the system to account for this loss, because light is reflected, or backscattered, in a manner not predicted by the theory, which works best for homogeneous, smooth spheres, unlike this material (see background section).

CONCLUSIONS

Use of inappropriate optical parameters in LD experiments can lead to large errors, such as displaced d(0.1), d(0.5), and d(0.9) and the appearance of artifactual peaks. Errors regarding the existence of extra size populations of fine or coarse particles can have serious consequences for pharmaceutical applications. For the spray-dried model excipients described above, a small change in the effective k_p can have a large impact on the calculated size results. It is therefore crucial for Mie theory–based LD particle sizing that the published procedures are followed (5), and the effective k_p should carefully be evaluated. With meticulous attention to measurement procedures and optical parameters, light scattering can yield size data that closely matches d(0.1), d(0.5), and d(0.9) from an independent method: microscopy with imaging. We further recommend IA as a method for spot-checking LD results.

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REFERENCES

1. F. M. Etzler. Particle size analysis: a comparison of methods. *Am. Pharm. Rev.* **7**:104–108 (2004).
2. H. Yoshida, H. Masuda, K. Fukui, and Y. Tokunaga. Particle size measurement with an improved sedimentation balance method and microscopic method together with computer simulation of necessary sample size. *Adv. Powder. Technol.* **12**:79–94 (2001).
3. C. Loisel, E. Vigneau, P. Cantoni, and M.-F. Devaux. Particle size distribution for food powders by image analysis: sample size determination and comparison with laser diffraction, *PARTEC98, 7th European Symposium Particle Characterization*, Nurnberg, Messe, Nurnberg, Germany, 10–12 March 1998, pp. 469–478.
4. C. Bosquillon, C. Lombry, V. Preat, and R. Vanbever. Comparison of particle sizing techniques in the case of inhalation dry powders. *J. Pharm. Sci.* **90**:2032–2041 (2001).
5. International Organization for Standardization. Particle Size Analysis-Laser Diffraction Methods-Part 1: General Principles, ISO 13320-1:1999(E), 1999.
6. USP. Light Diffraction Measurement of Particle Size. *Pharmacopeial Forum* **28**(4):1293–1298 (2002).
7. A. Lips, P. M. Hart, and I. D. Evans. Use of low angle light scattering in characterization of non-spherical particles and swelling of microgel particles. *Proceedings of the Fifth European Symposium in Particle Characterization*, Nurnberg Messe, Nurnberg, 1992, pp. 443–451.
8. C. F. Bohren and D. R. Huffman. *Absorption and Scattering of Light by Small Particles,* John Wiley & Sons, New York, 1983.
9. H. C. van de Hulst. *Light Scattering by Small Particles,* Dover Publications, New York, 1981.
10. B. van Ginneken, M. Stavridi, and J. J. Keonderink. Diffuse and specular reflectance from rough surfaces. *Appl. Opt.* **37**:130–139 (1998).
11. F. Rutley. *Rutley's Elements of Mineralogy,* 27th ed. Unwin Hyman, London, 1988, pp. 81–117.
12. P. F. Kerr. *Optical Mineralogy,* 4th ed., McGraw Hill, New York, 1977, pp 59–80.
13. R. Xu. *Particle Characterization: Light Scattering Methods,* Kluwer Academic Publishers, Boston, 2000, pp. 111–181.
14. International Organization for Standardization. Particle Size Analysis-Image Analysis Methods-Part 1: General Principles, Draft International Standard ISO/DIS 13322-1, 2003.
15. A. J. Paine. Error estimates in the sampling from particle size distributions. *Part. Part. Syst. Charact* **10**:26–32 (1993).
16. E. Vigneau, C. Loisel, M. F. Devaux, and P. Cantoni. Number of particles for the determination of size distribution from microscopic images. *Powder Technol.* **107**:243–250 (2000).