Microscopic Characterization of Particle Size and Shape: An Inexpensive and Versatile Method

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A variety of methods exists for measuring individual particle dimensions as a means of characterizing particle size, size distribution, and shape. The equipment described in this report belongs to the class of semiautomatic non-TV-interfaced analyzers. Unlike many existing image analysis systems, three-dimensional form measurements and texture data for the calculation of particle size and shape parameters can be determined easily and directly from each particle profile using this system. Essentially all data are collected directly from the particle and recorded by the computer with no intermediate steps. Much of the system consists of general-purpose and relatively inexpensive, commercially available hardware and software. Using this method, particle size, size distribution, and qualitative or quantitative shape information can easily and rapidly be obtained simultaneously. Particle length and width characterization, for example, can take less than 15 min. The equipment is versatile and flexible in measurements and calculations. The size and shape parameters to be measured are determined by the researcher and not the instrument. The ease with which this information can be obtained from small samples early in the development process makes it a valuable tool for the formulator.

KEY WORDS: particle size; shape analysis; instrumentation; light microscope.

INTRODUCTION

The quantitation of particle size and shape is problematic for researchers because descriptive single parameter measurements of particle morphology do not exist (1). For a very specific type of material, a single method of determining size and shape can be sufficient to describe differences between the individual particles of that material. However, a combination of methods is often required to provide more precise quantitation of the size and shape parameters (2).

There are many different methods available for particle size and shape analysis. Size characterization is simple for spherical particles; for irregular particles it is not, and the assigned size therefore depends on the method of measurement. Of the methods used for size characterization, microscopy is the only commonly used method in which individual particles are viewed and measured. An advantage of this method is that both size and qualitative or quantitative shape information can be obtained simultaneously.

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For particle shape, the methods used can be grouped by the type of parameter they yield: form, texture, or bulk property. Form is a function of the particle length, breadth, and thickness and describes the overall shape of the particle, whereas texture is a function of particle surface roughness (3,4). In contrast, bulk parameters are determined using information relating to the bulk properties of the material and not the individual particles (i.e., bulk density, porosity, specific surface area, particle size distribution, etc.) (2,5).

Various instrumentation exists for measuring individual particle dimensions. The most widely applied techniques involve the use of image analysis and range from semiautomatic, non-TV-interfaced analyzers to automatic, TV-interfaced models. The use of simple, inexpensive scanning systems coupled with computer software processing algorithms offer great flexibility for R&D applications. The shape analyzer described in this report belongs to the class of semiautomatic non-TV-interfaced analyzers. It differs from previously described systems (6,7) in several important ways. Unlike many existing image analysis systems, three-dimensional form measurements and texture data for the calculation of particle size and shape parameters can be determined easily and directly from each particle profile using this system alone. Essentially all data are collected directly from the particle and recorded by the computer with no intermediate steps. Much of the system consists of general-purpose and relatively inexpensive, commercially available hardware and software. Using this method, particle size, size distribution, and qualitative or quantitative shape information can be rapidly and easily obtained simultaneously.

MATERIALS AND METHODS

Equipment

A Nikon (Garden City, NY) Optiphot microscope with a Nikon Zoom Drawing Tube attachment is used to view the particles. A drawing tube provides a superimposed image of the graphics tablet and the particle in the microscope ocular to allow computerized data collection of information on the particle. A graphics tablet (Kurta, Phoenix, AZ) is used to digitize the individual particle measurements and profiles. An IBM-AT-compatible microcomputer is used to collect and process the particle measurements. A scientific measurement software package (Sigma-Scan, Jandel Scientific, Corte Madera, CA) is used to interface the graphics tablet and the microcomputer. Length and breadth measurements as well as perimeter length can easily be measured using this system. Additional software was written by the authors to calculate shape parameters from the completed set of digitized particle measurements.

System Calibration

A microscope slide micrometer is placed on the microscope stage. The microscope is then adjusted to provide Koehler illumination at the desired magnification. The draw-

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ing tube is focused to the surface of the brightly illuminated graphics tablet, providing a clear, superimposed image of the micrometer slide and the graphics tablet in the microscope ocular. The software is then calibrated for distance measurements using the micrometer scale. This process is repeated using a slide with a known rectangular area to calibrate the software for area measurements.

Particle Size and Shape Measurements

A random sample of the material to be studied is sprinkled on a microscope slide, allowing the particles to position themselves yielding the maximum projected area. The slide is then placed under the microscope and a particle to be measured is chosen randomly. The slide is rotated to align the longest particle dimension with the horizontal eyepiece micrometer. If size is to be determined, length measurements of the particle are taken by setting the software to measure distance and carefully marking the outermost horizontal edges of the particle. Repeating this procedure along the vertical axis yields the particle breadth. The particle thickness is measured by determining the difference in the vertical location of the fine focus marking when the focus is adjusted from the bottom of the particle (the top of the microscope slide) to the top of the particle. For particle shape information, the perimeter length and projected area as shown in Fig. 1 are also measured using the graphics tablet. Perimeter and area are determined by setting the software to measure the area and carefully tracing the particle profile with the cursor on the graphics tablet. The values for the length of the perimeter and the area enclosed by the perimeter are calculated and recorded by the computer. The parameters d_{min} and d_{max} can be determined by overlaying a series of calibrated circles on the particle image to determine the maximum inscribed circle (d_{min}) and the minimum circumscribed circle (d_{max}).

Numerous shape parameters have been defined and used in the literature. Several representative parameters defined in Table I have been calculated and reported in Table II using software written by the authors. The elongation and flatness ratios define the relative length-to-breadth and length-to-thickness values, while roundness, sphericity, and C/NC are different measures of the circularity (not necessarily the sphericity) of particles. The elongation ratio and flatness ratio lend themselves to the determination of the three-dimensional shape coefficients of Heywood (8,9).

Table I. Calculations for Particle Shape Parameters

Shape parameter	Equation
Elongation ratio	L/B
Flatness ratio[a]	B/T
Roundness	$(4\pi \times \text{area})/\text{perimeter}^2$
Sphericity	$[(4 \times \text{area})/\pi]^{1/2}/d_{max}$
C/NC	d_{min}/d_{max}

[a] T is the particle thickness determined by noting the difference in the vertical location of the fine focus marking when the focus was adjusted from the bottom of the particle (the top of the microscope slide) to the top of the particle.

Particle texture can also be characterized by recalibrating the software to yield positive x,y coordinates of the particle profiles. The perimeters of selected particles can be digitized (1000–2000 points per profile) for fractal analysis. An average fractal dimension can be calculated for each particle profile using the stride perimeter estimate technique (10,11).

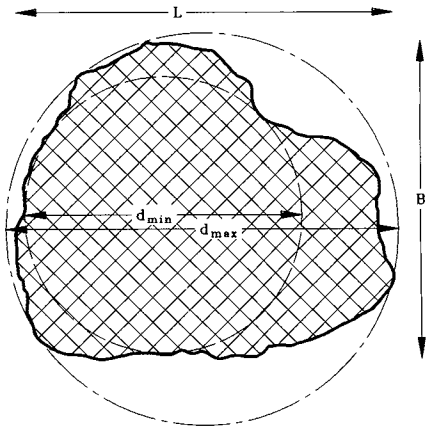

Fig. 1. Particle measurements made microscopically including particle perimeter and projected area. Measurements L and B are measured as Feret's diameters (1). (——) Sample particle; (– –) inscribed circle; (diam. = d_{min}); (– - –) circumscribed circle (diam. = d_{max}).

RESULTS AND DISCUSSION

Lot-to-lot variability in the particle size and/or shape of bulk drug can occur during development. Since such differences in particle size and shape can have an impact on solid and suspension formulations, three lots of an experimental drug were characterized to provide a data base from which to compare future bulk drug lots. Particle size and shape analyses were performed as described above. Particle measurements were made by mounting the bulk drug on a microscope slide using a 1.500-refractive index liquid and viewing at 250× magnification. The size of approximately 150 particles was measured and a frequency distribution established. The particle size is reported as the length of the rod-shaped crystal. The results are shown in Fig. 2 and Table III; the three lots appear to be log normally distributed, with significantly different geometric mean diameters. The geometric standard deviations, as determined from the scope in Fig. 2, also differ.

The collected data can also be viewed as shown in the box plot in Fig. 3. The box represents the 25th, 50th, and 75th percentiles of the distribution, while the dotted line in the box represents the arithmetic mean. Particles above the 90th percentile and below the 10th percentile are shown as open circles. From this plot, it is clear that lot C has much larger particles, with several particles in the sample exceeding 300 μm. The data from these three lab- and pilot-scale lots demonstrated that reactor size and agitation rate have a significant impact on particle size and distribution.

In addition to the particle length data discussed above,

Table II. Calculated Shape Parameters

Parameter	Emdex	Silica	Quartz	NuTab	Ibuprofen lot A	Ibuprofen lot B	Ibuprofen lot C	Ibuprofen lot D	Avg. % RSD
Number of particles	21	47	24	24	24	24	24	24	
Elongation	1.3	1.3	1.5	1.4	2.6	3.3	3.3	3.3	
% RSD	18	18	26	24	36	48	25	39	33
Flatness	1.0	1.2	1.5	1.6	1.1	0.95	0.87	0.93	
% RSD	17	18	32	30	38	33	30	31	32
Roundness	0.86	0.86	0.78	0.71	0.61	0.58	0.58	0.56	
% RSD	6	6	11	9	16	22	15	20	15
Sphericity	0.88	0.85	0.81	0.80	0.62	0.59	0.58	0.59	
% RSD	8	10	17	9	15	17	11	15	15
C/NC	0.78	0.70	0.65	0.64	0.38	0.33	0.30	0.32	
% RSD	15	16	25	16	32	36	26	31	28
Fractal dimension	1.018	1.016	1.026	1.036	1.042	1.031	1.030	1.021	
Literal description	Equant	Equant	Plate	Plate	Column	Column	Column	Column	

the elongation (length-to-width) ratio and roundness were determined on 25 particle samples of each of the three lots and the results are reported in Table III. All three lots appeared similar qualitatively as rod- or needle-shaped particles. Lots A and B had similar elongation ratios, while lot C had an elongation ratio of 21. This reflects the fact that the length of the crystals in lot C were greater than for lots A and B and the width did not increase in direct proportion to the length. Lot C was considered qualitatively as needle-shaped, while lots A and B were considered more rod-shaped.

The application of the method to determine particle shape parameters is demonstrated for two types of sand (silica and quartz), two excipients (Emdex and NuTab), and four lots of ibuprofen and the results are reported in Table II. Of the materials tested, Emdex appears to be the most spherical (roundness = 0.86, sphericity = 0.88). The quartz is somewhat more irregular than silica sand, as it has a greater elongation and flatness ratio. Quartz also has a greater fractal dimension, indicating that the surface is more textured than that of Emdex. Clearly, the experimental lots of ibuprofen (specially crystallized to result in different particle size) differ in shape as well. Lot A is somewhat more spherical than lots B, C, and D.

The relatively large standard deviations for the ibuprofen lots indicate that perhaps more particles should be evaluated to distinguish differences. Literature sources cite the use of sets ranging from 30 (2) to 500 (4) randomly chosen particles for shape analysis, with at least two sources (6,12) reporting the use of 100 particle sets. Statistical calculations done on the data sets show that the average relative standard deviation of the shape parameters calculated from a 25-particle set is about 25%. Increasing the particle set to 100 could help decrease the relative standard deviation of the shape parameters.

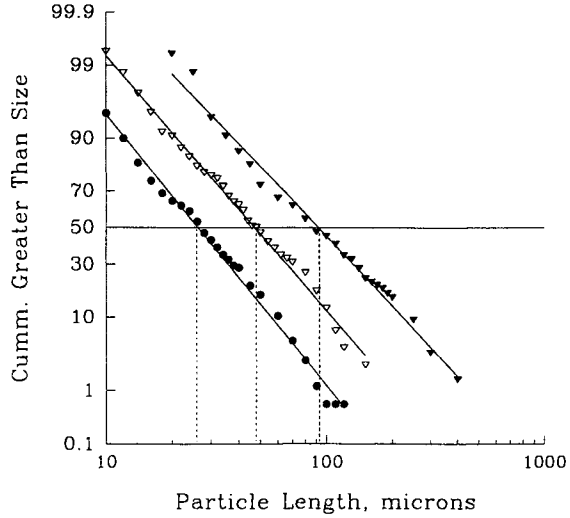

Fig. 2. Particle size distribution of three lots of an experimental drug. (●) Lot A; (▽) Lot B; (▼) Lot C.

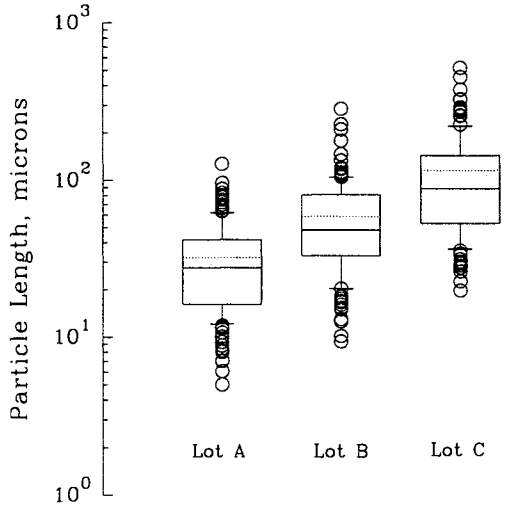

Fig. 3. Box plot representation of the particle size distribution of three lots of an experimental drug.

Table III. Size and Shape Characteristics of Experimental Drug

	Geometric		Shape		
Lot	Mean (μm)	SD	Roundness (* 4π)	Elongation ratio	Type
A	26.5	1.87	0.28	11	Rod
B	49.0	1.96	0.27	13	Rod
C	91	2.16	0.13	21	Needle

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