SHAPE DETAIL AND VOLUME CALCULATIONS IN MICRONIZED POWDERS

Accuracy of volume calculations is of critical importance in the assessment of active ingredients in dry powder formulation of medications. Automated Image Analysis allows the processing of practically an unlimited number of particles, giving access to unquestionable statistically significant results.

The main advantage of optical microscopy is that the user can observe individual particles in search of shape and size information, typically on particles ranging from 0.5 microns to 1000 microns. Detailed information on these parameters is of great value to the research and quality control scientists since the volume and size of active ingredients in the drug will affect dissolution rate and the strength of the medication. In some cases, a single oversized particle can cause a strong reaction in the patient. Failure to identify and accurately assess these particles can have serious consequences. Drug concentration, dissolution and release rate are effected not only by particle volume but also shape. Typically, powders will adopt three possible shapes; circular, flakes or needle-like (acicular).

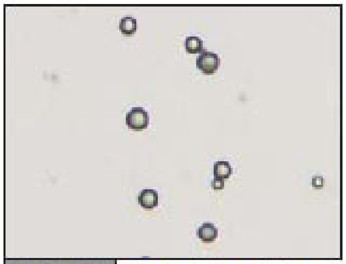

Figure 1 Spherical particles

Figure 2 Crystal particles

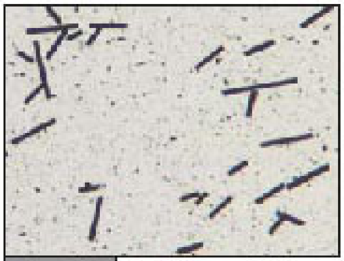

Figure 3 Acicular particles

Introduction

The purpose of this analysis is to demonstrate how the use of shape factors rather than only diameter (or main length) measurements can improve the accuracy of particle volume calculation in micronized powders by Automated Optical Microscopy. It has been demonstrated that particles as small as 0.5 microns can be accurately measured with this method, which offers the advantage that the individual particles can be viewed and verified. Standard methods use volume calculations assuming that the particles are spherical or near spherical. Volume calculations are carried out from the projected area, in the case of spherical particles, the projected area is a circle so the derived volume calculation is accurate. In the case of elongated, needle-like particles, the projected area is a rectangle; using the length as the diameter for spherical volume calculations will lead to increasingly false results, as the aspect ratio increases.

In other words, the more elongated the needle, the further from true shape will the result be. In the case of flakes, the projected area could be related to a circle, so that the average length could be expressed as the diameter but the thickness is far from corresponding to the diameter. In the case of particles exhibiting the shape of flakes, using a spherical equivalent diameter will unavoidably yield exaggerated volume. However, one would expect that spherical volume calculation is increasingly far from true volume as the shape of the particles differ from a sphere, either by being elongated or flat, hence volume calculations should take projected area and thickness into account.

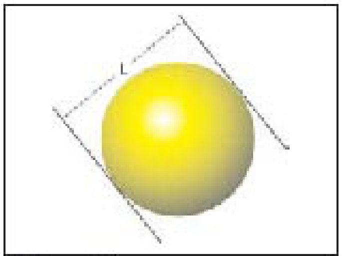

Figure 4 *Spherical and volume calculation*

$$V_{sph} = \frac{\pi}{6} \text{Circular diameter}^3$$

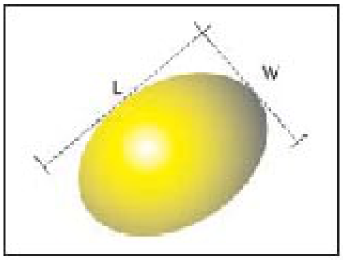

Figure 5 *Ellipsoid and volume calculation*

$$V_{el} = \frac{\pi}{6} \text{Main length x Width}^3$$

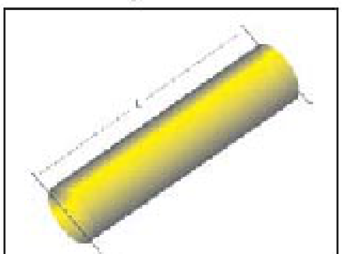

Figure 6 Cylinder and volume *calculation*

$$V_{cyl} = \frac{\pi}{4} \frac{\text{Area}^2}{\text{Main length}}$$

Figures 4, 5 and 6 illustrate the three volume shapes and corresponding mathematical formulas, which are used in this analysis. Fig 4 shows the spherical volume; Fig 5 shows the ellipsoidal volume which is used for the synthetic diamond sample; and Fig 6 shows the cylindrical volume, which is used for the elongated particle sample.

Analysis

Sample Preparation:

Three samples of micronized powders were analyzed, each exhibiting a particular shape of individual particles. In order to get the best possible statistical significance, each sample was prepared on four small glass slides rather than one large slide and the results were cumulated. The samples were prepared using a vacuum dispersing instrument in order to separate the agglomerated particles without breaking the individual ones.

Instrument Parameters:

Illumination, intensity, color balance and calibration were set and remained constant for all the runs. Two of the analyses were performed at 100x magnification with the third one at 200x, using a 1.4 MP 3 CCD camera. The resolution of the image on the screen was 0.65 microns per pixel.

Measurements:

Spherical Volume was measured on the three samples as well as a True Volume calculated with a shape factor corresponding to the samples. As described in the results summary.

The spherical sample consists of glass beads in the range of 10 to 20 microns in diameter. Figure 7a is a photomicrograph of the samples as observed under the microscope, at 200x magnification, or 0.2371 microns per pixel. Figure 7b illustrates that some particles remain clustered, they appear in red. Figure 7c illustrates that all the particles are separated; a different color is assigned to the red touching ones once they are separated.

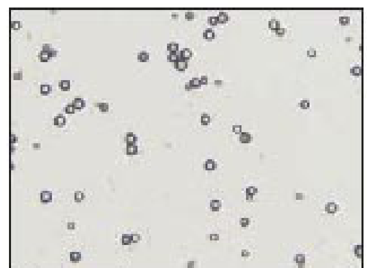

Figure 7a *Spherical particles*

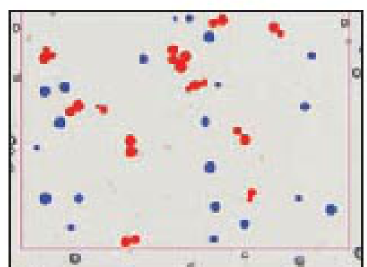

Figure 7b *Spherical particles detected*

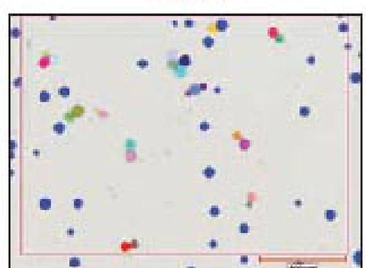

Figure 7c *Spherical particles seperated*

This ensures that the measurements will be done on single individuals rather than on clusters. The particles which are not colored will not be measured in this field because they are outside the Guard Frame, they will be taken into account in the adjacent field.

Figure 8a *Crystal particles*

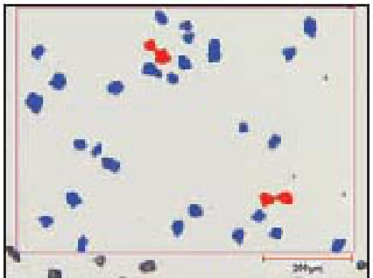

Figure 8b *Crystal particles detected*

Figure 8c *Crystal particles seperated*

The crystal shaped particles are synthetic diamonds in the range of 10 to 55 microns average diameter. Figure 8a is a photomicrograph of the samples as observed under the microscope, at 100x magnification, or 0.6541 microns per pixel. Figure 8b illustrates that some particles remain clustered, they appear in red. Figure 8c illustrates that all the particles are separated.

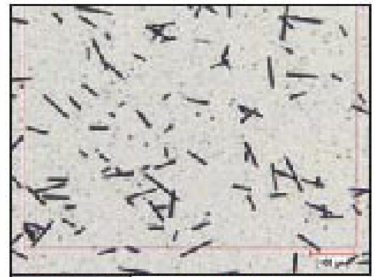

Figure 9a *Acicular particles*

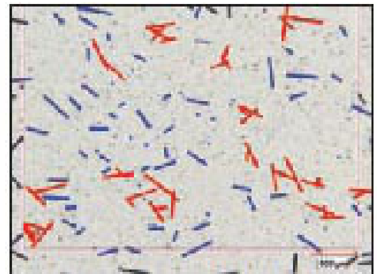

Figure 9b *Acicular particles detected*

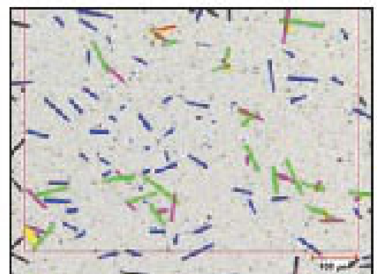

Figure 9c *Acicular particles seperated*

The acicular shaped particles are carbon fibers in the range of 10 to 140 microns average length. Figure 9a is a photomicrograph of the samples as observed under the microscope, at 100x magnification, or 0.6541 microns per pixel. Figure 9b illustrates that some particles remain clustered, they appear in red. Figure 9c illustrates that all the particles are separated.

Results & Discussion

Spherical Particles

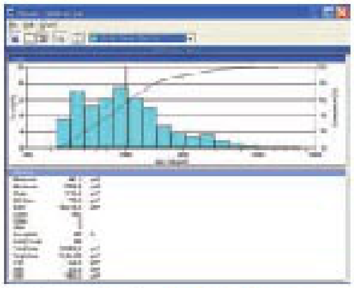

Figure 10a *Sperical volume*

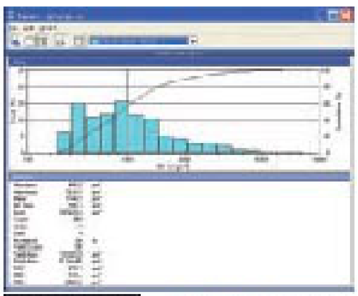

Figure 10b *Ellipsoidal volume*

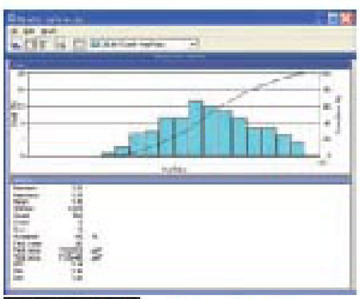

Figure 10c *Aspect ratio*

Figures 10a, 10b & 10c show the results of the volume and shape measurements on the glass beads. Histograms on Fig. 10a and 10b exhibit similar distributions when measuring either spherical volume or ellipsoidal volume. This is to be expected since the Main Length is equal to the Width. It is also reflected in the histogram on Fig 10c, which shows that the Aspect Ratio of the spheres is nearly equal to 1.

Crystal Particles

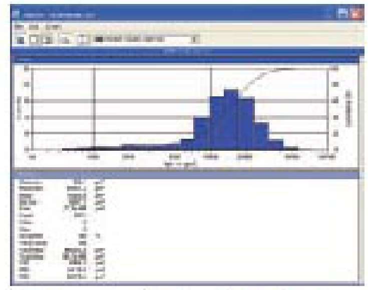

Figure 11a *Sperical volume*

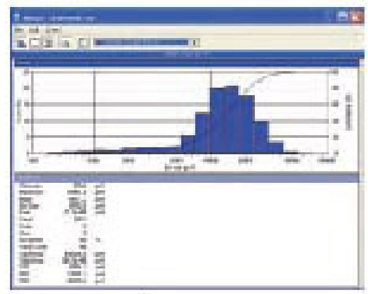

Figure 11b *Ellipsoidal volume*

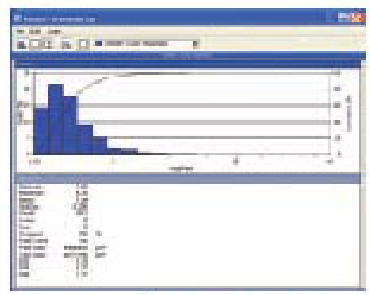

Figure 11c *Aspect ratio*

Figures 11a, 11b & 11c show the results of the volume and shape measurements on the synthetic diamond sample particles. Histograms on Fig. 11a and 11b exhibit very similar distribution when measuring either spherical volume or ellipsoidal volume. This is explained by the fact that since the average Main Length is close to the average Width since the diamond particles are evenly shaped. The histogram on Fig 11c, shows that the Aspect Ratio of the particles in the samples varies between 1.18 and 1.77, this reflects the variation in particle shape within the sample.

Acicular Particles

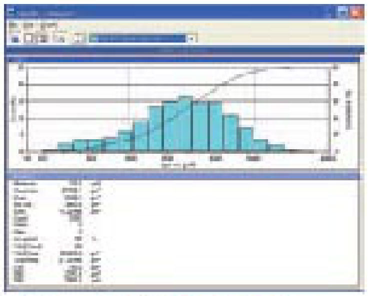

Figure 12a Sperical volume

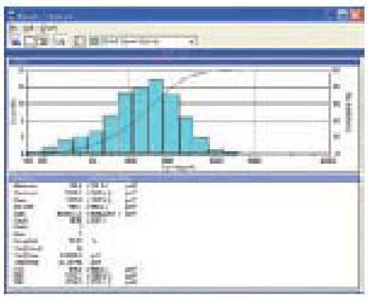

Figure 12b Ellipsoidal volume

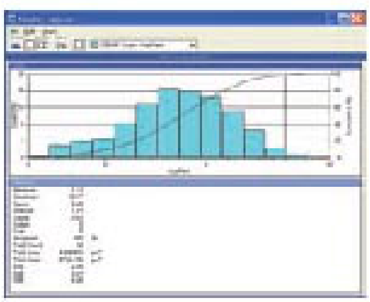

Figure 12c Aspect ratio

Figures 12a, 12b & 12c show the results of the volume and shape measurements on the acicular shaped particles. Histograms on Fig. 12a and 12b show that true volume for acicular particles (cylindrical volume) is overestimated by using the spherical volume. In the case of elongated particles, the Length is very different from the Width, which is also reflected by the histogram on Fig12c. This is explained by the fact that since the average Main Length is close to the average Width the diamond particles are evenly shaped. The histogram on Fig 11c shows that the Aspect Ratio of the particles in the samples varies between 1.18 and 1.77; this reflects the variation in particle shape within the sample.

Figure 13

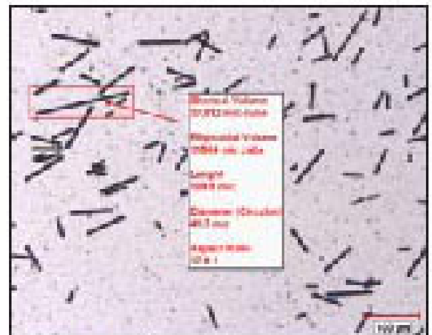

Figure 14

Figure 13 shows two particles from the same sample, both of which have similar spherical volume measurements even though they are visibly different in shape. Spherical volumes for the more elongated particle on the left and the regular shaped particle on the right are 12.022 micron^3 and 11.004 micron^3 respectively. Ellipsoidal volume better represents the elongated particle and is lower than spherical volume (7.415 micron^3 vs 12.022 micron^3). However, for the regular shaped particle, the ellipsoidal and spherical volume calculations give similar results (11.004 micron^3 vs 11.740 micron^3). Spherical diameter is similar for both particles since it is based on average diameter (mean chord), as opposed to Length, which is longer than for the elongated particles, and closer to spherical diameter for the evenly shaped particles. Aspect Ratio of the particles is 2.34 for the more elongated particle vs 1.15 for the more regular shaped particle.

Figure 14 shows the measurement of the same features as above, on a needle-shaped particle. In this case the spherical volume is 3.5 times the true volume (37.912 micron^3 vs 1.584 micron^3) and the Length is more than 4 times the spherical diameter (189.9 micron^3 vs 41.7 micron^3). The Aspect Ratio is 17.

This shows that volume accuracy depends on proper selection of shape factor. Shape volume calculations are based on length and width measurements which yield a more accurate representation of the true volume corresponding to the shape other than spherical.

Summary of Results

Measurement	Synthetic Diamonds (crystal shape)
Spherical Volume D50 (mic³)	14119
Ellipsoidal Volume D50 (mic³)	13580
Cylindrical Volume D50 (mic³)	-----
Aspect Ratio D10	1.18
Aspect Ratio D50	1.37
Aspect Ratio D90	1.77

Measurement	Glass Beads (spherical)
Spherical Volume D50 (mic³)	920
Ellipsoidal Volume D50 (mic³)	912
Cylindrical Volume D50 (mic³)	-----
Aspect Ratio D10	1.04
Aspect Ratio D50	1.06
Aspect Ratio D90	1.08

Measurement	Carbon Fibers (cylindrical)
Spherical Volume D50 (mic³)	2751
Ellipsoidal Volume D50 (mic³)	-----
Cylindrical Volume D50 (mic³)	1310
Aspect Ratio D10	2.05
Aspect Ratio D50	3.91
Aspect Ratio D90	6.85

Conclusions

It has been demonstrated in the analysis that using spherical volume calculations on all types of micronized particles can lead to misleading results. This is demonstrated by comparing spherical volume to "calculated volume" results for different shapes of particles, as observed in the result summary. For evenly shaped particles such as synthetic diamonds, the spherical volume is similar to the calculated ellipsoidal volume because the average length and width of the particles are nearly equal. However, in needle-like particles such as the carbon fibers used in this analysis, the spherical volume gives a significantly higher value than the calculated cylindrical volume, because the length and the width of the particles are very different.

For each type of sample, the variation of aspect ratio within each sample indicates the uniformity of the sample with respect to shape. For instance, in the spherical sample, the variation in aspect ratio is insignificant, however in the diamond sample, there is a 50% difference between the D10 and the D90 aspect ratio, which indicates less uniformity in the sample. In the acicular sample, there is a factor of 3 between the D10 and the D90 aspect ratios. In the latest, this is due to the fact that the shapes of the shortest needles are closer to spherical than the longer needle-like particles.

It is clear that the difference between spherical volume and true "calculated volume" increases with increasing aspect ratio, or as the difference between the length and the width of the particles increase. So in order to obtain accurate volume information on the sample, the shape of the individual particles must be known, so that corresponding volume calculations may be applied. In the case of samples composed of particles with different shapes, the particles must be isolated according to 2D shape factors such as aspect ratio. Only then can measurements be carried out on the different groups of particles with the appropriate volume calculations.