

APPLICATION NOTE

Characterization of Column Packing Materials Using Flow Imaging Microscopy

OVERVIEW

Chromatography is an important tool for separating and purifying complex mixtures. It involves forcing a sample that has been dissolved in a mobile phase through an immobile, immiscible stationary phase. The phases are chosen such that components of the sample have differing solubilities in each phase.

A component which is quite soluble in the stationary phase will take longer to travel through it than a component which

is not very soluble in the stationary phase but very soluble in the mobile phase. These mobility differences cause the sample components to separate as they travel through the stationary phase. Chromatography commonly uses columns in which the stationary phase is packed. The mobility of different components of a sample through a column is largely dependent on the size and shape of the particles in the packing material.

While common particle analysis techniques such as sieving, Coulter Counters, and laser scatter instruments will yield a distribution of particle sizes, they do not give any information regarding the shape of these particles. In the past, manual microscopy was used to take a closer look at particle shape. However, microscopy is extremely time consuming, and does not allow for inspection of statistically significant amounts of sample.

Digital image analysis with FlowCam provides critical size and shape information on column packing materials. This allows for tighter column density control, and in turn, better control of column performance. It also helps in the tracing of the damaged (non-spherical) particles that are often present in different lots of packing material.

METHOD

The FlowCam is ideally suited to rapidly characterize both particle size and shape of column packing materials. FlowCam captures and stores a digital image of each particle in the sample along with up to 26 different measurements for each particle image. The instrument can acquire, measure and store this particle data at speeds up to 50,000 particles/minute, yielding much more statistically significant data than manual methods in dramatically less time.

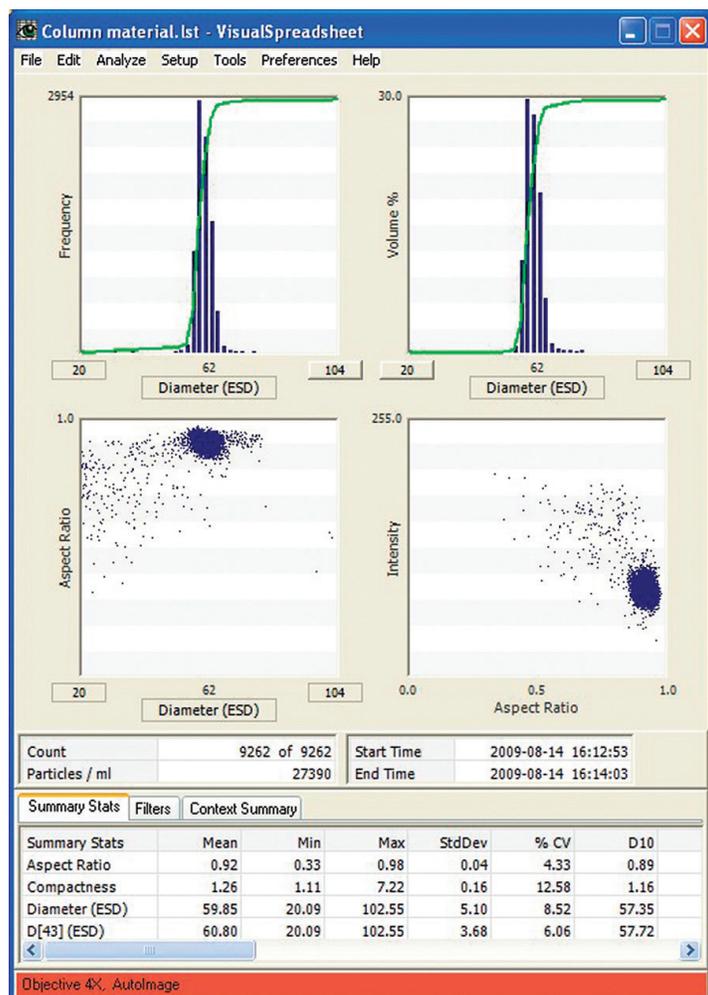


Figure 1: FlowCam results for polymer resin sample

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Figure 1 shows the results of a typical FlowCam run of a polymer resin sample. As can be seen from the frequency and volume distributions (upper two graphs), the sample shows a typical Gaussian size distribution with a mean Equivalent Spherical Diameter (ESD, volume based) of 60.80 μm .

The aspect ratio (width/length) scattergram in the lower left, however, indicates that the particles are not uniform in shape. Using the instrument's VisualSpreadsheet[®] software, we can display particles of different shapes and sizes easily.

Figures 2 and 3 are screen shots showing two sets of particles, which are considered to be *acceptable* and *unacceptable*. The first set of particles, Figure 2, have a round shape. Note from the ruler in the lower right hand corner that all of these particles have an ESD right around the mean for the sample, which was 60.92 μm . The second set of particles, Figure 3, are less round and appear to be mis-shapen beads, pieces of broken beads, or contamination.

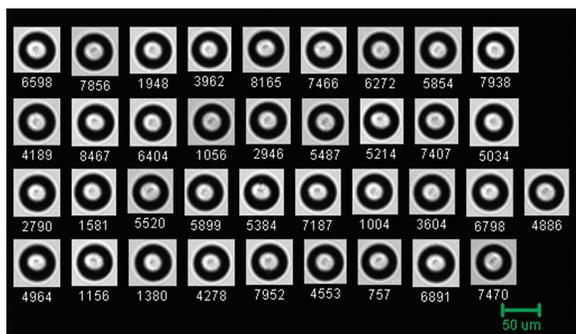


Figure 2: Round "acceptable" particle images

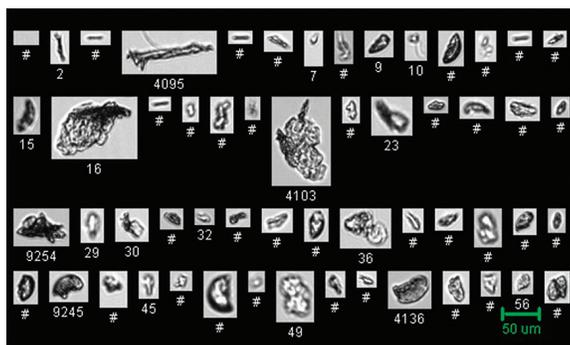


Figure 3: Less round "unacceptable" particle images

In this particular batch of polymer resin, for the intended application, it was desired that greater than 95% (by volume) of the batch be composed of the particles of the type shown in the first example (very round and within 15% of size mean).

Since the percentage allowable of the second type of particle (erose shape) is so low (5%), it would require a very time consuming manual microscope analysis to make a statistically significant decision on whether the batch meets specification.

Among the 26 measurements collected by FlowCam for each particle is aspect ratio. Aspect ratio is a measure of the particle's shape (of a 2D projected image) and is calculated as width/length. Using the interactive capabilities of VisualSpreadsheet, an operator familiar with the sample and its appearance under a microscope can quickly interrogate the particle images in order to find out at what aspect ratio value the particles should no longer be considered acceptable.

For this batch, it was determined that only particles within 15% of the mean in size and possessing an aspect ratio greater than .85 would be considered acceptable. The operator can then easily construct software filters for the acceptable and unacceptable particles.

Once the filters are defined in VisualSpreadsheet, they are added to the main statistics screen, and all particles are automatically characterized as either acceptable or unacceptable based upon those filters.

FlowCam will segment each particle into the two categories in real time during acquisition, yielding the desired statistics (% of each particle type by volume) immediately upon completion of the sample run. The filters can be saved and reused on new samples at any time in the future.

RESULTS AND CONCLUSIONS

Figure 4 shows the results of the sample run as before, this time with the filter statistics for acceptable and unacceptable particles displayed. The summary statistics show that for a total of 9,261 particles imaged, stored, and measured, 8,921 of them (97.26% by volume) were found to be acceptable and 340 of them (2.74% by volume) were found to be unacceptable. This means that this batch is characterized as acceptable since greater than 95% of the particles have acceptable shape. At this point the summary statistics can be exported to any database or LIMS system if desired. The operator can also view all particles as classified by clicking on the filtered results and instructing the software to display those images only.

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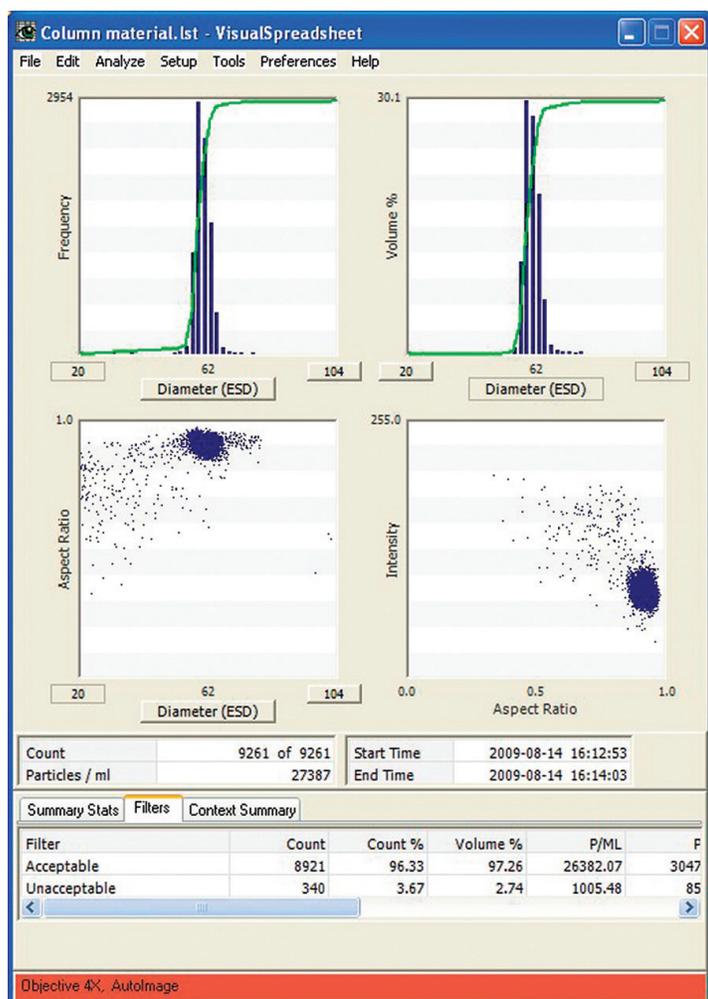


Figure 4: Automated filter results (bottom)

To summarize, the FlowCam and VisualSpreadsheet were able to characterize 9,261 particles automatically in a little over a minute in order to make a determination if this batch of polymer resin met the desired specification. It would have taken hours to make the same characterization using manual inspection through a microscope. In addition, this manual process would not have yielded results with anywhere near the same statistical significance or precision.