Particle Size Analysis of Active Pharmaceutical Ingredients (APIs)

AccuSizer® SPOS system

The particle size distribution of active pharmaceutical ingredients (APIs) is important for many reasons, including processability and performance. Most APIs have a specification for particle size that must be analyzed reliably and reproducibly before product can be integrated into the final dosage form. Many different techniques are used to perform particle size distribution analysis. This application note compares two techniques for particle size analysis: laser diffraction and single particle optical sizing (SPOS). A powder API was analyzed on both techniques and results were compared. The sample was then spiked with a 50 µm polystyrene latex standard, to test for sensitivity to tails outside of the main distribution.

INTRODUCTION

An API can be introduced to the patient via various dosage forms including oral, inhalation, parenteral, ophthalmic, topical, and suppository. Oral dosage forms can include: tablets, solutions, and suspensions. The particle size in tablets and suspensions is important for many reasons, including process characteristics such as powder flow, dissolution rate and content uniformity. With regards to content uniformity a few large particles can cause a dose to exceed safe limits and be detrimental to patient health.

Many techniques are used to measure particle size of active APIs for oral dosage forms including microscopy, sieves, laser diffraction and particle counting techniques. Microscopy is the most direct measurement and provides shape information. Sieves are often used for larger particle sizes (>50 µm) when analyzing powders. Laser diffraction may be the most common technique, because this method is fast, repeatable and covers a wide dynamic range. Counting techniques are inherently higher resolution and can provide quantitative concentration results.

Single particle optical sizing (SPOS) is a high resolution particle counter and particle size analyzer. Particles in liquid suspension flow through a photozone where they interact via extinction and/or scattering with a laser light source, see Figure 1. The extinction/scattering by the particle is related to particle size and concentration through the use of a pulse height analyzer and a calibration curve. The result generated is the concentration and particle size distribution of the particles in suspension.

Laser diffraction is a common particle size analysis technique used in many industries, including the pharmaceutical industry, see Figure 2. In Figure 2 we see that particles flow through a cell illuminated by one or more laser light sources. Laser/particle interactions create scattered light collected on multiple detectors and many angles. The particle size and light scattering angles have an inversely proportional relationship - larger particles scatter at low angles and smaller particles scatter at higher angles. The scattered light is converted to a particle size distribution using proprietary algorithms based on either Fraunhofer or Mie theory. Using Mie theory can generate more accurate results at smaller particle sizes (<20 µm), but requires accurate refractive index (RI) values for the dispersed phase of the particles.

Figure 1. SPOS technique

Figure 2. Laser diffraction technique
In this study, both SPOS and laser diffraction was used to determine the particle size distribution of an API powder suspended in liquid. Then the API suspension was spiked with 50 µm polystyrene latex (PSL) particles to compare the sensitivity of the two techniques to a second population outside from the main distribution.

**MATERIALS**

The API used in this study is Aripiprazole, in powder form. The sample was analyzed using the AccuSizer® A7000 AD SPOS system with the LE-400 sensor, dynamic range 0.5 – 400 µm and the Malvern Panalytical Mastersizer laser diffraction analyzer, dynamic range 0.2 – 2000 µm with the HydroS liquid sampler. IGEPAL® CA-630, octylphenoxypolyethoxyethanol, a nonionic surfactant, Sigma Aldrich product number I3021 was used to help wet and disperse the powder. The AccuSizer was tested using a 49.5 ± 0.7 µm PSL standard from calibration kit part number 075DT0F, lot no. RA06B-N from micro measurement laboratories. The Mastersizer was tested using a 50 µm PSL standard from Thermo Fisher cat no. 4250A, lot no. 44795, mean size = 49.5 ± 0.8 µm. Two different PSL standards were used because the SPOS technique can work at much lower concentrations than the laser diffraction technique.

**EXPERIMENTAL**

The API sample was prepared using the following procedure for the SPOS measurements:

- 0.05 g of API was weighed and placed into a 250 mL beaker
- 3 drops of 0.1% Igepal CA 630 was pipetted onto the powder
- 150 mL of DI water was poured into the beaker
- An ultrasonic probe was used for 60 seconds to disperse the powder

The API sample was prepared using the following procedure for the laser diffraction measurements:

- 3 drops of 0.1% Igepal CA 630 was pipetted onto the powder
- 100 mL of DI water was poured into the beaker
- An ultrasonic probe was used for 60 seconds to disperse the powder

These preparations were slightly different because as stated above, the SPOS technique can work at much lower concentrations than the laser diffraction technique.

The AccuSizer A7000 was flushed to reduce the background count to below 200 particles/mL. The measurement protocol used is shown below:

- Sample volume: 100 µL
- Flow rate: 60 mL/min
- Sensor mode: Summation
- Size threshold: 0.56 µm
- Stirrer speed: 60%
- Target concentration: 3500/mL
- Baseline offset*: 0
- Equilibration volume: 2 mL

* A 0 baseline offset means that all counts from all channels were included in the result calculations.

A summary of how the SPOS measurements were performed is shown below:

1. The beaker was placed on a stir plate to continuously mix the sample. This reduces the possible error from subsampling from the beaker into the analyzer.
2. Filtered DI water passes through the sensor until the background count of 200 particles/mL is achieved.
3. 100 µL of the sample was pipetted into the 60 mL mixing bowl in the AD sampler.
4. The sample experiences automated single stage exponential dilution until the count rate falls below the 3500 particles/mL target concentration.
5. The 2 mL equilibration volume is passed through the sensor before the measurement begins.
6. The sample is measured for 60 seconds.
7. The system flushes until the background count is again achieved.

The Mastersizer measurement protocol used is shown below:

- Analysis model: Multiple narrow modes*
- Sensitivity: Enhanced
• Particle RI: 1.590, 0.01**
• Dispersant RI: 1.33
• Sample time: 12 seconds
• Pump/stir speed: 2500 rpm
• Ultrasound = Off

*This model provides the highest resolution possible in order to resolve multiple peaks. It is rarely used for routine particle size analysis, but was chosen to best detect the 50 µm PSL spikes.
**These RI values produced the lowest weighted residual values - the suggested approach for selecting the RI of unknown samples (most APIs).

A summary of how the Mastersizer laser diffraction measurements were performed is shown below:

1. The beaker was placed on a stir plate to continuously mix the sample. This reduces the possible error from subsampling from the beaker into the analyzer.
2. Clean DI water was recirculated through the system while the optics were automatically aligned and the background was determined to be below 20 on the 20th detector.
3. Sample was pipetted into the HydroS sampler until the obscuration range was between 5 – 15%.
4. The sample was measured for 12 seconds.
5. The sampler was flushed twice to reduce the background to below 20 on the 20th detector.

RESULTS—BASIC PARTICLE SIZE ANALYSIS

A graph showing four SPOS repeat results of the API suspension is shown in Figure 3 and a table summarizing the results is shown in Table 1.

<table>
<thead>
<tr>
<th>SIZE</th>
<th>CUM PARTICLES/GRAM</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥0.63</td>
<td>5.46E+09</td>
</tr>
<tr>
<td>≥1.915</td>
<td>2.85E+09</td>
</tr>
<tr>
<td>≥5.366</td>
<td>8.77E+08</td>
</tr>
<tr>
<td>≥10.005</td>
<td>1.66E+08</td>
</tr>
</tbody>
</table>

Table 2. Cumulative number of particles/gram above given size

The AccuSizer software can also provide quantitative result calculations such as volume fraction, ppm/ppb, and absolute volume. For this study the tabular results were exported into excel where calculations were made to determine the number of particles (droplets)/gram greater than specified sizes. The results shown in Table 2 show cumulative number of particles/gram greater than 0.63, 1.9, 5.4, and 10 µm for measurement R2.

A graph showing three laser diffraction repeat results of the API suspension is shown in Figure 4 and a table summarizing the results is shown in Table 3.

Table 1. Overlay of four SPOS results

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Table 3. Overlay of three laser diffraction results
It is important for particle size results to be repeatable and reproducible. According to the USP <429> Light Diffraction Measurement of Particle Size, the expected repeatability for three measurements should agree within a coefficient of variation (COV) of less than 10%, at the D50 and less than 15% at the D10 and D90. The COV is defined as:

\[
\text{COV} = \left( \frac{\text{standard deviation}}{\text{mean}} \right) \times 100 \tag{Equation 2}
\]

The SPOS results were very repeatable, exceeding the requirements given in USP <429>. The SPOS results reported COVs of 2.85% at the D50, 2.68% at the D10 and 1.93% at the D90, as seen in Table 1. Although no official USP test exists yet for the SPOS technique, these kinds of results indicate this is a suitable technique for particle size analysis of APIs. The laser diffraction results reported COVs of 3.823% at the D50, 8.652% at the D10 and 3.944% at the D90 as seen in Table 3. These values all lie within the USP <429> guidelines.

<table>
<thead>
<tr>
<th></th>
<th>D10</th>
<th>D50</th>
<th>D90</th>
</tr>
</thead>
<tbody>
<tr>
<td>API R1</td>
<td>4.158</td>
<td>10.501</td>
<td>22.428</td>
</tr>
<tr>
<td>API R2</td>
<td>4.679</td>
<td>10.855</td>
<td>22.994</td>
</tr>
<tr>
<td>API R3</td>
<td>4.938</td>
<td>11.331</td>
<td>24.219</td>
</tr>
<tr>
<td>Mean</td>
<td>4.592</td>
<td>10.896</td>
<td>23.214</td>
</tr>
<tr>
<td>St Dev</td>
<td>0.397</td>
<td>0.416</td>
<td>0.915</td>
</tr>
<tr>
<td>COV (%)</td>
<td>6.652</td>
<td>3.823</td>
<td>3.944</td>
</tr>
</tbody>
</table>

Table 3. Laser diffraction result summary

**DISCUSSION: BASIC PARTICLE SIZE ANALYSIS**

The AccuSizer SPOS and laser diffraction results agree very well given that these are two entirely different techniques based on different principles. The AccuSizer reports a more narrow distribution than the Mastersizer. The span is a common way to report the width of the particle size distribution, defined as:

\[
\text{Span} = \frac{(D90 - D10)}{D50} \tag{Equation 1}
\]

The span for the AccuSizer = (18.752 – 4.893)/9.997 = 1.386

The span for the Mastersizer = (23.214 – 4.592)/10.896 = 1.709

The 23% increase in the span for the laser diffraction results is not unusual since this is a lower resolution technique than SPOS. The AccuSizer results are generated by converting individual pulses from particle/light interactions into a particle size based on a calibration curve. Thus each individual particle contributes evenly to the final reported distribution, creating an essentially unlimited resolution result. The Mastersizer results are generated by averaging the ensemble light scattering from all of the particles over a period of time. This averaged light scattering is then converted to the reported distribution using an algorithm that is inherently resolution limited. Two characteristics of the resolution limited laser diffraction results are a broadening of the distribution (increased span) as seen in these results, and reduced sensitivity to tails of distributions outside of the main population. The second effect is examined in the next section of this study.

SPOS provides several advantages over laser diffraction, including higher resolution results and greater sensitivity to tails. Previous studies have reported that SPOS is approximately 600 times more sensitive to tails than laser diffraction\(^6,7\). In this study the API suspension was spiked with a 50 µm polystyrene latex (PSL) standards, to test for sensitivity to small concentrations of tails outside of the main distribution.

First 100 µL of the same API suspension used to generate the results seen in Figures 2 and 3, were pipetted into the AccuSizer A7000AD. Next a small volume of 50 µm PSL standard was pipetted into the system. First 100 µL and then 10 µL of the 50 µm PSL standard were introduced to test the sensitivity of the system to the PSL spike. Figure 5 shows the volume distribution result from the 10 µL spike of 50 µm PSL. The AccuSizer A7000AD clearly had the sensitivity to detect the 10 µL spike of 50 µm particles. Figure 6 shows the same result plotted as counts on the Y axis using the full 1024 size channel resolution plus the defined region from 45 to 55 µm. Figure 6 also shows the statistics for the defined region. The “counts” value of 33 is extremely close to the theoretical recovery value of 26. The data available from the counts vs. size data may be helpful to better define the presence of fines in the sample that could negatively impact properties such as powder flow or tablet compression.
Next a similar spiking study was performed using the mastersizer laser diffraction system. Different volumes of a 50 µm PSL standard were added to the API suspension until the laser diffraction instrument was able to resolve the second peak. The 50 µm peak was resolved after 250 µL of the standard was added into 100 mL of the API suspension, see Figure 7 showing an overlay of results from a 150, 175 µL and 250 µL spike of the 50 µm particles. Although laser diffraction could resolve the second peak notice it is still not an entirely separate population as expected and as detected by SPOS.

Since the laser diffraction technique does not report actual concentration a direct, quantitative comparison of sensitivity to the spike of PSL particles was not attempted in this study, but a qualitative calculation indicated the SPOS technique to be approximately 700 times more sensitive to the presence of a second population than the laser diffraction technique. This compares well to other studies investigating the comparative sensitivity of these two techniques. 6, 7 Note that the laser diffraction results were calculated using the higher resolution “Multiple Narrow Modes” algorithm, not the “General Purpose” algorithm most customers would use for standard analysis. Therefore, the sensitivity could actually be much lower for standard operation of the laser diffraction analyzer.

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CONCLUSIONS

The SPOS technique provides a high accuracy, high resolution technique to measure both particle size and concentration. Compared to laser diffraction, the SPOS technique reports a more accurate width of the particle size distribution without false broadening. In addition, the technique is extremely sensitive to tails separated from the main distribution. This could be particularly useful to detect a few large particles that could result in content uniformity problems and over dosage in tablets.
References

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7 Nichols, K., et. al., perturbation detection analysis: a method for comparing instruments that can measure the presence of large particles in cmp slurry, report published by BOC Edwards, Chaska, MN