Particle characterization of pharmaceutical powder mixtures (count, size and chemistry)

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Abstract

For the study of a pharmaceutical drug product, particle characteristics (count, size and chemistry) and compound distributions (size and spatial) are among key parameters that strongly determine the quality of the product due to their huge impact on the dissolution rate (and thus bioavailability) for the final solid dosage form. Monitoring these characteristics is critical in raw materials, interim mixtures during processing, and final products. In this application note, a powder mixture similar to an interim mixture of a pharmaceutical product was used. Laser diffraction and Raman microscopy were applied to highlight the benefits of combining two technologies for particle characterization.

Keywords: Raman Microscopy, Granulometry, Pharmaceutical Powder, Size and Chemical Characterization

Introduction

One of the most important performance factors of a pharmaceutical product is the bioavailability, i.e. the release of the active compound in a human body. In a solid dosage product (e.g. tablets, powders or capsules), this is directly related to the dissolution rate, which in turn is a function of particle size. In a raw material, mainly found as powder samples, it is essential to determine the particle size and size distribution. During the pharmaceutical development, multiple ingredients are mixed. Therefore, it is critical to monitor particle size, size distribution and spatial distribution of these compounds in the intermediate/final products. Less than optimized processing, for example too little or too much mixing, can lead to agglomerates and uneven distribution, which can result in a poor content uniformity, a heterogeneity in the product, or an undesirable dissolution rate profile.

There are multiple analytical methods to characterize particle size and size distribution. Examples include laser diffraction, dynamic image scattering and static image analysis. Each has its own strength and weakness, but all share the same weakness – no chemical information. When dealing with a raw material, this is not a huge limitation – the chemistry is known. During processing dealing with mixtures, this could be a critical shortcoming as it can be very important to get chemical information on the samples.

There are multiple analytical methods to characterize chemistry. Examples include various spectroscopy technologies such as Raman and IR spectroscopy.

Each has its own strength and weakness, but all share the same weakness when it comes to particle analysis – no spatial distribution information. When dealing with a raw material, this is not a huge limitation – all particles are of the same ingredient. During processing dealing with mixtures, this could be a critical shortcoming.

Laser diffraction and Raman microscopy, which itself is a hybrid technology, Raman spectroscopy and optical microscopy, can be complementary. Both laser diffraction and Raman microscopy perform particle analysis, but in different ways (volume based vs area based). Utilizing and cross-checking results from both technologies can fortify the credibility of overall analysis.

- The advantages of laser diffraction include wide particle size range, high speed analysis, good statistical representation and high throughput. The typical output of laser diffraction is the distribution of volume equivalent sphere diameters.
- The advantages of Raman microscopy include dual analysis of static image analysis (optical microscopy) and chemical analysis (Raman spectroscopy). The typical output of Raman microscopy is the distribution of area equivalent circle diameters and chemical identification/confirmation.

In this application note, two ingredient mixtures of varying concentrations were selected and analyzed using laser diffraction and Raman microscopy. Results from each analytical technique were studied for complementary aspects.

Instruments and methods

Laser diffraction

The central idea in laser diffraction is that a particle will scatter the light at an angle determined by the particle's size. Large particles will scatter at small angles, and small particles at wide angles. A collection of particles will produce a pattern of scattered light defined by the intensity and the angle that can be transformed into a particle size distribution result. We used a LA-960 from HORIBA that can measure wet and dry samples from 10nm to 5mm.

Raman microscopy

Raman microscopy combines Raman spectroscopy and optical microscopy. Raman spectroscopy is a non-destructive and non-invasive vibrational spectroscopy that provides information on molecular structures, crystal phases, polymorphism, and much more. Optical microscopy visualizes and captures images of particles based on which static image analysis is performed. Various imaging modes (e.g. brightfield, darkfield, polarized light) are available to achieve the best contrast between particles and substrates. We used XploRA™ PLUS from HORIBA.

ParticleFinder™ is an application (or an App) in the LabSpec 6 Spectroscopy Suite that operates the XploRA™ PLUS. ParticleFinder™ automates static image analysis and Raman analysis with an intuitive user interface. ParticleFinder™ identifies particles in micrographs, counts and locates them, and characterizes their sizes and shapes before performing Raman analysis of each particle. The option is available to record a spectrum from the center of the particle, an average of multiple spectra from the particle, or a map of the particle.

Figure 1: Instruments used for analyses. Left is LA-960 laser diffraction particle size analyzer. Right is XploRA™ PLUS confocal Raman microscope.

Sample preparation

Three powder mixtures of an API and an excipient were prepared. API concentration of each mixture was 25%, 50% and 75%, respectively. The unit is wt/wt %. Pure API powder and pure excipient powder were analyzed as reference samples.

Results

Figure 2 shows the particle size distribution results from laser diffraction analysis using the LA-960. Red graph line represents the particle size distribution of pure API powder (or 100% API), pink 75% API, cyan 50%, black 25% API, and green excipient powder (or 0% API).
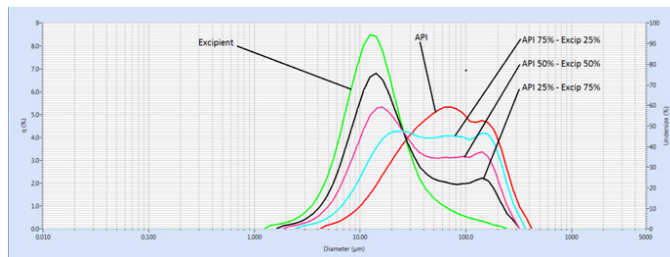

Figure 2: Particle size distributions of mixture powders of different mix ratios, pure API powder and placebo powder.

As it can be seen by observing the green curve, 32% (volume) of the excipient powder is smaller than 10µm, and 98% smaller than 100µm. The red curve highlights that 2% (volume) of the API powder is smaller than 10 µm and 67% smaller than 100µm. Percentages of particles smaller than 10µm and 100µm of each mixture are proportional to API ratio – higher the API ratio, smaller the percentage. As the percentages of particles smaller than 10µm (and 100µm) decrease, the median size of all particles in the sample increases. In other words, the median particle size increases as the API percentage increases for this set of samples.

The percentages are summarized in Table 1. The relationship between the percentage of particles smaller than 10µm and API % in the sample is plotted in Figure 3.

API (wt/wt %)	< 10 µm (Volume)	< 100 µm (Volume)	< 10 µm (Number)	< 100 µm (Number)
0 (pure Excipient)	32,0	98,2	95,3	100
25	21,4	87,5	92,1	100
50	14,2	80,9	89,3	100
75	7,3	73,2	82,7	100
100 (pure API)	2,6	67,2	60,9	100

Table 1: Percentages of particles of smaller than 10µm and 100µm of each powder as a function of API %
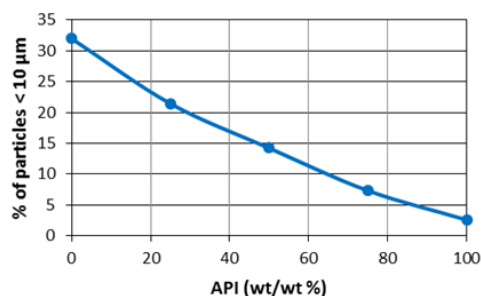

Figure 3: Result below 10µm versus API concentration.

Figures 4 to 6 show the results from XploRA™ PLUS using the ParticleFinder App. Each of three mixture powder samples was dispersed on a microscope slide. Using a 50× long working distance objective, a montage (capturing a series of images, and combined into one image) was recorded over approximately 2 mm × 2 mm. Based on the image contrast, areas of powder were identified as 'particles' on **Figure 4**. Morphological information about the observed 'particles' was directly available with ParticleFinder App. However, please note the 'particle' does not refer to individual particles, rather than clumps of them.
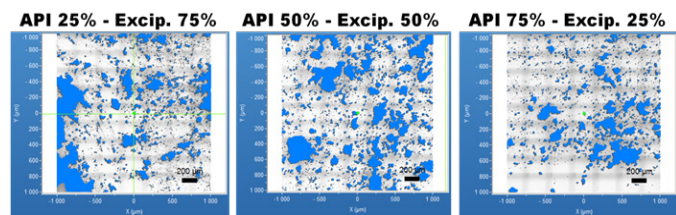

Figure 4: Micrographs of powder mixtures dispersed on a glass slide. Left: API 25%. Middle API 50%. Right: API 75%. Powders are colored in blue, separated from the substrate.

ParticleFinder recorded a spectrum from the center of each 'particle' automatically. Among spectra, two unique spectral species were identified (see **Figure 5**). Each presented an ingredient – red spectrum for the API, and green spectrum for the excipient.
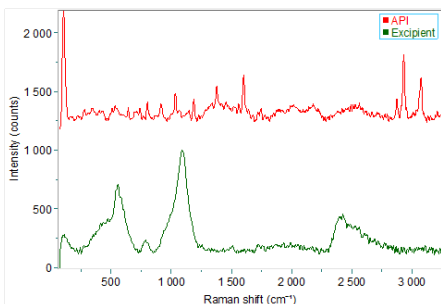

Figure 5: Reference spectra for particles identification.

Red: API Raman spectrum. Green: Excipient Raman spectrum.

Classical Least Square (CLS) analysis calculates the scores that are indicative of similarity of each particle spectrum with respect to reference spectra.

$$I = aA + bB + cC + ... + \varepsilon$$

$I = (i_1\ i_2\ ...\ i_{k-1}\ i_k)$ is the particle spectrum. A, B, C, … are reference spectra. ε is an error. a, b, c,… are scores, and determined by minimizing the error. For example, if a particle spectrum, I, is similar to a reference spectrum, A, the score for the reference spectrum, a, is close to 1, and those for other reference spectra, b, c,… are close to 0.

Figure 6 shows the results of applying CLS to all particle spectra using spectra in **Figure 5** as reference spectra. Red 'particles' indicate high score for API spectrum (or API 'particles'), and green for excipient spectrum (or excipient 'particles'). The gradient in each color (e.g. from bright red to dark red) represents the gradient of scores. Bright red or green represents high scores, and dark represents low.
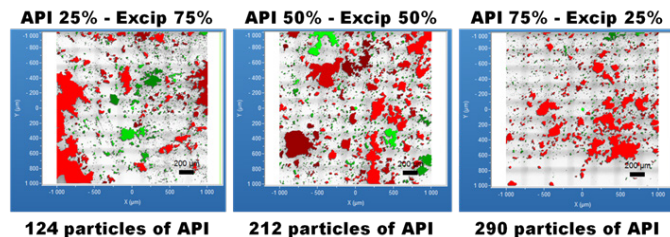

Figure 6: Micrographs of powder mixtures dispersed on a glass slide. Left: API 25%. Middle API 50%. Right: API 75%. Powders are colored in red when their spectra show high scores for API reference spectrum. Powders are colored in green when their spectra show high scores for excipient reference spectrum.

It was also possible to count the number of API particles observed and to correlate these results with the evolution of API's relative concentrations. As shown in **Figure 6**, it is clear that counting the API particle allowed a direct relative comparison between the different concentrations. As results are presented in number of particles, and not in volumic fraction, a clear correlation between the number of particles of API and its relative concentration is obtained. That confirms that mainly no aggregation is induced by the presence of the excipient. These results agree also with the assumption of non-interaction between the particles suggested by particle size analysis where the diameter distributions were equivalent for API's particles, independent of the excipient's relative concentrations.

Conclusion

We have demonstrated the possibility of syngeneic and complementary benefit of coupling laser diffraction (volume base, batch analysis, size distribution) and Raman microscopy (area base, individual analysis, chemistry based size distribution, potential spatial distribution). We have introduced ParticleFinder™ that combines and automates static image analysis for particle count, size and shape, and Raman spectroscopy for chemical identification. We hope to expand the applications of these combined and newly developed technologies to characterize particles and monitor their changes (e.g. physical aggregations, chemical reactions) throughout the pharmaceutical process.

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