Figure Out Any Particle with the Hound

Automated technology for particle characterization leads to more efficient analysis

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INTRODUCTION

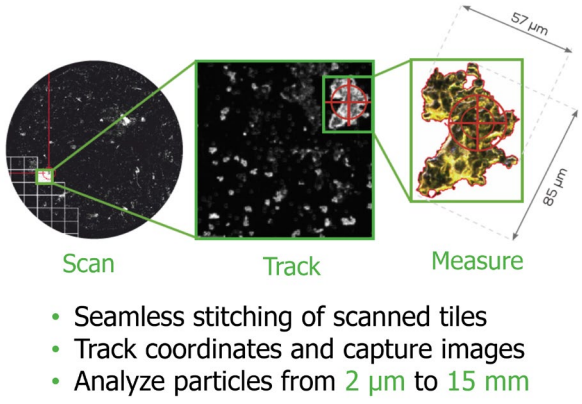
From drug product development to manufacturing, particle analysis is a critical quality attribute (CQA), which makes having the right tools for particle characterization essential. In development, characteristics such as morphology and size distribution are used to examine the solid forms of the active pharmaceutical ingredient (API). These properties are essential indicators for drug candidate selection because they can impact bioavailability, performance, and stability. Later stage particle identification can also be used to determine product purity and monitor particle changes in formulation mixtures. This information can help fast-track process development, monitor process variability, and be used in quality control (QC) for batch release.

PARTICLE SIZE ANALYSIS

There are four primary considerations for particle analysis techniques: function, throughput, ease of use, and flexibility. Function describes the ability to characterize the particle by count and size, morphology (shape), and identification of the particle. It is often necessary to analyze a large number of particles to get statistically significant data to draw the correct conclusions. Therefore, high throughput is essential. The analysis technique should also be easy to use and have a minimal impact on particles during sample preparation to prevent unnecessary stress, which could impact particle size or shape and affect conclusions. Finally, flexibility is required if the sample type will change. In drug development, the same API can be a solid powder, crystalline, in suspension, or in tablet form depending on the stage of drug development and analysis.

In many situations, it is not possible to characterize a sample solely by size distribution. This could occur if the sample has a complex mixture of materials, and those materials have

Figure 1: Fully automated particle analysis.



similar or identical size and morphology. In those situations, a more specific identification technique may be required. Those potential techniques include but are not limited to Scanning Electron Microscopy (SEM), Infrared (IR), or Raman spectroscopy. While each of these techniques have the ability to specifically identify materials, the requirement to test and identify thousands or tens of thousands of individual particles can be slow and cumbersome.

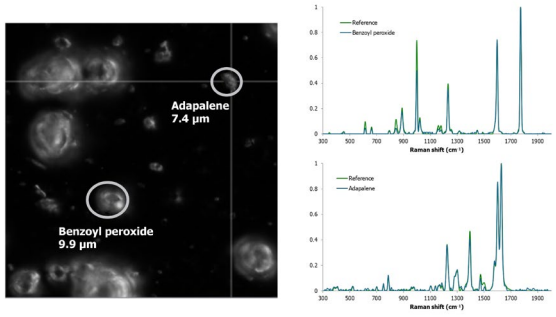
The Hound® instrument from Unchained Labs can characterize particles by count, size, and shape while also performing chemical and elemental identification on a single 21 *Code of Federal Regulations* (*CFR*) Part 11-compliant platform. Microscopy-based imaging automatically acquires images for count, shape, and size distribution for a wide variety of sample types—in solution or dry dispersed. As illustrated in FIGURE 1, the instrument can automatically scan and stitch a large area when it images a sample. It detects particles in each image, counts them, and stores size and shape information in sizes ranging from 2 µm to 15 mm, while also tracking the particle coordinates for future reference. The sample preparation is very straightforward. If particles are in solution, they are pipetted into a wet cell or filtered through a gold-coated membrane and directly analyzed. If the sample is a powder, it can be directly dispersed on the filter and analyzed.

Image-directed identification is accomplished using three laser options: Raman, at 785 nm and 532 nm, and laser-induced breakdown spectroscopy (LIBS). Dual Raman lasers are used to obtain the chemical fingerprint of a wide range of particles. The 785 nm laser can help identify fibers and contaminants such as laboratory wipes, while the 532 nm laser is optimal for identifying protein aggregates. Both lasers can ID particles down to 2 µm, and each has a built-in reference database of more than 150 contaminants that can be further customized.

Raman has several advantages for particle analyses. This technique provides a structural fingerprint based on unique Raman peaks that correlate to specific chemical bonds to identify a wide range of organic and inorganic materials. Consequently, by using Raman, it is possible to differentiate highly similar materials like different types of cellulose or other polymers, polymorphs, or proteins, and aqueous solutions can be analyzed because water does not interfere.

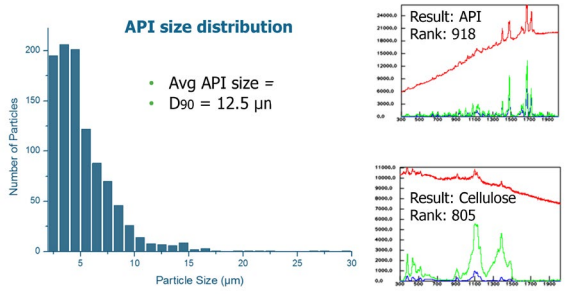
The LIBS option can be used to identify metal and other elemental particles down to 20 µm using a high-energy pulse that is laser-focused on the particle's surface, which promotes atoms to an excited state and creates a plasma. When the atoms and the plasma go back to the ground state, they emit an element-specific spectrum measured for material identification. Like Raman spectroscopy, LIBS identifies the particle material by comparing unknown spectra with those in a reference database, taking only one second per measurement with no additional sample preparation required.

Figure 2: Automated image analysis and ID.



- Raman spectra provides exact match
- Identified over 4000 particles in < 3 hours

Figure 3: Raman is performed to identify the API.



- 1,011 API/ 3000 particles analyzed
- Results 15X faster with morphology-directed Raman

The case studies below highlight how particle size distribution was supplemented with Raman and LIBS identification in cases where a size distribution alone was inadequate to fully characterize the sample.

TOPICAL CREAM ANALYSIS

API distribution in multi-API topical creams can be a challenging analysis, particularly if the two APIs are similar in size and shape. Using only particle size and shape alone cannot always distinguish between APIs or provide data on batch-to-batch variations. FIGURE 2 shows the Raman spectral analysis of a dual-API topical cream. Sample preparation is very straightforward, involving merely applying the cream directly to a gold-coated glass slide. As seen in FIGURE 2, the API particles in the cream are similar in size and shape. Using a traditional microscope, it is impossible to distinguish the API population by just size, count, and morphology alone. However, knowing the APIs are adapalene and benzoyl peroxide, particles are analyzed via Raman to get statistically meaningful size distributions for each API, and over 4000 particles can be identified and distinguished in less than three hours. Using Raman spectroscopy, combined with image analysis for size distribution, a distinct API size distribution from the two different APIs can be determined, and in this example, three times more benzoyl peroxide particles were found than adapalene particles.

NASAL SPRAY PARTICLE ANALYSIS

It is vital to characterize active ingredients by size distribution to ensure the API remains within specification for the best delivery and bioavailability. However, when the API is present in trace amounts (e.g., nasal sprays), several thousands of particles must be analyzed to find enough API particles to assess particle size distributions, which is highly inefficient. Since the count and size of every particle cannot distinguish filler materials from the API, Raman is the perfect technique to quickly identify API in the presence of a large number of excipients or filler. By combining rapid morphology classification with Raman identification, API identification and characterization can be easily and rapidly obtained. FIGURE 3 demonstrates how Raman can be used to identify an API by restricting identification to particles meeting specific size distribution, shape selectivity, and morphology grouping, which significantly reduces analysis time.

The challenge with this sample is that the API-content-to-excipient ratio is so low that looking for 1000 API particles is analogous to

Figure 4: Fibers caused batch failures.

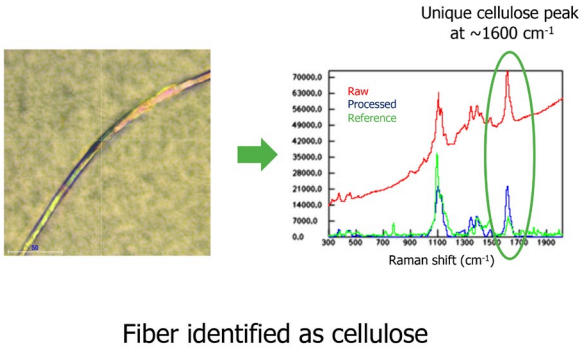
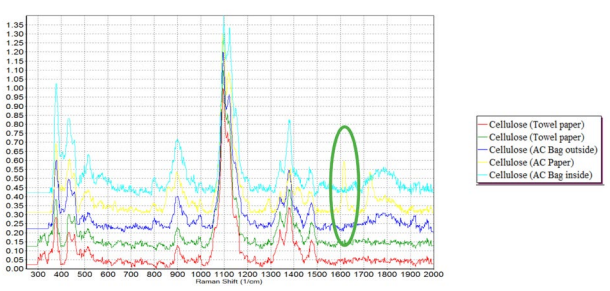


Figure 5: Moved upstream to manufacturing.



looking for a needle in a haystack, since the relative number of API particles in the sample was 2%, while the cellulose filler content was 98%. The initial analysis determined that the elongation factor of the API particles was always below 2.5, in contrast to the cellulose particles that were typically slightly elongated and had an aspect ratio of up to 5. A secondary selective analysis method was then set up to automatically direct Raman identifications to particles with elongation factors less than 2.5. This morphologically directed Raman analysis increased the API particle detection rate from 2% to 34%. As a result, only 3000 particles need to be identified to achieve 1000 API identifications instead of 45,000 particles needed without morphology classification beforehand.

CONTAMINANT CHARACTERIZATION

Particle contamination is a significant reason for batch failures in injectables. Visible particles can come from various sources, including materials that are intrinsic to the product, such as protein aggregates, or they can be an external contamination from the manufacturing process. When these contaminations occur, the source and criticality must be determined, and the root cause of the particle contamination eliminated.

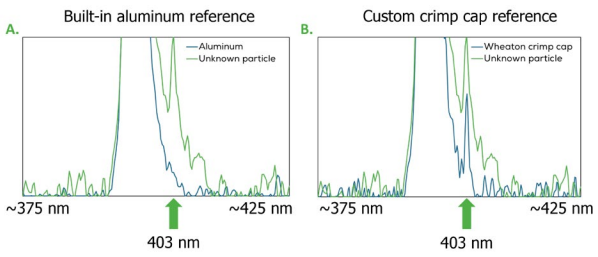
Raman and LIBS can be used to identify particle materials and compare them to production-specific particle sources. An example is illustrated in FIGURE 4, where visible cellulose particles were found to contribute to batch-failures. The Raman spectrum in FIGURE 4 shows an additional peak at 1600 cm^{-1} that is not present in most cellulose spectra and was used to find the specific source of the contaminant. Laboratory equipment and supplies were rinsed, and wash liquid was filtered to determine the source of the fiber. Rinse solutions from rubber stoppers used in vial capping clean-in-place equipment, and tubing used in the process were all analyzed, but none of these fibers had the characteristic peak seen in the contaminant.

Next, several types of cellulose material were collected from the laboratory, and the spectra were compared with the contaminant spectrum. However, again, none of them had the characteristic contaminant spectral peak. When the process was investigated further upstream, a spectrum belonging to cellulose laboratory wipes used to clean the process tanks matched the contamination fiber spectrum, as shown in FIGURE 5. Knowing the source and the pathway, the particle source could be easily removed.

METAL CONTAMINANT IDENTIFICATION

Metal contaminations like aluminum can be easily identified, but it is often essential to

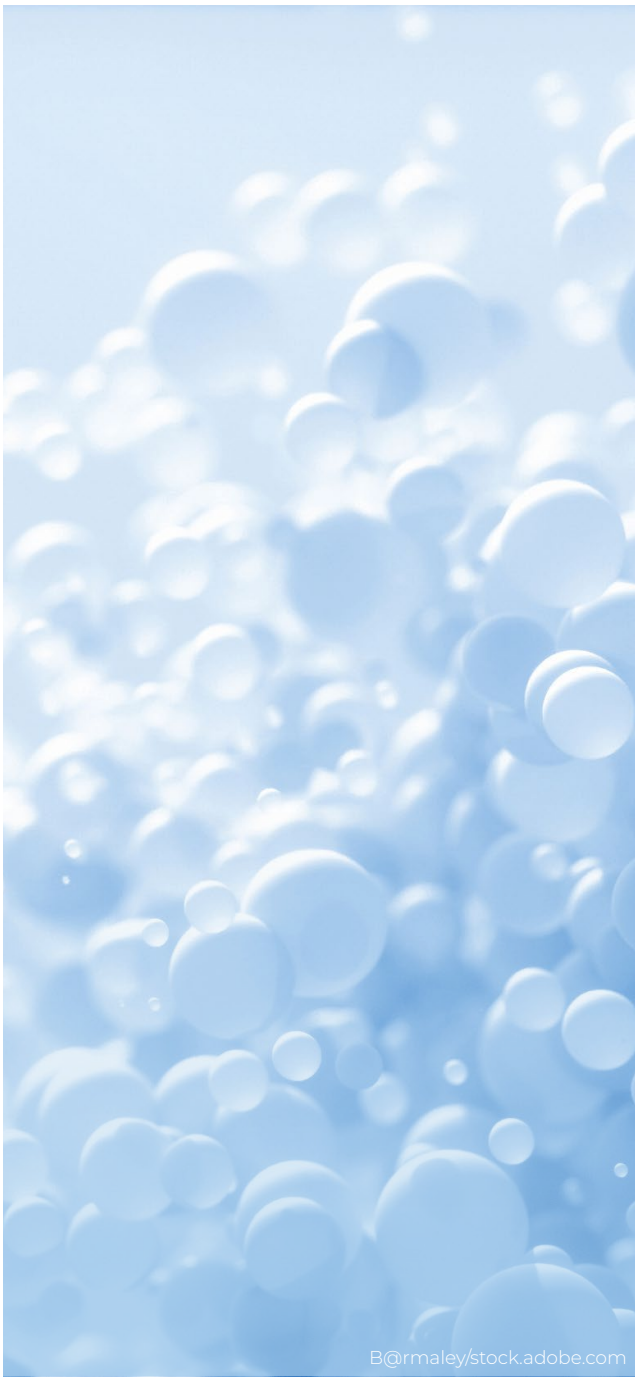
Figure 6: Precise matching capability.



discover the source. LIBS can be used to precisely match a material to determine the exact source of the particle, as presented in FIGURE 6. In this example, a precise match to a name-brand crimp cap was found by adding a custom crimp cap reference spectrum to the customizable database. As seen in FIGURE 6, LIBS is sensitive enough to catch the subtle difference in the 403-nm peak to identify the contaminant as not just an aluminum shard, but an aluminum crimp cap shard. This example illustrates the power of the customizable reference library that can identify a specific contaminant source.

CONCLUSION

Hound combines microscopy, Raman, and LIBS to characterize particles based on their count, size, shape ,and identification through chemical and/or elemental fingerprinting. With over 150 Raman reference spectra and 50 LIBS references, matches for unique particles of interest can be obtained in minutes. For increased specificity to find an exact match, Raman and LIBS databases can be fully customized with just a few minutes per material.



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