A Winning Combination

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Automated imaging systems have recently been combined with Raman spectroscopy to achieve a highly developed analysis of active ingredients that supports and accelerates the commercialisation of both innovative and generic products

Ongoing changes within the pharmaceutical industry provide a strong stimulus for the development of new analytical tools, such as instruments that are more precise, faster or more informative than their predecessors. The drive for greater efficiency, for example – from drug discovery through to manufacture – creates a demand for instruments that can efficiently provide the insight needed for fast and effective product development, while simultaneously putting the spotlight on systems that can monitor in real-time to support process optimisation. The blossoming generics sector is similarly thirsty for knowledge, but understanding and providing equivalent pharmaceutical product performance is a significant challenge.

For many years, pharmaceutical companies have used a variety of spectroscopic techniques for compositional analysis. Techniques such as near infrared (NIR) or Raman detect the vast majority of chemical species of interest, and the availability of online systems offers significant potential for real-time compositional monitoring.

Keywords

Raman spectroscopy
Automated particle imaging
Manual microscopy
Morphologically identical active ingredients

More recently, however, Raman spectroscopy has been combined with automated imaging to create systems that have significant potential for the most detailed and insightful product analysis and research. In this article we take a look at the capabilities of such systems, and at how the size, shape and compositional analysis they offer can support the development of pharmaceutical products, from dry powder inhalers to tablets.

Introducing Morphologically Directed Characterisation

The size and shape of drug particles have a defining impact on both in-process behaviour and finished product performance. For instance, during processing, these parameters influence characteristics like flowability, blending, aggregate formation and compaction behaviour in tableting. Crucial impacts on final product performance include changes to pharmacokinetic properties such as dissolution behaviour and bioavailability. The formulation of drug products involves blending active ingredients with many other components, including excipients, fillers and additives, but it is the behaviour of the active ingredient that remains of defining interest. This creates a need for component-specific information.

This need is typified by regulatory requirements for the testing of nasal sprays. Chemical manufacturing and control (CMC) guidance for this class of products, both New Drug Applications and Abbreviated New Drug Applications, states that drug producers must "provide information and data on the presence of large particles, changes in morphology of the drug substance particles, extent of agglomerates, and crystal growth" (1). Furthermore, it emphasises the need to define and measure the morphology of the active pharmaceutical ingredient (API) within the spray. Particle size measurements of the API in the formulation, before and after actuation of the spray, are also required for bioequivalence (BE) studies.

Conventionally, this need is met using microscopy techniques, but imaging methods increasingly represent a more efficient alternative. Fast, fully automated, and enabling rapid measurement and characterisation of diverse particle populations within a dispersion, these imaging systems are particularly well-suited to the application, especially when twinned with a spectroscopic technique, such as Raman, to provide chemical characterisation of the species of interest.

Particle Isolation

The requirement for component-specific analysis is far from isolated to the development of suspension and nasal sprays. For example, during tablet development, manufacturers often rely on a detailed understanding of the distribution of particle species within the finished product to achieve the necessary drug release profile. Such information may be an important aspect of quality control, or may be needed to fulfil regulatory requirements around the demonstration of BE, in the development of generic products.

Furthermore, for all types of pharmaceutical products, the detection of foreign particles within the formulation can be achieved in a strictly analogous way. Detecting and eliminating the source of foreign contaminants is a critical aspect of pharmaceutical product manufacture.

Raman spectroscopy is an established method within the pharmaceutical industry for measuring the chemical identity and structure of materials. It is also valued for its high chemical specificity and ability to differentiate between polymorphs. Morphologically directed imaging using Raman spectroscopy enables characterisation through the collection of spectral data for particle populations of interest. These can then be correlated with reference spectra to securely identify API particles and gather data uniquely for them. The ability to isolate a particle population on the basis of particle size and shape allows the efficient direction of spectroscopy to those particles of interest, providing complete and time-efficient characterisation.

In summary, combining morphological imaging with Raman spectroscopy offers drug manufacturers not only increasingly insightful particle characterisation for development, but it also crucially enables them to meet the stringent regulatory demands of the pharmaceutical industry in a more efficient manner.

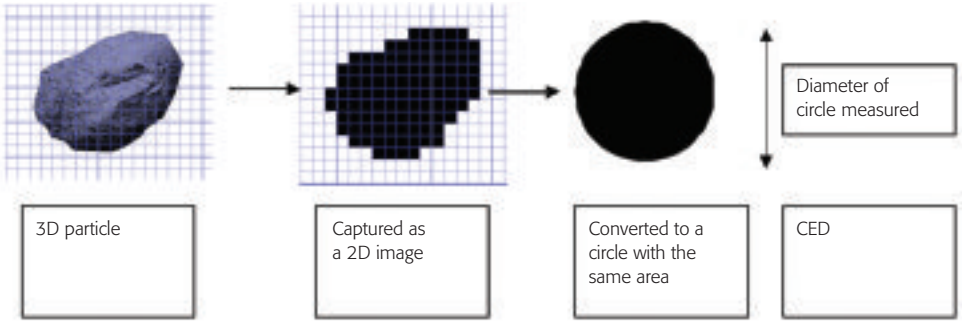

Figure 1: 3D images are captured of each particle. These are rendered as 2D representations which can be manipulated to give various particle data, such as the circle equivalent diameter (CED)

Source: Malvern Instruments

Understanding Particle Imaging

Not so long ago, the idea of analysing and characterising particles by collecting thousands of images in just a few minutes would seem somewhat fanciful. Today, however, considerable advances in digital cameras and imaging software have made this approach both feasible and accessible. Automated imaging is now an established technique for gathering statistically relevant particle size and shape data, and when combined with Raman spectroscopy it also provides comprehensive chemical analysis.

The forerunner, and alternative, to automated imaging is manual microscopy. This entails an analyst looking at a sample through a microscope and individually classifying a specific number of particles. The limitations associated with this method are obvious – the analysis is time-consuming, subjective and potentially inaccurate. As a result, manual microscopy is progressively being replaced with more efficient automated imaging for many applications. Automated imaging systems can analyse 10,000-500,000 in a single measurement within a matter of minutes, offering significant cost and efficiency savings relative to manual microscopy, in addition to greater data integrity.

Automated image analysis measures the size and shape of each particle within a sample. The technique involves dispersing a sample on a transparent plate, which can be automatically scanned under highly optimised digital microscope optics to capture a 2D image of each particle (see Figure 1). Metrics are derived from these images to describe particle size – via parameters such as circle equivalent diameter (CED) – and length and shape – via parameters such as convexity, elongation and circularity. Data for each particle is used to build number-based size and shape distributions for the sample, and can also be used to group particles based on their morphology to procure information for specific particle populations.

Combining automated particle imaging with Raman spectroscopy enables the differentiation of morphologically identical active ingredients within a dispersion. The techniques work symbiotically, with imaging serving to classify the sample such that chemical identification – a lengthier analysis – is applied only to those particles that cannot be differentiated on the basis of size and shape alone.

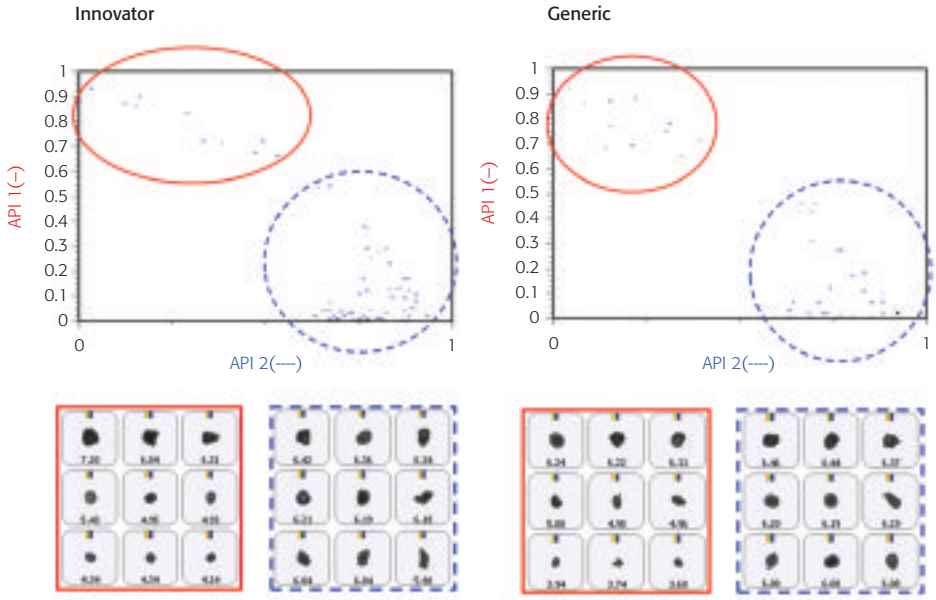

Figure 2:
By analysing a generic drug formulation with automated imaging software coupled to Raman spectroscopy, particles can be classified and characterised for direct comparison with the corresponding innovator product

New instruments streamline the application of these two techniques. They enable the user to rapidly and automatically find particles of interest, identified on the basis of size and shape, and then collect Raman spectra for each one. This allows enumeration of defined classes of particles, as well as the measurement of component-specific characteristics in a blend, such as the particle size distribution of an individual active ingredient. This has beneficial applications throughout the pharmaceutical industry when particle size data for a single ingredient, rather than for the complete formulation, are required, as highlighted previously.

The following case studies illustrate what can be achieved.

Case Study 1: Investigating the Composition of a Multiple Active Tablet

Two tablets – one innovator, one generic – were analysed after disintegration in an aqueous medium, using automated image analysis and Raman spectroscopy. A spectral reference library was created for the sample by taking representative spectra of the pure components, consisting of two different APIs and numerous excipients. The size range of interest for the analysis was between 1-10µm, where the majority of the API particles were expected to be, and Raman spectra were therefore acquired only from particles in this size fraction to minimise the analysis time. In this case, the components within the tablet could not be differentiated on the basis of morphology, as the particles of all the ingredients were similar in shape (irregular).

Figure 2 is a scattergram showing the chemical correlation values for the two API components for each product. In both formulations, particles of API 1 and API 2 are clearly separated via the Raman data, and once the two API populations are differentiated, it becomes possible to determine an individual particle size distribution for each one.

In the development of generic drugs, this ability to determine the composition of the innovator product to this extent, and demonstrate equivalence in a generic alternative, clearly has great financial value. Accessing this information quickly and efficiently helps to accelerate new products to commercialisation, and provides a secure basis for emulating drug performance.

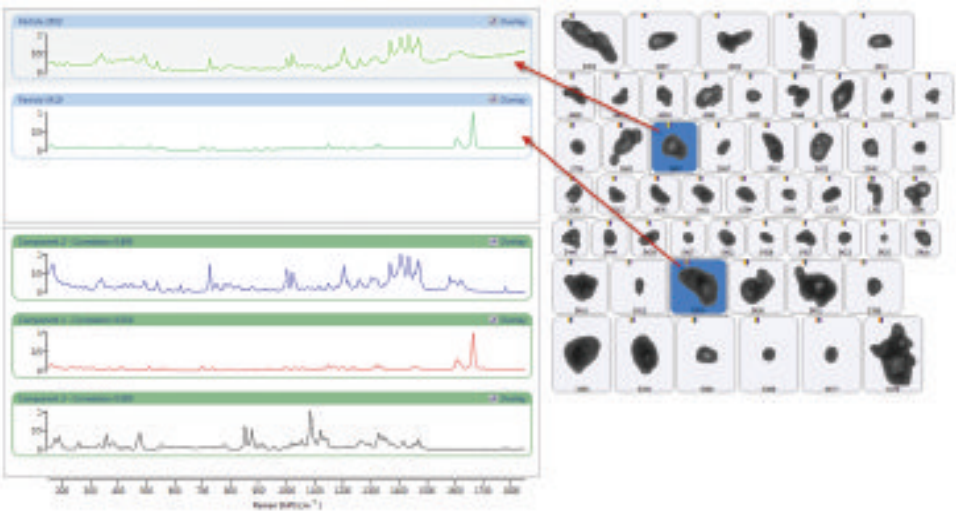

Figure 3:
Two unique particles within a dispersion distinguished by the correlation of their Raman spectra with reference spectra from a pure sample

Case Study 2: Investigating the Composition of a Dry Powder Inhaler

In another experimental study, a commercially available dry powder inhaler containing two active ingredients was characterised. Raman spectroscopy was applied to particles in the sub-ten micron fraction of the dose, as identified on the basis of size data, and the results were compared with reference spectra to identify particles as one active or the other. Here, the sub-ten micron fraction was the size range of interest because it is particles

within this region – especially the sub-five micron fraction – that tend to deposit in the lung. Again, particle size distribution data and shape parameters proved closely similar for both actives, so it was chemical differentiation that held the key to quantifying the amount of each active in the respirable dose (see Figure 3).

Spectroscopic analysis of particles in the sub-ten micron fraction yields a particle size distribution (PSD) for each active. Figure 4 shows the overlay of the CED distribution by number, of each API, from the chemically defined populations. The individual PSDs have been obtained from the blend by setting appropriate classes in the results. The results show that there is little variation between particle sizes of the two APIs.

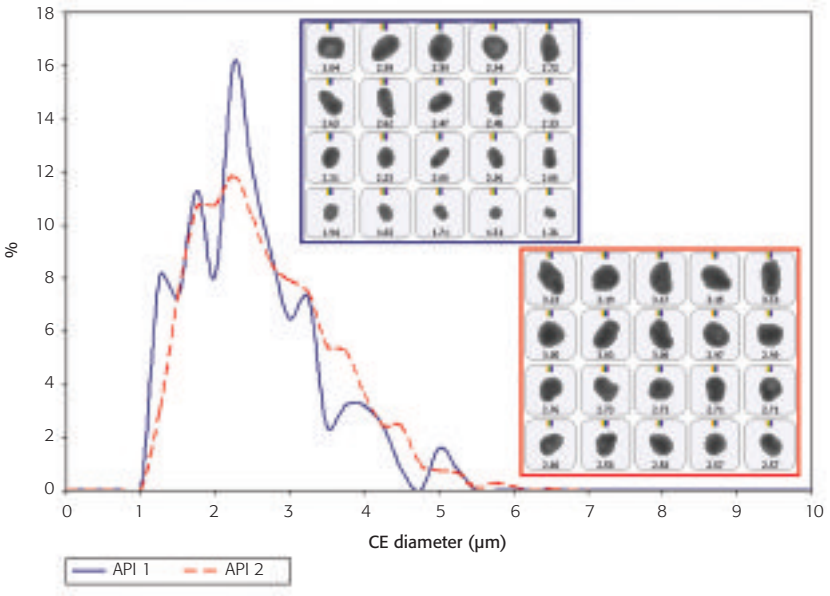

Figure 4: Overlay of particle size distributions for two APIs within a dry powder dispersion

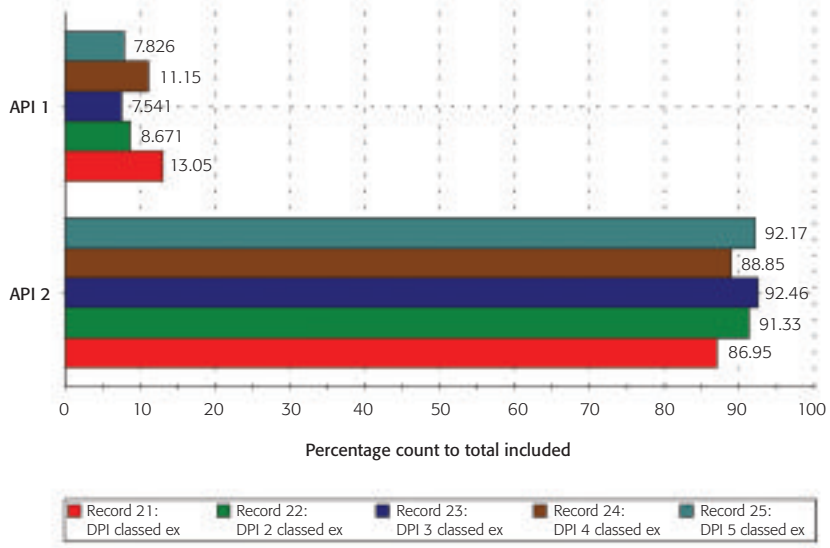

Figure 5: Classification chart of the two APIs in a DPI formulation, showing the relative proportion of each component present

However, once the particles have been chemical identified it becomes possible to quickly determine the relative proportion of each in the blend, and here a clear difference is observed. Figure 5 shows the amounts of the two APIs compared by percentage count of particles following classification based on chemical information. It is clear that the relative proportion of API 2 present in the samples of the formulation analysed is higher than that of API 1.

Conclusion

Across the pharmaceutical industry there are applications that call for the precise and reliable identification of particles within a blend that cannot be differentiated on the basis of size and shape alone. Building on the platform of robust image analysis, with the combined application of established spectroscopic analysis, delivers the powerful characterisation technology necessary to efficiently meet these requirements. From the determination of the particle size of an active in an inhalation product to the differentiation of morphologically identical actives in a multicomponent tablet, morphologically directed Raman spectroscopy supports the rapid and secure commercialisation of both innovator and generic products.

Reference

1. US Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER), July 2002

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