Gateway ANALYTICAL

White Paper

Analysis of Aggregates in Nasal Spray Suspensions

A recently introduced technique that uses Raman Chemical Imaging (RCI) for ingredient-specific particle sizing (ISPS) provides a greatly improved method of analyzing aggregates and agglomerates in nasal spray suspension formulations.

Performed in a GMP environment by skilled analysts who develop validated methods for each individual formulation, the RCI technique has the potential to address the FDA's Critical Path Opportunity (CPO) for direct measurement of particle size equivalence in nasal spray suspensions, possibly allowing for a waiver of *in vivo* biostudies and savings of millions of dollars in development costs and up to half of the time required for clinical studies.

Particle size distribution (PSD) of the active ingredient in a nasal spray suspension has a significant impact on its dissolution rate: larger particles will dissolve more slowly than smaller particles, affecting availability of the drug at the deposition site. As a result, establishing bioequivalence between an innovator and a reference drug requires the sponsor to establish that the drug PSDs of the two products are equivalent.

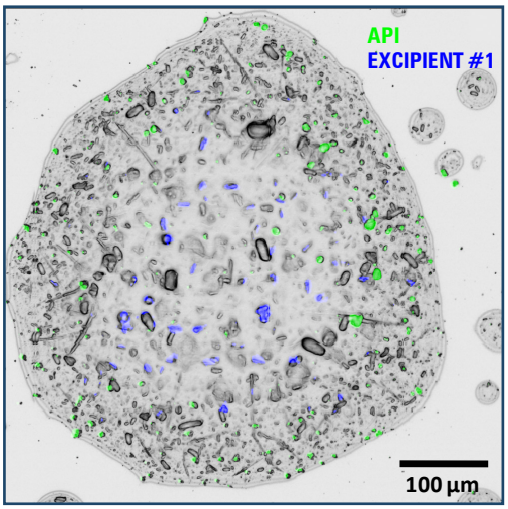

Figure 1. (A) Raman/brightfield image fusion of API and Excipient #1.

That task is complicated by the fact that suspension formulations usually contain one or more micronized active pharmaceutical ingredients (APIs) dispersed in an aqueous mixture that includes partially dissolved excipients such as microcrystalline cellulose, used as a suspending agent. Individual particles of API may agglomerate with each other and/or with the excipients, possibly distorting the drug-specific PSD data. The FDA guidance, therefore, says that that the sponsor should provide comparative data on the size and number of drug aggregates.

Until recently, the only way to assess the number and sizes of aggregates in nasal spray suspensions involved manual analysis of each sample using light microscopy, an extremely time-consuming and labor intensive method, especially since the FDA requires that studies include at least 10 samples from at least 3 batches each of both the test and reference products -- a minimum of 60 samples in total.

Another drawback of light microscopy is that the FDA considers this method "qualitative or semi-quantitative" and insufficient to establish bioavailability on its own. Because light microscopy cannot reliably determine the difference between aggregates of API and aggregates of excipients, and it can miss aggregates when two deposited particles are connected or oriented so that one is hidden underneath the other, sponsors using this method often struggle to establish the validity of their data. So, in order to definitively establish bioavailability for nasal spray suspensions, the FDA requests costly in vivo testing for these products.

Recognizing the difficulty of the approval process for suspension formulations, the FDA has stated in its guidance on CPOs for generic drugs that if the sponsor can establish equivalence between the particle size distribution (PSD) of a test drug to a reference product, including data on aggregates, "with sufficient accuracy and precision," the agency could then treat a nasal spray suspension like a solution formulation and waive *in vivo* testing requirements.

Use of the wide-field RCI technique not only requires significantly less time and labor than light microscopy, it also has the potential to meet those CPO requirements since the technique allows for full characterization of the PSD of one or more APIs within the formulation and reliably distinguishes drug-drug aggregates from excipient aggregates.

Unlike light microscopy, this RCI method for counting and sizing API and aggregates in nasal spray suspension formulations is reproducible and can be validated. Using sizing standards traceable to the National Institute of Standards and Technology (NIST) consisting of polystyrene microspheres (PSMS), analysts can demonstrate the accuracy of the system's measurements and its ability to produce reliable identification of aggregates.

In RCI, a Raman spectroscope analyzes the wavelengths of scattered light emitted by the molecules of every particle in a sample and matches up the spectral information with morphological information. Since each type of molecule emits a characteristic signature, its spectrum can definitively identify the makeup of each individual particle scanned.

The process includes the creation and implementation of a validation protocol prior to initiation of bioequivalence testing. Highly trained analysts first determine testing parameters, taking into consideration factors like fluorescence and Raman scattering signal strength, that are necessary for accurate identification of each active ingredient in the formulation. The method development process is completely documented, with extensive reports provided to the sponsor.

Once these parameters have been identified, analysts begin each ISPS and aggregate analysis by actuating a sample onto a slide so that the testing accounts for any de-agglomeration that might take place during aerosolization. The slide is covered to prevent any possible aggregation due to drying of the sample.

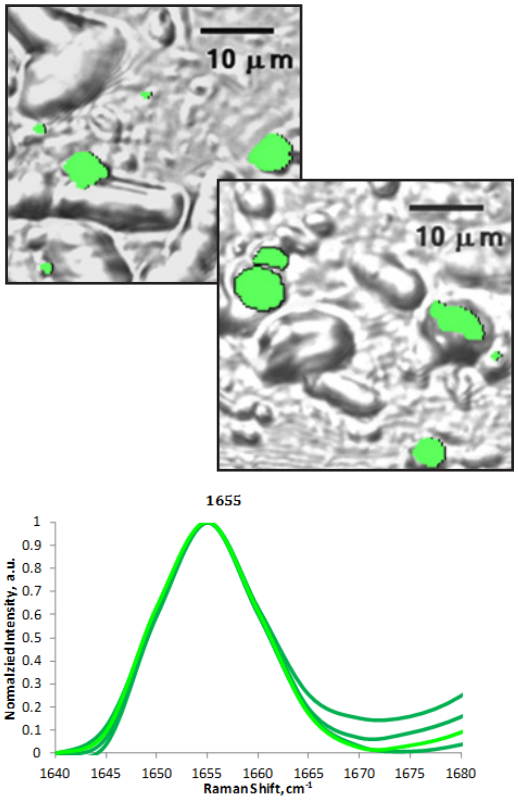

Figure 2. Representative fields of view of a droplet showing Brightfield reflectance/ processed RCI at 1655 cm^{-1} and normalized Raman spectra of the identified API particles. Spectra taken from the neighboring grouped pixels that poses high Raman signal at 1655 cm^{-1} for spectroscopic verification of each detected particle (false-colored in green).

The RCI microscope is then used to automatically collect data from approximately 1,000 randomly selected fields of view (FOVs) from across the entire area of the actuated sample and overlay the spectral data with an optical image of the sample. Using the resulting montage to correlate the data on a pixel-by-pixel basis, analysts can clearly see which particles consist of API and which are excipients. (Figure 1) The system's software then calculates particle counts and sizes the particles automatically. If the formulation contains more than one API, RCI can provide separate PSD data for each.

Analysis by RCI takes up to 90% less time for visual inspection time than light microscopy because, once the system has identified all of the API particles in the sample, the operator examines only the FOVs containing API particles to determine which are aggregates. Out of 1,000 FOVs, as few as 10% may contain API particles, so the operator may need to examine only 100-200 particles instead of thousands, resulting in significant savings in both labor costs and development time.

To assist the operator in determining whether a particle is an aggregate or not, the system generates a binary mask image that represents the API particles in white on a black background. The operator then compares the binary mask to a Raman image produced using a white light source (RWL) that provides a clear picture of the particles and an RWL image fused with an RCI image (Figure 2).

Once the operator has identified particles as aggregates, the system automatically counts and sizes them and provides a complete report of statistical data, as it does for the unaggregated, stand-alone API particles. (Table 1) Advances in image data processing will also soon allow the system to refer only particles that exceed certain size limits or those that have particular shape parameters to the operator for analysis, further reducing the labor involved in identifying aggregates and therefore the cost to the customer.

In addition to providing bioequivalence data for Abbreviated New Drug Applications (ANDAs) for generic nasal spray suspension formulations, the RCI technique for analyzing aggregates can also provide valuable performance information for New Drug Applications (NDAs) for novel nasal spray and metered dose inhaler (MDI) suspension formulations and for quality control in manufacturing situations.