Abstract:
Together the techniques of laser diffraction and automated particle imaging support the fast, cost-effective development of nasal spray products, simultaneously providing the data required for regulatory compliance; this article examines their application.
INTRODUCTION

Nasal spray products are increasingly valued for delivering systemic as well as locally-acting therapies, most notably hormone and migraine treatments. The large surface area and rich blood supply of the nasal passages aid the rapid absorption of drug entities, with their close proximity to the central nervous system being of particular benefit.

The regulatory guidance for nasal sprays emphasizes the importance of testing the device and the formulation together since it is in combination that they define the unique characteristics of the delivered dose. The success of drug delivery is strongly influenced by the particle size of the delivered droplets, and for suspension formulations the particle size of the suspended active as well.

According to the guidance, delivered droplet size ‘is an important property influencing the nasal deposition of aerosols and sprays’. Very fine droplets, lying in the sub-10 micron range, are likely to be drawn into the lungs while those that are excessively large may remain at the front of the nasal passages rather than depositing at the intended site. The particle size of a suspended active pharmaceutical ingredient (API) potentially influences dissolution rate in vivo, and also availability to sites of action within the nose. The recommendation is that particle size should be measured pre- and post- actuation to ensure that requirements for clinical efficacy are met and that the API particles are unchanged by the delivery process.

Laser diffraction is the recommended technique for measuring droplet size, while imaging or microscopy methods are the usual choice for analyzing suspended API, a task that can be complicated by the presence of visually similar excipient particles. This paper reviews the use of laser diffraction (Spraytec, Malvern Instruments) and automated imaging coupled with spectroscopic identification (Morphologi G3-ID, Malvern Instruments) in the development of nasal spray products, showing how the two techniques combine to provide the understanding and regulatory data required to develop and optimize nasal spray products in a cost and time-efficient way.

DEVELOPING NASAL SPRAYS

Conventional nasal sprays usually consist of an API which is dissolved or suspended in an aqueous medium. They are self-administered by the patient via the nasal cavity. Effective drug delivery depends on a number of factors: patient technique and physiology; the properties of the formulation; and the characteristics of the spray pump. Nasal spray development focuses on optimizing the device and formulation to deliver robust performance for the target user group, which may be very broad.

In terms of droplet size the target range tends to be 20-120μm. Droplets in this size range usually deposit beyond the nasal valve, in the posterior two thirds of the nasal cavity, thereby maximizing therapeutic effect. Particles smaller than 10 microns are prone to inhalation into the lungs,
so their generation or presence necessitates an assessment of the clinical risks associated with pulmonary delivery of the API concerned. Conversely, overly large droplets tend to remain in the front of the nose and fail to deliver API to the intended site.

In a nasal spray product, a metered spray pump atomizes and delivers the drug dose and its performance depends on the physical properties of the formulation. The nasal spray developer’s task is to understand and control device-formulation interactions and manipulate the parameters that dictate performance with the goal of better clinical efficacy.

In terms of the device, key variables include: the action of the pump and pre-compression ratio; and the length, geometry and orifice size of the actuator. Together these determine the shear force applied to the formulation during use. The response of the formulation to this applied shear is a function of its physical properties. Viscosity is a particularly important parameter and is routinely manipulated through the inclusion of modifiers and additives. By tuning some or all of these variables, product developers can tailor nasal spray devices to deliver the required droplet size under conditions that apply during patient use. Reliable and timely droplet size measurement supports this optimization process.

Developing suspension-based products is complicated by the fact that the size of both the API and the droplets in the delivered dose are important. Formulating a stable suspension with particles of a size that will ensure the required dissolution rate and bioavailability in vivo, is one part of the development challenge. The other is to make sure that particle size is unaffected by the delivery process, a check that relies on measuring the particle size of the active, before and after delivery.

INTRODUCING LASER DIFFRACTION

Laser diffraction is a non-destructive particle sizing technique, able to measure across a wide dynamic range (0.1 – 3000 microns), in real time. Suitable for characterizing both wet and dry spray samples, and with minimal calibration requirements, it is applicable in the study of both dispersed particle size and spray formation and dynamics. In the development and manufacture of nasal sprays, laser diffraction is used to ensure:

- Efficacy: to target a droplet size range that will maximize clinical efficacy.
- Quality: to verify the consistency of performance: over the lifetime of the product; from batch-to-batch; or after storage
- Safety: to detect and quantify the presence of a sub-10 micron fraction.

Using laser diffraction, particle size is determined from the scattering pattern produced as particles pass through a collimated light beam. Small particles scatter light weakly at wide angles while larger particles give a stronger signal at narrow angles. Laser diffraction analyzers detect the scattered light pattern produced by a sample and generate a complete particle size distribution from it, by applying the Mie theory of light.
Figure 1 shows typical nasal spray data measured using a laser diffraction particle size analyzer configured for spray measurement (Spraytec, Malvern Instruments). Data acquisition rates are very fast with one complete measurement occurring every 0.1ms. This is essential to capture the detail of the spray event, which lasts for just 160ms. Examining the results it is possible to observe three discrete phases in the delivery process: formation, fully developed/stable and dissipation.

Immediately post-actuation, all three size parameters and transmission decrease steeply. This is the formation phase. Transmission, which is shown by the green line in figure 2, is a measure of the source light that penetrates the sample, and therefore a measure of spray density. Transmission data during this phase therefore indicate a steady increase in spray density. Initially flow through the spray pump is low but it builds rapidly as the pump becomes primed. Droplet size decreases to a steady minimum as flow through the pump reaches a maximum steady rate, producing the stable, fully developed phase. This is the phase of most interest since most drug delivery occurs under these conditions. As the metering chamber of the device empties, flow rate decreases once more leading to an increase in droplet size during the dissipation phase that marks the end of the event.

For comparative studies, the US Food and Drug Administration (FDA) guidance recommends using data from the fully developed phase to provide statistically relevant droplet size information. Appropriate data-handling software enables precise definition of this phase making it easy to produce representative averaged data. Size information from the formation and dissipation phases can also be useful though to gain greater insight into how the device is working, a common aim being to reduce the duration of these two phases so that the fully developed phase lasts for as long as possible.
Case Study: Using laser diffraction data to investigate the impact of device and formulation properties.

Figure 2 shows some laser diffraction data from experimental studies carried out to assess the impact of formulation viscosity on atomization behavior and delivered droplet size. These results were gathered using a typical commercially-available nasal pump spray with actuation of the pump controlled using a velocity-controlled actuator (SprayVIEW NSx, Proveris Scientific), set to achieve a maximum velocity of 40 mm/s. The solutions tested differ in terms of polyvinylpyrrolidone (PVP) concentration, PVP being an additive routinely used to modify the viscosity of suspension formulations. Solutions in the range 0 to 1.5% by weight were assessed.

For solutions with a PVP concentration of 0.5% and below, the droplet size profile observed exhibits a clear fully developed phase. However, with the higher viscosity solutions atomization is less successful. Droplet size is much larger and, especially with the 1.5% solution, the fully developed phase is less well defined.

During each automatically controlled actuation, the pump applies the same levels of shear but with higher viscosity solutions this shear has less impact in terms of atomizing the dose. Solutions of higher viscosity require greater energy input to achieve successful atomization to an equivalent droplet size. One important reason for increasing viscosity for suspension-based products is to reduce the risk of settling during storage, another is to increase residence time in the nose. These results show that in the absence of any other measure such an action might have a negative impact on droplet formation and consequently on drug delivery.
Figure 3: The evolution of droplet size (Dv50) during delivery with an Equadel (Valois Pharma) nasal spray pump, for solutions of PVP in water

Figure 3 shows data for the same PVP solutions atomized using an Equadel pump (Aptar Pharma). These comparative data illustrate how changing the pump mechanism can be a successful strategy for achieving better atomization with a more viscous formulation. Equadel employs an energy storage mechanism to give closer control of the atomization process. As the pump is depressed, energy is stored within a spring. This energy is released when the pump reaches a pre-determined hydraulic pressure. This modifies the way in which the energy applied during actuation is released into the formulation, compared with a conventional pump, and has a pronounced effect on atomization behavior. The data in figure 4 show a long stable phase, with every solution, even those of high viscosity, suggesting that delivery would be more successful.

For the 1.0% and 1.5% solutions though, the Dv50 values reported for the stable phase are still relatively high. Possible steps to rectify this include reducing the diameter of the orifice over which atomization occurs and/or increasing actuation velocity, within the constraint of reflecting the capability of the target user group. Laser diffraction would efficiently support the evaluation of either strategy.
INTRODUCING AUTOMATED IMAGING

For suspension nasal sprays it is essential not only to measure droplet size but also to obtain a reliable measurement of the API particle size distribution. This creates a requirement for supplementary analysis that can differentiate the API from any other suspended solids present, and provide a statistically valid measure of its size. The traditional approach is to use manual microscopy but this technique is both labor intensive and operator dependent. Automated imaging is a more efficient alternative, a much faster way of gathering more statistically relevant data.

Over the last decade, automated imaging has benefited hugely from advances in camera technology and data analysis software. The technique involves capturing individual two-dimensional images of three-dimensional particles appropriately dispersed on a plate. Various size and shape parameters are then determined from the dimensions of each image. Principal among these, for size, is circle equivalent (CE) diameter, which is calculated by converting the captured image into a circle of equivalent area to give a single number (diameter) representation of particle size (figure 4).

Figure 4: Circle equivalent (CE) diameter is the diameter of a circle with the same area as the captured two-dimensional image of the particle

With respect to shape an array of parameters may be developed from the defining dimensions of the particle (see figure 5) to build a complete picture. Parameters such as convexity, elongation and circularity, describe not only the overall shape of particles but also the regularity of the shape, whether the perimeter is smooth or more convoluted.

Systems such as the Morphologi G3 from Malvern Instruments measure thousands of particles in just a few minutes to produce statistically relevant descriptors of size, shape, and transparency, that allow the identification and quantification of even very subtle differences.

Figure 5: The key dimensions of a particle can be used to generate a larger number of size and shape descriptors

For anyone looking to apply laser diffraction and imaging together, the preceding discussions highlight some important differences that need to be recognized and understood.
Laser diffraction analysis is an ensemble technique, meaning that it generates a single data point, a particle size distribution, for the entire sample. This distribution is volumetric i.e. it indicates the relative volume of sample in each size fraction. With imaging on the other hand, number-based distributions of size and shape are built-up from measurements of individual particles. These distributions show the relative number of particles in each size or shape fraction. Imaging and laser diffraction data and specifications can be directly compared but these fundamental characteristics of the techniques need to be taken into account when doing so.

Case study: Investigating the impact of drug delivery on API particle size

The particle size of the API in a nasal spray formulation was measured before and after drug delivery, using automated imaging, to determine whether the process of atomization through the nasal spray pump was causing any change. Samples were appropriately dispersed on to a measurement plate and then analyzed to gather both size and shape data for the formulation.

With this product the excipient and API are quite differently shaped and can be reliably differentiated on the basis of certain shape parameters. A shape classification filter was therefore applied to the data, to identify the API population. Particle size data, CE diameter values, were then generated just for these particles. The results are shown in figure 6.

![Figure 6: Particle size data for the API in a nasal spray formulation measured before and after spraying](image)

The profiles indicate that spraying causes a slight shift of the particle size distribution towards finer sizes, suggesting that the shear applied to atomize the dose causes some particle size reduction. Such an effect could have an impact on the deposition of the API and its uptake in vivo and could therefore be influential in determining clinical efficacy.
COMBINING AUTOMATED IMAGING AND CHEMICAL IDENTIFICATION TECHNIQUES

In nasal spray studies the parameters that imaging generates can be used to rapidly classify particle populations to identify and procure information for the API alone, as described. However, imaging and microscopy share the limitation of not being able to distinguish between API and excipients that are morphologically similar. One way of addressing this issue is to supplement imaging with a chemical identification technique such as a Raman spectroscopy. By collecting Raman spectra for particle populations of interest and correlating them with reference spectra it is possible to securely identify API particles and gather data uniquely for them.

An optimal way to apply such technology is to use the size, shape or transparency data to target the acquisition of Raman spectra – so called Morphologically Directed Raman spectroscopy. Doing so significantly reduces the time for measurements compared with conventional Raman mapping methods. In comparison with standard spectroscopy methods it also simplifies the measurement, as the position of the particles is automatically determined before analysis, removing any operator subjectivity.

Case study: Using morphologically directed Raman spectroscopy to verify the quantities of API in a formulation

Images for a suspension nasal spray are shown in Figure 7 alongside reference spectra for the API and excipient present. Chemical spectra data for 9000 particles from a single scan area were gathered and compared with these reference spectra to chemically identify the population of API particles present and verify the proportion of API in the formulation; two example spectra are shown.

Figure 7: Two unique particles distinguished by the correlation of their Raman spectra with reference spectra (shown in green)

Scatterplots of the correlation scores produced (Figure 8) chemically differentiate populations of API and excipient particles and show that of the 9000 particles analyzed, 450 are classified as API. This suggests that the amount ratio of API to excipient in the formulation is 1:20 by weight a figure consistent with the stated composition.
Laser diffraction and Automated imaging - complementary techniques for nasal spray development

Figure 8: Scatterplots for Raman correlation scores of API and excipient particles quantify the relative amounts of each constituent. Example images for the two classes indicate the potential benefit of shape classification.

These data make it possible to compare the ingredient-specific particle size and shape distributions for the API and excipient, an exercise that suggests that the bulk of the excipient can be excluded from any analysis on the basis of shape alone (see figure 9). Particles with an elongation greater than 0.4 can be securely identified as not being API and therefore do not require chemical analysis. Automatic classification on the basis of elongation would mean that only 3000 of the original 9000 particles would need to be chemically identified, reducing measurement times by 66%.

Directing chemical analysis on the basis of automated imaging in this way, efficiently streamlines measurement, enhancing the practicality of this approach for routine use in manufacturing and QC.

Figure 9: Comparison of particle shape distributions for API and excipient based on chemical classification shows that many particles can be secure identified as excipient on the basis of elongation (shape) alone, suggesting that morphological filtering will cut measurement times.
LOOKING FORWARD

The development and manufacture of clinically effective nasal sprays relies on understanding and controlling the atomization processes that drive delivery, to ensure successfully targeted deposition and consistent in vivo behaviour. Laser diffraction provides the real-time droplet size data required to understand the dynamics of atomization, supporting the optimization of both device and formulation. Automated imaging efficiently overlays such data with information relating to the particle size of any suspended active, and how it is affected by delivery. Supplemented with spectroscopy, imaging provides an extremely powerful tool for the insightful investigation of nasal spray products and enables the generation of objective, statistically sound data for the comparison of innovator and generic nasal sprays, as advocated by the regulators.

Together these techniques support drives towards the greater application of nasal drug delivery. One area of significant activity is exploitation of the nasal route for delivering a wider range of drug entities, another is the evolution of dry nasal powder products, a development that parallels the use of dry powder inhalers in the pulmonary area.

The potential benefits of delivery via dry nasal powders include: high patient compliance; good product stability and sterility; and closely targeted drug delivery. Research in this area is still in its infancy but the early indications are that here too laser diffraction and automated imaging will have a role to play in supporting advancement. The complementary application of these techniques holds value in all aspects of nasal spray development, as it does for many other pharmaceutical products, giving these technologies a central place in the industry’s analytical armory.