"Advanced Techniques for Particle Size Determination and Characterization in Aqueous Nasal Sprays: A Comprehensive Review"

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Abstract:
Particle size determination and characterization are crucial for optimizing the formulation and efficacy of aqueous nasal sprays. This comprehensive review explores the methodologies and instruments employed in the analysis of particle size within these pharmaceutical products. The review covers a range of techniques, including laser diffraction, dynamic light scattering (DLS), cascade impaction, spray particle size analyzers, optical microscopy and Morphology G3 ID. Each method's principles, advantages, limitations, and applications are discussed, providing insights into their suitability for different particle size ranges and product specifications. Key factors influencing the choice of method, such as the need for precision, sample preparation requirements, and regulatory considerations, are also addressed. This review aims to offer a detailed understanding of the capabilities and limitations of each technique, highlighting their roles in ensuring the quality and performance of nasal spray formulations. By synthesizing current practices and advancements, this review serves as a valuable resource for researchers, formulators, and quality control professionals involved in the development and analysis of aqueous nasal sprays.

Keywords: Particle size determination, Particle size Characterization, Nasal Spray products, Aerodynamic behavior of particle, Anderson Cascade Impactor, Therapeutic efficacy, Advancing the design and evaluation.

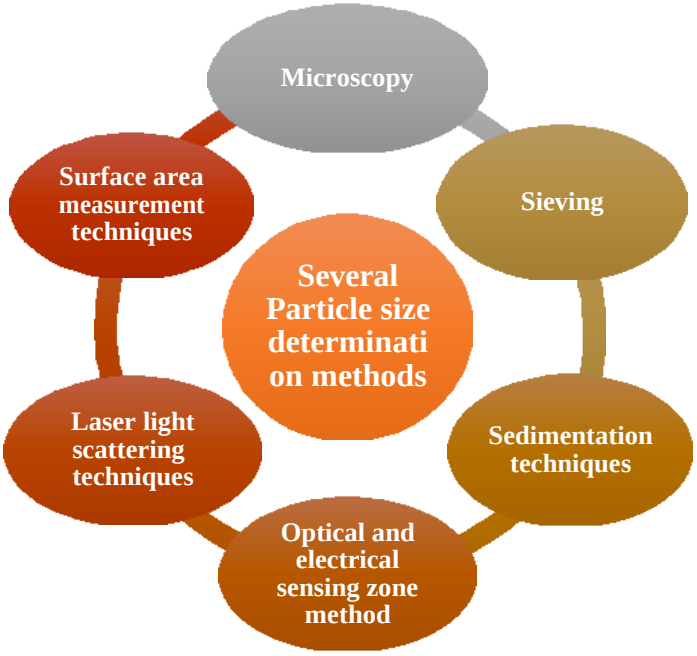
Introduction:
Patients have had access to topical vaccination administration to receptors in the nasal epithelium and inhaled medicine delivery through the nasal route for a long time. The most common method for characterizing aerosol clouds produced by medical aerosol generators in vitro is cascade impactor analysis. Drug fractions are categorized into aerodynamic size ranges pertinent to the deposition in the respiratory tract, which is one of the main justifications for applying this inertial separation concept [1]. Nonetheless, this mode of administration is becoming more popular for various reasons, chief among them being the ability to reach the brain through the olfactory bulb. Decongestants and antihistamines are frequently sprayed into the nose. Still, nasal administration of systemically active medications is gaining attraction. Decongestant and antihistamines are frequently sprayed into the nose. Still, nasal administration of systemically active medications is gaining traction.

However, since the nasal cavity is directly accessible by the spray,it is reasonable to treat the assessment of size distribution from nasal products separately, as done in the European Pharmacopoeia, given that the bulk of the droplets from nasal spray pumps typically have a size range of 20 to about 200 µm, which is more than an order of magnitude larger than the droplets from devices intended for oral delivery to the lungs[2]. Aqueous nasal spray droplet/particle size analysis has long been a part of normal quality control testing and product development. The measurement of suspension formulations is complex due to the presence of active pharmaceutical ingredient (API) particles within these droplets. It has been observed that a single droplet may contain multiple API particles, or may not contain any particles at all, as in the case of suspension pressurized metered dose inhalers (pMDIs). The presence of excipient particles further complicates the characterization of such goods. Droplet and particle size in aerosols can be measured using a variety of methods, thus when applying these measures to nasal medication products, it's critical to understand the constraints and underlying instrument method parameters [3].

Measurements of droplet and particle sizes have also developed as a means of identifying in vitro bioequivalence (BE). These methods form the basis of several regulatory BE guidelines for this reason. While laser diffraction (LD) is recommended as the preferred method for determining droplet size, particle size measurement methodology has developed to include novel techniques, such as Morphology Directed Raman spectroscopy (MDRS) and other advanced laser-based techniques like Phase Doppler Particle Analysis (PDPA), in order to develop more discriminatory methods to support BE guidance [4].

METHODS FOR DETERMINING PARTICLE SIZE:

There are several other techniques for determining particle size instead of Anderson Cascade Impactor like



KEY FACTORS FOR PARTICLE SIZING METHOD SELECTION:

- Nature of the material to be sized
- Estimated particle size and particle size range
- Solubility
- Ease of handling
- Toxicity
- Flowability
- Intended use
- Cost
- Capital
- Running
- Specification requirements
- Time restrictions

PARTICLE SIZE CHARACTERISATION TECHNIQUES:-

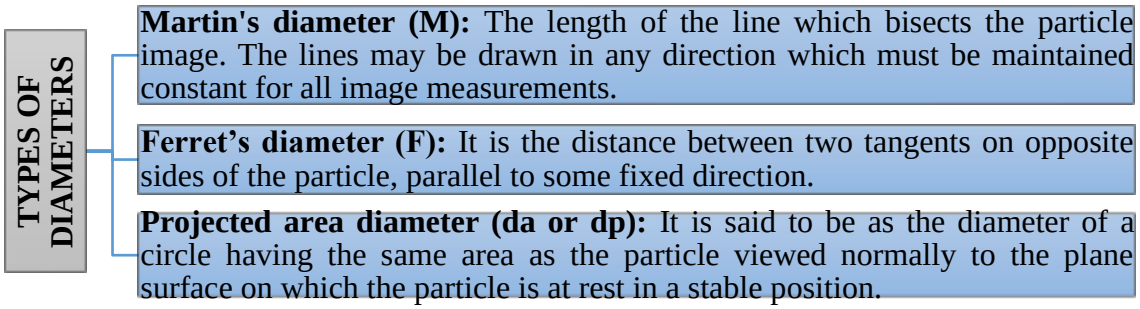
Microscopy-

- Electron microscopy is used for particles larger than 0.001 m while optical microscopy is used for particles between 1 and 150 m.
- The microscopy can look at each particle separately, it is now thought of as an exact way to quantify particle size.
- It can distinguish aggregates from single particles.
- Diffraction effects intensify with smaller particles, producing edge blurring; identification of particles smaller than 3 µm becomes increasingly uncertain [5].

- The primary constraint on optical microscopy is its shallow depth of focus, which is around 10µm at x100 and 0.5µm at x1000.
- For submicron particles it is necessary to use either
- TEM (Transmission Electron Microscopy)
- SEM (Scanning Electron Microscopy).

- TEM and SEM (0.001-5µm).

Types of Diameters-



Manual Optical Microscopy-

Advantages	Disadvantages
Relatively inexpensive.	It is time consuming - high operator fatigue - few particles examined.
Each particle individually examined - detect aggregates, 2D shape, colour, melting point etc.	Very low throughput
Permanent record – photograph.	No information on 3D shape.
Smaller sample sizes required.	Certain amount of subjectivity associated with sizing - operator bias.

Transmission and Scanning Electron Microscopy-

Advantages	Disadvantages
Particles are individually examined.	Very expensive
Visual means to see sub-micron specimens.	Time-consuming sample preparation.
Particle shape can be measured.	Materials such as emulsions difficult/impossible to prepare.
	Low throughput - Not for routine use.

Automatic and Image Analysis Microscopes-

Advantages	Disadvantages
Faster and less operator fatigue than manual	Can be very expensive
No operator bias	No human judgement retained e.g. to separate out aggregates, select or reject particles etc. (unlike semi-automatic)

Sieving-

- A nest or stack of sieves is used to perform sieve analysis, with each lower sieve having a smaller aperture size than the sieve above it.
- Sieves can be referred to either by their aperture size or by their mesh size (or sieve number).
- The mesh size is the number of wires per linear inch.
- Approx. size range : 5µm - ~3mm
  - Standard woven wire sieves
  - Electroformed micromesh sieves at the lower end or range (< 20µm)
  - Punch plate sieves at the upper range.
- Wet sieving
- Air-jet sieving
- Weight distribution

Advantages	Disadvantages
Easy to perform.	Known problems of reproducibility.
Wide size range.	Wear/damage in use or cleaning.
Inexpensive.	Irregular/agglomerated particles.
	Rod-like particles: overestimate of under-size.
	Labour intensive.

Coarse powder Selection-
They are not less than 95% by mass passes through a number 1400 sieve and not more than 40 % by mass passes through a number 355 sieve [6].

Moderately fine powder Selection-
- Number 355 sieves should pass 95% of their mass, whereas number 180 sieves pass 40%.
- Number 180 sieves should receive at least 95% of the fine powder, whereas number 125 sieves should get not more than 40%.
- Homogeneous suspensions settle in a cylinder and take samples at regular intervals at a fixed horizontal level to determine the size distribution.
- The concentration of solid in a sample taken at time (t) is determined by centrifugation of the sample followed by drying and weighing or simply by drying and weighing.
- This concentration expressed as a percentage of the initial concentration gives the percentage (w/w) of particles whose falling velocities are equal to or less than x/t. Substitution in the equation above gives the corresponding Stokes' diameter [7].

Stokes's Law: Stokes diameter (dst) is defined as the diameter of the sphere that would settle at the same rate as the particle settle. The particle size distribution of fine powders can be determined by examining a sedimenting suspension of the powder particle.

Two categories of Stokes's Law

Incremental	Cumulative
Changes with time in the concentration or density of the suspension at known depths can be determined. It can be either fixed time or fixed depth techniques.	The rate at which the powder is settling out of suspension is determined. i.e. the accumulated particles are measured at a fixed level after all particles between it and the fluid's surface have settled.

Advantages:
- Equipment required can be relatively simple and inexpensive.
- Can measure a wide range of sizes with considerable accuracy and reproducibility.

Disadvantages:
- Sedimentation analyses must be carried out at concentrations which are sufficiently low for interactive effects between particles to be negligible so that their terminal falling velocities can be taken as equal to those of isolated particles.
- Large particles create turbulence, are slowed and are recorded undersize.
- Careful temperature control is necessary to suppress convection currents.
- The lower limit of particle size is set by the increasing importance of Brownian motion for progressively smaller particles.
- Particle re-aggregation during extended measurements.
- Particles have to be completely insoluble in the suspending liquid.

CHARACTERIZATION OF NASAL SPRAY:

1) pH:
For both solution and suspension nasal sprays, the pH of the Formulation should be tested and an appropriate acceptance criterion established. The healthy human volunteers, overall range of pH of the anterior part of the nose was 5.17 to 8.13 while that of the posterior part was 5.20 to 8.00, indicating that an average baseline human nasal pH is approximately 6.3. Thus the stability can achieve by proper selection of pH of formulation. However, the pH of formulation should be near on human nasal mucosa (5.0-6.5) to prevent the sneezing.

2) Osmolality:
For formulations containing an agent to control the tonicity or for products having a label claim regarding tonicity, the osmolality of the formulation should be tested and controlled at release. The data from animal models has shown increased bioavailability for salmon calcitonin from nasal spray formulations with an osmolality of 100 or 600 mOsmol/Kg compared to isotonic formulations. Other studies have shown that hypotonic nasal spray formulations improved drug permeability through the nasal mucosa. Some existing marketed products have reported osmolality in the range of 300-700 mOsmol/Kg.

3) Viscosity:
For formulations containing an agent contributing to the viscosity, this parameter should be tested and controlled at release and on stability. The contact time between the drug and the nasal mucosa is increased by higher viscosity of formulation thereby increasing the time for permeation. Also high viscosity of formulations interferes with normal ciliary beating and/or MCC and, thus, increases the permeability of drugs.

4) Impurities and Degradation Products:
The levels of impurities and degradation products should be determined by a validated analytical procedure or procedures. Acceptance criteria should be set for individual and total impurities and degradation products. All related impurities appearing at levels of 0.1 percent or greater should be specified according to ICH guideline for impurities.

5) Preservatives and Stabilizing Excipients Assay:
If preservatives, antioxidants, chelating agents, or other stabilizing excipients (e.g., benzalkonium chloride, phenylethyl alcohol, edetate) are used in the formulation, there should be a specific assay for these components with associated acceptance criteria. Acceptance criteria for the chemical content of preservatives at the time of product release and through the product shelf life should be included in the drug product specification.

6) Pump Delivery:
A test to assess pump-to-pump reproducibility in terms of drug product performance and to evaluate the delivery from the pump should be performed. In general, pump spray weight delivery acceptance criteria should control the weight of the individual sprays to within 15 percent of the target weight and their mean weight to within 10 percent of the target weight.

7) Spray Content Uniformity (SCU):
The spray discharged from the nasal actuator should be thoroughly analyzed for the drug substance content of multiple sprays from beginning to the end of an individual container, among containers, and among batches of drug product. This test should provide an overall performance evaluation of a batch, assessing the formulation, the manufacturing process, and the pump. This test is designed to demonstrate the uniformity of medication per spray, consistent with the label claim, discharged from the nasal actuator, of an appropriate number (n = 10 from beginning and n = 10 from end) of containers from a batch.

The primary purpose is to ensure SCU within the same container and among multiple containers of a batch. For acceptance of a batch the amount of active ingredient per determination is not outside of 80 to 120 percent of label claim for more than 2 of 20 determinations 10 containers, none of the determinations is outside of 75 to 125 percent of the label claim, and the mean for each of the beginning and end determinations are not outside of 85 to 115 percent of label claim. If the above acceptance criteria are not met because 3 to 6 of the 20 determinations are outside of 80 to 120 percent of the label claim, but none are outside of 75 to 125 percent of label claim and the means for each of the beginning and end determinations are not outside of 85 to 115 percent of label claim, an additional 20 containers should be sampled for second tier testing for the second tier of testing of a batch, the acceptance criteria are met if the amount of active ingredient per determination is not outside of 80 to 120 percent of the label claim for more than 6 of all 60 determinations, none of the 60 determinations is outside of 75 to 125 percent of label claim, and the means for each of the beginning and end determinations are not outside of 85 to 115 percent of label claim.

8) Spray Pattern and Plume Geometry:
Characterization of spray pattern and plume geometry are important for evaluating the performance of the pump. Various factors can affect the spray pattern and plume geometry, including the size and shape of the nozzle, the design of the pump, the size of the metering chamber, and the characteristics of the formulation.
Plume geometry testing requires images taken from a sideward view of the emitted spray parallel to the axis of the plume, whereas for the evaluation of the spray pattern, an image of an axial cross-section of the plume at a defined distance to the nozzle is compulsory.
The evaluation of plume include plume angle, Width and height. The spray pattern is evaluated for maximum diameter (D_{max}) and minimum diameter (D_{min}), ovality ratio (D_{max}/D_{min}) measurements should be performed at two distances from the actuator tip, and the selected distances should be at least 3 cm apart within the range of 3 to 7 cm.

9) Particle/Droplet Size Distribution:

For suspension nasal sprays, the specification should include tests and acceptance criteria for the particle size distribution of the drug substance particles in the formulation. For example, microscopic evaluation can be used and such an examination can provide information and data on the presence of large particles, changes in morphology of the drug substance particles, extent of agglomerates, and crystal growth [8].

A. Electrical sensing zone method – Coulter Counter:

Instruments measure particle volume using dv, the diameter of a sphere with the same volume as the particle.
Particles in an electrolyte may be measured by pushing them through an aperture with an electrode on either side. Variations in electric impedance (resistance) create voltage pulses inversely proportional to particle volume when particles pass through the orifice [9].

Optical sensing zone method:

- Obscuration of light source relates to particle size (area).
- Advantage of not requiring medium to be an electrolyte.

Laser light scattering techniques:

- Laser Diffraction Particle Size Analysis Particle size range 0.02-2000µm
- Photon Correlation Spectroscopy Particle size range: 1nm to 5µm.

B. Laser diffraction:

Particles pass through a laser beam and the light scattered by them is collected over a range of angles in the forward direction. The angles of diffraction are, in the simplest case inversely related to the particle size. The particles pass through an expanded and collimated laser beam in front of a lens in whose focal plane is positioned a photosensitive detector consisting of a series of concentric rings.

Distribution of scattered intensity is analysed by computer to yield the particle size distribution. The droplet size distribution obtained from the laser diffraction method deviates for both small and large droplet sizes from the one obtained using the stroboscopic imaging technique. The small droplet population is overestimated for both laser diffraction and stroboscopic imaging, resulting in a peak at small droplet sizes [10].

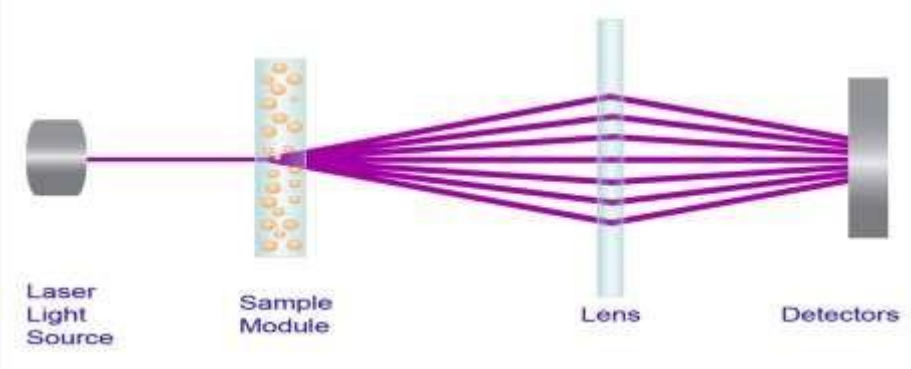


Fig. 1: Laser Diffraction

This is because they both yield a spatial distribution and they measure continuously for a given time frame, meaning that small droplets traveling at a slow speed will appear in a higher concentration in the sample volume. The deviation for large droplets is due to the fitting method of the Spraytec, which assumes a certain shape of the droplet size distribution. In fact, for the ultra-coarse spray nozzle data analysis of the raw images reveals that there are no droplets greater than 1700 µm. This means that the laser diffraction software "creates" droplets of diameters larger than 1700 µm to meet the expected shape of the distribution[11]. This correction of the raw data which compares the raw scatter data with the fit-corrected data. The largest deviations are at the first five detectors (smallest diffraction angles), where the largest droplets are detected. The laser diffraction data for all droplet size classifications appear more smoother than the results from the other methods.

Advantages:

- Non-intrusive: uses a low power laser beam
- Fast: typically, <3 minutes to take a measurement and analyse.
- Precise and wide range - up to 64 size bands can be displayed covering a range of up to 1000000:1 in size.
- Absolute measurement, no calibration is required. The instrument is based on fundamental physical properties.
- Simple to use.
- Highly versatile.

Disadvantages:

- Expensive.
- Volume measurement all other outputs are numerical transformations of this basic output form, assuming spherical particles.
- Must be a difference in refractive indices between particles and suspending medium.

Fig. 2: Laser diffraction set up for nasal spray analysis

C. Photon Correlation Spectroscopy (PCS)

Large particles move more slowly than small particles, so that the rate of fluctuation of the light scattered from them is also slower. PCS uses the rate of change of these light fluctuations to determine the size distribution of the particles scattering light. Comparison of a "snap-shot" of each speckle pattern with another taken at a very short time later (microseconds). The time dependent change in position of the speckles relates to the change of position of the particles and hence particle size. The dynamic light signal is sampled and correlated with itself at different time intervals using a digital correlator and associated computer software. The relationship of the auto-correlation function obtained to time intervals is processed to provide estimates of the particle size distribution.

Advantages:

- Non-intrusive.
- Fast.
- Nanometre size range.

Disadvantages:

- Sample preparation is critical.
- Vibration, temperature fluctuations can interfere with analysis.
- Restricted to solid in liquid or liquid in liquid samples.
- Expensive.
- Need to know R.I. values and viscosity.

TECHNIQUE OF NASAL DRUG DELIVERY SYSTEM:-

FDA guidance focuses on the two most well-established nasal drug delivery technologies: nasal sprays and nasal aerosols. Mechanical metered close nasal sprays currently dominate the nasal drug delivery market, having largely replaced droppers and squeeze bottles which were prone to inaccurate and inconsistent delivery. With a metered dose nasal spray, the active pharmaceutical ingredient (API or "active") is dissolved or suspended, usually in an aqueous medium, and a spray pump atomizes and delivers the dose.

These products are self-administered by the patient, with the efficiency of drug delivery influenced by a number of factors: patient technique and physiology; the physical properties of suspension/solution; and the design of the pump[12]. Multi-dose metered sprays are widely available but increasingly attention is turning to unit dose devices that deliver just one or two shots per nostril. Especially suitable for the delivery of pain relief and vaccines, unit dose systems avoid the microbiological contamination problems that necessitate the inclusion of preservatives in multi-dose products.

Propellant-based products, pressurized metered dose inhalers (pMDI) analogous to those used for pulmonary delivery, can also be formulated to deliver drugs via the nasal mucosa. These products deliver a "dry" nasal aerosol because the propellant evaporates rapidly during use, reducing drug losses attributable to dripping. Following the prohibition of chlorofluorocarbons, they are generally formulated with hydrofluoroalkane propellants. One criticism levelled at nasal aerosols is the force generated by the spray during use, so the trend here is towards using reduced actuation forces that give "softer" delivery.

Analytical data support systematic progression towards target bioavailability/ bioequivalence, and later, during manufacture, are also essential for quality control (QC). Laser diffraction and cascade imp action are both used to measure particle size, which is a critical parameter because of its influence on in vivo deposition, retention and uptake. Laser diffraction enables real-time measurement of the entire delivered dose while cascade impaction, in contrast, is a technique designed specifically for analysis of the particles in the sub-ten micron region, for which it provides API-specific data[13].

a) Development of nasal drug delivery:

In the development of nasal drug delivery products, performance targets are met by manipulating the design of the device or the properties of the formulation or both. Focusing on nasal sprays, for example, device parameters that can be varied include: the action of the pump and its pre-compression ratio; and the length, geometry and orifice size of the actuator. In terms of the formulation, its response to the shear applied by the pump during actuation can be tuned by varying physical properties such as viscosity, manipulated through the inclusion of modifiers and additives.

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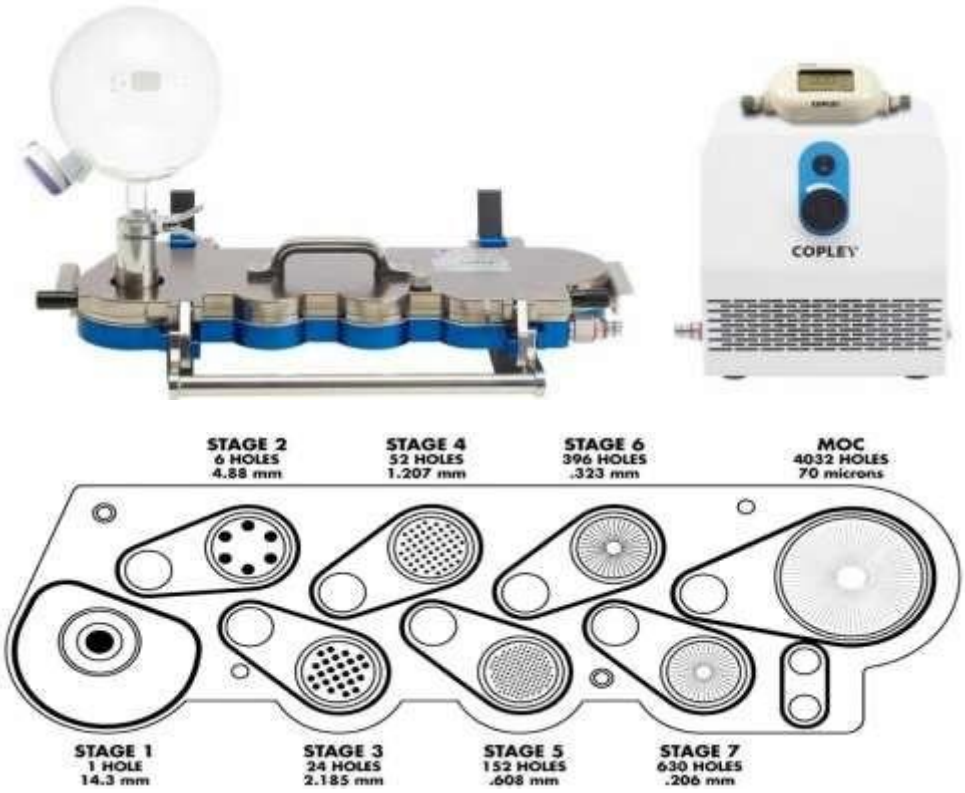

Fig. 3: Next generation Impactor (NGI)

For completeness, it is worth noting that when dealing with suspension formulations, the need for API-specific data extends to the entire dose. This is because of the influence of API particle size on dissolution and bioavailability [15]. Pre- and post-actuation measurements characterize particle size in order to confirm that it is unaltered by the drug delivery process. This regulatory requirement is usually met using microscopy, or increasingly and more efficiently with automated imaging, which comfortably spans the particle size range of interest [16].

b) Cascade Impactor:

Aerodynamic particle size largely correlates with regional deposition in the lungs and respiratory system, API should be inhaled in the fine particle fraction (FPF). Particles larger than 4-6 µm will lodge in the upper respiratory tract or trachea rather than the lungs. Pharmaceutical aerosol particle characterization is crucial, and the measurement range is generally less than 10µm.

Cascade impactors are the only particle size measurement technique that can distinguish API from other ingredients in a formulation, so all major pharmaceutical regulatory agencies require their use for inhaled product development and

quality assurance. Impactor testing needs time and money due to precision. Understanding the cascade impactor's design, operation, and settings may improve performance. A basic understanding of the design and operation of cascade impactors and their operating parameters can help achieve optimum performance in return.

Specifications for Andersen Cascade Impactor (ACI) Stages-

The challenge in defining in-use specifications for Andersen Cascade Impactor (ACI)'s that are linked in a meaningful way to the accuracy of an APSD determination is to relate variations in stage mensuration data to the corresponding changes in stage d_{50}. Given the proven link that exists between D_{eff} and d_{50} for a given CI stage.

By calculating extreme values of D_{eff} based on the existing manufacturing tolerances, assuming all the nozzles in the array are at the upper and lower extreme sizes, respectively is used to determine the corresponding range of d_{50} values about the nominal size reported from published calibration data for each CI system that have been determined at a given value of Q[17]. The following observations can be made from these data:

1. Although the relationship between D_{eff} and d_{50} for a given stage is non-linear, the changes in the latter performance measure brought about by movements in the D_{eff} to the extremes of the manufacturing tolerances for all stages of all these apparatuses are sufficiently small (<±10% of nominal under worst case conditions) and the shifts in $\Delta\ d_{50}$ are essentially symmetric about the nominal value for a given stage;
2. As a general rule, the magnitude of shifts in Δd_{50} increase as the size of individual nozzles in a given stage array decreases. This outcome is expected, given that there is a finite tolerance to which nozzles can be manufactured when they are individually smaller than about 1 mm in diameter;
3. The small increase in Δd_{50} for stage 5 of the ACI compared with the two following stages appears at first sight to be counter to the behaviour just described. However, these calculations rely on the precision to which the nominal stage d50 has been specified; stages for this impactor with d_{50} values <1.0 μm aerodynamic diameter have reported precision to two decimal places, in comparison with one-decimal-place precision for the remaining stages whose d_{50} values exceed this limit. In other words, the true Δd_{50} for stage 5 would likely be smaller than the reported value of ±9.1%, if calibration of the ACI was to be undertaken with greater precision. However, it should also be recognized that in absolute terms, the difference between the extreme upper and lower limits, as reported, is very small (0.2 μm);
4. The two-decimal-place precision for the stage d_{50} values for the NGI s justified by the quality of the archival calibration for this impactor. In this instance, the behaviour of Δd_{50} with increasing stage number (smaller D_{eff}) follows the expected pattern. Even in the worst case (stage 7), Δd_{50} was only ±8.8%, and it is notable that for all the other stages, values of Δd_{50} were substantially smaller, being close to ±1% for the upper stages 1 to 4. In absolute terms, these differences are very small (<0.05 μm).
5. The comparatively low susceptibility of both the MMI and MLSI to changes in D_{eff} reflects the fact that both apparatuses have fewer and larger diameter nozzles than would be the case for stages associated with the other impactors, having commensurate d_{50} values. Even though the manufacturing tolerances for the MSLI (±0.1 mm for stages 1 to 3 and ±0.05 mm for the outlet of stage 4) are significantly larger than the equivalent tolerances associated with the other CIs that are typically in the range from ±0.01 mm to ±0.05 mm, the larger nominal D_{eff} values for the MSLI offset the effect of shifts in D_{eff} on stage d_{50}.

These illustrations demonstrate that stage mensuration has an important role to play in the periodic validation of CI accuracy. However, provided that the measures of D_{eff} for all stages of a given CI lie within the manufacturer's tolerance range, the values of $\pm\Delta d_{50}$, expressed as a percentage of nominal d_{50}, indicate that effect on the accuracy of APSD-related measures will likely be sufficiently small even under worst-case conditions to be acceptable for the characterization of OIP-generated aerosols.

This outcome has to be appreciated in the context of the relative magnitudes of other sources of bias, in particular from the incorrect setting of volumetric flow rate to the CI, which should always be undertaken at the inlet to the IP by an appropriately calibrated flow meter, and also in the potential for leakage of ambient air into the measurement system through uncontrolled pathways, such as via defective inter-stage seals associated with some CI designs[18].

Requirements of Cascade Impaction-

The cascade impactor's inertial measuring approach outperforms particle time of flight (TOF), laser diffractometry (LD), and Phase-Doppler particle size analysis (FDA) for particle sizes < 10 μm. Only the cascade impactor's inertial mechanism distinguishes API from other formulation components; all other methods detect particle size distribution. The inertial approach also measures aerodynamic diameter, which is important for particle behaviour during inhalation, unlike most other methods.

Another benefit is that a cascade impactor collects the whole dose and allows comprehensive characterization. Other methods employ real-time measurements to get a quick picture of the dosage as it passes through the measuring instrument, which may not represent the whole amount. Inhalation products need resolution in the 0 to 5 μm particle size range, which is provided by the two most prevalent cascade impactor types.

The Next Generation Pharmaceutical Impactor (NGI) and Andersen Cascade Impactor (ACI) feature various stages with cut-off sizes that fit most operating conditions.

Fig. 4: Anderson Cascade Impactor (ACI)

Principle of cascade impaction-

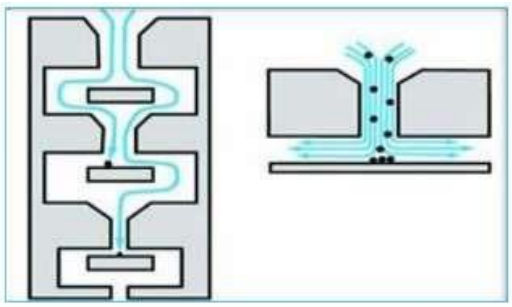

Fig. 5: Small particles with enough inertia to escape entrainment in the gas stream impact on a collection surface

Cascade Impactors separate a sample into fractions on the basis of differences in inertia, which is a function of particle density, shape, and velocity. A cascade impactor includes a number of stages, each machined with a specified number of nozzles of known diameter, with nozzle size and total nozzle area decreasing with stage number. A vacuum pump sample-laden air sequentially through the stages. At each stage, particles with sufficient inertia break out of the air stream and impact and collect on a surface located beneath the stage, while the remainder of the particles remains entrained in the air stream, passing onto the next stage. In the ACI, the collection surfaces consist of plates; the NGI uses removable cups.

The volumetric air flow remains constant, so velocity through the nozzles increases at each stage, meaning that smaller and smaller particles achieve sufficient inertia to reach the collection surface. Any residual material is captured in a final collector or filter, and the samples on each collection surface are recovered for analysis, usually by high pressure liquid chromatography (HPLC)[19]. Both the ACI and, in particular, the NGI are calibrated to provide a defined particle size fraction retained on the collection surfaces at any flow rate in the range of interest. The particles collected after each stage fall within a narrow size range for which the stage cut-off diameter[20].

Factors affecting Impactor performance-

- **Nozzle diameter -** the separation characteristics of an impactor are defined by this variable, which must be effectively specified, controlled, and maintained.
- **Air flow rate -** must be constant, reflect the conditions under which an inhaler device will operate, and be tightly controlled.
- **Other dimensions** (such as the distance between nozzle exit and collection surface) effective specification and control of these dimensions is vital.
- **Re-entrainment-** ultimately results in collection on the wrong stage, compromising accuracy; effective collection surface coating to retain impacted particles is often required.
- **Inter stage losses -** sample deposited on internal surfaces other than the collection surfaces will affect the results.
- **Leakage -** air entering into an impactor through points other than the inlet can affect its aerodynamic performance.
- **Temperature-** Extreme temperatures can affect the material properties and the impactor's performance. For instance, very cold temperatures might make materials more brittle.

- **Humidity-** High humidity can lead to increased moisture content in materials, which might make them more prone to clumping or affect their hardness. This can alter how effectively the impactor performs. Additionally, excessive moisture can lead to rust and corrosion on the impactor's components, reducing its longevity and efficiency.
- **Moisture Content-** High moisture levels can alter material properties and affect the impactor's effectiveness.
- **pH-** If the material or environment has an acidic (low pH) or alkaline (high pH) nature, it can lead to increased corrosion of the impactor's components. For instance, acidic environments can cause rust and deterioration of metal parts, while alkaline conditions can have similar effects.

Nasal spray testing-
Turning first to nasal sprays, the recommendation is that it is adequate to simply sum the amount of active collected beneath the first stage because nasal sprays tend to produce so little very fine material. A two litres or larger expansion chamber is suggested to minimize deposition on the walls and a test flow rate of 28.3 L/mm.

The need to measure the total amount of active in the fines, rather than a detailed APSD, enables testing to be simplified by using a reduced impactor stack. For example, combining stages 0, 2 and F of an Andersen Cascade Impactor gives three fractions: >9.0 microns; 4.7 to 9.0 microns; and 0.4 to 4.7 microns respectively, at a flow rate of 28.3 L/min. Such a stack can therefore be considered as indicating the fraction of the dose that may **(a)** be retained in the intranasal passage ways (>9.U microns), **(b)** be destined for the gastrointestinal tract [via the upper respiratory system] (4.7 to 9.0 microns), and **(c)** penetrate to the deep lungs (0.4 to 4.7 microns). This is more than adequate information for nasal spray bioequivalence testing[21].

Nasal aerosol-
In nasal aerosol testing, the guidance notes that the amount of drug deposited below the first stage of the impactor is "of the same order of magnitude as from orally inhaled products" leading to the recommendation that a full APSD is measured. Again, testing is carried out at 28.3 L/min but here smaller expansion chambers tend to be used, with a one litre chamber recommended, since these propellant based devices usually require smaller volumes for the aerosol to become fully developed.

For QC and bioequivalence applications, testing is always comparative and it can therefore be argued, the consistency of chamber size/test conditions is the crucial issue. More generally though, research into the impact of chamber size continues with the aim of ensuring that testing is more representative of in-use performance. Research has shown that reducing expansion chamber size clown below one litre, decreases the measured fine particle close so the one litre should be the worst case scenario for pulmonary deposition. However, there is on-going debate as to whether smaller chambers produce data that is more representative of activity in the nasal cavities, which have a volume of just 15ml[22].

D. Droplet Size Distribution (DSD) Analysis of Nasal Suspensions

DSD was measured using a Spraytec® (Malvern Panalytical, Worcestershire, UK) equipped with a 300-mm lens. The nasal spray was manually actuated at 3 cm from the laser in a carefully defined position with an extraction hood on top to capture the spray and prevent fall back of droplets through the beam. The RT Sizer software was used to capture the droplet size data at a frequency of 2.5 kHz for 0.6 s after the transmission dropped below 98%, while capturing the 0.1 s before dropping to this value. The average of 10%, 50% (volume median), and 90% of the cumulative volume undersize (d10, d50, and d90, respectively) during the fully developed phase of the spray was analyzed. All determinations were performed in triplicate after ensuring that the device was properly primed and by the same analyst to prevent any bias resulting from the different manual actuation profiles.

E. PSD by Morphologically-Directed Raman Spectroscopy (MDRS)

The morphology and particle size of the API within the manufactured nasal suspension formulations was characterized using a Morphologi G3-ID morphologically directed Raman spectroscopy system (Malvern Panalytical, Worcestershire, UK). The method development route for analysis and utilized the approach of optimizing sample preparation, imaging settings, applying imaging and API discriminatory morphological filters, and chemical analysis by Raman spectroscopy.

The method development started with the assessment of an optimized sample preparation method where the number of actuations and distance from the nozzle to the scintillation vial necessary to have a repeatable homogenous sample, volume, and pressure required to spread the suspension below the coverslip and settling time and actuation effect on the PSD were investigated. During this evaluation, it was determined that five actuations with the nozzle of the nasal spray inside the scintillation vial provide representative and repeatable measurements of particle size of the nasal product. Furthermore, pipetting 3.3 µL onto a microscope slide without applying any pressure was able to spread a thin layer of the sample on the entire coverslip area with repeatable size measurements and minimum input from the analyst. Moreover, leaving the sample to rest for at least 60 min before the analysis was considered essential to allow the particles to settle until no movement is observed. The PSD of pre- and post-actuations of the sample (e.g., sample taken from bottle and sample actuated from the bottle) were found to be comparable. After optimizing the sample preparation

method, a minimal amount of API-API or API excipient agglomerates were noted for all batches, suggesting that this preparation method with shaking and actuation successfully dispersed any loose agglomerates.

Fig. 6: Morphology G3 ID

During the microscopic measurement, the light settings and thresholds were defined in a Nasonex sample to ensure good contrast between particles and background and to capture the whole perimeter of the particles being analyzed. A 50× magnification was used to capture the micron size particles. Then, morphological filters, such as convexity <0.9, solidity <0.9, and intensity standard deviation <20.000, were used to remove poorly imaged particles and aggregates, as recommended by the FDA. An intensity standard deviation between 35.000 and 80.000 and a solidity higher than 0.8 were used to exclude air bubbles in the sample for chemical analysis. Before the chemical analysis, a nasal formulation was compared with a placebo. The main goal of this comparison was to identify particle morphology filters that could be used to improve the targeting of API particles for chemical analysis. Applying a filter based on elongation percentage within a range of 0.3–0.5 increased the sampling of many thousands of API particles compared to the analysis without any filter which captures mostly excipient particles. An elongation filter of 0.3 was used.

Upon applying these filters, the chemical analysis was carried out using the Kaiser Optical Systems RamanRxn1 Spectrometer integrated with the Morphologi G3-ID equipment. The Raman spectrum for each of the particles of the same scanning area was collected using 60 s of exposure time with excitation at a wavelength of 785 nm over the spectral range of 100–1825 cm−1 at a resolution of 6 cm−1. After the chemical analysis, the collected spectra from each particle were compared against the reference spectra of API and a correlation score was given to each particle. Particles with a score above 0.6 were classified as API. To facilitate the analysis of the collected spectra with minimum noise, only the spectra range between 1350 and 1750 cm−1 was used for correlation to the library spectra since the main identifiable peaks for the API (1397 cm−1, 1471 cm−1,1660 cm−1, and 1708 cm−1) are within this range. Moreover, a background subtraction from an area of the analyzed sample with no particles scaled to the signal based on its similarity, followed by the application of Savitsky-Golay filtering over 31 points (intermediate smoothing) and a second derivative of the signal were applied to reduce the noise in the spectrum while preserving the underlying signal. All determinations were performed in triplicate after ensuring that the device was primed. A minimum of 150 particles chemically identified as API was required per replicate[23].

F. Spray Pattern (SP) and Plume Geometry (PG) Measurements of Nasal Suspensions

SP and PG were determined by using Oxford Laser's Envision system. This system combines a laser sheet and high-speed camera specifically designed for the characterization of nasal sprays. While for SP, the laser sheet was positioned at 3 cm from the nasal pump nozzle tip, for PG analysis, the whole plume of the spray was captured. All actuations were actuated upward manually, and an extraction unit was positioned above the laser line to avoid fall back of droplets.

Data were analyzed with Oxford Lasers EnVision Patternate software. The plume width and angle were characterized for PG analysis, and the SP area and ratio of maximum and minimum diameter (ovality ratio) were calculated on a single frame during the fully developed phase. All determinations were performed in triplicate by evaluating one actuation per repetition after ensuring that the device was properly primed and by the same analyst to prevent any bias resulting from the different manual actuation profiles[24].

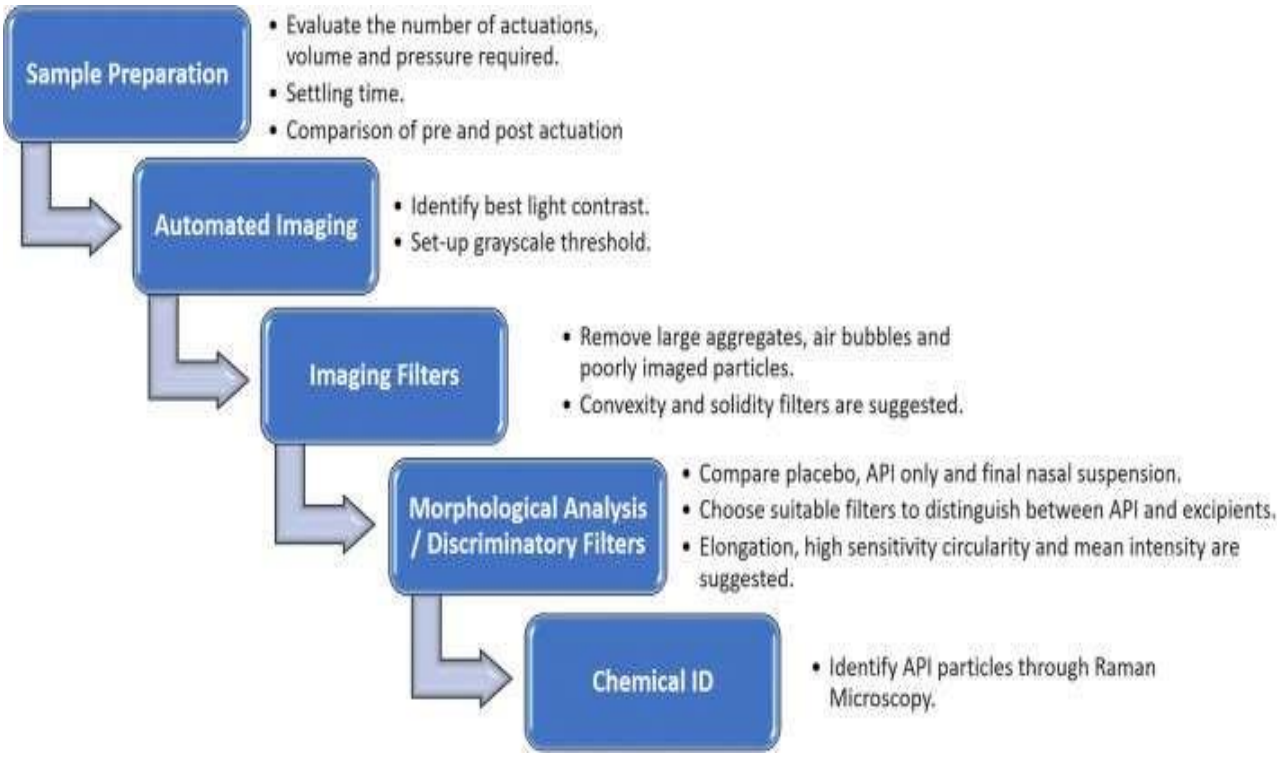

Fig. 7: Process diagram of the method development stages for the particle sizing of the API nasal suspension formulations

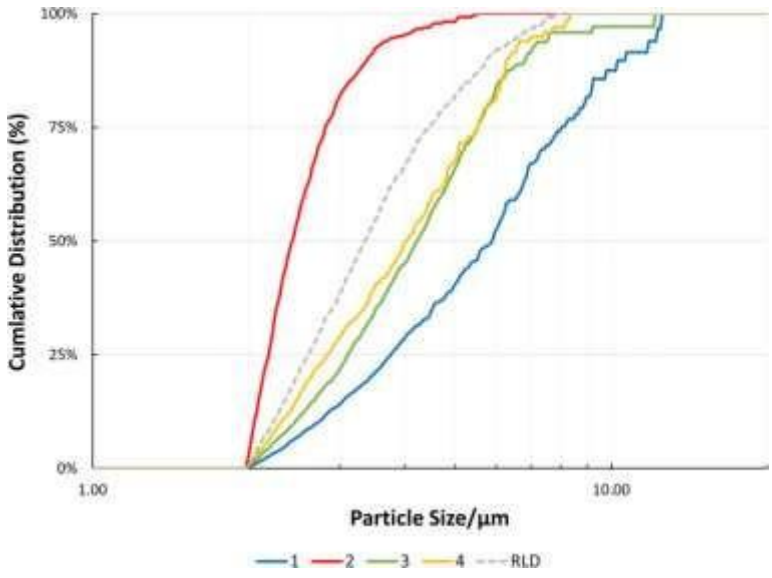

Fig. 8: Mean MDRS PSD in the volume distribution of four batches of API formulated into aqueous nasal suspension formulations

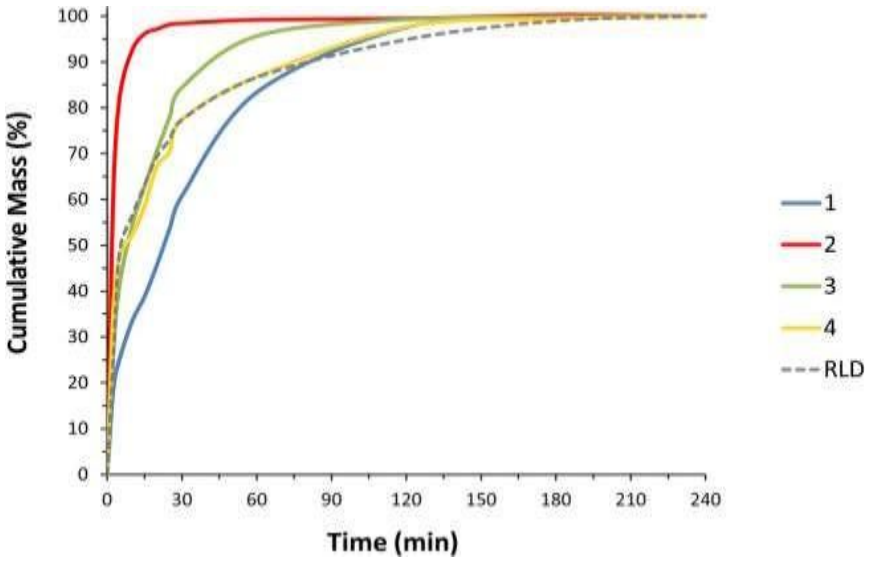

Fig. 9: Mean dissolution profile (n = 3) of four batches of API formulated into aqueous nasal suspension formulations

Validation of Raman Chemical Imaging as a Particle Sizing Method:
A blinded particle size standard study was performed using six polystyrene (PS) microsphere size standards in order to determine the accuracy of sizing micron dimension particles using RCI and optical microscopy. Initial efforts to characterize isolated, single PS microspheres resulted in a consistent overestimation of particle diameter for both optical and Raman chemical imaging measurements. The systematic overestimates were on the order of 43% for optical imaging and 24% for Raman chemical imaging relative to the particle diameter reported by the supplier. The overestimation in size may be attributable to the difficulty in determining the edge of the spherical particle, especially when approaching the diffraction limit of light (human error).

To minimize systematic overestimation, we prepared close packed hexagonal arrays of the microspheres. The resulting particle size can be determined more accurately by measuring multiple PS microspheres in a row and dividing by the total number of spheres, which effectively minimizes edge detection error. This method was used for all PS microsphere studies that formed close-packed arrays.

Using the hexagonal array sizing method, the six different PS standards were prepared by placing small drops of each of the size standard suspensions on standard glass microscope slides and allowing the suspension to dry. Bright- field and RCI measurements were made on particle size standards that formed a hexagonal close packing arrangement when deposited on a glass microscope slide.

Raman chemical imaging-based sizing results for size standards that formed a hexagonal close-packed arrangement are in good agreement with the nominal NIST-traceable size standard values. A two sided t-test (a=0.05) was performed to determine whether there was a statistical difference between the population means for the Raman measurements compared to the NIST-traceable optical microscopy results. The t-test results indicate that there is no statistically significant difference between the mean particle sizes determined by RCI and the NIST- traceable optical sizing method performed by the PS microsphere supplier.

The validation results are significant in that they demonstrate the feasibility of Raman chemical imaging for quantitative particle sizing. The 5.1 mm NIST traceable size standard could not be sized using the array method as it did not provide the necessary hexagonally close-packed arrangement of PS microspheres. Lack of formation of a close-packed arrangement may be due to the presence of impurities in the sample analyzed although this has not been examined. By measuring short "chains" of two to three particles, an average diameter within 2% of the nominal size was obtained.

The overestimation of particle diameter observed here is systematic and can be minimized through appropriate selection of binary thresholding criteria. These criteria may include edge detection based on the optical image where contrast exists and/or based on signal to noise ratios associated with each pixel (i.e., spectrum) in the Raman image. The image analysis procedures refined in the PS microsphere array studies led to the use of the optical-image guided protocol for establishing the binarization threshold levels of the API-specific particle sizing data[25].

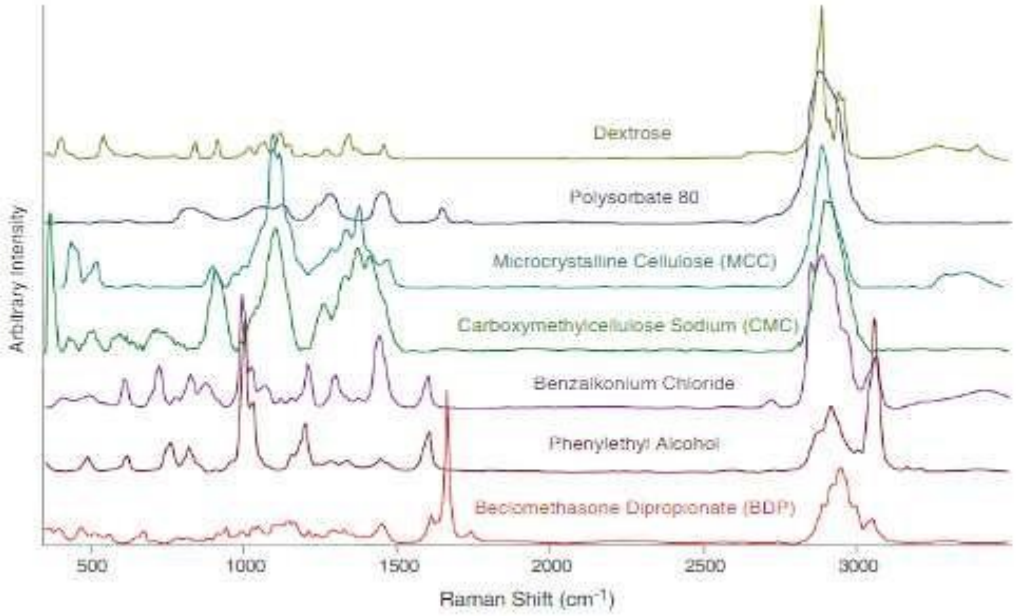

Fig. 10: Overlaid dispersive Raman spectra of all active and inactive ingredients.

G. Photon Correlation Spectroscopy (PCS):

Large particles scatter light slower than microscopic ones. PCS uses light fluctuation rate to determine light scattering particle size distribution. Each speckle pattern is matched to a "snap-shot" taken microseconds later. The speckles move with the particles and so alter particle size. A digital correlator and software sample and correlate the dynamic light signal at varied time intervals. The auto-correlation function's time interval connection estimates particle size distribution[26].

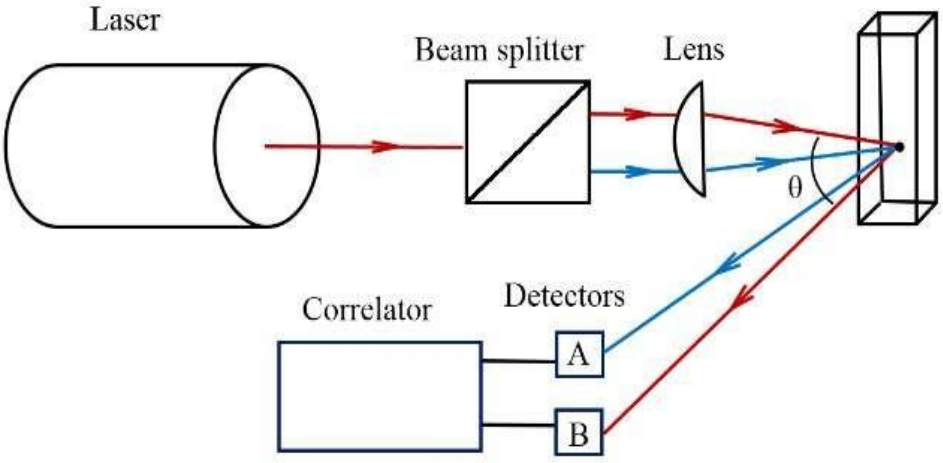

Fig. 11: Photon Correlation Spectroscopy

Advantages:

- Non-intrusive.
- Fast.
- Nanometre size range.

Disadvantages:

- Sample preparation is critical.
- Vibration, temperature fluctuations can interfere with analysis.
- Restricted to solid in liquid or liquid in liquid samples.
- Expensive.
- Need to know R.I. values and viscosity.

H. In vivo-Predictive Inlet Ports:

Glass inlet ports for nasal products can be as simple as inverted round flasks[27]. However, the use of more anatomically relevant devices such as models based on casts prepared at autopsy[28] have allowed more detailed Identification of deposition sites such as separate determination of API within the anterior or posterior cavities of the nose. Williams *et al.* through the work of the European Pharmaceutical Aerosol Group (EPAG) have more recently reported collaborative studies looking at other simple inlets (both glass and metal) aimed specifically at evaluating those nasal spray components that have the potential to reach the lungs[29].

Typically, regardless of material of construction, these inlets superficially resemble the USP induction port in design, but with an angle of 25° between the two sections allowing the nasal spray to be actuated in a more natural (i.e., per patient use) manner as opposed to horizontal actuation as is required when using the USP throat or other right-angle induction ports.

Ideally, the angle should be within the range specified in the patient use brochure. Initial findings testing an aqueous nasal spray were encouraging in terms of being able to detect azelastine, as model API, contained in small droplets[30]. However, further testing of the new inlet indicated that more work needed to be done to mitigate drip-back out of the inlet to enable more reproducible determinations of the low-level emissions from currently marketed nasal spray products. In addition, EPAG are evaluating these inlet ports, using the Fast-Screening Impactor (FSI) as an alternative to a full resolution impactor[31].

This inlet, referred to as the Kiel Nasal Inlet (KNI), slips onto the end of the USP throat. The inlet is covered with a lid that has air inlet holes in line with the axis of the throat allowing for the constant airflow required for the target cascade impactor. The nozzle of the nasal spray is inserted through an elastic seal at the bottom of the inlet. The seal allows for insertion at any angle between vertical and 60°. An assessment of a sodium cromoglycate nasal inhaler looked promising as it resulted in a sub 10 µm fractions which were comparable across analysis using a FSI with a 10-micron cut-of plate, an NGI, and a reduced NGI. Additional KNIs have been sent to other laboratories for further testing and acquisition of performance data[32].

The KNI was designed specifically for testing nasal sprays and additional evaluation will be required if it is to be applied for testing other dosage forms such as nasal aerosols or nasal powders. An idealized nasal inlet, the Alberta Idealized Nasal Inlet (AINI), has been developed to mimic in vivo deposition.

Currently, the use of anatomical nasal cavity-based models is expanding for the characterization of nasal drug products. Some commercial sources of relevant anatomical nasal models are those provided by the RDD Online organization, and Copley Scientific Ltd. although recent advances in 3D printing have enabled various research groups to create such models based on CT scans, while other groups have generated casts from cadavers, or other sources of anatomical data[33].

I. Optical/Imaging Techniques:

The most common technology for assessing particle/ droplet size in nasal spray plumes is laser diffraction (LD), which the FDA has recommended for over 20 years[34]. LD is based on the angular scattering of coherent light which passes through the medium containing the particles or droplets of interest. LD is an ensemble technique, in that the size analysis takes place simultaneously with all the particles or droplets in the light pathway, in contrast to single particle light scattering methods (optical particle counters). The scattering angle is inversely proportional to the diameter of the particle and a mathematical model (Lorenz-Mie or Fraunhofer) is applied to interpret the angular light scattering signal to produce the size distribution that is weighted in terms of volume. It should be noted that exact solutions to the Lorenz-Mie theory, that incorporates the effect of refractive index of the particles/droplets on the light scattering profile, assumes particle sphericity[35].

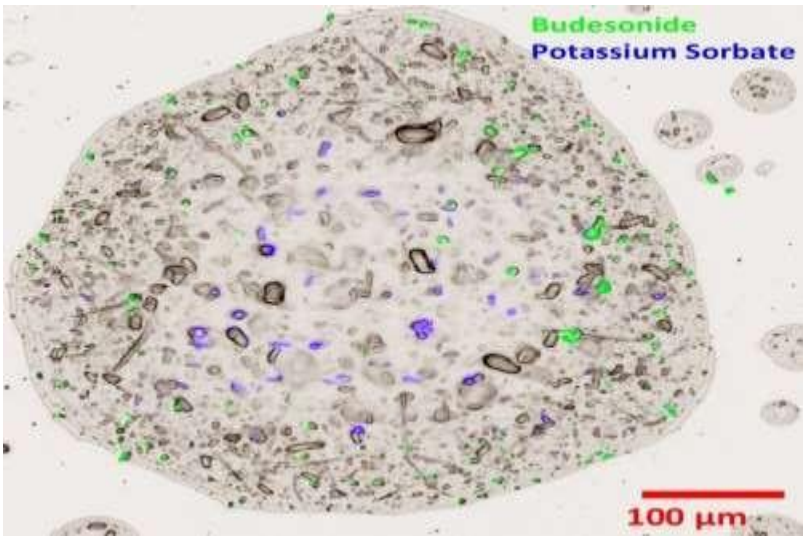

Fig. 12: Raman / brightfield image fusion of API (green) and excipient (blue) particle distribution in droplet of a nasal spray suspension.

The refractive indices for both the particles and the media need to be known and that there must be a difference between them and the refractive index has a greater effect than does the assumption of sphericity. Several thousand measurements per second are possible with current LD instruments such as the Spraytec. A major advantage of LD compared with other optical methods is that tens of thousands of particles can be measured. However, if the particle concentration is too high, secondary scattering can occur and must be avoided because the link between light scattering angle and particle size is lost. In addition to being affected by the fitting model selected (due to droplet deviation from the expected shape), LD tends to overestimate the number of small droplets because the technique yields a continuously measured spatial distribution such that "small droplets traveling at a slow speed will appear at a higher concentration in the sample volume"[36]. Making the assumption that the measured particles are spherical becomes especially problematic when the aerosol contains small agglomerates and aggregates. LD provides rapid analysis, especially compared to impaction methods, and does not require calibration as the light scattering-particle size relationship is absolute. Nevertheless, it requires rigorous method development and validation to ensure the resultant distribution is representative of the product.

Additional effort to circumvent limitations of using cascade impaction to characterize nasal spray drug products with respect to droplet/particle size and plume velocity have utilized various optical techniques other than LD. In a recent series of papers, Inthavong's research group used data from PDIA and particle image velocimetry (PIV) measurements for nasal spray delivery device optimization [37]. In an earlier study, this group used PIV combined with computational fluid dynamics (CFD) to optimize nasal spray delivery.

An important result from Inthavong's 2014 study with commercial nasal sprays is that, unless the inhalation is quite extreme, a user's breathing profile is insufficient to have an influence on the measured droplet size distribution. In a PDIA system, a high-power laser, used to illuminate the spray plume, is synchronized with a high-resolution CCD camera equipped with a long focal length microscope lens to obtain images of droplets and particles. Because the field of view (FOV) is small (approximately 3 mm × 4 mm), as many as 90 images (actuations) are required to image an entire plume out to 3 cm from the actuator. It is therefore reasonable to surmise that as many as 100 images may need to be collected for each FOV at each time point of plume life in order to obtain an adequate statistical average[38].

Paired images (separated by as little as 100 ns) are recorded and later analysed for PIV measurements, which typically make use of the same optical system as used for PDIA, to obtain an accurate velocity distribution. Renewed interest in PIV may be forthcoming as FDA has recently suggested use of this methodology to assess spray velocity for soft mist inhalers (SMIs, referred to as metered sprays for inhalation using FDA terminology). Despite the fact that plume velocity significantly impacts the efficacy and safety of nasal sprays by influencing the spray dispersion in the nasal cavity, plume velocity characterization is not (yet) a requirement for nasal sprays. One drawback of PIV, similar to LD, is that the presence of excessively high concentrations of API particles in the measurement zone can compromise the accuracy, and result in invalid data. Similar measurements can be made using plume geometry instrumentation.

Analysis of the interference patterns in the scattered light created by these intersecting laser beams provides droplet size. The measured volume is extremely small (1.29×10^{-3} mm^3) and the acceptance criteria have to be strict to ensure a valid light intensity profile at each detector, Therefore, mapping more than a few selected regions of the plume requires a very large number of measurements. As an example, PDA could be used as a discriminating parameter for in vitro testing of nasal sprays and later used this methodology to study the effect of actuation and formulation parameters on nasal spray

velocity. PDA to characterize nasal sprays and study differences in dose and deposition patterns between adult and paediatric nasal airway models. While regional deposition patterns were not statistically different between the paediatric and adult models, there were statistically different deposition amounts [39]. For best results using PDA, "droplets need to be homogeneous, transparent, and spherical." Non-spherical droplets tend to be slightly under-sized by PDA which also sees air bubbles within droplets as small droplets.
The particle trajectory criteria for a successful transition across the interference fringes set up by the intersecting light pathways are severe and may be a limiting factor for this technique as it can be difficult to assure that the sampled particles/droplets are representative [40].

J. Chemically Distinctive Imaging:

A major disadvantage of the optical/imaging methodologies discussed above is that, although particle/droplet size can be measured with varying degrees of precision and accuracy, there is no mechanism to provide the identity of those particles suspended within the sprayed droplets. Nasal spray suspension products contain solid excipients in addition to the API, so some means is essential to be able to differentiate and quantify API separately from excipient(s) and from API-excipient aggregates.

One way to accomplish this goal is to use a spectroscopic technique to provide the chemical identity of each particle. An early attempt to address this problem involved wide-field Raman micro-spectroscopy (Raman chemical imaging, RCI). For RCI, a relatively low power laser light source is used to illuminate the sample and the scattered light is magnified onto a two-dimensional CCD detector. Typically, liquid-crystal tuneable filter are used for wavelength selection. Optical (i.e., white light) images are also acquired which are fused with the Raman images, enabling differentiation between drug aggregates and individual particles [41].

The wide-field chemical imaging approach used in this study exhibits inherently higher spatial and spectral resolution than do other RCI techniques such as point mapping and line scanning. However, this methodology has not yet been widely adopted, likely because the instrumentation requires extensive operator training linking image and Raman spectra interpretation to make meaningful measurements.

Different version of Raman micro-spectroscopy has emerged in recent years and has gained sufficient favor within the FDA that, going forward, it will possibly become the method of choice for particle sizing. Note that LD will remain as a routine quality control test. This methodology, called Morphologically Directed Raman Spectroscopy (MDRS), first appeared in an FDA PSG for triamcinolone acetonide metered nasal spray (OTC)[42].

MDRS combines automated imaging+866 and Raman spectroscopy, where the individual particles are first identified using an integrated microscope, and then Raman spectra are collected for each particle. The correlations between the spectral information and the morphological data collected for individual particles provide insight into particle morphological characteristics (size and shape) and chemical properties. Morphological characteristics and the upper and lower limits associated with those parameters need to be optimized for best differentiation between API and excipients.

Microscopic images (white light) of all particles in multiple FOVs are obtained for the target formulation dispersed on a quartz or metalized microscope slide using a computer-controlled stage for the first stage of a measurement by MDRS. Sample preparation is somewhat complicated by the joint requirements that the dispersed formulation needs to remain in a liquid state, but the particles must remain immobile. Image analysis algorithms are then applied to all images to define a subset of particles based on specific shape and size parameters. For example, particles might be selected that have a specific circularity and convexity within a certain size range.

These parameters are chosen to select for the desired particle type, typically the API. Raman spectroscopy is then applied to the defined subset of particles. Typically, a full range spectrum is obtained for each particle. Because Raman signals are quite weak, this process can require as much as 20–30 s per particle such that acquisition of spectra for several thousand particles typically requires an over-night measurement [43]. However, optimization of the many parameters will require numerous experiments encompassing several days. The optimized parameters will be very much product specific and, if cohesive aggregates are to be considered, much longer times will be required for optimization and validation. Spectral results are processed using accepted spectroscopic methods and then matched against library spectra obtained using pure substances in a dry state. While there is currently no specific FDA guidance on MDRS, scientists from this regulatory body have published multiple papers on analytical method development and other considerations for this technique [44].

Conclusion:

The importance of accurate particle size determination and Characterisation of aqueous nasal sprays, examining various methods such as laser diffraction, dynamic light scattering (DLS), cascade impaction, spray particle size analyzers, optical microscopy, and Morphology G3 ID. Each technique offers unique benefits for measuring particle size and distribution, crucial for optimizing drug delivery and ensuring patient comfort. The review emphasizes the need for

method validation and adherence to regulatory standards, as well as the value of integrating multiple techniques for a comprehensive analysis. Advances in these methods continue to improve the precision and effectiveness of nasal spray formulations.

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