Aerosol Characterisation Techniques for Breath-Actuated Nebulisers

New technologies and regulatory requirements are calling upon companies to utilise different methods as they try to create the best product possible

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Assessing the size of particles generated by inhaled devices is one of the key steps to move forward in the development of inhalation treatment. Medical devices, such as inhalers and nebulisers, have been designed to create small particles containing active pharmaceutical ingredients (APIs) that are expected to spread across different sections of the respiratory airways to induce their therapeutic effect. To ensure that these particles are small enough to reach the targeted areas of the respiratory system, several *in vitro* studies have been developed and accepted by medical guidelines that intend to provide an understanding of how particle size distribution can define lung deposition in patients inhaling their medication. A droplet diameter of 5µm or below is generally considered within the ideal range to reach the central and peripheral lungs, which is stated as fine particle fraction (FPF) or fine particle dose (FPD) when referring to the dose of API.

When it comes to the analytical methods and instruments, cascade impactors are considered the standard for characterisation of aerodynamic particle size distribution (APSD) for inhalers and nebulisers, and are listed in both the United States Pharmacopeia (USP) and the European Pharmacopeia (Ph.Eur.). Laser diffraction particles size analysers are also able to provide particle size distribution data. However, although these instruments generate results in real time, their accuracy is still questioned, and therefore, their use is often proposed for faster assessments during development stages.

Characterisation of Nebuliser Droplets

Nebulisers are medical devices that transform liquid medication into aerosol, delivering droplets containing an API into the respiratory system. These devices

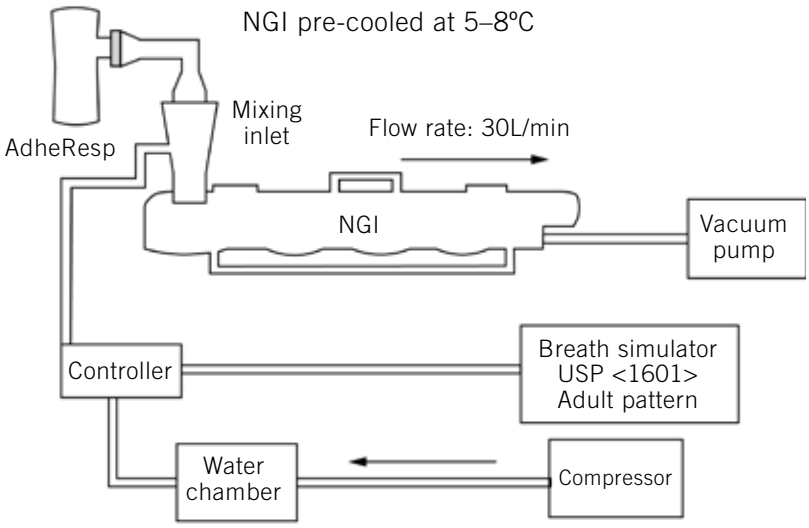

Figure 1: Configuration of the NGI + Mixing Inlet system used to test the breath-actuated mesh nebuliser AdheResp

(a)

Device	MMAD (µm)	FPF (%)	GSD
AdheResp	4.45±0.15	53.31±1.46	2.09±0.11

(b)

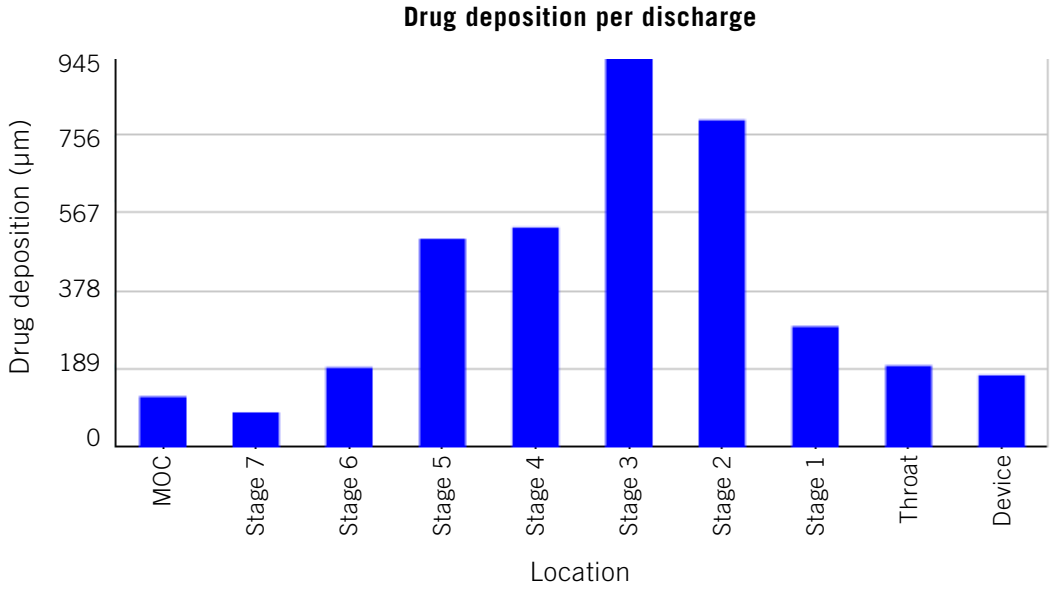

Figure 2: (a) NGI + mixing inlet test results with the AdheResp breath-actuated mesh nebuliser. MMAD, FPF, and GSD values presented as mean ± standard deviation. (b) Salbutamol distribution along the NGI system

– that were originally designed under a mechanism that allows air pressure to come into direct contact with a liquid substance to generate aerosol – have evolved to incorporate other aerosol generation mechanisms. Examples of these include ultrasonic vibrations and the addition of mesh membranes having micropores in the range of thousands to further optimise drug delivery. The quantitation of the droplets can be assessed by two major methods.

1. Cascade Impactors

The chapter <601> of the USP and the Ph.Eur. 2.9.44 have been harmonised to accept the next-generation impactor (NGI) at a volumetric flow rate of 15 L/min as the system to assess particle size distribution of all types of nebulisers (1). The seven-stage NGI is composed of trays that allow to collect API based on specific particle size ranges. The system further includes a micro-orifice collector (MOC) to capture the smallest particles, enabling calculation of API recovery when including the samples from the USP induction port and the residue in the device. The quantitation of the API is often performed using high liquid performance chromatography or ultraviolet-visible (UV-Vis) spectrophotometry. Another important factor mentioned in the pharmacopeias is that the NGI must be pre-cooled to 5ºC to conduct the APSD analysis. Besides the NGI, the guidelines also state that other cascade impactors can be applied to conduct the analysis as long as the methods are properly validated. Some of the key parameters obtained with these instruments are the mass median aerodynamic diameter (MMAD), FPF, and geometric standard deviation (GSD).

2. Laser Diffraction Particle Size Analysers

This method provides a much faster approach to measure the particle size of droplets. In the setup, the aerosolised liquid travels through an inhalation cell. Its purpose is to guide the aerosol through a channel that passes across the laser beam. The inhalation cell is often connected to a vacuum pump, operating at a flow rate of 15L/min. In some cases, the inhalation cell could be accessorised with a USP induction port that can be pre-cooled to recreate a similar environment to the NGI setup, or the nebulisers could also be connected directly to the inhalation cell by switching the vertical position of the cell to horizontal. Values such as FPF and GSD can be obtained with laser diffraction particle size analysers. However, the particle size distribution is not reported as MMAD, but instead as volume median diameter (VMD) or DV50. This variation is due to the droplet size being assessed according to the volume of the particle from a diffraction perspective, rather than the mass of API contained in the droplets of a specific size. The frequently followed guideline for the use of these instruments is based on the USP <429>.

Testing Variations and New Technologies

It does not come as a surprise that there could be variations when testing with different types of instruments. Although it would be desirable to achieve identical results when switching from a cascade impactor to a laser diffraction particle size analyser, and vice versa, to assess aerosol particle size distribution, testing can occasionally lead to variations for the same drug-device combinations, particularly if the liquid is not a solution. Nevertheless, in some instances, it is possible to obtain similar particle size distribution data when working with a specific drug, while in others a correlation between the outcome from the different techniques may be more attainable.

With the evolution of nebulisers, the introduction of breath actuation has brought new benefits to patients relying on nebulised drugs, since aerosol is triggered during the inhalation phase only. This mechanism reduces the amount of aerosol that is usually wasted during the exhalation phase, thus decreasing the emission of fugitive aerosols. Unfortunately, when it comes to aerosol characterisation studies, the breath actuation technology has planted new challenges. Most, if not all, validated test methods have been developed around nebulisers that operate under a continuous output mode. Therefore, instruments are similarly operated at a constant volumetric flow rate.

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To satisfy the new demand, additional implements such as the mixing inlet, have been developed and can nowadays be incorporated into the NGI system to conduct APSD analysis. Mixing inlets can be connected to a breathing simulator or another apparatus that mimics breathing patterns or creates purge flow rates in order to actuate the nebulisers accordingly. Neither the USP nor the Ph.Eur. has accepted the inclusion of the mixing inlet into its guidelines at this moment. Nonetheless, as more breath-actuated nebulisers are expected to enter the market in the years to come, this could gradually change. Laser diffraction particle size analysers have also incorporated new systems to help actuate breath-actuated devices, the mixing inlet being one of them. However, since the analysis with these instruments does not depend on the collected amount of API, there is no requirement regarding the amount of drug to be nebulised,

(a)

Device	DV50 (µm)	FPF (%)	GSD
AdheResp	4.77±0.01	53.14±0.16	2.12±0.18

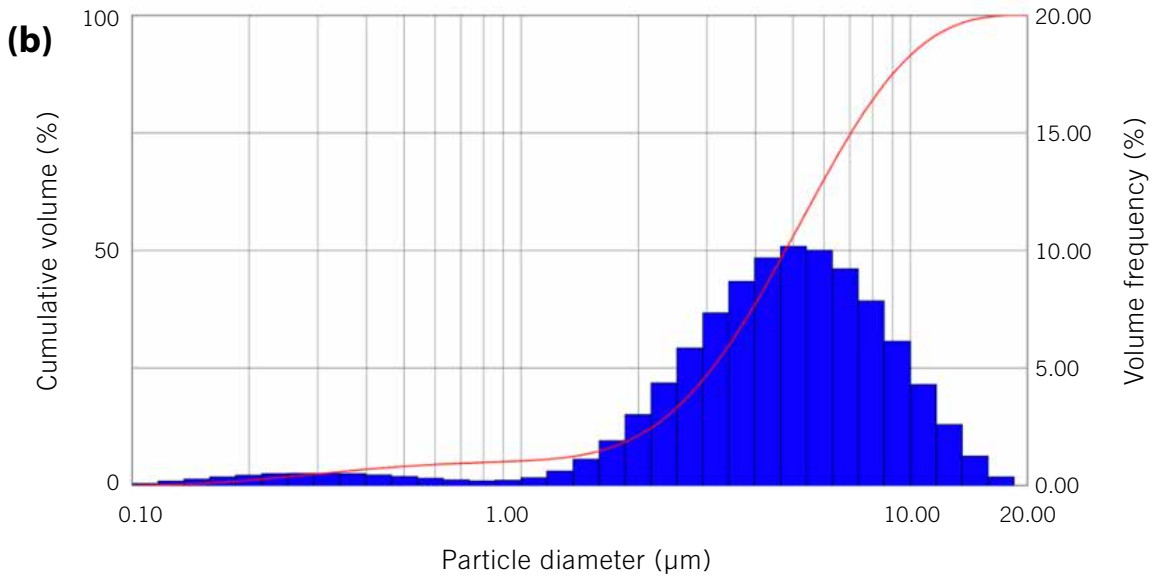

Figure 3: (a) Spraytec with inhalation cell test results with the AdheResp breath-actuated mesh nebuliser. DV50, FPF, and GSD values presented as mean ± standard deviation. (b) Particle size distribution of the aerosol detected from one of the replicates during the analysis

and a single actuation at constant flow rate can be sufficient to trigger several breath-actuated nebulisers.

Aerosol Characterisation of a Breath-Actuated Nebuliser

With the objective of comparing the particle size distribution of a newly developed breath-actuated mesh nebuliser, AdheResp® (HCmed Innovations, Taiwan), the nebuliser was tested using a short acting beta-2 agonist (Ventolin®, salbutamol, 5mg/2.5mL, GSK) (2).

For testing with a cascade impactor, the NGI (Copley Scientific, UK) was connected to a mixing inlet (Copley Scientific, UK), which in turn was linked to a breath simulator (BRS2100, Copley Scientific, UK), operating under the adult breathing pattern as stated in the USP <1601> (tidal volume: 500mL; frequency: 15 cycles/min; waveform: sinusoidal; inspiratory/expiratory ratio = 1:1) (3). The breath simulator was also linked to a controller from which a compressor working at a constant volumetric flow rate of 29.0±0.5L/min and relying on a water chamber to keep the humidity of the air above 60%, balanced the vacuum pump suction of the NGI, which operated at 30L/min (see **Figure 1, page 48**). The system was pre-cooled to 5–8ºC before performing the test.

After fully nebulising an entire ampoule of 2.5mL, the API was extracted from the trays, USP induction port, MOC, and device for quantitation purposes. A UV-Vis spectrophotometer was used to quantitate the amount of drug captured at each stage of the system. The average values from the analysis conducted in three replicates are summarised in **Figure 2**, see **page 49**, along with the particle size distribution of one of the tests.

The laser diffraction particle size analyser used in the study was the Spraytec with inhalation cell (Malvern, UK). A pre-cooled (5–8ºC) USP induction port was attached to the inhalation cell. The vacuum pump was operated at a constant flow of 15L/min, allowing for a single actuation of the nebuliser. The same nebuliser was tested during the laser diffraction analysis in triplicate. The results of the test and a graph displaying the particle size distribution of cumulative volume vs particle diameter are included in **Figure 3**.

The results of the testing of the breath-actuated mesh nebuliser revealed variations in the particle size distribution analysis with the two instruments. The median diameter of the droplets generated using the same drug and device match displayed a slightly larger particle size when using the laser diffraction analysis method than the cascade impactor with the mixing inlet, confirming the potential for divergence in testing with different methods. It is hypothesised that API dispersion homogeneity across the liquid could be considered as a driving factor to decrease or increase the result divergence in various types of liquids.

Developing and validating new analytical methods to assess aerosol characterisation of nebulisers is essential to guarantee their future improvements. As *in vitro* studies constitute one of the main pillars for the successful development of inhaled products, harmonising test methods and agreeing on guidelines for test procedures would greatly benefit and speed the process of these analyses, which could in turn allow discovery of new treatments for respiratory diseases.

References

1. *Youngren-Ortiz SR et al, Aerosol Delivery of siRNA to the Lungs. Part 1: Rationale for Gene Delivery Systems. Kona, 28;33: pp63–85, 2016*
2. *Visit: www.hcmed-inno.com/product/adp*
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Hernan Cuevas Brun is BD Manager and Aerosol Scientist at **HCmed Innovations**. He has over seven years of experience in the drug delivery field, holding a BS in Biomedical Engineering and a Master's in Business Administration. He is responsible for exploring the establishment of new partnerships with global pharmaceutical companies and coordinating HCmed products' branding, while also supporting the development of drug-nebuliser combination products. Moreover, he is involved in the development of inhaled and connected devices, assisting in the company's programmes, and establishing alliances with new partners.