Obscuring the message: A critical examination of laboratory test methods for orally inhaled products that could lead the user astray: Part 1—Aerodynamic Particle Size Distribution (APSD)

This is the first article in a two-part educational series discussing potential pitfalls in testing OIPs and ways to avoid them. The first article focuses on APSD and the second article will discuss emitted dose.

Jolyon P. Mitchell, PhD, FRSC(UK), CChem, CSci
Jolyon Mitchell Inhaler Consulting Services, Inc.

Mark W. Nagel, HBSc
Trudell Medical International

Jason A. Suggett, BPharm, PhD, MBA
Trudell Medical International

Dominic P. Coppolo, MBA, RRT, FAARC, AE-C
Monaghan Medical Corporation

Introduction

The laboratory testing of orally inhaled products (OIPs) for aerodynamic particle size distribution (APSD) is an important component of their overall characterization, because the methods employed are deemed suitable by regulatory agencies to provide insights into their likely performance in terms of safety and efficacy of medication delivery to the respiratory tract.[1,2]. This article is the first in a two-part series in which laboratory test procedures for the assessment of OIP performance are appraised. This first article focuses on APSD determinations with the purpose of identifying potential pitfalls in set-up, execution and analysis that could obscure the findings, and therefore the interpretation of data, that are derived from such measurements.

APSD is deemed a critical quality attribute by the regulatory agencies in North America and Europe,[1-3] and the methods by which it is obtained are therefore well-defined in both the US (USP) and European (PhEur) Pharmacopeias.[4,5] The purpose of these procedures is primarily to establish product quality based on emitted active pharmaceutical ingredient (API) mass, rather than to assess OIP performance in the hands of the patient. However, there has been a substantial effort in recent years to develop testing methods that address the latter issue, usually involving increased complexity in their execution.[6] Nevertheless, whatever the purpose for undertaking testing, it is important to be aware of the limitations of the selected procedure to avoid introducing bias and incorrect analysis/interpretation whose magnitude may often be unknown. Ultimately, the goal is to mitigate the risk that misleading information may be provided to potential users, caregivers and prescribers. The detailed differences between compendial methods compared with those more closely mimicking patient use have been addressed elsewhere.[6,7] Instead, this article provides guidance for the selection of the most suitable methods, identifying their limitations and associated potential pitfalls.

Compendial testing with a cascade impactor (CI)

Multi-stage cascade impactors (CIs) operate at a fixed flow rate, which defines the cut-point size or effective cut-off diameter (ECD) for each component stage.[8] ECD values are obtained by calibration with monodisperse particles of known aerodynamic diameter.[9-12] Table 1 summarizes the operating flow rates for commonly encountered CI systems used in the testing of OIPs, based on calibration data that have been published. It also identifies the most suitable class(es) of OIP for each apparatus.

It is possible to test OIPs at flow rates that are not identical with the flow rate(s) associated with such calibrations, as long as the flow rate is intermediate between maximum and minimum flow rates for which calibration data are available.[22] This flexibility is possible because the stage ECD values at the test (Qtest) and calibration (Qcal) flow rates are well-defined in terms of the following relationship:

$$ECD_{test} = ECD_{cal}\left[\frac{Q_{cal}}{Q_{test}}\right]^{1/2}$$

In this equation, ECD_{test} and ECD_{cal} represent the cut-point sizes linked with the flow rates associated with the test and calibration (reference) conditions, respectively. An example would be the use of the Next Generation Impactor (NGI) at a flow rate of 45 L/min, which is intermediate between calibration flow rates of 30 and 60 L/min.[16] However, when the test flow rate is less than the smallest flow rate for which calibration data are available, there is the possibility of bias associated with substantially decreased stage size selectivity. This bias can arise from the influence of gravity, rather than inertia, on the transport of the larger particles through the apparatus.[23] Even though this process predominantly affects the size-classification of particles > 5 µm aerodynamic diameter, it would, for example, be questionable to operate the Andersen Cascade Impactor (ACI) or the NGI at a flow rate less than 15 L/min, the smallest flow rate for which calibration data are currently published for both CIs.[9,16]

Extra care is required to operate at flow rates within the range for which there are calibration data specifically for the pre-separator, if this component is being used. This situation is most likely to arise in the assessment of carrier-based DPIs or high unit-dose pMDIs. In the admittedly unlikely instance when low flow rate testing of such products is needed, it is important to be aware that the pre-separator for the NGI should not be used at flow rates less than about 30 L/min. This is because there is evidence from the archival calibration at 15 L/min that gravitational sedimentation significantly degrades its performance.[16] Similarly, the pre-separator supplied with the ACI is unsuitable for use at flow rates less than the above value; its size-selectivity has been reported as being poor even at 28.3 L/min.[11]

Use of the Nephele Mixing Inlet in conjunction with a CI

There is a conflict between the requirement for a given CI to operate at a fixed flow rate and the variable flow rate-time patterns associated with the respiratory cycle.[6] In the past, several groups have tried to develop complex arrangements whereby the OIP-on-test is subjected to a breathing pattern, while the CI simultaneously operates at constant flow rate. The most successful of these methods involves the use of the Nephele Mixing Inlet,[24] in which a compressed air source can be added to the incoming aerosol from the inhaler. This enables the CI to sample at a constant flow rate, regardless of the breathing pattern to which the OIP-on-test is subjected. A detailed description of the procedure has been published elsewhere.[25] The outcomes from studies using this approach are encouraging in terms of providing *in vitro/in vivo* correlations for several different OIPs, each delivering budesonide as the API.[26] The mixing inlet approach has also been used to enable an ACI to operate at 28.3 L/min, yet evaluate DPIs at flow rates as low as 10 L/min.[27] However, the implementation of this methodology is quite complicated and has not yet been adopted into methodologies associated with the compendia/standards/regulatory guidelines. Furthermore, in a recently completed validation study using the Nephele Mixing Inlet, adult tidal breathing was simulated with vibrating membrane nebulizers delivering albuterol solution droplets. The results did not demonstrate equivalence, in terms of fine droplet mass < 5 µm aerodynamic diameter, to measures obtained using the chosen compendial method (NGI at 15 L/min).[28] Furthermore, there have so far been no data published confirming that sampling bias affecting the measured APSD does not exist with this arrangement, within the wide range of flow rates used to size-characterize the different classes of OIPs. Given the lack of guidance for this new approach by regulatory agencies, for the foreseeable future, most CI-based measurements of APSD are likely to be undertaken at constant flow rate using breath simulation or patient-generated breathing patterns and separately from determinations of emitted mass (dose).[6]

In addition, extraction of an aerosol sample from the flow emitted between the OIP and a CI, which must by its intrinsic design be operated at a very low flow rate, is difficult to achieve without introducing size-related bias. This type of set-up occurs in association with the testing of nebulizing systems following the European (CEN) standard.[19] Such an arrangement ideally requires measures to ensure that the flow velocity at the point at which the sample is extracted is identical to the velocity of the main flow (isokinetic sampling).[29]

Induction ports

The induction port inlet is the first part of the CI sampling system encountered by the OIP-generated aerosol. This inlet is present primarily for the purpose of product quality assessment.[30] It follows that the choice of induction port can have a significant impact on the measured APSD.[31] The compendial design of induction port[4,5] has become the most widely encountered inlet in this context. However, various designs have appeared during the past 20 years or so for a variety of purposes, most commonly the evaluation of different pMDI-based products.[32] Induction ports with larger internal volume, often shaped to enclose the expanding plume, will tend to capture less of the ballistic fraction as well as enhance evaporation of volatile species. This can result in a shift of the APSD to finer sizes, with an associated increase in measures such as fine particle fraction, typically chosen to be particles ≤ 5 µm aerodynamic diameter.[5] Unless the purpose of OIP testing is to mimic patient use more closely,[33] the compendial induction port should be selected as the inlet of choice. This approach will help avoid creating APSD data that might result in misleading comparisons at a future date.

Further considerations for specific OIP classes

Nebulizers

If OIP testing at low flow rates < 15 L/min is required, as a general rule, it is better to sample the entire aerosol with a CI such as the NGI or the Model 150P Marple-Miller

Table 1

Operating Flow Rate Ranges for Commonly Encountered Cascade Impactors Used for OIP Testing

Cascade Impactor	Suitable OIP Class	Flow rate for which calibration data are available (L/min)	Comment
Andersen 8-stage Non-Viable CI (ACI)	Nebulizer	15.0[9]	Standard configuration developed for use at 28.3 L/min; ECDs range from 0.6 µm (stage 7) to 12.4 µm (stage 0) – Not recognized by compendia, but could be used without pre-separator for nebulizer testing.
	SMI, pMDI and pMDI with S/VHC	28.3[10,11]	Standard configuration described as Apparatus 1 (USP) when used for pMDI testing without pre-separator or Apparatus 3 (USP) when used with pre-separator for DPI testing. Described as Apparatus D in the PhEur for use with pMDI (no pre-separator) or DPI evaluations (with pre-separator). ECDs range from 0.4 µm (stage 7) to 9.0 µm (stage 0).
	DPI (with or without pre-separator)	60.0[12]	Modified configuration created by removing stage 7 and inserting a new stage -1 above stage 0 (stage 0 is modified to accept the new stage). Described as Apparatus 3 in the USP and Apparatus D in the PhEur when used with pre-separator for DPI testing. ECDs range from 0.26 µm (stage 6) to 8.6 µm (stage -1).
		90.0[12]	Further modified configuration created by removing stage 6 and inserting a new stage -2 above stage -1 of configuration used at 60 L/min. Same purpose and compendial identity as configuration used at 60 L/min. ECDs range from 0.22 µm (stage 5) to 8.0 µm (stage -2).
Marple-Miller Impactor (MMI)	Model 150P: pMDI with Pediatric S/VHC and Nebulizer (12 L/min)	4.9 and 12[13]	Developed from the standard 5-stage Marple-Miller impactor. Not recognized by the compendia, but could be used for the evaluation of OIPs intended for infants/small children. Inlet nozzle diameter is varied for use at the different flow rates. ECDs range from 0.77 µm (stage 5) to 10.0 µm (stage 1) at 4.9 L/min and from 0.44 µm (stage 5) to 10.0 µm (stage 1) at 12 L/min.
	Model 150: SMI, pMDI and pMDI with S/VHC	30[14]	Not recognized in the compendia. ECDs range from 0.6 µm (stage 5) to 10.0 µm (stage 1).
	Model 160: DPI (has no pre-separator)	60, 90[14]	Standard configuration described as Apparatus 2 for DPI testing (USP) but is not recognized in the PhEur. Inlet nozzle diameter is varied for use at the different flow rates. ECDs range from 0.63 µm (stage 5) to 10.0 µm (stage 1) at 60 L/min and from 0.5 µm (stage 5) to 8.1 µm (stage 1) at 90 L/min.
Multi-Stage Liquid Impinger (MSLI)	pMDI or DPI (has no pre-separator)	60[15]	Four-stage liquid impinger described as Apparatus 4 (USP) and Apparatus C (PhEur). Is specified for use with either pMDIs or DPIs. ECDs range from 1.7 µm (stage 4) to 13.0 µm (stage 1).
Next Generation Impactor (NGI)	Nebulizer	15[16]	Seven impaction stages with back-up filter for use specifically to test nebulizers and not recognized by the compendia. The pre-separator is not used at 15 L/min and a back-up filter after stage 7 is preferred to the micro-orifice collector (MOC). ECDs range from 0.98 µm (stage 7) to 14.1 µm (stage 1).
	SMI, pMDI, pMDI and S/VHC DPI (with or without pre-separator)	30, 60, 100[17]	Same stage configuration as above but is used with pre-separator for DPI testing (Apparatus 5 (USP)/Apparatus E (PhEur)). The pre-separator also has defined ECD values so that the upper size limit for the mass of API collecting on stage 1 is defined. The pre-separator is not used for pMDI testing (Apparatus 6 (USP)/Apparatus E (PhEur). ECDs at 30 L/min range from 0.36 µm (stage 7) to 11.76 µm (stage 1); at 60 L/min from 0.14 µm (stage 7) to 8.06 µm (stage 1); at 100 L/min from 0.07 µm (stage 7) to 6.12 µm (stage 1). Corresponding ECD values for the pre-separator are: 14.9 µm (30 L/min), 12.7 µm (60 L/min) and 10.0 µm at 100 L/min.
Marple Model 290 Personal CI	Nebulizer	2.0[18]	This CI is not recognized by the compendia but operates at a low flow rate suitable for nebulizer testing. It is therefore specified in a European standard (CEN 13544-1:2001,[19] and is optional in an international standard (ISO 27427:2009.[20] Removing this low flow rate via a side-arm from the 15 L/min flow specified to be sampled ex nebulizer mouthpiece, as described in both standards, may result in significant bias to the APSD if steps to ensure isokinetic conditions are not taken. ECDs range from 0.53 µm (stage 8) to 21.3 µm (stage 1).
PC-2 Quartz Crystal Impactor (QCM)	pMDI Nebulizer	0.24[21] – Note: isokinetic sampling option is available to sample at 12.6 L/min and at higher flow rates (upon request)	This CI is not recognized by the compendia but has been used to evaluate a variety of OIP types because it offers rapid measurement capability, as there is no recovery/assay for API. ECDs range from 0.14 µm (stage 10) to 17.0 µm (stage 1). It has been used principally to assess pMDI-generated aerosols.

pMDI = pressurized metered dose inhaler; SMI = soft mist inhaler; DPI = dry powder inhaler; S = spacer; VHC = valved holding chamber

impactor. This is preferable to extracting a sample to make use of CIs that of necessity operate at very low flow rates, such as the Marple Model 290 personal sampler or the Quartz Crystal Microbalance Impactor (QCM) (Table 1). Both the NGI and Model 150P Marple-Miller impactor have demonstrated good size-selectivity for all stages, as shown by calibration data.[13, 16] A low flow rate ≤ 15 L/min is required for the evaluation of nebulizer-generated droplet APSD to minimize size-related bias from evaporation. Such evaporation is due to the entrainment of excessive amounts of drier ambient air, specifically where augmentation of output is facilitated by drawing in ambient air.[34] In recent years, 15 L/min has become recognized as the standard flow rate to test members of this diverse OIP class. This is partly because all the aerosol flow can be sampled and to minimize evaporative changes to the APSD that are more likely at higher flow rates.[19, 20, 35, 36] It is also necessary to chill the apparatus when evaluating nebulizers, to reduce heat-transfer-related evaporative bias because almost all impactors have significant thermal mass.[20, 37] Dennis, et al.[38]have recommended a temperature close to +5˚C when using the NGI, but the precise cooling regimen for the CI should be established for the particular nebulizer type being evaluated. Therefore, APSDs determined for nebulizers should be viewed with suspicion if both precautions (namely: cooling of the impactor and isokinetic sampling if the whole aerosol is not sized) have not been taken. Such APSDs are likely to be biased to finer sizes and so provide an inflated measure of important sub-fractions, in particular that relating to fine droplet content.

pMDI with spacers or valved holding chambers

Evaluation of pMDI/ spacers or valved holding chambers (S/VHC) combinations should always be undertaken with an appropriate delay interval between inhaler actuation and the onset of sampling. Such add-on devices are prescribed specifically for patients who cannot coordinate actuation with the onset of inhalation.[3, 39] The importance of this requirement is recognized by the European Medicines Agency in a guideline covering the requirements for demonstration of therapeutic equivalence between two inhaled products for use in the treatment of asthma and chronic obstructive pulmonary disease.[40] The length of the delay interval is not specified, but it is common for these add-on devices to be evaluated with delay intervals ranging from 2 to 10 seconds.[39] Delayed sampling of the emitted aerosol affects the measured APSD by preferentially removing larger particles. This is primarily due to gravitational sedimentation; its impact therefore also reduces measures of total emitted mass as well as pertinent sub-fractions. However, the magnitude of this effect has been shown to be both specific to the formulation and the S/VHC.[41] *In vitro* performance data for so-called "universal" add-on devices that are purported to be usable with any pMDI product, therefore cannot be predicted from limited measurements made with one or two formulations, even if the S/VHC is similar in appearance to a reference device.[42]

Alternatives to compendial CIs

Quartz Crystal Microbalance Impactor

It is well known that turnaround times per APSD determination with any of the 7- or 8- stage CIs described in the pharmacopeial compendia are lengthy, taking typically more than two hours per measurement.[43] Furthermore, CI-based methods are complex to execute time after time without errors.[44] Reducing the number of size-classifying stages (i.e., the Abbreviated Impactor Measurement (AIM) concept) is one approach that has been discussed elsewhere.[45-47] However if a full-resolution CI is preferred, the use of apparatuses in which a change in vibration frequency is detected as mass of particulate collects on the surface of a quartz crystal oscillator,[48, 49] is an attractive alternative approach. In addition, using a QCM impactor can reduce APSD measurement times to a few minutes. These impactors have widespread use in the measurement of environmental aerosols, where it may not be necessary to quantify the mass associated with a particular chemical species or where such analysis can be undertaken separately from the APSD determination.[49] However, care is needed in the interpretation of APSDs derived from such apparatuses because the QCM technique cannot discriminate between the chemical identity of the API(s) and any non-active excipient(s) that might be present.[22] Furthermore, these CIs may produce unpredictable results if precautions are not taken to mitigate electrostatic charge,[50] a feature commonly encountered with pMDI-generated aerosols.[51] One manufacturer supplies an isokinetic flow splitter so the impactor can sample from pMDIs and nebulizers at a total flow rate of 12.6 L/min, with options for sampling at higher flow rates.[52] Even so, the QCM technique is unsuitable for DPI evaluations because it is not possible to maintain isokinetic sampling while the flow rate from the inhaler is continually changing. Such is the case following compendial methodology for this class of OIPs.

Despite these limitations, several groups have made use of QCM technology to measure APSDs derived from nebulizers and pMDIs. For example, Tsou showed that the mass median aerodynamic diameters (MMADs) derived from APSDs of pMDI formulations without surfactant excipients were comparable to those obtained by full resolution CI.[53] Leach, et al. also reported using a QCM as a rapid aid when qualifying radiolabeled, pMDI-delivered beclomethasone dipropionate and fluticasone propionate aerosols, finding good agreement with ACI-generated APSDs.[54] In both instances, this outcome might be expected because the particular aerosols being sized contained only API. However, the lack of discriminating ability based on chemical identity, already mentioned, renders APSD measurements by this technique highly questionable if more than one API, or excipients, are also present in the formulation.

The QCM impactor has also been applied to the size analysis of nebulizer-generated aerosols. In one instance, relating to the evaluation of the droplet size distribution emitted by a continuously operating pneumatic nebulizer (Nebutech® HDN®, Salter Labs, Arvin, CA, US),

APSD data were provided, based on normal saline (0.9% w/v NaCl aq.), with no API present. The claim was made that more than 80% of the sized droplets were finer than 5 µm (aerodynamic) diameter, with mass median aerodynamic diameters ranging from 0.98 to 1.30 µm.[55] However, this assertion may not be realistic, since an assay was not undertaken and neither was any API present in their saline solution. This suspicion is reinforced by the outcome that the fine droplet fraction from this nebulizer was only about 60%. These results come from an independent study comparing the same nebulizer with similar jet nebulizers for the delivery of the API arformoterol in saline solution (15 µg/2 mL). Notably, the independent group determined their APSD data using an ACI followed by an assay for this API.[56]

Aerosol particle time-of-flight (TOF) methods

Time-of-flight (TOF) analysis is also an attractive alternative to APSD measurements made using a compendial CI because the former methodology is rapid; for example, one determination can be made in as short a time as 30 seconds. This approach also offers a higher degree of size resolution within the operating range of the instrumentation than that afforded by any of the CIs described in Table 1.[57] There are 52 size channels covering the range from 0.5 to 20 µm aerodynamic diameter for the only commercially instrument currently available in this class (Model 3321 Aerodynamic Particle Sizer (APS) Aerosol Spectrometer; TSI Corp., St Paul, MN, US). TOF analysis operates by accelerating individual particles in a highly defined flow field, in which the particles experience ultra-Stokesian motion as they pass through the measurement zone.[58] The time of flight of the particle between two well-defined locations in the measurement zone that are illuminated by laser light is determined. This value is a monotonic function of aerodynamic diameter. Longer flight times are associated with larger-sized particles due to enhanced drag in the accelerating flow field. TOF analysis is a particle counting technique, so that a number, rather than a mass-weighted APSD, is determined for each size channel. Transformation to the mass-weighted form is therefore required if comparisons with traditional CI-generated data are to be made. This process is undertaken by the software associated with the TOF analyzer, which multiplies the particle number in each size channel by the following factor:

$$\left[\pi {d_{mid}^3}/{6}\right]$$

where d_{mid} is the mid-point size of the channel under consideration. This conversion therefore tacitly assumes that the particles are spherical.[59] Although the transformation is carried out automatically, users need to be aware that the process inevitably introduces statistical noise towards the large particle size extreme of the APSD. This is because the presence or absence of just a few of the largest particles has a disproportionate influence on the outcome.[59] For example, a single particle that is 10 µm contains 1,000 times the mass of particles that are 1.0 µm aerodynamic diameter. A recent study by Wang, et al. in which an APS aerosol spectrometer was used to determine APSDs for several different pneumatic nebulizers, illustrates the effect on measured data.[60] Their TOF-determined, number-weighted APSDs illustrated for one particular device (Airlife® Misty Max®, CareFusion, Chicago, IL, US) are broad, occupying more than an order of magnitude in size from approximately 0.5 to 5.0 µm aerodynamic diameter. However, when transformed to a mass-weighted basis, the tail at the large size of the distributions extends well above 10 µm aerodynamic diameter, and is clearly based on a very small number of droplets that were collected. For this reason, even when sampling aqueous spherical droplets, TOF-generated measurements should always be supported by CI-generated data. This will ensure they are representative of the population of particles aerosol-sampled across the entire size range. Otherwise, measures such as fine particle/droplet mass could be significantly in error from their true values.

Another significant limitation of TOF analysis is the lack of a direct link to API mass through a chemical assay. In effect, the method cannot distinguish one API from another nor can it identify non-API particles associated with excipients.[59] The former limitation is becoming increasingly important as more combination API products become available. Recently, a modified version of TOF analysis has been developed. Using tandem, single particle mass spectrometry (SPAMS), particles are assayed immediately after passing through the TOF measurement zone.[61] However, this technique is still in its infancy and therefore its limitations are largely unknown.

TOF-based measurements by APS have been shown to produce APSDs from pMDIs that contain ethanol as a volatile excipient, which are very different from the API-related APSDs determined by CI. This can occur unless care is taken to ensure evaporation is complete, for instance, by inserting heated spacer-sections to extend the aerosol pathway through the inlet.[62,63] Recognizing the limitation of not having a direct link to API mass, the manufacturer of the APS has developed a single-stage impactor (SSI) that can make measurements of fine particle fraction in parallel to the TOF-based APSD determination. The SSI thereby provides a means of validating the TOF-measured APSD at a single size close to 5 µm aerodynamic diameter. Comparisons between SSI- and TOF-based measures have been shown to be good for several pMDI-based solution formulations.[63,64] Nonetheless, TOF-based APSD measurements should always be related back to those determined by multi-stage CI,[64] given the added potential for bias arising from differences in particle density from that of water[65] and deviations in shape from spherical.[65-67] This precaution is especially important when attempting to size non-spherical particles from DPIs. Control of changes to APSD caused by evaporation/condensation processes should also be considered when sampling aqueous droplets from nebulizing systems[59] or aerosols containing volatile species, e.g., ethanol, which is present as a solubilizer in some pMDI-solution formulations.[63]

Table 2

Key Considerations for Measurement Methods Used in the Determination of APSDs of Orally Inhaled Products

Measurement Method	Consideration
CI for All OIP Classes	Use the compendial induction port (inlet) to avoid introducing inlet-related differences in APSD measurements from one study to another.
CI Alone	Operate within the flow rate range for which calibration data are available.
CI with Pre-separator	Ensure that the flow rate is at least as great as the lowest value for which the pre-separator performance has been established.
CI with Mixing Inlet	Useful to enable inhaler to function as it would in practice. Be aware that equivalence in APSD-related metrics compared to pharmacopeial method needs validating on a product-by-product basis.
CI for Nebulizer Testing	Ideally sample entire flow from nebulizer at 15 L/min. If this is not possible, ensure sample to CI is extracted isokinetically from the main flow leaving the nebulizer.
	Chill the CI to mitigate the risk of heat-transfer-related droplet evaporation, especially for nebulizers without air entrainment.
CI for pMDI with Spacer/VHC	Ensure that at least one delay interval after pMDI actuation is simulated; typically performance should be assessed with the delay interval being at least 2 seconds in duration.
Quartz Crystal Microbalance Impactor (QCM)	For this non-compendial impactor, be aware there is no assay for API with this method; in some cases, there is a potential risk of under-estimation of MMAD.
	Take precautions to avoid heat-transfer-related evaporation if sampling aqueous droplets from a nebulizing system.
	Equivalence in APSD-related metrics compared to pharmacopeial method needs validating on a product-by-product basis.
Aerosol Particle Time-of-Flight (TOF)	Be aware there is no assay for API with this method.
	Statistical "noise" at the large particle tail of the APSD may affect magnitude of derived measures.
	Particle density and shape-related effects can bias APSD data; neither are well defined for most DPI-generated aerosols.
	Equivalence in APSD-related metrics compared to pharmacopeial method needs validating on a product-by-product basis.
Laser Diffractometry (LD)	Be aware there is no assay for API with this method.
	Particle shape-related effects can bias APSD data for DPI-generated aerosols.
	Equivalence in APSD-related metrics compared to pharmacopeial method needs validating on a product-by-product basis.

Click to read the remainder of the article and see the reference list.

Laser diffractometry (LD) applied to OIP aerosols

Laser diffractometry (LD) is the only other rapid measurement technique that is widely encountered in association with the size-characterization of OIP-generated aerosols. This technique is particularly widespread for the evaluation of nebulizer-generated droplet sizes, but is occasionally applied to other inhaler classes.[68] The operating principle underlying LD is the low-angle scattering of coherent light (so-called low-angle laser light scattering) that is described comprehensively in a recently updated ISO standard.[69] LD uses an ensemble approach (measuring all the particles in the aerosol cloud simultaneously) rather than a single-particle measurement technique. The volume- (mass)-weighted particle size distribution data is determined directly (with the underlying assumption that particle density is constant irrespective of particle size). Several thousand measurements are made per second with current instruments. Yet typically for OIP aerosol assessments, these data are combined over a period of several seconds to provide a single time-averaged particle size distribution. LD systems typically measure particle sizes covering more than two orders of magnitude in size, depending on optical configuration. Measurements can be made either in an open-bench arrangement, in which the aerosol is allowed to flow unimpeded across the measurement zone, or by using an enclosed cell. Like TOF-based systems, LD-analyzers have excellent resolution, providing as many as 15 channels per decade of size.

There are, however, some important limitations that users should be aware of when interpreting LD-generated data. Firstly, the size that is measured is related to the physical diameter (projected area/volume), not the aerodynamic diameter of the particles.[68] This distinction is unimportant for aqueous droplets because they are both spherical and have unit density (centimeter-gram-second (cgs) system), which are the reference conditions that define aerodynamic size in relation to physical (geometric) size scales. However, the distinction may be significant when using LD to size-analyze solid particles from pMDIs and DPIs, particularly if they are not spherical in shape. Secondly, like TOF-analysis, there is no assay for the API species that may be present. Nevertheless, some European[3] and US[70] regulatory guidance documents relating to OIP testing permit the use of LD in relation to OIP performance assessment, but only if supported by validation data obtained using the CI method. The manufacturer of the Spraytec LD, one of the more widely used, current LD systems (Malvern Instruments, Malvern, UK), has therefore developed a closed inhalation cell from which the OIP-generated aerosol can be sampled via a CI immediately following LD-assessment of particle size.[71]

Given these limitations, careful interpretation of LD-generated data is therefore advised, especially if the measurements are not supported by multi-stage-CI-generated data.

In the case of nebulizer-generated aqueous aerosols, similar precautions must be taken to avoid premature evaporation either by mixing with sub-saturated, ambient air, or as the result of heat transfer from associated sampling apparatus.[68] The use of the closed inhalation cell option, in which the ambient relative humidity can be controlled, is therefore a wise precaution. If evaporation is significant, LD-measured size distributions will be shifted to finer sizes, thereby inflating measures such as fine droplet fraction. An inhalation cell is always needed if LD is being used to size DPI-generated aerosols because an inhalation maneuver has to be simulated to operate the inhaler correctly.[69] Here, however, the concern is that bias not be introduced because the particles being sized are non-spherical. However, de Boer, et al. have shown that LD is applicable to the measurement of micronized powder aerosols in which fully dispersed single particles have near-to-spherical geometry,[72] such as is the case with powder aerosols delivered from the Turbuhaler® DPI (AstraZeneca, Mölndal, Sweden). In general, however, the relationship between the magnitude of bias in LD-based measurements associated with deviations from particle sphericity is unknown, re-emphasizing the desirability to link such assessments to CI-measured APSDs as a critical part of method validation.

Conclusions

The determination of APSD for OIP-generated aerosols requires considerable care in both choice of measurement apparatus and in the subsequent interpretation of the data. Table 2 lists considerations that are worthwhile evaluating, depending on the OIP class(es) under evaluation and the approach to measurement. The multi-stage CI is still the apparatus of choice for such work. Although relatively slow and labor-intensive compared with TOF and LD, it is the only approach that determines the therapeutically relevant, mass-weighted APSD directly and at the same time links these measurements to mass of API(s) that may be present in the formulation. This article has therefore focused on the CI, and it is the opinion of the authors that other techniques should only be used after having validated the methodology using a compendial CI-based method as the reference technique. However, it has also identified limitations associated with TOF and LD techniques that are most likely to be encountered because both techniques are attractive, alternative methods to the CI for acquiring OIP APSDs, due to their rapidity per measurement and higher size resolution.

The potential pitfalls of various techniques identified within this article are not exhaustive, but represent examples to avoid in the practice of measuring the critical APSD quality attribute for OIPs. The second article in this series will focus on the determination of total emitted mass, both by the compendial methods as well as scenarios in which patient use has been simulated.

References

1. United States Food and Drug Administration. Center for Drug Evaluation and Research (CDER): Draft guidance for industry metered dose inhaler (MDI) and dry powder inhaler (DPI) drug products chemistry, manufacturing, and controls documentation. 1998. http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM070573.pdf. Accessed December 19, 2016.

2. Health Canada: Pharmaceutical quality of inhalation and nasal products. 2006. http://www.hc-sc.gc.ca/dhp-mps/prodpharma/applic-demande/guide-ld/chem/inhalationnas_e.html. Accessed December 19, 2016.

3. European Medicines Agency: Guideline on the pharmaceutical quality of inhalation and nasal products. EMEA/CHMP/QWP/49313/2005 Corr. 2006. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003568.pdf. Accessed December 28, 2016.

4. US Pharmacopeial Convention: United States Pharmacopeia 39/National Formulary 34, Chapter <601> Aerosols, nasal sprays, metered-dose inhalers, and dry powder inhalers.Rockville, MD, 2016.

5. European Directorate for Quality in Medicines (EDQM): European pharmacopeia 9.0, monograph 2.9.18. Preparations for inhalations: Aerodynamic assessment of fine particles. Strasburg, France EDQM. 2017.

6. Mitchell JP, Suggett J, Nagel M. Clinically relevant *in vitro* testing of orally inhaled products—Bridging the gap between the lab and the patient. AAPS PharmSciTech. 2016; 17(4): 787-804.

7. Mitchell JP, Dolovich MB. Clinically relevant test methods to establish *in vitro* equivalence for spacers and valved holding chambers used with pressurized metered dose inhalers (pMDIs). J Aerosol Med Pulmon Deliv. 2012; 25: 217-242.

8. Marple VA, Liu BYH. Characteristics of laminar jet impactors. Environ Sci Technol. 1974; 8: 648-654.

9. Abdelrahim ME. Aerodynamic characteristics of nebulized terbutaline sulphate using the Andersen Cascade Impactor compared to the Next Generation Impactor. Pharm Dev Technol. 2011; 16(2): 137-145.

10. Vaughan NP. The Andersen impactor: Calibration, wall losses and numerical simulation. J Aerosol Sci. 1989; 20: 67-90.

11. Mitchell JP, Costa PA, Waters S. An assessment of an Andersen Mark-II cascade impactor. J Aerosol Sci. 1987; 19(2): 213-221.

12. Nichols SC, Brown DR, Smurthwaite M. New concept for the variable flow rate Andersen cascade impactor and calibration data. J. Aerosol Med. 1998; 11Suppl.1: 133-138.

13. Olson BA, Marple VA, Mitchell JP, Nagel MW. Development and calibration of a low-flow version of the Marple-Miller impactor. Aerosol Sci Technol. 1998; 29(4): 307-314.

14. Marple VA, Olson BA, Miller NC. A low-loss cascade impactor with stage collection cups: Calibration and pharmaceutical inhaler applications. Aerosol Sci. Technol. 1995; 22(1):124-134.

15. Asking L, Olsson B. Calibration at different flow rates of a multistage liquid impinger. Aerosol Sci. Technol. 1997; 27(1): 39-49.

16. Marple VA, Olson BA, Santhanakrishnan K, Mitchell JP, Murray SC, Hudson-Curtis BL. Next generation pharmaceutical impactor: A new impactor for pharmaceutical inhaler testing. Part III. Extension of archival calibration to 15 L/min. J Aerosol Med. 2003; 17(4): 335-343.

17. Marple VA, Olson BA, Santhanakrishnan K, Mitchell JP, Murray SC, Hudson-Curtis BL. Next generation pharmaceutical impactor. Part II. Calibration. J Aerosol Med. 2003; 16(3): 301-324.

18. Rubow KL., Marple VA, Olin J, McCawley MA. A personal cascade impactor: Design, evaluation and calibration. Am. Ind. Hyg. Assoc. J. 1987; 48(6): 532-538.

19. European Committee for Standardization: Respiratory therapy equipment—Part 1: Nebulizing systems and their components. EN 13544–1. CEN, Brussels, Belgium, 2001.

20. International Standards Organization: ISO27427: Anaesthetic and Respiratory Equipment—Nebulizer systems and components, Geneva, Switzerland, 2009.

21. Fairchild CI, Wheat LD. Calibration and evaluation of a real time cascade impactor. Am Ind Hyg Assoc J. 1984; 45: 205-211.

22. Mitchell JP, Nagel MW. Cascade impactors for the size characterization of aerosols from medical inhalers: Their uses and limitations. J Aerosol Med. 2003; 16(4): 341-377.

23. Rader DJ, Marple VA. Effect of particle gravitational forces on the calculation of impactor efficiency curves. In: Liu BYH, Pui DYH, Fissan HJ, (editors). Aerosols: Science, Technology and Industrial Application of Air borne Particles, Elsevier, New York, NY, US, 1984; pp.123-126.

24. Miller NC. Apparatus and process for aerosol size measurement at varying gas flow rates. US Patent 6,435,004-B1, 2002.

25. Olsson B, Berg E, Svensson M. Comparing aerosol size distribution that penetrates mouth–throat models under realistic inhalation conditions. In: Dalby RN, Byron PR, Peart J, Suman JD, Young PM, (editors). Respiratory Drug Delivery 2010, Davis Healthcare International Publishing LLC, River Grove, IL, US; 2010, pp. 225-234.

26. Olsson B, Borgström L, Lundbäck H. and Svensson M. Validation of a general *in vitro* approach for prediction of total lung deposition in healthy adults. J. Aerosol Med. Pulmon. Drug Deliv. 2013; 26(6): 355-369.

27. Nadarassan DK, Assi KH, Chrystyn H. Aerodynamic characteristics of a dry powder inhaler at low inhalation flows using a mixing inlet with an Andersen Cascade Impactor. Eur J Pharm Sci. 2010; 39(5): 348-354.

28. Svensson M, Berg E, Mitchell J, Sandell D. Comparison between pharmacopeial testing and testing based on mixing inlet methodology with application to nebuliser testing: Part 2–Main study. Drug Delivery to the Lungs-27, The Aerosol Society, Edinburgh, 2016: pp. 249-252.

29. Vincent JH. Aerosol Sampling and Practice, John Wiley & Sons, Chichester, UK, 1989.

30. Mitchell JP, Roberts DL. Current approaches to APSD measurement of OIPs based on inertial impaction. In: Tougas TP, Mitchell JP, Lyapustina SA, (editors). Good Cascade Impactor Practices, AIM and EDA for Orally Inhaled Products, Springer, New York, NY, US, 2013; pp. 15-55.

31. Olsson B, Borgström L, Asking L, Bondesson E. Effect of inlet throat on the correlation between measured fine particle dose and lung deposition. In: Dalby RN, Byron PR, Farr SJ, (editors). Respiratory Drug Delivery-V, Interpharm Press Inc., Buffalo Grove, IL, US, 1996, pp. 273-281.

32. Dolovich M, Rhem R. Impact of oropharyngeal deposition on inhaled dose. J. Aerosol Med. 1998; 11(S1): S112-S115.

33. Mitchell JP, Suggett J. Developing ways to evaluate in the laboratory how inhalation devices will be used by patients and caregivers: The need for clinically appropriate testing. AAPS PharmSciTech. 2014; 15(5): 1275-1291.

34. Dennis JH. Evolution of evaporative understanding within nebulizer standards. J Aerosol Med Pulmon Deliv. 2009; 22(1): 5-8.

35. US Pharmacopeial Convention: United States Pharmacopeia 39/National Formulary 34, Chapter <1601> Products for Nebulization. Rockville, MD. 2016.

36. European Directorate for Quality in Medicines (EDQM): European pharmacopeia 9.0, monograph 2.9.44. Preparations for nebulization. Strasburg, France EDQM. 2017.

37. Stapleton KW, Finlay WH. Undersizing of droplets from a vented nebuliser caused by aerosol heating during transit through an Andersen impactor. J Aerosol Sci. 1999; 30(1): 105-109.

38. Dennis J, Berg E, Sandell D, Ali A, Lamb P, Tservistas M, Karlsson M, Mitchell J. Cooling the NGI–An approach to size a nebulised aerosol more accurately. Pharm Europa Sci Notes. 2008; 1: 27-30.

39. Rubin BK, Fink JB. Optimizing aerosol delivery by pressurized metered-dose inhaler. Respir Care. 2005; 50: 1191-1200.

40. European Medicines Agency (EMA): Requirements for clinical documentation for orally inhaled products (OIP) including the requirements for demonstration of therapeutic equivalence between two inhaled products for use in the treatment of asthma and chronic obstructive pulmonary disease (COPD) in adults and for use in the treatment of asthma in children and adolescents. CPMP/ EWP/4151/00 Rev. 1, 2009. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003504.pdf. Accessed December 29, 2016.

41. Mitchell JP, Nagel MW, MacKay HA, Avvakoumova VA, Malpass J. Developing a ''universal'' valved holding chamber (VHC) platform with added patient benefits whilst maintaining consistent *in vitro* performance. In: Dalby RN, Byron PR, Peart J, Suman JD, and Young PM, (editors). Respiratory Drug Delivery Europe 2009. Davis Healthcare International Publishing LLC, River Grove, IL, US; 2009, pp. 383-386.

42. Suggett JA, Nagel MW, Doyle C, Avvakoumova V, Mitchell JP. Statistical performance evaluation of similar looking valved holding chambers: If it looks the same, does it perform the same? In: Dalby RN, Byron PR, Peart J, Farr SJ, Suman JD, Young PM, Traini D, (editors). Respiratory Drug Delivery 2016. Davis Healthcare International Publishing LLC, River Grove, IL, US; 2016, pp. 539-542.

43. Nichols SC, Mitchell JP. An assessment of the comparative efficiency of abbreviated versus full resolution cascade impactor measurements: A survey of European Pharmaceutical Aerosol Group (EPAG) members. Drug Delivery to the Lungs-24, The Aerosol Society, Edinburgh, 2013; pp. 237-240.

44. Christopher D, Curry P, Doub W, Furnkranz K, Lavery M, Lin K, Lyapustina S, Mitchell J, Rogers B, Strickland H, Tougas T, Tsong Y, Wyka B. Considerations for the development and practice of cascade impaction testing including a mass balance failure investigation tree. J Aerosol Med. 2003; 16(3): 235-247.

45. Copley M, Mitchell J, McAuley E, Russell-Graham D. Implementing the AIM concept: Streamlining the testing of inhaled drug products by using an Abbreviated Impactor Measurement method. Inhalation. 2010; 4(1): 7-11.

46. Mitchell JP, Tougas T, Lyapustina S. The Abbreviated Impactor Measurement (AIM) and Effective Data Analysis (EDA) concepts: Why they are important and how to work with them. Inhalation. 2012; 6(6): 13-19.

47. Tougas, TP, Mitchell, JP, Lyapustina, SA (editors): Good Cascade Impactor Practices, AIM and EDA for Orally Inhaled Products, Springer, New York, NY: 2013.

48. Chuan RL. Particulate mass measurement by piezo-electric crystal. NBS Spec. Publ. 1974; 412:137-148.

49. Chen R, Romay FJ, Li L, Naqui A, Marple VA. A novel quartz crystal cascade impactor for real-time aerosol mass distribution measurement. Aerosol Sci Technol. 2016; 50(9): 971-983.

50. Horton KD, Ball MHE, Mitchell JP. The calibration of a California Measurements PC-2 quartz crystal cascade impactor (QCM). J. Aerosol Sci. 1992; 23: 505-524.

51. Kwok PCL, Chan H-K. Electrostatic charge in pharmaceutical systems. In: Swarbrick J, (editor), Encyclopedia of Pharmaceutical Science and Technology (4th ed.), CRC Press, Boca Raton, FL, US, 2013; 2: pp. 1394-1407.

52. California Measurements. Cascade impactors for pharmaceutical aerosol analysis. California Measurements Inc., Sierra Madre, CA, US. Available at: http://www.californiameasurements.com/products.html. Accessed January 10, 2017.

53. Tsou TZ. Aerodynamic particle size of metered-dose inhalers determined by the quartz crystal microbalance and the Andersen cascade impactor. Int J Pharm. 1999; 186(1): 71-79.

54. Leach CL, Davidson PJ, Hasselquist BE, Boudreau RJ. Lung deposition of hydrofluoroalkane-134a beclomethasone is greater than that of chlorofluorocarbon fluticasone and chlorofluorocarbon beclomethasone. Chest. 2002; 122(2): 510-516.

55. Nebutech nebulizer from Salter, Salter Labs, Arvin, CA, US. 2016. Data on file at manufacturer.

56. Bauer A, McGlynn P, Bovet LL, Mims Pl, Curry LA, Hanrahan JP. Output and aerosol properties of 5 nebulizer/compressor systems with arformoterol inhalation solution. Respir Care. 2009; 54(10): 1342-1347.

57. Mitchell J, Bauer R, Lyapustina S, Tougas T, Glaab V. Non-impactor-based methods for sizing of aerosols emitted from orally inhaled and nasal drug products (OINDPs). AAPS PharmSciTech. 2011; 12(3): 965-988.

58. Baron PA, Mazumder MK, Cheng YS. Direct-reading techniques using optical particle detection. In: Willeke K, Baron PA, (editors). Aerosol measurement: Principles, techniques and applications. Van Nostrand Reinhold, New York, NY, US, 1993; pp. 381-409.

59. Mitchell JP, Nagel MW. Time-of-flight aerodynamic particle size analyzers: Their use and limitations for the evaluation of medical aerosols. J Aerosol Med. 1999; 12(4): 217-240.

60. Wang Y, Li J, Leavey A, O'Neil CO, Babcock HM, Biswas P. Comparative study on the size distributions, respiratory deposition and transport of particles generated from commonly used medical nebulizers. J Aerosol Med Pulmon Deliv. 2017; Fast Track. http://online.liebertpub.com/doi/pdfplus/10.1089/jamp.2016.1340. Accessed December 30, 2016.

61. Susz A, Morrical BD, Fergenson DP: New determination method of aerodynamic diameter size distribution in dry powder inhalers using SPAMS 3.0. In: Dalby RN, Byron PR, Peart J, Suman JD, Traini D, Young PM, (editors). Respiratory Drug Delivery 2014, Davis Healthcare International Publishing LLC, River Grove, IL, US; 2014, pp. 515-518.

62. Harris JA, Stein SW, Myrdal PB. Evaluation of the TSI aerosol impactor 3306/3321 system using a redesigned impactor stage with solution and suspension metered-dose inhalers. AAPS PharmSciTech. 2006; 7(1): Article 20.

63. Myrdal PB, Mogalian E, Mitchell JP, Nagel M, Wright C, Kiser B, Prell M, Woessner M, Stein SW. Application of heated inlet extensions to the TSI 3306/ 3321 system: Comparison with the Andersen cascade impactor and next generation impactor. J Aerosol Med. 2006; 19(4): 543-554.

64. Mitchell JP, Nagel MW, Wiersema KJ, Doyle CC. Aerodynamic particle size analysis of aerosols from pressurized metered-dose inhalers: Comparison of Andersen 8-stage cascade impactor, Next Generation Pharmaceutical Impactor, and model 3321 aerodynamic particle sizer aerosol spectrometer. AAPS PharmSciTech. 2003; 4: Article 54.

65. Cheng Y-S, Chen BT, Yeh HC. A study of density effect and droplet deformation in the TSI aerodynamic particle sizer. Aerosol Sci Technol. 1990; 12(2): 278-285.

66. Marshall IA, Mitchell JP, Griffiths WD. The behavior of regular-shaped, non-spherical particles in a TSI aerodynamic particle sizer. J Aerosol Sci. 1991;22(1):73-89.

67. Griffiths WD, Iles PJ, Vaughan NP. The behaviour of liquid droplet aerosols in an APS 3300. J Aerosol Sci. 1986; 17(6): 921-930.

68. Mitchell JP, Nagel MW, Nichols SC, Nerbrink O. Laser diffractometry as a technique for the rapid assessment of aerosol particle size from inhalers. J Aerosol Med. 2006; 19(4): 409-433.

69. International Standards Organization, Geneva, Switzerland: Particle size analysis—laser diffraction methods. ISO 13320; 2009.

70. United States Food and Drug Administration. Center for Drug Evaluation and Research (CDRH): Reviewer guidance for nebulizers, metered dose inhalers, spacers and actuators. Rockville: 1993. http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm081282.htm. Accessed December 30, 2016.

71. Krarup HG, Bumiller M, Stauffer T. The Malvern Spraytec applied to pharmaceutical spray analysis. In: Dalby RN, Byron PR, Peart J, Farr SJ, (editors). Respiratory Drug Delivery VIII, Davis Horwood International, Raleigh, NC, US; 2002, pp. 505-508.

72. de Boer AH, Gjaltema D, Hagedoorn P, Frijlink HW. Characterization of inhalation aerosols: A critical evaluation of cascade impactor and laser diffraction technique. Int J Pharm. 2000; 249(1-2): 219-231.

Jolyon P. Mitchell, PhD, FRSC(UK), CChem, CSci is an Adjunct Professor at the University of Western Ontario, an Affiliate Professor at University of Hawaii and the CEO of Jolyon Mitchell Inhaler Consulting Services, Inc. Mark W. Nagel, HBSc is Head of Aerosol Science at Trudell Medical International, London, Ontario, Canada. Jason A. Suggett, BPharm, PhD, MBA is Group Director, Science and Engineering at Trudell Medical International, London, Ontario, Canada. Dominic P. Coppolo, MBA, RRT, FAARC, AE-C is Vice President, Clinical Strategy Development at Monaghan Medical Corporation, Syracuse, NY, US. Corresponding author: Jolyon P. Mitchell, Jolyon Mitchell Inhaler Consulting Services, Inc., 1154 St. Anthony Road, London, Ontario, N6H 2R1, Canada, Tel: +1 519 472-5364, mitchelljolyon@gmail.com.