AAPS PharmSciTech (2019) 20: 206
DOI: 10.1208/s12249-019-1416-x

Research Article

Theme: Paul Myrdal Memorial Issue - Pharmaceutical Formulation and Aerosol Sciences
Guest Editors: Philip J. Kuehl and Stephen W. Stein

Determination of Passive Dry Powder Inhaler Aerodynamic Particle Size Distribution by Multi-Stage Cascade Impactor: International Pharmaceutical Aerosol Consortium on Regulation & Science (IPAC-RS) Recommendations to Support Both Product Quality Control and Clinical Programs

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Received 25 February 2019; accepted 9 May 2019; published online 30 May 2019

ABSTRACT. The multi-stage cascade impactor (CI) is the mainstay method for the determination of the aerodynamic particle size distribution (APSD) of aerosols emitted from orally inhaled products (OIPs). CIs are designed to operate at a constant flow rate throughout the measurement process. However, it is necessary to mimic an inhalation maneuver to disperse the powder into an aerosol when testing passive dry powder inhalers (DPIs), which constitute a significant portion of available products in this inhaler class. Methods in the pharmacopeial compendia intended for product quality assurance initiate sampling by applying a vacuum to the measurement apparatus using a timer-operated solenoid valve located downstream of the CI, resulting in a period when the flow rate through the impactor rapidly increases from zero towards the target flow rate. This article provides recommendations for achieving consistent APSD measurements, including selection of the CI, pre-separator, and flow control equipment, as well as reviewing considerations that relate to the shape of the flow rate-sampling time profile. Evidence from comparisons of different DPIs delivering the same active pharmaceutical ingredients (APIs) is indicative that the compendial method for APSD measurement is insensitive as a predictor of pharmacokinetic outcomes. Although inappropriate for product quality testing, guidance is therefore provided towards adopting a more clinically realistic methodology, including the use of an anatomically appropriate inlet and mimicking patient inhalation at the DPI while operating the CI at constant flow rate. Many of these recommendations are applicable to the testing of other OIP classes.

KEY WORDS: cascade impactor; dry powder inhaler; aerodynamic particle size analysis; quality control; clinical support testing.

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INTRODUCTION

Multi-stage cascade impactors (CIs) were initially developed in the 1940s to characterize the aerodynamic particle size of particles in aerosol clouds generated by chemical weapons (1,2). Since that time, CIs have evolved to become widely used to characterize atmospheric, workplace, industrial, and other aerosols (3). In the past 30 years or so, these apparatuses have been adopted for the characterization of the mass-weighted aerodynamic particle size distribution (APSD) as a critical quality attribute of therapeutic aerosols, such as those produced by the various classes of orally inhaled product (OIP), including dry powder inhalers (DPIs) (4). DPIs are widely prescribed devices for delivery by inhalation of formulations as a solid particle-based aerosol to the

respiratory tract of a patient (5,6). These formulations may comprise one or more active pharmaceutical ingredients (APIs), often with inactive components, such as lactose carrier particles. Currently, almost all DPIs are passive in nature; that is, they do not contain their own energy source to disperse the aerosol for inhalation, but instead rely on the patient's inhalation for this purpose (5). The signing of the Montreal Protocol in 1987 restricting the use of chlorofluorocarbon (CFC) propellants in pressurized metered-dose inhalers (pMDIs), another major class of OIP, as well as market dynamics and prescribing preferences, has led to a dramatic increase in the popularity of passive DPIs (7), with worldwide sales reaching $17 billion in 2014 (8).

The deposition of airborne particles in the human respiratory tract is significantly influenced by the size of the inhaled airborne particles (9,10). As a result, the APSD of the delivered API, or APIs for a multi-component formulation, is a critical quality attribute of all classes of OIP. The situation is complicated by the fact that the size distribution of the particles consisting of substantially non-active components, such as carrier particles when present, is often quite different compared with that for the particles largely comprised of API (5,6). Quantitative, API-specific analysis of the APSD delivered from inhaler devices is therefore required in order to quantify fully the aerodynamic performance of DPI-generated aerosols. Such testing to characterize the APSD of the API(s) present on a mass-weighted basis is typically required by regulatory agencies in order to obtain regulatory approval (11–13) and is expected as an element of the quality control strategy. The methods in the pharmacopeial compendia are appropriate for within-product assessment to detect important changes in APSD, but, as will be shown later in this article, they may not be suitable for discerning between-product differences in APSD relevant to pharmacokinetic and pharmacodynamic outcomes.

The nature of aerosol generation for DPIs causes inherent difficulties with the determination of the resulting aerosol APSD. In the case of passive devices that are the focus of this article, the aerosol is formed by the energy imparted by the patient inhaling through the device after a dose has been prepared for delivery, either from a capsule or blister containing the formulation or by removing an aliquot of powder from a bulk reservoir (6). Patient inhalation profiles through the DPI vary considerably depending upon patient age and disease type/severity (14). Recently, Azouz *et al.* (15) provided indicative values of several parameters affecting the aerosol formation and dispersion process, including peak inspiratory flow rate (PIF), pressure drop (ΔP), the inhalation volume (IV), and duration of inhalation (T_i) for asthmatic children ($n = 16$), asthmatic adults ($n = 53$), and adults with chronic obstructive pulmonary disease (COPD, $n = 29$). These measures were determined with their patients inhaling through different passive commercially available DPIs (Aerolizer®, Diskus®, Turbuhaler®, and Easyhaler®) representing low, medium, medium/high, and high resistance devices, respectively (Table I). They showed that their inhalation profile parameters depended not only on the patient age and disease type but also on the intrinsic flow resistance of the DPI device. The importance of DPI resistance confirmed the earlier observations of Clark and Hollingworth (16), who determined a monotonic decrease in PIF and device-specific resistance in healthy adult volunteers, inhaling with either maximum or comfortable effort.

In laboratory testing, it is self-evident that in order to measure the aerosol APSD, air must be drawn through a passive DPI during the measurement process. Chavan and Dalby (17) showed that the resulting APSD is dependent on both the flow rate-time profile and total volume drawn through the device. The methodologies in the pharmacopeial compendia, whose purpose is primarily for ascertaining product quality, streamline the process by adopting standardized flow rate-time profiles, as well as tightly constraining the total air volume sampled at 4 L, in order to minimize measurement variability (18,19). There are therefore reductions in method complexity compared with the clinical reality. Such simplifications can introduce significant challenges associated with obtaining results that are predictive of pharmacokinetic and pharmacodynamic outcomes, when such data are used to predict clinical performance related to the pulmonary dose received (20). Such concerns are particularly pertinent in the comparison for bioequivalence of different DPIs delivering the same formulation. Here, the United States Food and Drug Administration (FDA) requires evidence of equivalence based on data from all three aspects, namely equivalent APSD and other physical properties, equivalent systemic exposure (pharmacokinetic study), and equivalent local delivery to receptors in the lungs (pharmacodynamic study) (21–23). There is a largely unmet need to provide guidance on steps that can be taken to make the CI-based measurement procedure more similar to the physical processes that take place when the inhaler is used by a patient, in terms of aerosol generation and transport to the point at which size-classification takes place.

Given the foregoing, this article has the following overarching purposes:

1. to review the methodologies in the pharmacopeial compendia for the APSD measurement of passive DPI-emitted aerosols;
2. to indicate where the basic methodology might be adapted to provide more pertinent APSD measurements in support of the clinical program;
3. to establish best procedures (good cascade impactor practices (GCIP)) for obtaining reproducible and meaningful results from cascade measurements of any passive DPI-generated aerosol following the procedures in the pharmacopeial compendia in the context of product quality control.

A key feature of this article is the summary of recommendations from the cross-industry International Pharmaceutical Consortium on Regulation & Science (IPAC-RS) for optimizing the chances of successful APSD measurements in association with DPI performance testing (Table II).

METHODOLOGIES FOR PERFORMANCE TESTING PASSIVE DPI AEROSOLS BY CASCADE IMPACTOR

Role of the CI in the Pharmacopeial Compendia

The methods in the pharmacopeial compendia aimed at testing passive DPIs have been designed to provide a standardized approximation to patient use (18,19). This strategy results in a

Table I. Selected Patient-Generated Inhalation Profile Parameters (Mean ± S.D.) for Passive DPIs Having Different Resistances from Study by Azouz *et al.* (15) with Asthmatic Children ($n = 16$), Asthmatic Adults ($n = 53$), and Adults with Chronic Obstructive Pulmonary Disease (COPD, $n = 29$)

Disease	Patient age category	Parameter	Aerolizer® Low resistance	Diskus® Medium resistance	Turbuhaler® Medium/high resistance	Easyhaler® High resistance
Asthma	Child	PIF (L/min)	71.4 ± 21.5	53.3 ± 24.2	44.8 ± 16.0	45.5 ± 13.2
		ΔP (kPa)	2.37 ± 1.33	2.10 ± 1.70	2.55 ± 1.79	4.02 ± 2.21
		IV (L)	1.22 ± 0.68	1.19 ± 0.76	1.00 ± 0.73	1.00 ± 0.46
		T_i (s)	1.69 ± 0.38	1.50 ± 0.46	1.52 ± 0.17	1.62 ± 0.23
	Adult	PIF (L/min)	93.7 ± 25.9	76.3 ± 23.8	60.2 ± 17.0	58.3 ± 14.4
		ΔP (kPa)	4.05 ± 2.09	3.96 ± 2.39	4.44 ± 2.39	6.67 ± 2.28
		IV (L)	1.96 ± 0.77	1.89 ± 0.74	1.63 ± 0.74	1.68 ± 0.81
		T_i (s)	1.54 ± 0.34	1.61 ± 0.56	1.63 ± 0.45	1.55 ± 0.47
COPD	Adult	PIF (L/min)	81.8 ± 25.4	62.0 ± 22.4	50.9 ± 15.3	49.6 ± 15.0
		ΔP (kPa)	3.13 ± 1.88	2.68 ± 1.80	3.19 ± 1.94	4.80 ± 2.71
		IV (L)	1.71 ± 0.83	1.79 ± 0.87	1.50 ± 0.80	1.52 ± 0.80
		T_i (s)	1.71 ± 0.46	1.53 ± 0.24	1.57 ± 0.20	1.68 ± 0.60

COPD = chronic obstructive pulmonary disease; *S.D.* = standard deviation; *DPIs* = dry powder inhalers; *PIF* = peak inspiratory flow rate; ΔP = pressure drop; *IV* = inhalation volume; T_i = duration of inhalation

procedure in which the flow rate of air entering the inhaler starts from zero when vacuum is applied to the CI by operation of a solenoid gate valve, rises rapidly to reach the final target value during the measurement procedure and then quickly returns back to zero after a fixed volume of air has been sampled. It is self-evident from the challenges previously identified for such a testing methodology; the nature of the measurement procedure itself has the potential to influence the size properties of the sampled aerosol by controlling the energetics of both the initial powder dispersion and later the transfer of the resulting aerosol from the inhaler *via* the inlet to the pre-separator (if used) and subsequently to the size-fractionating stages of the CI (24,25). These methods utilize standardized profiles of sampling flow rate *versus* time that are not intended to be perfect representations of patient inhalation profiles (26). Instead, method robustness is perceived to be of greater importance in the quality control environment (27). Here, the focus is on achieving a high degree of data reproducibility associated with good precision, so that product performance against pre-determined specifications can be reliably assured to minimize patient risk (11–13).

Impactor Selection

There are currently three different CI apparatus types cited in the European Pharmacopeia that are identified as being suitable for DPI aerosol assessment (18) and four apparatuses described in the United States Pharmacopeia (USP) (19) (Table III). These impactors have been described in detail by Mitchell and Nagel (4) for use in testing all classes of OIP, so only those aspects pertinent to DPI performance evaluation are covered in this article. The Andersen 8-stage non-viable cascade impactor (ACI) and 7-stage next generation impactor (NGI) are currently the most commonly used apparatuses. Both the ACI and NGI operate within approximately the same flow rate ranges that are suitable for DPI-derived aerosol APSD measurements (ACI: 28.3 to 90 L/min; NGI 30 to 100 L/min). The Marple-Miller impactor (MMI) (28), which was the predecessor of the NGI, is seldom encountered, because it does not have an associated pre-separator and only contains five stages. This CI is not recognized by the European Pharmacopeia. Likewise, the multi-stage liquid impinger (MSLI) only affords five sub-fractions and does not have an associated pre-separator. Furthermore, the size selectivity of its stages, based on a calibration published by Asking and Olsson (29), is relatively poor. However, even with these limitations, this apparatus was widely used in Europe before 2000. The MSLI was attractive partly because bias from particle bounce can be avoided altogether by collecting the size-fractionated particles under an impingement fluid, rather than allowing them to impact directly onto a hard surface coated with a substrate to mitigate particle bounce and re-entrainment (4). Both the MMI and the MSLI are currently proposed to be removed from the options offered in the USP for APSD determination (30). This decision was made to simplify available choices. If the proposed change is eventually adopted into official text, only methods in widespread use for current FDA submissions will be referenced, namely the ACI with and without pre-separator and the NGI also with or without pre-separator (4).

The following considerations are important when selecting a CI for DPI testing:

1. the flow rate range for which calibration data are available to define the cut-point size associated with each size fractionating stage;
2. the span of the overall size range associated with the APSD;
3. the number of size-fractionated components of the APSD (size resolution);
4. the intrinsic resolution of each stage (size selectivity), often defined as the square root of the ratio of the sizes at which the stage is 84 and 15% efficient at collecting particles (this ratio would equal 1.00 for a stage with ideal performance exhibited by a step change in collection efficiency from zero to 100% at the cut-point size).

Tables IV and V contain the pertinent cut-point sizes ($D_{a,50\text{-stage}}$) for the various ACI and NGI apparatus configurations, respectively. It is important to note that the ACI

Table II. Summary of IPAC-RS Recommendations for Determining the APSD of DPI-Generated Aerosols from Passive DPIs

Aspect of testing DPIs by CI	Recommendation
Testing for product quality control	Use one of the apparatuses in the pharmacopeial compendia following the instructions therein for flow rate control and sample volume. Consider also the following: 1. Include a pre-separator only if the formulation requires it to be present (*i.e.*, if the active pharmaceutical ingredient is present with larger carrier particles). 2. Do not reduce the sample volume below the specified 4 L, especially if using the NGI whose internal volume is relatively large even without a pre-separator. 3. The minimum number of repeated actuations should be made, justified by the analytical procedure sensitivity used to quantitate the drug deposited on each component of the apparatus.
Testing in product development for support to the clinical program	Consider a more patient-use focused approach by: 1. Replacing the USP/PhEur induction port with either an age-appropriate idealized or anatomically correct naso-/oropharyngeal model inlet. 2. Operating the DPI by breathing simulator with either age-appropriate standardized or patient acquired inhalation profiles. 3. Using a Nephele mixing inlet to enable the cascade impactor to operate at constant flow rate throughout the measurement. 4. Sampling for long enough to ensure complete aerosol bolus transfer to the distal region of the cascade impactor; the 4-L limit specified in the pharmacopeial compendia is a good lower limit for calculating measurement duration. 5. Making the minimum number of repeated actuations based on the clinical dose, but increased if necessary to meet analytical procedure sensitivity requirements used to quantitate the drug deposited on each component of the apparatus. These measures, while recommended for product development, are not recommendable for routine quality control testing, because they significantly increase the complexity of APSD measurements without adding any useful information about quality aspects.
Cascade impactor selection	1. Either the ACI or NGI are suitable cascade impactors for use in the aerodynamic particle size characterization of all types of DPI-generated aerosols, as well as with aerosols from other classes of OIP. However, the NGI has some important advantages, most notably uniform spacing of the cut-point sizes of the stages, excellent size selectivity of each stage, as well as size-characterization of the pre-separator and stages throughout the operating range from 30 to 100 L/min. 2. The Marple-Miller cascade impactor (MMI) has been superseded by the NGI and the multi-stage liquid impinger (MSLI) should only be considered for very high unit dose products, where the elimination of the potential for particle bounce may be important. Users should be aware that the 5-stage size resolution afforded by either impactor may not meet FDA requirements.
Inlet selection	1. Use the PhEur/USP induction port for measurements associated with product registration and quality control. 2. Use either an anatomically accurate or "idealized" inlet for measurements in support of the clinical program. 3. Undertake clinically appropriate APSD measurements with more than one inlet if the indication for the product is wider than one age range (adult, small child, infant). 4. Coat the inlets to prevent de-aggregation and re-entrainment unless data are available to indicate that these processed do not occur.
Nephele mixing inlet	Consider using this inlet when attempting APSD measurements in support of the clinical program where the DPI is to be actuated mimicking patient inhalation profiles
Use of a pre-separator	1. Always use a pre-separator if the formulation makes use of carrier particles. 2. In cases where the powder is dispersed without carrier particles, consider using the NGI with its pre-separator in order to take advantage of having the upper-bound size for stage 1 of the impactor defined. 3. Always fill the central cup with at least 15-ml of fluid when using the NGI pre-separator. 4. Experiment to establish if and how much interior surface coating or liquid is needed to minimize de-aggregation and re-entrainment of large particles in the ACI pre-separator.
Flow controller and associated components when testing for product quality control	1. Always use a flow controller system to minimize measurement-to-measurement variability and ensure that the pipework dimensions (length and diameter) between the exit of the impactor and flow control valve are standardized for all apparatuses allocated to the measurement of APSD for a particular DPI product. 2. Flow controller systems with minimal time required to reach the target velocity are to be preferred.
Impactor maintenance	Develop a strategy for apparatus maintenance that is appropriate for the amount of use that these components are likely to receive in service. An annual stage mensuration is

Table II. (continued)

Aspect of testing DPIs by CI	Recommendation
	recommended as a minimum requirement in order to have assurance that aerodynamic performance has remained within the specifications in the pharmacopeial compendia for stage and pre-separator cut-points.
Control of particle bounce	1. Coating of the induction port with a suitable tacky substance should be followed unless the measurements indicate that bounce and re-entrainment are not evident. 2. Coating all collection surfaces of the CI with a suitable tacky agent should be undertaken as a routine measure to mitigate particle bounce.
Control of electrostatic effects	A careful assessment should be undertaken to establish the level of electrostatic control measures that are needed as part of the preparative work before undertaking testing of a new DPI product. Operation of the apparatus in a climate-controlled environment with relative humidity in excess of 35% year-round is likely to be required in most instances.
In use GCIP measures	Implement all aspects that are relevant to the particular DPI testing regimen that is being undertaken.

IPAC-RS = International Pharmaceutical Aerosol Consortium on Regulation & Science; *APSD* = aerodynamic particle size distribution; *DPI* = dry powder inhaler; *CI* = cascade impactor; *NGI* = next generation impactor; *USP* = United States Pharmacopeia; *PhEur* = The European Pharmacopoeia (Pharmacopoeia Europaea); *FDA* = the Food and Drug Administration (of the U.S.); *GCIP* = good cascade impactor practices

requires reconfiguration if it is to be used at higher flow rates than the original apparatus that was designed to operate at 28.3 L/min (31). At 60 L/min, stage 7 is removed from the bottom of the stack and an externally modified stage "− 0" is inserted above stage "1." This change enables a new stage "− 1" to be mounted on top instead of the inlet cone. At 90 L/min, the process is repeated, this time removing stage "6" and mounting stage "− 2" above stage "1." The spacing in cut-point sizes from one stage to the next in the ACI is irregular, whichever configuration is chosen.

The size selectivity of the pre-separator when used at 28.3 L/min (part (a) in Table IV) is about 1.5, based on the calibration data of Vaughan (32), and the collection efficiency-aerodynamic size curve of this component therefore overlaps with those of stages 0 and to a lesser extent with stages 1 and 2. This pre-separator can be used at flow rates up to 60 L/min without resulting in sonic (choked) flow. However, separate purpose-designed pre-separators are available (Copley Scientific Ltd., Nottingham, UK) for use at 60 and 90 L/min. The upper-bound size of the uppermost stage of the ACI (stage 0, − 1, or − 2, depending upon the configuration used (Table IVa–c)) is defined in terms of the size finer than the cut-point of the appropriate pre-separator. The cut-point sizes supplied by a manufacturer for the three different pre-separators available for use with this CI (8.9-μm aerodynamic diameter for the configuration used at 28.3 L/min (33), 9.8-μm aerodynamic diameter for the configuration used at 60 L/min (34), or 10.2-μm aerodynamic diameter for the configuration used at 90 L/min (35). However, full collection efficiency-aerodynamic size profiles of the two high flow rate pre-separators have not been published, so that the size selectivity of either pre-separator, in relation to the upper stages of the ACI configured for use at these flow rates, remains unknown. Nichols *et al.* (36), who introduced the concept of altering the ACI stage configuration to enable comparable cut-points to be achieved at 60 L/min to those of the standard 28.3 L/min configuration, did not report pre-separator collection efficiency-aerodynamic size data, because this component was not present as part of their apparatus assembly. Its size selectivity may be better than that of the standard pre-separator operated at 28.3 L/min, because of the increased importance of particle inertia in the size-separation process at higher flow Reynolds numbers (4). However, in

Table III. Cascade Impactor Types Currently Recommended in the Pharmacopeial Compendia

USP	PhEur	Suitability for DPI testing
Andersen 8-stage cascade impactor (non-viable configuration) (ACI)	Andersen 8-stage cascade impactor (non-viable configuration) (ACI)	Suitable with or without pre-separator, although compendial method for DPI testing assumes a pre-separator is present; standard flow rate is 28.3 L/min, but configurations are available for use at 60 and 90 L/min
Next generation impactor (NGI)	Next generation impactor (NGI)	Suitable with or without pre-separator, although compendial method for DPI testing assumes a pre-separator is present
Marple-Miller impactor (MMI)—proposed removal because largely unused	Not listed	Does not have a pre-separator and is rarely used now that the NGI is available
Multi-stage liquid impinger (MSLI)—proposed removal because of lack of FDA acceptance	Multi-stage liquid impinger (MSLI)	Does not have a pre-separator but has been widely used in Europe; has insufficient size resolution for USFDA submissions; now largely replaced by the NGI

USP = United States Pharmacopeia; *PhEur* = European Pharmacopeia; *DPI* = dry powder inhaler; *FDA* = Food and Drug Administration (of the USA)

general, the lack of calibration data in the public domain for the ACI pre-separators designed specifically for use at 60 and 90 L/min makes it difficult to judge whether overlap exists with the upper-most size fractionating stages with either of these configurations. Furthermore, in contrast with the stage cut-point data for the ACI (Table IV), there are no corresponding specifications for these pre-separators in the pharmacopeial compendia. In view of this situation, if the decision is taken not to refer to the cut-point size of the particular pre-separator for the ACI configuration in use, the upper-bound size for the impactor is defined as the size that penetrates the uppermost impaction stage, effectively reducing the number of size values associated with ACI-determined APSDs by one.

Roberts *et al.* (37) recently reported a redesign of the ACI pre-separator base intended for use at 28.3 L/min, replacing the three stand-pipes by annular slits at the periphery (Fig. 1), resulting in three times more area for air flow in the modified base. The air flow approaching stage 0 (at 28.3 L/min) has an insignificant momentum compared with that which would be required for the flow to penetrate the nozzles of the underlying stage 0 of the ACI. In contrast, the reduced area for flow *via* the original pre-separator base imparts sufficient momentum to each of the three jets that enables the flow to penetrate the stage 0 nozzles, thereby interfering with the normal size fractionation process that takes place there. Roberts *et al.* evaluated the new and original pre-separator designs above an ACI operated at both 28.3 L/min (conventional stage configuration (part a in Table IV)) and at 60 L/min (higher flow rate stage configuration (part b in Table IV)) with a commercially available DPI (Spiriva® Handihaler®, Boehringer Ingelheim Pharmaceuticals Inc., Ridgefield, CT, USA). Although the number of replicate measurements at each condition was small ($n = 5$), they nevertheless observed significantly less variability in the mass-per-stage data from the inhaler with the modified pre-separator, expressed as fine particle mass at 28.3 L/min (Table V). However, the reduced variability was just below significance threshold ($p < 0.05$) at 60 L/min. Measurements of impactor sized mass at either flow rate were comparable with either pre-separator. Importantly, however, they visually observed more uniform deposition of deposits on the uppermost (stage −1) of the ACI, suggestive that the flow entering the impactor was also more uniform. Although these findings are promising, further repeatability measurements are needed with larger sample sizes as well as with other DPIs before the prototype pre-separator is likely to be adopted as an improvement to the current design of pre-separators for this CI. In addition, there are no calibration data published for the modified pre-separator, so that its size selectivity remains unknown. Once its cut-point size is established by calibration, it will be possible to assign an upper-bound size to the uppermost stage of the ACI, thereby increasing the number of size fractions that can be determined from eight (Table III) to nine, but perhaps more importantly, slightly extending the operating size range in the direction of larger sizes.

In contrast with the ACI, the stage cut-point sizes for the NGI are designed to be spaced at equal intervals on a logarithmic scale, in terms of aerodynamic diameter (Table VI) (38). This uniform spacing of cut sizes aids in the intuitive understanding of mass distribution data that are often presented on a stage-by-stage basis as mass deposition profiles (39). It is important to note that the upper size bound of stage 1 of this CI is always known when the pre-separator is used, because this pre-separator was evaluated in the archival calibration undertaken at 30, 60, and 100 L/min (40) and therefore has its cut-point size defined at these calibration flow rates and also at flow rates that are intermediate. Eight size fractions can therefore be obtained at any flow rate in the range 30 to 100 L/min if the pre-separator is used with this impactor (Table VI).

In general, the stage size selectivity of the NGI at 60 L/min is somewhat better than the corresponding stages of the ACI. However, in practice, this difference is unlikely to be of importance, except in the unlikely scenario in which the same DPI is being assessed by the two different impactor types.

The ability to determine the stage (and pre-separator) cut-point size at flow rates intermediate between those for which calibration data are available is an important aspect when testing DPIs. In the case of the ACI, the relationship:

$$D_{\mathrm{a,50,stage-i,Q_1}} = D_{\mathrm{a,50,stage-i,Q_2}} \left[\frac{Q_2}{Q_1}\right]^{1/2} \quad (1)$$

can be used to estimate the cut-point of a given stage, i, ($D_{\mathrm{a,50,stage-i,Q1}}$) at the test flow rate, Q_1, when the cut-point aerodynamic diameter of the same stage ($D_{\mathrm{a,50,stage-i,Q2}}$) is known by calibration at flow rate, Q_2. However, this relationship is an approximation that makes no allowance for non-ideal factors, such as the influence of gravity on the size-separation process (41). The designers of the NGI therefore provided a more accurate way to undertake the same calculation for stages 1 to 7 of that particular apparatus (38):

$$D_{\mathrm{a,50,stage-i,Q_1}} = A \left[\frac{60}{Q_1}\right]^{\mathrm{B}} \quad (2)$$

A and B are the empirical constants (Table VII) with their values determined by weighted non-linear least-square regression of the stage calibration data subjected to the following constraints:

1. Q_1 and 60 are expressed in L/min
2. $D_{\mathrm{a,50,stage-i,Q1}}$ and "A" are expressed in μm
3. the number of decimal places to the right of the decimal point in the values of "A," "B," and, $ECD_{\mathrm{stage-i,Q1}}$ is equal to two.

A value of 0.50 for B would represent the equivalent flow rate-stage cut-point size relationship, as defined by Eq. (1). Marple *et al.* (40) also developed the following relationship for calculating the cut-point size for the pre-separator ($D_{\mathrm{a,50-ps}}$):

$$D_{\mathrm{a,50,ps}} = 12.8 - 0.07(Q_1 - 60) \quad (3)$$

The micro-orifice collector (MOC) stage of the NGI was intended to be a substitute for a back-up filter by Marple *et al.* (38). In consequence, the MOC has been characterized in

Table IV. Stage Cut-Point Sizes ($d_{a,50\text{-stage}}$) of the Configurations of the Andersen Cascade Impactor for operation at 28.3 and 60 L/min

Stage	Upper-bound size entering stage (μm)	Cut-point size (μm)	Size selectivity[a] (dimensionless)
(a) Operation at 28.3 L/min		Ref[a, b]	
Pre-separator	–	8.9[c]	*ca.* 1.5
0	8.9	9.0	1.15
1	9.0	5.8	1.17
2	5.8	4.7	1.20
3	4.7	3.3	1.22
4	3.3	2.1	1.20
5	2.1	1.1	1.23
6	1.1	0.7	1.21
7	0.7	0.4	1.21
filter	0.4		Not applicable
(b) Operation at 60 L/min		Ref[e]	
Pre-separator	–	9.8[f]	Unknown
−1	9.8	8.6	1.3
0[d]	8.6	6.5	1.3
1	6.5	4.4	1.3
2	4.4	3.2	1.3
3	3.2	1.9	1.3
4	1.9	1.2	1.3
5	1.2	0.55	1.3–1.4
6	0.55	0.26	1.3–1.4
Filter	0.26	Not applicable	Not applicable

[a] The size selectivity data are from Vaughan (32), as is the cut-point size of the pre-separator
[b] The cut-points for the stages are manufacturer-specified nominal values
[c] The pre-separator cut-point size is from Copley Scientific Ltd. (33)
[d] Stage "0" has an external modification permitting another stage (−1) to be inserted above it, rather than the inlet adapter cone that now locates above stage "−1"
[e] The cut-point sizes are from Nichols *et al.* (36), and the corresponding size selectivity values are estimated from their calibration data
[f] The pre-separator cut-point size is from Copley Scientific Ltd. (34)

terms of its collection efficiency at 80% (40). It should therefore not be treated as a size-fractionating stage as its size selectivity has not been determined from a complete collection efficiency profile at the three calibration flow rates. Instead, assurance that any fine particles of the aerosol that penetrate as far as the MOC should be verified as being collected efficiently. If this is not the case, either the MOC should be substituted with the internal filter option or an external filter should be used with the MOC.

The IPAC-RS position (Table II) regarding the ACI and NGI apparatus mirrors the situation in the European and United States pharmacopeial compendia (18,19), in that either impactor is suitable for DPI and other OIP assessments for the determination of aerosol APSD.

ADDITIONAL COMPONENTS

Pre-separator

As mentioned previously, at least one manufacturer of ACI components (Copley Scientific Ltd., Nottingham, UK) offers three different pre-separators for use at 28.3, 60, and 90 L/min, so that some care will be needed to choose the appropriate option depending upon the target flow rate for testing of the DPI that will depend upon its flow resistance. No liquid is required to be inserted into these pre-separators for them to function as designed, although some users prefer to add a small amount of liquid to avoid particle bounce and re-entrainment. However, if a particle impingement liquid is not utilized, as an alternative strategy, Sethuraman and Hickey (42) reported that coating the interior surfaces with a variety of agents that produce a tacky surface after solvent evaporation reduced large particle (> 8.7-μm aerodynamic diameter) transfer to the ACI when operated at 60 L/min. They reported that coating mitigates the tendency for de-aggregation of incoming particles that would otherwise break-up on impact with uncoated surfaces. If a new product is being evaluated with the ACI, experimentation is therefore advised to establish whether interior coating is needed for the pre-separator.

There is only one pre-separator design for the NGI for use throughout the flow rate range from 30 to 100 L/min for which archival calibration data are available for this component (40). It is important to ensure that the central cup of this pre-separator contains sufficient liquid to maintain its intended collection efficiency performance. The designers

Table V. Fine Particle Measures Reported by Roberts *et al.* (37) for Spiriva® Handihaler® DPI-Derived Aerosols Sampled at 28.3 and 60 L/min by Andersen 8-Stage CI with Standard Pre-separator and a New Pre-separator with a Modified Base ($n = 5$ Replicates)

	Flow rate (L/min)	Size range (μm)	Conventional pre-separator (μg/actuation) mean (%RSD)	Modified pre-separator (μg/actuation) mean (%RSD)	*p* value for un-paired *t* test
For fine particle mass					
	28.3	0.7–4.7	1.84 (10%)	2.27 (5%)	0.005[a]
	60	1.1–4.4	2.16 (13%)	2.32 (10%)	0.42
For impactor sized mass fraction					
	28.3	< 5.8	0.69 (5%)	0.73 (1%)	0.06
	60	< 6.5	0.76 (5%)	0.77 (4%)	0.70

DPI = dry powder inhaler; *CI* = cascade impactor
[a] Significant difference

Fig. 1. Improvement by Roberts *et al.* (37) to the design of the base-plate of the Andersen cascade impactor. **a** The original design has three stand pipes oriented at 120°. **b** The stand-pipes are replaced by annular slits at the periphery of the base-plate in the redesign. Used with permission from Daryl Roberts

claim that pre-separator performance in terms of particle capture efficiency should be insensitive to the exact volume of solvent in the cup (38), but most users load it with about 15 mL of solvent in accordance with the advice given in the instruction manual.

The IPAC-RS position (Table II) regarding pre-separator use is:

1. always include this component if the formulation contains carrier particles larger than 20-μm aerodynamic diameter;
2. in cases where the powder is dispersed without carrier particles, consider using the NGI with its pre-separator in order to take advantage of having the upper-bound size for stage 1 of the impactor defined;
3. when using the NGI pre-separator, always fill the central cup with 15 mL of a suitable liquid, compatible with the API recovery and assay methods, to prevent bias from particle bounce and subsequent entrainment in the flow entering the impactor;
4. when using the ACI with any of the pre-separator options, experimentally establish whether and, if so, how much interior surface coating is needed to mitigate potential bias in the measured APSD, arising from large particle de-aggregation and re-entrainment in this component.

Flow Controllers and Related Equipment

In the methodologies described in the European and United States pharmacopeial compendia (18,19), a vacuum source is needed to draw air through and from the DPI. The vacuum imparts energy that is necessary to disperse the powder into aerosol, either from a sample taken from a powder reservoir or from an opened blister or capsule. In practice, critical (sonic) flow conditions ($P3/P2 \leq 0.5$) are established by applying the vacuum to the exit of the CI by rapidly opening a control valve (Fig. 2). A further pressure tap (P1), not shown in Fig. 2, measures ambient atmospheric pressure. Ensuring critical flow across the flow control valve results in a stabilized flow through the system by eliminating the impact of pressure from the vacuum source.

The type of flow controller is not specified in detail in pharmacopeial compendia; however, critical flow controller apparatuses, such as the TPK-series (Copley Scientific, Nottingham, UK) are widely used for this purpose. Once vacuum is applied, the decrease in pressure rapidly

Table VI. Stage Cut-Point Sizes ($d_{a,50\text{-stage}}$) of the NGI at Flow Rates for Which Archival Calibration Data Are Available (40)

Flow rate	30 L/min	30 L/min	30 L/min	60 L/min	60 L/min	60 L/min	100 L/min	100 L/min	100 L/min
Stage	Upper-bound size entering stage (μm)	Cut-point size (μm)	Size selectivity (dimensionless)	Upper-bound size entering stage (μm)	Cut-point size (μm)	Size selectivity (dimensionless)	Upper-bound size entering stage (μm)	Cut-point size (μm)	Size selectivity (dimensionless)
PS	–	14.9	1.49	–	12.7	1.35	–	10.0	1.33
1	14.9	11.4	1.31	12.7	8.3	1.33	10.0	6.0	1.34
1[a]	–	11.6	1.34	–	8.1	1.31	–	6.1	1.35
2	11.4/11.6[b]	6.4	1.19	8.3/8.1[b]	4.5	1.21	6.0/6.1[b]	3.4	1.26
3	6.4	4.0	1.21	4.5	2.9	1.24	3.4	2.2	1.27
4	4.0	2.3	1.11	2.9	1.7	1.17	2.2	1.3	1.22
5	2.3	1.4	1.11	1.7	1.0	1.17	1.3	0.71	1.20
6	1.4	0.81	1.14	1.0	0.56	1.15	0.71	0.39	1.28
7	0.81	0.54	1.17	0.56	0.34	1.20	0.39	0.24	1.38
MOC/filter	0.54	Not applicable	Not applicable	0.34	Not applicable	Not applicable	0.24	Not applicable	Not applicable

NGI = next generation impactor; *PS* = pre-separator; *MOC* = micro-orifice collector
[a] If no pre-separator used
[b] The first value applies if the pre-separator is present; the second value applies if this component is not used

propagates back through to the inhaler-on-test *via* the CI, pre-separator (if present) and inlet. However, before the APSD measurement can be made, the target volumetric flow rate has to be determined at the entry to the induction port (43), since its magnitude affects the cut-point sizes of the size-fractionating stages of the CI, as has previously been described by Eqs. (1) to (3). Target flow rate setting initially requires careful adjustment of the flow control valve with the DPI in place at the entry to the induction port (but not actuated), in order to ensure that the pressure drop across the inhaler is fixed at 4 kPa. The inhaler is then replaced with a flow meter for volumetric flow rate determination. With low resistance DPIs, this method can enable very high flow rates to be achieved, so the pharmacopeias specify an upper limit of 100 L/min. Test duration is calculated from the measured volumetric flow rate, assuming this to be constant throughout the duration of the sampling period, considering that the total volume should be 4 L. Olsson and Asking have shown that when critical flow conditions are maintained at the regulating valve, as is the case with the pharmacopeial compendial methods; the volumetric flow rate downstream from a variable inlet resistance mimicking a DPI is constant, although the mass flow varies with pressure (43). However, quantifying the mass flow is unimportant because impaction efficiency defined by the stage cut-point sizes is related to the Stokes number, which in terms of air properties depends only on velocity and viscosity in accordance with the following expression:

$$d_{a,50-\text{stage}} = \sqrt{\frac{9Stk_{50}\eta\pi ND_{\text{eff}}^3}{4\rho_0 QC_c}} \tag{4}$$

where Stk_{50} is the value of the Stokes parameter at 50% particle collection efficiency ($\sqrt{(Stk_{50})} = 0.49$ for most impactor stages with circular orifices), η is the air viscosity, D_{eff} is the effective nozzle diameter (44), N is the number of nozzles on the stage, ρ_0 is the reference density (1 g cm^{-3}), Q is the volumetric flow rate measured at the inlet to the apparatus, and C_c is the Cunningham slip correction factor that is close to unity for micrometer-sized particles that are typically produced by passive DPIs.

Table VII. Coefficients "A" and "B" for the Next Generation Impactor (NGI) to Enable Stage Cut-Points (μm) to be Calculated at Flow Rates Intermediate from the Reference Value of 60 L/min (Data from Marple *et al.* (40))

Stage	A	B
1	8.06	0.54
2	4.46	0.52
3	2.82	0.50
4	1.66	0.47
5	0.94	0.53
6	0.55	0.60
7	0.34	0.67

The internal dead volume of the pipework between the exit of the CI and the control valve, where the pressure is at its lowest during the measurement of APSD, should be standardized. This precaution is particularly important if there is the need for method transfer from one apparatus to another or from one measurement location to another with different measurement apparatuses. The consideration is not mentioned explicitly in the compendial methodologies; however, variations in this volume associated with differences in pipework length and/or diameter will likely contribute to the rate at which the flow rate rises from zero to the target value, as is discussed further in the next section.

The IPAC-RS position (Table II) is to recommend that a self-contained, automated flow controller system is preferred, rather than a manually operated arrangement in order to minimize measurement-to-measurement variability. Care should also be taken to ensure that the connection pipe length and diameter, which control the internal volume

between the exit of the impactor and flow control valve, are standardized for all apparatuses allocated to the measurement of APSD for a particular DPI product.

Flow Rate Stability and Sample Volume

All types of CI are designed to operate at a fixed flow rate throughout the measurement, because the size fractionating performance of each stage as well as the pre-separator, defined by equations (1–3) in terms of the cut-point diameter ($D_{a,\ 50\text{-stage}}$), that varies with the flow velocity through the nozzles associated with the stage (45,46). The Stokes relationship (Eq. (4)) that governs the operation of all types of CI relates the aerodynamic cut-point of the stage ($d_{a,50\text{-stage}}$) to the volumetric flow rate (Q). According to this equation, the flow rate through the impactor must be kept constant to maintain consistent size classification of an aerosol during CI measurement.

Recent work from the European Pharmaceutical Aerosol Group (EPAG) has shown that, regardless of DPI flow resistance, this ideal situation is never realized during the early part of the entire measurement sequence. Instead, a finite time is always required for the flow rate to increase from zero at vacuum initiation to its final and stable value (47). While the flow rate through the apparatus is below this target value, the cut-points of the size fractionating components are larger than intended, resulting in a potential to bias the APSD measurements in the direction of larger size. In the EPAG study that used three different sizes of orifice plate to mimic low-, medium-, and high-resistance DPIs, it was shown that if a pre-separator is present with either the ACI or NGI, the duration of the interval before constant flow rate is achieved can exceed 250 ms after application of vacuum to the measurement apparatus, based on the time needed for the flow rate to attain 90% of the target value (t_{90}). For some DPIs, this corresponds to the time interval where the majority of the powder is aerosolized (Fig. 3) (48). The magnitude of this delay increases with internal dead volume (Fig. 4) and is greatest for high resistance DPIs where the target flow rate is lowest (Table VIII).

The time-dependent trends in flow rate at the location of the DPI, associated with the EPAG study (47), are consistent with the findings of Beron *et al.* (49), who also investigated the flow rate-rise time behavior for two (unspecified) passive DPIs with both ACI and NGI, but sampling at target flow rates of 60 and 100 L/min. This group made their measurements by inserting buffer volumes of varying size between a dose uniformity sampling apparatus (DUSA) and the flow controller to avoid additional variability due to assay of API from multiple CI components (49). Beron *et al.* reported large differences in times to 90% of the target flow rate (t_{90}) between internal volumes representing ACI and NGI configurations each with pre-separator. This group also determined flow rate acceleration across a range from 20 to 80% of the target value for both 60- and 100-L/min target flow rates (Table IX). By focusing on the portion earlier in the flow rate-rise time profile, they argued that the flow rate acceleration parameter likely relates more closely than does t_{90} to the physical processes taking place during aerosol formation. Its magnitude may therefore be more indicative of the initial powder dispersion behavior. Unfortunately, they did not extend their initial investigation to relate these acceleration measures to changes in APSD. They highlighted that the shape of the time-dependent flow profile during this early part of the measurement process has the potential to influence the measurement of delivered dose uniformity, reducing it from 100% label claim when the apparatus internal volume was 456 mL to between 85 and 90% label claim for larger internal volumes (1436 and 2012 mL) that are closer to those associated with the ACI and NGI, respectively. It should be noted, however, that the effect was only observed where the flow acceleration was less than values typically observed for measurements following compendial procedures. Both these observations, as well as the findings from the EPAG study, provide support for maintaining a consistent connection pipe length and diameter from the CI exit to the flow controller as discussed in the previous section.

The consequences for APSD measurement, in which the internal volume is greatly increased by the addition of a pre-separator and induction port so that in total this volume is 1137 and 2007 mL for the ACI and NGI configurations, respectively, are therefore currently uncertain. However, it is important to be aware that this effect on flow rate rise kinetics could be DPI design-dependent, if powder-to-aerosol dispersion is affected. Since the internal volume of the apparatuses is increased by the addition of a pre-separator (180 and 780 mL for the ACI and NGI, respectively), it follows that a pre-separator should only be used if the nature of the formulation being dispersed requires it to be present, for example, in all instances in which the API is formulated with larger carrier particles.

It is also important to pay attention to the total volume sampled as well as the control of the flow rate through the apparatus during the entire measurement. Attempts have been made to reduce the sampled volume to mirror the IV of a patient to make the measurements more clinically relevant (50,51). However, there is evidence from another EPAG-led laboratory study by Mohammed *et al.* (24,25), in which the sample volume was progressively reduced from 4 L, that significant bias in the APSD to larger sizes can occur at smaller sample volumes, because not all of the aerosol bolus from the DPI has had time to penetrate through the entire set of size-fractionating stages. In their first study, Mohammed *et al.* found that this behavior was particularly apparent with the NGI, whose internal volume is almost twice that of the ACI. It follows that if the sample volume is reduced, the proportion of the period during which the flow rate has not stabilized at the target value (previously discussed) compared with the measurement duration is also increased. In a follow-on study, Mohammed *et al.* (25), by studying the behavior of low and high resistance DPIs, were able to show that incomplete capsule emptying took place with low resistance Cyclohaler® DPIs only at the smallest sample volume investigated (1 L). Furthermore, capsule emptying was apparently efficient, regardless of comparable reductions in sample volume, with the higher resistance HandiHaler® DPIs that were also evaluated in the same investigation. Although, based on the outcome from the EPAG studies, smaller volume sampling may be possible with the ACI, caution should be exercised. The recommendation should be followed in the compendial methodologies to set the flow

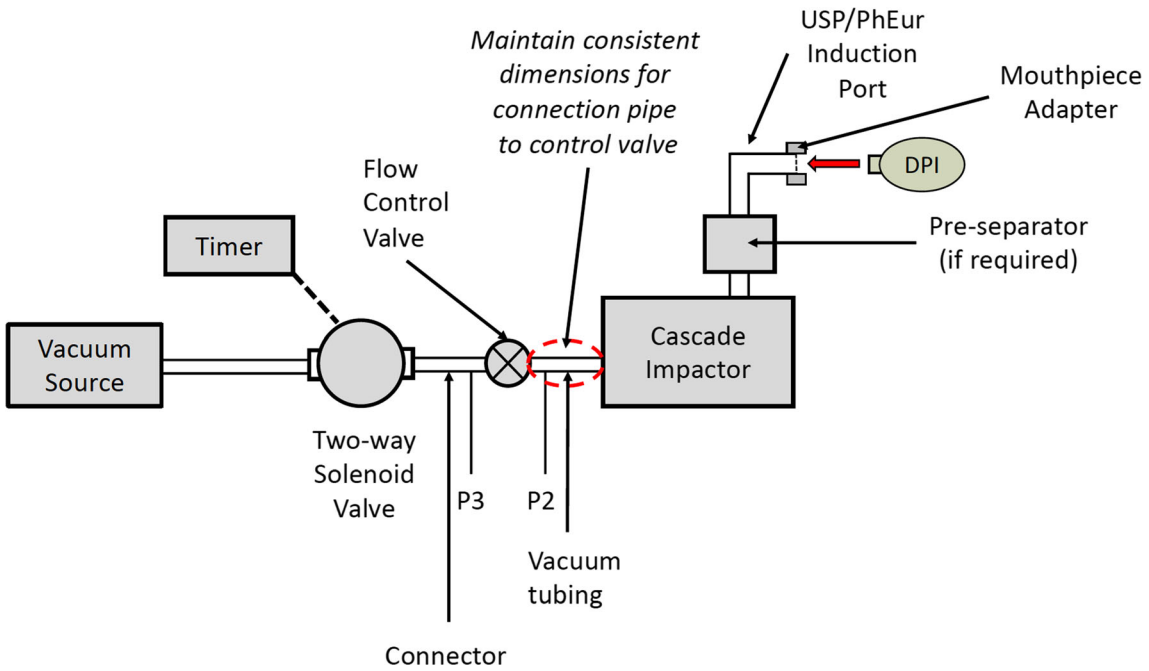

Fig. 2. Configuration of flow control for testing DPIs for aerosol APSD measurement in accordance with pharmacopeial compendia procedures (18,19)

control timer, so that a 4-L sample volume is always withdrawn from the DPI-on-test.

ENHANCEMENTS TO THE CI METHOD TO SUPPORT THE CLINICAL PROGRAM

From the previous section, it is apparent that the scope of compendial methods for APSD measurement of aerosols from DPIs is likely to be quite limited in what these methodologies can reveal about how the product will perform in use with patients, even if the inhaler is operated in compliance with the Instructions for Use. This situation was highlighted quantitatively by Daley-Yates *et al.* (52), in which they established near-to-identical APSDs by ACI measurements at 60 L/min following the methodology in the pharmacopeial compendia for salmeterol xinafoate (SX)/fluticasone propionate (FP) combination therapy (50-μg/actuation SX/250-μg/actuation FP) delivered either *via* the Diskus® multi-dose DPI or a prototype reservoir powder inhalation device (RPID) under development at the time. They also undertook two clinical studies, hoping to establish bioequivalence with the same formulation and inhalers:

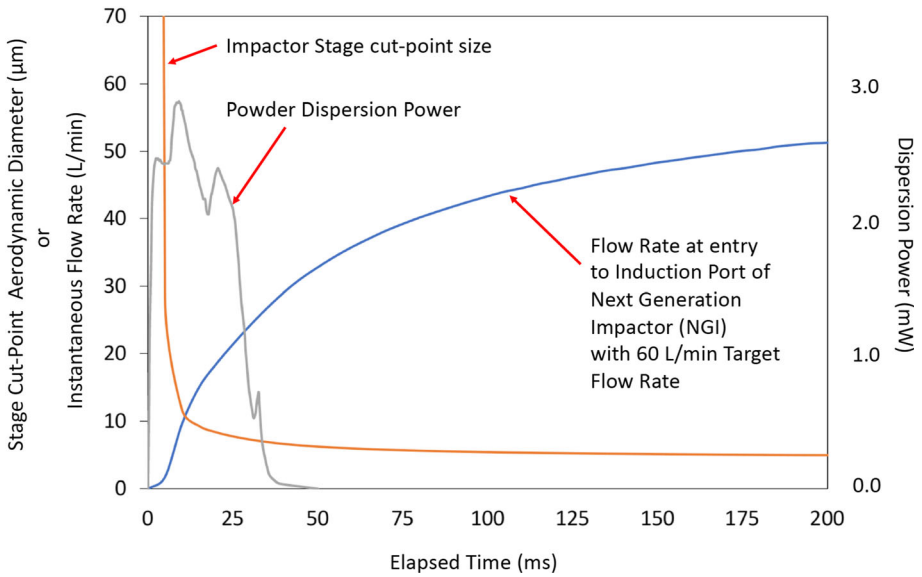

Fig. 3. Time-dependent dispersion power and instantaneous flow rate for Diskus® DPI estimated from data of Tibbats *et al.* (2010), following initiation of sampling by NGI with a target flow rate of 60 L/min. The cut-point size of the second stage of the NGI is also shown for comparison. Used with permission from Christopher Shelton

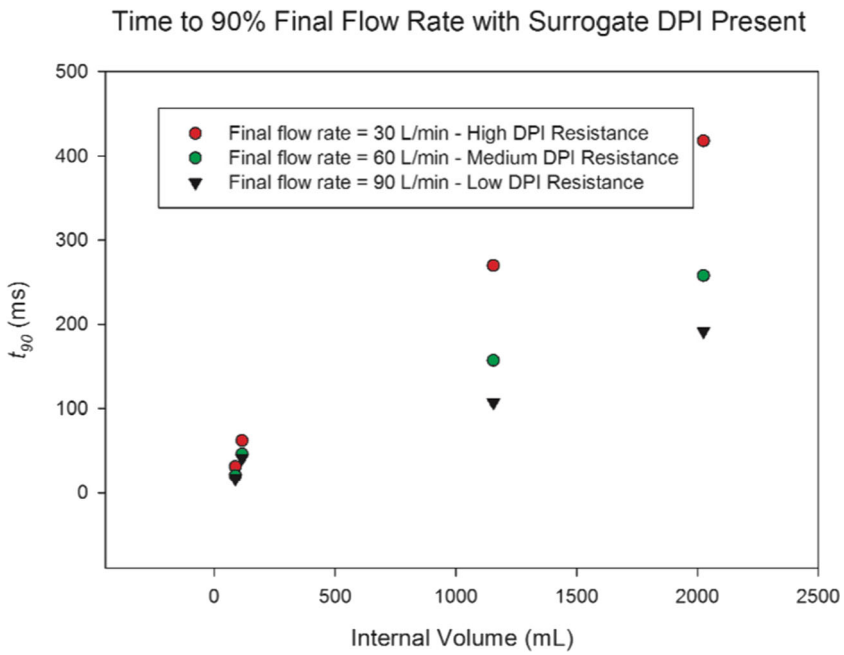

Fig. 4. EPAG study (47) correlated apparatus internal volume with time for flow rate to achieve 90% of the final target value. The shortest times were achieved with flow meter test systems and dose uniformity sampling apparatuses having the lowest internal volumes. The mid-range times were obtained with Andersen 8-stage cascade impactors and the longest times with next generation impactors—all impactors were configured with a pre-separator and USP/PhEur induction port entry

1. a pharmacokinetic/pharmacodynamic study administered in two 14-day crossover treatment periods to 22 adults with moderate, persistent asthma, to determine the equivalence of both inhalers in terms of topical drug delivery to receptors in the lungs and systemic exposure;
2. a 12-week clinical efficacy and safety study of 50-μg/actuation SX//250-μg/actuation FP in 270 patients > 12 years of age with moderate, persistent asthma to assess the equivalence of the RPID and Diskus® inhaler based on peak expiratory flow (PEF) rates.

Daley-Yates *et al.* found that the two inhalers did not meet the criteria for declaring bioequivalence because the estimated ratios (RPID: Diskus®) were 2.00 (90% CI, 1.56 to 2.55) for FP area-under curve (AUC) up to the time point of next dosing and 1.92 (90% CI, 1.64 to 2.25) for SX maximum observed plasma concentration at the end of the dosing interval (at steady state). Urine cortisol (0–24 h) was significantly lower for the RPID than for the Diskus® inhaler (ratio, 0.74 (95% CI, 0.57 to 0.96); $p = 0.026$). The conclusion was that the *in vitro* APSD measurements, following procedures in the pharmacopeial compendia, indicating equivalence between the two DPIs were not predictive of the clinical outcomes.

A similar finding was obtained later in another study by Daley-Yates *et al.* (53), this time in a comparison of the Diskus® multi-dose DPI with its predecessor Rotahaler® capsule-based DPI, sampling 50-μg/actuation SX//250-μg/actuation FP by ACI and NGI at 60 L/min. They established that for both APIs, the *in vitro* aerodynamic particle size profiles for the Rotahaler® were within −15% of Diskus® DPI for the fine particle mass < 5-μm aerodynamic diameter (FPM) and emitted dose (ED), using an ACI, and also for emitted mass/actuation, mass median aerodynamic diameter, and geometric standard deviation, using a NGI. This was also the case for the fine particle component of FP in the size

Table VIII. Mean Values of t_{90} for Andersen Cascade Impactor (ACI) and Next Generation Impactor (NGI) With and Without Pre-separator at Target Flow Rates of 30, 60, and 90 L/min from a Cross-Industry Study by the European Pharmaceutical Aerosol Group (Data Adapted from Mitchell *et al.* (47))

Apparatus (all equipped with USP/PhEur induction port)	Internal volume (mL)	Number of measurements	Target flow rate 30 L/min	Target flow rate 60 L/min	Target flow rate 90 L/min
ACI	957	3	245	154	101
ACI + pre-separator	1137	18	274	157	108
NGI	1227	3	304	182	150
NGI + pre-separator	2007	27	431	266	197

Table IX. Measured Mean Flow Rate Acceleration from Flow Profiles Generated by Andersen Cascade Impactor (ACI) and Next Generation Impactor (NGI) with Target Flow Rates of 60 and 100 L/min (Data from Beron *et al.* (49))

Apparatus	Flow rate acceleration over 20–80% range of 60 L/min ($L\ s^{-2}$)	Flow rate acceleration over 20–80% range of 100 L/min ($L\ s^{-2}$)
Dose uniformity sampling apparatus (DUSA)	30.0	41.5
ACI with pre-separator	17.3	18.7
NGI with pre-separator	6.5	8.1

range between 0.9- and 8.1-μm aerodynamic diameters. The divergence between these two fine particle mass measures for the two inhalers was only slightly larger than 15% for the SX component. In their investigation, Daley-Yates *et al.* recruited adult patients with either moderate asthma or moderate-to-severe COPD for their clinical studies, and similar pharmacokinetic measures were assessed to those investigated in the 2009 study. For the combined subjects, the plasma AUC and maximum blood plasma concentration (C_{max}) for both FP and SX were higher for the Rotahaler® DPI. Thus, the Rotahaler®/Diskus® geometric mean ratios (90% confidence intervals) for AUC were 1.52 (1.37–1.67) and 1.15 (1.09–1.21) for FP and SX components, respectively. The corresponding C_{max} ratios were 1.94 (1.75–2.10) and 1.56 (1.42–1.67). In this investigation, they concluded that although their *in vitro* measurements and other systemic pharmacodynamic endpoints revealed no major differences between the two inhaler types, they lacked predictive power and sensitivity to guide *in vivo* drug delivery performance and systemic exposure.

In an attempt to address the need for more clinically appropriate inhaler testing, Mitchell and Suggett (26) identified the following measures that can be readily undertaken to make inhaler measurements, including DPIs that are likely to be more appropriate in support of a clinical program, in particular in their potential to improve the predictive capability for *in vivo* bioequivalence:

1. replace the compendial induction port by an anatomically accurate (or idealized) inlet as the entry to the CI sampling apparatus;
2. operate the inhaler using a breathing simulator in conjunction with a mixing inlet so that the CI samples at a constant flow rate (the way this situation can be achieved is discussed later in this section).

Under these conditions, there is no need to restrict the volume sampled by the CI, although the volume through the DPI is controlled *via* the inhalation profile derived from the simulator.

Clinically relevant inhaler testing by CI is currently an active area of research. Further *in vitro-in vivo* comparison studies, similar to those undertaken by Daley-Yates and colleagues, need to be undertaken with a variety of formulations and DPI types before firm conclusions can be drawn as to the effectiveness of these suggested modifications. A further caveat is that both measures identified by Mitchell and Suggett (26) add complexity to the APSD measurement procedure. In consequence, IPAC-RS recommends that they not be implemented in product quality control (Table II).

When introducing clinically appropriate enhancements, users should be aware that the modified measurement apparatuses retain fixed dimensions, whereas the airways of the lungs are flexible with variable diameters and length and possibly changing angles of bifurcation upon inhalation (54). Furthermore, obstructive lung disease has an influence on airway morphology (55). In consequence, the predictive capability for such CI-based measurements with the modifications suggested by Mitchell and Suggett (26) for the purpose of correlating *in vitro* with *in vivo* outcomes will likely still be limited (56).

Regardless of the choice of such methodological enhancements whose purpose is to improve support for clinical studies, as with measurements made for product quality control purposes, it is important to sample the DPI-generated aerosol for sufficient time to ensure complete penetration through the apparatus to the MOC (NGI) and/or back-up filter (ACI or NGI). This consideration is especially important if only a single inhalation is being made per APSD measurement. The 4-L sample volume guidance provided in the pharmacopeial compendia (18,19) therefore remains relevant when calculating the duration of each determination per inhaler actuation. It should also be noted that the clinical dose may be as little as a single actuation for some products. Under these circumstances, it may not be possible to reduce the number of repeated actuations per APSD determination to one in order to mimic clinical use, for example, with a high potency formulation. This consideration is especially important if the mass of API dispersed throughout the apparatus is insufficient for the assay method to enable accurate measurements, especially at stages where particles at the extremes of the APSD are collected. Under such circumstances, the IPAC-RS position (Table II) is to recommend actuating the DPI the minimum number of times for adequate API detection throughout the apparatus, justified by the sensitivity of the analytical procedure used to quantitate the deposited drug. This position essentially follows current FDA guidance on APSD measurements for product quality control (13).

Paying attention to the choice of inlet is essential towards the goal of mimicking clinical reality, as the incoming aerosol from the DPI has initially to pass through this component before size fractionation can take place. The particle deposition characteristics of the inlet modify the aerosol that is subsequently transported to the CI *via* the pre-separator (if

present). The right-angled PhEur./USP Induction port, although an advance in terms of standardization, compared with the variety of entries that had previously been available (57), is well known to be unrepresentative of the complex geometry of the adult oropharynx (58). Laboratory studies sampling both pMDI- and DPI-derived aerosols *via* a CI equipped with either the compendial inlet or an adult "idealized" induction port, in which the particle deposition characteristics more closely mirror anatomic reality, have shown that resulting APSDs are shifted to finer sizes with decreased "spread" (58,59). In recent years, several studies have demonstrated that, irrespective of inhaler type, replacing the compendial inlet with either an "idealized" design or one that is an age-appropriate anatomically correct representation of the oropharyngeal airway will result in a more accurate measure of the APSD (60–63). However, given the natural variability in upper airway dimensions apart from the influence of age, establishing what is "accurate" for a patient cohort in a given age range (pediatric/adult) is a challenge. Magnetic resonance imaging studies with adults by the Oropharyngeal Consortium (61,64) resulting in the development of small, medium, and large adult oropharynx models offer a way forward. However, their work was not extended to the development of similar pediatric models, which are currently lacking. Given that some DPIs are now seen as viable treatment alternatives to pressurized metered dose inhaler or nebulizer-delivered inhaled medication for preschool age children (65), the internal geometry of the inlet should be chosen to be age-appropriate, since the internal dimensions of the naso- or oro-pharyngeal airway increase as the upper respiratory tract develops in childhood (66). This requirement may require repeating APSD measurements with more than one inlet if the indication for the product is wider than one age range (*e.g.*, infant, small child, adult).

The use of a breathing simulator to operate the DPI-on-test is at first sight an obvious improvement in terms of mimicking patient use. However, here, the practicality of doing so is complicated by the requirement of keeping the CI operating at constant flow rate throughout the entire measurement period. Early attempts to achieve this goal involved quite complex arrangements, including solenoid valves to control the transfer of the aerosol from the inhaler to the impactor (67,68). Such flow control systems introduced the possibility of transient pressure pulses that may have affected the resulting measured APSD (4). Even in attempts in which the aerosol from the inhaler was transferred without the use of solenoid valves (69,70), careful flow control was required to avoid losing aerosol in transit from the inhaler to the CI. Some of these arrangements were also limited to testing the inhaler at flow rates that were lower than the flow rate required by the CI (4). The Nephele mixing inlet, patented by Miller (71), and available commercially (RDD On-Line, Richmond, VA, USA; Copley Scientific, Nottingham, UK) offers a solution to the problem by bringing in additional constant flow from a compressed air source through a tapered structure in which the flow is gently merged with the variable flow from the inhaler-on-test, thereby minimizing internal losses of the airborne particles caused by turbulent deposition (Fig. 5). The CI is located downstream of the mixing inlet exit and samples the mixed flows containing the aerosol at a constant flow rate throughout the measurement process. This arrangement has since been widely adopted (64,72–74). In particular, Olsson *et al.*, simulating adult use, demonstrated using a Nephele inlet in conjunction with an NGI that consistent *in vitro-in vivo* correlations (IVIVCs) are potentially possible for the inhaled corticosteroid, budesonide, delivered by a variety of inhaler platforms, including three different passive DPIs (64). Despite this promising outcome, particle aerodynamic size-related internal losses in the mixing inlet as a function of flow rate have not yet been reported. Even in the likely event that such losses are small because the internal geometry has been designed to minimize particle deposition, there is a need for them to be quantified, as the present lack of information is likely to hinder adoption of the mixing inlet into standardized methodologies associated with inhaler testing. Nevertheless, it is reasonable to assume that they are small, to judge from the ability of the various study groups who have used the device to achieve acceptable material balances close to the label claim dose/actuation associated with these measurements. However, it is recommended that the performance of this inlet as a function of particle aerodynamic size should be evaluated with monodisperse calibration particles of known aerodynamic diameter in the range associated with inhaler testing (0.5- to 15-μm aerodynamic diameter), in order to provide reassurance of its capability to avoid bias in connection with APSD measurements.

Standardized inhalation breathing patterns may be useful to compare the performance of DPIs having different flow resistances (75). However, most groups generally prefer to use patient-generated inhalation waveforms (76–80), now that breathing pattern recording apparatuses are widely available. Whatever approach is taken, the inhalation profiles should encompass the entire age range that is indicated for use on the label for the DPI product.

GOOD CASCADE IMPACTOR PRACTICES

Whether or not CI measurements of DPIs are being undertaken for product quality control or in support of the clinical program, there are several aspects associated with their use that fall under the general title of GCIP. The purpose of GCIP is to ensure that sources of bias are avoided or mitigated (81), and there are three components to consider (Fig. 6):

1. apparatus maintenance;
2. method development;
3. in-use considerations.

A new informative chapter proposed for the USP (82) contains comprehensive guidance on how GCIP might be implemented, including a flow chart that addresses the particularly important aspect of ensuring that the critical components, namely the nozzle diameters of each stage, are within specification, which is a key aspect of apparatus maintenance that is discussed further below.

Apparatus maintenance includes the induction port, preseparator, and induction port, and its focus is chiefly on periodic (*i.e.*, annual) CI performance validation through stage mensuration (83), whereby optical microscopy combined with automated image analysis is used to determine the individual nozzle diameters of each stage. Mechanical go-no

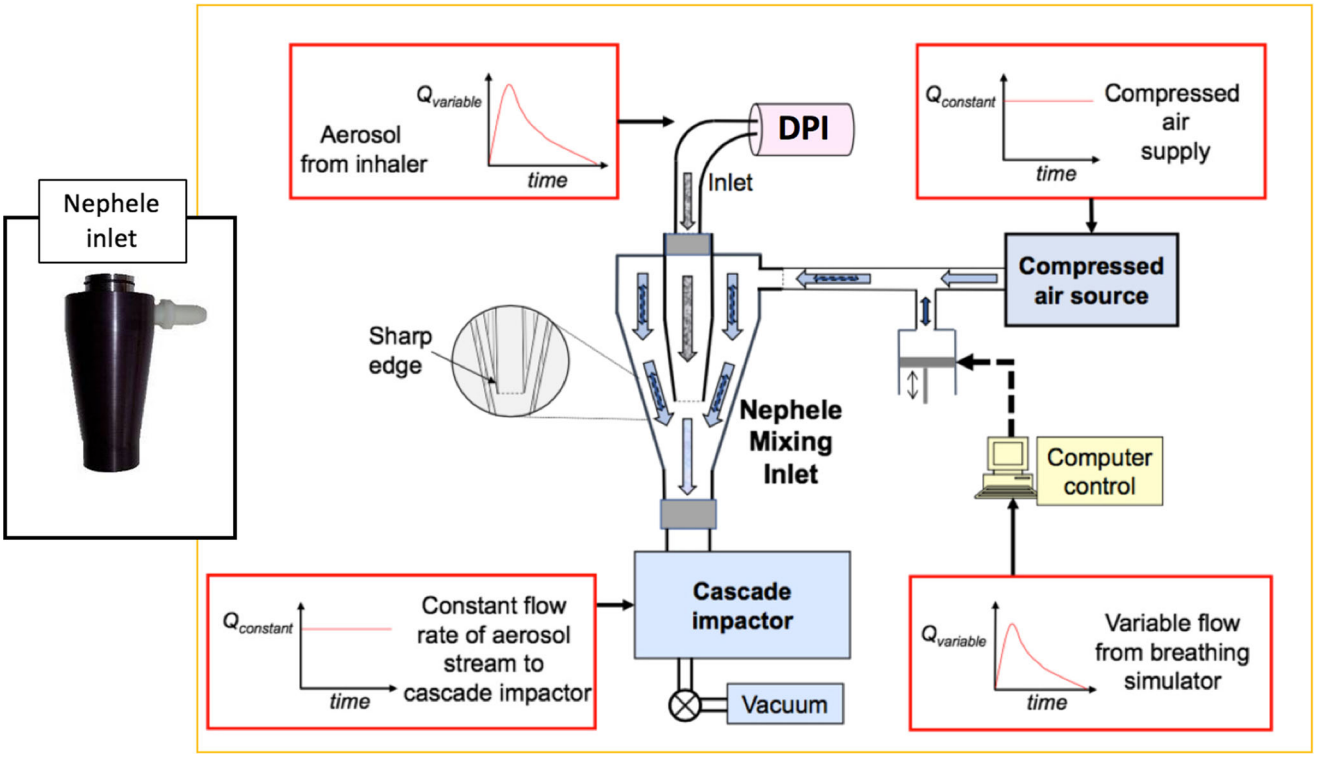

Fig. 5. The "Nephele" mixing inlet (70) in a configuration in which a DPI can be subjected to variable flow-time profile, such as a patient-generated waveform, while the cascade impactor samples the mixed flow from the inhaler together with make-up air from a separate supply

go gauges can be used for checking nozzles $>$*ca.* 2-mm diameter. D_{eff} is then calculated as described by Roberts (44) and used to determine whether the stage remains suitable for continued use on the basis of Eq. (4) that links this measure with the stage cut-point size ($d_{a,50\text{-stage}}$). Nozzle diameters of the size-separating components in the pre-separator should also be checked as part of this process. At the same time, the induction port and collection plates or cups for the CI should be inspected for visible damage and replaced as needed. The IPAC-RS position regarding maintenance under GCIP (Table II) is to recommend that the laboratory manager develop a strategy for apparatus maintenance that is appropriate for the amount of use and the chemistry of the API recovery solvents that these components are likely to receive in service. An annual stage mensuration is recommended as a minimum requirement in order to have assurance that aerodynamic performance has remained within the specifications provided in the pharmacopeial compendia for stage and pre-separator cut-points.

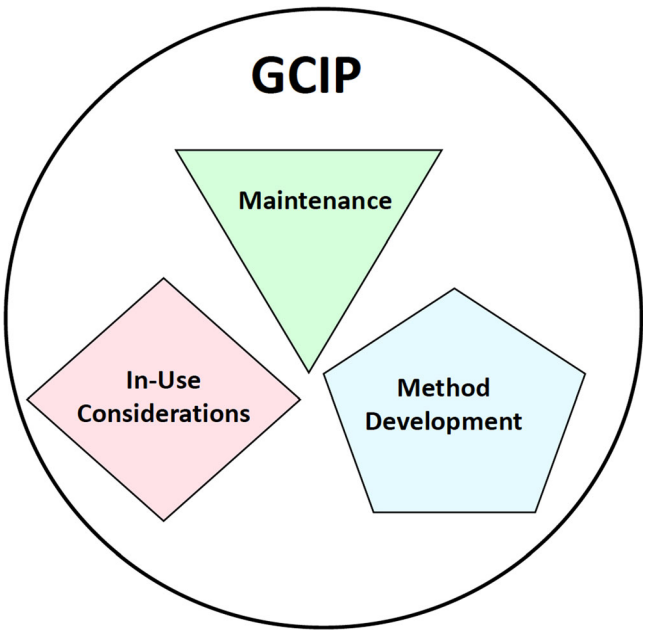

Fig. 6. The three elements of good cascade impactor practices (GCIP)

A recent development to GCIP has been the provision of general advice applicable to all classes of OIP when creating a new method for inhaler testing, or in method transfer (81). Guidance is given on optimizing the number of inhaler actuations/inhalations per determination to achieve adequate recovery of API for the assay method in use and avoidance of an excessive number that might conceal underlying measurement-to-measurement variability. Advice is also given on the number of replicate determinations per measurement. The suggestions regarding the control of particle bounce and re-entrainment and electrostatic charge accumulation/ transfer are particularly pertinent for DPI testing. Coating of CI stage surfaces with an agent to improve particle adhesion is well understood to be necessary to avoid particle bounce and associated re-entrainment in the high velocity flow passing from a particular stage to the remainder of the apparatus (84,85). Particle bounce and re-entrainment bias the measured APSD to finer sizes. Bias from this effect is almost inevitable in the case of the solid particles associated with DPI-generated aerosols, unless some form of coating, such as silicone oil or more commonly a polyoxyethylene

lauryl ether (such as Brij-35 (86)), is applied. The coating of the induction port to mitigate bounce of high inertia (large) particles that should otherwise deposit therein, especially prevalent with carrier-based formulations, is a further precaution (87). This practice should be followed unless the measurements indicate that bounce and re-entrainment are not evident (Table II). The effect is readily diagnosed by the presence of a larger-than-expected recovery of API from the back-up filter (or MOC with the NGI). The IPAC-RS position regarding control of particle bounce within the CI (Table II) is to advocate coating all collection surfaces with a suitable tacky agent as a routine measure when sampling aerosols from DPIs.

Finally, it is well-known that DPI-generated aerosols can carry significant electrostatic charge (6,88). Electrostatic charge accumulation can result in highly variable APSD measurement outcomes, which are still poorly understood in comparison with other physical processes associated with aerosol formation by OIPs. Part of the problem is associated with the limited availability of suitable measurement equipment having the capability to make relevant and reproducible measurements (89). Implementation of electrostatic controls, including grounding both the analyst and the equipment, using an ionizing air blower and anti-static gun to discharge the air surrounding the apparatus, and having the analyst not wear gloves or touch the induction port during testing have been shown to be effective for the mitigation of electrostatic charge-related effects in the context of APSD determination with pMDIs (89). A further precaution is to undertake the measurements in an ambient environment with controlled relative humidity in excess of 35% (90). The IPAC-RS position (Table II) is that a careful assessment be undertaken to establish the level of electrostatic control measures that are needed as part of the preparative work before undertaking testing of a new DPI product. Operation of the apparatus in a climate-controlled environment with relative humidity in excess of 35% year-round is likely to be required in most instances. These controls would be appropriate for testing either for inhaler product quality or in support of the clinical program.

The final aspect of GCIP relates to the precautions "in-use" that should be taken in association with each APSD determination. This includes inspection of critical components, such as seal rings, for damage or wear; assurance of stage collection coating; assertion of correct assembly of the stages and their collection surfaces (plates for the ACI, cups for the NGI); a check for ambient air leakage into the assembled CI with pre-separator (if used) and induction port; and setting of volumetric flow rate at the inlet to the induction port and cleaning/storage after use. There is widespread experience that paying attention to these details will result in fewer erroneous CI-based measurements of APSD. The IPAC-RS position (Table II) is to recommend the implementation of all these aspects of in-use GCIP that are relevant to the particular DPI testing regimen that is being undertaken.

CONCLUSIONS

In the context of product quality control, the performance testing of passive DPIs by CIs for the determination of aerosol APSD presents significant challenges, because it is necessary to use the flow through the multi-stage CI and associated equipment as an energy source in order both to aerosolize the powder presented for inhalation and subsequently transport the airborne particles through the apparatus efficiently for size fractionation. This article has provided recommendations for achieving consistent APSD measurements in the context of product quality control, including the selection of the CI, pre-separator and flow control equipment, as well as considerations that relate to the shape of the flow rate-sampling time profile that is intimately associated with the aerosol creation and transport processes. Although inappropriate for product quality testing, guidance has also been given towards adopting a more realistic methodology for DPI testing when measuring APSD measurements in support of the clinical program. Such enhancements are likely to be appropriate where it is sought to demonstrate *in vitro* equivalence. For clinically relevant use, in addition to replacing the USP/PhEur induction port with either an idealized or anatomically accurate inlet, it is advisable to use a breathing simulator in order to operate the DPI-on-test with patient age-appropriate profiles. At the same time, the air flow through the CI with/without pre-separator can be maintained constant during the measurement process by means of the Nephele mixing inlet. Although the focus is on DPIs, many of the recommendations herein are applicable to the testing of other OIP classes.

ACKNOWLEDGMENTS

The authors gratefully acknowledge input at various times from members of the Cascade Impactor Working Group of IPAC-RS as well as wider support from the IPAC-RS Board of Directors. IPAC-RS is a non-profit association of companies involved in developing and manufacturing of OIPs, focused on advancing scientific and regulatory approaches for these products.

COMPLIANCE WITH ETHICAL STANDARDS

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