Significant advances in laboratory testing of orally inhaled products during the ten-year life of *Inhalation*

Testing for dose content uniformity and aerodynamic particle size distribution has shifted from pharmacopeial quality control testing to more clinically appropriate methods.

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Introduction

The laboratory testing of orally inhaled products (OIPs) for dose content uniformity (DCU) and aerodynamic particle size distribution (APSD) forms the backbone of the evaluation of these products. A function that is undertaken throughout their lifecycles, from design concept through the design development and registration processes and later during post-marketing surveillance. In particular, the past ten years have seen increasing emphasis on methods that are appropriate for verifying the design intent as part of a quality-by-design approach. Such testing has been driven by regulatory recommendations[1, 2] and was incorporated in an international standard in 2009, whose focus is specifically on OIP development.[3] In parallel, there has been increased recognition that understanding how a product behaves in the hands of the patient should be a key component of the regulatory package.[4] As a result, the relatively straightforward compendial methods described in the European (Ph.Eur.)[5] and United States (USP)[6] Pharmacopeias for dose content uniformity and aerodynamic particle size distribution have been increasingly augmented by testing methods that seek to be more clinically aligned in their purpose. This article covers the evolution of OIP laboratory testing strategies that have taken place in the past decade, wherein the industry has seen a noticeable shift from standard pharmacopeial quality control testing to more realistic end-user-driven testing by incorporating clinically appropriate methods. It also looks into development of more advanced technologies for OIP-generated particle size distribution assessment. Finally, it briefly mentions testing of APSD for OIPs containing formulations that incorporate nanoparticles.

Table 1 summarizes the more significant events that have taken place, classifying them into the following categories: compendial, clinically realistic and advanced methods. Progress within each of these categories is the focus of this article.

Compendial methods

The methodologies associated with determining OIP aerosol properties, aimed particularly at design verification and quality control, have undergone significant development, principally associated with the assessment of APSD. Here, the drivers have been the desire to reduce both the complexity and quantity of testing. Slightly before the beginning of the ten-year period with which this article is concerned, the development of the Next Generation Impactor (NGI)[7] marked the creation of the first impactor purpose-designed for use in OIP testing. The subsequent archival calibration of a representative impactor, whose stage nozzle diameters were intentionally manufactured to be as close as possible to nominal, avoided the need for individual NGIs to be calibrated with monodisperse particles, as had been the practice with previous impactors.[8, 9]

Table 1

Significant Recent Developments in the Testing of OIPs for Dose Content Uniformity and Aerodynamic Particle Size Distribution

Event	Type of Testing	Importance to the Industry
Development of the NGI	Compendial	The first multi-stage cascade impactor purpose designed for use in OIP testing
Cascade impactor traceability	Compendial	Extension of theory linking the stage cut-point size to "effective diameter" as a single size measure for a multi-nozzle stage
EDA concept	Compendial	Offers new way to assess both full resolution impactor- and AIM-derived data with potential for improved decision-making in product quality control
Semi-automated ancillary equipment	Compendial	Speeds up analytical processes associated with both DC and APSD determinations
Automated inhaler actuation	Compendial	Increasingly prevalent in use because such equipment, once set up and validated, eliminates operator-induced variability
Automated cascade impactors	Compendial	Avoids inter-operator variability when many APSD measurements are needed
AIM concept	Compendial/ Clinically realistic	Reduces complexity of cascade impactor method, more rapid and uses fewer resources (supports green chemistry)
"Alberta" idealized inlet	Clinically realistic	Affords the opportunity to make clinically appropriate measurements with realistic upper airway particle deposition, without the complexity of an anatomically correct inlet
Oropharyngeal Consortium adult models	Clinically realistic	Makes possible clinically appropriate measurements with small, medium and large anatomically correct upper airway geometries
Nephele mixing inlet	Clinically realistic	Enables an OIP to be tested with realistic breathing patterns while the cascade impactor is operated at the required constant flow rate for APSD determination
Laser diffraction (LD) simultaneous with cascade impactor measurement	Advanced	Links LD-measured particle size distributions to the aerodynamic diameter size scale; supports validation of LD as a more rapid technique than cascade impaction, especially for aqueous droplet aerosol characterization
Time-of-flight aerodynamic particle sizing combined with single particle mass spectrometry (SPAMS)	Advanced	A more rapid alternative than cascade impaction for APSD determinations, with potential for traceability to drug mass; still a research tool
Phase-Doppler anemometry (PDA)	Advanced	Affords rapid, high resolution droplet size analysis but without traceability to drug mass, but only technique giving particle velocity simultaneously; still a research tool
Raman chemical imaging (RCI) with microscopy-image analysis	Advanced	Permits chemical species identification associated with individual particles; especially useful for multi-component formulation development

The next significant development was the extension of inertial impactor theory to include the concept of effective nozzle diameter quantified for multi-nozzle stages by the process of mensuration using appropriate techniques.[10] This development was subsequently extended by formalizing the link between effective diameter and stage cut-point size.[11] Both advances have enabled the achievement of traceability in terms of the international length standard for APSD measurements made with the NGI and, by extension, to all multi-stage cascade impactors (CIs) where an archival calibration has been performed.[12]

In addition, the concepts of Abbreviated Impactor Measurement (AIM)[13] and Efficient Data Analysis (EDA)[14, 15] were developed and have reached a high degree of maturity. AIM simplifies the measurement of large and small particle mass fractions related to a chosen boundary size (often, but not necessarily chosen to be 5.0 µm aerodynamic diameter). EDA relates their sum, which corresponds to the impactor-sized mass and the ratio of large/small particle mass, to shifts in the underlying APSD. The AIM and EDA concepts are independent of each other and have shown promise in support of traditional, full resolution, cascade-impactor-based testing of all classes of OIP. However, their acceptance as part of dossiers accepted by the regulatory agencies is uncertain.

The evolution of improved semi-automated methods for both DCU and APSD determinations has also proceeded in parallel with these method-based developments. Indeed, the largely horizontal profile of the NGI was chosen to assist in the implementation of such methods.[7] Such ancillary equipment includes: the induction port and pre-separator rinsing apparatuses; the "gentle rocker" device for recovering particle deposits from the collection cups of the NGI; and equipment such as the NGI Assistant, which enables the process of sample recovery to take place on a larger scale; all are available from MSP Corporation (St. Paul, MN, US). The development of automated inhaler actuation stations by Novi Systems, Ltd., Gloucester, UK) and Proveris Scientific (Marlborough, MA, US) has improved measurement precision by eliminating variability associated with manual operation of OIPs. The apex of such developments has been fully automated solutions for both DCU and APSD by companies such as Astech Projects, Ltd. (Cheshire, UK).

More than one purpose for OIP testing: Clinically appropriate methods

The traditional paradigm of quality control testing based on simplified methods that assume ideal inhaler operation has gradually been found by stakeholders to be inadequate on its own as a descriptor of product function. The need for testing that simulates both realistic and sub-optimal conditions of use has therefore gained wider acceptance. Purewal, in 2002, was the one of the first to propose that laboratory testing of pressurized metered dose inhalers (pMDIs) and dry powder inhalers (DPIs) could be conducted under conditions of operation intended in the Instructions for Use and also by mimicking sub-optimal use (misuse).[16] The type of testing he described was focused largely on evaluating product robustness in normal use, such as priming the inhaler (for pMDIs), cleaning between uses and checking for inhaler exhaustion. However, a category of tests termed "Simulated Patient Use"[16] was also included, with the purpose of evaluating how an inhaler would perform throughout its design life when used as instructed. This category of testing included the incorporation of representative storage periods between dosing sessions. Despite this advance with respect to patient-inhaler interaction, the testing methods advocated for DCU and APSD were based on those in the pharmacopeial compendia at the time.

Since Purewal's work, there has been increasing awareness that OIPs should be evaluated in ways that more closely mimic actual patient use. It has been known for some time, since its creation in the mid-1990s, that the compendial inlet (USP/Ph.Eur. induction port) allows more of the coarse fraction of the dose emitted by OIPs to be assessed by cascade impaction for APSD.[17] The use of anatomically correct inlets had previously been accepted as providing a more realistic measure (than can be obtained with the compendial induction port) of the aerosol likely to penetrate the oropharynx to the airways of the lungs. However, casts prepared from cadavers did not always provide the correct dimensions due to *post mortem* tissue collapse. Two developments took place in parallel to remedy the situation. Firstly, the Oropharyngeal Consortium, a European-based group of academic and pharmaceutical industry researchers, funded the collection of a dataset of three-dimensional oropharyngeal (OP) images from healthy adults by magnetic resonance imaging (MRI).[18] They went on to create small, medium and large adult oropharyngeal models that are now commercially available from Emmace Consulting AB (Lund, Sweden). Secondly, the group at the Alberta Aerosol Research Laboratory developed the adult idealized inlet,[19] followed shortly afterwards by child[20] and infant[21] versions. The infant inlet is a nasal, rather than an oropharyngeal model, reflecting the fact that infants are generally obligate nose-breathers. These inlets are easier to manufacture than an anatomically correct upper airway, yet their aerosol transport properties mirror those of the corresponding anatomic upper airways. The Alberta Idealized Throats (AITs) are now commercially available from Copley Scientific, Limited. (Nottingham, UK).

Mimicking the moist surfaces of the mucosa with the use of surfactant agents as well as simulating

body temperature and relative humidity are important aspects affecting aerosol transport through either anatomically accurate or idealized inlets. These have been addressed in order to achieve closer realization to clinically relevant conditions.[22]

The next major step in achieving more clinically appropriate testing was the development of the Nephele aerosol mixing inlet by Miller in 2002,[23] as a means of merging the aerosol flow from an OIP-on-test with a supply of clean make-up air. This mixing inlet is commercially available from Copley Scientific, Limited. and RDD Online (Richmond, VA, US.) An OIP can be evaluated mimicking either a standardized or patient-derived breathing profile. At the same time, a cascade impactor positioned downstream of the mixing inlet can determine the APSD of the "inhaled" aerosol. This impactor can be operated at the required constant flow rate, in order to function in accordance with inertial impaction theory, by controlling a supply of clean make-up air fed to the mixing inlet.[24]

These enhancements to the existing compendial test methods for OIPs have been spurred on by regulatory guidance. The advice to industry introduced in 2009 by the European Medicines Agency (EMA) concerning second entry pMDIs is an important example of such developments.[25] This guidance suggests that such second entry products be evaluated with add-on devices (spacers/valved holding chambers (VHCs)) because these aids are widely prescribed to assist patients achieve optimum medication delivery.[25] In this guideline, the important step was taken to specify a more clinically appropriate approach to laboratory testing in the following clause: *"…that the* in vitro *testing should be carried out by preparing the spacer and setting up the apparatus in a clinically relevant manner which may influence the performance of the product, for example, inserting a time delay between actuation and inhalation to simulate tidal breathing."* This recognition of the importance of mimicking clinical use more closely when a spacer/VHC is present is in harmony with the guidance on Pharmaceutical Quality of Inhalation and Nasal Products, also published by the EMA in 2005,[26] and harmonized with a Health Canada guideline published the next year,[27] where the following statement is provided: *"The fine particle mass test used for routine testing of the product may be altered to mimic patient performance with the spacer or holding chamber (e.g., a 2 second delay, tidal breathing)."*

Evaluation of sub-optimal OIP use: A further refinement to clinically appropriate testing

Today, there is widespread recognition that clinically appropriate testing should be extended to investigate the robustness of an OIP used in sub-optimal ways. The driver for this development has been the extensive clinical evidence that OIPs are widely used incorrectly,[28-30] even after training by professional caregivers.[31] Sub-optimal use can include inhaler performance deterioration caused by either unintentional or intentional actions. One widely known example of unintentional sub-optimal use is the patient who delays inhaling for an indeterminate period following pMDI actuation, even when a spacer/VHC is used with the inhaler.[29] This scenario can be realized in the laboratory by the use of an apparatus that imposes a temporary shutter between the mouthpiece of the add-on device and the aerosol measurement equipment during the simulated delay interval.[22] Another example is a patient experiencing a severe asthma attack or exacerbation associated with chronic obstructive pulmonary disease, who can only attain sub-optimal flow during inhalation when using a dry powder inhaler.[32] Patient-generated breathing patterns played back through a simulator have proven effective in mimicking this situation.[33] A further example, but this time involving intentional misuse, would arise when a patient receiving therapy by nebulizer removes the interface (mouthpiece or facemask) for a time, perhaps to hold a conversation with a neighboring patient. This type of behavior has been evaluated in the laboratory by playing back a recorded, patient-generated, tidal-breathing pattern in which a significant pause exists between successive cycles.[22] Under such a circumstance, it is self-evident that little or no medication would be delivered. However, non-breath-actuated nebulizers would continue to generate droplets containing drug that would go to waste [22] if not captured and held in a reservoir, and if the pause is sufficiently short that losses due to gravitational sedimentation in the reservoir are minimal. The clinical and economic implications of such sub-optimal use remain to be evaluated.

More advanced developments in support of OIP testing

During the past ten years, methodology for determination of DCU has remained unchanged, with the exception of the move towards automation already mentioned. This is largely because the dose uniformity sampling apparatus (DUSA) is relatively simple and rapid to use in comparison with methods for measuring APSD. In contrast, there have been numerous attempts to replace the multi-stage cascade impactor with a more rapid technique, preferably affording improved size resolution. Such developments have focused principally on low-angle light scattering, also termed laser diffractometry (LD), where several thousand volume (mass)-weighted size distribution measurements can be made per second.[34] This attribute makes this technique potentially powerful for investigating transient behavior associated with the aerosol generation process. However, LD does not determine particle aerodynamic diameter directly. Fortunately, the divergence

between LD-measured size and the aerodynamic diameter scale vanish for homogeneous aqueous droplets, since they are spherical and of unit density (centimeter-gram-second units).[34] Nevertheless, this technique does not relate particle/droplet size directly to the mass of active pharmaceutical ingredient (API), because there is no chemical assay performed. For these reasons, LD has been traditionally used for the evaluation of nebulizing systems delivering aqueous solutions, rather than suspension-based formulations.[35-37] Despite this significant limitation, a number of studies have been undertaken with OIPs other than nebulizers over the past ten years,[38-40] possibly as a result of the commercialization of LD instruments with improved capabilities, in terms of sample presentation as well as extending the sizing capabilities well into the sub-micron range. For example, the recent capability of operating an LD-based system in parallel with a multi-stage CI when testing the same inhaler (Table 1), has an advantage in that the impactor-generated data then enable the LD-based measurements to be directly related to the aerodynamic diameter size scale.[41]

Phase Doppler anemometry (PDA) has, for some time, been used to characterize simultaneously API particle velocity and size distribution from OIPs.[42] However, as with LD, this light-based measurement method cannot capture API mass-specific, size-related information. Rather, the results provide a number-weighted distribution. The reported particle or droplet size reflects the magnitude of the phase shift between the two photodetectors. The shift is caused by individual scattering particles or droplets that cross the measurement volume, which is defined by two intersecting beams of monochromatic laser-generated light. Although still a research technique, the ability to determine velocity as well as size data is nevertheless useful as a tool to explore the aerosol development process, for instance, associated with the actuation of pMDIs.[43]

An alternative approach has been to replace the cascade impactor altogether with a technique that is capable of determining particle size directly, in terms of the aerodynamic diameter scale. Instruments based on particle time-of-flight (TOF) analysis in accelerating motion between two well-defined locations have been available since the 1980s.[44] However, like LD, these instruments do not determine API mass directly. This limitation has prompted one manufacturer (TSI, Inc., St. Paul, MN, US) to incorporate an abbreviated impactor as part of the TOF apparatus so that a measure, (typically fine particle mass defined at a well-defined aerodynamic diameter, such as 5 μm), can be related to the full TOF analysis-generated APSD. However, TOF analysis combined with single particle mass spectrometry (SPAMS) has recently offered to overcome lack of API identity in terms of APSD data, and has recently been applied to the assessment of DPI-generated aerosols.[45] The technique is expensive compared with cascade impaction and is still at the research stage, rather than in general use.

The advent of Raman chemical imaging (RCI) in combination with optical microscopy-automated image analysis has been a significant development during the past ten years. It is helping elucidate the underlying chemical structure of the aerosol that is likely to be inhaled from a given OIP.[46] Although largely a research tool that is applied to support classical APSD measurements by cascade impactor, RCI-microscopy enables the user to distinguish drug from excipient in particles containing a single API.[46] Moreover, this technique can separate each of the APIs in multi-component formulations that have been and are being developed. This facilitates inhaled therapy for multiple aspects of disease management by a single OIP, for instance, bronchodilation and treatment of underlying inflammation.[47]

Nanoparticle-formulated OIPs

During the ten-year period discusses in this article, nanoparticle-based formulations, including inorganic nanocrystals, dendrimers, nanotubes and liposomes, have become increasingly important as vehicles for the delivery of stable drug products. This is due to their increased surface area per unit mass, which therefore offers the potential for greater bioavailabilty.[48] Dynamic light scattering (photon correlation spectroscopy) is widely used in the rapid and non-invasive *in situ* assessment of such formulations.[49] In practice, these formulations are most often delivered as aqueous suspensions in micrometer-sized droplets by a suitable nebulizing system.[50] However, at least one group of researchers has considered the potential delivery of such particles to the lung airways via micrometer-sized soluble carrier particles, using a DPI-based device.[51] The choice of methods used to assess the inhaled aerosol particle size distribution of nanoparticle-formulated products for inhalation is driven by consideration of the delivery device and is therefore based on the techniques previously described in this article.

Conclusions

The ten-year lifetime of *Inhalation* has seen significant developments in the laboratory testing methodology for all types of OIPs. Existing compendial test methods for DCU and APSD are increasingly becoming augmented by automation, especially for actuation of the inhaler itself. This development has become supported by semi-automated apparatuses intended to simplify and reduce the intrinsic variability associated with equipment operation and the recovery of collected drug mass.

The multi-stage CI, particularly the NGI, remains the mainstay for the determination of APSD in the context of quality control as well as in product devel-

opment for all classes of OIP. Alternative particle sizing techniques affording greater resolution and measurement speed will most probably continue to remain research tools unless means can be found both to reduce their cost and relate their size-based measurements to the aerodynamic diameter size scale. Therefore, AIM and EDA are respectively likely to continue to attract interest as potential ways to simplify the APSD measurement process and assess the resulting data. More advanced techniques, particularly involving LD, have become routine in their use, especially for rapidly sizing aerosols from nebulizer-generated solutions with high size resolution. Moreover, where a requirement for aerodynamic diameter traceability needs to be demonstrated, this light-scattering technique can be used in conjunction with a cascade impactor as the reference technique. Such a capability is important, particularly with dry powder inhaler-generated aerosols, where particle density and shape are not precisely known.

Development of more clinically appropriate methodologies to evaluate OIP performance was evident more than ten years ago. It has blossomed to become recognized by regulatory agencies as a key aspect of the performance data package associated with product registration. Cascade impactors are increasingly being used simultaneously with breath simulation at the inhaler patient interface (mouthpiece or facemask). A variety of approaches have been tried to ensure that the CI is operated at the required constant flow rate, but the Nephele mixing inlet has been the most successful to date. In the future, more emphasis is likely to be placed on the acquisition of patient-generated rather than standardized breathing patterns, especially in the context of passive DPI evaluations, where the inhalation flow rate/time profile is critical for effective device function. It is likely that this trend will continue in response to the evidence from a multitude of clinical studies that show patients commonly do not use their inhaler(s) in accordance with the Instructions for Use, and that the robustness of an inhaler can be evaluated in the laboratory simulating many of these sub-optimal uses.

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