Improving Inhaled Product Testing

Methods for Obtaining Better In vitro–In vivo Relationships

Mark Copley

Even in an industry in which all product development is complicated by the intricacies of human biology, orally inhaled products (OIP) stand out as singularly demanding. Clinical response is a particularly complex issue, but other areas are challenging too. Both patient technique and individual capability can have a major impact on the effectiveness of drug delivery and development is complicated by the interplay between device and formulation. The author discusses testing equipment and information-collection techniques used to improve aerodynamic particle-size distribution measurement and the relationship between in vitro test data and in vivo behaviour.

The technique of cascade impaction is used to measure the aerodynamic particle size distribution (APSD) of all orally inhaled products (OIPs). The resulting data are broadly indicative of likely deposition behaviour in the respiratory tract and support development of a target drug-delivery efficiency. Cascade impaction is widely acknowledged as being unable to completely replicate the complex aerodynamics and deposition behaviours taking place in the throat and lungs. The lungs operate under high humidity conditions and within them volumetric flow rate decelerates with each bifurcation, establishing complex velocity profiles across the lung structure. The resulting mechanisms of particle deposition, which include sedimentation and diffusion, as well as impaction, are difficult to comprehensively simulate. Improving the relationship between in vitro test data and in vivo behaviour, however, is becoming increasingly important for a number of reasons, including the successful implementation of quality by design (QbD), the need to reduce the costs of OIP development and the desire to achieve in vitro bioequivalence for generic products.

Steps to modify cascade impaction to secure better in vitro–in vivo relationships (IVIVRs) range from the use of new testing equipment, such as the Alberta Idealised Throat (AIT) and state-of-the-art breathing simulators, to the adoption of more efficient information-gathering techniques, including Abbreviated Impactor Measurement (AIM).

Multistage cascade impaction

Multistage impactors consist of a series of stages each made up of a nozzle plate, with a specific nozzle arrangement and a collection surface. Sample-laden air is drawn into the impactor, at a constant volumetric flow rate, and passes sequentially through the stages. Because nozzle size and total nozzle area decrease with stage number, the particles are progressively accelerated. At each stage, particles with sufficient inertia break free of the prevailing air flow and impact on the collection surface, thus producing a series of mass fractions that can be analysed to determine how the active is distributed with respect to size.

**Figure 1** shows a typical pharmacopeial cascade impactor setup for dry-powder inhaler (DPI) testing that includes a number of essential ancillaries. The device is interfaced to the cascade impactor using an appropriate mouthpiece adapter and an induction port. Flow rate through the cascade

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Submitted: 28 June 2012. Accepted: 12 Oct 2012.

**Citation:** When referring to this article, please cite it as M. Copley, “Improving Inhaled Product Testing: Methods for Obtaining Better In vitro–In vivo Relationships,” *Pharmaceutical Technology, 37* (2) (2013).
Inhaled Drug Development

Assessing established practice

Assessing the established setup against the information requirements for knowledge-led product development highlights some of its limitations. The standard United States Pharmacopeia (USP)/European Pharmacopoeia (Ph.Eur.) induction port described in USP General Chapter <601> "Aerosols, Nasal Sprays, Metered-Dose Inhalers, and Dry Powder Inhalers" and Ph. Eur. Section 2.9.18 “Preparations for Inhalation”, for example, is well-suited to precision manufacture and delivers reliable, consistent performance, but it is now widely recognised as having a tendency to significantly underpredict the amount of emitted dose captured by the upper respiratory tract for some products, relative to clinical data (1). Thus, irrespective of deposition behaviour within the lung, the USP induction port tends to overestimate the extent of whole-lung deposition.

Of equal importance is the fact that this setup requires the application of a constant flow rate through both the device and the cascade impactor, whereas in clinical use, inhaled products are subject to a range of user breathing profiles. The constant flow rate required by cascade impactors results in a square wave form, rather than the infinitely variable, broadly bell-shaped patterns of real patients. Furthermore, impactors ideally require multiple volume changes to guarantee reliable and complete sizing of the aerosol. This requirement invariably results in a test volume that is greater than the inhalation volume of at least part of the intended patient population.

Finally, there is the overarching concern of productivity. Cascade impaction has long been recognised as a time-consuming, manually intensive task, which becomes even more limiting as demands for more valuable and discriminating data grow. Alleviating the burden of analysis is, therefore, important for continued advancement, especially in R&D environments in which budgets are being increasingly cut.

**Better representation of throat deposition**

The goal of more closely simulating deposition in the mouth and throat focuses attention on alternatives to the standard USP/Ph.Eur. induction port. One such is a human throat cast (2–5). These alternatives offer the advantage of accurately reflecting the physiology of a throat. Experimental work, however, has shown significant differences in deposition behaviour between different throat casts (1). Although these differences are to be expected given the variability in human anatomy, they complicate results interpretation within the context of standardisation and routine analysis. Furthermore, reproducible, precision manufacturing of such casts is complex, which makes them difficult to mensurate and qualify. Because casts are also not easy to handle or to interface with the impactor, they are less than ideal from a practical standpoint.

These limitations have stimulated interest in developing solutions that fall between a throat cast and the standard induction port (6). These solutions include the Alberta Idealised Throat (AIT). Developed by researchers at the Aerosol Research Laboratory of Alberta (University of Alberta, Canada), the AIT has a standardised and highly reproducible but human-like internal geometry that lends itself to precision manufacture. Its performance is independent of flow rate and, with both pressurised metered-dose inhalers (pMDI) and DPs, it has been shown to collect more of the emitted dose than the USP induction port (1, 7, 8). Indeed, the ability of the AIT to more closely replicate in vivo deposition behaviour in the throat compared with the USP induction port has been directly confirmed in a number of studies, some of which include marketed OIPs (9–11). AIT geometries are also available in child and infant forms, which widens their appeal for in vitro testing (12).

**Experimental study: Assessing AIT performance**

The APSD of a pMDI (active ingredient salbutamol) was measured in a collaborative project with Melbourn Scientific (UK) using an impactor (Next Generation Impactor, Copley

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**Figure 1: A typical cascade impactor test setup for dry-powder inhaler (DPI) testing.**

![Diagram of cascade impactor test setup](image.png)

Note: This diagram is a general guide to the test set-up for DPs. Please refer to the individual test monographs (e.g., United States Pharmacopeia and European Pharmacopoeia) for detailed instructions. Not drawn to scale.
Scientific) with both the USP induction port and an AIT (7). Testing was carried out at a flow rate of 30 L/min, according to pharmacopeial guidance for MDIs, with six replicate measurements conducted in each case. All collection stages of the NGI and the AIT were coated with silicone oil applied in n-hexane solution (1% w/v), which is a standard practice that reduces particle bounce (i.e., the re-entrainment of particles impacting at high velocities). The USP induction port was left uncoated as is done for routine testing.

Table I shows averaged collection data for each component of the test equipment including a combined mass recorded for the throat and inhaler mouthpiece. Values for fine-particle dose (FPD), fine-particle fraction (FPF), geometric standard-deviation (GSD) and mass median aerodynamic diameter (MMAD) were determined from these data. Calculations were based on total emitted mass/actuation and the assumption of a 5-micron upper limit for FPD and FPF.

Deposition data for the throat/mouthpiece shows that the AIT captures more of the dose, thus reducing the mass of drug entering the cascade impactor. This result is attributed to differences in the geometry of the two interfaces, rather than any coating effect (7).

Figure 2 shows averaged cumulative APSDs based on the amount of material exiting the mouthpiece/throat. Here, use of the AIT shifts the APSD to finer sizes across the entire size range, thus suggesting that not all particle sizes are equally retained relative to the standard induction port. In a parallel experiment with a DPI, the same effect was observed (7). These are early results and further research is required. The results, however, underline the fact that better representation of mouth/throat deposition may influence assessments of regional deposition in the lung, which is a function of particle size, as well as estimates of whole lung deposition. From an in vitro perspective, use of the AIT ensures that only the portion of the aerosol that would actually deposit in the lung is sized by the cascade impactor, therefore providing more relevant data.

### Table I: Summary collection data for a pressurised metered-dose inhaler (pMDI) tested using a United States Pharmacopeia (USP) induction port and Alberta Idealised Throat (SD is standard deviation, T/MP is throat/mouthpiece, μg a/ac is μg active/actuation, MOC is micro orifice collector, FPD is fine particle dose, FPF is fine particle fraction, GSD is geometric standard deviation, MMAD is mass median aerodynamic diameter).

<table>
<thead>
<tr>
<th>Stage</th>
<th>USP Induction Port</th>
<th></th>
<th>Alberta Idealised Throat</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>T/MP (μg a/ac)</td>
<td>54.2</td>
<td>1.6</td>
<td>66.8</td>
<td>5.1</td>
</tr>
<tr>
<td>1 (μg a/ac)</td>
<td>2.9</td>
<td>0.6</td>
<td>1.1</td>
<td>0.2</td>
</tr>
<tr>
<td>2 (μg a/ac)</td>
<td>1.8</td>
<td>0.1</td>
<td>0.7</td>
<td>0.1</td>
</tr>
<tr>
<td>3 (μg a/ac)</td>
<td>4</td>
<td>0.3</td>
<td>2.2</td>
<td>0.6</td>
</tr>
<tr>
<td>4 (μg a/ac)</td>
<td>16</td>
<td>1.9</td>
<td>12.7</td>
<td>2</td>
</tr>
<tr>
<td>5 (μg a/ac)</td>
<td>14.2</td>
<td>1.5</td>
<td>15.5</td>
<td>0.9</td>
</tr>
<tr>
<td>6 (μg a/ac)</td>
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<td>0.6</td>
<td>4.3</td>
<td>0.5</td>
</tr>
<tr>
<td>7 (μg a/ac)</td>
<td>0.7</td>
<td>0.2</td>
<td>0.7</td>
<td>0.1</td>
</tr>
<tr>
<td>MOC (μg a/ac)</td>
<td>0.5</td>
<td>0.1</td>
<td>0.5</td>
<td>0.1</td>
</tr>
<tr>
<td>TOTAL</td>
<td>98</td>
<td>3.6</td>
<td>104.4</td>
<td>8.4</td>
</tr>
<tr>
<td>FPD (μg a/ac)</td>
<td>37.3</td>
<td>2.6</td>
<td>34.8</td>
<td>3.3</td>
</tr>
<tr>
<td>FPF (%)</td>
<td>38</td>
<td>1.4</td>
<td>33.4</td>
<td>1.3</td>
</tr>
<tr>
<td>GSD</td>
<td>1.9</td>
<td>0.2</td>
<td>1.6</td>
<td>0</td>
</tr>
<tr>
<td>MMAD (μm)</td>
<td>2.5</td>
<td>0.1</td>
<td>2.2</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Figure 2: For this pressurised metered-dose inhaler, use of the Alberta Idealised Throat shifts the measured, cumulative, aerodynamic particle size distribution to finer size distributions relative to the standard United States Pharmacopeia (USP) induction port.
Applying representative breathing profiles

Because multistage-cascade impactors require a constant air-flow rate for successful operation, the issue of applying breathing profiles that better represent real life demands a two-stage solution: decoupling the flow rate and volume through the device from the flow rate and volume through the impactor and identifying the most suitable profile for testing.

The mixing inlet is an established option for decoupling flow rates. Designed to fit between the inlet of a range of cascade impactors and a USP induction port or AIT, it allows the cascade impactor to be operated under steady-state conditions, as a constant flow-rate aerosol sampler (in the way that it was designed), while at the same time permitting application of a reduced or variable flow rate through the inhaler. A gentle mixing action ensures effective, turbulence-free mixing of the make-up (i.e., sheath) and sample-laden air streams prior to introduction into the impactor, resulting in minimal internal losses (4, 13).

Assessment of the most appropriate breathing profile for testing is an ongoing and more complex question. For inhalers with an active delivery mechanism, such as pMDIs and some DPIs, there is experimental evidence to suggest relatively low sensitivity to test flow rate (13, 14). Nebuliser testing, on the other hand, in which dose aerolisation relies on patient tidal breathing, has already undergone significant revision with the release of two, new, harmonised monographs: Ph.Eur. 2.9.44 and USP 1601 (15, 16). These came into force in January 2012 and August 2011, respectively, and include four different breathing profiles for assessing dose delivery for different patient groups: adult, child, infant and neonate.

For the majority of DPIs, the motive force driving the device is supplied solely by the patient, and so they are widely referred to as passive DPIs. The breathing profile selected, therefore, impacts the dose dispersion process as well as inhalation of the resulting particles. Here, the application of more representative breathing profiles is generally acknowledged as being important, but there is considerable debate about what is actually required.

Focusing on DPIs

Pharmacopeial monographs for testing DPIs specify the application of a flow rate that results in a pressure drop of 4 kPa across the device, which is considered broadly representative of an adult patient’s inhalation strength. DPIs vary considerably in terms of flow resistance so, in practice, test flow rates span a broad range of values, from around 30–40 L/min for a high resistance device up to the imposed limit of 100 L/min for those with much lower resistance. The defined flow rate is applied for the time taken to draw 4 L of air through the device if following pharmacopeial specifications or 2 L if following FDA guidance, in the form of a square wave profile (see Figure 3).

While this procedure is logical as part of a standardised, routine, quality-control test, some evidence has been presented to suggest that using a 4-kPa pressure drop gives a flow rate that is unrealistically high for certain patient groups with impaired lung function. Likewise, the total inspired volume has also been questioned. On this basis, suggestions have been made that lower flow-rate testing should be carried out, possibly with smaller total volumes, in the development of products for paediatric use, for example, or for the treatment of chronic obstructive pulmonary disease (COPD) or asthma (17).

On the other hand, as DPI technology spreads into the delivery of systemic therapies, contrary arguments have also been proposed (18). Research suggests that, in a group of adult users with no lung impairment, an 8-kPa pressure drop could be achieved, which suggests that test flow-rates should be much higher if the same inhalation manoeuvre is applied during use. This latter point is crucial. If, for systemic delivery, patients are instructed to inhale in a different way to optimise the deposition profile of the active, then testing needs to reflect this. Equally important, beyond these considerations of test flow rate, there is a broader issue of the shape of the applied breathing profile during testing.
During patient use, air is drawn through the DPI to fluidise and aerosolise the dose, thus forming a cloud of particles, of which a large proportion is fine enough to be drawn into the lung. The mechanisms involved in this aerosolisation process are complex, but it can be postulated that the acceleration of air through the powder plug results in the application of shear forces, thus giving rise to dispersion and device emptying. If the device does not empty, or the force applied is insufficient to disperse the dose to a respirable size (typically taken to be less than 5 microns), then drug delivery efficiency is impaired (18).

This proposed mechanism has led to the suggestion that it is not only the flow rate applied during testing that is important but also the rate at which that flow rate ramps up from zero. In conventional, pharmacopeial testing, the air is effectively “on” or “off”, with near instantaneous acceleration. In contrast, data for healthy adults indicates that it may take up to 0.5 seconds to achieve peak inspiratory flow, thus giving an appreciably slower acceleration rate (18).

These debates have resulted in the application of electronic or artificial lungs and the introduction of new breathing simulators that enable detailed exploration of the impact of breathing profile on device performance during routine testing (2, 19). Capable of applying profiles typical of the intended patient populations and ramping up air flows in a variable but closely defined way, these systems allow researchers to determine how critical quality attributes may be affected by patient-to-patient variability in this area. This research is an ongoing but critical area in the development of DPI technology (see Figure 4).

Improving productivity using AIM

In recent years, the search for improved productivity in inhaler product testing has fuelled interest in AIM (20). A key focus is QC testing, but the approach also holds promise for R&D. AIM involves separation of a sample into far fewer fractions than a multistage cascade impactor, thereby delivering some important benefits:

- Faster analytical throughput
- Less complicated analysis, which results in reduced likelihood of error
- Ease of automation.

In R&D, the proposal is that requirements for better productivity may be met by applying the human respiratory tract (HRT) version of the AIM concept, which focuses attention on those parts of the APSD that are crucial for the assessment of drug-delivery performance (see Figure 5). Published studies highlight industrial interest in this approach (21–23). The potential that AIM offers for reducing human error and enhancing data integrity may also support the development of better IVIVRs that are based on more secure results.

AIM-HRT consists of two impaction stages, a filter and a spacer to modify aerodynamic performance. It separates the dose into a coarse particle mass (>5 microns), a fine particle mass (<5 microns) and an extra-fine particle mass (<1 micron). These fractions would typically be associated with mouth/throat deposition, lung deposition and, potentially, loss via exhalation, respectively. The technology, however, enables the cut-off diameter of the stages to be varied, as necessary, to produce results that more closely correlate with measured in vivo data, if available. Some studies indicate that this can be achieved by using a cut-off diameter closer to 3 microns rather than 5 microns (24). In the future, AIM may, therefore, enable the acceleration of design of experiments and screening trials although there is considerable debate about its uptake.

Looking ahead

The conventional test cascade-impactor setup used to measure the APSD of all OIPs was originally developed with QC testing in mind. It fulfills this function well because it allows a highly discriminating and reproducible measure of product quality. The conventional setup has drawbacks, however, for researchers trying to access a more detailed understanding of inhalation technology and obtain better IVIVRs that will further development. One concern is better representation of deposition behaviour in the mouth and throat and another is the application of more representative breathing profiles during testing, in which they may have an impact on dose emission and the resulting aerosol APSD generated. The issue of analytical productivity remains pressing in an environment of ever deeper cost-cutting.

Instrument suppliers are responding positively to these requirements by introducing new products such
as the mixing inlet, sophisticated breathing simulators, new interfaces such as the AIM and AIM equipment that streamlines testing. These new products potentially allow for significant advances in the area of inhaled-product testing within the R&D environment. In the future, such innovations offer the opportunity to shape in vitro testing into a far more effective tool for the rapid advancement of inhaler technology for the widest possible range of drug therapies.

References

Article reprinted from February 2013 issue of Pharmaceutical Technology EUERPEADVANSTAR

PTH021398