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Performance Testing for Dry Powder Inhaler Products: Towards Clinical Relevance †

Sara E. Maloney, Jeffrey B. Mecham and Anthony J. Hickey *

RTI International, Research Triangle Park, USA

Abstract

It is well established that the critical performance metrics for aerosol products are aerodynamic particle size distribution (APSD) and delivered dose uniformity (DDU). In broad terms, these performance characteristics dictate the efficiency and reproducibility with which an aerosol is administered clinically. However, these properties alone do not support in-vitro, in-vivo correlations. There have been numerous publications attempting to more directly link product performance testing to physiological relevance or further to draw direct correlations of relevance to bioequivalence testing for the development of generic products. While these novel methods have been employed in product development activity, their suitability for compendial testing has yet to be established. This paper explores the potential to establish biologically relevant compendial standards for dry powder inhaler products while maintaining accuracy and reproducibility of data collected to support the quality and performance of the product.

Keywords: dry powder inhaler, therapeutic aerosol, aerodynamic particle size distribution, cascade impaction, in vitro/in vivo correlation, bioequivalence

1. Introduction

Modern pharmaceutical aerosol products have been available for the treatment of pulmonary diseases, such as asthma, chronic obstructive pulmonary disease (COPD), and cystic fibrosis (CF), since the middle of the last century (Cheng Y.S., 2014). Attention was given to the control of the quality of these products by the manufacturing industry and government regulators. The importance of accurately and reproducibly controlling the dose of the active pharmaceutical ingredient (API) and key properties that influence therapeutic effect, notably aerodynamic particle size distribution (APSD), was seen as the key to assuring the desired therapeutic outcomes while minimizing any adverse effects. To demonstrate the quality of the product, which underpins its safety and efficacy, various tools were required for sampling. These tools, when combined with instruments in analytical chemistry, could support the specifications to which process controls could be tuned to assure the quality of these products.

There are several major categories of pharmaceutical aerosol products, namely pressurized metered dose inhalers (MDIs), dry powder inhalers (DPIs), nebulizers, and soft mist inhalers (Cheng Y.S., 2014), which consist of an aerosol formulation and device. Each product presents unique requirements for establishing quality and performance specifications (Uddin S. et al., 2016). The development and use of DPIs over the last three decades was largely driven by the global phase-out of chlorofluorocarbon propellants in medical products due to their deleterious effects on the ozone layer (Wu X. et al., 2010) and by the need for an alternative dosage form and route of administration for the products of biotechnology. Dry powder inhalers offer many advantages, such as their propellant-free dispersal mechanisms, product stability, portability, and ease of use. The characterization of quality and performance of DPIs will therefore be the focus of this review.

1.1 Development tools

Development tools for solid state products include those that measure the physicochemical properties of the materials incorporated in the formulation, establishing the quality foundation. For DPI formulations, analysis of these physicochemical properties via analytical methods is imperative, as the dispersion of powders, and therefore efficiency of the DPI product, is highly influenced by such properties. These properties, such as particle size, crystallinity, surface rugosity, shape factor, moisture content, and chemical composition, all contribute to interparticulate forces (e.g., electrostatic, capillary, and van der Waals forces), which correlate to particle flow and dispersion behavior (Hickey A.J., 2018a). Beyond physicochemical properties, the

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* Corresponding author: Anthony J. Hickey;
Add: 3040 Cornwallis Road, Research Triangle Park, NC 27709, USA
E-mail: ahickey@rti.org
TEL: +1 (919) 541-6771

performance of the product must be established in the final form, which includes formulation, metering system, and device (Uddin S. et al., 2016).

1.2 Regulatory considerations

To establish the quality and performance of inhaled drug products, a variety of considerations are required. The drug formulation must be controlled according to ICH Q1 and Q2 which govern the components (Q1) and their quantitative proportion (Q2) in the preparation. The device is accompanied by a drug master file establishing specifications and controls on its manufacture. Critical quality attributes of DPI formulations as specified by the United States Food and Drug Administration (US FDA) include assay, impurities and degradants, leachables, foreign particulate matter, moisture content, net content, microbial load, and device characteristics (US-FDA, 2018). The drug product (formulation and device) is also subject to performance testing to evaluate the APSD and delivered dose uniformity (DDU) as described in compendia (United States Pharmacopeia [USP], European Pharmacopeia [EP], and Japanese Pharmacopoeia [JP]) and regulatory guidance documents, such as those promulgated by the United States FDA (Kuribayashi R. et al., 2017; 2019).

1.3 Dry powder inhaler products

It is generally accepted that particles with an aerodynamic size of 1–5 μm can enter the lungs (Adams W.P. et al., 2007; Alagusundaram M. et al., 2010; Hickey A.J., 2018a). However, the way that the particles are prepared affect their performance characteristics (Hickey A.J., 2018a). High energy processes used to prepare micron-sized particles, such as jet milling, lead to the formation of particles with high specific surface areas (i.e., surface area with respect to mass) and surface energies. These attributes generally result in poor flow, fluidization, and deaggregation (Alagusundaram M. et al., 2010; Chaurasiya B. and Zhao Y.-Y., 2021; Hickey A.J., 2018a). Formulation strategies, such as those described below, aim to mitigate these issues.

1.3.1 Lactose blends

Drug products intended for the treatment of asthma and COPD are primarily lactose blends. These products include α-lactose monohydrate, a monoclinic crystalline substance, as a large carrier particle onto which respirable drug particles are attached at very low concentrations. The drug is prepared in respirable sizes, often by jet milling, and is predominantly crystalline. The drug is then uniformly distributed in the lactose powder using a tertiary blending process (Hickey A.J., 2018a). The large carrier particles occupy the high energy sites of the drug, reducing drug cohesiveness and improving disaggregation and dispersal (Hickey A.J., 2018b; Wu X. et al., 2010). When the formulation is released from the device by patient inhalation, the drug detaches from the lactose and enters the lungs. The lactose (typically 50–150 μm) is too large to be inhaled (Hickey A.J., 2016). This process of detachment occurs through shear, turbulence, and impacts with the walls of the mouthpiece of the device (Hickey A.J., 2018a; b). Of note, while lactose is most common, alternative carrier molecules, such as mannitol, glucose, trehalose, erythritol, and sorbitol have all been proposed as large carrier particles to serve a similar purpose (Rahimpour Y. et al., 2014; Wu X. et al., 2010).

1.3.2 Spray dried formulations

Spray dried formulations of drug, accomplished by atomizing a liquid drug-containing solution or suspension into a hot drying medium, have been used for high dose dry powder formulations (Hickey A.J., 2018a). Notably, tobramycin, a drug used to treat *Pseudomonas aeruginosa* infection, is delivered at a dose of 112 mg (VanDevanter D.R. and Geller D.E., 2011), a dose not easily achievable through lactose blending. The spray drying process allows for manipulation of the drug into particles of pure or near pure drug content that are amorphous in nature with lower densities than a crystalline solid particle (Hickey A.J., 2018b; Wu X. et al., 2010). It is also possible to prepare hollow particles or particles with very low density and high roughness (Gradon L. and Sosnowski T.R., 2014). These attributes confer lower interparticulate forces than those observed with milled particles, and the powder requires much less energy input from the patient for high efficiency dispersion.

1.3.3 Other formulations

Jet milling/lactose blending and spray drying represent the most common techniques for developing DPI formulations. However, a variety of alternative methods have been considered for the preparation of dry powder formulations. Particles prepared through thin film formation occur through dropwise deposition of the drug solution or suspension onto a frozen surface to form a thin film, which is then subject to lyophilization for solvent removal. The result is a porous, brittle, interconnected matrix that can be dispersed to small particles upon aerosolization (Hufnagel S. et al., 2022). This process has been used to prepare various aerosol particles containing both small and large molecules, including tacrolimus, lactate dehydrogenase, and lysozyme (Engstrom J.D. et al., 2008; Sahakijpijarn S. et al., 2020). Controlled aggregation of carrier-free microparticles following jet milling, such that dispersion is efficient and reproducible, has been utilized to prepare clofazimine particles (Brunaugh A.D. et al., 2017) and ibuprofen particles (Yazdi A.K. and Smyth H.D.C., 2016) and has also been employed in the Mometasone Furoate Twisthaler product (Yang T.T. et al., 2001). Heat-sensitive biological formulations can be prepared using either spray

freeze drying or supercritical fluid drying in methods that avoid high critical temperatures. Both methods are more complicated and expensive and are typically only used when necessary for bioactive molecules (Chaurasiya B. and Zhao Y.-Y., 2021; Gradon L. and Sosnowski T.R., 2014; Wu X. et al., 2010).

2. Data generation

Characterization of dry powder aerosol formations includes aspects of both quality and performance. Quality evaluation, including composition and physicochemical characteristics, is performed on the dry powder formulation to monitor properties that are known to contribute to aerosol dispersion efficiency, stability, and potency. In combination with the device and metering system, performance characterizations are then investigated to monitor the aerodynamic properties of the combined product.

2.1 Qualitative and quantitative composition

To establish quality measures of the drug formulation, it is important to note the nature of the drug and any additives that are employed. In the first instance, this relates to the form in which each of the components is supplied with measures of purity (Hickey A.J., 2018b). Once the nature of the components has been established, the amounts employed must be defined and specifications set to assure accuracy and reproducibility of the drug dose. The quantity of each drug should be reported both in terms of concentration (amount of ingredient per unit formulation) and net content in each blister or capsule (US-FDA, 2018). Analytical instrumentation and techniques, such as X-ray microanalysis, X-ray photoelectron spectroscopy (XPS), and inverse gas chromatography (IGC) can be utilized to determine composition and evaluate homogeneity of the composition throughout the formulation (Hickey A.J., 2018b; Hickey A.J. et al., 2007; Wu X. et al., 2010). The US FDA defines composition metrics as Q1, or qualitative composition, and Q2, or quantitative composition. These metrics are used when defining bioequivalence for the contents of a drug formulation, where Q1 would inform whether two formulations contained the same active and inactive ingredients, and Q2 would inform whether those ingredients were in the same concentration for each formulation (Hickey A.J., 2018b).

2.2 Physicochemical characteristics

Since the drug and any additives for a DPI formulation will be in the solid state, the structure and morphology must be defined to allow for replication of the formulation by adopting adequate controls. The typical properties that must be measured include morphology and particle size, crystallinity, polymorphism, and moisture content (Dunbar C.A. et al., 1998). Dispersion of powders is highly influenced by the size distribution of the particles as well as their shape, surface rugosity, hardness, and porosity due to the role of these properties on interparticulate interactions, as noted above (Hickey A.J., 2018a). Particle morphology and geometric size is often visualized using scanning electron microscopy (SEM), where size is manually measured from images and gives rise to a size distribution in terms of number of particles. In contrast, laser diffraction is a population-based method that is typically representative of the true geometric particle size of regularly shaped particles, and the size distribution can be reported as a function of particle volume (Hickey A.J., 2018b). Surface geometries can be visualized with both SEM and atomic force microscopy (AFM), where AFM can also provide information on specific particle interactions between drug and carrier particles or between two drug particles (Hickey A.J. et al., 2007; Wu X. et al., 2010). Surface roughness, such as that associated with corrugated particles, is often reported to increase surface dispersibility due to decreased van der Waals forces (Chaurasiya B. and Zhao Y.-Y., 2021; Wu X. et al., 2010). However, there is also a drawback to surface irregularities, as they can promote interlocking between particles (Wu X. et al., 2010). The effect of these properties must be evaluated to determine an optimal surface roughness and shape.

Bulk crystallinity properties are often evaluated using X-ray powder diffraction analysis (XRPD). The resulting diffractogram shows a series of peaks, corresponding to crystalline structural features; the absence of such features indicates that the material is amorphous (Hickey A.J., 2018b). Crystalline solids exhibit long-range molecular order, whereas amorphous solids show no long-range molecular order. Polymorphism refers to the ability of a crystalline material to exhibit more than one crystal system. To quantify the extent to which polymorphism is present, thermal analysis using differential scanning calorimetry (DSC) can be employed, as polymorphs exhibit different melting points (Hickey A.J., 2018b). Due to their lack of order, amorphous solids often exhibit greater solubility, molecular mobility, and bioavailability, as well as faster degradation kinetics (Wu X. et al., 2010). As a consequence, amorphous materials readily take up small amounts of water vapor. This moisture can induce solid-state phase transitions, chemical degradation, and physical instability due to capillary forces and can alter the bulk density of the particles, surface charge, and aerodynamic properties (Chaurasiya B. and Zhao Y.-Y., 2021; Hickey A.J., 2016; Wu X. et al., 2010). As a result, it is necessary to monitor moisture content using either Karl Fischer titrimetric analysis or thermogravimetric analysis (TGA) (Hickey A.J., 2018b). Further, the FDA recommends evaluating the effect of storage on moisture content, including storage at 25 °C/60 % relative humidity (RH) and 30 °C/65 % RH (Lyapustina S., 2018; US-FDA, 2018). Lack of change in moisture content is considered a measure of stability.

2.3 Performance

Once the foundational quality of the formulation has been established, the performance of the drug should be assured. However, the product must be assembled (formulation and device) and testing performed to establish performance uniformity, as it cannot be assumed. Current compendial standards include evaluating performance via monitoring the aerodynamic particle size distribution of the resulting aerosol and delivered dose uniformity. However, additional methodologies have been proposed to increase physiological relevance and thus clinical translation.

2.3.1 Aerodynamic particle size distribution

The aerodynamic particle size distribution (APSD) is the property that defines the performance of inhaled products. As lung deposition is a function of the APSD, it is clear that the proportion of the distribution in the desired size range dictates the dose delivered, and thereby, safe and efficacious treatment (Hickey A.J., 2018b). Generally, it is proposed that large particles (>5 μm) are deposited via inertial impaction in the oropharynx and large airways. Smaller particles (2–5 μm) are likely deposited in the bronchioles by gravitational sedimentation, and the smallest particles (< 2 μm) are deposited by diffusion in the terminal bronchioles and alveolar region (Chaurasiya B. and Zhao Y.-Y., 2021; Hickey A.J., 2016; Lee S.L. et al., 2009). By determining the APSD, particle deposition in the lungs can be speculated. However, it is important to note that this is not a direct indicator of lung deposition, as the methodologies employed to determine APSD utilize a uniform flow rate rather than varying time-flow profiles such as those exhibited in vivo (Mitchell J. et al., 2007; Mitchell J.P. and Roberts D.L., 2013).

The APSD is determined by sampling the aerosol generated by the product using a calibrated cascade impactor. Particles released from an inhaler device are subjected to changes in flow direction under laminar conditions, where the inertia of small particles causes them to stay in the flow stream upon directional change and particles of a greater size impact on the surface. Several stages are sequentially arranged in a cascade impactor, with each stage collecting particles of a progressively smaller size (Mitchell J. et al., 2007). The US FDA accepted methods for APSD via cascade impaction include the 8-stage Anderson cascade impactor operated at 28.3 L/min, the 5-stage Marple-Miller impactor operated at 60 L/min, and the 7-stage next generation impactor (NGI) with pre-separator in place operated at 60 or 100 L/min (Frohlich E., 2019; US-FDA, 2018). The cutoff diameters for each stage of the three listed methods are presented in **Table 1**. The length of collection should be adjusted based on the flow rate to allow for the collection of 4 L of air (US-FDA, 2018). Of note, the flow utilized for analysis via NGI affects the stage diameter cutoffs, and this must be considered (Weers J. and Clark A., 2017). Once the aerosol has been sampled, data can be depicted as mass collected on each stage according to the cutoff diameter for that stage from calibration. Importantly, the APSD can be presented as a mass distribution, where mass relates directly to the dose of the drug and is the most relevant metric, rather than a number distribution (from SEM) or volume distribution (from laser diffraction) (Hickey A.J., 2018b).

Table 1 Stage cutoff diameters for common cascade impactor types. [a]

Stage	Stage Cutoff Diameter (D_{50}) (μm)		
	Anderson Cascade Impactor [b]	Marple-Miller Impactor [c]	Next Generation Impactor [c]
Stage 0	—	—	—
Stage 1	9.0	—	—
Stage 2	5.8	10.0	8.06
Stage 3	4.7	5.0	4.46
Stage 4	3.3	2.5	2.82
Stage 5	2.1	1.25	1.66
Stage 6	1.1	—	0.94
Stage 7	0.7	—	0.55
Filter	0.4	0.625	0.34

[a] Stage cutoff diameters are reported according to USP General Chapter <601>. [b] Cutoff diameters are valid at a flow rate of 28.3 L/min. [c] Cutoff diameters are valid at a flow rate of 60 L/min.

2.3.2 Delivered dose uniformity

A separate technique is utilized to monitor delivered dose uniformity (DDU) to minimize analytical errors resulting from dividing the dose into recovered fractions and summing measurements, as would be done with cascade impaction. Contrarily, DDU is established by sampling aerosol from the drug product into a Teflon tube with a filter under vacuum (Hickey A.J., 2018b). The delivered dose, or emitted dose, refers to the proportion of the nominal dose that leaves the mouthpiece of the inhaler (Hickey A.J., 2018b). This sampling technique will allow the amount delivered to be measured, as well as the amount remaining in the device or metering system (e.g., capsule, blister). Importantly, as the resistance varies highly between inhaler devices, the compendial standard specifies a 4 kPa pressure drop rather than a specific flow rate. Further, the time of collection should be set to not exceed a 2-L collected volume at a constant flow rate (Hickey A.J., 2018b; US-FDA, 2018).

2.3.3 Aerosol delivery rate

Dry powder inhalers are unique among pulmonary drug

delivery devices in that the aerosol is administered on the inspiratory flow of the patient. Consequently, the release of the drug from the device depends on the response of the formulation and device to the energy imparted by the inspiratory flow. The temporal response resulting from this sequence of events gives rise to the aerosol being delivered at a particular rate with respect to the inspiratory flow, which in turn, can affect the site of deposition in the lungs. Multiple studies have compared parameters such as aerodynamic diameter and emitted dose as a function of flow rate. For example, Louey M.D. et al (2006) compared the response of powder dispersion released from a standardized entrainment tube with a flow rate of 28.3 and 60 L/min. The greater flow rate (i.e., 60 L/min) produced increased delivered doses and fine particle fractions (i.e., percentage of particles < 5 μm) and decreased mass median aerodynamic diameters (MMAD) (Louey M.D. et al., 2006). Similarly, Coates M.S. et al. (2005) discovered that powder dispersion and throat deposition increased with increasing air flow. Recently, 25 healthy volunteers were chosen to use an inhaler to provide data for the development of idealized inspiratory waveforms. Using a numerical statistical model, Kugler Sz. et al. (2019) determined that peak inhalation flow and total volume inhaled were the main factors that affect the efficiency of deposition. In another study, flow rates of 30, 40, 60, and 90 L/min were compared across multiple dry powder inhalers (Buttini F. et al., 2016). Here, the differences found due to flow rate were suggested to be inhaler dependent. NEXThaler® and Diskus® inhaler devices were relatively unaffected by flow rate, whereas the Turbohaler® demonstrated a large decrease in emitted dose when the device was operated at 30 or 40 L/min (Buttini F. et al., 2016). The specific resistance of an inhaler is known to control the inspiratory flow rate and dispersion (Clark A.R. and Hollingworth A.M., 1993), which likely causes these discrepancies between different inhaler models.

Due to its important influence in APSD and lung deposition, the aerosol delivery rate can be studied using light obscuration methods that are designed specifically for this purpose or can employ existing methods such as laser diffraction. Ziffels S. et al. (2015) reported a methodology for monitoring aerosol release through a cascade impaction inlet via light obscuration. Briefly, the inlet of the cascade impactor was equipped with a clear tube, a photodiode (940 nm), and a phototransistor (**Fig. 1**). While air flowed through the tube, reductions in voltage between the diode and transistor resulted from obscuration by particles (**Fig. 1A–D**). It was determined that the aerosol transit time through the tube, amplitude of obscuration, and area under the response curve were all influenced by shear conditions (i.e., 1.41 and 4.34 N/m^2) and carrier (**Fig. 1E–F**). These

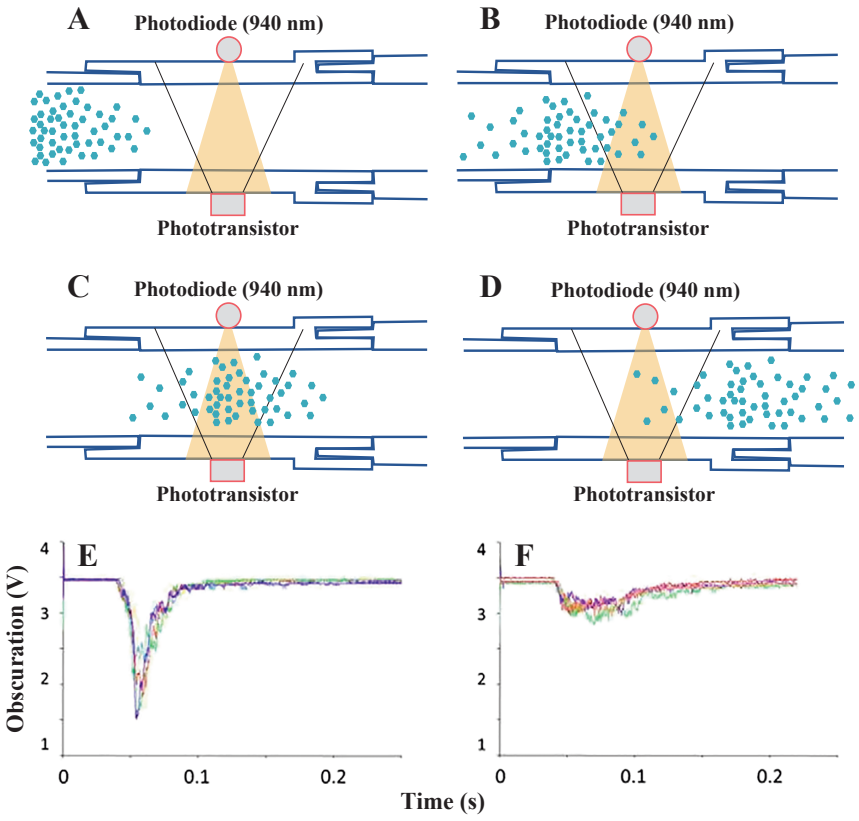

Fig. 1 Schematic of obscuration of the phototransistor by the powder upon **(A)** initial generation, **(B)** entry to, **(C)** peak powder density in, and **(D)** departure from the optical sensing volume. Obscuration of the photodetector by albuterol sulfate delivered with lactose carrier with shear conditions of **(E)** 1.41 N/m^2 and **(F)** 4.34 N/m^2. Figure adapted with permission from Ref. (Ziffels S. et al., 2015). Copyright: (2015) Elsevier.

same variables (i.e., shear conditions and carrier) did not affect the MMAD (Ziffels S. et al., 2015). By only evaluating metrics of APSD and DDU, the role of shear conditions and carrier would be lost, even though they may exhibit an effect in vivo. In a different approach, de Boer A.H. et al. (2002) presented the use of laser diffraction to monitor aerosol dispersion. When the apparatus was combined with a pre-separator to remove large carrier materials, dry powder inhaler formulations were able to be analyzed rapidly with high accuracy and reproducibility. Importantly, it is possible using this method to follow the size distribution in the aerosol cloud as a function of time, facilitating aerosol delivery rate measurements (de Boer A.H. et al., 2002). As the rate of delivery is likely to affect the lung deposition dose as well as the site of deposition, these methodologies allow for in vitro evaluation of aerosol delivery rate.

2.3.4 Physiologically relevant measures

Interest has increased in linking traditional quality measures to predictions of lung deposition as it applies to bioequivalence. Initial observations found that the fine particle fraction of drug aerosol below 3 μm correlated to lung deposition as monitored by gamma scintigraphy (Newman S.P. and Chan H.-K., 2008). This method has since been superseded by sampling through anatomically correct inlets to the cascade impactor and/or physiologically accurate inspiratory flow cycles representing healthy and diseased lung function.

The USP sampling inlet for a cascade impactor, as is described in USP General Chapter <601>, was the first standardized inlet and allowed for meaningful comparisons between labs. However, the USP sampling inlet is a right-angled tube that does not accurately mimic airway geometry (Kaviratna A. et al., 2019). Several approaches have been used to develop realistic mouth-throat models as a sampling inlet to a cascade impactor, including geometries based on cadaver casts or CT/MRI data or idealized geometries using critical airway dimensions (Newman S.P. and Chan H.-K., 2020). Zhang Y. et al. (2007) reported a comparison on mouth-throat deposition from a DPI between the USP sampling inlet, an idealized mouth-throat model, and a highly idealized mouth-throat model. Following analysis, it was discovered that the USP inlet had the lowest mouth-throat deposition (57.3 ± 4.5 %), showing that both the idealized and highly idealized models, whose depositions (67.8 ± 2.2 % and 69.3 ± 1.1 %, respectively) were much closer to the reported in vivo deposition (65.8 ± 10.1 %), improved accuracy of predicting in vivo deposition from in vitro analyses (Zhang Y. et al., 2007). From this study, it was realized that additional factors must be considered when developing and testing mouth-throat models, namely age, peak inspiratory flow, and disease state.

A study reported by Below A. et al. (2013) considered the role of age on mouth-throat geometry. An idealized pediatric upper airway model, approximating age 4–5, was used to evaluate DPI performance comparing three inspiratory flow rates (28–75 L/min). High quantities of drug, up to 80 %, were deposited in the airway model, facilitating potential pulmonary doses of 29 % and 8–12 % for Easyhaler® and Novolizer® devices, respectively (Below A. et al., 2013). With a USP sampling inlet, it is likely that the potential pulmonary dose would be overestimated. Dolovich M.B. et al. (2019) evaluated three mouth-throat models specifically designed from CT scans of three COPD patients using constant inspiratory flow rates of 30, 60, 90, and 120 L/min. In this study, anatomical differences between the three patient models were found to be a major source of variability in lung deposition. The lung dose for each model was relatively consistent across all flow rates with the exception of a slight decrease in delivery at the higher inspiratory flow rates (Dolovich M.B. et al., 2019). These reports highlight the influence of mouth-throat geometry on predicting lung deposition, describing the need for more realistic sampling inlets than the current USP standard. However, an additional factor not considered in these evaluations includes the reality that the inspiratory flow is not a constant value but instead increases and then decreases in rate for each breath.

This dynamic nature of the inspiratory flow is likely to affect lung deposition, necessitating the incorporation of time-dependent breathing profiles to current in vitro analyses (Dorosz A. et al., 2020). In combining the roles of anatomy and breathing profile, Olsson B. et al. (2013) evaluated three throat models, including high, medium, and low filtering efficiency, and three inspiratory profiles. The three profiles were chosen to represent the 5th, 50th, and 95th percentiles from 74 inspiratory flow patterns from healthy adults (**Fig. 2B**). For APSD measurements, the mouth-throat model was utilized as a sampling inlet to a cascade impactor, which was complete with a mixing inlet connecting to both a pressurized air source (to maintain a constant flow across the cascade impactor) and a breath profile generator (to simulate an inspiratory flow cycle for the inhaler) (**Fig. 2A**). Three DPIs were investigated, and it was determined that the mouth-throat geometry had a significant effect for all devices, with smaller throat models facilitating less drug penetrating the model. Further, the flow profile was found to significantly affect two out of three DPIs, where the amount of drug penetrating the model, an estimate of potential lung deposition, was lower for weaker flow profiles (Olsson B. et al., 2013). In another comprehensive analysis, Wei X. et al. (2018) compared eight mouth-throat models, including small, medium, and large Virginia Commonwealth University (VCU) models, small, medium, and large Oropharyngeal Consortium (OPC) models, a medium adult Alberta Idealized Throat, and a USP sampling inlet, as displayed in **Fig. 3**. In addition to the many models, weak, medium, and strong

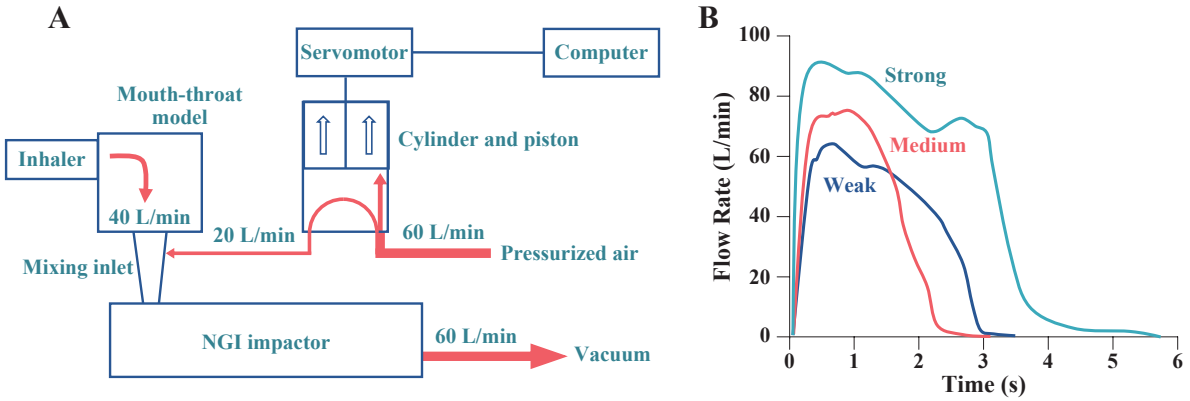

Fig. 2 **(A)** Schematic demonstrating mixing inlet, breath profile generator, and impactor setup. Piston movement allows for flow through inhaler as set using the breath profile generator but holds flow in impactor constant. **(B)** Approximate flow profiles representing weak, medium, and strong flow. Figure adapted with permission from Ref. (Olsson B. et al., 2013). Copyright (2013) Mary Ann Liebert Inc.

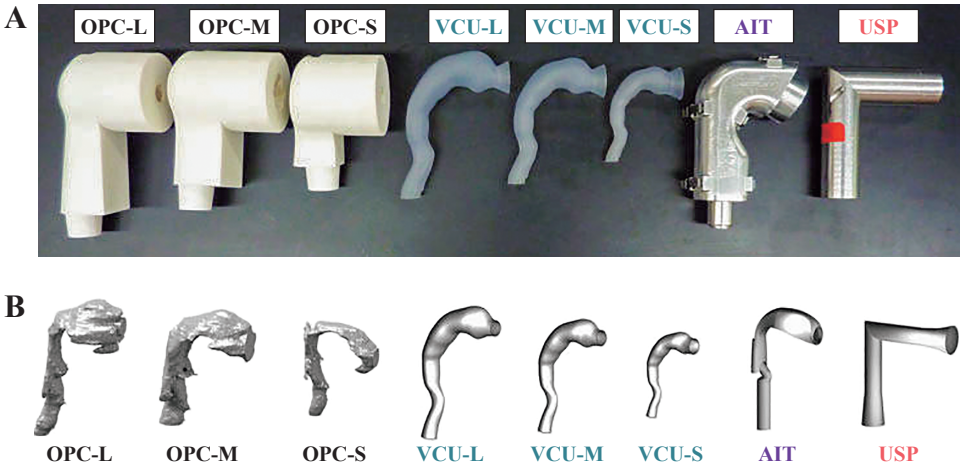

Fig. 3 Realistic mouth-throat models developed for inhaler in vitro testing: OPC large (OPC-L), OPC medium (OPC-M), OPC small (OPC-S), VCU large (VCU-L), VCU medium (VCU-M), VCU small (VCU-S), Alberta Idealized Throat (AIT), and USP sampling inlet. **(A)** side view; **(B)** internal geometry. Figure adapted with permission from Ref. (Wei X. et al., 2018). Copyright (2018) Mary Ann Liebert Inc.

inspiratory flow profiles were included using a breath simulator. Both the model geometry and flow condition had significant effects on mouth-throat deposition; however, the effect of flow condition was stronger than that of the geometry. Consistent with previous reports, the USP throat retained the least amount of drug (Wei X. et al., 2018). In all described studies, it is noted that the mouth-throat geometry and inspiratory flow cycle affect mouth-throat and lung depositions. To enhance correlation between in vitro analyses and in vivo outcomes, the incorporation of these variables into in vitro analyses is essential.

3. Data analysis

Following the collection of data through the methods presented above, further analysis must be performed to extract meaningful conclusions. Further, analytical methods for comparing these results between different formulations and devices, such as in the case of preparing generic versions of brand-name drugs, must be implemented. These necessities give rise to statistical descriptors for understanding the data and profile comparison techniques, such as the chi-square comparison and multivariate statistical analysis techniques, as described below.

3.1 Statistical descriptors

Historically, cascade impactor data was described in terms of population statistics. Each particle deposits according to its aerodynamic diameter, which is defined as a unit density sphere with the same terminal settling velocity as the real particle according to Stokes' Law. This aerodynamic diameter encompasses particle attributes including shape, density, and physical size (Hickey A.J., 2004). However, particles exist in populations, powders, that can be described in terms of a central tendency of the distribution and its breadth. Many distributions conform to a log-normal mathematical function and consequently can be described by a median according to the mass deposited on each stage, thereby giving rise to a MMAD. The MMAD also represents a degree of deaggregation, where smaller MMAD values (i.e., decreased median aerodynamic diameter)

represent greater particle deaggregation (Louey M.D. et al., 2006). As the MMAD is calculated using the logarithm of particle size in a geometric rather than arithmetic function, the geometric standard deviation (GSD) represents the variance of the distribution as a unitless dimension. The relationship between GSD and MMAD is as follows:

$$\mathrm{GSD}=\frac{\mathrm{MMAD}}{d_{16}}=\frac{d_{84}}{\mathrm{MMAD}}=\sqrt{\frac{d_{84}}{d_{16}}} \quad (1)$$

Where d_{16} represents the aerodynamic diameter one standard deviation below the median (at the 16th percentile) and d_{84} represents the aerodynamic diameter one standard deviation above the median (at the 84th percentile) (Chaurasiya B. and Zhao Y.-Y., 2021; Finlay W.H. and Darquenne C., 2020; Hickey A.J., 2004). Aerosols with a GSD greater than 1.15 are considered to be polydisperse, where larger values indicate greater levels of heterogeneity in the size distribution (Chaurasiya B. and Zhao Y.-Y., 2021; Pleasants R.A. and Hess D.R., 2018). Most therapeutic aerosols exhibit GSDs in the range of 2–3 (Pleasants R.A. and Hess D.R., 2018).

Oftentimes, conversion of the APSD to something that may indicate potential lung deposition is beneficial. In these cases, the fine particle dose (FPD) and fine particle fraction (FPF) are defined. While exact cutoffs vary, the FPD is generally referred to as the total dose of dry powder, in terms of mass, that is below 3–5 µm (Chaurasiya B. and Zhao Y.-Y., 2021; Dunbar C.A. et al., 1998). The FPF normalizes the FPD to the total emitted dose (Pleasants R.A. and Hess D.R., 2018). Further, this metric represents a degree of drug deaggregation, where a higher FPF indicates greater deaggregation (Louey M.D. et al., 2006).

3.2 Profile comparisons

While statistical descriptors as described above are useful for data interpretation, the US FDA advises that it is inadequate to characterize the APSD only in terms of MMAD, GSD, and FPD/FPF (US-FDA, 2018), especially when trying to compare profiles for bioequivalence determinations. As such, multiple techniques in comparing APSD profiles have been investigated. The major goals of a profile comparison test, as specified by the Product Quality Research Institute (PQRI) and Orally Inhaled and Nasal Drug Products Technical Committee (OINDP-TC) include: (1) the test is sensitive to differences at each impactor deposition site; (2) the test is based on a single metric that incorporates all differences at all sites to minimize the number of in vitro tests that must be performed; (3) the test is applicable to all inhalation products; and, (4) the test is independent of impactor type (Adams W.P. et al., 2007). With these goals in mind, multiple approaches have been presented.

3.2.1 Stage-by-stage and stage-grouping comparison

The most straightforward method of profile comparisons involves comparing the mass of drug deposited on each stage individually or as groups of stages (EMEA, 2009; Taki M. et al., 2011). This methodology is based on the premise that by comparing only MMAD, GSD, and FPD/FPF, changes to the distribution in the 1–5 µm range may be overlooked in vitro but could affect the drug deposition site in the lungs. The European Medicines Agency (EMEA) specified that two products must be within 15 % of each other at each deposition stage, or at 4 justified grouped stages, when tested at all flow rates to be considered equivalent (EMEA, 2009). While this method is sensitive to differences in mass at each impactor stage, it does not provide a single metric for testing equivalence and does not allow for comparisons between different impactor configurations or types.

3.2.2 Chi-square comparison

The chi-square ratio test does not ascribe meaning to the data under consideration but does allow comparison of two data sets which historically were considered the test and reference profile. The chi-square statistic, calculated as the sum of the squared differences in deposition at each impaction site between the two profiles, scaled by the average deposition on that site, provides a measure of distance between two profiles (Adams W.P. et al., 2007). The developed singular test metric is a comparison of the chi-square statistics characterizing both products (Adams W.P. et al., 2007). However, both products must be tested the same way with the same impactor configuration. This test allows statistical inferences to be drawn but requires subjective input to assure that meaning ascribed to the particle size distribution (i.e., two profiles are the same), is correct by observation.

The PQRI working group conducted an assessment in which they separated the impactor sized mass from the fine particle fraction and applied chi-square analysis. Subsequently, experts in the field were asked to observe the profile being compared to confirm that the chi-square ratio test was predicting similarity as would be defined by simply comparing the data. The goal of this assessment was to determine a critical value of the chi-square ratio that would separate identical profiles from those that are different (Adams W.P. et al., 2007). Unfortunately, it was discovered that choosing a singular value to separate equivalent from different profile pairs was difficult (Christopher D. et al., 2007b). Proposed critical values included 7.66 and 2.75. While 2.75 provided better discriminatory power, it resulted in less consistency when compared to the judgment of experts in the working group. While a promising method, no guidelines have been set at this time for evaluating using this test (Christopher D. et al., 2007a).

3.2.3 Multivariate analysis

Cascade impaction data is complicated by the fact that deposition on each stage is accompanied by variability from each replicate, while the distribution itself represents information on the variability of particle sizes, carrying the consequences for the behavior of the aerosol when inhaled. Multivariate analysis techniques, such as principal component analysis (PCA), allow the simplification of data while retaining as much information as possible. PCA is an orthogonal linear transformation that transforms the data to a new coordinate system, where the largest variance is accounted for on the first coordinate, the second greatest variance is accounted for on the second coordinate, and so on (Christopher J.D. et al., 2013). This process reduces the number of variables that must be considered by developing principal components through a purely statistical model. The threshold for equivalence using this method is related to a specified confidence interval (Christopher J.D. et al., 2013).

Another method based on multivariate analysis involves orthogonal partial least squares analysis (OPLS). While PCA is an unsupervised statistical method and is generally used as a pattern recognition tool prior to OPLS, OPLS itself is a supervised method (Shi S. and Hickey A.J., 2009). Importantly, OPLS is not a statistical test but is an analytical tool. The resulting OPLS score plot provides a visual representation of the comparison between a test and reference profile. The larger the visual difference, the further they are separated based on principal components. However, this is a qualitative test; to derive a semi-quantitative parameter, R^2 can be employed. Two identical profiles would give an R^2 value of zero whereas two completely different profiles would give an R^2 value of one (Shi S. and Hickey A.J., 2009). The defined equivalence parameter as reported by Shi S. and Hickey A.J. (2009) is termed Eq, where one subtracted by the R^2 value gives this measure of equivalence. While not a purely statistical method, this proposed metric allows for semi-quantitative analysis when comparing two APSD profiles.

3.3 Efficient data analysis

Full APSD evaluations from a cascade impactor require time and resources that may not be necessary for formulation and device optimization as well as routine quality control analyses. While these evaluations need to be accurate and precise, it is highly desired that throughput be increased compared to full cascade impactor and data analyses (Tougas T.P. et al., 2011). Two concepts have been introduced to expedite data acquisition and subsequent analysis: the abbreviated impactor measurement (AIM) concept and the efficient data analysis (EDA) concept.

The AIM concept involves altering the configuration of the cascade impactor to streamline analysis. Rather than provide each stage's measurement, unnecessary stages are removed depending on the goal of the analysis. For example, a configuration for quality control (AIM-QC) to monitor the MMAD includes a sampling inlet and initial stage, an intermediate stage near the MMAD, and a final filter. The intermediate stage as a boundary should split the powder in half to a small particle mass (SPM) and large particle mass (LPM). If the SPM and LPM portions do not each contain 50 % of the impactor-sized powder mass, it can be determined that the MMAD has shifted (Tougas T.P. et al., 2011). A different configuration has been proposed for predicting human respiratory tract deposition (AIM-pHRT), containing two intermediate stages in addition to a sampling inlet and final filter. With this method, the first stage sets a boundary at approximately 5 µm, where the mass deposited after that stage is referred to as the fine particle mass, or FPM. The second stage is set at a boundary of approximately 1 µm, where the mass of particles deposited after that stage denotes the extra-fine particle mass (EPM) (Tougas T.P. et al., 2011). Quantification of the EPM may be desired as this portion may be related to systemic absorption or may be exhaled before deposition can occur, especially if one's breath is not held following inhalation (Tougas T.P. et al., 2011). However, at a minimum for the AIM-pHRT configuration, it is necessary to distinguish the FPM, which describes the mass of particles with the potential to carry drug into the respiratory tract, from the coarse particle mass, which will likely not penetrate past the oropharyngeal region (Mitchell J.P. and Tougas T.P., 2013).

Efficient data analysis represents a way to simplify AIM data as well as full-resolution cascade impaction data. Two EDA metrics are defined: (1) the large and small particle mass (LPM + SPM), and (2) their ratio (LPM/SPM) (Tougas et al., 2011). These metrics signify the amplitude of the mass distribution profile and the position of the

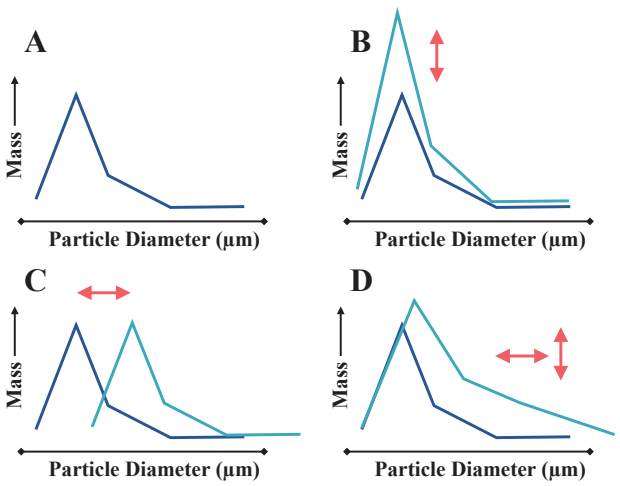

Fig. 4 Changes in aerodynamic particle size distribution. **(A)** Nominal APSD, **(B)** shift in area under the curve **(C)** shift in central tendency (i.e., mean), and **(D)** shift in both mean and area. Figure adapted with permission from Ref. (Tougas T.P. et al, 2009). Copyright (2009) Springer Nature.

profile on the size scale and can detect changes in position or total area independently of each other (**Fig. 4**) (Tougas T.P. et al., 2009). When EDA is combined with an AIM approach, the time per measurement is drastically reduced, increasing throughput, and sensitivity to APSD shifts is enhanced (Mitchell J.P. and Tougas T.P., 2013). A general recommendation proposed by Mitchell J.P. and Tougas T.P. (2013) includes full resolution cascade impaction for initial screening and developing, AIM-QC for quality control measures on a smaller list of candidates; and AIM-pHRT with an anatomically correct sampling inlet for in vitro equivalence comparisons.

4. Data interpretation

The implications of data obtained from in vitro testing for safety and efficacy in vivo is an important potential application, but so far, a true in vitro/in vivo correlation has been difficult to establish. The following sections describe some considerations that have been given to this topic and illustrate the significance that success in this endeavor would have for pharmaceutical product development.

4.1 Inhaled biopharmaceutical classification system

The gastro-intestinal biopharmaceutical classification system (giBCS), which defines the desirability of developing a drug for oral delivery based on its solubility and permeability, has been a working guide for drug discovery and delivery since the 1990s (Hastedt J.E. et al., 2016). The approach is usually depicted as a quadrant schematic in which drugs that are highly soluble and permeable are logical candidates for further development, drugs that have low solubility and high permeability, or vice versa, may be considered candidates for formulation development to improve their properties, and those with low solubility and permeability are considered poor candidates for further development (Hickey A.J., 2018b). Using these criteria, candidate selection can be addressed early in the discovery process where many analogues are available for consideration.

In 2015, a workshop cosponsored by the American Association of Pharmaceutical Sciences (AAPS), US FDA, and USP was held to address the possibility of developing an inhaled biopharmaceutical classification system (iBCS) (Bäckman P. et al., 2022; Hastedt J.E. et al., 2016; 2022). It is important to recognize that the majority of inhaled pharmaceutical agents are intended for localized action. Consequently, permeability as defined for the giBCS may not be relevant. In addition, unlike the gastro-intestinal tract that is essentially a tube through which substances pass by entry and exit, the lungs are a closed container in which clearance mechanisms are required to remove any deposited substances. These differences suggest that alternate or additional metrics must be adopted when developing an iBCS. Considerations for developing an iBCS include lung physiology, regional aerosol deposition, clearance mechanisms, particle dissolution, permeability, and absorption (Bäckman P. et al., 2022; Frohlich E., 2019; Hastedt J.E. et al., 2016; 2022). From this workshop, it was determined that there is an opportunity in creating this model, and additional work on this topic is forthcoming.

4.2 Bioequivalence and in vitro/in vivo correlation

The goal of predicting in vivo deposition from in vitro data has been an objective of pharmaceutical scientists for decades. With the significant increase in new products over the last 20 years and the desire to bring forward generic versions, this objective has taken on urgency. When performing a comparison for bioequivalence, the goal is to demonstrate equivalent performance (i.e., not better, not worse) (Lyapustina S., 2018). However, the complexity of aerosol dosage forms makes them difficult to mimic and raises many questions about what defines equivalence (Adams W.P. et al., 2010; Apiou-Sbirlea G. et al., 2013). In oral formulations, the main way to analyze bioequivalence is through serum concentration over time; however, with inhaled formulations, the lung is the target and systemic absorption is typically not desired. One metric that has been suggested for inhaled drug bioequivalence studies is to look at the concentration of drug in exhaled breath condensate (Khoubnasabjafari M. et al., 2019). Though no direct guidance has been placed forward as of yet for inhaled therapeutics bioequivalence comparisons (Apiou-Sbirlea G. et al., 2013), profile comparisons based on cascade impactor data have garnered the most interest, as described above. However, regulatory statures for comparing inhaled drugs for bioequivalence both in vitro and in vivo are urgently needed.

From the foregoing discussion, it can be seen that in vitro methods have evolved from standards that would establish therapeutic aerosol quality in terms of accuracy and reproducibility of performance to physiologically relevant methods that would produce data predictive of lung deposition and disposition. Current compendial in vitro standards have not consistently correlated with pharmacokinetics and efficacy in vivo (Hickey A.J., 2018b). However, if physiologically relevant advances can be made to these standards, then there is the possibility that in vivo studies can be limited to the first instance of drug testing and perhaps, in the future, eliminated as a requirement for regulatory approval.

For dry powder inhalers, physiologically relevant measures of APSD and DDU would seem to be the first step towards in vitro/in vivo correlation (IVIVC). The goal of IVIVC is to construct a model that can be used to predict in vivo outcomes, such as lung deposition and peripheral-to-central deposition ratio, from in vitro cascade impactor data (Chow M.Y.T. et al., 2021). In vivo, peripheral-to-central deposition ratios have been shown to

qualitatively deviate between healthy patients and those with asthma or COPD, where patients with lung diseases exhibit slightly less drug deposition in the lung periphery. It was also proposed that differences in peripheral-to-central deposition ratios in vivo may be due to intersubject variability caused by differences in airway dimensions, which occurred to a stronger extent than variability resulting from flow rate or delivery modality (Clark A.R., 2012). As such, additional parameters incorporated within cascade impaction analysis, such as physiological throat models and representative inspiratory flow cycles of healthy and diseased individuals, may prove beneficial in such correlations. As noted above, since the point at which the aerosol is dispersed on the inspiratory flow may also affect deposition, a measure of the aerosol delivery rate should be considered as an additional in vitro analysis for the prediction of in vivo outcomes.

5. Conclusions

Pharmaceutical inhalation aerosols are widely used to treat diseases, notably asthma, COPD, infectious diseases, and rare diseases, such as cystic fibrosis. Dry powder inhaler products represent a significant proportion of the market. To assure safety and efficacy of these products, their quality and performance must be assured in terms of the accuracy and reproducibility of drug delivery. Since the effective dose depends on efficient aerosol generation and deposition in the lungs, properties that guarantee these outcomes must be measured. Standardized methods have been available for decades to define properties that establish quality, but recent efforts have focused on rendering these quality measures relevant to in vivo deposition and link to safety and efficacy. As these methods emerge, the role of compendial methods and regulatory guidance may progress towards in vivo relevance. Ultimately, this may support considerations of an inhaled biopharmaceutical classification system, which can be extrapolated to bioequivalence testing in a manner similar to drugs delivered by the gastro-intestinal route of administration.

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Nomenclature

AAPS	American Association of Pharmaceutical Sciences
AFM	Atomic force microscopy
AIM	Abbreviated impactor measurement
API	Active pharmaceutical ingredient
APSD	Aerodynamic particle size distribution
CF	Cystic fibrosis
COPD	Chronic obstructive pulmonary disease
CT	Computed tomography
DDU	Delivered dose uniformity
DPI	Dry powder inhaler
DSC	Differential scanning calorimetry
EDA	Efficient data analysis
EP	European Pharmacopeia
EPM	Extra-fine particle mass (μg, mg)
Eq	Equivalence parameter
FPD	Fine particle dose (μg, mg)
FPF	Fine particle fraction (%)
FPM	Fine particle mass (μg, mg)
giBCS	Gastro-intestinal biopharmaceutical classification system
GSD	Geometric standard deviation
iBCS	Inhaled biopharmaceutical classification system
ICH	International Conference on Harmonization
IGC	Inverse gas chromatography
JP	Japanese Pharmacopeia
LPM	Large particle mass (μg, mg)
MDI	Metered dose inhaler
MMAD	Mass median aerodynamic diameter (μm)
MRI	Magnetic resonance imaging
NGI	Next generation impactor
OINDP	Orally inhaled and nasal drug products
OPC	Oropharyngeal Consortium
OPLS	Orthogonal partial least squares analysis
PCA	Principal component analysis
PQRI	Product Quality Research Institute
RH	Relative humidity (%)
SEM	Scanning electron microscopy
SPM	Small particle mass (μg, mg)
TGA	Thermogravimetric analysis
US FDA	United States Food and Drug Administration
USP	United States Pharmacopeia
VCU	Virginia Commonwealth University
XPS	X-ray photoelectron spectroscopy
XRPD	X-ray powder diffraction

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Authors' Short Biographies

Sara E. Maloney

Dr. Sara Maloney is currently a postdoctoral fellow at RTI International in Research Triangle Park, NC. She obtained her B.S. in chemistry and forensic chemistry from Towson University and her Ph.D. in analytical chemistry from the University of North Carolina at Chapel Hill under the direction of Dr. Mark H. Schoenfisch. Her current research interests include therapeutic aerosol formulation, characterization, and delivery as well as development of antibacterial biomaterials for lung infections.

Jeffrey B. Mecham

Dr. Mecham is a Research Chemist in RTI International's Center for Engineered systems, in the Technology Advancement and Commercialization Division. In this capacity, he is responsible for the design and synthesis of monomers, polymers, and nanoparticles (at the micro- and nano-scale) with targeted performance capabilities for a wide array of applications. His team are contributors to the Center's successful development of particle-based controlled release technologies for triggered release of therapeutics and chemistries for environmental remediation. The development of such materials over the last several years at RTI has resulted in IP that has been licensed by interested parties for further development and subsequent commercialization of particles with controlled release properties.

Anthony J. Hickey

Dr. Hickey is a Distinguished Fellow at RTI International, Professor Emeritus of Pharmaceo-engineering and Molecular Pharmaceutics, and Adjunct Professor of Biomedical Engineering at the University of North Carolina at Chapel Hill. He obtained Ph.D. and D.Sc. degrees in pharmaceutical sciences from Aston University, Birmingham, UK. He is a Fellow of the American Association for the Advancement of Science, the American Association of Pharmaceutical Scientists, the Royal Society of Biology, and the Royal Society of Medicine, and member of the Pharmaceutical Dosage Forms Expert Committee (DFEC) and Aerosols Sub-Committee of the DFEC of the United States Pharmacopeia. Dr. Hickey is a highly published (over 300 papers) and cited author having edited or authored 16 books on pharmaceutical aerosols, particulates, manufacturing and complex systems. He holds over 25 patents and conducts multidisciplinary research programs in the field of pulmonary drug and vaccine delivery for the treatment and prevention of a variety of diseases.